Biohydrogen production from fermentation of organic waste, storage and applications

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\begin{abstract}
Biohydrogen is a carbon-free alternative energy source, that can be obtained from fermentation of organic waste, biomass-derived sugars, and wastewater. This article reviews the current processes for fermentative biohydrogen production from biomass including its appropriate storage and transport challenges. The review showed that a comparison of fermentation pretreatment methods across the literature is complicated and that fermentability tests are necessary to determine the best combination of pretreatment/feedstock. Operational parameters, such as temperature, pH, macro/micronutrients addition are widely dependent on the type of fermentation and microorganisms used and hence their content need to be tailored to the process. For immobilized cells, the range of hydrogen production rate values reported for granulation processes using mixed microbial cultures, were higher (13–297 mmol H\textsubscript{2}/L h) than those reported for entrapment (1–115 mmol H\textsubscript{2}/L h) and adsorption (3–83 mmol H\textsubscript{2}/L h), suggesting an achievable and sustainable route for full-scale applications. A purification phase is mandatory before the final use of biohydrogens. Sorption techniques and the use of membranes are the most widely used approaches. Pressure swing adsorption has the highest recovery rate (it reaches 96%). In addition, storage of biohydrogen can have several forms with varying storage capacities (depending on the form and/or storage materials used). The transport of biohydrogen often faces technical and economic challenges requiring optimization to contribute to the development of a biohydrogen economy.
\end{abstract}

\section{Introduction}

The Net-Zero targets recommended by the Intergovernmental Panel on Climate Change (2018) and the Paris agreement (2015) targets accelerate the search for carbon-free, non-polluting and low-cost alternative energy sources. The use of fossil fuels has two main downsides: the depletion of oil reserves and its greenhouse gases (GHG) emissions responsible for global warming and climate change (Mishra et al., 2019). A carbon-free alternative not only will help achieve net-zero targets but can also increase countries autonomy and energy security. Hence the urgency to look for these alternative processes. In this context, biohydrogen (BioH\textsubscript{2}) is an alternative renewable energy source that can be obtained from anaerobic fermentation of organic waste, biomass, and wastewater.

For example, the anaerobic digestion (AD) process is usually used to recover methane from these feedstocks (Habchi et al., 2022). However, hydrogen is an intermediate product of this process and in recent years there has been an increasing interest in optimizing further this step and achieving its production from organic waste. With an energy content of 143 kJ/g hydrogen has a much higher energy content than methane 56 kJ/g hence a better fuel and energy carrier (Khanal, 2008).

\begin{acknowledgement}
Abbreviations: AD, Anaerobic digestion; ANOVA, variance analysis; BioH\textsubscript{2}, Biohydrogen; BNG, boron-nitrogen codoped graphene; C/N, Carbon/ Nitrogen; COD, Chemical Oxygen Demand; CSS, cyclic steady state; CSTR, Continuous Stirred Tank Reactor; CSTR, continuous stirred tank reactor; DD, disperser disintegration; DF, Dark fermentation; DGGE, Denaturing Gradient Gel Electrophoresis; DTD, dispersion thermal disintegration; ECP, Extracellular Polymers; FCEV, fuel cells in electric vehicles; GaS, Galliumsulfide; GHG, greenhouse gases; HEG, hydrogen-exfoliated graphene; HES, Hydrogen energy systems; HRT, Hydraulic Retention Time; IEA, International Energy Agency; MOF, metal–organic framework; MTH, Methylcyclohexane-Toluene-Hydrogen; MWCNT, materials with predetermined functionality; PF, Photo-fermentation; PMMA, polymethyl methacrylate; PSA, Pressure Swing Adsorption; PVA, Polyvinyl Alcohol; rGO, reduced graphite oxide; SC, Silicone gel; TS, Total solide; TSA, Temperature Swing Adsorption; TSI, Two stages integrated; US, ultrasound; VFA, Volatile Fatty Acids; VPSA, Vacuum Pressure Swing Adsorption; VSA, vacuum swing adsorption
\end{acknowledgement}

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Several types of biomasses are a suitable substrate for BioH₂ production because of their high organic matter content, low nutrient dependency and high energy potential; these include: wastewater sludge (Banu et al., 2020), food waste (Dinesh et al., 2018), microalgae (Show et al., 2019) and lignocellulosic biomass (Soares et al., 2020). Substrate composition, its pretreatment methods, environmental conditions (i.e., pH, temperature) and contaminants (e.g., metal ions) are some of the parameters that can influence the BioH₂ process (Dinesh et al., 2018).

The aim of this paper is to provide an up-to-date review of the challenges in BioH₂ production, storage and use, including the appropriate pretreatment methods, the processes and parameters of biohydrogen production, and the microbiological aspects for different processes. In addition, this work will describe storage and transport challenges of biogas and its main applications.

Most reviews of studies in the literature do not address the multidisciplinary aspect of biohydrogen production. In this context, the originality of this article is that the reader will not only learn how biohydrogen is produced from biomass but also how to optimize it by a proper choice of the pretreatment method as well as the proper selection of microorganisms. In addition, this article provides an insight into the challenges and recent methods of purification, storage and different applications.

2. Substrate pretreatment

A variety of biomasses that, due to their high organic matter content, low nutrient dependency, and high energy potential, are appropriate substrates for the synthesis of BioH₂. These biomasses include: food waste, lignocellulosic biomass, wastewater sludge, and microalgae. BioH₂ from biomass faces obstacles related to low rate of production and low rate of substrate degradation, or hydrolysis step. The latter in particular is seen as the limiting step in the overall process. The addition of a pretreatment step can improve both the rate of biomass degradation and the performance of H₂ production (Hay et al., 2015).

One of the most recalcitrant components in waste biomass is lignocellulose, and a pretreatment step is usually used to help the degradation of the macro-molecular crystal structure of its components: cellulose and lignin. The pretreatment is used to reduce the degree of polymerization of these two main components to transform lignocellulosic waste into fermentable substances accessible to most microorganisms. The choice of the pretreatment method generally depends on the composition of the substrates to be fermented and includes physical, chemical, biological and combined pretreatments. A summary of different pretreatments and their effect on biomass and on BioH₂ yield is presented in Table 1. It shows clearly the positive effects of several pretreatment methods on BioH₂ yield and lignin removal.

