HYDROGEN, FUEL CELL & ENERGY STORAGE

A Critical Review of Continuous Biohydrogen Production

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Abstract

Biohydrogen is a feasible and environmentally sustainable fuel for the world's growing energy demand. As an alternative renewable energy source, hydrogen contains 2.75 times more energy per gram than any other known source. Biohydrogen is produced from various microorganisms and renewable materials through anaerobic dark fermentation and phototrophic bacteria, operating in batch, semi-continuous, and continuous modes. Anaerobic dark fermentation has a higher production rate and yield compared to photofermentation. In this review, the dark fermentation of hydrogen is discussed concerning key influential parameters and environmental factors such as temperature, pH, hydraulic retention time, mass transfer coefficient, and recycle ratio. A low initial pH affects metabolic pathways, prolongs the lag phase, and enhances Fe-hydrogenase activity. Mesophilic, thermophilic and ultrathermophilic strains could tolerate maximum operating temperature of 40, 65 or 108 °C, respectively. A varity of fermenters are continuously used for biohydrogen production, with a few discussed here. Tower-type fermenters are more feasible than CSTRs as they provide a higher hydraulic retention time for continuous biohydrogen production. Fluidized beds are used for both short- and long-term hydraulic retention time operations. Hydraulic retention time (HRT) typically depends on the bioreactor type, geometry, and feed composition, particularly the carbon source. When wastewater is used, HRT generally ranges from 8 to 14 hours. However, the process requires more detailed data to fully understand and overcome thermodynamic limitations. These limitations could be addressed through genetic modification or metabolic pathway alterations to enhance biohydrogen yield, making large-scale and commercial production more feasible.

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1 Introduction

Greenhouse gas emissions from the use and combustion of carbon-based resources such as wood, fossil fuels, and natural gases, along with the increasing release of greenhouse gases, have significantly contributed to climate change, raising Earth's temperature by approximately 1.64 °C since the Industrial Revolution. As a result, the world is becoming an increasingly insecure place to live, with severe climate conditions and freshwater scarcity. A key solution to addressing climate challenges is the production of biohydrogen from renewable and sustainable resources. Technological limitations in certain renewable energy production methods are key challenges hindering global progress and the improvement of social welfare. Ultimately, the world's most polluting nations should contribute in various ways, including financial investments, to develop affordable infrastructure and technologies for green hydrogen production. This effort is essential to restoring Earth's health, creating a less strained environment, and ensuring a safer planet for future generations. Further, non-renewable fossile fuels like crude oil, are becoming heavier and more sulfur-rich in reservoirs due to continuous extraction. The uncertainty of a stable supply is exacerbated by factors such as war, terrorist attacks, and political instability. Therefore, now more than ever, it is crucial to prioritize the goal of achieving net-zero emissions by 2050 and ultimately replace carbon-based energy sources with safer alternativeslike green hydrogen [1].

Renewable and sustainable energy sources, including solar, wind, ocean waves, geothermal, hydropower, biomass, and bioprocesses such as dark fermentation, need to be produced on a large scale with economic advantages. Bio-based renewable energy sources are particularly attractive due to their low greenhouse gas emissions, ease of handling, and environmentally friendly nature. Furthermore, some biofuels are produced through fermentation processes that are not dependent on seasonal or geographical conditions, and a few utilize low-cost ingredients to reduce production costs. Biomass is also processed to produce bioethanol and biogases, including biohydrogen [2]. Energy sources that do not emit greenhouse gases (GHGs) during combustion, do not compete with water and food resources for humans and animals, and help reduce greenhouse gas emissions are preferred. However, for a long time, green hydrogen has been recognized as a key energy source for addressing decarbonization concerns [3]. Hydrogen is a promising energy carrier due to its high energy content per unit weight (122 kJ/g), which is approximately 2.75 times higher than any known hydrocarbon-based resource. Moreover, its combustion does not emit CO_2, producing only energy, water vapor, and a minimal amount of NO_x [4, 5].

At commercial scale, hydrogen is purified by pressure swing adsorption (PSA), achieving purity levels of up to 99.999%, making it suitable as feedstock for hydrogen fuel cells to generate electricity. Furthermore, fermentative hydrogen production is independent of geographical location and climate variations.In addition to glucose, sucorose, various carbohydrates feed stocks including organic wastes, molasses consuming agro-bio industrial wastewater, saccharified cellulosic materials, and animal wastes, have potential to be used as feed. Moreover, dark fermentation (DF) offers a higher production rate than photo-production and provides satisfactory yeilds. Furthermore, contineuous dark fermentation offers advantages of ease of operation and simple process control. It requires cheaper experimental facilities and can utilize a wide range of temperature-tolerant species, such as mesophilic, thermophilic, extremophilic, and hyperthermophilic organisms. Biohydrogen production is renewable, sustainable, and easily accessible due to its independence from geo-fossil fuel sources, making it more beneficial than methane production.Moreover, biological processes are often carried out at ambient temperature and pressure, using renewable sustainable substrates. When compared to thermal cracking, steam reforming of methane, and electrochemical processes, biological methods require less energy for production and offer significant economic advantages [6]. Over the past 15 years, hydrogen production through dark fermentation by anaerobic bacteria has seen significant advancements, enhancing its economic and commercial viability. In 2009, aerobic biohydrogen production by bacteria was also reported [7].

The feasibility of biohydrogen production through different bioreactor operation modes, including batch, semi-continuous, and continuous processes, is examined in this review. Continuous bioreactor operation at a steady state is generally preferred, as it requires less manpower for feed and product handling, sterilization, and operation. Additionally, it offers a simpler control system, ease of cleaning, and long-term operational stability [8]. Further, various aspects of biohydrogen production by continuous bioreactor operation are comprehensively reviewed. The yield of biohydrogen in relation to energy consumption and source, as well as the effects of several parameters, is discussed. Key influencing factors, such as bioreactor type, geometry, and operational conditions – including temperature, pH, recycle ratio, and mass transfer – are examined. The advantages of specific bioreactor types used for biohydrogen production are highlighted. Addition-

ally, recommendations on factors influencing continuous biohydrogen production are provided to pave the way for economic feasibility and eventual commercialization.

2 Modes of Biohydrogen Production by Dark Fermentation

2.1 Batch mode of operation

Batch mode of anaerobic operation is typically conducted by sterilizing the fermenter and defined substrates, cooling the bioreactor to the specified room temperature, inoculating it as per protocol, and sparging with nitrogen to remove as much oxygen from the medium as possible. Moreover, after a lag phase of more than three hours, the log phase follows, as biohydrogen production is biomass-based. The limiting reactants are typically carbon-rich substrates, such as glucose, which are usually supplied in excess. Biogas is collected from the top of reactors, including bubble, fluidized, packed, slurry, air-lift, and mechanically agitated types, while acids and alcohols are removed from the bottom. Batch- mode anaerobic operation for biohydrogen production via dark fermentation is a more traditional and widely practiced approach. Extensive research in this field has focused on studying the effects of different substrates at appropriate concentrations, various types of microorganisms, including bacteria, and the optimization of operating parameters in batch mode, all aimed at enhancing production rate and yield. To understand the kinetics of substrate metabolism and product formation during dark fermentation in batch mode, the Monod-type equation is generally used. This equation effectively determines the specific growth rate and maximum growth rate, as presented by the following Equation (1):

$$\mu = \mu_m \frac{S}{K_s} + S \tag{1}$$

where S corresponds to carbon species concentration, and supplied in excess ($S \gg K_s$), a zero-order reaction like Equation (2) is obtained:

$$\mu = \mu_m \tag{2}$$

where μ is the specific growth rate (h), μ_m is the maximum growth rate (h), S is the substrate concentrationan (kg/m^3) and K_s (kg/m^3) is the concentration coefficient or the affinity of the microorganism for the substrate. Among various kienetic models available, the modified Gompertz and logistic models are commonly usedwith the former being preferred for estimating the lag phase duration, as described by Equation (3) [9].

$$H = H_m \exp\left\{-\exp\left[\frac{R_m e}{H_m}(\lambda - t) + 1\right]\right\} \tag{3}$$

where H (mL), H_m (mL), and R_m (mL/h) represent the cumulative hydrogen production, hydrogen production potential, and maximum hydrogen production rate, respectively. λ (h) and t (h) refer to the lag time and incubation time and e is 2.71828. The kinetic parameters (H_m, R_m and λ) are assessed using various softwere including the `Origin 9.1` [1, 2, 9]. Since biohydrogen production is growth-dependent, the maximum hydrogen production occurs during the log phase, approaching the stationary phase when using batch mode of operation. During this process, the pH of the medium often decreases due to the accumulation of organic acids, which in turn reduces the reaction rate until it finally reaches the stationary phase. The yield of hydrogen production in batch mode is reported to range between 1.5 and 2.5 moles of hydrogen per mole of glucose [1, 10]. Provided that an inhibiting concentration prevails, a kinetic model proposed by Haldane is given by the following Equation (4):

$$\mu = \frac{\mu_m S}{K_s + S + \frac{S^2}{K_i}} \tag{4}$$

where μ is the specific growth rate (h), μ_m is the maximum growth rate (h), S is the substrate concentrationan (kg/m^3) and K_s (kg/m^3) is the concentration coefficient or the affinity of the microorganism for the substrate, K_i is the inhibition constant (kg/m^3). The model is a useful technique for understanding the effects of both low and high substrate concentrations, with the latter often being the focus of studies [11]. Batch mode of operation is investiged with eitherfree or immobilized cells to examine the effect of parameters on the rate and yeid of biohydrogen production. Continuous bioreactor mode of operation is often preferd by employing immobilized cells on microbially friendly materials, which is durable and economical.

2.2 Cells immobilizations

Immobilization methods are diverse and include entrapment, electrochemical bonding (such as covalent, ionic), physical adsorption, biofilm formation, granulation, and floc formation [12]. Immobilizing cells increases their resistance to fluctuations in concentration, temperature, and pH, thereby enhancing their ability to function continuously in biological processes, particularly in the dark fermentation of biohydrogen production. Furthermore, immobilized processes, such as those involving cells and enzymes, offer several advantages across various scales. Some of these include ease

of operation, higher stability, efficient product separation, space savings, relatively simpler control and automation, reduced manpower requirements, and ultimately lower overall costs. However, during operation, it is essential to maintain an appropriate cell density during the log phase to ensure optimal performance [13]. Fluidized and fixed bed bioreactors are irregularly filled with various immobilized fillers. These fillers are typically chosen to allow microorganisms to grow within their cavities, forming biofilms, or to be immobilized through electrochemical charges. Additionally, the fillers should be non-toxic, exhibit good mechanical, thermal, and chemical stability, be inexpensive, reusable, scalable, and resistant to fermentation products. The main role of fillers is to provide a high surface area, maintaining a suitable population density of microorganisms in the bioreactor. This, in turn, increases the production rate per unit area and contributes to a more stable mode of continuous operation, offering economical benefits [14, 15].

