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Process parameters affecting the sustainability of fermentative hydrogen production: A short-review

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Abstract
Anaerobic fermentation is regarded as the least energy intensive method for H_2 production. Extensive literature on experimental attempts to achieve the highest possible theoretical yield (e.g. 4 mol H_2/mol glucose) is available. All published steady state, mixed culture studies show yields much lower than the theoretical maximums for the substrates applied. This article summarizes the influence of key process parameters (pH and buffer systems, temperature, H_2 partial pressure, feed stock, and reactor configuration) on fermentative hydrogen production. The following three requirements for successful Bio-H_2 fermentation in mixed cultures are identified: (1) Maintain environmental conditions for the formation of oxidized products; (2) Optimize the relationship between biomass and hydrogen yields; and (3) Maintain unfavorable conditions for hydrogen consuming organisms. Fulfilling these requirements has not yet been achieved in stable continuous cultures, and it may not be achievable do to some fundamental limitation.

Keywords: Biohydrogen; Fermentation; Process parameters; Homoacetogenesis.

1. Introduction

A global system using hydrogen as an energy carrier to supply the energy needs in transport, heating and diverse public activities has been proposed [1, 2]. Such extended role of hydrogen breaks down when examined in the light of engineering, physics, and chemistry [3], but H_2 can be an important energy carrier in various renewable energy schemes.

Hydrogen can be produced from fossil fuels, water and biomass by a number of processes [4]. Biological production is assumed to be more environmental friendly than the alternatives [5]. Biological hydrogen production includes direct and indirect bio-photolysis, photo-fermentations, dark fermentation and hybrid systems [6]. Dark fermentation processes are simpler than photo-processes and can be based on a wide range of carbon sources [7], and are therefore the focus of this review.

Low hydrogen yield is a limitation, caused by the fact that fermentation has been optimized by evolution to produce biomass and not hydrogen. Fermentative bacteria have diverse metabolic capabilities. In mixed cultures, microorganisms can oxidize sugar to: formate, acetate, lactate, propionate, succinate, butyrate, ethanol, propanol, butanol and acetone (Figure 1). To achieve the highest possible hydrogen yield, however, all sugar (e.g. glucose) must be fermented to acetate. In this way electrons will end up with the electron-carrier ferrodoxin, a direct electron donor for proton (H^+) reduction in the generation of molecular hydrogen (H_2). Additionally, reactions involving H_2 and NADH generation compete for Fd_{red}

(Figure 1). NADH is the main electron donor for the oxidation of pyruvic acid, and in the formation of bio-molecules. Several authors have discussed that pH is the main factor coupling the oxidation of Fd_{red} with NAD^+ or H^+ to form NADH or H_2, as summarized by Lee et al. [8].
Molecular hydrogen acts as an intermediate metabolite that is quickly consumed by acetogens and hydrogenotrophic methanogenic bacteria to form acetate and methane in standard anaerobic digestion for methane production. It is therefore mandatory to eliminate hydrogen consumers in bio-hydrogen reactors, where similar mixed cultures are used. pH drop is also a problem in bio-hydrogen fermentation since it also produces organic acids (pka ≈ 4.76). In laboratory experiments this problem is overcome by the use of buffers, which is an expensive solution for commercial scale. Fe^{2+} and NH_4^+concentrations, organic loads and other nutrients have also been observed to affect the specific hydrogen production rate [9, 10]. Bio-H_2 production is also dependent on hydrogen partial pressure and hydraulic residence time (HRT), factors that directly affect the metabolic balance. Thus products distribution depends on the environmental conditions surrounding bacteria. To maximize hydrogen yield, metabolism need to be directed away from solventogenesis and reduced acids production towards acetate and/or butyrate. Requirements for successful hydrogen fermentation can therefore be summarized as follows: 1) To create the environmental conditions for the formation of oxidized products; 2) To optimize the relationship between biomass and hydrogen yields ($Y_{X/S}$ and $Y_{H2/S}$); and 3) To maintain unfavorable conditions for the development of hydrogen consuming organisms.

