

## Design of bioreactors for biohydrogen production

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Using dark fermentation, hydrogen can be generated from renewable organics including waste materials. Key to successful application of anaerobic fermentation is to uncouple liquid and biomass retention times in reactor system. This paper reviews reactor configurations (fixed-bed, fluidized-bed, upflow anaerobic sludge blanket and continuous stirred tank reactors) and operating processes (batch, semi-continuous and continuous). Immobilized- and suspended- cell systems are compared based on biomass growth in forms of granular, biofilm, gel-entrapped bioparticle and flocs.

**Keywords:** Anaerobic fermentation, Biohydrogen, Bioreactors, Reactor configuration

### Introduction

Hydrogen ( $H_2$ ) is a promising alternative to conventional fossil fuels, because it release energy explosively in heat engines or generate electricity quietly in fuel cells while producing water as the only by-product.  $H_2$  is also raw material for synthesis of ammonia, alcohols, and aldehydes, and for hydrogenation of various petroleum and edible oils, coal, and shale oil<sup>1</sup>.  $H_2$  is proposed as ultimate transport fuel for vehicles because of its non-polluting characteristics, it enables use of highly efficient fuel cells to convert chemical energy to electricity<sup>2</sup>. Fig. 1 shows shares of alternative fuels compared to total automotive fuel consumption in the world<sup>3</sup>.

Most of  $H_2$  is being generated from fossil fuels through thermochemical processes (hydrocarbon reforming, coal gasification and partial oxidation of heavier hydrocarbons)<sup>4,5</sup>. Biohydrogen production studies have focused on biophotolysis of water using algae and *cyanobacteria*, photo-decomposition of organic compounds by photosynthetic bacteria and dark fermentation of organic compounds with anaerobes. Under anaerobic fermentation,  $H_2$  is produced in first

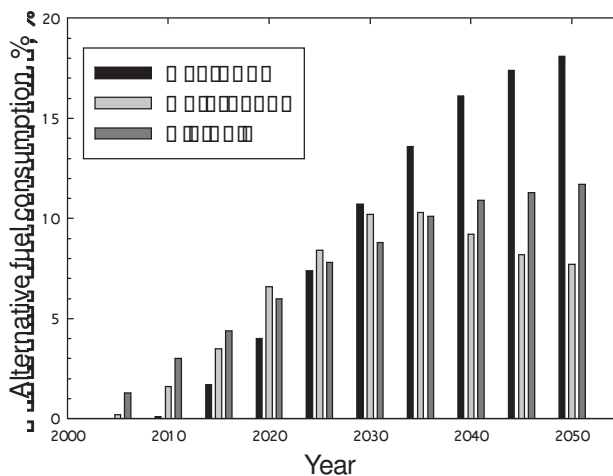


Fig. 1—Shares of alternative fuels compared to total automotive fuel consumption in the world<sup>3</sup>

stage as an intermediate product, which at second stage is used as an electron donor by methanogens. Microbial consortia, mainly methanogenic archaea, acetogenic bacteria and sulphate-reducing bacteria, utilize  $H_2$ . It is possible to harvest  $H_2$  at acidification stage of anaerobic fermentation, leaving remaining acidification products for further methanogenic treatment. A possible approach is by promoting acidogens to produce  $H_2$ ,  $CO_2$  and volatile fatty acids (VFAs) in first stage, while final stage or methanogenesis and other  $H_2$ -consuming biochemical reactions are inhibited. This can be achieved through

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regulating biohydrogen cultures at a low pH, and/or short hydraulic retention time (HRT)<sup>6,7</sup>, or through inactivating H<sub>2</sub> consumers by heat treatment<sup>8,9</sup> and chemical inhibitors<sup>10,11</sup>. Anaerobic H<sub>2</sub> fermentation, which has several positive features (high production rate, low energy demand, easy operation and sustainability), has to compete with commercial H<sub>2</sub> production processes from fossil fuels in terms of cost, efficiency and reliability.