2.1. Acid and alkali pretreatment

Due to their ease of use and low energy demands chemical pretreatments are the most studied for optimizing BioH₂ production. Chemical degradation of lignocellulosic biomass to enhance BioH₂ production can be achieved by chemical reagents such as acids, alkalies, organo-sols, ionic liquids, metal chlorides, etc. Both acid and alkaline methods are very effective for cellulose biomass degradation, having an ability to convert mainly cellulose biomass into soluble sugars, which facilitates their use by microorganisms to produce BioH₂. Furthermore, for both pretreatments, the initial pH of the substrate must be considered, as it can affect the BioH₂ yield (Xiao and Liu, 2009). Acid pretreatment of lignocellulosic biomass has been done using sulfuric acid, hydrochloric acid, boric acid and nitric acid, where as sodium hydroxide (NaOH), potassium hydroxide (KOH) and calcium hydroxide (Ca(OH)₂) are the most adopted alkalies. Results showed that a chemical pretreatment with 4% HCl of grass substrate increased considerably the maximum cumulative BioH₂ yield, where sometimes acid pretreatment is considered superior to alkaline pretreatment for the improvement of BioH₂ production (Cui and Shen, 2012).

In another study, using a ratio of 1:10 (w/v), 10% ammonium hydroxide solution and rice straw particles were completely mixed before being autoclaved at 121 °C for a variety of periods. The solid fractions were then collected for the following pretreatment step by vacuum filtration after being rinsed with water; then the water-washed solid fractions were hydrolyzed with 1.0% sulfuric acid under autoclaving conditions at 121 °C for 50 min as the following pretreatment step (Nguyen et al., 2010). The results show that the combined acid with ammonia showed a good performance of the rice straw in BioH₂ yield production (increase of 17%). Otherwise, the maximum cumulative BioH₂ yield of 137.76 mL/gTS was obtained from corn straw pretreated with 2% NaOH, which was 31% higher than that of the control; this result suggests that an appropriate pretreatment can effectively destroy the structure of the corn straw improving its BioH₂ production potential (Zhang et al., 2020).

2.2. Biological pretreatment

Biological pretreatment methods have some advantages over chemical and physical methods because they do not require a great deal of energy or the use of harsh chemicals. On the other hand, the control and monitoring of biological processes makes this technology less suitable for industrial applications because of the longer retention time. Biological pretreatment methods include fungal pretreatment, enzymatic hydrolysis and aeration. Their main benefit is the efficient and specific decomposition of lignocellulosic substrates. For example, Fungi produce specific enzymes that significantly improve the rate of hydrogen by increasing BioH₂ yield by 209% from corn stalk fermentation (Keskın et al., 2019). In fact, industrial enzymes can be directly applied for hydrolysis such as α-amylase, hemicellulase, arabinase, and xylanase.

Immobilized laccase showed desirable results in delignification, although fermentation of biomass for BioH₂ production in CSTR gave a yield of 2.8 mol H₂/mol substrate, which makes this enzyme the capacity of catalyzing the removal of 76.93% of lignin from sweet sorghum stalks (Shanmugam et al., 2018). The hemicellulose present in biomass can be effectively degraded by enzymes such as xylanases into simple sugars; thus cellulases act on cellulose-producing byproducts that promote the production of BioH₂ (Banu et al., 2020).

2.3. Physical pretreatment

Mechanical and thermal processes can be used to disrupt the lignocellulosic structure. Mechanical methods such as grinding, ball mill, screw press, microwaves and sonication are the most used for biomass. The cellular disintegration and solubilization of the particles is carried out by ultrasonic waves in the frequency range of 10–20 kHz, having the power to degrade the lignocellulosic structure. For example, the best ultrasonic pretreatment of pulp and paper mill effluent was obtained at an amplitude of 60% and for a period of 45 min and has been considerably improved by 424% BioH₂ yield (Hay et al., 2015).

Thermal pretreatment are simple and easy to operate and can be done at high temperature or low temperature. In hydrogen production heat treatment is used for wastewater sludge (treating range 100–175 °C) and algae biomass (treating range 65–180 °C) whereas for lignocellulose biomass the thermal process is usually combined with other treatment methods (Wang and Yin, 2018).

2.4. Combined pretreatment

The combination of microwave pretreatment with other pretreatments showed a successful impact on BioH₂ production (Mishra et al., 2020). The combined acid-microwave pretreatment is characterized by a short duration and a higher sugar yield (Khamtib et al., 2011). At 140 °C and 2450 MHz with 1% H₂SO₄ for 15 min, combining microwave pretreatment of macroagal could increase BioH₂ yield up to 87%,
<table>
<thead>
<tr>
<th>Feedstock</th>
<th>Process scale</th>
<th>Pretreatment parameters</th>
<th>BioH₂ yield method</th>
<th>Increase in BioH₂ Yield (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass</td>
<td>Laboratory scale</td>
<td>4% HCl for 30 min</td>
<td>gas chromatograph</td>
<td>1544</td>
<td>(Cai and Shen, 2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9% NaOH for 30 min</td>
<td></td>
<td>338</td>
<td></td>
</tr>
<tr>
<td>Rice straw</td>
<td>Laboratory scale</td>
<td>1.0% H₂SO₄ for 30 min</td>
<td>gas chromatograph</td>
<td>17</td>
<td>(Nguyen et al., 2010)</td>
</tr>
<tr>
<td>Corn straw</td>
<td>Laboratory scale</td>
<td>10% NH₃ for 30 min</td>
<td>gas chromatograph</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological</td>
<td>Laboratory scale</td>
<td>2% NaOH for 60 min</td>
<td>gas chromatograph</td>
<td>31</td>
<td>(Zhang et al., 2020)</td>
</tr>
<tr>
<td>Corn stalk</td>
<td>Laboratory scale</td>
<td>Fungi (Trichoderma reesei Rut C-30) autoclaving at 121 °C for 25 min</td>
<td>gas chromatograph</td>
<td>209</td>
<td>(Cheng and Liu, 2012)</td>
</tr>
<tr>
<td>Sorghum stalks</td>
<td>Laboratory scale</td>
<td>Laccase from T. asperellum strain BPLM6T1</td>
<td></td>
<td>3.26</td>
<td>(Shanmugam et al., 2018)</td>
</tr>
<tr>
<td>Physical (thermal and mechanical)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp and paper mill effluent</td>
<td>Laboratory scale</td>
<td>Ultrasound (amplitude of 60% and for 45 min)</td>
<td>Water displacement</td>
<td>424</td>
<td>(Hay et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>Laboratory scale</td>
<td>Ultrasound at 195 J/mL</td>
<td>60-mL polypropylene syringe to bring the pressure inside serum vials to ambient pressure</td>
<td>38</td>
<td>(Leafo et al., 2012)</td>
</tr>
<tr>
<td>Sugar waste</td>
<td>Laboratory scale</td>
<td>Microwave (320 W for 5 min)</td>
<td>gas chromatograph</td>
<td>113</td>
<td>(Singhal and Singh, 2014)</td>
</tr>
<tr>
<td>Food waste</td>
<td>Laboratory scale</td>
<td>Heat 90 °C, 20 min</td>
<td>gas chromatograph</td>
<td>497</td>
<td>(Kim et al., 2009)</td>
</tr>
<tr>
<td>Microalgae (C. vulgaris)</td>
<td>Laboratory scale</td>
<td>Hot air oven at 100 °C for 60 min</td>
<td>plastic syringes</td>
<td>476</td>
<td>(Stanikaus et al., 2018)</td>
</tr>
<tr>
<td>Combined</td>
<td>Laboratory scale</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macroalgae (Laminaria japonica)</td>
<td>Laboratory scale</td>
<td>Microwave (1.40 °C and 2450 MHz) with acid (1% H₂SO₄ for 15 min)</td>
<td></td>
<td>87</td>
<td>(Yin and Wang, 2018)</td>
</tr>
<tr>
<td>Rice straw</td>
<td>Laboratory scale</td>
<td>Dispersion Disintegration rpm ( 12,000), heat (80 °C) and fixing pH at 10 using 1 N NaOH</td>
<td>Syringe displacement method</td>
<td>1512</td>
<td>(Yukesh et al., 2019)</td>
</tr>
</tbody>
</table>

