Clean – Soil, Air, Water 2012, 40 (11), 1297–1305

DOI: 10.1002/clen.201100007

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# Influences of Environmental and Operational Factors on Dark Fermentative Hydrogen Production: A Review

## Introduction

Emissions emanating from the combustion of fossil fuels lead to some adverse environmental impacts. Furthermore, fossil fuels are finite energy resources and non-renewable. Therefore, a number of alternative and renewable energy resources have been explored. Hydrogen ($H_2$) is one of such renewable energy sources known for its non-polluting and environmentally friendly nature, as its end combustion product is water ($H_2O$). Furthermore, $H_2$ is a promising fuel due to its high energy yield (122 kJ/g) which is 2.4, 2.8, and 4 times higher than energy yields of methane, gasoline, and coal, respectively [1–11]. Different $H_2$ production methods have been reported; some of which are fossil fuel reforming [12], biological processes of biomass [3, 13], and electrolysis of water [14]. Biological production of $H_2$ is a less energy intensive alternative where processes can be operated at ambient temperature and pressure [10].

The three main biological processes that have been applied are biophotolysis of water using algae and cyanobacteria, photodecomposition of organic compounds by photosynthetic bacteria, and fermentative $H_2$ production from organic matters [15–26]. Amongst aforementioned biological methods, fermentative $H_2$ production became more favorable due to some outstanding advantages such as high $H_2$ production rate (HPR), low energy requirement, relatively easy operation, and high sustainability [27–30]. This technique provides a certain condition under which acidogens (hydrogen producing bacteria) and methanogens ($H_2$ consuming bacteria) exhibit an imbalance in their activities resulting in accumulation of $H_2$ [10]. Dark fermentation in the acidogenic phase utilizing obligate and facultative anaerobes leads to $H_2$ production. This method usually achieves a much higher HPR than other biological processes [17, 24, 27].

Previous studies depicted four fermentation reactions that take place in the anaerobic $H_2$ production systems, viz. acetic acid fermentation, propionic acid fermentation, butyric acid fermentation, and ethanol fermentation as given, respectively, in Eqs. (1)–(4) [31–34]. Among these reactions, $H_2$ is generated from acetic acid (Eq. (1)), butyric acid (Eq. (3)), and ethanol fermentations (Eq. (4)); however, the propionic acid fermentation (Eq. (2)) may be more energetically favorable than other fermentation types due to the lower Gibbs free-energy change ($\Delta G^0$) [32, 35–39].

*Please refer to the full text*

$H_2$ producing bacteria can utilize various forms of substrates. Reported studies showed that glucose, sucrose, and starch were mainly used as substrates for fermentative $H_2$ production [40]. However, numerous works also made use of wastewater as substrates (e.g., rice winery wastewater, palm oil mill effluent (POME), food waste, and dairy wastewater) due to their ready availability, low cost, high carbohydrate content, and biodegradability in the biological processes of $H_2$ production [5, 8, 9, 26, 41]. In addition to the energy generation from waste, it simultaneously contributes to treatment of the various wastes [41–43]. Diverse reactor designs have been introduced for accommodating bacteria and providing them with the required conditions to accomplish the $H_2$ fermentative production process. Some of them are: anaerobic sequencing batch reactor (AnSBR) [26, 44–46], batch reactor fermentation [47, 48], up flow anaerobic sludge blanket (UASB) [49, 50], granule-based continuous stirred tank reactor (CSTR) [51], fixed or packed bed reactor [10, 13, 26, 52], anaerobic fluidized bed reactor (AnFBR) [53, 54], and trickling bio-filter [55, 56]. The environmental and

operational conditions were varied in the reactors in order to achieve better efficiency in the anaerobic $H_2$ production process. The environmental conditions included temperature, pH, hydraulic retention time (HRT), type and concentration of substrate (e.g., organic matter, nitrogen, phosphorous, and metal ions), inoculums, and organic loading rate (OLR) [17, 40, 57–59].

This review examines the previous studies on the fermentative $H_2$ production, and specifically discusses the environmental/operating variables important in enhancing biohydrogen production.

## Effect of organic loading rate (OLR)

Organic loading rate is one of the most significant parameters extensively studied to investigate the effects of various substrate loadings when either a real or a synthetic wastewater was utilized as the substrate(s) [58]. Organic matter is utilized by microorganisms to produce hydrogen (e.g., glucose was used in Eqs. 1–4) and it is measured by OLR (g/(L day)) which represents the magnitude of chemical oxygen demand (COD) (g/L) fed within a certain period. With regard to the relationship defining the loading rate, two independent parameters viz. HRT and COD concentration can be considered to evaluate the effect of the loading rate [45, 58, 60]. The range of OLR applied to the process is important in order to obtain optimal microbial growth and hydrogen production; however, the higher OLR does not necessarily lead to higher hydrogen yield. Therefore, optimization of operational variables is essential to gain the higher production efficiency. Nevertheless, optimization of OLR can only be implemented when the microbes are well acclimatized to the applied OLR or kinetically at their highest affinity towards the substrate(s) [60, 61]. Thus, gradual exposure of the microorganism population to the final OLR is usually of fundamental importance, and acclimatization is thoroughly achieved when experiments reach a steady state in HPR.