A carrier must meet the following prerequisites to be used for the immobilization of biological hydrogen-producing microorganisms. It should have a large surface area, functional groups and electochemical charges to immobilze large amount of cells. It is preferred that the carrier possesses good mechanical, chemical, and thermal stability, in addition to being inexpensive, reusable, and scalable. The carrier should also resist fermentation by-products and maintain uniformity within the bioreactor, ensuring uniform permeability to allow free diffusion of nutrients, gases, cofactors, and products . The advantages of continuous operation can be summarized as follows: product formation is flow rate-dependent. Several modes of continuous operations are used to produce biohydrogen through dark fermentation, including suspended cells, immobilized fluidized bed, and fixed bed systems. The most widely empolyed modes are anaerobic sequential batch bioreactors (ASBR) and continuously stirred tank bioreactors (CSTBR). The latter type is more commonly used to achieve proper mixing, improve mass transfer, and maintain a homogeneous environment. However, cell concentration in ASBR and CSTR is limited because suspended microorganisms have a retention time equal to the hydraulic retention time and may be carried out with the outflow. Consequently, the washout of microorganisms from the bioreactor reduces the efficiency of these fermenters at high feeding rates for biohydrogen production, requiring optimization [16].

Unlike suspended cell bioreactors, immobilized cell bioreactors do not face the same complications. Cell immobilization is typically achieved through cell seeding, physical microbial biofilm formation, and electrochemical interactions. Furthermore, bioreactors used for continuous biohydrogen production can be classified into various types, including up-flow fluidized beds, packed beds, up-flow anaerobic sludge beds (UASB), expanded granular sludge beds (EGSB), carrier-induced granular sludge beds (CIGSB), immobilized cell-seeded anaerobic reactors (ICSAB), trickling biofilter reactors (TBR), and membrane bioreactors (MBR). Furthermore, microorganism's immobilization techniques play a crucial role in governing the bioreactor process [17]. When continuous processing remains uncontaminated, it offers the advantage of lower production costs. If the limiting substrate is available in excess ($S \gg K_s$), the bioreactor volume remains constant, and the inlet and outlet flow rates are approximately uniform, the specific growth rate, maximum growth rate, and dilution rate in the bioreactor reach a steady state concerning the microorganisms, as described by the following Equation (5):

$$\mu = \mu_m = D \tag{5}$$

where D is dilution rate (h^{-1}) is given by Equation (6):

$$D = \frac{F}{V} = \frac{1}{\tau} \tag{6}$$

where F is volmetric flow rate (m^3/h), V stands for volume of reactor (m^3) and τ is retention time (h). By rearranging Equation (5) and applying mass balance for continuous operation at steady state Equation (7) is obtained for limiting substrate:

$$S = D\frac{K_s}{\mu_m} - D \tag{7}$$

where K_s is concentration coefficient (kg/m^3) and S is limiting substrate concentration (kg/m^3). In a continuous processing of biomass, yield can be obtained by Equations (8) and (9):

$$X = Y_{x/s}\frac{S_i - DK_s}{\mu_m - D} \tag{8}$$

Rearranging Equation (8) for yield

$$Y_{x/s} = X\frac{\mu_m - D}{S_i - DK_s} \tag{9}$$

where S_i and X are initial substrate and biomass concentration (kg/m^3), respectively. Continuous bioreactors are chassified in two major catagories as mechanically mixed and plug flow reactors.

2.2.1 Mechanically stirred bioreactor HERE18-12

Stirred tank bioreactors are commonly used for continuous biohydrogen production. Continuous mixing, provides homogenuous medium for biohydrogen-producing

bacteria suspended in liquid. These reactors facilitate proper mixing by maintaining uniform contact between substrates, bacteria, and products, while increased shear enhances mass transfer rates. However, excessive shear stress generated by stirrers may lead to potential cell damage.Moreover, the extent of damage depends on the type of microorganism, its stability, and the surrounding environment. Consequently, the biohydrogen production rate in CSTBR is lower compared to micro-sludge bed bioreactors and cell bed bioreactors due to the stresses imposed. The washout of free cells from the bioreactor further reduces efficiency, as the suspended microbial mixture may not function optimally at high feeding rates. In general, stirred bioreactors exhibit lower performance in biohydrogen production over short retention times [18–20]. However, Paillet [21] examined the effect of ionic strength on the stability of microorganisms and found that ammonia nitrogen in its non-ionic form (NH_3) can inhibit hydrogen production. This occurs because NH_3 easily penetrates bacterial cell membranes, leading to a decrease in overall metabolite production. Characterization of hydrogen-producing microorganisms suggests that a low ionic strength environment is generally more favorable for their growth and activity. Further increase in ionic strength may adversly influence hydrogen-producing bacteria that are not well-adopted to high-ionic environments. Therefore, maintaining an optimized ionic strength in the medium is essential for achieving efficient conversion [21]. Table 1 illustrates a wide range of inoculum sources, reactor volumes, hydraulic retention times (HRT), and feedstocks used for biohydrogen production in stirred tank bioreactors. These feedstocks include various types of sugars and sugar-containing effluents, starch, molasses, whey, animal feed, and complex compounds such as cellulosic materials.

Table 1. Biohydrogen production using stirred bioreactors.

Substrate	Inoculum	Reactor working volume (L)	HRT (h)	Hydrogen production efficiency	Reference
Concentrated molasses	Seed sludge	4	12	132.2 grams of hydrogen/kg COD	[22]
Sucrose	Activated Sludge	-	8	23.5 grams of hydrogen/kg COD	[23]
Whey	Anaerobic sludge	3	6	14.6 grams of hydrogen/kg COD	[24]
Glucose	Granular sludge	6	0.5	18.8 grams of hydrogen/kg COD	[19]
Sweet sorghum	Anaerobic sludge	0.5	12	8.81 liters of hydrogen/kg of substrate	[25]
Sweet sorghum	Anaerobic sludge	0.5	12	0.87 mol of hydrogen/mol of glucose	[26]
Starch	Anaerobic sludge	3	12	0.92 mol of hydrogen/mol of glucose	[27]
Sewage slurry of pig breeding place	Pig sewage slurry	0.75	24	3.65 cubic centimeters of hydrogen/volatile solid	[28]
Food waste	Granule obtained from UASB bioreactor	0.7	60	261 mL of hydrogen/gram of volatile solid	[29]
Whey	Granule obtained from UASB bioreactor	3	6	2.8 moles of hydrogen/mol of lactose	[24]

2.2.2 Packed immobilized bioreactor

Packed immobilized reactors are weidly used across various sectors of the chemical and biochemical industries, including distillation, liquid-liquid extraction, adsorption, biosorption, and immobilization. They are also employed for research, process development, and scale-up studies. Packed bed reactors are typically filled either regularly or randomly with various support materials to immobilize microorganisms, cells, and enzymes – either physically or through electrochemical interactions. These reactors facilitate the production of biohydrogen, organic acids, and alcohols under different operational modes. The packings used in packed bed reactors come in various materials, shapes, and surface properties. The extent of mixing in this type of bioreactor is typically less than in CSTBR, which results in higher resistance to mass transfer, leading to reduced conversion rates and hydrogen production [14]. Packed bed reactors can be classified into two subtypes: packed fixed beds and fluidized beds. When designing, constructing, and fabricating packed bioreactors, several factors must be considered to achieve a high rate and yield of hydrogen production. These factors include the choice of packing materials, reactor geometry, flow dynamics, and operational parameters that affect the efficiency of mass transfer and microbial activity [14, 15].

In addition, the distribution of pH gradient along the column walls contributes to reduced microbial activities. To address this issue, it is recommended to use recycled flow to enhance the feed conversion rate and increase hydrogen production yield [15]. The factors affecting the performance of a packed bed bioreactor also include the type of packing used and its specific surface area (including porosity and charges), shape factor, surface properties, nontoxicity, and wetting characteristics, concerning immobilization materials. Packings with specific surface areas of 190 and 365 m^2 per m^3 have a significant influence, contributing 30% or 75%, respectively, to the production of biological hydrogen. The use of packings with a larger specific surface area increases microbial adsorption, thereby enhancing the production rate of biological hydrogen. However, a high microbial population may hinder flow distribution and lead to an increase in pressure drops, potentially causing blockages in the bioreactor and ultimately reducing the mass transfer rate. Additionally, this can adversely affect the distribution of nutrients to the microorganisms in the packed tower, resulting in lower product excretion [30]. The advantages of fixed-bed bioreactors containing immobilized microorganisms include ease of operation, flexibility in feed variation, simpler scale-up, and effective performance. The productivity of immobilized microorganisms is typically higher than that of free microorganisms across a wide range of submerged fermentations. This enhanced productivity can be attributed to the microenvironments created by the carrier, which help retain the stability of biomolecules. Microorganism's immobilization allows fixed-bed bioreactors to operate in a manner that approximates a plug flow regime, with dilution rates exceeding the maximum specific growth rate of the strain. As a result, the productivity of these bioreactors is enhanced. When designing, constructing, and fabricating packed bioreactors, several factors must be considered to achieve high rates and yields of hydrogen production. These factors include packing material selection, specific surface area, porosity, microbial retention capacity, and the overall design to optimize mass transfer and minimize resistance [14, 15]. However detailed process development and significant design concepts are required for operating large-scale fixed-bed reactors [31]. Table 2 shows several packing materials and shapes used to fill bioreactors, with different working volumes, HRT (hydraulic retention times), and feed compositions for producing biohydrogen. As significant variations are observed in the table, a more detailed, classified approach is necessary to facilitate the scale-up process and ensure optimal performance at larger scales.

Fluidized bed bioreactors. Fluidized beds are well known for their role in process development across laboratory, pilot plant, and industrial scales. They are conventionally used in industries for various applications such as hydrometallurgical operations, crystallization, ion-exchange, adsorption, sedimentation, wastewater treatment, particle classification, and more. These reactors are characterized by high mass and heat transfer rates, minimal wash-out, and high productivity, making them highly efficient for continuous processes like biohydrogen production. In fluidized beds, biological materials are immobilized in the form of biofilms, pellets, or usingelectrochemical charges. The support materials must have several desirable characteristics, including medium friendly, high adhesion properties, density difference ($\Delta\rho$, $kg\,m^{-3}$) slightly higher than the medium This density difference allows the support materials to fluidize based on the gases generated when the specific growth $\mu = \mu_m = D$, (h^{-1}). The rate of degradation of suspended substrates is higher in fluidized bed bioreactors compared to packed bed bioreactors. Fluidized beds are desirable for operation with both short- and long-term hydrolytic retention times [32]. Moreover, fluidized bed bioreactors have high efficiency due to low microbial leaching, making them suitable for bio-hydrogen production.

Table 2. Biohydrogen production employing packed bioreactors.