* Increase in BioH₂ Yield (%) calculated as: \(\frac{\text{BioH}_2 \text{ Yield}_{\text{Pretreated}} - \text{BioH}_2 \text{ Yield}_{\text{Control}}}{\text{BioH}_2 \text{ Yield}_{\text{Control}}} \times 100\).
and energy conversion efficiency increased from 9.5% to 23.8% (Yin et al., 2018a).

In recent research, the efficiency of the combined pretreatment dispersion, thermochemical disintegration (DTCD) in terms of the degree of disintegration and the production of BioH₂ from rice straw was investigated and compared to dispersion thermal disintegration (DTD) and disperser disintegration (DD). A higher BioH₂ improvement of about 1512% was observed in DTCD when compared to DTD pretreatment (912%) and to DD pretreatment (625%). These results were obtained under the optimal conditions for combinative pretreatments (pH 10, temperature 80 °C, rpm 12,000 and disintegration time 30 min) (Yukesh et al., 2019).

Many authors have demonstrated the importance of performing fermentability tests to determine the best combination of pretreatment-/feedstock for hydrogen production (Panagiotopoulos et al., 2009). Indeed, the pretreatment not only has an effect on the components released in the media but also on the microbial communities composition and their fermentative pathways (Mohammadi et al., 2011), favoring butyric-acid type fermentation or mixed-acid type fermentation (Ren et al., 2008). The different types of pretreatment and their advantages and disadvantages are listed in Table 2.

### 3. Processes and parameters of BioH₂ production

#### 3.1. Dark fermentation

Dark fermentation (DF) allows the degradation of organic matter by anaerobic bacteria in the absence of oxygen and light. It is an interesting technological process as it can use mixed biomass waste as raw material. DF is frequently demonstrated by its straightforward method, independence from light, and ability to use renewable substrates, in contrast to other biotechnological processes. In addition, the process is relatively simple in design and it can have high production. For instance, the maximum BioH₂ production via DF of rice straw was between 0.08 and 0.09 mmol H₂/L.h (Sen et al., 2016). According to a recent study (Gonzales et al., 2016), the BioH₂ production rate from empty palm fruit bunch ranges between 0.25 and 0.32 mmolH₂/L.h.

However, the process has an inhibitory factor that can influence BioH₂ production. These include, inorganic inhibitors (light metal ions, heavy metal ions, ammonia and sulfate); organic inhibitors (volatile fatty acids (VFA), phenolic components, and furan derivatives) and bio-inhibitors (bacteriocins and thiolsulfonates) (Chen et al., 2021). The different types of pretreatment and their advantages and disadvantages are listed in Table 2.

**Table 2**

Comparison of pretreatment methods for improving BioH₂ production.

<table>
<thead>
<tr>
<th>Pretreatment method</th>
<th>Process</th>
<th>Strengths</th>
<th>Weakness</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical pretreatment</strong></td>
<td>Acids and alkaline, ozonation, and ionic liquids</td>
<td>Reduced energy demand, Easy process, Decrease crystalline structure.</td>
<td>Costly chemicals, Risk of inhibitors formation.</td>
<td>(Atelge et al., 2020)</td>
</tr>
<tr>
<td><strong>Biological pretreatment</strong></td>
<td>Utilizing microbial whole cells, enzymes, and fungi pretreatment.</td>
<td>Environmentally friendly, No chemicals required, Easy process.</td>
<td>High price of enzymes, Slow process.</td>
<td>(Atelge et al., 2020)</td>
</tr>
<tr>
<td><strong>Physical pretreatment</strong></td>
<td>Mechanical (ultrasonic, microwave, grinding, milling and shearing) thermal pretreatment</td>
<td>Low energy requirements, No toxic by-products, No additional chemicals added.</td>
<td>High demand for heat and electricity, High capital cost.</td>
<td>(Singhal and Singh, 2014)</td>
</tr>
<tr>
<td><strong>Combined pretreatment</strong></td>
<td>Combination of two or more different pretreatment processes</td>
<td>Fast process, High biodegradability, More efficiency.</td>
<td>Risk of producing non-biodegradable components</td>
<td>(Nguyen et al., 2010)</td>
</tr>
</tbody>
</table>

#### 3.2. Photo fermentation

Photo-fermentation (PF) produces BioH₂ during the decomposition of organic compounds by photosynthetic bacteria via a nitrogenase reaction catalyzed by light energy (Kumar and Das, 2001). PF is a very promising process for BioH₂ production due to its sustainability, environment-friendly features and potential for the simultaneous production of high value compounds (Sun et al., 2019). The process uses purple unsulphured photosynthetic bacteria which are well known for their ability to produce BioH₂ from organic acids when grown under phototrophic conditions with nitrogen limitation. The evolution of BioH₂ under these conditions is catalyzed by nitrogenase, which normally functions to catalyze the reduction of dinitrogen to ammonia with the release of H₂ from reduced N₂. In the absence of other reducible substrates, nitrogenase continues to transform protons into BioH₂.