This paper reviews bioreactor designs (fixed-bed, fluidized-bed, upflow anaerobic sludge blanket and continuous stirred tank reactors) and operating modes (batch, semi-continuous and continuous processes). Also, immobilized-cell systems and suspended-cell systems are compared based on biomass growth in forms of granular, biofilm, gel-entrapped bioparticle and flocs.

## Reactor Operation

### Batch and Semi-Continuous Processes

Under laboratory experiments<sup>12-15</sup> conducted in batch mode to examine characteristics of H<sub>2</sub>-producing bacteria and to optimize culture-operating conditions, microbial cultures were found to perform inefficiently, leading to lower H<sub>2</sub> production rates (0.06-0.66 l/l.h). Enhancing H<sub>2</sub> production efficiency, stability and sustainability is thus a major challenge to batch hydrogen systems. Han & Shin<sup>12</sup> developed a semi-continuous mode for anaerobic H<sub>2</sub> production from food waste. Pretreated seed sludge and food waste were loaded into an anaerobic leaching-bed reactor, and dilution water was continuously fed to reactor by a peristaltic pump at different dilution rates. Microbial reaction was considered accomplished as biogas production ceases, which generally took around 7 days. Appropriate control of dilution rate could enhance H<sub>2</sub> fermentation efficiency by improving degradation of not readily degradable matters. Also, dilution rate might delay shift of predominant metabolic flow from H<sub>2</sub>- and acid-forming pathway to solvent-forming pathway. Valdez-Vazquez *et al*<sup>16</sup> examined H<sub>2</sub> production from municipal solid wastes in another semi-continuous pattern. Reactors, which were fed with substrate twice a week in a draw-and-fill mode in an anaerobic chamber and operated continuously at 35°C and 55°C for 40 days, demonstrated that H<sub>2</sub> was produced steadily.

A high-rate anaerobic sequencing batch reactor (ASBR) has been used to evaluate H<sub>2</sub> productivity of an acid-enriched sewage sludge microflora at 35°C<sup>17</sup>.

A 4 h cycle, including feed, reaction, settle, and decant steps, was operated on 5-l ASBR. Sucrose substrate concentration was kept at 20 g COD/l, and HRT was maintained initially at 12-120 h and thereafter at 4-12 h. Reaction/settle period ratio was maintained at 1.7. Hydrogenic activity of sludge microflora was found HRT-dependent, and that proper pH control was necessary for a stable operation of bioreactor. Peak hydrogenic activity was noted at an HRT of 8 h and an organic loading rate (OLR) of 80 kg COD/m<sup>3</sup>.day. Each mole of sucrose in reactor produced 2.8 mol of H<sub>2</sub> and each gram of biomass produced 39 mmol of H<sub>2</sub> per day. Very low HRT might deteriorate H<sub>2</sub> productivity. Concentration ratios of butyric acid to acetic acid, as well as VFA and soluble microbial products to alkalinity can be used as monitoring indicators for hydrogenic bioreactor.

### Continuous Suspended Sludge Processes

#### Continuous Stirred Tank Reactor (CSTR)

In CSTR, which is frequently used for continuous H<sub>2</sub> production<sup>18-20</sup>, H<sub>2</sub>-producing bacteria are well suspended in mixed liquor and less suffered from mass transfer resistance. Because of its intrinsic structure and operating pattern, a CSTR is unable to maintain high levels of biomass inventory. Depending on operating HRTs, biomass measured in terms of volatile suspended solids (1-4 g-VSS/l) is commonly reported<sup>18,21-23</sup>. Washout of biomass may occur at short HRTs, and thus H<sub>2</sub> production rates are considerably restricted. Highest H<sub>2</sub> production rate of CSTR culture fermenting sucrose with a mixed H<sub>2</sub>-producing culture was reported as 1.12 l/l.h<sup>18</sup>.

