Effect of polyhydroxyalkanoates on dark fermentative hydrogen production from waste activated sludge

Dongbo Wang, Guangming Zeng, Yinguang Chen, Xiaoming Li

A B S T R A C T

Polyhydroxyalkanoates (PHA), an intracellular energy and carbon storage polymer, can be accumulated in activated sludge in substantial quantities under wastewater dynamic treatment (i.e., substrate feast-famine) conditions. However, its influence on hydrogen production has never been investigated before. This study therefore evaluated the influences of PHA level and composition in waste activated sludge (WAS) on hydrogen production. The results showed that with the increase of sludge PHA content from 25 to 178 mg per gram volatile suspended solids (VSS) hydrogen production from WAS alkaline anaerobic fermentation increased from 26.5 to 58.7 mL/g VSS. The composition of PHA was also found to affect hydrogen production. When the dominant composition shifted from polyhydroxybutyrate (PHB) to polyhydroxyvalerate (PHV), the amount of generated hydrogen decreased from 51.2 to 41.1 mL/g VSS even under the same PHA level (around 130 mg/g VSS). The mechanism studies exhibited that the increased PHA content accelerated both the cell solubilization and the hydrolysis process of solubilized substrates. Compared with the PHB-dominant sludge, the increased PHV fraction not only slowed the hydrolysis process but also caused more propionic acid production, with less theoretical hydrogen generation in this fermentation type. It was also found that the increased PHA content enhanced the soluble protein conversion of non-PHA biomass. Further investigations with enzyme analyses showed that both the key hydrolytic enzyme activities and hydrogen-forming enzyme activities were in the sequence of the PHB-dominant sludge > the PHV-dominant sludge > the low PHA sludge, which was in accord with the observed order of hydrogen yield.

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1. Introduction

The usage of fossil fuel is generally considered as unsustainable due to its diminishing supply and large contribution to greenhouse gas generation (Lingampalli et al., 2013). Meanwhile, waste activated sludge (WAS), which is a byproduct of biological wastewater treatment, is inevitably produced in huge quantities (Xu et al., 2012). Therefore, biological production of hydrogen from WAS has attracted much attention (Cai et al., 2004; Li et al., 2009; Zhao et al., 2010), by which fossil fuel is saved, WAS is reduced and reused, and the important renewable energy hydrogen is also achieved.

In general, the rate of hydrogen production from WAS is low, thus most of previous studies to date have focused on the enhancement of hydrogen generation efficiency via pretreating sludge (Yang et al., 2012; Assawamongkolsiri et al., 2013; Kim et al., 2013), controlling operational parameter (Saady, 2013; Gioannis et al., 2013; Zhou et al., 2013), and improving reactor design (Saady, 2013; Jung et al., 2011). For example, it was found that hydrogen production from WAS could be significantly enhanced by controlling fermentation pH at constant 10, because this strategy not only improved the hydrolysis process but also inhibited the activities of hydrogen consuming bacteria of both methanogens and acetobacteria (Zhao et al., 2010). Besides, it is known that WAS is a nitrogen-rich substrate with low carbon to nitrogen ratios (around 7/1) whereas the recommended C/N ratio for anaerobic fermentation system is 20/1 to 30/1 (Kim et al., 2012). Hence, several researches were performed to improve hydrogen yield through optimizing co-fermentation substrates. It was reported that the bioconversion of sludge protein and the yield of hydrogen could be largely increased by pertinent addition of carbohydrate-rich substrates, such as primary sludge, food wastes, and agricultural wastes to WAS fermentation reactors (Saady, 2013; Zhou et al., 2013; Kim et al., 2012; Chen et al., 2012; Liu et al., 2013). Despite these important progressions, the enhancement of hydrogen production from WAS by improving the self-characteristic of sludge has been seldom documented in the literature.

Polyhydroxyalkanoates (PHA), an intracellular metabolic intermediate and energy and carbon storage polymer in wastewater treatment processes, has the ability of rapid and complete degradation under anaerobic conditions (Reischwitz et al., 1998; Chen and Wang, 2002). PHA can be accumulated in the external substrate feast stage, but the accumulated PHA is easily consumed in the subsequent famine stage. As a result, its content in WAS wasted from the traditional wastewater treatment plants (WWTPs) is usually at low levels (Fig. S1, Supporting Information). When using this WAS for anaerobic fermentation, as mentioned above, the rate of hydrogen production is low. Recently, there have been increasing evidences showing that WAS with high levels of PHA can be obtained in WWTPs either by process improvement or by operation optimization. Takabatake et al. (2002) reported that activated sludge biomass from 4 real WWTPs had the capability to accumulate PHA up to 18.8% of dry cell weight on average, with the range of 6.0%–29.5%. Coats et al. (2007) found activated sludge consortiums capable of synthesizing PHA at 10–25% when fed with primary solid fermented liquors. Based on the results, they further proposed a side-stream process for both PHA production and wastewater treatment. In our recent studies, it was observed that PHA content in WAS withdrawn from a biological phosphorus removal reactor reached 116 ± 5 mg per gram volatile suspended solids (VSS) by wasting sludge at 1 h of aeration (Wang et al., 2013).

The increase of PHA content in WAS might cause the changes of sludge characteristics, which further affected the subsequent anaerobic fermentation. To date, however, the influence of PHA on hydrogen production from WAS has never been reported. Some scientists suggested that the microbial cells would become more fragile with the increase of intracellular PHA (Budwill et al., 1992; Page and Cornish, 1993; Lee, 1996). Thus, it is presumed that the increased PHA in WAS might be beneficial to hydrogen production. If this hypothesis is clearly supported by experimental evidences, a new door may be opened for both wastewater treatment and hydrogen production from WAS. That is, organic pollutants in wastewaters are designed to be primarily removed via PHA accumulation, and then the WAS with high levels of PHA is used for hydrogen production, by which aeration cost in wastewater treatment process is saved, WAS amount is reduced, and hydrogen yield in WAS anaerobic fermentation is enhanced.

The aim of this paper was to provide a deep understanding of PHA associated with hydrogen production in dark fermentation. First, the influences of PHA level and composition in WAS on anaerobic hydrogen production were investigated in batch tests at pH 10. It was reported that alkaline conditions (especially pH 10) were beneficial to hydrogen production from WAS (Cai et al., 2004; Zhao et al., 2010), because this method not only enhanced the hydrolysis process but also inhibited the activities of hydrogen consuming bacteria (Zhao et al., 2010). Then, the reasons for PHA affecting the yield of hydrogen production were explored from the aspects of the microbial cell disruption, solubilized substrate hydrolysis, acidification of hydrolyzed products, fermentation type, mass balance, and activities of key enzymes.