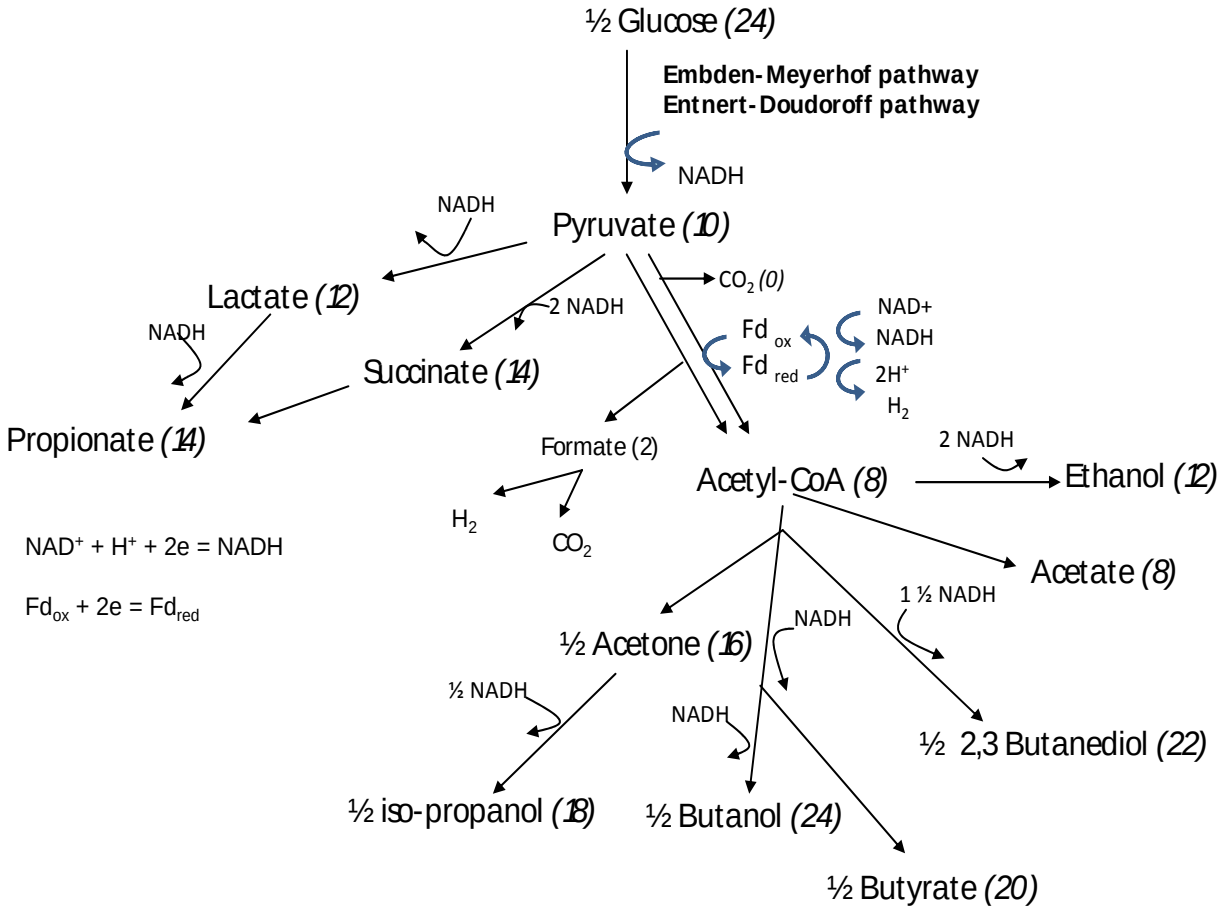

Figure 1. Products from glucose fermentation. The degree of reduction per mol of substance is presented in parentheses. Modified from Evans and Furlong [11]; Temudo et al. [12]

The results reported are far from the maximum theoretical yield (4 moles H_2/ mol glucose), implying that nobody has yet found a way to combine the mentioned requirements. This article describes the state of fermentative hydrogen research considering the main process parameters: pH, temperature, H_2 partial pressure, feed stock and reactor configuration. Additionally, efforts made in the field of metabolic engineering are shortly describe. Factors associated with the physiology and ecology of the organisms

involved are not discussed, and remains a future task. Focus here is on the fundamental obstacles for mixed culture sustainable hydrogen production by dark fermentation.

2. PH

The concentration of H^+ in the extracellular environment is used in bio-H_2 studies to both control the general directions of the metabolic pathways (towards oxidized products) and to suppress the activity of methanogenic bacteria. Literature clearly shows that pH has a strong effect on hydrogen production [12-15], and despite of observed variations, the majority of the articles cited here agree on an optimal pH of 5.5.