Like other biological processes, PF is influenced by physico-chemical parameters such as the C/N ratio, temperature, pH, and light intensity. Furthermore, several studies have proven that the addition of a few chemicals, such as iron and molybdenum, ethylene diamine, tetra-acetic acid (EDTA), vitamins, buffer solutions and other chemicals, can increase the production rates and BioH₂ yields of appreciable value (Budiman, 2021). The applicability and relevance of the production of BioH₂ and poly-β-hydroxybutyrate in a single step PF of celler wastewater was demonstrated in the Policastro et al. study (Policastro et al., 2020). For an initial chemical oxygen demand of 1500 mg/L, the maximum amount of hydrogen and poly-β-hydroxybutyrate produced were 468 mL/L and 203 mg L/1 respectively (Policastro et al., 2020).

#### 3.3. Combined dark fermentation and photo fermentation

A recent review suggests that, for industrial applications, the partially light-driven system (PF) with a dark fermentative initial reaction...
4. Processes conditions

4.1. Culture conditions

Most DF studies focus on mixed cultures, yet, a number of mesophilic and thermophilic microorganisms have been widely applied in DF. *Clostridium* spp. and *Enterobacterium* spp. are the two most common mesophilic species used in the process (Osman et al., 2020) whilst *Thermoanaerobacterium* spp and *Thermotoga* spp. are the thermophilic ones (Osman et al., 2020). Pure cultures grown on specific substrates, are important to improve our knowledge on how to improve yields and production rates also in mixed culture, by identifying shift in metabolic pathways. Elshamouby et al. (2013) provided a comprehensive review on biohydrogen production from pure culture. Mixed cultures (mesophilic or thermophilic) are easier to use, have low operational costs and can use a broader range of feedstocks, but have a number of drawbacks including the presence of *H₂* consuming bacteria, producing undesirable products (Bundhoo and Mohee, 2016). Isolation strategies, such as heat (Liu et al., 2020) oxygen and pH (Shamurad et al., 2020) have been successful in suppressing the growth of unwanted species present in mixed culture inoculum (Soares et al., 2020). Increased yields of mixed culture have also been achieved with bioadditions of one or two *H₂*-producing species to the process (Kumar et al., 2016). Process parameters like pH, temperature and HRT have been shown to play an important role in BioH₂ yields (Arimi et al., 2015).

Temperature can control the growth rate of microorganisms and increase *H₂* production by mediating the enzymatic reactions (Sinha et al., 2015) and its optimal range is dictated by the microbial species/group involved in the process. An increase in temperature can positively impact the *H₂* production both at mesophilic and thermophilic conditions. Mesophilic cultures are likely to produce higher volumetric production rates whereas higher BioH₂ yields are likely to be achieved with thermophilic ones (Lukajits et al., 2018). A likely reason is that the higher gas(es) solubility and concentration at lower temperatures inhibit microbial activity and thus decrease the efficiency of *H₂* production (Silva et al., 2019). Mu et al., showed that *H₂* yields and biomass growth for mesophilic cultures was higher at 41 °C compared to 33 °C (Mu et al., 2006). However, the same study showed that the specific *H₂* production started to decline, as a result of enzymes denaturation, at 39 °C. Specific conditions should therefore be identified for each application to increase *H₂* yields.

Variation in pH can influence the microbial growth and metabolic pathways of *H₂*-producing bacteria and hence substrate degradation and *H₂* production yields (Arimi et al., 2015). The control of pH in a favorable range throughout the process is a strategy to prevent methanogenesis and solventogenesis (Kumar et al., 2016). Process optimization using multi-factor variance analysis (ANOVA), linear regression models and response
surface plots for different process variables, showed that the pH needed to be adjusted to pH 5.8 for optimal H₂ production from Agave tequilana vinasses (Espinosa-Escalante et al., 2009). Similarly, Phowan and Danvirutai showed that more than double H₂ (209 mmol H₂/L·h) was produced at pH of 5.5 compared to pH 8 (72.9 mmol H₂/L·h) (Phowan and Danvirutai, 2014). A wider range of initial pH (3–9) was examined by using sugarcane bagasse hydrolysate and H₂ yields doubled (1.97 mmol H₂/L·h) when pH was increased from 3 (0.81 mmol H₂/L·h) to 5, to decrease again at pH 9. (1.16 mmol H₂/L·h) (Reddy et al., 2017). Optimal pH, however, need to be assess for each process as it showed to be different (between 4 and 7.5) for different substrates and source of inoculum (Soares et al., 2020).

Hydraulic retention time (HRT) describes the average residence time of the feedstock in the bioreactor. Optimal HRT is a necessary for achieving high H₂ production rate and minimize unfavorable microbial pathways and the formation of undesired by-products (Tomczak et al., 2018). Short HRTs have proven beneficial for H₂ production (Hafez, 2010). It is believed HRTs shorter than the growth rate of the methanogens restricts their activity (Ueno et al., 2001). To illustrate, H₂ yields doubled (30 mmol H₂/L·h) as a result of a decrease in HRT from 4 to 1 h (Rosa et al., 2014).

4.2. Effects of macronutrients and micronutrients

 Macronutrients availability, such as nitrogen and phosphorus, is required for optimal growth of the targeted microorganisms and H₂ production. Correct ration of C and N is critical for microbial growth in all processes. An optimal carbon/nitrogen (C/N) ratio of 47 was shown to increase H₂ production rate by 80% compared to the blank using acclimated sewage sludge (Lin et al., 2004). Excess nitrogen however, could induce ammonification and cause toxicity problems (Arimi et al., 2015) or have an impact on the intracellular pH of the microorganisms and inhibits their activity (Chandrasekhar et al., 2015).

In addition, a proper phosphate concentration, which function as alkalinity mitigator and phosphorus donor, is necessary for the process (Lin and Lay, 2004). Na₂HPO₄ showed a bell-shaped dose-response and both higher or lower concentrations of 600 mg/L resulted in lower H₂ production (Lin and Lay, 2004).