2. Materials and methods

2.1. The source of sludges with different PHA contents

The following activated sludge bioreactors were performed to culture the sludges with different PHA contents as such characteristic sludges are not available now in real WWTPs. Seed sludge was taken from the secondary sedimentation tank of a municipal WWTP in Shanghai, China, and was concurrently inoculated into five identical sequencing batch reactors with a working volume of 40 L each. All reactors were carried out the same and operated with four cycles (6 h per cycle) daily. Each cycle consisted of a 240 min aerobic period, a 55 min settling period, a 5 min decanting period, and a 60 min idle period. During the aerobic period, air was supplied into all reactors at a flowrate of 20 L/min. To obtain sludges with different PHA contents, these reactors received 200, 400, 600, 800, and 1000 mg/L of influent chemical oxygen demand (COD)
concentrations (acetate was the sole carbon source), respectively. The concentrations of other nutrients in these synthetic media were the same and were presented as below (per liter): 0.1 g NH₄Cl, 0.04 g KH₂PO₄, 0.01 g MgSO₄·7H₂O, 0.005 g CaCl₂, and 0.5 mL of a trace element solution. The composition of trace element solution was documented in previous publication (Wang et al., 2008). After settling period 30 L supernatant was discharged from each reactor and replaced with 30 L respective medium during the first 6 min of subsequent aerobic period. About 5.7 L mixture was daily wasted from each reactor at 1.5 h aeration of the second cycle, thus the sludge retention time was maintained at approximately 7 d. After operation for about 60 d the five reactors reached stable, and then the wasted sludges were used in the following anaerobic fermentation tests. These wasted sludges were concentrated at 4 °C for 12 h before use.

2.2. The source of sludges with different PHA compositions

To obtain the sludges with different polyhydroxybutyrate (PHB)/polyhydroxyvalerate (PHV) fractions but similar total PHA amount, the following activated sludge bioreactors were conducted. Five identical reactors, as described above, were performed and received the synthetic media with different ratios of acetate to propionate but the same COD concentration. It is widely accepted that the composition of wastewater can affect PHB-PHV fraction (Li and Yu, 2011), and the pertinent increase of propionate concentration in wastewaters will increase the PHV fraction of PHA (Chen et al., 2004). These reactors received media with 800 mg/L of influent COD concentration, which were prepared with 100% acetate, 85% acetate +15% propionate, 70% acetate +30% propionate, 55% acetate +45% propionate, and 40% acetate +60% propionate, respectively. Hereinafter, the sludges withdrawn from these reactors were defined as sludge-I, sludge-II, sludge-III, sludge-IV, and sludge V, respectively. On each day, about 5.7 L of sludge-I, sludge-II, sludge-III, sludge-IV, and sludge V mixtures were respectively withdrawn from these reactors at proximately 100, 90, 80, 70, and 65 min of aeration, because it was measured via batch tests that these sludges contained similar PHA content at these times. All the other operations were the same as those depicted above. It took 54 d before these reactors achieved stable characteristic of wasted sludge, and then the wasted sludges began to be used for anaerobic fermentation trials. Before use, these wasted sludges were also concentrated at 4 °C for 12 h.

2.3. The effect of PHA content and composition on hydrogen production

The batch tests were performed in ten serum bottles with a working volume of 0.6 L each. Ten serum bottles were divided into two groups with five in each. One group (group-I) was used to evaluate the effect of PHA content on hydrogen production while the other group (group-II) was employed to investigate the PHA composition’s influence. Five serum bottles of group-I were respectively fed with 300 mL of sludge-200, sludge-400, sludge-600, sludge-800, and sludge-1000, and meanwhile the other five serum bottles (group-II) were fed with 300 mL of sludge-I, sludge-II, sludge-III, sludge-IV, and sludge-V, respectively. The pH value of sludge mixtures in both group-I and group-II was adjusted to 10 by adding 4 M hydrochloric acid (HCl) or 4 M sodium hydroxide (NaOH). Oxygen in the bottles was removed from the headspace by nitrogen gas sparging for 30 s. After that, all bottles were capped with rubber stoppers, sealed, and placed in an air-bath shaker (120 rpm) at 37 ± 1 °C. During the whole fermentation period (10 d), the pH value in all bottles was controlled to 10.0 ± 0.1 by adding 4 M HCl or 4 M NaOH with an automatic titrator. It should be noted that no extra inoculum was added into these fermentation reactors, and therefore WAS was used for both substrate and inoculum in this study. The total gas volume was determined via releasing the pressure in the bottle using a glass syringe (300 mL) to equilibrate with the room pressure according to the method documented in the literature (Owen et al., 1979). As the syringe was always in the bottle, the accumulative volume was followed with time. The cumulative volume of hydrogen gas was calculated by the following equation described in previous publications (Zhao et al., 2010; Oh et al., 2003).

\[
v_{H_2}^{i} = v_{H_2}^{i-1} + c_{H_2}^{i} \times v_{C_1} - c_{H_2}^{i+1} \times v_{C_1}^{i-1} \quad (1)
\]

Where, \(v_{H_2}^{i}\) and \(v_{H_2}^{i-1}\) are respectively the cumulative volumes of hydrogen gas in the current (i) and previous (i-1) time intervals, \(v_{C_1}\) and \(v_{C_1}^{i-1}\) are respectively the total gas volumes in the current and previous time intervals, and \(c_{H_2}\) and \(c_{H_2}^{i-1}\) are the fractions of hydrogen gas measured by gas chromatography in the current and previous time intervals, respectively.

2.4. Long-term semi-continuous reactor operation for the analysis of key enzymes

Three typical sludges, which were respectively the low PHA sludge (sludge-200), the PHB-dominant sludge (sludge-I), and the PHV-dominant sludge (sludge-V), were selected to be fed to three semi-continuous reactors for the analysis of key enzymes relevant to hydrogen production. The three semi-continuous reactors were identical with a working volume of 0.6 L each. The three reactors received 400 mL of sludge-200, sludge-I, and sludge-V, respectively, and the fermentation conditions were the same as described above. According to the results obtained from the above batch tests the sludge retention time was maintained at 7, 4.5, and 5.5 d in the sludge-200, sludge-I, and sludge-V fermentation reactors, respectively. Every day, 57, 89, and 73 mL of fermentation mixtures were manually withdrawn from the sludge-200, sludge-I, and sludge-V fermentation reactors, respectively. Then, the same amounts of new sludge-200, sludge-I, and sludge-V were respectively added into these reactors, which resulted in the VSS loading rate of 1.73 kg/(m³ d) in the sludge-200 fermentation reactor, 2.63 kg/(m³ d) in the sludge-I fermentation reactor, and 2.22 kg/(m³ d) in the sludge-V fermentation reactor. After that, all reactors were sparged with nitrogen gas for 30 s to remove oxygen before they were re-capped and re-sealed. After operation for about 80 days, hydrogen yield reached stable, and then the assay of key enzyme activities was performed.
Table 1 – The main characteristics of the sludges withdrawn from the five reactors fed with different influent COD concentrations.a