Substrate	Carrier	Reactor working volume (L)	HRT (h)	Hydrogen production efficiency	Reference
Glucose	Granular activated carbon	0.6	0.25	17.7 grams of hydrogen/kg COD	[32]
Sucrose	Modified alginate gel	10	2	28 grams of hydrogen/kg of COD	[33]
Glucose	Polyethylene-octene elastomer	3.861	4	10.4 grams of hydrogen/kg COD	[34]
Sucrose	Synthetic polymer (silicone gel)	8	8.9	28 grams of hydrogen/kg of COD	[35]
Sucrose	Spherical activated carbon	1	0.5	15.6 grams of hydrogen/kg COD	[36]
Glucose	Expanded clay grains	0.85	0.5	26 grams of hydrogen/kg COD	[37]
Sugars	Clay seeds	0.3	4	9.4 grams of hydrogen/kg COD	[38]
Refinery effluent	Polyurethane sheets	15	36	109 ml/g COD	[39]
Glucose	Polyurethane rings	2.5	24	0.67 mol/mol of glucose	[40]
Glycerol	Porous ceramic beads	1	24	0.5 mol/mol glycerol	[41]
Sucrose	Polyurethane	1	10	0.86 mol/mol of sucrose	[42]
Glucose	Low density polyurethane	2.3	8	2.1 mol/mol of glucose	[43]
Sugarcane residue	Low density polyurethane	2.3	5.7	3.4 mol/mol of carbohydrates	[44]
Sucrose	Pumice stone	-	3	4 mol/mol of sucrose	[45]
Dehydrated wheat powder	Metal mesh covered with sponge layers	2.1	2	1.6 mol/mol of total sugar	[46]
Glucose	Polyurethane rings	2.5	2	0.98 mol/mol of glucose	[47]
Sucrose	Ceramic rings	-	5.1	5 mol/mol of sucrose	[45]
Sugar beet molasses	Fibers	0.35	25.1	16.82 mmol/g COD	[48]
Beverage factory effluent	Polyurethane parts	2.37	5	3.47 mol/g of sucrose	[49]
Sugarcane residue	Polyurethane parts	2.3	10	2.4 mol/mol of total carbohydrate	[50]
Beverage factory effluent	Economical flex metal filters	2	4	1.4 mol/mol of hexose	[30]

Additionally, anaerobic fluidized bed reactors (AFBR) are commonly used for the immobilization of biomolecules in the production of bio-products such as hydrogen. Various materials, including activated carbon, alginate, ethylene-vinyl acetate copolymer, powdered tire, polyethylene terephthalate, polystyrene, expanded clay, and zeolite, are used as support materials for biofilm formation in submerged H_2 fermentation [51]. In addition to conventional geometry, there are certain modification of fluidized bed reactors such as draft-tube fluidized beds and counter-current fluidization. An average laboratory scale AFBR includes a vertical column with inert support or granules suspended by the upward flow, where microorganisms exist in the form of biofilm. This type of bioreactor is commonly applied for bioprocess development and wastew-

ater treatment, known for its effectiveness and the utilization of short hydraulic retention times. The biogas build-up and particle settling typically occur in separate compartments within the column. While AFBR may show promising characteristics for generating biogases like H_2. Stratification of biological molecules in a tower bioreactor can occur due to size distribution; however, in immobilized fluidized beds, where microorganisms are attached to solid supports, such constraints usually do not happen. Further fluidized bed bioreactors offer advantages, including overcoming concentration gradients, handling sudden temperature and pH shocks, and providing low shear stress for sensitive microorganisms due to controlled turbulence intensity. They enable a high density of microorganisms, leading to a high rate of volumetric mass transfer and heat transfer. This results in enhanced productivity and reliable performance during both short and long hydraulic retention times (HRT). The disadvantages of fluidized bed reactors include the energy required for pumping the substrate to fluidize the immobilized medium, which is particularly influenced by the density of the support materials and the amount of biogas produced [32, 52]. In particular, when $\rho_S \gg \rho_L$, the minimum fluidization velocity becomes relatively high, which can negatively impact shear-sensitive microorganisms.

2.2.3 Upflow anerobic sludge blancket reactor

The UASBR is one of the most widely used bioreactor to enhance microbial populations and treat wastewater. The feed stream is introduced from the bottom of the bioreactor, where the kinetic energy of the feed and produced gases lift and suspend the granules, promoting efficient reaction and wastewater treatment. As a result, biogases such as CO_2, H_2S, and biohydrogen are produced.The gases are collected from the top of the bioreactor. Further, microbial consertcia emerge, multiply, grow and float to digest organic material and produce gases. As the granules reach the expanded zone, they encounter stationary surfaces, causing their speed to decrease to zero. They then descend along the bioreactor wall, with many granules settling due to gravity. Conventionally these bioreactors are used to treat wastewater and produce biological methane. A particular limitation of the bioreactor is its long start-up time, which may take several months for the formation and growth of microorganisms. This process depends on factors such as the type of self-immobilized granules, temperature, pH, and the characteristics of the wastewater [53]. However, this type of bioreactor is more resistant to microbial wash-out than continuouly stirred bioreactor and can be operated with shorter hydraulic retention times than the mixed reactor. In addition, the bioreactor is less susceptible to fluctuations in feed concentration, temperature, pH, and hydraulic retention time compared to mixed-type bioreactors [54]. Povided untreated sludge with high solids is processed in these types of bioreactors, it would increase the growth of methanogenic bacteria. To address this issue, the initial sludge is generally heat-treated, which adds to the economic constraints. The rapid growth of hydrogen-producing bacteria leads to reduced plant size, lower construction expenses, and shorter operational times. However, managing such a complex process technically requires experience and expertise in the field. Moreover, the application of this type of bioreactor for biohydrogen production is gaining popularity compared to biological methane processing [55]. The UASB bioreactor is less susceptible to washout than the stirred type; in the stirred bioreactor, as the solid retention time equals the hydraulic retention time, washout occurs [36]. Table 3 shows several research studies conducted on hydrogen production using upflow anaerobic sludge blanket bioreactors.

2.2.4 Expanded granular sludge bed bioreactor

The UASB bioreactors is often modified to a different geometrical structure, such as an expanded granular sludge bed (EGSB). In this type of bioreactor, the inflow is increased to the point where the granules become suspended, which increases the contact area. As a result, the mass transfer rate achieved in EGSB bioreactors is higher than in UASB bioreactors.However, this type of construction increases the possibility of wasing-out of microorganisms. In processing biological hydrogen production, using EGSB bioreactors is less common, due to the high energy requirements for pumping and suspending the medium [64]. Table 4 shows certain applications of EGSB bioreactors for hydrogen production.

3 Effect of Process Parameters

The most effective method to increase biohydrogen production while using a mixed medium and untreated sludge is to halt methane formation. In an anaerobic microbial environment, the hydrogen produced is consumed by methanogenic activity, resulting primarily in methane production. Hydrogen is generated as the final product of the metabolic process. Sporulating hydrogen-producing bacteria, such as Clostridium species, can form protective spores under unfavorable conditions, whereas methanogens cannot survive. The key factors limiting the rate of anaerobic hydrogen pro-

duction include temperature, pH, volatile fatty acids, residence time, nutrient composition and type (organic substrate loading), sparingly soluble hydrogen partial pressure, and the presence of oxygen in the environment. By modifying some of these factors in the microbial culture, it is possible to create a suitable environment for producing either hydrogen or methane [67].

Parameters such as microorganism type and immobilization technique can influence mass transfer in biological hydrogen production under continuous operation. As scale-up processes advance, various technologies are being developed, including downstream processes such as purification, storage, and transportation, to achieve economic benefits.

Table 3. Biohydrogen production in UASB bioreactor.

Substrate	Inoculum	Reactor working volume (L)	HRT (h)	Hydrogen production efficiency	Reference
Sucrose	Anaerobic sludge granules	3	8	27 grams of hydrogen/kg COD	[56]
Glucose	Methanogenic sludge	4.01	17	1.51 moles of hydrogen/mol of glucose	[57]
Glucose	Heat-treated sludge	4.01	17	1.19 moles of hydrogen/mol of glucose	[57]
Whey	Seed sludge	4.6	12	0.2 grams of hydrogen/kg COD	[54]
Citric acid wastewater	Acidic wastewater with anaerobic bacteria	50,000	12	8.3 grams of hydrogen/kg of COD	[58]
Caffeine waste	Seed sludge	3.5	6	13.5 grams of hydrogen/kg COD	[59]
Glucose	Sludge from the stirred bioreactor	0.22	24	2.47 moles of hydrogen/mol of glucose	[60]
Whey powder	UASB bioreactor granule of confectionery factory	1.3	6	1.31 moles of hydrogen/mol of hexose	[61]
Glycerol	Klebsiella	1	4	44.27 mmoles of hydrogen/gram of glycerol	[62]
Glucose	Anaerobic sludge	2.5	8	1.47 moles of hydrogen/mol of glucose	[47]
Sugar industry waste	Municipal sewage sludge	20	30	2240 ml of hydrogen/day	[63]

Table 4. Biohydrogen production using EGSB bioreactors

Substrate	Inoculum	Reactor working volume (L)	HRT (h)	The hydrogen production efficiency	Reference
Sugar beet molasses (glucose)	Activated sludge	3/35	2	3.47 moles of hydrogen/ mole of sucrose	[65]
Sugar beet molasses (glucose)	Municipal sewage treatment plant sludge	2	10	0.92 mol of hydrogen/mol of substrate	[66]

3.1 Effect of temperature

The optimal temperature for hydrogen production largely depends on the type of microorganisms, medium pH, and substrate used. The rate of bacterial growth and the extent of substrate metabolism into hydrogen and by-products are influenced by the operating temperature during dark fermentation. Dark fermentation is typically conducted within a temperature range spanning from mesophilic to ultra-thermophilic conditions [68]. However, high temperatures can lead to inactivity of enzymes catalising hydrogen fermentation [69]. Although, according to the Arrhenius equation, higher temperatures generally enhance reaction rates, this effect is limited for microorganisms. The enzymatic properties of microorganisms are highly influenced by pH and temperature, and excessively high temperatures should not compromise enzyme proteins or alter the properties of the fermentation broth [70]. A high-temperature thermophilic process offers several advantages: (1) Under extreme thermophilic conditions, hydrogen yield can approach the theoretical maximum of 4 moles of hydrogen per mole of glucose; (2) Operating at high temperatures effectively eliminates pathogenic organisms. However, anaerobic hydrogen fermentation is an endothermic process, typically producing slightly more than 2 moles of hydrogen per mole of glucose. Additionally, hydrogen-consuming microorganisms, such as methane-forming organisms and solvent producers, are minimized. [71]. However, as the medium temperature increases, reactions associated with hydrogen consumption often intensify [72, 73]. Extreme thermophilic bacteria exhibit higher tolerance to elevated hydrogen partial pressures, which can result in metabolic shifts toward non-hydrogen-producing pathways, such as solvent formation. From a thermodynamic perspective, high-temperature fermentation is advantageous for hydrogen production, as it increases thermo-entropy and the energy yield, theoretically reaching 4 moles of hydrogen per mole of glucose.

3.2 Effect of pH

The pH of the fermentation medium is a crucial factor influencing metabolic pathways, enzyme activity, Fe-hydrogenase function, lag phase duration, and biohydrogen yield. All microorganisms and microbial enzymes function within a specific pH range, with their maximum activity occurring at an optimal pH value [74]. Various studies about the impact of pH on biohydrogen production consistently indicate that the optimal pH range for maximizing hydrogen production in both pure and mixed bacterial cultures is generally between 6.5 and 7. A significantly high initial pH may induce a metabolic shift from acidogenesis to solventogenesis; however, it can also reduce the lag phase duration, ultimately leading to a lower yield of biohydrogen production. In contrast, a low pH of 4.0-4.5 prolongs the lag phase and often has a negative impact on substrate decomposition and metabolism. Maintaining a stable pH throughout fermentation can stimulate microbial activity for optimal hydrogen production. Conversely, significant pH fluctuations during fermentation may lead to the inactivation of hydrogenase and related enzymes [69, 75].