The influence of pH on the hydrogen potential (HP) and hydrogen production rate (HPR) have been studied in batch experiments [9, 16-18], and in continuous reactors [13, 19, 20, 22]. The reported optimal pH for hydrogen production is conflicting, varying from pH 9 to pH 6.5 for batch fermentation of sucrose [17] and xylose [21] to pH 4.0-4.5 and pH 4.7-5.7, for the continuous fermentation of sucrose [19] and starch [20], respectively. Van Vinkel et al. [16] and Fang and Liu [13] all found an optimal pH of 5.5 for the production of hydrogen from sucrose and glucose in batch and CSTR. Khanal et al. [18] concluded that the initial pH did have an effect on both hydrogen production potential (HPP) and hydrogen production rate (HPR), where HPP and HPR were parameters estimated from the modified Gompertz equation [23]. Khanal and collaborators also estimated pH 5.5 as optimal for hydrogen production. Such discrepancies between these studies can be related to different feedstock, microbial populations and operational conditions. It is recognized that a change in pH produce a metabolic shift that change the proportions between oxidized and reduced substances, of significance for the ultimate hydrogen yield. pH also influence the activity of hydrogenase that catalyze the reversible half-cell reaction $2\ H^+ / H_2$. The activity of this iron-containing enzyme is inhibited by low pH, reducing the ability to generate molecular hydrogen [24-26].

Low pH has also been used to avoid the activity of methanogenic cultures. Such archea grow optimally at pH between 7.0 and 7.5. Acetoclastic methanogens are completely inhibited at pH < 6 while hydrogenotrophic methanogens are not, according to the anaerobic digestion model number one ADM1 [27]. The ability of methanogens to endure low pH has been observed in semi-continuous fermentative reactors [28] and in batch methanogenic cultures [29, 30] both at pH lower than 5.0. This has been explained by the establishment of micro-niches with neutral pH [31]. The only effective way to avoid hydrogenotrophic methanogens appears to be by means of sludge pre-treatment. Oh and collaborators [32] found that not pre-treated sludge have the capacity of consuming H_2 at pH of 6.2, while sludge pre-treated with heat or acid consistently eliminated the production of measurable methane. Acid and base treatment of the sludge by Chen and collaborators [33] also positively enhanced the hydrogen production by inhibiting methanogenesis.

Eliminating methanogenesis is, however, not sufficient to avoid H_2 consumption, since homoacetogenic bacteria can also consume H_2. Homoacetogenic bacteria have been found in a wide range of pH; 4-8 [34]. The consumption of H_2 by these microorganisms, to produce acetate, formate or methanol, may in part explain some of the variations in H_2 yields observed, especially in reactors with long sludge retention time [35, 36]. PH management is ineffective method to stop the activity of H_2 consuming autotrophs.

Heat and/or acid treatment may not be enough to avoid molecular hydrogen consumption because of the presence of spore forming homoacetogenic organisms. Methods to excise their activity need to be found to completely eliminate hydrogen consumption. The negative effect of homoacetogens has been reduce in laboratory scale experiments by the removal of CO_2 from the headspace using a chemical scavenger, KOH [37] or sparging gas [38], successfully inhibiting the action of acetogenic bacteria and increasing hydrogen yield by 43 %.

2.1 Buffer systems

The use of chemicals to control pH becomes very important when treating organic material with low alkalinity, as for example fruit waste. Buffers may also be necessary in high performance reactors, characterized by high organic loads and short hydraulic retention time. In that sense Lin and Lay [39] showed in batch experiments that adding phosphate instead of carbonate as buffer can enhance hydrogen production. It can be speculated that reducing the concentration of HCO_3^- diminished the activity of homoacetogens. Effects of buffer systems in hydrogen fermentation have not been studied much, but it may be a relevant issue especially when wastes rich in carbohydrates are feeds for hydrogen production.

Co-digestion of organic wastes with high buffer capacity, such as sewage sludge, is therefore an interesting option. Protein-rich feeds are also interesting as they produce an increase in $NH4^+$-N, conducing to a higher pH, as observed by Lay et al. [40], in a study on the influence of the chemical nature of organic wastes on H_2 production.

