Enhancement strategies for hydrogen production from wastewater: a review

Periyasamy Sivagurunathan[1,2], Gopalakrishnan Kumar[2,3], Sang-Hyoun Kim[1], Takuro Kobayashi[2], Kai-Qin Xu[2], Wenshan Guo[4], Huu Hao Ngo[4*]

[1] Department of Environmental Engineering, Daegu University, Gyeongsan, Gyeongbuk 712-714, Republic of Korea

[2]Center for materials cycles and waste management research, National Institute for Environmental Studies, Tsukuba, Japan

[3]Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Vietnam

[4]Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology, Sydney, Broadway, NSW 2007, Australia

*Corresponding Author's Address: h.ngo@uts.edu.au

Abstract

This mini review focuses on the current developments in the field of dark fermentation technologies using wastewater as carbon and nutrient source in batch reactors. Besides, the major microbiota (pure, enriched mixed, co and mixed cultures) involved in the process have been emphasized. Additionally, problems associated with the lower production performances and the overcoming strategies applied to enhance the production rate (HPR) and yield (HY) in ways of bio-augmentation, immobilization, enrichment technique and nano particles (NP) addition were also discussed. This mini review provides more insights about the recent developments of the dark fermentative hydrogen production (DHFP) process and their advantages in a brief manner. The perspective towards the development of sustainable society by using bioH_2 technology is enlightened.

Key words: wastewater, hydrogen, production rate, fermentation, hydrogen yield

1. Introduction

The deterioration of fossil fuels due to rapid consumption has caused environmental issues and its associated pollution leads to an alternative renewable energy source that can reduce the impact of the pollution during the combustion process. In recent years, the energy research has focused on biologically oriented fuels production which includes bioethanol, biomethane, biodiesel and biohydrogen owing to the fact that production of these carriers in a moderate temperature and pressure conditions and also from renewable organic matters which are abundant in various waste streams such as lignocelluloses, algae and wastewaters. Among the biofuels, hydrogen production meets the environmental pollution standards with no pollution formation during its consumption. Additionally it also possesses a 2.5 times higher energy yield (122 kJ/g) than hydrocarbon fuels and can be directly involved in power generation via fuel cells implementation.

The light independent fermentative hydrogen production is popular due to facultative anaerobes/aerobes and strict anaerobes by the way of pure cultures, co-cultures and mixed consortia. Among them, mixed microbial consortia provide distinct advantages, such as handling complex organic wastes, resilience to metabolic product inhibition, and high hydrogen production rates. Besides, the mixed consortia operation seems to be a favorable option towards industrial scale applications, in which it can be conducted in non-sterile conditions, reducing the additional operational cost for feedstock purity, and provide a suitable platform for harnessing energy from various complex organic waste materials. Additionally, the seed inocula for hydrogen production can be obtained from soil, sewage sludge, compost, etc. Apart, from inoculum issues, the hydrogen production can be conducted in the reactor by batch, fed-batch, repeated batch and continuous mode of operation. Among them, continuous mode of operation is widely adopted due to its stable and higher hydrogen production rates as well as efficient utilization of organic wastes to generate hydrogen [1].

1.1 Biohydrogen production methods

At present, the production of hydrogen (about 90%) mainly acquired from non-renewable sources through the conversion of methane and oil/naphtha which is neither sustainable nor environmental-friendly [2,3]. Thus, clean technologies for making hydrogen energy carrier should be developed. For this purpose, biological approaches take the leading role as emerging opportunities. The biological hydrogen production can be achieved by different taxonomic, physiologic types of microorganisms in an anaerobic environment, while the methods are classified as direct or indirect biophotolysis, light-dependent **photo fermentation, light-independent dark fermentation** and **microbial** electrolysis **cells.** The pros and cons of light dependent and independent technologies are documented in Table 1.

In direct and indirect photolysis, light energy is used to split water and transfer electron to generate hydrogen and oxygen by green algae and cyanobacteria, respectively. However, the light conversion efficiency is relatively low, and oxygen presence also inhibits the key enzyme hydrogenease and nitrogenase for hydrogen production [4,5]. In photo-fermentation, photosynthetic bacteria utilize light energy and organic acids to produce hydrogen, whereas lower **light conversion ability,** and **high energy requirement** are **the** major limitations of this process result in lower hydrogen production rate [6,7]. Additionally the elimination of competing microorganisms while using the dark fermentation effluent, ammonia removal and the size of the photo bioreactors are the challenging task to improve the efficiency of the process [8].

As for microbial electrolysis cells (MEC), the low amount of voltage is applied to degrade the volatile fatty acids and further utilized by acidophilic populations with the release of electrons/protons to generate hydrogen [5,9]. **The** dark fermentation can be carried out by fermentative bacteria. It can produce hydrogen without any external light source and utilize variety of carbon sources as substrates. Although the co-generation of volatile fatty acids and alcohols relatively lowers the hydrogen yield, this hurdle can be overcome by integrating approaches such as photo fermentation, and MEC via two-step process for potential conversion of acid-rich effluent into hydrogen with additional recovered energy from the process [10,11].

1.2 Dark fermentative hydrogen production

Dark fermentation hydrogen producing bacteria are principally facultative or strict anaerobes, which produce hydrogen during the degradation of carbohydrates molecules. The major secreted by products formed during the fermentation reactions are acetate, butyrate, propionate, lactate and ethanol. The fermentative hydrogen production is a spontaneous reaction, however depending on the bacterial groups and the reaction operational conditions, the secreted by products varied remarkably. It is widely observed that three major fermentation pathways such as butyrate-type, propionic-type, and ethanol-type occurred via dark fermentation reactions classified based on the major by products formation [12]. The butyrate-type pathway dominated with H_2, CO_2, butyrate and acetate involved in major hydrogen production reactions, on the other hand, the propionic-type pathway involved in hydrogen consuming reactions with the formation of propionate and acetate. Thus propionic-type pathway should be avoided for efficient hydrogen production [13]. The ethanol-type fermentation pathway involved in the hydrogen producing reactions at low pH 4.5 with the formation of ethanol, acetic acid, H_2, CO_2 [14].

Several species of bacteria can produce hydrogen under anaerobic condition, including the species of *Clostridium, Enterobacter, Bacillus,* and *E.coli*. These bacteria are found in different environmental condition and can utilize variety of substrates.