Vanderhaegen *et al*<sup>24</sup> found that granular sludge disappeared within three weeks when CSTRs were incubated statically instead of being shaken. Spontaneous granulation of H<sub>2</sub>-producing bacteria can occur with reduced HRT in CSTR<sup>25-27</sup>. In such a conventional system, H<sub>2</sub>-producing bacteria are well suspended in mixed liquor and less suffered from mass transfer resistance, but washout of biomass may occur at shorter HRTs. H<sub>2</sub> production rates are thus restricted considerably by a low CSTR biomass retention and low hydraulic loading<sup>28,29</sup>. Show *et al*<sup>30</sup> and Zhang *et al*<sup>31</sup> found that formation of granular sludge significantly increased overall reactor biomass to as much as 16.0 g-VSS/l, which enabled CSTR to operate at an OLR of up to 20 g-glucose/l.h and hence enhanced performance in H<sub>2</sub> production.

#### Membrane Bioreactor

One method for increasing reactor biomass concentration is the use of a membrane in a chemostat to

control biomass concentration. At a HRT of 3.3 h, Oh *et al.*<sup>32</sup> demonstrated that biomass concentration increased from 2.2 g/l in a control reactor (no membrane chemostat) to 5.8 g/l in an anaerobic membrane bioreactor (MBR). This was achieved by controlling sludge retention time (SRT) at 12 h, corresponding to a slight increase in H<sub>2</sub> production rate from 0.50 to 0.64 l/l.h. Increasing SRT can further enhance biomass retention, which favors substrate utilization, but may result in a decrease in H<sub>2</sub> production rate. By summarizing several studies of H<sub>2</sub> production by MBR, Li & Fang<sup>13</sup> found that H<sub>2</sub> production rates were achieved between 0.25-0.69 l/l.h in MBR systems. This process has not shown any advantage compared to other high-efficiency H<sub>2</sub> production systems. In addition, membrane fouling and high operating cost would limit the use of MBR process in anaerobic H<sub>2</sub> fermentation.

#### Immobilized-Cell Processes and Methods

Immobilized-cell systems, in comparison to suspended-cell systems in continuous operations, are more capable of maintaining higher biomass concentration and can be operated at high dilution rates without biomass washout<sup>30,33</sup>. Biomass immobilization can be achieved through forming granules, biofilm or gel-entrapped bioparticles. Many researchers immobilized pure or mixed cultures of H<sub>2</sub>-producing bacteria by gel entrapment in a form of biogels such as *C. butyricum* strain IFO13949 in agar gel<sup>34</sup>, *E. aerogenes* strain HO-39 in k-carrageenan, calcium alginate or agar gel<sup>35</sup>, sewage sludge in calcium alginate beads, or alginate bead with adding activated carbon powder, polyurethane and acrylic latex/silicone<sup>36</sup>, and sewage sludge and activated carbon powder fixed by ethylene-vinyl acetate copolymer<sup>37</sup>. Peak H<sub>2</sub> production rates obtained by continuous gel-immobilized sludge ranged from 0.090 l/l.h<sup>35</sup> in a chemostat with stirring to 0.93 l/l.h<sup>38</sup> in a fluidized bed reactor.

Biofilm attachment on solid and porous support carriers seems to be superior to gel-entrapped bioparticles in continuous H<sub>2</sub> production. In continuous cultures without any pH control, H<sub>2</sub> production and glucose consumption rates<sup>34</sup> with *C. butyricum* immobilized on porous glass beads were higher than corresponding values with cells immobilized in agar gel at HRTs of 3 h and 5 h. In another study<sup>35</sup> with *E. aerogenes* strain HO-39 culture, H<sub>2</sub> production rate for agar gel and porous glass beads increased with decreasing HRT; this did not occur in free cells. H<sub>2</sub> production rate of glass beads was superior

to that of agar gels at HRT < 3 h, and was maximum (0.85 l/l.h) at HRT of 1 h. Biofilm attached on solid or porous supports had an advantage in improving H<sub>2</sub> production rate compared to that of gel-entrapped bioparticles, wherein inferior performance is attributed to low mass transfer efficiency, and stability and durability of bioparticles. Hence, this may not be technology of choice for fermentative H<sub>2</sub> production.