Micronutrients such as trace elements and metal ions (Na⁺, Mg²⁺, Zn²⁺, and Fe⁺) can stimulate the activity of the enzymes thus facilitate H₂ synthesis. Each of the micronutrients has a specific effect on the bacterial cell during fermentation and can change the function of enzymes. Iron and nickel are enzymatic co-factors able to enhance H₂ production (Baeyens et al., 2020). Optimal concentration of Ni²⁺ and Fe⁺ can produce a positive effect on the active site of [NiFe] H₂ase which improves its catalytic activity (Bao et al., 2013). Iron and molibdenum are essential nutrients for the activation of nitrogenase, as a key enzyme in P₄ of H₂ production (Zhu et al., 2007). In addition, iron and sulfur have an important role in the functioning of proteins by transferring electrons during the oxidation process of pyruvate to acetyl-CoA, CO₂, and H₂ (Baeyens et al., 2020). The presence of micronutrients is essential for microbial metabolism and H₂ production yet, like for other parameters, the identification of the correct quantities to add to the systems is pivotal in process optimization.

4.3. Cell immobilization

Notwithstanding all potential process improvement, traditional CSTR fermentative H₂ production is limited by process pathways (Misbra et al., 2019) and operational problems such as low cellular density and biomass retention (Dzul Rashidi et al., 2020). Cell immobilization, by decoupling cell growth from H₂ production, therefore offers a sustainable and cheap solution to improve process yields (Wu et al., 2002). Immobilization defines a wide range of physical-chemical methods for increasing cell density in processes which can be divided in four main mechanisms: entrapment using a porous matrix; adsorption on solid carrier by physical adsorption or by covalent binding; self-aggregation by flocculation (i.e. granulation) or using cross-linking agents; and mechanical containment by means of a barrier (i.e. microporous membrane or a microencapsulation) (Mitropoulou et al., 2013). Each technique has been used in different sectors for different fermentations or remediation process and offers specific advantages for each application. The selection of the immobilization mechanism and material are significant in dictating the overall performance of the process. It is therefore necessary to find a simple and inexpensive immobilization technique for H₂ production that would also provide high cell viability over time and hence high operational activity and stability (Gotovtsev et al., 2015).

Natural polymers like alginate, agarose, carrageenan and chitosan are some of the natural gelling polysaccharides used for entrapment in fermentative processes due to their non-toxic, cheap and versatile nature (Kosseva, 2011). Whilst entrapped and protected by the matrix, the cells are unable to diffuse in the media but have the ability to grow and often present an increased metabolic activity (Gotovtsev et al., 2015). The entrapment method has also the advantage to allow the addition in the matrix of nanomaterial (Yang and Wang, 2018), supplements (Dzul Rashidi et al., 2020) or supporting media (such as activated carbon) to enhance the process yields or provide strength to the beads (Wu et al., 2003).

Entrapment has been used for single microbial species fermentation or mix cultures, in batch or continuous and with or without the addition of carriers or metals. In the adsorption process, the supporting material surface charge (zeta potential) and surface-to-volume ratios play a significant role in the establishment of the microbial biofilm, along with the cell charge and its wall composition (Kosseva, 2011). The material surface provides protection to the cells, helping biomass retention, and a structure to regulate and support cell growth. The process allows a better mass transfer and substrate utilization with shorter HRT (Kumar et al., 2016).

Microorganisms tend to form flocks in specific conditions thanks to the production of extracellular polymers or ECP (Show et al., 2019). In H₂ fermentation the polymer is composed mainly of polysaccharides, which have the role providing structural integrity to the granules and protect the cells (Liu and Sung, 2002). A confocal image analysis of the internal structure of H₂-producing granules showed that the cores was mainly comprised of proteins whereas the polymer and cells were mostly distributed on the outer layers of the granules (Zhang et al., 2008). This suggests that these granules are less likely to limit the mass transfer than other immobilization systems. In addition, granular processes have shown higher resistance to extreme conditions such as fluctuation in temperature, pH, influent concentration as well as high salinity (Owusu-Agyeman et al., 2019).

Summaries of these work has been reported in specific reviews (Show et al., 2020) and in Table 4. The data in the table comprises both batch and continuous fermentation and single or mixed microorganisms and was aimed at providing a broad overview of the work available related to the three immobilization methods. When plotted on a Box-Whisker graph (Fig. 1) the data for biogasification process rate showed that the range of values reported for granulation processes, were higher (13–297 mmolH₂/L·h) than those reported for entrapment (1–115 mmolH₂/L·h) and adsorption (3–83 mmolH₂/L·h). This is quite an interesting finding as granulation has also shown higher production yields in the methanisation process (Owusu-Agyeman et al., 2019). Bioractors favouring granule formation are also favored for the treatment of high strength wastewaters (van Lier, 2005), suggesting that the granule-based DF for H₂ production could be successfully implemented at scale.

5. Storage and transport challenge of BioH₂

5.1. BioH₂ purification

Unlike for other processes, H₂ produced via biological pathways requires purification/separation steps. Fermentation process such as
Table 4
H₂ yields of immobilized reactors.