Glucose yields different quantities of hydrogen depending on the fermentation type and by products formation. With obligate anaerobes, a maximum yield (Eq. 1) of 4 moles H_2 per mole of glucose is obtained:

$$C_6H_{12}O_6 + 4H_2O \rightarrow 2\,CH_3COO^- + 2HCO_3^- + 4H^+ + 4H_2 \qquad (1)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2CH_2\,COO^- + 2HCO_3^- + 3H^+ + 2H_2 \qquad (2)$$

However, if the main producers are facultative anaerobes such as *E. coli* (Eq. 2), maximum 2 moles of H_2 is formed.

The dark fermentation processes share similar concepts with anaerobic digestion, which is widely used in wastewater treatment. The most common feature is the separation of the gaseous products from the treated water [9]. In a typical anaerobic digestion process, methane (Fig. 1) and several organic acids (e.g. acetate, butyrate, propionate) and solvents (ethanol, propanol, butanol) are produced as the end products. The process generally involves three groups of microorganisms which coexist and work as a consortia. First, hydrolytic bacteria transform the complex polymers into simple monomers. Then, fermentative bacteria produce organic acids, H_2 and CO_2 from monomeric molecules, while acetogens degrade some volatile fatty acids (VFA) (e.g. propionate, butyrate) to produce acetate and hydrogen. Finally, acetate and hydrogen are used to produce methane by methanogens [15].

Hydrogen is produced as an intermediate product of methane production. Thus, to produce hydrogen as the main product, methanogenesis has to be blocked. Pretreatments such as heat treatment, chemical treatment and pH treatment will block or repress methanogenic activity during biohydrogen production [16]. Among them, heat treatment was a widely adopted method to suppress the hydrogen-consuming bacteria and enrich the spore-forming hydrogen producers. However, heat treatment was not successful in eliminating homoacetogenic bacteria. Homoacetogens can be suppressed by the removal of CO_2 from the medium using some strong alkaline chemicals like KOH [17].

2. Microbiome involved in dark fermentative hydrogen production (DFHP) from wastewaters

Microbial consortia in the seed sludge or the inoculum is the main factor arbitrates the production performance. Microbiomes in the DFHP process vary accordingly, some are naturally occurring (sewage sludge, soil, etc) and some are fabricated to have the desired population (for example, enriched mixed cultures). The diversity in the source could also be enhanced by some pretreatment methods, such as heat pretreatment, which selectively enrich the clostridia species, which are known as good hydrogen producers. Major microbiome involving in the DFHP is discussed further. As various wastewaters are rich in organic content, the application of wastewater for hydrogen generation is a prospering way for producing the clean energy with effective waste treatment. Moreover, the industrial wastewaters containing easily hydrolysable carbohydrates, unlike the lignocellulosic counterparts, with moderate nutrient and mild pretreatment requirement (to remove co-existing hydrogen-consuming bacteria) and positive energy gain [18,19].

Table 2 summarizes the microbiome involved in the DHFP process of various types of industrial wastewaters. Taken into the view of importance of wastewater treatment and co-generation of hydrogen gas, various types of microbiome, viz., pure cultures, co-cultures, mixed cultures and enriched cultures were investigated for stable and efficient hydrogen production from wastewaters. Generation of hydrogen from industrial wastewaters was significantly influenced by the inoculum type, composition and biodegradability nature [18]. Among them, inoculum source portrayed a major factor in deciding the performances of hydrogen production. Since, the prevailing fermentation metabolic end products influenced by the activity of the hydrogen-producing bacteria population. The microbiome used for the hydrogen production from wastewater include: mixed consortium of anaerobic bacteria obtained from soil, compost, anaerobic digester sludge and pure cultures of isolated hydrogen producing bacteria [20].

The main advantage of pure cultures over the mixed cultures is relatively high yield, however the chance of contamination and difficulties in maintenance are the major aspects need to be considered, besides the shift in metabolic pathway are easily detected due to the limited microbial diversity abundance in the system[20]. Additionally, the improvement of hydrogen production rate and yield can also be done by using the metabolic engineering applications[10]. On, the other hand, exploiting the usage of mixed culture is considered as a practical approach to maximize the hydrogen production in large scale industrial process. The mixed culture operation posses a robust performance over the pure cultures for wide range of organic utilization, non – sterile nature of the feedstock (direct utilization of the industrial wastewater streams) and resistance to the environmental factors (pH, Temperature, organic loading rate and so on) [1].

However, the key challenges relied with the application of mixed culture operation is the co-existence of other non-hydrogen producing populations and monitoring the dynamic behavior of the bacterial shifts. For instance as mentioned by Chu et al. [21], the population dynamics of the hydrogen producing bacteria from sugary wastewater was affected with the predominance of non-hydrogen production bacteria during the process disturbances of pump failure deteriorates the H_2 production, further heat-treatment of the inoculum leads to the substantial improvement in the hydrogen production.

2.1 Pure culture

Various pure bacterial species have been investigated in recently to produce hydrogen from industrial wastewaters. Starchy and carbohydrate rich wastewaters such as cassava, distillery effluent, rice mill wastewater were used as a substrate and their hydrogen production indexes hydrogen production rate (HPR) and hydrogen yield (HY) lied in the range of 0.6 to 1.9 L/L-d and 1.40 to 2.41 mol/mol substrate , respectively. A study by Ramprakash and Muthukumar [22] indicated that the hydrogen production efficiency depends on the type of bacterial species. The peak **hydrogen yield of 1.** 74 **mol/mol** sugar **obtained** from enzymatically pretreated **rice mill wastewater** by *Enterobacter aerogenes* is superior to the other facultative anaerobe *Citrobacter freundii* with an achievable HY of 1.40 mol/mol sugar, respectively. In another study, by the same group [23], mentioned that the pretreatment of substrate played a key role in improving the hydrogen production performances from **rice mill wastewater.** The combined two-step acid and enzyme hydrolysis of rice mill wastewater leads to the significant improvement in the specific hydrogen production rate (SHPR) with a value of 35.4 mmol/g cell .h than the individual pretreatment of acid and enzyme hydrolysis of 32.4 mmol /g cell .h and 32. 6 mmol/g cell .h, which shows that appropriate pretreatment and or combination is appropriate for efficient hydrogen production from complex wastewater such as rice mill wastewater, respectively [23].