Granular sludge has some advantages over biofilm sludge in continuous dark H<sub>2</sub> fermentation. Firstly, fast-growing characteristics of H<sub>2</sub>-producing cultures might cause system upset of fixed-bed biofilm processes. Maximum specific growth rate<sup>21,39,40</sup> (0.17-0.5 h<sup>-1</sup>) and biomass growth yield<sup>25,27,39,41</sup> (0.08-0.33 g-VSS/g-COD) of H<sub>2</sub>-producing bacteria indicated that H<sub>2</sub>-producing bacteria would increase rapidly if a higher OLR was employed. OLR for immobilized sludge H<sub>2</sub> production was reported as high as 80 g-glucose/l.h<sup>42</sup>. Nicoletta<sup>43</sup> mentioned that biofilm reactors are not particularly useful when dealing with fast-growing organisms with a maximum specific growth rate faster than 0.1 h<sup>-1</sup>. Rapid buildup of H<sub>2</sub>-producing biofilms could result in system upset due to mass transfer limitation. Oh *et al.*<sup>26</sup> reported microbial growth of H<sub>2</sub>-producing bacteria too excessive under mesophilic condition, causing system upset just after one week of operation. On the other hand, a packed-bed reactor using cylindrical activated carbon as support matrix exhibited steady and efficient H<sub>2</sub> production. Fed with synthetic sucrose at 20 g COD/l, system was operated at 0.5-5 h HRT and 35°C for 15 days<sup>44</sup>. Reduction of bed porosity from 90% to 70% would result in a decrease in H<sub>2</sub> production performance, and pressure drop was higher when bed porosity was lower. System stability of such a biofilm-based process may be challenged by long-term operation. System upset might occur once interstitial void spaces in pack-bed reactor are clogged with biomass.

Washout of support carriers might be an intrinsic drawback of biofilm processes. Zhang *et al.*<sup>45</sup> investigated H<sub>2</sub> production by granular sludge and biofilm sludge growing on granular activated carbon in two fluidized bed reactors at a pH of 5.5 and an OLR of 40 g-glucose/l.h. A similar performance in H<sub>2</sub> production was observed with two immobilized cultures, both were tested at different HRTs (0.125-3 h) and influent substrate concentrations (5-120 g/l). Biofilm sludge was washed out substantially and reactor biomass was replaced by granular sludge after 50 days of operation. But H<sub>2</sub>

Table 1—System performance of batch hydrogen production by pure cultures of dark fermentative microorganisms

