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Review Article

Innovation indicators in biohydrogen production by dark fermentation based on patent analysis

Indicadores de innovación sobre la producción de biohidrógeno por fermentación oscura mediante análisis de patentes

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Abstract

Introduction: Given the urgency of transforming the global energy system to achieve carbon neutrality, microorganism-mediated hydrogen (H_2) production has emerged as a key technology toward a low-carbon society. In particular, dark fermentation (DF) stands out due to its low energy requirements and its ability to utilize diverse organic substrates.
Objectives: To present a critical review of the technological evolution of dark fermentation through the analysis of patents and scientific publications registered between 2002 and 2022.
Methodology: A total of 106 patents and 469 scientific publications were analyzed, evaluating keywords, temporal trends, country leadership, technological classification, citation levels, and technological behavior using S-curves.
Results: The most frequent keywords were "fermentation," "dark fermentation," and "H_2 production," with a transition toward terms such as "metabolites," "food waste," and "biological fuel cells," suggesting a shift toward technological applications and bioelectrochemical production systems. Since 2006, a growing disparity between scientific publications and patents has been observed, reflecting a gap between research and innovation. The United States and China lead in expired patents, indicating early development but commercial slowdown. The C12P3/00 code and the "Biotechnology" domain concentrate most inventions. The low citation level of patents highlights accessibility challenges and the predominance of theoretical knowledge. S-curve analysis reveals technological stagnation associated with efficiency and scalability barriers.
Conclusions: Although DF shows high potential as a sustainable route for H_2 production, its industrial implementation and competitiveness require overcoming technical limitations and strengthening effective knowledge transfer toward mature technological applications.

Keywords: Bio-H2, Dark fermentation , Bio-reactors, Patent analysis.

Spanish version

Resumen

Introducción: Ante la urgencia de transformar el sistema energético global para alcanzar la neutralidad de carbono, la producción de hidrógeno (H_2) mediada por microorganismos se consolida como una tecnología clave hacia una sociedad baja en carbono. En particular, la fermentación oscura (FO) destaca por su bajo requerimiento energético y su capacidad de utilizar diversos sustratos orgánicos.
Objetivos: Presentar una revisión crítica de la evolución tecnológica de la fermentación oscura mediante el análisis de patentes y publicaciones científicas registradas entre 2002 y 2022.
Metodología: Se analizaron 106 patentes y 469 publicaciones científicas, evaluando palabras clave, tendencias temporales, liderazgo por países, clasificación tecnológica, nivel de citación y comportamiento tecnológico mediante curvas en S.
Resultados: Las palabras clave más frecuentes fueron "fermentación", "fermentación oscura" y "producción de H_2", evidenciándose una transición hacia términos como "metabolitos", "residuos alimentarios" y "celdas de combustible biológicas", lo que sugiere un desplazamiento hacia aplicaciones tecnológicas y sistemas bio-electroquímicos. Desde 2006 se observa una creciente disparidad entre publicaciones y patentes, reflejando una brecha entre investigación e innovación. Estados Unidos y China lideran en patentes vencidas, indicando desarrollo temprano pero ralentización comercial. El código C12P3/00 y el dominio "Biotecnología" concentran la mayoría de las invenciones. El bajo nivel de citación de patentes evidencia desafíos de accesibilidad y predominancia del conocimiento teórico. Las curvas en S revelan estancamiento tecnológico asociado a barreras de eficiencia y escalabilidad.
Conclusiones: Aunque la FO posee alto potencial como ruta sostenible para producir H_2, su implementación industrial y competitividad requieren superar limitaciones técnicas y fortalecer la transferencia efectiva hacia aplicaciones tecnológicas maduras.