Cappeletti et al. [24], investigated the strict anaerobic bacteria *Clostridium acetobuytlicum* ATCC824 for biohydrogen production from starch-rich cassava wastewater. Their results showed that the lower chemical oxygen demand (COD) concentrations of 5 g/L favored the higher hydrogen yield with a value of 2.41 mol/mol glucose; further increment in the substrate concentration substantially affected the hydrogen yield. Mishra and Das [25], showed that the addition of supplementary nutrients (yeast extract, malt extract, Fe++, Cu++ and Mg++) showed a 2.2 times higher yield (165.3 mL/ g COD) than the non-supplemented distillery effluent. These studies, showed that types of inoculum, initial pretreatment of wastewater, supplementation of external nutrients majorly influenced the overall performances of hydrogen production from industrial wastewater streams by pure culture.

2.2 Co-cultures

Another variant of the pure culture mediated hydrogen production is the co-culture of hydrogen producing bacteria. The co-culture or bioaugmentation term has been widely used for the enhancement of hydrogen production performances. Co-cultures can aids to improve the species richness within the hydrogen-producing microbial community. The detailed information on the co-culture addition on improvement of H_2 productions were discussed in a recent review [20].

The Co-culture mediated hydrogen production from wastewaters are quite few and for instance in a study by Sivagurunathan et al. [26], the addition of facultative anaerobes (*Enterobacter cloacae* (DSM 16657) and *Escherichia coli* XL1-BLUE) with anaerobically enriched mixed cultures (with *Clostridium* sp, being dominant) promotes the effective substrate utilization and improvement in HPR to a value of 2.2 L/L-d than the individual anaerobic enriched mixed culture of 1.81 L/L-d, respectively. In another study, by Goud et al. [27], addition of potent acidogenic isolates with the native anaerobic acclimatized culture, showed an improvement in hydrogen production from real field food WW. Addition of native inoculum with *Bacillus subtilis* elevated the maximum HPR value with 2.1 L, which is about 175 fold higher than the native inoculum with a value of 0.12 L at an initial COD of 50 g/L, which showed that the augmentation with potent acidogenic isolates is an effective strategy for enhancing the biohydrogen production.

2.3 Enriched mixed culture

In recent years, the application of enriched mixed cultures (EMC) has been grown significantly to improve the hydrogen production performances due to the enriched populations of the definitive species from complex ecosystem of the microbial niche. The ideal background, related with the enrichment of hydrogen producers is to select the most reliable and stable functional consortium, which can able to perform the efficient hydrogen production with short adaptation period. In general, repeated batch mode operation was widely adopted to obtain a selective enrichment of efficient hydrogen producers, or in other words, during the repeated batch transfer, the dominant microorganism allowed to grow with a significant elimination of other populations from the ecosystem. For example, as indicated by Hasyim et al. [28], during the repeated batch operation over six transfers, the increment of hydrogen yield during repeated batch transfers from starch processing wastewater by enriched mixed cultures (geothermal hot spring) occurred with a peak value of 442 mL/g starch at substrate concentration of 2.5 g/L attained at the end of the sixth transfer.

In another study by O-Thong et al. [29], it is indicated that the selection of inoculum source plays a significant role in the enrichment of hydrogen producers from raw cassava starch processing wastewater. Among the tested hot-spring inoculum (Klong Pai Poo hot spring (PK) , Romani hot spring (PR), Phang Nga Province and Wat Than Nam (SW)) for culture enrichment , the enriched mixed culture obtained from PK hot spring provided a 26-44% maximum hydrogen yield during the repeated batch operation and provided a stable value of 236 mL/ g starch, which is higher than the other two inoculums SW and PR with a value of 180 and 128.4 mL/ g starch ,respectively. The observed variation of hydrogen yield is attributed by the variations in the microbial community. Besides, the enriched mixed cultures can be also obtained using pure substrates such as glucose and starch and latter applied with the real wastewaters. For instance, Sen and Suttar, [30] enriched the hydrogen producing bacteria from sago starch, afterwards the initial enrichment or adaptation with the sago starch, the selective populations were cultivated with real starch processing wastewater and showed a higher HY 456 mL/g starch than sago starch 412.6 mL/g starch, which showed that the enriched mixed 11 cultures possess an rapid acclimatization for the synthesis of metabolic intermediates which favors the efficient hydrogen production. In another study by Sivagurunathan et al. [31] , the enriched cultures obtained from compost fed with glucose was also successfully assessed with beverage wastewater for hydrogen production and showed a stable HY of 1.1 mol/mol hexose.

2.4 Mixed microbiota

The mixed cultures of anaerobic communities obtained from anaerobic sludge, soil, slaughter house sludge, anaerobic digester sludge has been successfully employed for hydrogen production from wastewaters. Among the studied wastewaters dairy wastewater was widely investigated (5 studies) with a HY range from 13.54 to 29.91 mmol/g COD, followed by distillery wastewater (3 studies) with a range in HY from 8.83 to 10.95 mmol/g COD, respectively. Sugar beet juice sugar beet juice provides a maximum HY of 3.2 mol/mol hexose [32], followed by organic wastewater 2.32 mol/mol hexose [33] and textile wastewater of 1.37 mol/mol hexose [34], respectively. The variations in the hydrogen production are mediated by the composition of wastewater characteristics, inoculum, and operational conditions and so on. More detailed parameters were discussed elsewhere [1]. The microbiomes involved in DFHP process are heterogenic in nature due to their origination of the seed source.

3. Perspectives and challenges

The surpassing growth of the DFHP from WW research seems a promising way towards future commercial applications, the substrate degradation/growth of competitor microorganisms and lower hydrogen yield are the major challenging aspects has to be overcome using appropriate possible strategies [35] for the enhancement of hydrogen production performances from wastewaters.

In general, wastewaters are a rich source of organic carbon, thus it supports not only the DFHP microorganism's growth, but also promotes the growth of the other unwanted microorganisms during the storage/transportation. The presence of other microbial populations could be the possible reason for the competition towards the substrate, besides, hinders the activity of hydrogen producing bacteria and resulting in the lower production performances. Hence, removal or suppressing the activity of these hydrogen consumers and other microbiomes in the reaction is essential for the enhanced hydrogen productivity. Another notable challenge in the DHFP of WW feedstock is the enhancing activity of hydrogenase enzyme of the hydrogen producers, which requires many practices, while using the mixed cultures, since the population and proportion differs widely in this aspect. The lower hydrogen yield obtained from DHFP via

WW feedstock can be improved by other possible strategies which will be discussed in the upcoming sections.

In this review, based on the points discussed above, which mainly focused on the microbiomes involved in the DHFP, the main attempts made towards the enhancement possibilities are as follows, immobilization, bioaugmentation, nanoparticles (NP) addition which are discussed in the coming sessions. The possible way of integration systems and pathway is presented in Fig 2.