The primary challenge in maintaining stable operation in co-culture processes is the accumulation of organic acids, which ultimately reduces hydrogen production. The optimal initial pH for dark fermentation (DF) of biohydrogen is approximately 7.0 and should ideally be maintained throughout the process. A major obstacle to achieving higher hydrogen yields arises when the pH drops to around 4.5-5.5 in the fermentation medium. This occurs because the production rate of volatile fatty acids (VFAs) during dark fermentation exceeds their consumption. Therefore, continuous pH regulation is essential for sustaining efficient fermentation.Maintaining the concentration of phosphate buffer at a specific ionic strength supports hydrogen production by preventing rapid pH reduction. A co-culture medium is more susceptible to pH fluctuations compared to a single culture. However, there is limited literature on the use of pH-controlled media for co-culture fermentation [76]. Most facultative anaerobic bacteria produce hydrogen by breaking down glucose into pyruvate through the glycolysis pathway. The efficiency of hydrogen production depends on the metabolites formed from pyruvate decomposition. Metabolites such as ethanol and other alcohols contain more hydrogen atoms than their corresponding acids. Major studies investigating the effect of pH on continuous hydrogen fermentation are summarized in Table 5.

3.3 Effect of metal ions

During dark fermentation for biological hydrogen production, microorganisms require metal ions such as iron, magnesium, copper, chromium, sodium, zinc, and nickel. These ions support bacterial metabolism, cell growth, and the activation of enzymes and coenzymes. The bacterial enzyme [Fe-Fe] hydrogenase specifically requires iron to facilitate electron transfer processes. The iron-sulfur combination plays a crucial role in the function of primary proteins and contributes to hydrogen production. Iron concentration is a crucial and influential factor in the metabolic pathways of hydrogen fermentation. Its required amount varies depending on the bacterial strain and cell density [83].

Table 5. Effect of pH on hydrogen production by dark fermentation in continuous systems

Substrate	Inoculum type	Reactor working volume (L)	HRT (h)	pH		The hydrogen production efficiency	Reference
				Checked range	The optimal value		
A mixture of past dairy products (93% milk, 5% yogurt, 2% cheese w/w)	Anaerobic sludge	0.75	144	4-5.7	5	0.84 mol/mol carbohydrate	[77]
Expired baby food	Food industry waste	0.4	12	5.9-5	5.4	141.47 liters of hydrogen / kilogram of food waste	[78]
Sucrose	Anaerobic sludge	2	13	3.4-6.3	4.2	1.61 moles/mol of glucose	[74]
Sucrose	Anaerobic sludge	2.01	13	6.1-9.5	7	1.61 moles/mol of glucose	[79]
Rice wine factory wastewater	Bacterial mixture	3.01	2	4-6	5/5	1.74 moles/mol of hexose	[80]
Glucose	Anaerobic sludge	1.7	6	4-7	5/5	2.1 moles/mol of glucose	[81]
Liquid pig manure with glucose	Anaerobic sludge	4	16	4.7-5.9	5	1.48 liters of hydrogen / liter of substrate	[82]

The aforementioned ions are classified into two categories: light and heavy metal ions. Heavy metal ions like iron, nickel, zinc, copper, chromium, lead, cadmium, and manganese and light metal ions such as calcium, sodium, and magnesium play essential roles in microbial metabolism. However, excessive concentrations of these ions can inhibit and suppress biohydrogen production [84]. Among the heavy metal ions involved in biohydrogen production through dark fermentation, nickel and, more notably, iron have received significant attention. This is due to their crucial role in the production of ferredoxin, which regulates electron transfer during the oxidation of pyruvate to acetyl coenzyme A [85]. The addition of Fe in the culture medium is widely used to enhance biohydrogen production by acting as catalizer for hydrogenases and other enzymes. Trace Fe is an essential element to form the metal content at the active sites of hydrogenase ([FeFe], [FeNi], and [Fe]), thus catalyzing the reduction reaction of H^+ to H_2. In addition, the presence of Fe increases the activity of ferredoxin oxidoreductase by reducing the dissolved oxygen (DO) content and increasing electron transfer due to the surface and quantum size effects. Further, Fe-based components could enhance the microbial community and growth of H_2-producing bacteria. However, at higher Fe concentrations, oxidative stress increases, which leads to the formation of numerous oxidative radicals, which can deactivate or degrade essential enzymes, ultimately inhibiting hydrogen production [86]. Similarly, nickel ions or Ni-based nanoparticles have been widely studied for their significant role in enhancing biohydrogen production during dark fermentation.

The mechanisms between Ni-ion/Ni-based nanoparticles and Fe-ion/Fe-based nanoparticles are largely identical, though there are minor differences. The main mechanisms of action of Ni include (a) facilitating [FeNi] hydroase synthesis, (b) increasing ferredoxin oxidoreductase activity, and (c) Ni nanoparticles controlling the concentration of Ni^{2+} to an optimal level. In addition, it should be noted that [NiFe] hydrogenase is found in more bacteria than [FeFe] hydrogenase. Therefore, Ni can promote H_2-producing bacteria in the dark fermentation process to some extent [86]. Li, et al. [87] studied the impact of various metal ion concentrations on the hydrogen production efficiency of bacterium R3 sp.no v. The results showed that $CoCl_2$ levels between 1.00 and 2.00 mg/L led to decreased hydrogen production, relative to cellular concentration that imposed significant concentration inhibition. They also found iron ions to play a role in gene expression of key enzymes related to hydrogen production metabolism, and low concentrations of iron ions can stimulating hydrogen production [88]. Lee et al. [89] found that critical concentration of iron ions was 10.9 mg/L. They reported that concentrations ex-

ceeding this value had an adverse effect on hydrogenase activity, ultimately reducing the rate of hydrogen production. Lee et al. [90] found that iron levels affected hydrogen production using digester sludge as inoculum. The highest hydrogen production rate observed was 24 mL/gVSS/h with 4000 mg/L $FeCl_2$. Dabrock et al. [91] showed that the growth of Clostridium pasteurianum hindered at iron concentrations below 10 mmol/L, adversely affecting product yieldsduring glucose fermentation. At 5.7 mmol/L iron, lactate replaced butyrate as the main product, while hydrogen production was sustained. Nonlimiting iron levels (up to 25 mmol/L) resulted in acidogenic metabolism, with hydrogen as the primary metabolite for C. acetobutyricum [92]. In a study by Zhang and Shen [93], the combined impact of temperature and iron levels on hydrogen production was examined. They found that the influence of iron on hydrogen generation decreased as reactor temperature was increased. At 25 and 35 °C, highest hydrogen production was observed at about 800 and 200 mg/L $FeSO_4$ concentration, respectively. The results suggested that at lower temperatures, bacteria require more ferrous ions to activate hydrogenase, which enhances hydrogen production by oxidizing reduced ferredoxin. Additionally, the addition of a certain number of ions is essential for the long-term preservation of bacteria and for improving hydrogen generation.

3.4 Effect of hydraulic retention time

A short residence time alters the fermentation pattern and inhibits the growth of methanogens that consume the generated hydrogen, although it generally takes a longer period for acidogens to develop. In a continuous fermentation process, it is essential to determine the minimum residence time, which refers to the time the system remains at the microbial washout point. Methanogenic activity can be controlled by maintaining the hydraulic retention time between 2 and 10 hours, provided that the hydrogen-producing bacteria are in the logarithmic growth phase. From an economic prespetive, a shorter retention time and smaller bioreactor volume are preferable due to lower total investment costs. For optimal hydrogen production, the hydraulic retention time is mainly reported to be between 8 to 14 hrs. Reasonable hydrogen production has been achieved using various wastewaters as feedstock, with HRT ranging from zero to 14 hours. While a short retention time can help eliminate methane and acetate-producing bacteria, it is important to note that an excessively short retention time may also eliminate hydrogen-producing microorganisms.

Furthermore, at low retention time, the overall conversion efficiency of the process decreases because the amount of substrate used is hampered due to inappropriate feed-to-microorganism ratio [94]. Continuous hydrogen production has been reported using glucose or sucrose with mixed microflora in 6-8 hrs HRT [81, 95, 96]. Ueno et al. [97] achieved a hydrogen yield of 2.52 mol mol-1 hexose at 60 °C using sugar factory wastewater. Fang et al. [81] showed that a mixed microflora at 26 °C and 6 hrs HRT could create granules with a hydrogen yield of 2.25 mol/mol hexose. However, there is limited information on converting low-cost agricultural and organic waste to hydrogen in continuous processes. Yokoi et al. [98] showed that hydrogen can be produced from sweet potato starch waste by adding corn steep liquor to a defined culture of Clostridium butyricum or Enterobacter aerogenes in a 12-day fed-batch culture. In an earlier experimental verification, Yokoi et al. [99] used corn steep liquor and polypeptone for hydrogen production.

In Lay's study [100], a mix of microflora with clostridial traits produced hydrogen continuously from soluble starch, achieving a hydrogen yield of 1 mol/mol hexose in an enriched culture at a pH about 4.5 and 22 hrs HRT. By applying a factorial-design experiment with this culture, optimal conditions for the unspecified starch were found to be at about pH 5.2 and 17 hrs HRT, resulting in a hydrogen yield of 2.4 mol/mol hexose. Furthermore, HRT is influenced by type and geometry of fermenter used to produce biohydrogen.

3.5 Effect of partial pressure of hydrogen

The influence of hydrogen partial pressure on the metabolism of anaerobic bacteria during dark fermentation process of hydrogen production and its redissolution in the medium is presented here. The partial pressure of biohydrogen could be a limiting factor affecting the rate of its production, as it influences the activities of the hydrogenase enzyme and, consequently, the yield. Therefore, by removing the biogases from the bioreactor headspace, the efficiency of the system can be improved [71]. Hydrogen production is limited by the thermodynamics of the hydrogenase reaction, which involves the enzyme-catalyzed transfer of an electron from an intracellular electron carrier molecule to a proton. As the concentration of hydrogen in the liquid phase increases, the oxidation of ferredoxin decreases, and hydrogenase reversibly oxidizes and reduces ferredoxin. When hydrogen in the liquid phase is oxidized to protons, it results in a reduction in the rate of hydrogen production. At high hydrogen concentrations, the metabolic pathway shifts from acidification to solubilization, leading to the production of reduced substrates primarily in the form of lactate,

ethanol, butanol, or alanine, which in turn reduces hydrogen production. From a thermodynamic perspective, producing hydrogen at a partial pressure above 60 Pascals is not desirable. Bacteria that produce propionic acid, ethanol, and other hemogenic bacteria can maximize hydrogen production by maintaining optimal conditions for their activity.In addition, injecting gases such as nitrogen and argon reduces the concentration of dissolved hydrogen, consequently increasing the efficiency of biohydrogen generation. Membrane sorption technologies can be used to purify and remove hydrogen from the top of the bioreactor, further enhancing the overall process by maintaining optimal hydrogen production conditions [7].