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sludge-200</th>
<th>Sludge-400</th>
<th>Sludge-600</th>
<th>Sludge-800</th>
<th>Sludge-1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>13,595 ± 306</td>
<td>13,608 ± 280</td>
<td>13,549 ± 393</td>
<td>13,835 ± 328</td>
<td>13,710 ± 375</td>
</tr>
<tr>
<td>VSS</td>
<td>12,120 ± 285</td>
<td>12,128 ± 271</td>
<td>12,174 ± 332</td>
<td>12,310 ± 410</td>
<td>12,243 ± 296</td>
</tr>
<tr>
<td>Total COD</td>
<td>14,290 ± 240</td>
<td>14,310 ± 310</td>
<td>14,220 ± 420</td>
<td>14,550 ± 370</td>
<td>14,370 ± 390</td>
</tr>
<tr>
<td>Total proteinb</td>
<td>575 ± 29</td>
<td>556 ± 23</td>
<td>542 ± 20</td>
<td>517 ± 27</td>
<td>491 ± 18</td>
</tr>
<tr>
<td>Total carbohydratec</td>
<td>231 ± 14</td>
<td>202 ± 11</td>
<td>181 ± 9</td>
<td>165 ± 13</td>
<td>142 ± 7</td>
</tr>
<tr>
<td>PHA</td>
<td>25 ± 3</td>
<td>87 ± 7</td>
<td>107 ± 8</td>
<td>146 ± 12</td>
<td>178 ± 11</td>
</tr>
<tr>
<td>Lipid and oil</td>
<td>5.8 ± 0.4</td>
<td>5.5 ± 0.8</td>
<td>5.7 ± 0.9</td>
<td>5.3 ± 0.8</td>
<td>5.2 ± 0.5</td>
</tr>
</tbody>
</table>

a Results are the averages and their standard deviations of triplicate measurements. Sludge-X represents the sludge withdrawn from the reactor which is fed with X mg/L COD. The unit for total suspended solids, VSS, and total COD is mg/L while the remainder is expressed in mg/g VSS.
b EPS protein content in sludge-200, sludge-400, sludge-600, sludge-800, and sludge-1000 was respectively 56 ± 4, 52 ± 5, 49 ± 3, 55 ± 7, and 51 ± 5 mg/g VSS.
c EPS carbohydrate content in sludge-200, sludge-400, sludge-600, sludge-800, and sludge-1000 was respectively 26 ± 2, 28 ± 3, 24 ± 3, 25 ± 5 and 23 ± 2 mg/g VSS.
d The percentages of PHB, PHV, and PH2MV are 77.3%, 19.5%, and 3.2% in the sludge-200, and 79.5%, 18.6%, and 1.9% in the sludge-400, and 77.6%, 18.9%, and 3.5% in the sludge-600, and 80.1%, 17.5%, and 2.4% in the sludge-800, and 78.3%, 20.2%, and 1.5% in the sludge-1000, respectively.

2.5. Batch fermentation test of the effect of sludge PHA content on cell disruption

To eliminate the potential impact of microbial composition on cell disruption, the following batch fermentation test was conducted. Two fermentation reactors were performed. The sludges fed to the two fermentation reactors were withdrawn from the same activated sludge bioreactor (i.e., 1000 mg/L of influent COD fed reactor) but at different aerobic times. One was fed with the sludge wasted at 1.5 h aeration (i.e., sludge-1000) while the other was fed with the sludge withdrawn at the end of aerobicis (this sludge was defined as sludge-1000-l). After concentrating at 4 °C for 12 h, it was measured that the sludge-1000-l contained 12,280 ± 360 mg/L VSS, 14340 ± 340 mg/L total COD, 570 ± 31 mg/g VSS total protein, 224 ± 15 mg/g VSS total carbohydrate, and 31 ± 5 mg/g VSS PHA. The fermentation conditions were the same as those described in the section 2.3.

2.6. Comparison of protein consumption and hydrogen production among non-PHA sludge, intracellular-PHA sludge, and exogenous-PHA sludge

To evaluate the potential effect of PHA on non-PHA biomass during fermentation, we performed the following batch fermentation experiment. In this batch experiment, three fermentation reactors were carried out and were respectively fed with 300 mL non-PHA sludge, 300 mL intracellular-PHA sludge (i.e., sludge-1000), and 224 mL non-PHA sludge + 654 mg exogenous PHA (88% PHB and 12% PHV). The non-PHA sludge was collected from 1000 mg/L of influent COD fed reactor at 6 h of aeration, because it was found that PHA content was non-detectable after 6 h of aeration. The fermentation conditions were also the same as those depicted in the section 2.3. It took about 156, 144, and 108 h for these reactors to reach the maximal hydrogen production, respectively. At this time, PHA was non-detectable in all fermentation reactors.

2.7. Analytical methods

Hydrogen fraction in the generated gas was measured via a gastight syringe with 0.2 mL injection volume and a gas chromatograph (GC112A, China) equipped with a thermal conductivity detector and a 6 mm × 32 m stainless column (Zhao et al., 2010; Xiao et al., 2014). The temperatures of the injection port, column, and detector were set at 40, 40, and 80 °C, respectively. Nitrogen was used as the carrier gas at a flowrate of 30 mL/min. The determinations of COD, VSS, and total suspended solids (TSS) were conducted in accordance with standard methods (APHA, 1998). The measurements of sludge PHA, protein, carbohydrate, lipid, and short-chain fatty acids (SCFA) were the same as depicted in previous publications (Wang et al., 2009; Yuan et al., 2006). Carbon, hydrogen, and nitrogen elemental compositions of fermentation substrates were analyzed by an elemental analyzer (Elemental Analyzer NA 2500). Microbial extracellular polymeric substances (EPS) containing loosely bound EPS and tightly bound EPS of activated sludge were measured according to the method documented in the literature (Mu et al., 2012). Molecular weight (Mw) distribution of the fermentation liquid was measured via gel-filtration chromatography analyzer (Shimadzu Co., Japan) according to the literature (Zhao and Chen, 2011). The activities of key hydrolytic enzymes (alpha-glucosidase and protease) were measured the same as described by Goel et al. (1998). One enzyme unit of alpha-glucosidase was defined to produce 1 μM of p-nitrophenol in 1 h while one enzyme unit of protease was defined to hydrolyze 1 mg of azocasein per hour (Goel et al., 1998). The measurement of [FeFe] hydrogenase activity was performed according to the method reported in the publications with minor revision (the debris was centrifuged at 15,000 or 20,000 g in the publications while it was centrifuged at 12,000 g in this study due to the limit of available centrifuge), and one unit of [FeFe] hydrogenase was defined as the amount of hydrogenase evolving 1 M hydrogen gas from sodium dithionite reduced methylviolemin per min (Khanna et al., 2011; Bai et al., 2012). Briefly, fermentation mixtures were harvested
and washed for 3 times with 50 mM Tris–HCl (pH 7.5) containing 2 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride. Then, the resuspended cells were sonicated at 20 kHz for 45 min in an ice bath to break down the cell structure. The debris was centrifuged at 12,000 g and 4 °C for 30 min, and the crude extracts in supernatant were obtained for [FeFe] hydrogenase activity measurement. The analysis was performed in a 5 mL plain tube. A volume of 100 µL crude extracts was added to start the reaction in the tube containing 50 mM Tris–HCl (pH 7.5), 25 mM sodium dithionite, and 1.5 mM methylviologen in a final volume of 2 mL. The assay mixtures were bubbled with argon to remove traces of dissolved oxygen before addition of the crude extracts. The reaction mixture was incubated in a shaker at 25 °C for 10 min.