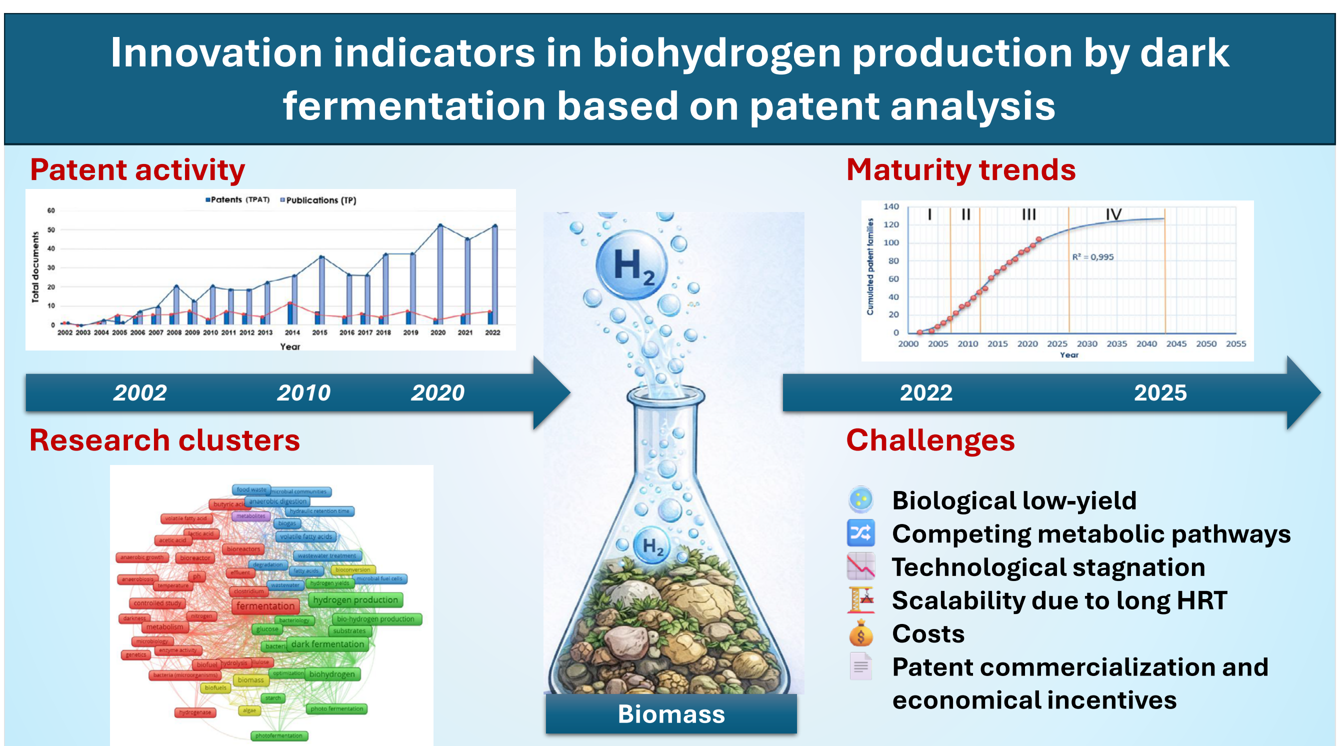
Palabras clave: Bio-H2, Fermentación oscura, Bio-reactor, Análisis de patentes.

Why was the article written?
The article was conducted with the objective of analyzing the technological and scientific landscape of hydrogen production via dark fermentation (DF), a biological route for H2 generation. Specifically, the study aimed to assess the technological maturity of this pathway through a systematic Patent Landscape Report (PLR), supported by bibliometric analysis, IPC code classification, co-occurrence mapping, international cooperation network analysis, and S-curve modeling of technological evolution and maturity. The primary motivation was to evaluate the potential of DF as a sustainable and economically viable alternative and complementary technology to more established technologies such as electrolysis powered by renewable energy sources.

What were the most relevant findings?
1. Advanced technological maturity but limited industrial impact: Using the Gompertz model and S-curve analysis, it was identified that dark fermentation (DF) is currently in a phase of technological maturity (Technological Maturity Rate, TMR ≈ 83%), with an Expected Remaining Life (ERL) of 33 years. However, this maturity has not translated into commercial consolidation or strong competitiveness compared to other hydrogen production technologies. The authors suggest that the number of data points influenced the calculation of these indicators, which are sensitive to the size of the analyzed dataset.
2. Disconnect between scientific and technological production: A moderate correlation (r = 0.549) was found between scientific publications and patents, indicating a gap between academic research and applied innovation. This gap may be influenced by both technical factors (such as feasibility and scalability) and business-related factors (such as market incentives and public policy frameworks). 3. Dominance of the biotechnology domain in patents: Most protected technological developments fall within the Biotechnology domain, particularly in relation to the use of microorganisms and the optimization of fermentation processes. 4. Low international cooperation in certain patent offices While countries such as the United States and South Africa showed high levels of international collaboration (RC), other jurisdictions, such as China, demonstrated a more domestic-oriented innovation strategy. 5. Stagnation in exploratory innovation: The analysis of the exploration (Rt) and exploitation (It) indicators suggests a shift toward technological consolidation and exploitation, rather than the exploration of new concepts and IPC codes—potentially limiting the emergence of disruptive inventions.

What do these findings contribute to?
These results allow for: Identification of technological bottlenecks that hinder the commercial deployment of DF for H2 production. Guidance for R&D and innovation strategies, particularly in underdeveloped areas such as scalable bioreactor design, integration with electrochemical technologies, and enhancement of biological catalysts. Informed policymaking by providing evidence-based insights on the real maturity and competitiveness of biological hydrogen routes, emphasizing the need for targeted incentives if DF is to play a role in the energy transition. Regional relevance for Latin America, showing how such technologies can be incorporated into energy systems using locally available residual resources.

Graphical Abstract



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Introduction

The growing dependence on fossil fuels, such as coal, oil, and natural gas, responsible for approximately 80% of global energy demand, raises serious environmental concerns due to global warming and the emission of greenhouse gases (GHG) (1–3). Although various renewable sources such as solar, hydroelectric, wind, biogas, among others, are being explored, their adoption faces operational limitations that make them viable only in certain regions and times of the year (4) as well as regulatory limitations that restrict their large-scale impact (5). In this context, hydrogen (H_2) is presented as a strategic energy alternative, given its high energy density (with a lower heating value of 120 MJ/kg, equivalent to 2.4 times that of methane and nearly three times that of gasoline) and the possibility of generating carbon-neutral emissions (6). However, around 95% of the annual H_2 production is based on fossil fuels (7), which has driven interest in biotechnological methods such as photo-fermentation (PF), anaerobic digestion, dark fermentation (DF), and microbial electrolysis (2,8,9).