3.1 Improvement strategies

Fig 3, illustrates the possible attempts made towards the improvement of production performances in the DHFP process in batch reactions. This includes, active inoculum preparation via Enrichment method and augmentation with other cultures (especially, facultative anaerobes). In other words, enriched mixed cultures reduced the recovery period of the bioreactor in case of process disturbances/upset due to the functional consortium. Apart from enrichment, bioaugmentation strategy also proclaimed to induce the performance of HPR and HY. And recently, immobilization (hybrid material) and Nano particles (NP) such as Fe_2O_3 plus NiO, addition also enlightened in the further sections.

3.1.1 Enrichment

Enrichment is an operational strategy towards the selection/enrichment of particular microbial consortium, in this case, hydrogen producing *Clostridium* and other bacterial population. In a study by Sen and Datar [30], employed EMC to enhance the production performance from sago-processing wastewater feedstock, and they reported that peak HY of 126.5 mL/g COD, while using the Peptone, yeast extract and agar (PYG medium) for the enrichment of heat treated cultures [30]. Similarly, the PYG medium was employed for the enrichment strategy by another studies, by Sivagurunathan et al. [26], reported that, HPR value as 1.8 L/L-d, while using beverage WW as carbon source. Another investigation by the same author [31], utilized the cow dung compost as a seeding microbiota for the enrichment and employed beverage WW as feedstock in the DHFP, reported the HY of 1.92 mol/mol. These above mentioned reports are very few to be mentioned regarding the EMC usage and boosting performances of H_2 production.

3.1.2 Bio-augmentation

Bioaugmentation is reported widely an excellent method to promote the performances of bacterial populations bearing different capacities. While they are working together, there is a synergy/symbiotic relationship evolved and thus results in the enhanced levels of end products. A recent report by Kumar et al. [36], narrated that, bioaugmentation of facultative anaerobic strains with mixed cultures have enhanced the production performances, augmenting the mixed cultures with *E.coli* XL1 blue, a facultative anaerobic bacterium improved the performances by creating strict anaerobic conditions for the *Clostridium* species, which is well known as hydrogen producer. The peak HPR and HY values were 1.75 L/L-d and 260 mL/g COD added, while the bioaugmentation with *E. coli* XL1 blue, and the PCR-DGGE results have proved the same. Another report by Sivagurunathan et al. [26], investigated the promotion strategy using the enriched mixed cultures (EMC) using statistical approach for the optimization factors. In that report authors have implemented the co-cultures of *E.cloacae* and E. *coli* XL1 blue as well. The results have achieved 2.25 L/L-d as HPR as peak production performances, while mixing the EMC with *E. cloacae*.

3.1.3 Immobilization

Immobilization of hydrogen producers has been put forth as an efficient way to overcome the loss of biomass in the system. It could be done in various ways as encapsulation, entrapment, adsorption and recently hybrid via combining 2 or 3 methods together. A study by Sivagurunathan et al. [37], reported that using immobilized consortia aided in the improvement of hydrogen production from beverage wastewater (BWW), and the improvements were from 2660 ml/L of suspended cells to 2866 ml/L of immobilized systems in the HPR and 1.07 to 1.12 mol H_2/mol hexose, in HY, respectively.

3.1.4 Nano particles (NP) addition

Addition of metal co-factors such as Fe has been explored as enhancement way towards the higher production performances in DHFP process [16]. However, very recently, another approach called NP addition has gained much attention due to the significant contribution in the 15 improvement of production performances. A study by Gadhe et al. [38], investigated the effects of nano particles in the BHP process, using dairy wastewater as feedstock, the addition of Fe_2O_3 and NiO, NP has significantly increased the production performances over 1.5 folds and resulted in HY and SHPR of about 17.2 mmol/g COD, and 47.67 mmol/g VSS.d, respectively, and also authors have reported that intensified activity of the ferredoxin oxidoreductase, ferredoxin, and hydrogenase enzymes observed by the NPs addition is responsible for the improvement. Another study, by Gadhe et al, [39], investigated the nano sized particles and their effects on bio H2 production. In that report, HY and SHPR were achieved as 8.83 mmol/g COD, and 18.14 mmol/g VSS.d, while co-addition of Fe_2O_3 (200 mg/L) and NiO NP (5 mg/L) showed 1.2-4.5 order more effective towards the improvement.

4. Conclusions

This review comprehended the wastewater to hydrogen as an emerging biofuel technology towards the green and sustainable environment in batch reactors. Major microbiome involved in the reaction are highlighted. The microbial diversity either naturally occurring or engineered in the lab are evaluated based on their performances. It has been turned out that selection of the microbial source and the enrichment conditions are of important factors towards the success and stability of the hydrogen producers in the batch reaction. Furthermore, enhancing strategies such as the addition of nanoparticles (activating the active sites of hydrogenase enzyme) and augmenting with facultative anaerobes (symbiotic relationship and maintain the strict anaerobic conditions) are suggested to enhance the production performance significantly.

Acknowledgements

Authors would like to acknowledge the financial support from the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2014R1A2A2A04005475) and Japan Society for Promotion of Science: JSPS ID: 26-04209 and JSPS ID: 25740056 for the financial support of this study.

References

1. Lin, C.Y.; Lay, C.H.; Sen, B.; Chu, C.Y.; Kumar, G.; Chen, C.C.; Chang, J.S. Fermentative hydrogen production from wastewaters: A review and prognosis. *Int. J. Hydrogen Energy.,* **2012,** *37* (20), 15632-15642.
2. Ewan, B. C. R.; Allen, R. W. K. A figure of merit assessment of the routes to hydrogen. *Int. J. Hydrogen Energy.,* **2005,** *30* (8), 809-819.

3. Chaubey, R.; Sahu, S.; James, O. O.; Maity, S. A review on development of industrial processes and emerging techniques for production of hydrogen from renewable and sustainable sources. *Renew. Sust. Energy Rev.,* **2013,** *23*, 443-462.

4. Das, D.; Khanna, N.; Nejat Veziroğlu, T. Recent developments in biological hydrogen production processes. *Chem. Ind. Chem. Eng. Quart.,* **2008,** *14* (2), 57-67.

5. Khanna, N.; Das, D. Biohydrogen production by dark fermentation. *Wiley Interdisciplinary Reviews: Energ. Environ.,* **2013,** *2* (4), 401-421.

6. Ghimire, A.; Frunzo, L.; Pirozzi, F.; Trably, E.; Escudie, R.; Lens, P. N. L.; Esposito, G. A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. *Appl. Energy.,* **2015,** *144*, 73-95.