2.8. Statistical analysis

All measurements were conducted in triplicate. An analysis of variance was used to evaluate the significance of results, and p < 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. The effect of PHA content and composition in WAS on hydrogen production

Table 1 presents the main characteristics of sludges with different PHA contents. It can be seen from Table 1 that protein, carbohydrate, and PHA are the top three organic compounds in these sludges. With the increase of PHA content, both protein and carbohydrate contents are decreased. Nevertheless, the increased PHA content does not result in significant increase of total COD concentration (p > 0.05). In addition, PHB is found to be the dominant fraction of PHA in all sludges, and the percentages of PHB, PHV, and poly-3-hydroxy-2-methylvalerate (PH2MV) are almost the same among these sludges.

Fig. 1a shows the time curve of cumulative hydrogen production using sludges with different PHA contents. It can be seen that the behavior of hydrogen production at different PHA contents was similar. The volume of generated hydrogen first increased with the increase of fermentation time and then kept almost constant in the remainder of fermentation period. No hydrogen consumption was observed in all the fermentation reactors due to the strong alkaline condition controlled in these reactors (pH 10). It was proven that constant pH 10 could effectively inhibit the activities of methanogens and acetobacteria (Zhao et al., 2010). All these observations made in Fig. 1a were similar to those reported in the literature at constant pH 10 (Zhao et al., 2010). It can be also found in Fig. 1a that the maximal hydrogen yield was affected by PHA content. With the increase of PHA content, hydrogen yield increased. For instance, the hydrogen production in the Sludge-200 (with PHA content of 25 ± 3 mg/g VSS, Table 1) fed reactor increased gradually with fermentation time during the initial 156 h, and no significant increase was found after that time (p > 0.05). At time of 156 h the hydrogen generation was 26.5 mL/g VSS, which was in accord with the datum reported in the literature (Zhao et al., 2010). Nevertheless, the maximal hydrogen production in the Sludge-1000 (with PHA content of 178 ± 11 mg/g VSS, Table 1) fed reactor was observed at fermentation time of 108 h, and the corresponding hydrogen production was 58.7 mL/g VSS, which was 2.2-fold higher than that detected in the Sludge-200 fed reactor. It should be emphasized that the optimal fermentation time in the lowest PHA sludge (i.e., Sludge-200) was 156 h whereas this value was 108 h in the highest PHA one (i.e., Sludge-1000). Clearly, the sludge containing higher PHA produced more hydrogen but required less fermentation time.

PHA contains several compositions, it is therefore necessary to investigate the effect of PHA composition on hydrogen production to gain a comprehensive understanding of PHA associated with hydrogen production. However, since PHB and PHV are the main compositions of PHA in activated sludge involved in WWTPs with their contents usually above 90%, we only focus on these two compositions in this study. Table 2 outlined the main characteristics of sludges with different PHA compositions. From Table 2, it can be found that the main difference among the five sludges was PHA composition. With the increase of influent propionate ratio, PHV fraction increased. For example, PHB was the dominant composition of PHA with its percent up to 79.1 ± 8.6% of PHA (i.e., sludge-I) when influent COD was prepared with 100% acetate. When the reactor received 40% acetate + 60% propionate, PHV was

![Fig. 1](image-url) -- Effect of sludge PHA content (a) and composition (b) on hydrogen production during sludge dark fermentation. Error bars represent standard deviations of triplicate tests.
The main characteristics of the sludges wasted from the five reactors fed with the same COD concentration but different acetate to propionate ratios were not the same. When the sludge, hydrolysis of solubilized substrates, acidification of following four steps are usually included: solubilization of protein and carbohydrate, as seen in Tables 1 and 2, PHA was also one of the primary organic compounds in these sludges tested in this study, and the changes of its content and composition affecting hydrogen production was mainly focused on the three former steps. Since no hydrogen consumption was observed in all reactors, PHA’s influence on hydrogen production was mainly focused on the three former steps.

Several researchers showed that PHA as an exogenous plastic material could be completely decomposed under anaerobic conditions (Reischwitz et al., 1998; Chen and Wang, 2002). However, compared with the exogenous PHA materials, PHA is an intracellular polymer in this study. This indicates that the microbial cell needs to be disrupted before it can be further degraded. Therefore, its potential influence on cell disruption was investigated first. In the literature, cell disruption is usually estimated by the determination of intracellular substrate release (Wang et al., 2013; Tam et al., 2012). In this study, the variations of soluble protein and carbohydrate were applied to indicate cell breakage, because the sludges used in this study contained almost the same protein and carbohydrate contents in EPS (Tables 1 and 2). It can be seen from Fig. 2a that both the ratios of soluble protein to total protein and soluble carbohydrate to total carbohydrate at 1 d of fermentation time increased with the increase of intracellular PHA content. When PHA content increased from 25 ± 3 (Sludge-200) to 30.5 ± 2 (Sludge-300) to 31 ± 4 (Sludge-1000) mg/g VSS, the ratio of soluble protein to total protein gradually increased from 14.6 ± 0.9% (4.7 ± 0.4%) to 30.5 ± 2.4% (16.2 ± 1.9%). PHA has associated proteins in its biogenesis and degradation, and the degradation of PHA will increase protein solubilization, which thereby affecting the assessment of cell disruption. Thus, we further determined the VSS reduction ratio among these reactors at 1 d of fermentation, and the results are shown in Fig. 2b. It was observed that the VSS reduction in the high PHA contained sludge was also greater than that in the low PHA sludge. Since both the decomposition of PHA and solubilization of other cell compositions will contribute the VSS reduction, it is necessary to figure out whether the increased VSS reduction in the high PHA contained sludge is caused by the PHA decomposition. Based on the following equation, it was necessary to investigate how hydrogen production was affected by PHA. During sludge anaerobic digestion, the following four steps are usually included: solubilization of sludge, hydrolysis of solubilized substrates, acidification of hydrolyzed products, and methane production (Zhao et al., 2010). Hydrogen is mainly generated in the acidification step.