Unlike other methods of bio-H_2 production, DF stands out for its independence from light (enabling 24/7 production), substrate versatility (9), and higher volumetric production rates compared to PF (10) clean-burning, and renewability, hydrogen is a fuel believed to be able to change energy structure worldwide. Biohydrogen production technologies effectively utilize waste biomass resources and produce high-purity hydrogen. Improvements have been made in the biohydrogen production process in recent years. However, there is a lack of operational data and sustainability analysis from pilot plants to provide a reference for commercial operations. In this report, based on spectrum coupling, thermal effect, and multiphase flow properties of hydrogen production, continuous pilot-scale biohydrogen production systems (dark and photo-fermentation. However, it simultaneously generates significant amounts of volatile fatty acids (VFAs), which act to the detriment of bio-H_2 production (6,11–13). Over the past 20 years, numerous articles have been published on DF aiming to increase H_2 production by optimizing key factors such as pH, temperature, nutrients, and substrates (11,14,15). Nevertheless, DF still faces significant technical challenges related to the low efficiency of organic matter conversion, accumulation of by-products, and process stability at pilot scale, especially influenced by reactor design and process configuration (10)clean-burning, and renewability, hydrogen is a fuel believed to be able to change energy structure worldwide. Biohydrogen production technologies effectively utilize waste biomass resources and produce high-purity hydrogen. Improvements have been made in the biohydrogen production process in recent years. However, there is a lack of operational data and sustainability analysis from pilot plants to provide a reference for commercial operations. In this report, based on spectrum coupling, thermal effect, and multiphase flow properties of hydrogen production, continuous pilot-scale biohydrogen production systems (dark and photo-fermentation.

While scientific literature has advanced in the optimization of biotechnological pathways and genetic improvement of microorganisms (16), the challenge of scaling the process to industrial levels persists. The integration of patent analysis becomes essential, as it enables the identification of the technological maturity of DF, the main research and scaling trends, and the detection of critical innovation gaps that must be addressed for commercial implementation.

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This analysis covers two decades of patenting and scientific publication (2002–2022), a period considered representative for capturing the technological evolution in the field of bio-H_2 production via DF. This choice is based on two fundamental reasons. First, from a methodological perspective, selecting 2022 as the cutoff year ensures data set integrity, as many patent applications are subject to the "18-month rule", meaning they are not published immediately after submission. Analyzing more recent patents could introduce bias due to underreporting of still-confidential documents. Second, from a technological standpoint, bio-H_2 production began to gain scientific and technical relevance around 2004. The 20-year range not only allows the evaluation of historical knowledge development but also the exploration of correlations between academic research and patenting activity. This parallel between scientific and technological evolution is key to identifying gaps, emerging trends, and innovation opportunities. Finally, this analysis may also yield results that provide valuable information to promote research and development (R&D) in the field, as well as to guide decision-making in the context of bio-H_2 production via DF, both in Colombia and globally.

Methods

Keyword search, search equation, data collection and refinement

A systematic search was conducted for information related to DF for H_2 production, covering the period 2002–2022. Data were obtained from two main sources: the Orbit Intelligence patent database (17) and ScienceDirect to gather relevant articles and keywords for the search equations (see Table 1). Research and review articles were primarily considered. To maximize thematic coverage, tools such as the WIPO Pearl concept map (18) were used. The search equations were refined with Boolean operators to ensure relevance and precision of the results. Regarding patents, search results were filtered up to the year 2022, including all patents registered during that year; however, due to the 18-month latency period between patent filing and publication, it is possible that some of these patents were published during 2023. Figure 1 outlines the process.

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Table 1. Search query for patents and publications.

Database	**Search query**	**Results**	**Estimated search date**
Orbit	$= ((DARK + 1D\ FERMENT+)/TI/AB/CLMS/DESC/ODES/ADB/KEYW\ AND(+HYDROGEN)/TI/AB/CLMS/DESC/ODES/ADB/KEYW\ AND\ (???REACTOR)/TI/AB/CLMS/DESC/ODES/ADB/KEYW)\ AND"C12P-003"/IC$	124 patents	06/28/2023
Science Direct	$= TITTLE - ABS - KEY((dark * W/1\ ferment *)AND\ (* hydrogen)AND(;??reactor))\ AND\ PUBYEAR > 2001\ AND\ PUBYEAR < 2023\ AND\ (EXCLUDE(DOCTYPE))$	469 publications	06/28/2023