7. Hallenbeck, P. C.; Benemann, J. R. Biological hydrogen production; fundamentals and limiting processes. *Int. J. Hydrogen Energy.,* **2002,** *27* (11–12), 1185-1193.

8. Levin, D. B.; Pitt, L.; Love, M. Biohydrogen production: prospects and limitations to practical application. *Int. J. Hydrogen Energy.,* **2004,** *29* (2), 173-185.

9. Azwar, M. Y.; Hussain, M. A.; Abdul-Wahab, A. K. Development of biohydrogen production by photobiological, fermentation and electrochemical processes: A review. *Renew. Sust. Energy Rev.,* **2014,** *31*, 158-173.

10. Kumar Gupta, S.; Kumari, S.; Reddy, K.; Bux, F. Trends in biohydrogen production: Major challenges and state-of-the-art developments. *Env. Technol.,* **2013,** *34* (13-14), 1653-1670.

11. Valdez-Vazquez, I.; Poggi-Varaldo, H. M. Hydrogen production by fermentative consortia. *Renew. Sust. Energy Rev.,* **2009,** *13* (5), 1000-1013.

12. Cohen, A.; Breure, A. M.; van Andel, J. G.; van Deursen, A. Influence of phase separation on the anaerobic digestion of glucose—I maximum COD-turnover rate during continuous operation. *Water Res.,* **1980,** *14* (10), 1439-1448.

13. Cohen, A.; Breure, A. M.; van Andel, J. G.; van Deursen, A. Influence of phase separation on the anaerobic digestion of glucose—II: Stability, and kinetic responses to shock loadings. *Water Res.,* **1982,** *16* (4), 449-455.

14. Ren, N.; Wang, B.; Huang, J.-C. Ethanol-type fermentation from carbohydrate in high rate acidogenic reactor. *Biotechnol. Bioeng.,* **1997,** *54* (5), 428-433.

15. Hawkes, F. R.; Dinsdale, R.; Hawkes, D. L.; Hussy, I. Sustainable fermentative hydrogen production: Challenges for process optimisation. *Int. J. Hydrogen Energy.,* **2002,** *27* (11-12), 1339-1347.

16. Wang, J.; Wan, W. Factors influencing fermentative hydrogen production: A review. *Int. J. Hydrogen Energy.,* **2009,** *34* (2), 799-811.

17. Park, W.; Hyun, S. H.; Oh, S. E.; Logan, B. E.; Kim, I. S. Removal of headspace CO2 increases biological hydrogen production. *Env. Sci. Technol.,* **2005,** *39* (12), 4416-4420.

18. Arimi, M. M.; Knodel, J.; Kiprop, A.; Namango, S. S.; Zhang, Y.; Geißen, S.-U. Strategies for improvement of biohydrogen production from organic-rich wastewater: A review. *Biomass. Bioenergy.,* **2015,** *75*, 101-118.

19. Barca, C.; Soric, A.; Ranava, D.; Giudici-Orticoni, M.-T.; Ferrasse, J.-H. Anaerobic biofilm reactors for dark fermentative hydrogen production from wastewater: A review. *Bioresour. Technol.,* **2015,** *185*, 386-398.

20. Elsharnouby, O.; Hafez, H.; Nakhla, G.; El Naggar, M. H. A critical literature review on biohydrogen production by pure cultures. *Int. J. Hydrogen Energy.,* **2013,** *38* (12), 4945-4966.

21. Chu, C.-Y.; Hastuti, Z. D.; Dewi, E. L.; Purwanto, W. W.; Priyanto, U. Enhancing strategy on renewable hydrogen production in a continuous bioreactor with packed biofilter from sugary wastewater. *Int. J. Hydrogen Energy.,* **2016**, 41(7), 4404-4412.

22. Ramprakash, B.; Muthukumar, K. Comparative study on the production of biohydrogen from rice mill wastewater. *Int. J. Hydrogen Energy.,* **2014,** *39* (27), 14613-14621.

23. Ramprakash, B.; Muthukumar, K. Comparative study on the performance of various pretreatment and hydrolysis methods for the production of biohydrogen using *Enterobacter aerogenes* RM 08 from rice mill wastewater. *Int. J. Hydrogen Energy.,* **2015,** *40* (30), 9106-9112.

24. Cappelletti, B. M.; Reginatto, V.; Amante, E. R.; Antônio, R. V. Fermentative production of hydrogen from cassava processing wastewater by *Clostridium acetobutylicum*. *Renewable Energy* **2011,** *36* (12), 3367-3372.

25. Mishra, P.; Das, D. Biohydrogen production from *Enterobacter cloacae* IIT-BT 08 using distillery effluent. *Int. J. Hydrogen Energy.,* **2014,** *39* (14), 7496-7507.

26. Sivagurunathan, P.; Gopalakrishnan, K.; Lin, C.Y. Enhancement of fermentative hydrogen production from beverage wastewater via bioaugmentation and statistical optimization. *Cur. Biochem. Eng.,* **2014,** *1* (2), 92-98.

27. Goud, R. K.; Sarkar, O.; Chiranjeevi, P.; Venkata Mohan, S. Bioaugmentation of potent acidogenic isolates: A strategy for enhancing biohydrogen production at elevated organic load. *Bioresour. Technol.,* **2014,** *165*, 223-232.

28. Hasyim, R.; Imai, T.; O-Thong, S.; Sulistyowati, L. Biohydrogen production from sago starch in wastewater using an enriched thermophilic mixed culture from hot spring. *Int. J. Hydrogen Energy.,* **2011,** *36* (21), 14162-14171.

29. O-Thong, S.; Hniman, A.; Prasertsan, P.; Imai, T. Biohydrogen production from cassava starch processing wastewater by thermophilic mixed cultures. *Int. J. Hydrogen Energy.,* **2011,** *36* (5), 3409-3416.

30. Sen, B.; Suttar, R. R. Mesophilic fermentative hydrogen production from sago starch-processing wastewater using enriched mixed cultures. *Int. J. Hydrogen Energy.,* **2012,** *37* (20), 15588-15597.

31. Sivagurunathan, P.; Sen, B.; Lin, C.-Y. Batch fermentative hydrogen production by enriched mixed culture: Combination strategy and their microbial composition. *J.Biosci. Bioeng.,* **2014,** *117* (2), 222-228.