<table>
<thead>
<tr>
<th>Culture type</th>
<th>Average production rate (mmol H₂/L·h)</th>
<th>Process conditions and scale</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biofilm on PVA</td>
<td>Mixed culture</td>
<td>8.9</td>
<td>(Jeong et al., 2005)</td>
</tr>
<tr>
<td>Granular activated carbon</td>
<td>Mixed culture</td>
<td>7.8</td>
<td>(Jamali et al., 2019a)</td>
</tr>
<tr>
<td>Glass Beads</td>
<td>Mixed culture</td>
<td>5.2</td>
<td>(Jamali et al., 2019a)</td>
</tr>
<tr>
<td>Ceramic ball</td>
<td>Mixed culture</td>
<td>5.0</td>
<td>(Keskin et al., 2012)</td>
</tr>
<tr>
<td>Granular activated carbon</td>
<td>Mixed culture</td>
<td>5.4</td>
<td>(Wu et al., 2012)</td>
</tr>
<tr>
<td>Glass Beads</td>
<td>Mixed culture</td>
<td>5.5</td>
<td>(Pekguzel, 2016)</td>
</tr>
<tr>
<td>Granular activated carbon</td>
<td>Mixed culture</td>
<td>15.8</td>
<td>(Hau et al., 2015)</td>
</tr>
<tr>
<td>Granular activated carbon</td>
<td>Mixed culture</td>
<td>12.3</td>
<td>(Wang et al., 2013)</td>
</tr>
<tr>
<td>Granular activated carbon</td>
<td>Mixed culture</td>
<td>2.7</td>
<td>(Juppi et al., 2016)</td>
</tr>
<tr>
<td>Porous glass beads</td>
<td>C. butyricum</td>
<td>51.3</td>
<td>(Yokoi et al., 1997)</td>
</tr>
<tr>
<td>Gau</td>
<td>E. Chocaee</td>
<td>82.6</td>
<td>(Kumar and Das, 2001)</td>
</tr>
<tr>
<td>Polyurethane foam</td>
<td>C. tyrobutyricum</td>
<td>13.4</td>
<td>(Zhang et al., 2008)</td>
</tr>
<tr>
<td>Expanded clay</td>
<td>Mixed culture</td>
<td>43.3</td>
<td>(Amorim et al., 2009)</td>
</tr>
<tr>
<td>Entrapment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated carbon + polymer</td>
<td>Mixed culture</td>
<td>14.1</td>
<td>(Chu et al., 2011)</td>
</tr>
<tr>
<td>Polyvinyl Alcohol (PVA) Gels</td>
<td>Mixed culture</td>
<td>3.4</td>
<td>(Yin et al., 2018a, 2018b)</td>
</tr>
<tr>
<td>Alginate gel beads (+ Fe) immobilized cells</td>
<td>Mixed culture</td>
<td>12.3</td>
<td>(Sekkou et al., 2018)</td>
</tr>
<tr>
<td>Alginate gel beads (+ Mg) immobilized cells</td>
<td>Mixed culture</td>
<td>7.3</td>
<td>(Sekkou et al., 2018)</td>
</tr>
<tr>
<td>Silicone gel (SC)</td>
<td>Mixed culture</td>
<td>115.6</td>
<td>(Chu et al., 2011)</td>
</tr>
<tr>
<td>Polyethylene-octene-estamer (POE)</td>
<td>Mixed culture</td>
<td>80</td>
<td>(Wu et al., 2007)</td>
</tr>
<tr>
<td>Alginate + chitosan</td>
<td>Mixed culture</td>
<td>21.3</td>
<td>(Wu et al., 2002)</td>
</tr>
<tr>
<td>Agar</td>
<td>Mixed culture</td>
<td>23.1</td>
<td>(Ishikawa et al., 2008)</td>
</tr>
<tr>
<td>Alginate/Acrylic/latex/Silicone</td>
<td>Mixed culture</td>
<td>47.5</td>
<td>(Wu et al., 2002)</td>
</tr>
<tr>
<td>GAC-Alg beads</td>
<td>Mixed culture</td>
<td>2.5</td>
<td>(Izul Rashidi et al., 2020)</td>
</tr>
<tr>
<td>GAC with Alg and chitosan (GAC-AlgC)</td>
<td>Mixed culture</td>
<td>0.93</td>
<td>(Izul Rashidi et al., 2020)</td>
</tr>
<tr>
<td>Sodium alginate and polymethyl methacrylate</td>
<td>Mixed culture</td>
<td>37.4</td>
<td>(Wu et al., 2007)</td>
</tr>
<tr>
<td>Granulation</td>
<td>Mixed culture</td>
<td>13.4</td>
<td>(Jeong et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture</td>
<td>227</td>
<td>(Lee et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture</td>
<td>285</td>
<td>(Zhang et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture</td>
<td>145</td>
<td>(Show et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture</td>
<td>294</td>
<td>(Zhang et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>E. Larrogenes</td>
<td>58</td>
<td>(Bachman et al., 1990)</td>
</tr>
<tr>
<td></td>
<td>Mixed culture</td>
<td>297</td>
<td>(Lee et al., 2004)</td>
</tr>
</tbody>
</table>

Fig. 1. Box and Whiskers plots of the data reported in Table 4, H₂ production rate of different immobilization methods.

DF, PF or DF-PF yields a gaseous mixture. BioH₂ purification, in its role, represents a critical challenge for the implementation of a sustainable and profitable BioH₂ economy (Gupta et al., 2013). The drawbacks that each method suffers from summarize BioH₂ economy setting up main issues. A high loss of gas following pressure release during desorption is the main challenge of Pressure Swing Adsorption (PSA) (Chowdhury and Sarkar, 2016). Temperature Swing Adsorption (TSA) is an energy-intensive process requiring very large adsorbent stocks (Bonjour et al., 2002). TSA uses heating of the adsorbent used by means of a hot gas. Table 5 illustrates the main methods used for the purification and separation of BioH₂ from the fermentation gas mix. Adsorption is one of the best-known approaches in the field of gas separation. Depending on the parameter used (temperature or pressure), the separation is carried out based on an adsorbent. Absorption is another separation method that can be used; the idea is to exploit the solubility of H₂ via a solvent. It is about the use of a suitable solvent to absorb the existing gases with the H₂. It exploits hydrogen solubility in water, which is on the order of 1.8 g/cm³ with P = Patm and T = 20 °C (Gupta et al., 2013). Finally, membrane separation is based on the difference between gaseous components speeds to extract the BioH₂.

6. Biohydrogen storage: methods, challenges and potential for enhancement

It is important to mention that BioH₂ produced by biological pathways storage methods do not differ from those of H₂ from other processes (Table 6). Compressed gaseous storage is carried out under significant pressure tanks (200 – 500 bar). This method is beneficial and easy to use at an affordable cost (Du et al., 2021). Another option is to store H₂ in liquid form. The liquefaction ("Linde-Hampson" liquefaction cycle) consists of passing the gaseous H₂ through a series of
interventions, namely compression and heat exchange ($T = -253 \, ^\circ\text{C}$) (Yin et al., 2018a, b). Cryo-compressed storage is used to maintain significant energy density and reduce evaporation losses following liquefaction (Lively et al., 2012). It is possible to store $H_2$ in solid form. It can be stored via physisorption on a large surface area substrate or via metal hydrides (e.g. NaAlH$_4$, AlH$_3$, LiBH$_4$, MgH$_2$, NaBH$_4$) (Xu et al., 2018a). These have a $H_2$ storage capacity of 5–7% by weight (Yin et al., 2018a, b). Also, there is the possibility of underground storage (e.g. salt caverns). This technique relies on the accumulation of gas at a very significant depth, several meters, or even more (Agueda et al., 2013). The diversity of BioH$_2$ storage methods has not been able to prevent several challenges disrupting the development of a sustainable BioH$_2$ economy. Therefore, improving and optimizing storage remains a major challenge (Banu et al., 2013). Currently, the improvement of physisorption for $H_2$ storage is the subject of advanced research. For example, hydrogen storage properties of co-functionalized 2D Gallium sulfide (GaS) monolayers have been systematically investigated by first-principles calculations. Table 7 shows the impact of functionalized 2D GaS on hydrogen storage.