Source: own elaboration

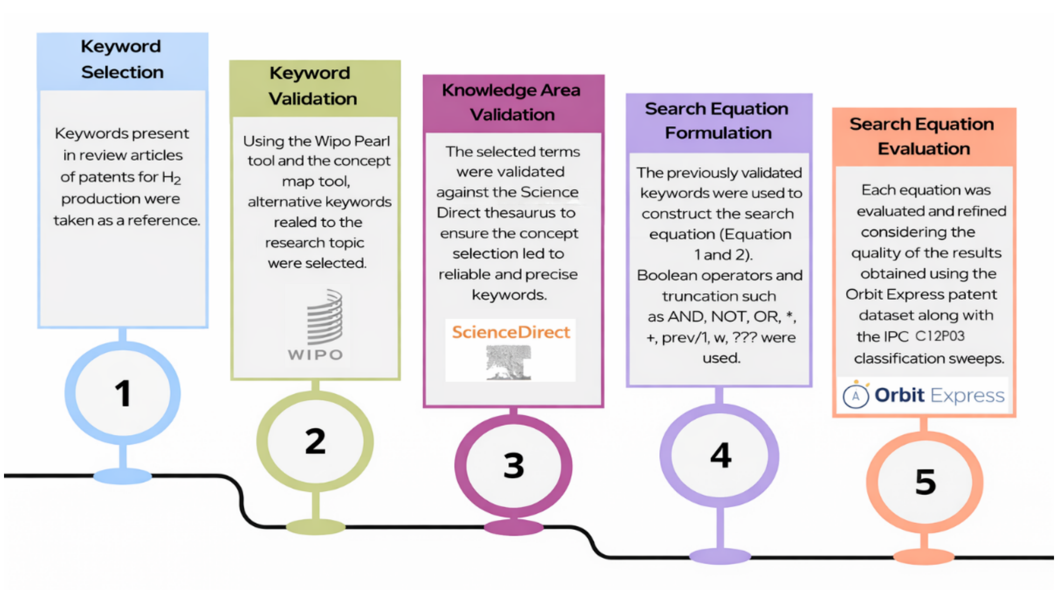

Figure 1. General scheme for patent search. Source: Author's own elaboration

Bibliometric analysis of keywords

A co-occurrence analysis of keywords was performed using the records obtained with VOSviewer (19), allowing the identification of thematic groupings through clusters. Concept maps were visualized based on the average year of publication to detect emerging research lines and their temporal evolution. This approach makes it possible to recognize areas of greatest research activity and their dynamism, without making qualitative judgments about specific technologies

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(20). Additionally, the evolution of IPC codes (International Patent Classification) and technological domains (TDs) were analyzed, enabling the identification of technological hotspots and their relationship with the evolution of predominant scientific concepts in the DF field.

Patent indicators and trend analysis

Patent analysis was structured into three levels: performance, technological mapping, and trend analysis (see Figure 2). Performance was assessed using indicators such as number of publications, citations, and technological relevance, where publications are associated with productivity and citations reflect the impact and influence of the documents (21). Technological mapping allowed the exploration of structural relationships between patents using tools such as Intellixir and VOSviewer. The exploration (R_t) and exploitation (I_t) indicators were used, where the former describes the ratio of recently used IPCs in period r () to the total IPCs in the study period t (), and the latter is defined as the ratio of the total number of IPCs used in periods t and t–1 () to the total IPCs in period t (). Other indicators used included cooperation networks (RC), technological relevance (TR), and technological activity (TA), as they allow the estimation of the activity and relevance of a patent portfolio in a specific technology. Table 2 describes the equations used in this analysis.

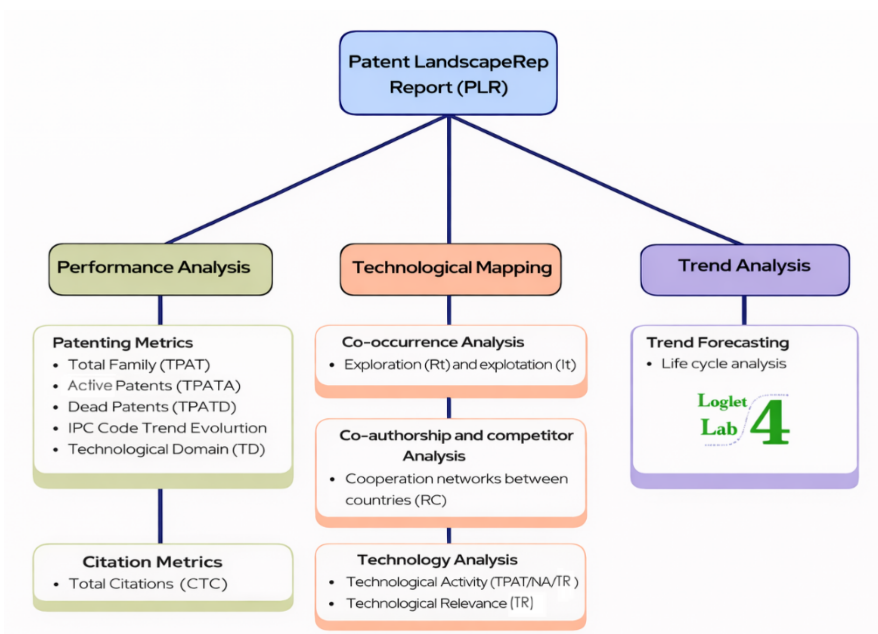

Figure 2. Indicators for patent analysis. Source: (22) and based on information from (21)

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Table 2. Technology mapping equations

Indicators	Abbreviation	Units	Equation
Exploration in period t	R_t	%	$R_t = \frac{n_t^r * 100}{n_t}$
Exploitation in period t	I_t	%	$I_t = \frac{n_t^i * 100}{n_t}$
Cooperation networks	RC	$\frac{No\ cooperation\ networks}{Patents}$	$RC = \frac{No.nodes}{TPAT}$
Technological relevance	TR	$\frac{Active\ patents}{Year}$	$TR = \frac{TPATA}{NAYT}$
Technological activity	TA	$\frac{Total\ families}{Patent/Year}$	$TA = \frac{TPAT}{NAYT}$

Source: own elaboration

For technological trend analysis, mathematical S-curve models were applied to characterize the life cycle of DF. The most commonly used approaches in literature are the Gompertz and Logistic models. Following the methodology proposed by Ampah et al. (23), the Gompertz curve model (Eq. 1) was selected in this study to analyze the life cycle of accumulated patents and publications. Loglet Lab software, version 4 from 2022-12-04 (24), was used to perform the forecasting analysis, with the fundamental coefficients of both curves determined through Monte Carlo iterations.