Genus classification	Substrate	Temperature	Hydrogen yield mol-H <sub>2</sub> /mol hexose	Hydrogen production rate l/l h
<b>Facultative anaerobes</b>				
<i>Escherichia coli</i> NCIMB 11943 <sup>57</sup>	Starch hydrolysate	37	1.8	0.33
<i>E. aerogenes</i> NCIMB 10102 <sup>57</sup>	Starch hydrolysate	40	1.1	0.24
<i>E. cloacae</i> IIT-BT08 <sup>58</sup>	Glucose	36	2.2	0.45
<i>E. cloacae</i> IIT-BT08 <sup>58</sup>	Sucrose	36	3.0	0.66
<i>E. cloacae</i> IIT-BT08 <sup>58</sup>	Cellobiose	36	2.7	0.65
<i>E. cloacae</i> IIT-BT08 <sup>58</sup>	L-Arabinose	36	1.5	0.36
<i>E. cloacae</i> IIT-BT08 <sup>58</sup>	Fructose	36	1.6	0.44
<i>E. cloacae</i> IIT-BT08 <sup>58</sup>	Maltose	36	1.4	0.18
<i>E. cloacae</i> IIT-BT08 <sup>58</sup>	Potato starch	36	-	0.20
<i>E. cloacae</i> IIT-BT08 <sup>58</sup>	C.M. cellulose	36	-	0.09
<i>E. aerogenes</i> HU-101 AY2 <sup>59</sup>	Glucose	37	1.2	-
<i>Enterobacter aerogenes</i> DM11 <sup>60</sup>	Glucos	36	3.80	-
<i>Rhodospseudomonas palustris</i> P4 <sup>61</sup>	Glucose	37	2.76	-
<i>Citrobacter</i> sp. Y19 <sup>62</sup>	Glucose	36	2.49	-
<b>Strict anaerobes</b>				
<i>C. beijerinchi</i> AM21B <sup>63</sup>	Glucose	36	2.0	0.66
<i>C. beijerinchi</i> AM21B <sup>63</sup>	Starch	36	1.8	0.41
<i>C. paraputrificum</i> M-21 <sup>64</sup>	N-acetyl-v-glucosamine (GlcNAc)	45	2.4 <sup>a</sup>	-
<i>C. paraputrificum</i> M-21 <sup>64</sup>	Ball-milled	45	1.5 <sup>a</sup>	0.21
<i>C. paraputrificum</i> M-21 <sup>64</sup>	raw shrimp and lobster shells	45	1.2 <sup>a</sup>	0.06
<i>C. paraputrificum</i> M-21 <sup>64</sup>	Acid/alkali treated raw shrimp and lobster shells	45	1.3 <sup>a</sup>	0.1
<i>C. paraputrificum</i> M-21 <sup>64</sup>	Corn fiber	45	1.1	-
<i>C. paraputrificum</i> M-21 <sup>64</sup>	Cellobiose	45	1.4	-
<i>C. paraputrificum</i> M-21 <sup>64</sup>	Glucose	45	1.1	-
<i>C. bif fermentans</i> <sup>65</sup>	Wastewater sludge	35	2.1 <sup>b</sup>	-
<i>C. butyricum</i> CGS5 <sup>66</sup>	Sucrose	37	1.39	0.21
<i>C. saccharoperbutylacetonicum</i> ATCC 27021 <sup>67</sup>	Lactose	30	1.41	0.12
<i>C. saccharoperbutylacetonicum</i> ATCC 27021 <sup>67</sup>	Sucrose	30	1.42	0.20
<i>C. saccharoperbutylacetonicum</i> ATCC 27021 <sup>67</sup>	Maltose <sup>6</sup>	30	1.39	0.12
<i>C. saccharoperbutylacetonicum</i> ATCC 27021 <sup>67</sup>	Glucose <sup>6</sup>	30	1.37	0.16
<i>C. saccharoperbutylacetonicum</i> ATCC 27021 <sup>67</sup>	Fructose	30	1.20	0.14
<i>C. saccharoperbutylacetonicum</i> ATCC 27021 <sup>67</sup>	Cheese whey	30	1.35	0.14
<b>(Hyper)thermophiles</b>				
<i>Thermotoga maritima</i> <sup>68</sup>	Glucose	80	4.00	-
<i>Caldicellulosiruptor saccharolyticus</i> <sup>69</sup>	Paper sludge hydrolysate	70	3.84	0.12
<i>Caldicellulosiruptor saccharolyticus</i> <sup>70</sup>	Sucrose	70	3.33	0.20
<i>Thermotoga elfi</i> <sup>70</sup>	Glucose	65	3.33	-
<i>Thermotoga neapolitana</i> <sup>71, 72</sup>	Glucose/soluble starch	70	-	0.01
<b>Co-cultures</b>				
<i>C. butyricum</i> and <i>E. aerogenes</i> <sup>73</sup>	Starch residue		1.7	
<i>C. butyricum</i> IFO13949 and <i>E.</i> <i>aerogenes</i> HO-39 <sup>73</sup>	Starch residue	37	1.7	-

<sup>a</sup>mol/mol-GlcNAc; <sup>b</sup>mmol H<sub>2</sub>/g-COD

production was not affected during transition. Severe washout of support carriers is presumably attributed to fast-growing characteristics of H<sub>2</sub>-producing bacteria, wherein maximum specific growth rate and cell yield coefficient were determined to be 0.5 h<sup>-1</sup> and 0.12 g-VSS/g-glucose, respectively<sup>33</sup>. A large amount of support carriers is normally required to support microorganism growth in biofilm processes. Carriers occupy a considerable space in reactor and reduce effective volume for biomass-substrate interactions, resulting in lower reactor performance and efficiency. Supporting carriers need to be replaced periodically due to wear and tear. Cost of material replacement could be a major economic consideration in maintenance.