### 6.1. Biohydrogen storage advances

Currently, there are no specific storage techniques for biohydrogen. We always talk about hydrogen storage even though it is the source of production. In terms of obstacles, the storage of biohydrogen and other types of hydrogen suffer from the same obstacles including low density

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**Table 5**

<table>
<thead>
<tr>
<th>Method</th>
<th>Process ideas</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Swing Adsorption (PSA)</td>
<td>PSA is gas separation technique relies on the adsorption of unwanted gas on a porous adsorbent (high pressure regime). The recovery of the desired gas happens under low pressure conditions. The performance of PSA systems is a function of the number of adsorption beds, the bed dimensions, the layers, the cycle configuration and the operating conditions (Xu et al., 2018a). Many adsorbents are allowed to be used (e.g., zeolites, activated carbon). The nature of the adsorbent has a considerable effect on the recovery rate (Du et al., 2021).</td>
<td>93–96 (metal-organic framework (MOF) “UTSA-16” as adsorbent) (Kuroda et al., 2018) 88.1 (Hollow fiber sorbent as adsorbent) (Lively et al., 2012) 88.43 (palm kernel shell activated carbon as adsorbent) (Shamsudin et al., 2019) 69.6 (CaX zeolite as adsorbent) (Agueda et al., 2015) + 75 (Cu-AC-2 as adsorbent) (Banu et al., 2013) 71–85 (Activated carbon/zeolite 5 A as adsorbent) (Rotava et al., 2018) + 75 (Activated carbon as adsorbent) (Ahn et al., 2012) 89.7 (Activated carbon/zeolite LiX as adsorbent) (Abdeljalil et al., 2018) 85 – 90 (Sirosh et al., 2002)</td>
</tr>
<tr>
<td>Membrane separation</td>
<td>Membrane separation is a process where we separate the components in a solution by rejecting unwanted substances and allowing the others to pass through the membrane.</td>
<td></td>
</tr>
</tbody>
</table>

**Table 6**

<table>
<thead>
<tr>
<th>Storage method</th>
<th>Challenge overview</th>
<th>Maximum storage capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compressed gaseous $H_2$</td>
<td>✓ Density issue: a liter of $H_2 = 0.21$ gasoline at the most ✓ Lack of volumetric and gravimetric efficiency ✓ High investment cost (e.g. $850 /Kg H_2$ storable for low pressure uses) ✓ Material requirements</td>
<td>The storage capacity varies according to the nature of the vessels (Rivard et al., 2019): [Type 1: all metal construction: 1.7% wt] (Rivard et al., 2019) [Type 2: Mostly metal, composite overlap in the hoop direction: 1.1% wt] (Rivard et al., 2019) [Type 3: Metal liner, full composite overlap: 4.2% wt] (Ijka et al., 2011) [Type 4: all composite construction: 5.7% wt] (Law, 2011) 7.5% wt (Siros et al., 2002) 5.4% wt (Könze and Kircher, 2012)</td>
</tr>
<tr>
<td>Liquid storage $H_2$</td>
<td>Requires 30% of stored energy</td>
<td></td>
</tr>
<tr>
<td>Physisorption</td>
<td>✓ Carbon materials, MOF and zeolites has shown an adsorption limitation ✓ Optimization of deH$_2$ation/reH$_2$ation kinetics: technical challenges to reach short times ✓ Released heat post-storage management ✓ Heat transfer and storage capacity challenge: caused by changes during charge/discharge cycles.</td>
<td>MOF: 8–10% wt (Blankenship et al., 2017) Graphite: 3% wt (Ströbel et al., 2006) Carbon nanotubes: 4.5% wt (Ströbel et al., 2006) Zeolites: 1.54% wt (NaA = 1.89% wt (CaA) (Langmi et al., 2005) 6% wt(wt: gravimetric density) (Nogita et al., 2009) 7.6% wt (Crivello et al., 2016)</td>
</tr>
<tr>
<td>Metal hybrids</td>
<td>✓ Operating pressure inside the tank system must be high ✓ Sensitive and demanding stock in terms of filling time (due to kinetics and heat transfer challenges) ✓ Heat transfer and storage capacity challenge: caused by changes during charge/discharge cycles.</td>
<td></td>
</tr>
<tr>
<td>Underground storage</td>
<td>✓ Geological site choice ✓ $H_2$ escape and migration risks ✓ For deep aquifers case: Adaptation of existing boreholes for hydrogen storage may be feasible. The availability of suitable technology and equipment for the construction and operation of the storage system</td>
<td>It depends on the geometry of the storage (ex: If the cavern roof is about 1000 m deep and the cavern has a geometric volume of 700,000 m$^3$, the net storage capacity - also called working gas - will be about 6000 t.) (Crotogino et al., 2010)</td>
</tr>
</tbody>
</table>
for physical storage via compression, loss via evaporation and high cost for physical storage via liquefaction, the specificity of catalysts for chemical storage via organic liquids and others.

6.2. BioH₂ transportation

BioH₂ transportation is carried out in various ways depending on the desired duration of this transfer; the mass of H₂ involved, geographic features also need to be considered plus the technical and economic parameters. At present, three ways for H₂ transportation exist, namely rail or road transportation, ocean transportation, and transportation via pipelines (Boucher and Alleau, 2016). The integration of H₂ into the worldwide energy loop faces several technical and economic obstacles (Gerboni, 2016). Table 8 details and classifies the challenges of BioH₂ transport into 3 types of challenges. Regarding transport, it is still affected by the challenges we have presented in the table. Unlike storage, transport has not seen much progress at this time.