(Eq. 1) $$y = ke^{-e^{-r(t-tm)}}$$

where $y\ (t)$ represents the dependent variable of the S-curve, i.e., the cumulative annual publications, t represents the time variable, r is a model parameter, tm is the mean time, and K is the maximum number of publications.

Finally, based on these models, indicators such as the Technological Maturity Rate (TMR), Expected Remaining Life (ERL), and Potential Patent Applications (PPA) were calculated with Eqs. 2-4 (25), providing a quantitative view of the state and projection of the technologies without making critical comparisons among them. The TMR, with values between 0 and 1, indicates how close a technology is to reaching its maximum development. When the TMR crosses the development threshold of 0.5, the technology is considered to have reached maturity. The ERL is used to estimate how long it will take to reach saturation. The equations used to estimate TMR, ERL, and PPA are shown below.

(Eq. 2) $$TMR(\%) = \frac{k_0}{k} * 100$$

(Eq. 3) $$ERL\ (t) = t_k - t_0$$

(Eq. 4) $$PPA\ (N°\ de\ documentos) = k - k_0$$

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where k_0 and k represent the cumulative number of patents at time t_0 and at the saturation point t_k, respectively.

Results

Performance analysis

Patent Metrics

In the analysis of the temporal evolution of patent families (TPAT) and total publications (TP) on Bio-H_2 production via DF, 469 scientific articles and 106 patents were examined, compiling data from scientific publications and patents registered from the year 2000 to 2023. The growing interest in sustainable development and waste management has driven research in Bio-H_2 over the past two decades, leading to the emergence of three main technologies for Bio-H_2 generation: (a) bio-fermentation (including DF and PF), (b) bio-photolysis (direct and indirect), and (c) bio-electrochemical systems such as microbial electrolysis cells (MEC). Figure 3 presents a comprehensive map of these three Bio-H_2 production pathways.

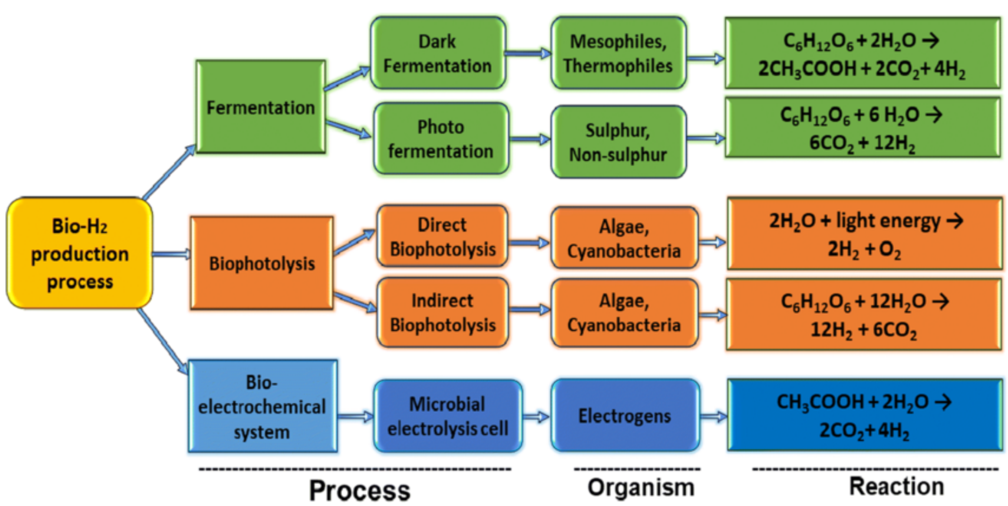

Figure 3. General map of the biological production processes of bio-H_2. Source: (26).

Within the fermentation route, in addition to DF, PF also enables Bio-H_2 generation. However, PF is carried out using photosynthetic bacteria (light-dependent purple bacteria), which in the presence of natural or artificial sunlight are capable of degrading various carbon substrates such as carbohydrates, organic matter, biowaste, and organic acids to generate H_2 and CO_2 (8). It has been shown that some parameters, such as the high energy demand of the process, H_2 re-oxidation, low enzyme activity of nitrogenase, and limited availability of suitable organic matter, are major limitations of PF (2). For this reason, this method is often coupled with DF in two-stage or multi-stage processes to increase H_2 production yield, leveraging PF's ability to generate H_2 from VFAs that are generated during DF (27).

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Bio-photolysis known also as water-splitting photosynthesis, is a biological process in which photosynthetic microorganisms like cyanobacteria and green microalgae are used to convert water into H_2 in the presence of sunlight. In this method, the FeFe-hydrogenase enzyme is essential for green microalgae, while heterocysts cyanobacteria use nitrogenase (8). These microorganisms use only water and light, and in the case of indirect bio-photolysis, they can also use atmospheric CO_2. However, indirect bio-photolysis remains in a very early development stage and requires more practical applications (28).