### Table 2 – The main characteristics of the sludges wasted from the five reactors fed with the same COD concentration but different acetate to propionate ratios

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sludge-I</th>
<th>Sludge-II</th>
<th>Sludge-III</th>
<th>Sludge-IV</th>
<th>Sludge-V</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (mg/g VSS)</td>
<td>13,340 ± 410</td>
<td>13,125 ± 376</td>
<td>13,037 ± 423</td>
<td>13,295 ± 357</td>
<td>13,453 ± 442</td>
</tr>
<tr>
<td>VSS (mg/g VSS)</td>
<td>11,810 ± 265</td>
<td>11,776 ± 293</td>
<td>11,485 ± 316</td>
<td>11,995 ± 325</td>
<td>12,160 ± 384</td>
</tr>
<tr>
<td>Total COD (mg/g VSS)</td>
<td>14,130 ± 370</td>
<td>13,680 ± 340</td>
<td>13,550 ± 310</td>
<td>14,160 ± 330</td>
<td>14,190 ± 350</td>
</tr>
<tr>
<td>Total protein (mg/g VSS)</td>
<td>505 ± 32</td>
<td>510 ± 27</td>
<td>507 ± 23</td>
<td>501 ± 30</td>
<td>505 ± 26</td>
</tr>
<tr>
<td>Total carbohydrate (mg/g VSS)</td>
<td>169 ± 15</td>
<td>162 ± 13</td>
<td>172 ± 15</td>
<td>166 ± 11</td>
<td>164 ± 12</td>
</tr>
<tr>
<td>PHA (mg/g VSS)</td>
<td>132 ± 11</td>
<td>135 ± 9</td>
<td>130 ± 14</td>
<td>127 ± 15</td>
<td>129 ± 10</td>
</tr>
<tr>
<td>PHB fraction (%)</td>
<td>79.1 ± 8.6</td>
<td>52.9 ± 4.7</td>
<td>31.5 ± 5.9</td>
<td>20.3 ± 5.2</td>
<td>13.7 ± 1.6</td>
</tr>
<tr>
<td>PHV fraction (%)</td>
<td>18.2 ± 1.5</td>
<td>41.5 ± 3.8</td>
<td>62.3 ± 5.4</td>
<td>76.1 ± 7.9</td>
<td>82.8 ± 7.7</td>
</tr>
<tr>
<td>Lipid and oil (%)</td>
<td>5.1 ± 0.5</td>
<td>5.3 ± 0.7</td>
<td>4.8 ± 0.7</td>
<td>5.2 ± 0.4</td>
<td>4.3 ± 0.9</td>
</tr>
</tbody>
</table>

* Results are the averages and their standard deviations of triplicate measurements. The unit for total suspended solids, VSS, and total COD is mg/L; PHB and PHV fractions are expressed in % of total PHA; the remainder is expressed in mg/g VSS.

** Notes:**
- **b EPS carbohydrate content in sludge-I, sludge-II, sludge-III, sludge-IV, and sludge-V was respectively 31 ± 4, 33 ± 5, 29 ± 2, 35 ± 6, and 30 ± 2 mg/g VSS.
- **c EPS protein content in sludge-I, sludge-II, sludge-III, sludge-IV, and sludge-V was respectively 63 ± 4, 59 ± 6, 62 ± 7, and 65 ± 8 mg/g VSS.
- **e EPS carbohydrate content in sludge-I, sludge-II, sludge-III, sludge-IV, and sludge-V was respectively 31 ± 4, 33 ± 5, 29 ± 2, 35 ± 6, and 30 ± 2 mg/g VSS.
Total VSS (g) × the measured VSS reduction ratio(%) = PHA reduction (g) + non-PHA cell composition (g) × the solubilization ratio of non-PHA cell composition(%)

(2)

Where, total VSS is calculated by initial VSS concentration (Table 1) × 0.3 L, the measured VSS reduction ratio is shown in Fig. 2b, PHA reduction is calculated by initial PHA (according to Table 1) – remnant PHA (according to Fig. 2b and S2), non-PHA cell composition is calculated by total VSS - initial PHA.

It should be noted that the sludges used in above fermentation tests might have different microbial compositions due to the fact that they were wasted from different activated sludge bioreactors with different influent COD concentrations. Different organisms may have different disruption thresholds. To eliminate this potential influence, a batch fermentation experiment was carried out using the sludges withdrawn from the same activated sludge bioreactor but at different aerobic times (Table S1, Supporting Information).

All these results showed that the increased PHA content accelerated cell solubilization, which thereby caused more soluble substrates for subsequent hydrolysis and acidification stages (Fig. S3a, Supporting Information). Some researchers reported that the microbial cell became more fragile with the increased intracellular PHA level (Budwill et al., 1992; Page and Cornish, 1993; Lee, 1996), it can be understood that the increased PHA could accelerate the solubilization of PHA contained biomass. However, as all fermentation sludges used in this study contained PHA-biomass and non-PHA biomass, it is unclear whether the increased PHA is beneficial to the cell solubilization of non-PHA biomass according to the above data, which will be further analyzed below. Figs. S3b and S4 (Supporting Information) present the soluble COD and the ratio of soluble protein (carbohydrate) to total protein (carbohydrate) in the fermentation systems fed with different PHA compositions at 1 d of fermentation time. Further analysis found that compared with the PHB dominant sludge (i.e., sludge-I), the increased PHV fraction did not significantly affect the ratio of soluble protein (carbohydrate) to total protein (carbohydrate), VSS reduction, and soluble COD concentration (p > 0.05, Table S2, Supporting Information), which suggested that PHA composition (i.e., PHB/PHV fraction) caused insignificant impact on sludge solubilization.