To understand the phenomena, a simple mass balance was presented by Beckers (Equation (10)):

$$Q = k_L a \times \mathrm{He} \times (P_G^0 - P_G) \tag{10}$$

where the rate of gaseous mass flow Q passing through a gas-liquid boundary, measured in terms of time and liquid volume (mol/Lh), primarily relies on the overall mass transfer coefficient $k_L a$ (in h^{-1}, influenced by the mixing condition and interfacial area) and further by the transfer potential $P_G^0 - P_G$ (with respects to gas partial pressure P_G and dissolved gas concentration at equilibrium $C_L^0 = (\mathrm{He} \times P_G^0)$ where 'He' is influenced by the type of gas and liquid present [101].

3.6 Mass transfer

Efficient mass transfer is a key parameter in bioreactors operation, especially during hydrogen production. Agitation is typically employed to ensure effective contact between microorganisms and substrates, as well as to facilitate the separation of gases from a homogeneous medium. However, stagnation and the formation of gas pockets can lead to a reduction in hydrogen production in certain types of bioreactors, such as packed bed bioreactors, where gas flow and mixing are less efficient.The mass transfer rate can be increased by selecting an appropriate bioreactor geometry and optimizing its dimensions, such as the height-to-diameter ratio of the tank, the impeller-to-tank diameter, impeller tip speed, and other factors. To remove oxygen from the medium in a mixed fermenter, the type, configuration, and speed of the agitator are important. Additionally, the backflow of gas or liquid can be used to improve the mixing of the medium [102]. The gas-to-liquid or liquid-to-gas mass transfer rate can be described using the mass transfer coefficient k_L (s^{-1}), the gas-liquid interfacial area a (m^{-1}), and the concentration driving force for the transfer is:

$$\frac{dC_L}{dt} = k_L a(C^* - C_L) \tag{11}$$

where C_L, is the concentration of specie (s) in the liquid phase and C^* is its saturation concentration (i.e. saturation or equilibrium value) in the liquid.

Mass transfer in gas-liquid reactors including airlift devices has frequently been stated in terms of an overall volumetric mass transfer coefficient $k_L a_L$ or $k_L a_D$, based on unaerated liquid volume and gas-liquid dispersion volume, respectively [103]. Najafpour et al. [104] investigated biological hydrogen production using Rhodospirillum rubrum in both batch and continuous mode of operations, with synthesis gas as substrate, using stirred reactor. They estimated the gas-liquid mass transfer coefficients and developed correlations for batch mode of operation. The system was operated at ambient temperature and pressure. According to the material balance proposed by Levenspiel for the stirred tank contactor, it is assumed that the composition of the gases is uniform throughout the fermentation. Therefore, the material balance for the rate of CO absorption, transferred from the gas phase, is considered to be equal to the CO involved in the biotransformation process:

$$\mathrm{CO}_{\text{transferred from gas phase}} - \mathrm{CO}_{\text{uptake rate}} = \mathrm{CO}_{\text{consumption in the bioconversion}} \tag{12}$$

$$\frac{F_g}{\pi}(P_{\mathrm{CO,in}} - P_{\mathrm{CO,out}}) - \frac{k_L a}{H} P_{\mathrm{CO,out}} V_r = (-r_{\mathrm{CO}})V_r \tag{13}$$

where $k_L a$, F_g and H are volumetric mass transfer coefficient (min^{-1}), gas molar flow rate (mol/min), and Henry's constant (atm L/mol), respectively. PCO, in is Partial pressure ratio of CO at inlet (atm) and PCO, out is Partial pressure ratio of CO at outlet (atm), r_{CO} is rate of reaction (mole/Lh), V_r (L) is the working volume of the reactor and π (atm) is the total pressure of the gas phase

$$\pi = P_{\mathrm{H_2}} + P_{\mathrm{CO}} + P_{\mathrm{CO_2}} + P_{\mathrm{Ar}} \tag{14}$$

The rate of loss of CO by the reaction is shown as follows:

$$\frac{dX}{dt}\frac{dP_{\mathrm{CO,out}}}{dX}\frac{1}{V_r} = \frac{dP_{\mathrm{CO,out}}}{dt}\frac{1}{V_r} = -r_{\mathrm{CO}} \tag{15}$$

where X (g/L) the cell density of the microorganism and t is time (min). By multiplying the above equation by V_r:

$$(-r_{\mathrm{CO}})V_r = \frac{dP_{\mathrm{CO,out}}}{dt} = \frac{dP_{\mathrm{CO,out}}}{dX}\frac{dX}{dt} \tag{16}$$

Microbial growth can be described by the Monod equation:

$$\mu X = \frac{dX}{dt} = \frac{\mu_m X P_{\mathrm{CO,out}}}{K_m + P_{\mathrm{CO,out}}} \tag{17}$$

By replacing this equation with Equation (5), Equation (7) is obtained:

$$-r_{\mathrm{CO}}(V_r) = \frac{dP_{\mathrm{CO}}}{dX}\,\frac{\mu_m X P_{\mathrm{CO,out}}}{K_m + P_{\mathrm{CO,out}}} \tag{18}$$

where K_M, μ, X and μ_m are the Monod constants, specific growth rate, cell density, and maximum specific growth rate, respectively. Substituting the above equation in the mass balance results:

$$\frac{F_g}{\pi}(P_{\mathrm{CO,in}} - P_{\mathrm{CO,out}}) - \frac{k_L a}{H} V_r P_{\mathrm{CO,out}} = \frac{\mu_m\, X\, P_{\mathrm{CO,out}}}{K_m + P_{\mathrm{CO,out}}}\,\frac{dP_{\mathrm{CO}}}{dX} \tag{19}$$

At a steady state, the rate of CO transfer from the gas phase is equal to its absorption and losses by the reaction. Therefore, both sides of the above equation are divided by $P_{\mathrm{CO,out}}$. In steady state $dP_{\mathrm{CO}}/dX = 0$ as a result:

$$\frac{F_g}{\pi}\left(\frac{P_{\mathrm{CO,in}}}{P_{\mathrm{CO,out}}} - 1\right) = V_r \frac{k_L a}{H} \tag{20}$$

Rearranging the above equation leads to a linear form and its slope indicates the mass transfer coefficient as follows:

$$\left(\frac{P_{\mathrm{CO,in}}}{P_{\mathrm{CO,out}}} - 1\right) = \frac{\pi V_r}{F_g}\,\frac{k_L a}{H} \tag{21}$$

Using the ideal gas law has a correlation in terms of gas pressure:

$$P_g v_g = F_g RT \quad \text{or} \quad F_g = \frac{P_g v_g}{RT} \tag{22}$$

where P_g is Partial pressure ratio (atm), v_g is volumetric gas flow rate (ml/min), F_g is Molar flow rate (mole/min), R is ideal gas law constant (L/mole K) and T is absolute temperature (K). So that the subscript g refers to the inert gas:

$$\left(\frac{P_{\mathrm{CO,in}}}{P_{\mathrm{CO,out}}} - 1\right)\frac{k_L a}{H}\,\frac{\pi V_r RT}{P_g v_g} = k_L a \frac{V_r RT}{H_v} \tag{23}$$

$k_L a$ is estimated by plotting $\frac{P_{\mathrm{CO,in}}}{P_{\mathrm{CO,out}}} - 1$ versus $\frac{V_r RT}{H_v}$ as slope [104].

The mass transfer resistance is generally reduced by agitator's speed, volumetric flow rate, if packed with selection of proper packing size or static mixer type to generate maximum ΔP and dispersion, Additionally, if trays are used, they should provide a sufficient percentage of free area and optimal downcomer geometry. The extent of mixing by the agitator should be within the range of safe shear to avoid creating turbulence in the medium, as excessive shear can adversely affect the growth and productivity of sensitive microorganisms. The safe range of mixing helps reduce bubble size, which in turn affects the terminal rise velocity and increases the effective interfacial area (a, m^{-1}). This improvement leads to an increase in the overall volumetric mass transfer coefficient ($k_L a$, s^{-1}). Further, by increasing dilution rate the overall volumetric mass transfer coefficients increases, provided that the cell density, CO concentration, and required nutrients are well balanced, and the hydraulic retention time (HRT) is within the optimal range.

3.7 Recycled flow

One of the parameters affecting the efficiency of biohydrogen production in bioreactors is the recycle ratio. By introducing a recycled flow within the bioreactor, the concentration gradient of the incoming raw material can be more predictable. If the feed enters the bioreactor directly, the biofilm may be exposed to a high feed concentration, which could shock or inhibit the microorganisms, potentially affecting by-product formation. Furthermore, the absence of a recycled flow may contribute to an uneven feed concentration gradient, which can have negative effects on the biofilm's performance in the bioreactor. Furthermore, the advantages of recycled flow include increased mass transfer between the microorganisms and the broth, which positively impacts the process by facilitating the transfer of gases from the liquid phase to the gaseous phase, thereby preventing the excessive accumulation of hydrogen in the liquid phase during processing. Additionally, the removal of hydrogen from the liquid phase helps prevent its conversion into secondary metabolites, thus improving the overall efficiency of the production process. However, it is important to note that an excessive increase in the recycled flow rate could lead to the removal of microorganisms from the bioreactor, as well as increased energy requirements for pumping, which would ultimately add to the production cost [105]. The recycle ratio R is:

$$R = \frac{\text{volume of fluid returned to the reactor inlet}}{\text{volume in the outlet}} \tag{24}$$

The recycle ratio may range from ($R = 0$) to ($R = \infty$), with behavior shifting from plug flow to mixed flow as the ratio increases. Different level of backmixing is achieved in a recycled plug flow reactor [106]. Considering a recycling reactor with the nomenclature shown in Figure 1. For a plug flow reactor mass balance is shown by Equation (25):

$$\frac{V}{F'_{A0}} = \int_{X_{A1}}^{X_{A2}=X_{Af}} \frac{dX_A}{-r_A} \tag{25}$$

where F'_{A0} is the initial feed rate of A (mol m^3/s) entering the reactor and V is volume (m^3). Since F'_{A0} and X_{A1} are not known directly, they should be written in terms of known quantities before using Equation (25).