Finally, bio-electrochemical systems for Bio-H_2 production, particularly microbial electrolysis cells (MECs), have emerged as an innovative technology gaining interest in recent years. Also known as bio-catalyzed electrolysis cells or electro-fermentation, these systems can be configured as single-chamber or dual-chamber setups separated by a proton exchange membrane, in which microorganisms oxidize organic matter at the anode, generating electrons that are transferred to the cathode, where they combine with protons to form molecular hydrogen (H_2). This technology allows the use of organic wastewater as a substrate, offering an efficient and sustainable route for Bio-H_2 production (8).

In recent years, DF has been favored over bio-photolysis and PF or used in combination with them due to the reliance of the latter two on light and microorganisms that may be sensitive to oxidative conditions, increasing process costs. Figure 4 shows the scientific (TP) and technological (TPAT) evolution of DF, highlighting the yearly patent publication trend (red line). Between 2002 and 2005, both publications and patents were relatively low. However, from 2006 onward, a notable increase in TP was observed, with the highest growth recorded between 2020 and 2022. This trend aligns with growing global interest in renewable energy sources promoting a circular bioeconomy and reducing environmental impact (29). In comparison, the patent production trend (TPAT) remained relatively low and steady. Between 2005 and 2022, a notable divergence is observed between the evolution of indicators TP and TPAT, suggesting that this technology still faces barriers to implementation beyond controlled laboratory environments.

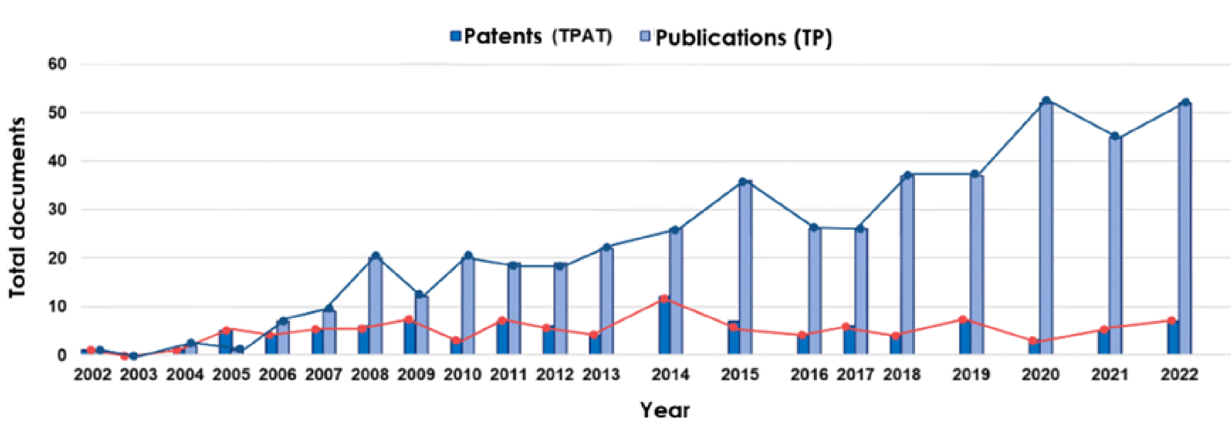

Figure 4. Temporal evolution of the indicators TP and TPAT in dark fermentation. Source: own elaboration

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The state of the art indicates that this difficulty is associated with process scaling and bioreactor configuration, primarily due to the complexity of the biological kinetics involved. The Gompertz equation has traditionally been used to model H_2 production, although its empirical nature limits predictive capacity under variable conditions, such as different substrate types and concentrations, temperature, and pH (30). In response, mechanistic models such as ADM1, originally designed for methanogenesis, have been successfully adapted to describe H_2 and VFA production in DF (31), integrating key physicochemical and biochemical parameters. This transition toward more robust models reflects efforts to overcome technical limitations that have hindered technology transfer and commercial exploitation of Bio-H_2.

In general, most inventions focus on biological approaches to Bio-H_2 production using various raw materials (pretreated biomass, starch-rich organic matter, cellulose, sludge, kitchen waste, winery industry biomass, and xylose) and processes such as recirculated fermentation, two-stage fermentation with mixed strains, and fermentation with embedded microorganisms to maximize efficiency and reduce environmental impact (32–34). For instance, the Organic Fraction of Municipal Solid Waste (OFMSW) is among the most researched and patented feedstocks. It comprises food waste containing a large proportion of biodegradable carbohydrates. Wastewater treatment plants also generate large volumes of residual sludge or biosolids, which contain carbohydrates and proteins and have been used in H_2 generation. However, sludge requires pretreatment such as alkaline conditioning or sterilization to facilitate generation. Notably, patent CN104372030, the most cited in 2014, involves a method for producing H_2 and CH_4 through combined fermentation of sludge and kitchen waste (33).

Agricultural residues such as rice and wheat straw, corncobs, husks, bagasse, stubble, and plant biomass waste, which generally contain cellulose, hemicellulose, and lignin, as well as livestock waste including forage residues, solids from animal manure, and wastewater containing urine and feces, are also used (13,14,35). For example, the invention by Fanhe et al. (36), patent number CN107012195B, describes a method for H_2 production via fermentation of agricultural straw using a mixed bacterial population. In this process, the straw concentration in the reactor was maintained between 10 and 40 g/L, at a controlled temperature of 36 °C and a constant agitation of 100 rpm. Under these conditions, maximum H_2 production ranged between 65 and 186 mL of H_2 per 100 mL of fermentation medium, demonstrating the potential of agricultural residues as a viable substrate for bio-H_2 generation via DF.