It is known that before the solubilized substrates can be directly utilized to produce SCFA and hydrogen, the solubilized substrates with large Mw require to be hydrolyzed. The hydrolysis rate is closely relevant to the yield and fermentation time of hydrogen production. Thus we further investigated the effect of PHA on hydrolysis step. In this study, PHA content was observed to decrease with fermentation time rapidly (Fig. S2, Supporting Information). Although PHA degradation rate was affected by PHB/PHV fraction, more than 94% of sludge PHA polymer was degraded in the initial 72 h of fermentation time no matter what the original PHA content and composition in sludge were (Fig. S2, Supporting Information). Using bovine serum albumin (model protein compound with average Mw of 67,000) and dextran (model polysaccharide compound with average Mw of 23,800) as model substrates of protein and carbohydrate, Zhao et al. (2010) observed that only 54.5% of protein and 84% of carbohydrate were respectively decomposed under alkaline anaerobic fermentation (pH 10) even in 84 h of fermentation time. It seems that the anaerobic hydrolysis rate of PHA might be faster than that of protein and carbohydrate, which are the main compositions of traditional cell.

This hypothesis can be strongly supported by the Mw distribution of solubilized substrate shown in Fig. 3. It can be clearly observed that solubilized substrate with low Mw were in the sequence of the PHB-dominant sludge (i.e., Sludge-I) > PHV-dominant sludge (i.e., Sludge-V) > the low PHA sludge (i.e., Sludge-200) at 2 d of fermentation time (Fig. 3a). Further investigations revealed that the percentages of small soluble substrates (Mw < 1000) at this fermentation time showed well positive correlation with PHA level (R² = 0.972, Fig. 3b) but exhibited negative correlation with PHV fraction (R² = 0.9124, Fig. 3c). Considering that the SCFA generation at
this fermentation time was found to be unaffected by both sludge PHA content and composition (Table S3, Supporting Information), it can be concluded that the increased PHA content benefited the hydrolysis process of solubilized substrates, and PHB was more beneficial to hydrolysis step, as compared with PHV. Since the sludge with higher PHA content had lower protein and carbohydrate (Table 1), it can be further inferred that PHA had faster anaerobic hydrolysis rate than protein and carbohydrate.

It is reported that both PHB and PHV can be anaerobically converted to SCFA under anaerobic conditions through a series of biochemical degradations, and the metabolic pathways are summarized in Fig. S5 (Supporting Information). PHB and PHV are first degraded to their monomers 3-hydroxybutyrate and 3-hydroxyvalerate by depolymerases, respectively. Then, 3-hydroxybutyrate and 3-hydroxyvalerate are activated by CoA transfer and generated the resultant 3-hydroxybutyryl-CoA and 3-hydroxyvaleryl-CoA, which are further bio-converted to acetyl-CoA, propionyl-CoA, butyryl-CoA, and valeryl-CoA. Finally, acetate, propionate, butyrate, and valerate are produced in the acidification process. As hydrogen is generated in acidification step concurrently, accelerating hydrolysis process by PHA indicates that more hydrolyzed substrates can be provided for subsequent hydrogen production. Meanwhile, fermentation time will be also saved if hydrolysis rate is accelerated. It can be therefore understood that the hydrogen production was in the sequence of the PHB-dominant sludge > the PHV-dominant sludge > the low PHA sludge but the required fermentation time was on the opposite sequence, because PHB was more beneficial to hydrolysis process than PHV while both PHB and PHV had faster anaerobic hydrolysis rate than other main cell compositions, such as protein and carbohydrate.

Hydrogen is primarily produced in acidification step, and the fermentation type is also reported to affect hydrogen generation (Khanal et al., 2004; Li et al., 2009). Thus, we also compared with the total and individual SCFA production among these reactors. Table 3 illustrates the total and individual SCFA produced from the three typical reactors at the time of maximal hydrogen production, and the detailed SCFA information in other reactors is listed in Table S4 (Supporting Information). As seen from Table 3, the fraction of individual SCFA between the low PHA sludge (25 ± 3 mg/g VSS, Table 1) and PHB-dominant sludge (132 ± 11 mg/g VSS, Table 2) fed reactors was similar. Acetic acid, propionic acid, and isobutyric acid were the top three SCFA. The total SCFA production in the PHB-dominant sludge, however, was much higher than that in the low PHA sludge fed reactor (375.0 ± 17.1 vs 194.3 ± 11.1 mg COD/g VSS). With the increase of PHA content the total SCFA yield increased. Similar results were also observed in other sludges with different PHA levels but similar PHA composition (Table S4, Supporting Information). Therefore, improvement of acidification of hydrolyzed products was another reason for the increased PHA sludge showing greater hydrogen production.

From Table 3, it can be also observed that even under the same total PHA level (Table 2) both individual SCFA fraction and total SCFA production varied significantly (p < 0.05) when PHA composition changed. The dominant PHA monomer shifted from PHB (sludge-I, Table 2) to PHV (sludge-V, Table 2), the top three SCFA were propionic acid, acetate acid, and n-valeric acid instead of acetic acid, propionic acid, and isobutyric acid. It was reported that the fermentation type also affected hydrogen production, and the higher the acetic or the lower the propionic were generated, the greater hydrogen was produced (Khanal et al., 2004; Li et al., 2009). When 1 mol monomeric 3-hydroxybutyrate (or 3-hydroxyvalerate) is fermented, 1 mol NADH and 1 mol H⁺ will be generated in the step of 3-hydroxybutyryl-CoA (or 3-hydroxyvaleryl-CoA) degradation, which can be further formed 1 mol H₂ (Janssen and Schink, 1993; de María and Domínguez, 2010). That is, if 1 mol-C (or 1 g) of PHB and 1 mol-C (or 1 g) of PHV are respectively fermented, the theoretical hydrogen production from PHB will be higher than that from PHV (1 mol-C: 0.25 vs 0.2 mol H₂; 1 g: 11.6 vs 10 mmol H₂). Moreover, the PHV-dominant sludge produced less total SCFA than the PHB-dominant sludge. Similar observation was made in other sludges with different PHB/PHV fractions (Table S4, Supporting Information). Thus, it can be understood that the PHV-dominant sludge produced less hydrogen than the PHB-
dominant sludge. From the metabolic pathways for anaerobically converting PHB and PHV to SCFA presented in Fig. S5 (Supporting Information), we can see that acetic acid and butyric acid are the resultant of PHB fermentation whereas acetic acid, propionic acid, and valeric acid are the resultant of PHV fermentation, which might be the reason for more propionic acid generated in the PHV-dominant sludge fed reactor.

According to the above analysis, it is clear that biomass containing higher PHA is easier to be disrupted, which is thereby beneficial to dark fermentative hydrogen production. After the disruption of PHA contained biomass, PHA will be released to the fermentation system. Thus, one might want to know whether the released PHA affects the fermentation of non-PHA biomass. To evaluate this potential impact, one batch fermentation test using non-PHA sludge, intracellular-PHA sludge, and non-PHA sludge + exogenous PHA (with equivalent PHA content to intracellular-PHA sludge) was carried out, and the results are shown in Table 4. It can be seen that the protein consumption ratio, SCFA production, and hydrogen production in the exogenous-PHA sludge reactor were much higher than those in the non-PHA sludge reactor. Since the exogenous-PHA reactor was fed with the same sludge (i.e., non-PHA sludge) as the non-PHA reactor, and the added exogenous PHA did not contain any protein, the increased protein consumption rate in the exogenous-PHA reactor indicated that the presence of PHA benefited the fermentation of non-PHA biomass.