Granular sludge processes generally exhibit long startup. A complete development of H<sub>2</sub>-producing granules may take several months<sup>25,27</sup>. During startup of an UASB H<sub>2</sub>-producing reactor, Mu & Yu<sup>47</sup> found that small granules (diam 400-500 μm) were formed at reactor bottom after 140 days of operation. Granules developed further to sizes larger than 2.0 mm upon 200 days. Although, reactor reached steady-state H<sub>2</sub> production and substrate degradation after 5 months of startup operation, development and accumulation of mature and stable granular sludge were only completed beyond 8 month of operation. Chang & Lin<sup>55</sup> noted that a UASB reactor took 39 days to achieve constant gas production at a HRT of 24 h and granules become visible after 120 days of operation. A longer period (180 days), however, was required for further development of granules.

Granulation of H<sub>2</sub>-producing cultures can be markedly accelerated. Packing of a small quantity of carrier matrices significantly accomplished sludge granulation within 80-290 h in a novel carrier-induced granular sludge bed (CIGSB) bioreactor<sup>56</sup>. Column reactors were initially packed with cylindrical activated carbon, spherical activated carbon, sand or filter sponge at a bed height of 4-8 cm and with bed porosities of 90-99%. Granulation of seed sludge could take place in all carrier-packed reactors as HRTs were shortened to 4-8 h, dependent on carrier type.

By adding cationic polymer (cationic polyacrylamide) and anionic polymer (silica sol), rapid granulation of H<sub>2</sub>-producing culture could be accomplished within 5 min<sup>50</sup>. As sludge has a negative charge of -26 mV, high molecular weight cationic polymer (MW, 15,000,000) with 0.7% (w/w) of dry sewage digester sludge was added and stirred at 200 rpm for 2 min to neutralise sludge.

Since residual cations may cause detrimental effect on microorganisms, anionic silica sol of 0.7% (w/w) of dry sewage digester sludge were added and stirred at 200 rpm for 2 min. Total time required for granulation was about 5 min. When granular sludge was operated in a stirring reactor, granular shape was maintained stably, its size ranged from 1.0 to 3.0 mm and maximum concentration granular sludge was found to be approx. 7 g/l. Zhang *et al*<sup>45</sup> developed an approach of acid incubation to initiating formation of H<sub>2</sub>-producing granules rapidly in a CSTR. H<sub>2</sub>-producing granules were formed rapidly within 114 h as seed microbial culture was subjected to a 24 h period of acid incubation at a pH of 2.0. Changing culture pH would result in improvement in surface physicochemical properties of culture favouring microbial granulation.

## Reactor Type

### Fixed-bed Reactor

Fixed- or packed-bed reactor is operated under lesser extent of hydraulic turbulence, thus its microbial cultures usually encounter mass transfer resistance resulting in lowered rates of substrate conversion and H<sub>2</sub> production. Kumar & Das<sup>51</sup> investigated H<sub>2</sub> production by *Enterobacter cloacae* attaching on coir in packed-bed reactors at a HRT of 1.08 h, and found that rhomboid bioreactor with convergent-divergent configuration gave maximum H<sub>2</sub> production (1.60 l/h) as compared with tapered reactor (1.46 l/h) and tubular reactor (1.40 l/h), attributed to higher turbulence created by reactor geometry favouring mass transfer and reduced gas hold-up.

Rachman *et al*<sup>52</sup> found that high H<sub>2</sub> molar yield could not be maintained consistently in a packed-bed reactor, although pH in effluent was controlled at > 6.0. This is because pH gradient distribution along reactor column resulted in a heterogeneous distribution of microbial activity. In order to overcome mass transfer resistance and pH heterogeneous distribution, fluidized-bed or expanded-bed reactor system with recirculation flow was recommended to be more appropriate in further enhancing H<sub>2</sub> production rate and yield. Increasing slurry recycle ratio can alleviate mass transfer resistance in a packed-bed reactor. Kumar & Das<sup>51</sup> observed that both H<sub>2</sub> production and substrate conversion rates of a packed-bed reactor increased with recycling ratio. Maximum H<sub>2</sub> production rate (1.69 l/h) was noted at a recirculation ratio of 6.4.