32. Dhar, B. R.; Elbeshbishy, E.; Hafez, H.; Lee, H.-S. Hydrogen production from sugar beet juice using an integrated biohydrogen process of dark fermentation and microbial electrolysis cell. *Bioresour. Technol.,* **2015,** *198*, 223-230.

33. Sharma, Y.; Li, B. Optimizing hydrogen production from organic wastewater treatment in batch reactors through experimental and kinetic analysis. *Int. J. Hydrogen Energy.,* **2009,** *34* (15), 6171-6180.

34. Li, Y.-C.; Chu, C.-Y.; Wu, S.-Y.; Tsai, C.-Y.; Wang, C.-C.; Hung, C.-H.; Lin, C.-Y. Feasible pretreatment of textile wastewater for dark fermentative hydrogen production. *Int. J. Hydrogen Energy.,* **2012,** *37* (20), 15511-15517.

35. Sivagurunathan, P.; Kumar, G.; Bakonyi, P.; Kim, S.H.; Kobayashi, T.; Xu, K. Q.; Lakner, G.; Tóth, G.; Nemestóthy, N.; Bélafi-Bakó, K. A critical review on issues and overcoming strategies for the enhancement of dark fermentative hydrogen production in continuous systems. *Int. J. Hydrogen Energy.,* **2016,** *41* (6), 3820-3836.

36. Kumar, G.; Bakonyi, P.; Sivagurunathan, P.; Kim, S.H.; Nemestóthy, N.; Bélafi-Bakó, K.; Lin, C.-Y. Enhanced biohydrogen production from beverage industrial wastewater using external

nitrogen sources and bioaugmentation with facultative anaerobic strains. *J.Biosci. Bioeng.,* **2015,** *120* (2), 155-160.

37. Sivagurunathan, P.; Kumar, G.; Sen, B.; Lin, C.Y. Development of a Novel Hybrid Immobilization Material (HY-IM) for Fermentative Biohydrogen Production from Beverage Wastewater. *J. Chin. Chem. Soc.,* **2014,** *61* (7), 827-830.

38. Gadhe, A.; Sonawane, S. S.; Varma, M. N. Enhancement effect of hematite and nickel nanoparticles on biohydrogen production from dairy wastewater. *Int. J. Hydrogen Energy.,* **2015,** *40* (13), 4502-4511.

39. Gadhe, A.; Sonawane, S. S.; Varma, M. N. Influence of nickel and hematite nanoparticle powder on the production of biohydrogen from complex distillery wastewater in batch fermentation. *Int. J. Hydrogen Energy.,* **2015,** *40* (34), 10734-10743.

40. Yunus, N.; Jahim, J. M.; Anuar, N.; S. Abdullah, S. R.; Kofli, N. T. Batch fermentative hydrogen production utilising sago (*Metroxylon* sp.) starch processing effluent by enriched sago sludge consortia. *Int. J. Hydrogen Energy.,* **2014,** *39* (35), 19937-19946.

41. Lazaro, C. Z.; Perna, V.; Etchebehere, C.; Varesche, M. B. A. Sugarcane vinasse as substrate for fermentative hydrogen production: The effects of temperature and substrate concentration. *Int. J. Hydrogen Energy.,* **2014,** *39* (12), 6407-6418.

42. Yang, P.; Zhang, R.; McGarvey, J. A.; Benemann, J. R. Biohydrogen production from cheese processing wastewater by anaerobic fermentation using mixed microbial communities. *Int. J. Hydrogen Energy.,* **2007,** *32* (18), 4761-4771.

43. Leaño, E. P.; Babel, S. Effects of pretreatment methods on cassava wastewater for biohydrogen production optimization. *Renew. Energy.,* **2012,** *39* (1), 339-346.

44. Gadhe, A.; Sonawane, S. S.; Varma, M. N. Kinetic analysis of biohydrogen production from complex dairy wastewater under optimized condition. *Int. J. Hydrogen Energy.,* **2014,** *39* (3), 1306-1314.

45. Gadhe, A.; Sonawane, S. S.; Varma, M. N. Optimization of conditions for hydrogen production from complex dairy wastewater by anaerobic sludge using desirability function approach. *Int. J. Hydrogen Energy.,* **2013,** *38* (16), 6607-6617.

46. Gadhe, A.; Sonawane, S. S.; Varma, M. N. Enhanced biohydrogen production from dark fermentation of complex dairy wastewater by sonolysis. *Int. J. Hydrogen Energy.,* **2015,** *40* (32), 9942-9951.

47. Wicher, E.; Seifert, K.; Zagrodnik, R.; Pietrzyk, B.; Laniecki, M. Hydrogen gas production from distillery wastewater by dark fermentation. *Int. J. Hydrogen Energy.,* **2013,** *38* (19), 7767-7773.

48. Gonçalves, M. R.; Costa, J. C.; Pereira, M. A.; Abreu, A. A.; Alves, M. M. On the independence of hydrogen production from methanogenic suppressor in olive mill wastewater. *Int. J. Hydrogen Energy.,* **2014,** *39* (12), 6402-6406.

49. Sivaramakrishna, D.; Sreekanth, D.; Sivaramakrishnan, M.; Sathish Kumar, B.; Himabindu, V.; Narasu, M. L. Effect of system optimizing conditions on biohydrogen production from herbal wastewater by slaughterhouse sludge. *Int. J. Hydrogen Energy.,* **2014,** *39* (14), 7526-7533.

50. Moreno-Andrade, I.; Moreno, G.; Kumar, G.; Buitrón, G. Biohydrogen production from industrial wastewaters. *Water. sci. technol.,* **2015,** *71* (1), 105-110.