In order to assess the potential correlation between scientific publication output and patent activity related to DF, a correlation analysis was conducted (see Figure 5). This analysis aids in understanding the relationship between research activity, represented by the trend in scientific publications, and efforts in invention, innovation, or commercialization, which are typically associated with patent filings. While scientific articles generally reflect fundamental research and theoretical advancements, patents are more indicative of applied research and innovations that may or may not have commercial potential. Identifying a correlation between these two variables allows for the evaluation of how closely scientific knowledge advances are linked to practical applications or market-oriented innovations.

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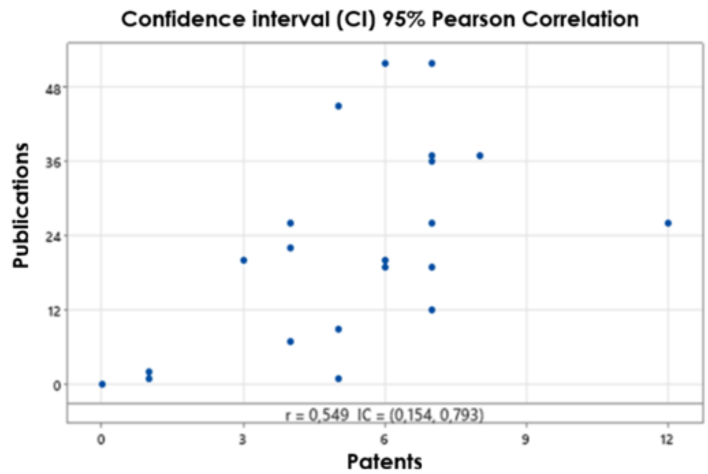

Figure 5. Correlation analysis between the number of new publications and patents per year. Source: own elaboration.

The analysis revealed a moderate linear correlation, with a coefficient of $r_{xy} = 0.549$, indicating a positive but moderate relationship between scientific publications and patent activity ($0.30 < r_{xy} < 0.60$). This suggests that both variables tend to increase concurrently, albeit not strongly or in a fully synchronized manner. Therefore, although there is some degree of connection between scientific production and innovation efforts, the relationship is not directly proportional. Furthermore, this connection is mediated by several external factors, including market demand, technological feasibility, regulatory frameworks, availability of funding, and strategic priorities. The relationship is also influenced by a well-documented disconnect between the academic and industrial sectors (37). The moderate correlation may additionally be attributed to a temporal lag between the generation of scientific knowledge and its translation into patents, given that basic research often requires several years before resulting in patentable developments. This lag implies that, despite the sustained increase in scientific publications (TP), the technology may not yet have achieved sufficient maturity or applicability due to specific limitations such as technological efficiency, scalability, or industrial adoption levels (37).

Currently, grey H_2 production from fossil fuels (primarily via steam methane reforming (SMR) accounts for the majority of global H_2 demand. Between 2011 and 2023, approximately 70% of global electricity was generated from fossil sources, while renewables contributed only 15–30%, with bioenergy representing less than 2%, compared to hydroelectric, solar, and wind energy (see Figure 6). By 2024, the share of renewables in the global electricity supply has steadily increased, surpassing 30%; however, bioenergy has not experienced a recent exponential growth sufficient to significantly alter its proportion in the global energy mix (38).

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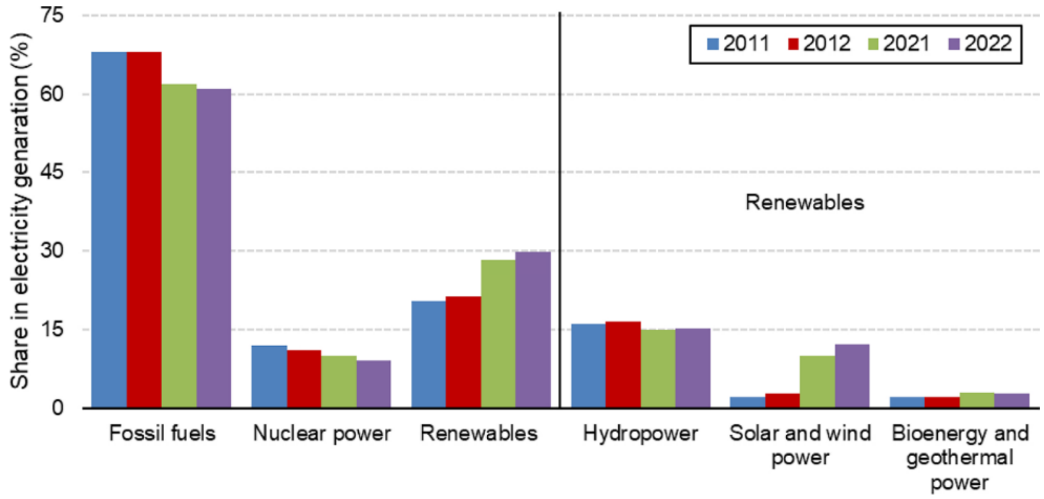

Figure 6. Comparison of the global energy mix from 2011 to 2022. Source: (39) and the consequences of climate change on the environment and public health have now become visible.