3. Temperature

Both microbial kinetics and thermodynamics of equilibrium reactions are affected by temperature [41]. Fermentation reactors can be operated at psychrophilic (5 - 25 °C), mesophilic (25 - 40 °C), thermophilic (40 - 65 °C), extreme thermophilic (65 - 80 °C) or hyperthermophilic (> 80 °C) temperatures [5]. Enzymes work optimally at a specific temperature. Increases in temperature will double the enzymatic activity for every ten degrees Celsius until the optimal temperature is reached, above which the enzymatic activity will rapidly decrease. Hydrogenase enzymes, characterized by their thermostability, are widely distributed in bacteria enabling microorganism to either use H_2 as source of energy or H^+ as a terminal electron acceptor. Increasing temperature has also a positive effect on the hydrolysis rate for the anaerobic digestion of complex particles [42] that could enhance the yield and rate of hydrogen production. Reactor operation at temperatures above 70 ^oC is not only beneficial to the reaction kinetics and stoichiometry. It also increases the solubility of many polymeric substrates, decreases viscosity, increases bioavailability and decreases the risk for contamination by methanogenic bacteria [43].

Temperature effect on the hydrogen production potential have been mostly described for mesophilic [10] and thermophilic [44, 45] conditions. An increase in temperature from 33 °C to 41 °C in anaerobic fermentors resulted in increased substrate consumption rate, H_2 yield and biomass growth rate [46]. Lee and collaborators [47] studied the hydrogen yield in a granular sludge bed reactor operated between 30 °C and 45 °C. The optimal working temperature was found at 40 °C, higher temperatures resulted in reduced biomass by inhibiting granules formation.

In recent years the interest for extreme thermophilic fermentation has increased much. Such cultures are more suitable for biomass containing lignocelluloses, due to higher hydrolysis rates compared to mesophilic and thermophilic cultures [48]. Above 75 % of the theoretical maximum yield could be achieved in extreme thermophilic cultures [49, 50]. Furthermore, an 83 % yield of the theoretical maximum was obtained by Van Niel et al. [51] fermenting glucose with the extreme thermophiles *Caldicellulosiruptor Saccharaolyticus* and *Thermotoga elffi*. Kongjan et al. [52] studied bio-H_2 production in mixed culture bacteria at 70 °C fed on xylose. The results (H_2 partial pressure = 0.14 atm, and yield = 1.62 mol H_2/mol xylose) may however not justify the use of energy for heating in a practical application. Hyperthermophilic organisms as *Thermococcus kodakaraensis* grown at 85 °C on sucrose [53], and *Pyrococcus furiosus* grown optimally at 100 °C [54], have also been used to successfully produce H_2. Bioreactor configuration is important because of the difficulties in maintaining the required biomass of high temperature cultures. Kongjan and Angelidaki [55] studied the hydrogen yield of extreme thermophiles (70 °C) in three reactor configurations CSTR (continuous flow stirred tank reactor), UASB (up-flow anaerobic sludge bed) and AF (anaerobic filter). They observed cell mass washout at HRT of 2.5 days in the CSTR, and reducing hydrogen production at less than 1 day HRT for the UASB and AF reactors, pointing out the operation challenges of extreme thermophilic cultures.

The cited literature shows that cultivation at high temperature (i.e. hyperthermophilic) can be a viable strategy to maximize hydrogen yield, obtain high production rates and inhibit hydrogen consumers (hydrogenotrophic methanogens and homoacetogens). The required energy for the reactor operation needs, however, to be considerably less than the energy recovered as hydrogen to make the processes economically feasible and sustainable. This goal is specially complicated when large volumes of fermentation broth need to be heated up avoiding, at the same time, heat losses. Additionally, energy is also required in the separation of the H_2 from CO_2. Fermentative hydrogen production at higher temperatures may then be limited by operational costs for practical purposes. Extreme and Hyper-thermophilic reactors are also technically more demanding than thermophilic and mesophilic cases since bacteria may not grow to high densities [56], a requirement for high rate processes.

4. H_2 partial pressure

H_2 partial pressure is key parameter in the production of hydrogen by fermentative bacteria [57], but difficult to control. Hydrogen synthesis pathways are very sensitive to hydrogen concentration and are subject to end-product inhibition. Increasing hydrogen concentrations trigger a metabolic shift towards a more reduced metabolism. Also, high hydrogen partial pressure in the reactor will, in theory, make the

production of hydrogen thermodynamically unfavorable. 20 kpa seems to be the limiting headspace pressure when it is in equilibrium with the liquid phase [58]. Hydrogen production has, however, frequently been observed up to about 60 kpa [59].
The effect of H_2 partial pressure on products distribution in fermentors has been studied mostly through models [60]. Head space partial pressure may not be a direct indication of the thermodynamic state of reactions governed by H_2 concentration due to mass transfer limitations, concentration gradients and the existence of micro-niches and bacterial associations [61]. Therefore complex models, including 3D models, have been developed to evaluate such situations at a molecular level [62].
Gas sparging is one of the useful methods to reduce hydrogen partial pressure, and has been shown to increase hydrogen yield in batch experiments [63] and in continuous cultures [64]. Other methods to remove hydrogen include: Absorption of H_2 by metals and stripping hydrogen either by boiling or evaporation at a large surface [65]. Only the last alternative has been found possible in practice, but requiring ~25 % of the energy obtained by the combustion of the hydrogen produced.