Table captions

Table 1: Biohydrogen production using various biological routes

Table 2: Microbiome involved in batch hydrogen production of wastewaters

Table 1

Process	**Types**	**Pros**	**Cons**
Light dependent	Photo-fermentation	+Efficient substrate utilization and able to catabolise the effluents (organic acids) generated from dark fermentation.	-Low hydrogen production rates -Inefficient light-conversion -Expensive bioreactor design
	Biophotolysis	+Abundant and inexpensive substrate (water) for generation of hydrogen	-Oxygen liberation affects the hydrogen-producing catalyst
Light Independent	Dark fermentation	+Utilization of wide range of organic waste streams +Less energy input Simple reactor design	- Considerably none except low hydrogen yield at times

Table 2

Wastewater type	Inoculum source	Peak Hydrogen production rate (HPR) (L/L- d)	Hydrogen Yield (HY) (mol/mol hexose added)	References
Cassava WW	*C. acetobuytlicum* ATCC824	0.6	2.41 mol/mol glucose	[24]
Distillery effluent	*Enterobacter Cloacae*	1.92	165.3 mL/ g COD	[25]
Rice mill WW	*Enterobacter aerogens*	SHPR:35.5 mmol/g cell ·h	1.74 mol/mol sugar	[22]
Rice mill WW	*Citrobacter ferundii*	SHPR:33.2 mmol/g cell ·h	1.40 mol/mol sugar	[22]
Rice mill WW	*Enterobacter aerogens* RM08	SHPR:35.4 mmol/g cell ·h	1.97 mol/mol	[23]
BWW	EMC+ *E. coli* XL1 blue+ *E. cloacae*	1.68	nr	[26]
BWW	EMC+ *E. coli* XL1 blue	1.22	nr	[26]
BWW	EMC+ *E.cloacae*	2.25	nr	[26]
BWW	EMC- compost	1.81	nr	[26]

BWW	EMC+*E. coli* XL1 blue	1.75	260 mL (0.01 mol)	[36]
Real field food WW	Anaerobic consortium	0.12	nr	[27]
Real field food WW	Anaerobic consortium + *Bacillus subtilis*	2.1	nr	[27]
Real field food WW	Anaerobic consortium + *Pseudomonas stutzeri*	0.8	nr	[27]
Real field food WW	Anaerobic consortium + *Lysinibacillus fusiformis*	1.0	nr	[27]
Sago starch in WW	EMC-hot spring	nr	442 mL/g starch	[28]
Cassava starch processing WW	EMC- Klong Pai Poo hot spring	nr	236 mL/g starch	[29]
Cassava starch processing WW	EMC- Romani hot spring	nr	128.4 mL/g starch	[29]
Cassava starch processing WW	EMC- Phang Nga Province and Wat Than Nam Ron Hot spring	nr	180 mL/g starch	[29]
Sago starch effluent	EMC	0.50	0.44	[40]

Sago starch effluent	EMC	nr	126.5 mL/g COD	[30]
Sugarcane vinnase	EMC	17.52 mmol/L-d	2.23 mmol/g COD	[41]
BWW	EMC- compost	2.6	1.12	[31]
Cheese processing WW	Mixed cultures	nr	10.2mM/g COD	[42]
Cassava WW	Anaerobic sludge	nr	4.24 mol/g COD	[43]
Complex dairy WW	Anaerobic sludge	13.54 mM/g COD	29.91 mM/g COD	
Complex dairy WW	Anaerobic sludge	185 mM/g COD	Nr	[44]
Dairy WW	Anaerobic sludge	SHPR: 29.91 mmol/g-VSS ·d	13.54 mmol/g COD	[45]
Dairy WW	Anaerobic sludge	SHPR:31.38 mmol/g-VSS ·d	15.33 mmol/g COD	[46]
Dairy WW	Anaerobic sludge	SHPR:47.7 mmol/g VSS ·d	17.2 mmol/g COD	[38]
Distillery WW	Anaerobic sludge	2.88	nr	[47]
Distillery WW	Anaerobic sludge	SHPR:18.14 mmol/g-VSS ·d	8.83mmol/g COD	[39]
Distillery WW	Anaerobic sludge	nr	10.95 mmol/g COD	[48]

Herbal WW	Slaughter house sludge	nr	165 mL/g COD	[49]
Organic WW	Soil	0.32 L/d	2.32 mol/mol	[33]
Olive mill wastewater	Anaerobic sludge	0.0106 mmol/ g COD	nr	[48]
Physico-chemical treated plastic industry	Anaerobic sludge	109	nr	[50]
Raw plastic WW	Anaerobic sludge	281	nr	[50]
Sugar beet juice	Anaerobic digested sludge	2.0	3.2	[32]
Textile WW	Anaerobic sludge	4.32	1.37	[34]
Toilet aircraft	Anaerobic sludge	280	nr	[50]

BWW, beverage wastewater; WW- wastewater; EMC- enriched mixed culture; SHPR- specific hydrogen production rate; nr- not reported

Figure captions

Figure 1 General anaerobic digestion pathway of methane generation.

Figure 2: Consolidated scheme for BioH_2 production from WW streams (BES: bio-electrochemical systems)

Figure 3: Wastewater to H_2 batch fermentation

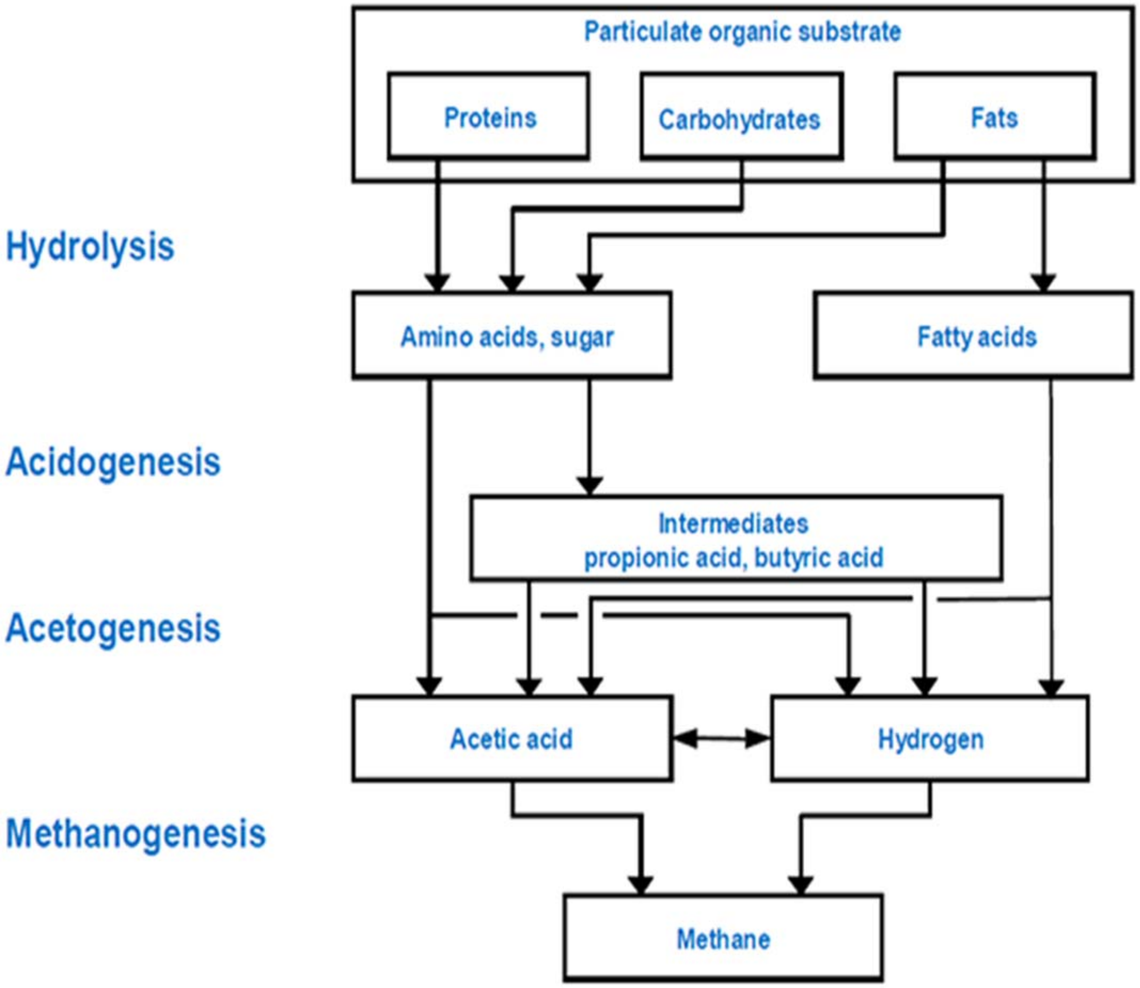
Particulate organic substrate
Proteins
Carbohydrates
Fats
Hydrolysis
Amino acids, sugar
Fatty acids
Acidogenesis
Intermediates
propionic acid, butyric acid
Acetogenesis
Acetic acid
Hydrogen
Methanogenesis
Methane