Support materials have important effects on biomass retention and consequently  $H_2$  production in fixed-bed reactors. Chang *et al*<sup>53</sup> immobilized acclimated sewage sludge on surfaces of porous supports using loofah sponge, expanded clay, and activated carbon for continuous  $H_2$  fermentation in fixed bed reactor. Besides loofah sponge, other carriers exhibited better biomass yields. By comparing two support carriers favouring biomass yield, activated carbon was found a better choice of support carriers used in  $H_2$ -producing fixed-bed reactors, with which maximum  $H_2$  production rate (1.32 l/l.h) was reached at a HRT of 1 h and a sucrose concentration of 20 g/l.

Kumar & Das<sup>51</sup> assessed effect of support materials on immobilization of *Enterobacter cloacae* IIT-BT 08 in packed-bed reactors and found that coir, with bigger surface area due to its fibrous and corrugated properties, is best carrier compared to rice straw and bagasse in terms of cell retention (0.44 g dry cell/g dry carrier), packing density (100 g/l reactor volume), cell loading (44 g dry cell/l reactor volume) and  $H_2$  production rate (62 mmol/l h). Therefore, packing materials of higher surface area are preferred in packed-bed reactors for  $H_2$  production.

#### Fluidized Bed Reactor (FBR)

In gel-immobilized sewage sludge<sup>38</sup>, immobilized culture was able to produce  $H_2$  efficiently in a three-phase FBR operated at a HRT between 1-6 h with a maximal steady-state rate (0.93 l/l.h) and an optimal yield of  $H_2$  (2.67 mol/mol sucrose), which was highest value reported in gel-immobilized culture systems. Zhang *et al*<sup>31</sup> obtained higher  $H_2$  production by biofilm culture (pH 4.0) growing on granular activated carbon in an anaerobic FBR at HRTs of 0.5-4 h and influent glucose concentrations of 10-30 g/l. At operating pH, biofilm sludge concentration was retained up to 21.5 g-VSS/l.  $H_2$  might be produced efficiently in an anaerobic FBR as  $H_2$  production rate reached a maximum rate of 2.36 l/l.h.

#### UASB Reactor

UASB reactor system has been applied in  $H_2$  production due to its potential of high biomass concentration and treatment efficiency. Chang & Lin<sup>55</sup> found that  $H_2$  yield stabilized at 1.5 mol  $H_2$ /mol sucrose at HRT of 8-20 h in a UASB granular reactor. The yield drastically decreased at a HRT of 4 or 24 h.  $H_2$  production rate (0.25 l/l.h) and specific  $H_2$  production rate

(53.5 mmol  $H_2$ /g-VSS.day) peaked at a HRT of 8 h. Biomass concentration reached maximum value of 7.2 g-VSS/l at a HRT of 24 h, but decreased to 5.0 g/l at optimum HRT of 8 h. Yu & Mu<sup>27</sup> studied  $H_2$  production (yield 0.49-1.44 mol- $H_2$ /mol-glucose) from synthetic sucrose wastewater in a UASB reactor with granular sludge operated at 38°C and a pH of 4.4±0.1 for over 3 years.  $H_2$  production rate increased with increasing substrate concentration from 5.33 to 28.07 g-COD/l, but decreased with increasing HRT from 3 to 30 h. However, optimum operating conditions only gave rise to a low  $H_2$  production rate (0.2 l/l.h).

Yu *et al*<sup>29</sup> investigated  $H_2$  production from rice winery wastewater in an upflow anaerobic reactor inoculated with mixed anaerobic cultures at various HRTs (2-24 h), substrate concentrations (14-36 g-COD/l) and temperatures (20-55°C).  $H_2$  yield (1.37-2.14 mol- $H_2$ /mol-hexose) attained optimum  $H_2$  production rate (0.16 l/l.h) and specific  $H_2$  production rate (8.02 l  $H_2$ /g VSS d) under following testing conditions: biomass concentration, 2.50 g-VSS/l; HRT, 2 h; COD, 34 g/l; and temp., 55°C. Due to a low level of biomass retention, UASB granular system with or without granular sludge did not show advantages in  $H_2$  production rate or specific  $H_2$  production rate compared over other systems such as fixed-bed reactor or FBR.