5. Feed stock

Organic wastes are inexpensive sources of substrate for fermentative hydrogen production, by which reduction and stabilization of waste is also to some extent accomplished. Bio-conversion of biomass to hydrogen has been study using anaerobic fermentation of the organic fraction of municipal solid waste [66, 67], sewage sludge [68], food waste [69-72], paper mill waste [73, 74], co-digested food waste with sewage sludge [75] and some well defined compounds such as cellulose [45, 76], xylose [73], lactose [77], starch [9, 45], sucrose [17, 78, 79] and glucose [13, 80, 81].
A broad range of organic substrates can be utilized by hydrogen producing bacteria, as described above, but carbohydrates are preferred because it yields the highest amount of hydrogen per mol of substrate. The carbohydrates can be monosaccharides or polymers such as starch and cellulose. The large number of hydrogen producing microbial species, such as *Clostridia, Enterobacter* and *Archea*, suggests that most carbohydrates are suitable feedstock for dark hydrogen fermentation. Proteins, peptides, and aminoacids are less suitable for dark reduction, however, work reported by Okamoto et al. [66], confirm that hydrogen can be produced (2.47 mL/g-VS) from a lean meat protein base.
Studies carried out on food processing waste [26, 82, 83] have demonstrated the feasibility of bio-hydrogen production from industrial waste. The biogas produced contained between 53-64 % of hydrogen, 37-40 % of carbon dioxide and almost no methane at hydraulic retention times between 2-72 Hrs. Han and Shin [69] indicate that among various reaction constrains affecting the fermentation of food waste, a key factor is the adjustment of environmental conditions during the fermentation, because various components of food waste have different characteristics of degradation. This also implies that automatic monitoring and control of such processes is required. The food processing industry produce highly concentrated waste rich in carbohydrate with potential for fermentative hydrogen production. In some cases the low nutrient concentration can be the limiting factor, but standard fertilizing procedures can handle this. Co-digestion with sewage sludge, which provides bacteria and nutrients, with industrial waste as the feed also seems possible. A general guideline on optimum conditions, such as water content, carbohydrate concentration, carbohydrate/protein balance and environmental condition for mixed microflora is still not available.

6. Reactor configurations

Different types of bioreactors have been used for continuous fermentative hydrogen production. CSTR is the most common [84-87] because it allows simple control of pH and temperature, making it ideal for lab-scale studies. High rate reactors are used to achieve higher biomass densities trough the formation of granules, biofilm or flocs. In such reactors shorter HRT, higher loading and volumetric H_2 production rates are possible, without risk for washout [88]. High density continuous flow reactors tested for biohydrogen production include packed bed [89-92], fluidized bed [93, 94], trickling biofilter [95, 96] and membrane bio-reactors, MBR [97-99]. The best results reported so far are obtained in a MBR, a chemostat connected to a cross-flow membrane [97]. Reactor operation resulted in 60 % H_2 in the headspace; however, increasing HRT higher than 3.3 hours resulted in lower hydrogen yields.
In spite of the obvious advantages of using high rate, high density reactors, observations indicated that hydrogen yield decrease with increasing HRT and sludge age. Camilli and Pedroni [100] studied three reactor configuration; CSTR, up-flow fixed bed reactor (UFBR) and an up-flow anaerobic sludge blanket reactor (UASB). All reactors where operated at similar conditions and feed strategies. In all cases the