7. Applications of BioH₂

7.1. Industrial applications

Biohydrogen could be used as a feedstock in many industrial applications, we can cite: Chemical industries like refineries, ammonia and methanol synthesis, also in steelmaking process. According to the International Energy Agency (IEA), Hydrogen demand reaches almost 90 million tons per year in 2020, almost 38 million tons per year in 2020 for oil refining industry, and 51 million tons per year for other industries including Ammonia and methanol synthesis and steel making, as shown in Table 9 (Nazir et al., 2020).

To achieve a higher climate change ambition, hydrogen can be one of the key elements that offers a clean, sustainable and flexible option, contributing to reaching a low-carbon economy. The industry sector is the most consuming sector of Hydrogen. Indeed, the global Hydrogen production market for industrial uses was valued at $115.5 billion in 2017 and is projected to reach $154.1 billion by 2022 (Boateng et al., 2020). Hydrogen used in ammonia synthesis exceeds 27% of the worldwide hydrogen produced; 33% in refineries; methanol producers use almost 10%, 23% are used in the transport sector, and over 6% are used by other industries (Crotogino et al., 2010). So there is significant potential for GHG emissions reductions in using clean Hydrogen as a feedstock for all those industries.

Fig. 2 shows the present hydrogen demand.

As explained in different studies, the major problems in bioH₂ production from wastes or in biological sources are the low rates and yields of H₂ formation (Kapdan and Kargi, 2006). The ability of the systems to scale up to volumes large enough to generate the requisite rate is the most important of these issues. Any of the processes has insufficient H₂ production and yield for commercial use. Future research must make a number of enhancements to get beyond these limitations.

7.2. Transport applications

Other interesting opportunities for hydrogen usage are associated with transportation applications, using fuel cells in electric vehicles (FCEV), trucks, buses, trains, and ships. Fuel cells for mobility have excellent performance in driving range, and they offer shorter refueling times (from 3 to 5 min) (In fact, FCEV delivers electrical energy using hydrogen as a feedstock with zero CO₂ emissions and water as the only byproduct on the downstream process. Commercialization of hydrogen cars has been launched by several automotive manufacturers. In addition to road transport, hydrogen also is contributing to decarbonizing the rail sector. The first Hydrogen powered FC train was developed and successfully tested in 2018 in Northern Germany, to replace diesel trains on no-electrified lines. Now there is big dynamic to move toward Hydrogen trains in the next few years, especially in some European countries. Adoption of hydrogen in transportation applications is limited by fuel cell costs of infrastructure, namely refueling stations, safety aspects, and maintenance costs (Crotogino et al., 2010). In different studies, the integration system between the bioH₂ production technologies, the biohydrogen purification system and the application of Proton Exchange Membrane (PEM) fuel cell to generate electricity have been reviewed and discussed (Rahman et al., 2016). The previous cited papers highlight that some technical barriers must be examined, such as the efficiency of the bioreactor so that it can be scaled up to high volumes to provide high flow rates of the required H₂. Furthermore, other production processes, such as biomass feedstock pre-conditioning, waste processing and H₂ separation and purification must also be considered before this system can generate Hydrogen to power the fuel cells.

7.3. Building and electricity

Hydrogen from renewable sources can be used in stationary applications such as power generation and energy demand in buildings. The advantage of those two applications is their lowering of CO₂ emissions. BioH₂ can be stored and then converted into electrical energy via fuel cells when needed (Sazelee et al., 2018). That makes sense where large amounts of abundant biomass are produced. Hydrogen energy systems
buildings. This solution could avoid the whole transformation in the gas storage and pumped hydro range from 10 MWh to 10 GWh. The degree of infrastructures, while respecting hydrogen concentration in the gas works at low concentration to overcome heat and cooking demand in safety, and economic issues (Yao et al., 2020). In residential applications, hydrogen could be blended into existing natural gas networks at low concentration to overcome heat and cooking demand in buildings. This solution could avoid the whole transformation in the gas infrastructures, while respecting hydrogen concentration in the gas network which must not exceed 12% according to countries legislation (Yao et al., 2020).

The Microbial fuel cells (MFC) are proven to be at least 50% and as much as 98% effective at treating wastewater. Depending on the design and feedstock, MFCs have been reported to produce 30 W/m2 of energy and 1 m3/d of bioH2. Till now, this technology is facing some limitations, due to the gap in knowledge between laboratory and commercial-scale applications (Ahmed et al., 2022).

More research needs to be done to enhance and optimize the current production technologies with the goal of raising H2 yield and simultaneously lowering prices.

8. Large scale challenges and advances

The nature of the industrial application determines the need for hydrogen. Current laboratory scale biohydrogen production quantities may be suitable for small applications but are insufficient for large scale industrial applications. Secondly, unlike other hydrogens, it is not possible to determine a precise and generalized quantity for all organic waste (or biomass in general). If we take the example of water electrolysis, a daily quantity of 9 liters of water is sufficient to generate 1 kg of hydrogen (Reddy et al., 2019).

9. Conclusion

The process of producing BioH2 from biomass faces obstacles related to the low rate of BioH2 production and the low rate of substrate degradation. Both dark fermentation and photo-fermentation have positive and negative features, suggesting that one-solution fits all should not be used for these processes. For example, DF, a more robust process, could be a suitable solution to treat mixed organic waste in addition, or as an alternative, to biomethane. Whereas PF, which requires a cleaner feedstock and light, could be used in the treatment of specific waste materials, with the simultaneous production of other high-value compounds or in countries where light variation is not an issue. It is important to mention that BioH2 produced by biological pathways storage methods do not differ from those of BioH2 from other processes (e.g. electrolysis). Therefore, reflection towards BioH2 purification/separation precedes its storage, which represents a critical challenge for the implementation of a sustainable and profitable BioH2 economy.

The improvement and optimization of storage remains a major challenge. BioH2 transportation is carried out in various ways depending on the desired duration of this transfer, the mass of BioH2 involved, geographic features are considered plus the technical and economic parameters. The energy consumption for compression, the security and economic aspects are today the challenges of BioH2 transport.

Conflict of interest

We confirm that we have no conflict of interest to declare, and this work is original and has not been published elsewhere or is it currently under consideration for publication elsewhere.

References

Akroum-Amrouch et al., 2013a. Biohydrogen Production by Dark and Photo-fermentation Processes.


Overview of Dark, Photo and Integrated Dark-photo Fermentative Approach to Biomass.