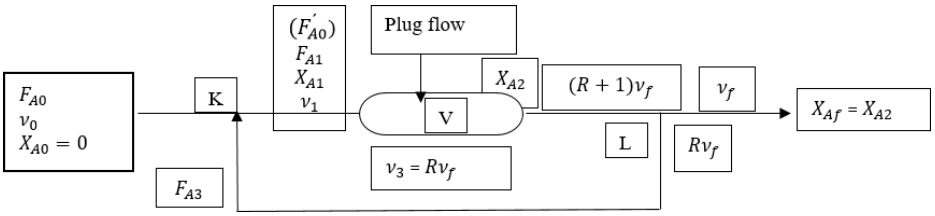

Fig. 1. Schematic of recycle flow in plug reactor (Adopted from Levenspiel 1999)

The stream entering the reactor includes both fresh feed and the recycled stream. Measuring the splitted flow at point L (provided flow at point K is $\mathcal{E} \neq 0$) then

$$\begin{aligned} F'_{A0} &= (A \text{ enters in an unconverted recycle stream}) \\ &\quad + (A \text{ in the fresh feed}) \\ &= RF_{A0} + F_{A0} \\ &= (R+1)F_{A0} \end{aligned} \tag{26}$$

Evaluation of X_{A1} from equation $X_A = \frac{C_{A0}-C_A}{C_{A0}-\mathcal{E}_A C_A}$

$$X_{A1} = \frac{1 - \frac{C_A}{C_{A0}}}{1 + \mathcal{E}_A \frac{C_A}{C_{A0}}} \tag{27}$$

Since pressure is taken to be constant, the streams at point K may be added directly. In the form of an equation as Equation (28):

$$\begin{aligned} C_{A1} &= \frac{F_{A1}}{v_1} = \frac{F_{A0} + F_{A3}}{v_0 + Rv_f} \\ &= \frac{F_{A0} + RF_{A0}(1 - X_{Af})}{v_0 + Rv_0(1 + \mathcal{E}_A X_{Af})} \\ &= C_{A0}\left(\frac{1 + R - RX_{Af}}{1 + R + R\mathcal{E}_A X_{Af}}\right) \end{aligned} \tag{28}$$

Combining Equation (27) and Equation (28) gives X_{A1}, is a measured quantity, or

$$X_{A1} = \frac{R}{R+1} X_{Af} \tag{29}$$

Finally, by replacing Equations (26) and **??** in Equation (29), the performance equation for the recycle reactors fit any kinetics equation, any $\mathcal{E}$ value, with $X_{A0} = 0$ considered.

$$\frac{V}{F_{A0}} = (R+1) \int_{\left(\frac{R}{R+1}\right) X_{Af}}^{X_{Af}} \frac{dX_A}{-r_A} \qquad \text{for any } \mathcal{E}_A \tag{30}$$

For defined case where density changes are negligible, the equation can be written in terms of concentrations [106]:

$$\tau = \frac{C_{A0}V}{F_{A0}} = -(R+1) \int_{\frac{C_{A0}+RC_{Af}}{R+1}}^{C_{Af}} \frac{dC_A}{-r_A} \qquad \mathcal{E}_A = 0 \tag{31}$$

where τ is space-time (s).

The rate of hydrogen production is higher in a continuous compared to a batch process. A faster dilution rate (i.e. shorter HRT) results in wash-out of active cells. Therefore, recycling cells is necessary to maintain consistent active cell concentrations in the reactor, maximizing hydrogen production. Therefore, the mass transfer rates from the bulk liquid to the immobilized microorganisms are also influenced by the recycle ratio. Variations in the recycle ratio affect the liquid-to-gas mass transfer for sparingly soluble gases, such as hydrogen and carbon dioxide. The byproducts of the hydrogen fermentation process, namely volatile fatty acids (VFAs), are also recycled, which influences the biological status of the microbes in the bioreactor. As the recycle ratio and the liquid superficial velocity within the immobilized packed bed reactor are increased, both liquid and solid side mass transfer improve. However, limited data exist regarding the physiological condition of the microorganisms in the reactor while hydrogen is being produced. To assess the physiological state of the microorganisms, flow cytometry combined with fluorescent dyes is used at a specific substrate concentration [48].

3.8 Comparision of performance of biofilm and granule bioreactors

Zhang et al. [107] used two types of AFBRs to load with biofilm and granules for biohydrogen production at a constant organic loading rate (OLR) of 40 g/Lh, varying HRTs (0.125-3 hrs) and glucose concentrations

of (5-120 g/L). Figure 2 depicted that the hydrogen production remained consistent at each HRT in both granule and biofilm bioreactors. Although the hydrogen yield was similar for both bioreactors, the biofilm reactor showed a sightly higher rate of hydrogen production. The biofilm thickness gradually decreased due to weak attachment of microorganisms to immobilization media. As a result of particle collisions, the biofilm in the reactor detached from the support media, leading to fragmented biofilm. After 50 days of operation, the disjointed biofilm transformed into a granule bioreactor. Around 80% glucose conversion rates were reached in biofilm reactor and granule reactor with HRT between 0.25 and 1.0 h, for glucose concentrations of 10-40 g/L. The glucose conversion rate decreased as the HRT and the glucose concentration increased to 3 hrs and to 120 g/L, respectively. The optimal HRT and glucose concentration for both biofilm and granule reactors were were found to be between 0.25-0.75 hrs and 10-30 g/L, based on hydrogen yield and production rate. Stable hydrogen production was maintained in both bioreactors by controlling the HRT between 0.25-0.75 h and glucose concentrations about 10-30 g/L. The hydrogen yield ranged from 0.4 to 1.7 mol-H_2/mol glucose in both bioreactors, with the highest yield observed at HRT of 0.25h and glucose concentration of 10 g/L. Under these conditions, the hydrogen production rate reached maximum values of 6.6 and 7.6 L-H_2/Lh in the granule reactor and biofilm reactor, respectively.

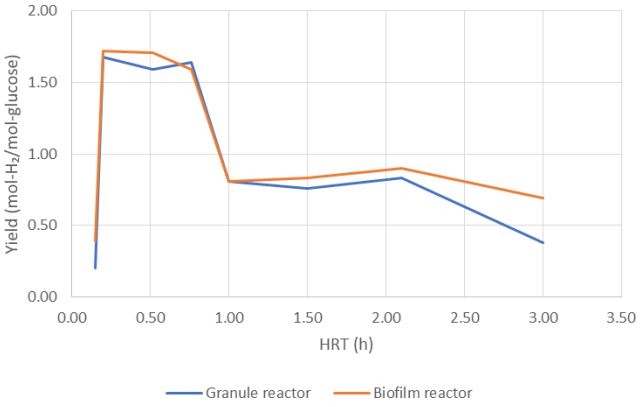

Fig. 2. Presenting yield of biohydrogen production by two types of bioreactors at various HRTs and glucose concentrations

4 Conclusion

Microbial hydrogen production empolying renewable sustainable resources is independent of geographical locations and seasonals changes, with dark fermentation offering a higher production rate compared to photo-fermentation. The energy in the form of hydrogen, produced by renewable fermentative sources, can be puriefied, stored and transported to end users via pipelines, ships or other suitable arrangments. Hydrogen contains 122 kJ/g of energy, making it the highest energy source – 2.75 times more than any known hydrocarbons – positioning biohydrogen as a strong candidate to replace traditional fuels and decarbonize the energy sector in pursuit of environmental targets. Furthermore, when hydrogen is used in an internal combustion engine, it only produces energy and water vapor, with no CO_2 emissions.

To generate electricity using hydrogen fuel cells, hydrogen with a purity of 99.99% is required, which is typically purified through Pressure Swing Adsorption (PSA) technology. This review aims to highlight the advantages of continuous hydrogen production through bioreactors, addressing the limitations associated with batch-mode operations. Additionally, it reports that the yield and rate of hydrogen production are influenced by various factors, including the type of microorganisms, inoculum ratio, feed composition and concentration, added cofactors and trace elements, environmental parameters such as pH and temperature, immobilization methods, mode of operation, bioreactor geometry, hydraulic retention time, and more.

The substrates concentration should be maintained within the optimal range to avoid the following scenarios: (1) Concentrations higher than the optimum value leads to overproduction of volatile fatty acids and alcohols resulting in a decreased hydrogen production rate: (2) Concentrations lower than the optimal level reduce biomass concentration, ultimately lowering the hydrogen production rate.To start fermentation, a pH range of 6.5 to 7 is ideal, and it is preferable to regulate pH during the fermentation process for biohydrogen production. The temperature range for efficient fermentation depends on the species used, with 32 to 37 °C being effective for mesophilic species, and 55 to 90 °C for thermophilic species involved in biohydrogen production. Additionally, certain cofactors such as Fe, Ni, Mg, Co, and Cu are essential for the enzymes, like hydrogenase, in microorganisms to enhance biohydrogen production.

The amount of iron ions required varies depending on the microorganism type and the temperature range used. At higher temperatures, lower concentrations of cofactors are generally needed. As biogases accumulate in the headspace of the bioreactor, the partial pressure of these gases increases and they moderately re-dissolve in the medium. Therefore, it should be continuously removed. The dark hydrogen fermentation in continuous mode of operation is velocity-driven, and various types of bioreactors, such as CSTBR, packed bed, fluidized bed, USABR, and recycled systems, are com-

monly used. Additionally, controlling continuous bioreactor operation is generally easier and more economical compared to batch systems. Furthermore, hydrogen-producing and biofilm-forming bacteria should exhibit synergistic effects to prevent leaching out and maintain a sufficient population density to ensure high biohydrogen yields throughout the process. If sludges are used, they should undergo pretreatment, which increases the operating costs.

Generally, CSTBR is preferred for basic investigations and process development, particularly in continuous fermentation, due to their ability to generate a well-mixed medium. However, the stress caused by impellers can adversely affect the productivity of sensitive microorganisms, and CSTBRs do not perform as well with short retention times for biohydrogen production. FBR and UASB bioreactors are more resistant to microbial leaching than stirred vessels and can better withstand sudden changes in temperature, pH, and short hydraulic retention times. Fluidized bed bioreactors are more favorable for biohydrogen production, especially with short HRTs. Therefore, the HRT value is influenced not only by the type of microorganism used but also by fermenter geometry and feed properties. Proper control of the recycle ratio and the discharge of solids from continuous bioreactor operation is crucial and depends on several factors. For large-scale dark biohydrogen production, FBRs and packed-bed immobilized bioreactors are preferred. This work has analyzed and emphasized continuous biohydrogen production through various fermenter geometries to help lower production costs.

Currently, the main technological gap lies in the thermodynamic conversion of sugar to hydrogen through dark fermentation using Enterobacteria or Clostridium. A hydrogen yield of more than 2.4 moles of H_2 per mole of glucose can be achieved if the initial pH is maintained at around 7 and regulated throughout the process, with the medium temperature kept at 37 °C using mesophilic Enterobacteria. Thermophilic microorganisms, operating at temperatures of 55 °C or higher, can produce 4 moles of hydrogen per mole of glucose, approaching the theoretical maximum yield of 4 moles. Closteridum species generally produce only 4 moles of hydrogen per mole of glucose. The mass transfer within the system becomes quite complex when no gas is added to the medium. Therefore, more detailed studies should be conducted and made available in the open literature to help differentiate the performance of various fermenters and provide insights into scale-up phenomena. As a result, there is a need for time and research to develop species with higher performance to achieve 12 moles of H_2 per mole of glucose. Furthermore, tireless efforts are underway in many laboratories worldwide to make breakthroughs in enhancing biohydrogen yield through the use of modified microorganisms and inexpensive substrates, with the aim of achieving economic goals and commercial production.

Conflict of interest

The authors declared that no interest of conflict..

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Ethical Approval

Not applicable.

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References

[1] Ni M, Leung DYC, Leung MKH, Sumathy K. An overview of hydrogen production from biomass. Fuel Processing Technology. 2006;87:461-72.