hydrogen yield diminished with increasing HRT, while higher yield and stable operation were obtain in the CSTR. Shorter HRT resulted in washout at 17.4, 13.1 and 5.2 hours respectively. Gavala et al. [101], studied hydrogen production in two different reactor configurations, CSTR and UASB. They observed that the produced H_2 correspond close with the acids concentrations for the CSTR ($Y_{H2/Hac+Hbu} = 2$). However for the UASB reactor, the measured hydrogen gas correspond just between 23 % and 37 % with the total acids production ($Y_{H2/Hac+Hbu} \sim 0.6$, and no significant reduced products were detected). Zhang et al. [94], studied hydrogen production in a fluidized bed reactor packed with granular activated carbon. They report up to 60 % H_2 in the headspace and a maximal H_2 yield of 1.6 mol H_2/glucose. However, increasing the HRT from 1 to 4 hours reduce both the hydrogen production rate and H_2 yield by 50 %, followed by a higher acetic acid concentration in the effluent. These and other publications [92, 97, 102-105] show a correlation between longer sludge retention time and reduced hydrogen yield. These observations can be explained by the activity of homoacetoacetogenic bacteria [35, 36, 106].

7. Metabolic engineering to enhance bio-hydrogen production

Diverse metabolic engineering strategies are being used, experimentally, to overcome the limitations of hydrogen production in fermentative processes. Here a few examples are cited, while an extensive review can be found in Mathews and Wang [107]. *Clostridum acetobutylicum* and *Escherichia coli* are the preferred microorganisms for such modifications. One of the used techniques is to avoid genetically the production of butyrate; this will result in higher rates of acetate, and therefore hydrogen, production [108] Over-expression of the hydrogenase gene has also been shown to be a successful method to increase the hydrogen production due to the over-oxidation of NADH in *C. paraputrificum* [109]. A similar strategy can also be applied to *C. butyricum*. Other genetic modifications are directed to the improvement of the degrading capability of extracellular enzymes to achieve more free monomers for the conversion to H_2 [107].

Engineering and regulation of hydrogenase in *E. coli* have the capability of producing large quantities of H_2 [110]. Hydrogen production has also been improved in *E. coli* by eliminating competing pathways, thereby increasing the flow of carbon trough the hydrogen producing paths (formate production). Such strategies include the inhibition of the lactate and succinate pathways [111], and the reduction of formate consumption or export [112, 113]. Studies show that H_2 production can be increased from 70 % [114] up to 280 % [113] compared to the hydrogen yield of the wild type *E. Coli* by the use of these approaches.

Monocultures of genetically modified bacteria will however have a very limited application in H_2 production because sterilized substrates are needed to maintain the cultures. Besides, the use of complex substrates such as organic wastewaters is probably not suitable for such bacteria due to their narrow physiological capabilities.

8. Final remarks

In spite of all the efforts, hydrogen yields obtained in the cited studies are far from the theoretical maximum (4 mol H_2/mol glucose). This shows that nobody has managed to fulfill the stated requirements for successful Bio-H_2 fermentation with the use of mixed cultures: (1) To create the environmental conditions for the formation of oxidized products; 2) To optimize the relationship between biomass and hydrogen yields; and 3) To maintain unfavorable conditions for the development of hydrogen consuming organisms. Reasons for this include: Studies on pH, to control the metabolic pathways, are conflicting, and generally show low level of control. Extreme thermophilic systems give good results, but such solutions have practical limitations regarding energy cost and low bacterial densities. Avoiding methane production requires energy for heat-treating the sludge and that still is no guaranty for avoiding latter colonization by Archea. The highest yields reported are obtained by genetic manipulations, but stable continuous operation will require feed sterilization, and may not be suitable for complex substrates.

In addition to these limitations comes the persistent homoacetogenic hydrogen consumption, that has not been well described in the literature for biohydrogen fermentation, even when several authors have identified it as a possible hindrance for successful hydrogen production [32, 63, 37, 115]. Homoacetogenesis has evident negative effects on hydrogen production [35, 36, 106], implying that hydrogen production by dark fermentation may not become an important renewable energy process.

The knowledge generated in the cited articles can be useful with respect to renewable energy processes even if hydrogen production by dark fermentation may not generally be sustainable as a single process. Coupled systems with two or more staged reactors for biogas production may include both hydrogen and methane production [70, 116-118]. The obtained information can also be used to design and operate two

step processes for methane production only, in which case minimizing hydrogen production is desirable. The same strategies may also be used to avoid hydrogen in fermentation as pretreatment for bio-electrochemical hydrogen production. The aim of the second stage in this case is VFAs conversion to hydrogen and CO_2 using electrochemical principles [119]. This principle has the potential to become a sustainable method for hydrogen production, as less than 10 % of the recovered energy is needed to sustain the electrochemistry. Hydrogen fermentation combined with photo-fermentation [120] is another interesting alternative.

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