To obtain a deeper understanding, COD mass balance among these fermentation systems was assessed, and the results are displayed in Fig. 4. It was found that the COD ratios of both SCFA and hydrogen in the exogenous-PHA reactor were higher than those in the non-PHA reactor, which were consistent with the lower VSS and soluble protein remained in the fermentation system. It should be highlighted that the average COD ratio of soluble protein in the exogenous-PHA reactor was only 61.1% of that in the non-PHA reactor (8.0% vs. 13.1% of total COD). Although the average COD ratio of VSS remained in the former was also lower as compared with that in the latter (49.3% vs 63.0%), VSS reduction from the aspect of sludge solubilization was approximately the same in the two fermentation systems. In the non-PHA reactor, all VSS reduction was ascribed to sludge solubilization, and the average ratio of VSS reduction was 37.0% (1–63.0% = 37.0%). In the exogenous-PHA reactor, however, both the exogenous PHA decomposition and sludge solubilization contributed to VSS reduction. Considering that the exogenous PHA, which was completely decomposed during fermentation, accounted for about 22.3% (as COD) of total VSS [(0.576 g PHB / 1.92)/(0.576 g PHB / 1.92)] = 0.223], the VSS

### Table 3 – Comparison of individual and total SCFA production from the low PHA sludge (Sludge-200), PHB-dominant sludge (Sludge-I), and PHV-dominant sludge (Sludge-V) at the time of maximal hydrogen production. a

<table>
<thead>
<tr>
<th></th>
<th>Low PHA sludge</th>
<th>PHB-dominant sludge</th>
<th>PHV-dominant sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Concentration</td>
<td>114.2 ± 8.2</td>
<td>211.0 ± 12.0</td>
<td>109.3 ± 1.9</td>
</tr>
<tr>
<td>Acetic Fraction</td>
<td>58.7 ± 2.2</td>
<td>56.3 ± 1.5</td>
<td>33.6 ± 0.6</td>
</tr>
<tr>
<td>Propionic Concentration</td>
<td>32.3 ± 2.9</td>
<td>59.3 ± 2.6</td>
<td>122.1 ± 5.9</td>
</tr>
<tr>
<td>Propionic Fraction</td>
<td>16.6 ± 1.0</td>
<td>15.8 ± 0.3</td>
<td>37.5 ± 1.2</td>
</tr>
<tr>
<td>n-Butyric Concentration</td>
<td>7.5 ± 2.0</td>
<td>16.0 ± 0.3</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>n-Butyric Fraction</td>
<td>3.8 ± 1.0</td>
<td>4.3 ± 0.2</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>Isobutyric Concentration</td>
<td>20.3 ± 9.0</td>
<td>46.1 ± 5.7</td>
<td>25.6 ± 3.6</td>
</tr>
<tr>
<td>Isobutyric Fraction</td>
<td>10.5 ± 4.6</td>
<td>12.3 ± 1.6</td>
<td>7.9 ± 1.1</td>
</tr>
<tr>
<td>n-Valeric Concentration</td>
<td>13.2 ± 1.1</td>
<td>29.4 ± 4.5</td>
<td>42.7 ± 3.7</td>
</tr>
<tr>
<td>n-Valeric Fraction</td>
<td>6.8 ± 0.4</td>
<td>7.8 ± 1.0</td>
<td>13.1 ± 1.0</td>
</tr>
<tr>
<td>Isovaleric Concentration</td>
<td>6.8 ± 0.5</td>
<td>13.2 ± 2.3</td>
<td>20.3 ± 1.1</td>
</tr>
<tr>
<td>Isovaleric Fraction</td>
<td>3.5 ± 0.4</td>
<td>3.5 ± 0.6</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Total SCFA Concentration</td>
<td>194.3 ± 11.1</td>
<td>375.0 ± 17.1</td>
<td>325.3 ± 7.4</td>
</tr>
</tbody>
</table>

a The data are the averages and their standard deviations of triplicate measurements. The unit for concentration is mg COD/g VSS while fraction is expressed as % of total SCFA.

### Table 4 – Comparison of protein consumption and hydrogen production among non-PHA sludge, exogenous-PHA sludge, and intracellular-PHA sludge at the time of maximal hydrogen production. a

<table>
<thead>
<tr>
<th></th>
<th>Protein consumption ratio (%)</th>
<th>SCFA production (mg COD/g VSS)</th>
<th>H2 production (mL/g VSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-PHA sludge a</td>
<td>15.8 ± 1.1</td>
<td>189.7 ± 13.2</td>
<td>26.1 ± 1.5</td>
</tr>
<tr>
<td>Exogenous-PHA sludge a</td>
<td>23.6 ± 1.5</td>
<td>326.5 ± 18.5</td>
<td>47.2 ± 1.9</td>
</tr>
<tr>
<td>Intracellular-PHA sludge</td>
<td>27.9 ± 2.3</td>
<td>387.4 ± 21.9</td>
<td>59.3 ± 2.8</td>
</tr>
</tbody>
</table>

a Results are the averages and their standard deviations of triplicate measurements. The calculated total COD in the non-PHA sludge, exogenous-PHA sludge, and intracellular-PHA sludge reactor was respectively 4.28, 4.31, and 4.31 g based on the characteristics of these sludges, while the measured C/N ratios of these sludges were 7.08, 9.39, and 9.37, respectively.

b After 12 h concentration, it was detected that the non-PHA sludge contained 12,160 ± 390 mg/L VSS, 14250 ± 370 mg/L total COD, 586 ± 37 mg/g VSS total protein, and 239 ± 18 mg/g VSS total carbohydrate.

c The calculation of VSS included both the biomass and exogenous PHA.
reduction come from sludge degradation should be 36.6% \(\left(\frac{(1-49.3\%) - 22.3\%}{1-22.3\%}\right) = 0.366\). These results indicated that the presence of PHA could not enhance non-PHA sludge solubilization but promote the soluble protein conversion. From Table 4, it was observed that the C/N ratio of exogenous-PHA system was 9.39, which was higher than that of non-PHA system (7.08). It was reported that pertinent increase of C/N ratio benefited the conversion of protein and the production of SCFA and hydrogen (Chen et al., 2012; Feng et al., 2009). Therefore, the increased C/N ratio may be the main reason for the presence of PHA enhancing soluble protein conversion and hydrogen production.