[2] Cui M, Yuan Z, Zhi X, Wei L, Shen J. Biohydrogen production from poplar leaves pretreated by different methods using anaerobic mixed bacteria. International Journal of Hydrogen Energy. 2010;35:4041-7.

[3] Boshagh F, Rostami K. A review of application of experimental design techniques related to dark fermentative hydrogen production. Journal of Renewable Energy and Environment. 2020;7(2):27-42.

[4] Ghimire A, Frunzo L, Pirozzi F, Trably E, Escudie R, Lens PNL, et al. A review on dark fermentative biohydrogen production from organic biomass: process parameters and use of by-products. Applied Energy. 2015;144:73-95.

[5] Das D, Veziroğlu T. Hydrogen production by biological processes: a survey of literature. International Journal of Hydrogen Energy. 2001;26:13-28.

[6] Tamburic B, Zemichael FW, Maitland GC, Hellgardt K. Parameters affecting the growth and hydrogen production of the green alga Chlamydomonas reinhardtii. International Journal of Hydrogen Energy. 2011;36:7872-6.

[7] Chong M, Sabaratnam V, Shirai Y, Hassan MA. Biohydrogen production from biomass and industrial wastes by dark fermentation. International Journal of Hydrogen Energy. 2009;34:3277-87.

[8] Förberg C, Häggström L. Control of cell adhesion and activity during continuous production of acetone and butanol with adsorbed cells. Enzyme and microbial technology. 1985;7(5):230-4.

[9] Budiman P, Wu T. Ultrasonication pretreatment of combined effluents from palm oil, pulp and paper mills for improving photofermentative biohydrogen production. Energy Convers Manag. 2016;119:142-50.

[10] Zhang Y, Liu G, Shen J. Hydrogen Production in batch culture of mixed bacteria with sucrose under different iron concentrations. International Journal of Hydrogen Energy. 2005;30:855-60.

[11] Muloiwa M, Nyende-Byakika S, Dinka M. Comparison of unstructured kinetic bacterial growth models. South African Journal of Chemical Engineering. 2020;33:141-50.

[12] Boshagh F, Rostami K, Moazami N. Immobilization of Enterobacter aerogenes on carbon fiber and activated carbon to study hydrogen production enhancement. Biochemical Engineering Journal. 2019;144:64-72.

[13] Boshagh F, Rostami K, Moazami N. Biohydrogen production by immobilized Enterobacter aerogenes on functionalized multi-walled carbon nanotube. International Journal of Hydrogen Energy. 2019;44:14395-405.

[14] Chen C, Lin C, Lin M. Acid-base enrichment enhances anaerobic hydrogen production process. Applied Microbiology and Biotechnology. 2002;58:224-8.

[15] Kumar N, Das D. Continuous hydrogen production by immobilized Enterobacter cloacae IIT-BT 08 using lignocellulosic materials as solid matrices. Enzyme and Microbial Technology. 2001;29:280-7.

[16] Stoodley P, Sauer K, Davies D, Costerton J. Biofilms as complex differentiated communities. Annual Review of Microbiology. 2002;56:187-209.

[17] Fang H, Liu H, Zhang T. Characterization of a hydrogen production granular sludge. Biotechnology and Bioengineering. 2002;78:44-52.

[18] Show K, Zhang Z, Tay J, Liang D, Lee D, Ren N, et al. Critical assessment of anaerobic processes for continuous biohydrogen production from organic wastewater. International Journal of Hydrogen Energy. 2010;35:13350-5.

[19] Show K, Zhang Z, Tay J, Liang D, Lee D, Ji W. Production of hydrogen in a granular sludge-based anaerobic continuous stirred tank reactor. International Journal of Hydrogen Energy. 2007;32:4744-53.

[20] Zhang M, Fan Y, Xing Y, Pan C, Zhang G, Lay J. Enhanced biohydrogen production from cornstalk wastes with acidification pretreatment by mixed anaerobic cultures. Biomass and Bioenergy. 2007;31:250-4.

[21] Paillet F, Barrau C, Escudié R, Trably E. Inhibition by the ionic strength of hydrogen production from the organic fraction of municipal solid waste. International Journal of Hydrogen Energy. 2020;45:5854-63.

[22] Lay C, Wu J, Hsiao C, Chang J, Chen C, Lin C. Biohydrogen production from soluble condensed molasses fermentation using anaerobic fermentation. International Journal of Hydrogen Energy. 2010;35:13445-51.

[23] Chen C, Lin C. Using sucrose as a substrate in an anaerobic hydrogen-producing reactor. Advances in Environmental Research. 2003;7:695-9.

[24] Davila-Vazquez G, Cota-Navarro C, Rosales-Colu L, León-Rodríguez A, Razo-Flores E. Continuous biohydrogen production using cheese whey: Improving the hydrogen production rate. International Journal of Hydrogen Energy. 2009;34:4296-304.

[25] Antonopoulou G, Gavala H, Skiadas I, Lyberatos G. Effect of substrate concentration on fermentative hydrogen production from sweet sorghum extract. International Journal of Hydrogen Energy. 2011;36:4843-51.

[26] Antonopoulou G, Gavala H, Skiadas I, Lyberatos G. Influence of pH on fermentative hydrogen production from sweet sorghum extract. International Journal of Hydrogen Energy. 2010;35:1921-8.

[27] Arooj M, Han S, Kim S, Kim D, Shin H. Continuous biohydrogen production in a CSTR using starch as a substrate. International journal of hydrogen energy. 2008;33:3289-94.

[28] Kotsopoulos T, Fotidis I, Tsolakis N, Martzop G. Biohydrogen production from pig slurry in a CSTR reactor system with mixed cultures under hyper-thermophilic temperature (70 C). Biomass Bioenergy. 2009;33:1168-74.

[29] Reungsang A, Sreela-or C, Plangklang P. Non-sterile bio-hydrogen fermentation from food waste in a continuous stirred tank reactor (CSTR): Performance and population analysis. International Journal of Hydrogen Energy. 2013;38:15630-7.

[30] Hastuti Z, Chu C, Rachman M, Purwanto W, Dewi E, Lin C. Effect of concentration on biohydrogen production in a continuous stirred bioreactor using biofilm induced packed-carrier. International Journal of Hydrogen Energy. 2016;41:21649-56.

[31] P´ortner R, Faschian R. Design and Operation of Fixed-Bed Bioreactors for Immobilized Bacterial Culture. IntechOpen; 2019.

[32] Zhang Z, Show K, Tay J, Liang D, Lee D. Biohydrogen production with anaerobic fluidized bed reactors-A comparison of biofilm-based and granule-based systems. International Journal of Hydrogen Energy. 2008;33:1559-64.

[33] Wu S, Lin C, Chang J. Hydrogen Production with Immobilized Sewage Sludge in Three-Phase FluidizedBed Bioreactors. Biotechnology Progress. 2003;19:828-32.

[34] Wu K, Chang C, Chang J. Simultaneous production of biohydrogen and bioethanol with fluidized-bed and packed-bed bioreactors containing immobilized anaerobic sludge. Process Biochemistry. 2007;42:1165-71.

[35] Lin C, Wu S, Chang J. Fermentative hydrogen production with a draft tube fluidized bed reactor containing silicone-gel-immobilized anaerobic sludge. International Journal of Hydrogen Energy. 2006;31:2200-10.

[36] Lee K, Wu J, Lo Y, Lo Y, Lin P, Chang J. Anaerobic hydrogen production with an efficient carrier-induced granular sludge bed bioreactor. Biotechnology Bioengineering. 2004;87:648-57.

[37] Leite J, Fernandes B, Pozzi E, Barboza M, Zaiat M. Application of an anaerobic packed-bed bioreactor for the production of hydrogen and organic acids. International Journal of Hydrogen Energy. 2008;33:579-86.

[38] Fritsch M, Hartmeier W, Chang J. Enhancing hydrogen production of Clostridium butyricum using a column reactor with square-structured ceramic fittings. International Journal of Hydrogen Energy. 2008;33:6549-57.

[39] Elreedy A, Tawfik A, Enitan A, Kumari S, Bux F. Pathways of 3-biofules (hydrogen, ethanol and methane) production from petrochemical industry wastewater via anaerobic packed bed baffled reactor inoculated with mixed culture bacteria. Energy Conversion Management. 2016;12:119-30.

[40] Si B, Liu Z, Zhang Y, Li J, Xing X, Li B, et al. Effect of reaction mode on biohydrogen production and its microbial diversity. International Journal of Hydrogen Energy. 2015;40:3191-200.

[41] Dounavis A, Ntaikou I, Lyberatos G. Production of biohydrogen from crude glycerol in an up-flow column bioreactor. Bioresource Technology. 2015;198:701-8.

[42] Carrillo-Reyes J, Cortés-Carmona M, Bárcenas-R C. Cell wash-out enrichment increases the stability and performance of biohydrogen producing packed-bed reactors and the community transition along the operation time. Renewable Energy. 2016;97:266-73.

[43] Júnior A, Zaiat M, Gupta M, Elbeshbishy E, Hafez H, Nakhla G. Impact of organic loading rate on biohydrogen production in an up-flow anaerobic packed bed reactor (UAnPBR). Bioresource Technology. 2014;164:371-9.

[44] Fuess L, Kiyuna L, Garcia M, Zaiat M. Operational strategies for long-term biohydrogen production from sugarcane stillage in a continuous acidogenic packed-bed reactor. International Journal of Hydrogen Energy. 2016;41:8132-45.

[45] Azbar N, Kapdan IK. State of the Art and Progress in Production of Biohydrogen, first ed., canada: Bentham Science; 2012.

[46] B K, I K. The effect of HRT on biohydrogen production from acid hydrolyzed waste wheat in a continuously operated packed bed reactor. International Journal of Hydrogen Energy. 2018;43(23):10678-85.

[47] Si B, Li J, Li B, Zhu Z, Shen R, Zhang Y, et al. The role of hydraulic retention time on controlling methanogenesis and homoacetogenesis in biohydrogen production using upflow anaerobic sludge blanket (UASB) reactor and packed bed reactor (PBR). International Journal of Hydrogen Energy. 2015;40:11414-21.

[48] Roy S, Vishnuvardhan M, Das D. Continuous thermophilic biohydrogen production in packed bed reactor. Applied Energy. 2014;136:51-8.

[49] Peixoto G, Saavedra N, Varesche M, Zaiat M. Hydrogen production from soft-drink wastewater in an upflow anaerobic packed-bed reactor. International Journal of Hydrogen Energy. 2011;36:8953-66.

[50] Júnior A, Wenzel J, Etchebehere C, Zaiat M. Effect of organic loading rate on hydrogen production from sugarcane vinasse in thermophilic acidogenic packed bed reactors. International Journal of Hydrogen Energy. 2014;39:16852-62.

[51] noz Páez KM, Ruiz-Ordaz N, García-Mena J, Ponce-Noyola M, Ramos-Valdivia A, Robles-González I, et al. Comparison of biohydrogen production in fluidized bed bioreactors at room temperature and 35C. International Journal of Hydrogen Energy. 2013;38:12570-9.

[52] Zhang Z, Tay J, Show K, Yan R, Liang D, Lee D, et al. Biohydrogen production in a granular activated carbon anaerobic fluidized bed reactor. International Journal of Hydrogen Energy. 2007;32:185-91.