Fig .1

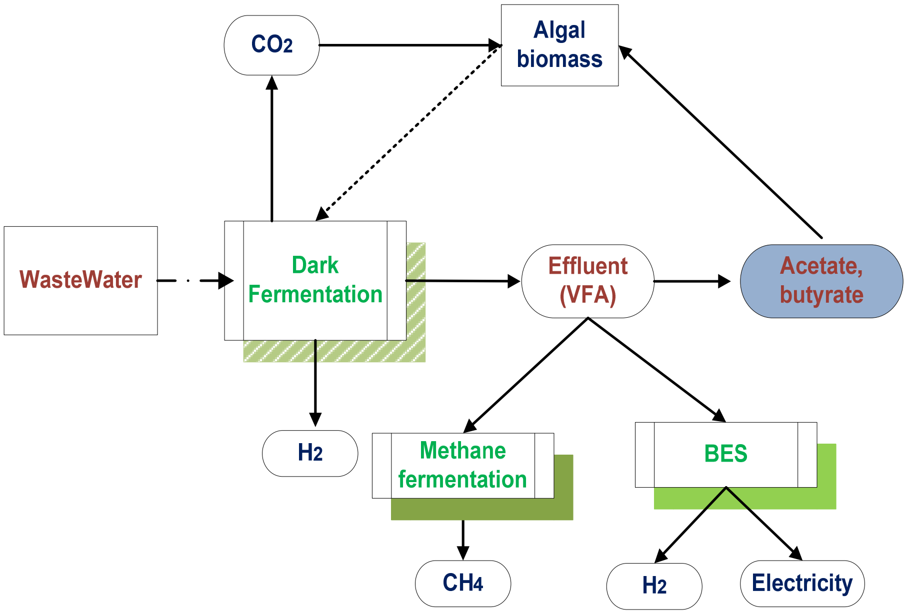
CO2
Algal biomass
WasteWater
Dark Fermentation
Effluent (VFA)
Acetate, butyrate
H2
Methane fermentation
BES
CH4
H2
Electricity

Fig.2

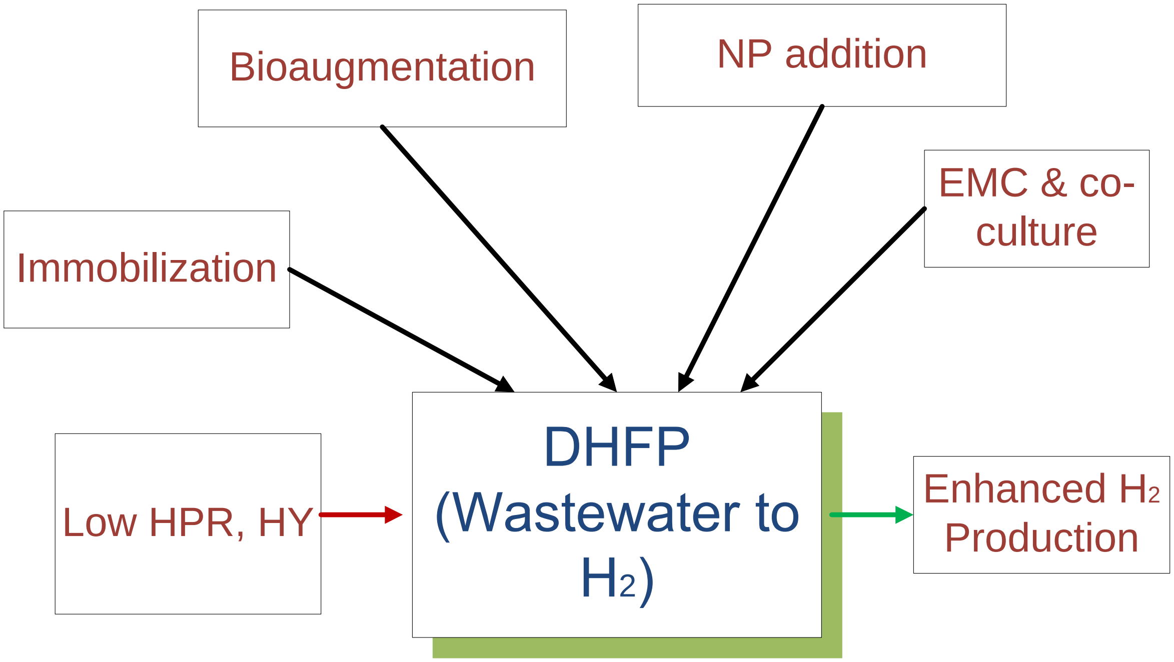
Bioaugmentation
NP addition
EMC & co-culture
Immobilization
Low HPR, HY
DHFP (Wastewater to H2)
Enhanced H2 Production

Fig.3