It should be also noted that the intracellular-PHA sludge reactor showed higher hydrogen yield, SCFA production, and VSS reduction as compared with the exogenous-PHA reactor, though they had the similar PHA content and C/N ratio (Table 4 and Fig. 4). As demonstrated above, intracellular PHA could accelerate sludge solubilization whereas exogenous PHA could not enhance it, which may be the main reason for the increased VSS reduction and hydrogen and SCFA production in the intracellular-PHA sludge reactor. Another possibility might be that exogenous PHA would tend to be crystallized, which would be more resistant to enzymatic attack. Moreover, due to the limit of our available exogenous PHA, the compositions of exogenous PHA (88.0% PHB + 12.0% PHV) and intracellular PHA (78.3% PHB + 20.2% PHV + 1.5% PH2MV) were not completely the same, which might also affect hydrogen yield and SCFA production. The results of COD mass balance analysis also showed that with the increase of sludge PHA content, the COD ratios of produced hydrogen and SCFA increased (Fig. S6a, Supporting Information). When the dominant composition of PHA shifted from PHB to PHV, both ratios decreased (Fig. S6b, Supporting Information).

The production of hydrogen during sludge anaerobic fermentation at pH 10 is primarily related to sludge hydrolysis and acidification because both methanogens and acetobacteria are inhibited (Zhao et al., 2010). Determination of enzyme activities is an alternative method to evaluate microbial activities (Nybroe et al., 1992). Thus, the activities of key hydrolytic and hydrogen-forming enzymes were finally measured to reflect the microbial activities of key hydrolytic and hydrogen-producing microbes. Protease and \(\alpha\)-glucosidase are the key enzymes for protein and carbohydrate hydrolysis, respectively, while [FeFe] hydrogenase is the key enzyme in the biochemical metabolism for the production of molecular hydrogen (Goel et al., 1998; Khanna et al., 2011; Bai et al., 2012). As seen in Fig. 5, the Sludge-I (i.e., PHB-dominant sludge) fed reactor had the highest activities of both key hydrolytic enzymes and hydrogen-forming enzyme while the Sludge-200 (i.e., low PHA sludge) fed reactor had the lowest ones. The activities of protease, \(\alpha\)-glucosidase, and [FeFe] hydrogenase in the Sludge-V (i.e., PHV-dominant sludge) fed reactor were lower than those in the Sludge-I (i.e., PHB-dominant sludge) fed reactor but much higher than those in the Sludge-200 (i.e., low PHA sludge) fed reactor. All these observations were consistent with the detected order of hydrogen production.

### 3.3. Implications for wastewater and WAS treatments

This study reveals for the first time that enhanced hydrogen production can be achieved from increased PHA sludge. The findings obtained in this work have important implications to sludge fermentation systems for hydrogen production. Although numerous studies have been performed in the field, the strategy to enhance hydrogen generation from the aspect of improving the self-characteristic of sludge has never been reported. Thus, the findings of this study can provide a new solution to enhance hydrogen generation. More importantly, a

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**Fig. 4** — COD mass balance analysis of the non-PHA sludge, exogenous-PHA sludge, and intracellular-PHA sludge fermentation reactors at the time of maximal hydrogen production. The data reported are the averages of triplicate measurements. The COD conversion coefficients are 1.42 g COD/g VSS, 1.67 g COD/g PHB, 1.92 g COD/g PHV, 2.11 g COD/g PH2MV, 8 g COD/g H2, 1.5 g COD/g protein, 1.06 g COD/g carbohydrate, 1.07 g COD/g acetic, 1.51 g COD/g propionic, 1.82 g COD/g butyric, and 2.04 g COD/g valeric.

**Fig. 5** — Comparison of the relative activities of key hydrolytic and acid-forming enzymes between the three semi-continuous fermentation reactors after stable operation. Error bars represent standard deviations of triplicate measurements.
new door may be opened for both wastewater treatment and hydrogen production from WAS based on the findings achieved in this work. In the conventional WWTPs, wastewaters are first influent into the anaerobic phase, where carbon sources (e.g., acetate) will be taken up and further converted to intracellular PHA. In the subsequent aerobic phase, the accumulated PHA will enter into the tricarboxylic acid cycle and be oxidized to provide carbon and energy (oxygen is consumed and CO₂ is formed) for cell growth and nutrient removal (Fig. S1, Supporting Information). Therefore, if the wasted WAS contained higher PHA by either process improvement or operation optimization as mentioned in the “Introduction” section, less PHA will be oxidized in the bioreactors of WWTPs. As a result, less oxygen is required (i.e., aeration is saving), less CO₂ is formed, and less cell growth is also occurred. Furthermore, the increase of sludge PHA level can accelerate cell solubilization and solubilized substrate hydrolysis processes, as demonstrated in this work, which thereby enhances the subsequent hydrogen production from WAS.

Several strategies have been verified to promote hydrogen production from WAS, such as ozone or ultrasound pretreatment (Yang et al., 2012), heat pretreatment (Assawamongkholsiri et al., 2013), acid or alkaline pretreatment (Cai et al., 2004; Assawamongkholsiri et al., 2013), and co-digestion of WAS and other biosolids (Zhou et al., 2013; Kim et al., 2012; Chen et al., 2012; Liu et al., 2013). However, these strategies require either consumption of energy, addition of chemicals, or transportation of other substrates. In comparison, the PHA based technology developed on the basis of the findings in this work does not have these limitations, because this strategy enhances hydrogen production by improving the self-characteristic of sludge in wastewater treatment step. It should be noted that this PHA accumulation based method can be integrated with other strategies (e.g., alkaline condition applied in this study). Thus, hydrogen production may be further enhanced if we combine the PHA accumulation based method with other strategies, such as heat, ozone or ultrasound pretreatment and co-fermentation substrate optimization, which remains to be investigated in future.

4. Conclusions

This study evaluated the influences of intracellular PHA level and composition on hydrogen production from anaerobic WAS fermentation. The results showed that the sludge containing higher PHA not only promoted hydrogen yield but also shortened fermentation time. Compared with PHV, PHB was more beneficial to hydrogen production. The increased PHA content accelerated cell solubilization and solubilized substrate hydrolysis processes and enhanced soluble protein conversion. Compared with the PHB-dominant sludge, the increased PHV fraction not only slowed the hydrolysis process but also caused more propionic acid production, with less theoretical hydrogen generation in this fermentation type. Enzyme analyses further showed that both the key hydrolytic enzyme activities and hydrogen-forming enzyme activities were in the sequence of the PHB-dominant sludge > the low PHA sludge, which was consistent with the observed order of hydrogen production.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2015.01.017.

References