[53] Mu Y, Yu H. Biological hydrogen in a UASB reactor with granules: Physicochemical characteristics of hydrogen producing granules. Biotechnology. 2006;94:980-7.

[54] Castelló E, C Y Santos TI, Paolino G, Wenzel J, Borzacconi L, Etchebehere C. Feasibility of biohydrogen production from cheese whey using a UASB reactor: links between microbial community and reactor performance. International Journal of Hydrogen Energy. 2009;34:5674-82.

[55] Liu D, Liu D, Zeng R, Angelidaki I. Hydrogen and methane production from household solid waste in the two-stage fermentation process. Water Research. 2006;40:2230-6.

[56] Chang F, Lin C. Biohydrogen production using an up-flow anaerobic sludge blanket reactor. International Journal of Hydrogen Energy. 2004;29:33-9.

[57] Wang Y, Mu Y, Yu H. Comparative performance of two upflow anaerobic biohydrogen-producing reactors seeded with different sludges. International Journal of Hydrogen Energy. 2007;32:1086-94.

[58] Yang H, Shao P, Lu T, Shen J, Wang D, Xu Z, et al. Continuous biohydrogen production from citric acid wastewater via facultative anaerobic bacteria. International Journal of Hydrogen Energy. 2006;31:1306-13.

[59] Jung K, Kim D, Shin H. Continuous fermentative hydrogen production from coffee drink manufacturing wastewater by applying UASB reactor. International Journal of Hydrogen Energy. 2010;35:13370-8.

[60] Kotsopoulos T, Zeng R, Angelidaki I. Biohydrogen production in granular up-flow anaerobic sludge blanket (UASB) reactors with mixed cultures under hyper-thermophilic temperature (70 °C). Biotechnology Bioengineering. 2006;94:296-302.

[61] Carrillo-Reyes J, Celis L, Alatriste-Mondragón F, Razo-Flores E. Different start-up strategies to enhance biohydrogen production from cheese whey in UASB reactors. International Journal of Hydrogen Energy. 2012;37:5591-601.

[62] Chookaew T, Sompong O, Prasertsan P. Biohydrogen production from crude glycerol by immobilized Klebsiella sp. TR17 in a UASB reactor and bacterial quantification under non-sterile conditions. International Journal of Hydrogen Energy. 2014;39:9580-7.

[63] Radjaram B, Saravanane R. Start up study of UASB reactor treating press mud for biohydrogen production. Biomass Bioenergy. 2011;35:2721-8.

[64] Puyol D, Mohedano A, Sanz J, Rodriguez J. Comparison of UASB and EGSB performance on the anaerobic biodegradation of 2, 4-dichlorophenol. Chemosphere. 2009;76:1192-8.

[65] Guo W, Ren N, Wang X, Xiang W, Meng Z, Ding J, et al. Biohydrogen production from ethanol-type fermentation of molasses in an expanded granular sludge bed (EGSB) reactor. International Journal of Hydrogen Energy. 2008;33:4981-8.

[66] Cisneros-Pérez C, Carrillo-Reyes J, Celis L, Alatriste-Mondragón F, Etchebehere C, Razo-Flores E. Inoculum pretreatment promotes differences in hydrogen production performance in EGSB reactors. International Journal of Hydrogen Energy. 2015;40:6329-39.

[67] Li C, Fang HH. Fermentative hydrogen production from wastewater and solid wastes by mixed cultures. Critical reviews in environmental science and technology. 2007;37(1):1-39.

[68] Guo X, Trably E, Latrille E, Carràre H, Steyer J. Hydrogen production from agricultural waste by dark fermentation: a review. International Journal of Hydrogen Energy. 2010;35:10660-73.

[69] Balachandar G, Khanna N, Das D. Biohydrogen, first ed., India: Elsevier; 2013.

[70] Wang J, Wan W. Factors influencing fermentative hydrogen production: A review. International Journal of Hydrogen Energy. 2009;34:799-811.

[71] Levin D, Chahine R. Challenges for renewable hydrogen production from biomass. International Journal of Hydrogen Energy. 2010;35:4962-9.

[72] Lin C, Lay C. Carbon/nitrogen-ratio effect on fermentative hydrogen production by mixed microflora. International Journal of Hydrogen Energy. 2004;29:41-5.

[73] Ren N, Gong M. Acclimation strategy of a biohydrogen producing population in acontinuousflow reactor with carbohydrate fermentation. Engineering Life Science. 2006;4:403-9.

[74] Mu Y, Yu H, Wang Y. The role of pH in the fermentative H2 production from an acidogenic granule-based reactor. Chemosphere. 2006;64:350-8.

[75] Kim M, Cha J, Kim D. Biohydrogen, first ed., Republic of Korea: Elsevier; 2013.

[76] Zagrodnik R, Laniecki M. The role of pH control on biohydrogen production by single stage hybrid dark- and photo-fermentation. Bioresource Technology. 2015;194:187-95.

[77] Stavropoulos K, Kopsahelis A, Zafiri C, Kornaro M. Effect of pH on Continuous Biohydrogen Production from End-of-Life Dairy Products (EoL-DPs) via Dark Fermentation. Waste and Biomass Valorization volume. 2016;7.

[78] Alexandropoulou M, Antonopoulou G, Trably E, Carrere H, Lyberatos G. Continuous biohydrogen production from a food industry waste: Influence of operational parameters and microbial community analysis. Cleaner Production. 2018;174:1054-63.

[79] Zhao Q, Yu H. Fermentative H_2 production in an upflow anaerobic sludge blanket reactor at various pH values. Bioresource Technology. 2008;99:1353-8.

[80] Yu H, Zhu Z, Hu W, Zhang H. Hydrogen production from rice winery wastewater in an upfow anaerobic reactor by using mixed anaerobic cultures. International Journal of Hydrogen Energy. 2002;27:1359-65.

[81] Fang H, Liu H. Effect of pH on hydrogen production from glucose by a mixed culture. Bioresource Technology. 2002;82:87-93.

[82] Li Y, Zhu J, Wu X, Miller C, Wang L. The Effect of pH on Continuous Biohydrogen Production from Swine Wastewater Supplemented with Glucose. Applied Biochemistry and Biotechnology. 2010;162:1286-96.

[83] Sepehri S, Rostami K, Azin M. A Study on the Role of Clostridium Saccharoperbutylacetonicum N1-4 (ATCC 13564) in Producing Fermentative Hydrogen. International Journal of Chemical Reactor Engineering. 2018;17:1-12.

[84] Boshagh F, Rostami K, Niel EV. Application of kinetic models in dark fermentative hydrogen productioneA critical review. International Journal of Hydrogen Energy. 2022;47:21952-68.

[85] Bundhoo M, Mohee R. Inhibition of dark fermentative bio-hydrogen. International Journal of Hydrogen Energy. 2016;41:6713-33.

[86] Liu Y, Liu J, He H, Yang S, Wang Y, Hu J, et al. A Review of Enhancement of Biohydrogen Productions by Chemical Addition Using a Supervised Machine Learning Method. Energies. 2021;41:1-16.

[87] Li Y, Chen H, Zhao T, Xiong X, Yao X, Han W, et al. Effects of different metal ions on the hydrogen production capacity of Biohydrogenbac-

terium R3. Solar Energy Journal. 2013;34:1280-7.

[88] Zhang J, Zhang Y, Quan X, Chen S. Effects of ferric ions on hydrogen production efficiency of anaerobic fermentation in UASB reactor. Environmental Science & Policy. 2013;34:2290-4.

[89] Lee D, Li Y, Oh Y, Kim M, Noike T. Effect of iron concentration on continuous H2 production using membrane bioreactor. International Journal of Hydrogen Energy. 2009;34:1244-52.

[90] Lee Y, Miyahara T, Noike T. Effect of iron concentration on hydrogen fermentation. Bioresource Technology. 2001;80:227-31.

[91] Dabrock B, Bahl H, Gottschalk G. Parameters affecting solvent production by Clostridium pasteurianum. Applied and Environmental Microbiology. 1992;58:1233-9.

[92] Peguin S, Soucaille P. Modulation of carbon and electron flow in Clostridium acetobutylicum by iron limitation and methyl viologen addition. Applied and Environmental Microbiology. 1995;61:403-5.

[93] Zhang Y, Shen J. Effect of temperature and iron concentration on the growth and hydrogen production of mixed bacteria. International Journal of Hydrogen Energy. 2006;31:441-6.

[94] Hussy I, Hawkes F, Dinsdale R, Hawkes D. Continuous fermentative hydrogen production from a wheat starch co-product by mixed microflora. Biotechnology and Bioengineering. 2003;84:619-26.

[95] Chen C, Lin C, Chang J. Kinetics of hydrogen production with continuous anaerobic cultures utilizing sucrose as the limiting substrate. Applied Microbiology and Biotechnology. 2001;57:56-64.

[96] Mizuno O, Dinsdale R, Hawkes F, Hawkes D, Noik T. Enhancement of hydrogen production from glucose by nitrogen gas sparging. Bioresource Technology. 2000;73:59-65.

[97] Ueno Y, Otsuka S, Morimoto M. Hydrogen production from ndustrial wastewater by anaerobic microflora in chemostat culture. Journal of Fermentation and Bioengineering. 1996;82:194-7.

[98] Yokoi H, Maki R, Hirose J, Hayashi S. Microbial production of hydrogen from starch-manufacturing wastes. Biomass Bioenergy. 2002;22:389-95.

[99] Yokoi H, Saitsu A, Uchida H, Hirose J, Hayashi S, Takasaki Y. Microbial hydrogen production from sweet potato starch residue. Journal of Bioscience and Bioengineering. 2001;91:58-63.

[100] Lay J. Modelling and optimization of anaerobic digested sludge converting starch to hydrogen. Biotechnology and Bioengineering. 2000;68:269-78.

[101] Beckers L, Masset J, Hamilton C, Delvigne F, Toye D, Crine M, et al. Investigation of the links between mass transfer conditions, dissolved hydrogen concentration and biohydrogen production by the pure strain Clostridium butyricum. Biochemical Engineering Journal. 2015;98:18-28.

[102] Jung K, Kim D, Kim S, Shin H. Bioreactor design for continuous dark fermentative hydrogen production. Bioresource Technology. 2011;102:8612-20.

[103] Chisti M, Moo-Young M. Airlift reactors: characteristics, applications and design considerations. Chemical Engineering Communications. 1987;60:195-242.

[104] Najafpour G, KU I, Younesi H, Mohamed A, Kamaruddin A. Performance of biological hydrogen production process from synthesis gas, mass transfer in batch and continuous bioreactors. International Journal of Engineering. 2004;17:105-20.

[105] Lima D, Zaiat M. The influence of the degree of back-mixing on hydrogen production in an anaerobic fixed-bed reactor. International Journal of Hydrogen Energy. 2012;37:9630-5.

[106] Levenspiel O. Chemical reaction engineering. 3rd ed. Wiley; 1999.

[107] Zhang Z, Show K, Tay J, Liang D, Lee D. Enhanced continuous biohydrogen production by immobilized anaerobic microflora. Energy & Fuels. 2008;22:87-92.