#### CSTR Granular Sludge Reactor

Fang *et al*<sup>25</sup> demonstrated that  $H_2$ -producing acidogenic sludge could agglutinate into granules in a well-mixed CSTR reactor treating a synthetic sucrose-containing wastewater at 26°C, pH 5.5 and HRT of 6 h. Formation of granular sludge enhanced biomass growth up to 20 g/l and consequently  $H_2$  production rate up to 0.54 l/l.h with 97% sucrose being degraded. In a similar CSTR system<sup>46</sup> with granular sludge fermenting glucose wastewater (10 g/l) at a pH of 5.5 and 37°C, a maximum  $H_2$  yield (1.81 mol- $H_2$ /mol-glucose) and a maximum  $H_2$  production rate (3.20 l/l.h) were obtained at a HRT of 0.5 h. Wu *et al*<sup>42</sup> further developed such a granular-sludge based CSTR system. CSTR system was initially seeded with silicone-immobilized sludge at 40°C and pH 6.6±0.2, and reactor performance was examined at a HRT of 0.5-6 h and an influent sucrose concentration of 10-40 g-COD/l. Self-flocculated granular sludge occurred at a HRT of 0.5 h, reached a concentration of up to 35.4 g-VSS/l, and resulted in a significant increase in  $H_2$  production rate (15 l/l.h). A two-fold increase in specific  $H_2$  production rate was found after formation of

self-flocculated granular sludge due to transition in bacterial community structure.

Several other high-rate H<sub>2</sub>-producing systems based on granular sludge techniques have been developed. Packing of a small quantity of carrier matrices at the bottom of upflow reactor significantly stimulated sludge granulation that can be accomplished within 100 h in a novel carrier-induced granular sludge bed (CIGSB) bioreactor<sup>49</sup>. CIGSB bioreactor, started up with a low HRT of 4-8 h (corresponding to an OLR of 2.5-5 g COD/l-h), enabled stable operation at an extremely low HRTs (0.5 h) without experiencing biomass washout. Granular sludge was rapidly formed in CIGSB supported with activated carbon, reaching a maximum concentration of 26 g/l at a HRT of 0.5 h. Ability to maintain high biomass concentration at low HRTs corresponding to high OLRs highlights remarkable H<sub>2</sub> production efficiency of CIGSB processes. Reactor achieved an optimum volumetric H<sub>2</sub> production rate at 7.3 l/l-h (7.15 mol/l-d) and a maximum H<sub>2</sub> yield (3.03 mol H<sub>2</sub>/mol sucrose), when operated at a HRT of 0.5 h on an influent sucrose concentration of 20 g COD/l. Under optimum conditions, H<sub>2</sub> content and substrate conversion exceeded to 40 and 90%, respectively. H<sub>2</sub> production rate of CIGSB system further improved<sup>56</sup> (9.3 l/l.h) by optimizing reactor column height and diameter at a ratio of 12 and with agitation. After altering physical configuration of CIGSB bioreactor, concentration of granular sludge increased to 40 g-VSS/l.

### Conclusions

With respect to cell immobilization approaches, granular sludge processes are most suitable for dark H<sub>2</sub> fermentation. Granular sludge has proved feasible in CSTR, UASB reactor, packed-bed reactor and FBR. A reactor system with adequate hydrodynamic turbulence is preferred. All immobilized-cell systems for continuous H<sub>2</sub> production were investigated only in laboratory scale on soluble substrates rather than on particulate wastewaters, and scale-up studies have yet to be reported. Commercial methane-producing granule-based UASB reactors are undesirable when operating on substrates with high suspended solids. Same rationale might be applicable to H<sub>2</sub>-producing granular reactors. Producing H<sub>2</sub> from organic particulate wastewaters and solid wastes has to rely on reactor system in either a continuous mode or a batch mode. Enhancing volumetric H<sub>2</sub> production rates is thus a major challenge to such biohydrogen-producing systems while fermenting particulate wastewaters or solid wastes.

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