On the other hand, with the same OLR, the substrate composition is an imperative factor impacting on the $H_2$ generation rate. According to Monod relation between growth rate and substrate concentration, a hyperbolic function for hydrogen yield is increased by increasing the substrate concentration until the maximum specific growth rate ($m_{max}$) is reached. Thus, as the refractory fraction of the COD content in the wastewater increases, the $H_2$ yield will remain constant or even decreases because of the substrate inhibition effect [27, 45]. At a constant OLR with different HRT and substrate concentrations, hydrogen yield was sensitive to HRT but insensitive to the substrate concentration. Hence, as HRT increases the hydrogen content of the biogas as well as the hydrogen yield decrease [58].

Moreover, it must be noted that in the case of real wastewater where the composition is varied, the increase in OLR causes proliferation of microbial diversity and competition which

inhibits the $H_2$-producing ability of the mixed bacterial consortium used, exceeding themaximumcapacity that the $H_2$ producers are able to handle efficiently [62, 63]. From several studies, high substrate concentration showed the metabolic pathway change [63–65]; whereas, metabolic activity is the effectual key for $H_2$ yield in fermentative hydrogen production [26]. Therefore, metabolic activity of the microorganisms must be improved through gradual exposure and adaptation to high substrates concentration [56].

The diverse optimum OLRs for maximum $H_2$ production utilizing wastewater found in literature (Tab. 1) is attributed to the differences in the reactor configuration, types of wastewater, inoculums, range of OLR applied, temperature, pH, etc. A wide range of OLR from 0.87 to 160 g COD/(L day) was investigated utilizing either batch or continuous reactors with various organic substrates such as glucose, sucrose, starch, and few cases with organics available in real wastewaters. As shown, there is no definitive range or optimum OLR in fermentative hydrogen production and sometimes the achieved results are extremely divergent. For instance, Lin et al. [62] found the maximum hydrogen yield at an optimum OLR 120 g COD/(L day), while Ozmihci and Kargi [68] reported 1.93 g COD/(L day) as the optimum OLR. This phenomenon could be due to the diverse environmental/ operating conditions or ranges of the variables applied in the studies. The applied ranges and obtained results for some of the studies were illustrated in Fig. 1. Lin et al. [62] reported an increase of $H_2$ production to 3.46 mol $H_2$/(gCODday) with an increase in OLR from 20 to 120 g COD/(L day); however, the HPR dropped to 1.96 mol $H_2$/(gCODday) as a higher OLR of 160 g COD/(L day) was applied in a fluidized bed reactor operation where heat pre-treated seed sludge was used. This seems to suggest that a high substrate concentration of 40 g COD/L may inhibit the $H_2$-producing ability of the mixed bacterial consortium used. The same ascending–descending trend was observed by Show et al. [58] and Liu et al. [63] in the range of 2.5–20 and 3–18 g COD/(L day), respectively, when glucose was used as sole carbon and energy source in microbial biohydrogen production at 378C. Nevertheless, results achieved by Wu et al. [60] and Wu and Chang [27] showed negligible variation in HPR through alteration of OLR since approximately constant values were reported for $H_2$ production when immobilized sludge was used in batch system (358C, pH 6–7). The results suggest that the cell immobilization procedures did not considerably alter the $H_2$ production properties of the bacterial population in the sludge. Contradictory results which occur sometimes for the same OLR ranges could be due to dissimilarity in other effective factors such as pH, temperature, substrate, reactor design, pre-conditioning of inocula, or types and population of microorganisms [40]. For example, Wu et al. [60] and Wu and Chang [27] examined similar ranges of 10–40 and 5–30 g COD/(L day), respectively, in batch experiments with

sucrose as the organic source; however their processes differed in the applied pH, preparation of the synthetic wastewater, and reactor design. As a result, trace amount of hydrogen was produced by Wu and Chang [27], while Wu et al. [60] attained 1.42 mol $H_2$/(gCODday), and it was apparently due to employing a new synthetic polymer (ethylene–vinyl acetate copolymer) as the bio-carrier which resulted in betterment of microbial growth and activity.

## Effect of hydraulic retention time (HRT)

Hydraulic retention time is the time period for a specific volume of liquid to be retained in the working volume of the reactor, and is in inverse relation to food-to-microbe ratio (F/M). Therefore, too long or too short HRT will result in unfavorable metabolic activities of the microbes. The hydrogen production increased to a higher value with increase in HRT when adequate time was given to microbes for processing substrates [70]. However, decline in HPR was seen when HRT was too long. It was attributed to a shift of metabolic flux that might occur after transition from a high to a shorter HRT where more substrate is shifted to reaction end products instead of bacterial growth or maintenance, leading to an increased hydrogen yield [54, 58, 73–75]. In processes without sludge pre-treatment and pH adjustment, for instance where OLRs are high and/or HRTs are low, the methanogenic activities will be inhibited due to the higher acidogenic rate. Concomitantly, the drop in pH (<5.0) due to acidogenic activities will favor hydrogen generation process [61]. On the other hand, the hydrogen content of the biogas decreases by the increase in HRT due to diversion from hydrogen production reactions. However, in some cases, the low substrate concentration resulted in the yield increment when HRT was constant [62, 76].