The increase in greenhouse gas emissions resulting from human activities, which is the main cause of global climate change, caused the global surface temperature to be 1.1 °C higher between 2011 and 2020 compared to 1850–1900. In parallel with this global problem, the transition to clean energy has increased significantly with Russia's invasion of Ukraine, more aggressive energy and climate policies, technological developments, and increasing concerns about energy security. In this study, global climate change indicators, including land and sea surface air temperatures, sea level rise, sea ice extent, ocean heat content, surface humidity, and total column water vapor, are reviewed and updated in parallel with a comprehensive analysis of the progress in renewable energy. The results showed that if no measures are taken to reduce human-induced greenhouse gas emissions, the global average temperature will increase further in the coming years and the negative effects of other climate parameters will be felt even more. It has been emphasized that limiting human-induced global warming requires renewable and sustainable energy sources and net zero CO\n 2\n emissions and that the simultaneous adoption of emission reduction and adaptation strategies will be the most effective economic and technical solution to the global warming problem.","container-title":"Arabian Journal for Science and Engineering","DOI":"10.1007/s13369-024-09390-y","ISSN":"2193-567X, 2191-4281","issue":"11","journalAbbreviation":"Arab J Sci Eng","language":"en","page":"14503-14531","source":"DOI.org (Crossref.

In this context, bio-H_2 production pathways such as DF and PF must compete with more mature technologies, particularly electrolysis powered by solar and wind electricity, commonly referred to as green H_2. The share of renewables in the global H_2 production market remains below 1%, despite the rapid expansion of installed capacity worldwide. This low penetration is primarily due to the lack of economic competitiveness in comparison to fossil-based H_2, as evaluated through the levelized cost of energy (LCOE). While grey H_2 has an LCOE ranging from approximately USD 0.70 to 2.50 per kg (depending on the country), green H_2 ranges from USD 3.50 to 6.00 per kg or higher, which limits its large-scale adoption (40). This economic disparity directly impacts the relationship between scientific knowledge generation and technological production, ultimately manifesting as a disconnect between science, technology and economy in this field, as previously illustrated in Figures 4 and 5.

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Citation Metrics

Figure 7 illustrates the annual evolution of the total citation count (CTC) for both scientific publications and patents. The slow growth observed in the patent CTC is also related to the TPAT indicator (see Figure 4), which reflects a lower number of patent filings per year. As a general empirical rule, the higher the number of documents, the greater the expected citation count. Accordingly, between 2002 and 2007, the growth of the CTC was gradual. Beginning in 2008, an almost exponential increase in citations of TP was observed, with the highest citation counts recorded in 2021 and 2022. This trend is similarly reflected in Figure 4. However, the gap between TP and TPAT, as evidenced in Figures 4 and 5, is also mirrored in Figure 7 by the slower, but steady, increase in the citation count for patents. Overall, these results demonstrate a growing interest in scientific research on DF. Although patents have received fewer citations over time, the upward trend in their citation rate also reflects increasing technological interest in DF-related developments. This aligns with the trend over the past two decades toward the development of transitional technologies for alternative renewable energy sources with low environmental impact, particularly within the field of biotechnology (41).

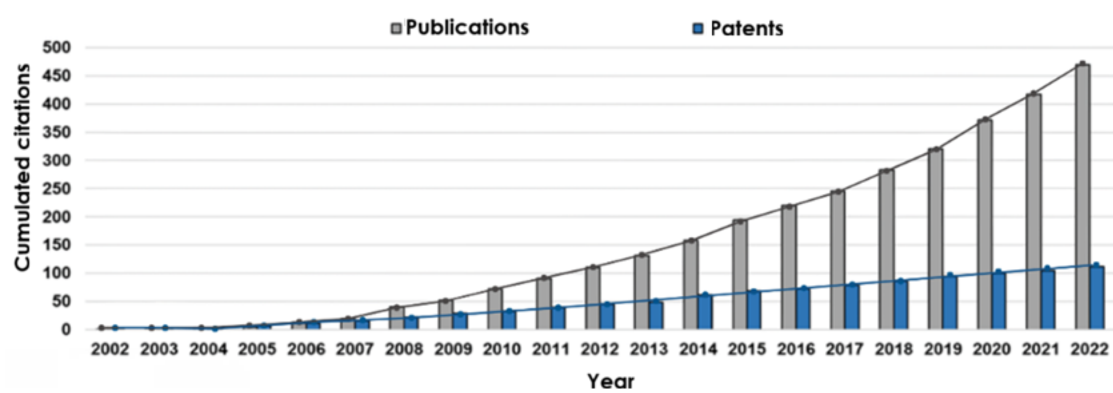

Figure 7. Cumulative CTC by year for publications and patents. Source: own elaboration

Technological Mapping

Co-occurrence analysis and Temporal Evolution of Key Scientific Concepts

Co-occurrence analysis and temporal evolution of terms is a fundamental tool in bibliometric studies to identify thematic cores, emerging patterns, and research dynamics within a field. In this section, VOSviewer was used to generate conceptual maps based on keywords extracted from the reviewed documents. Figures 8 and 9 illustrate the results of this analysis, revealing the conceptual structure and its temporal evolution surrounding bio-H_2 production through DF.

Figure 8 displays a co-occurrence map where terms are grouped into five clusters differentiated by color, each representing a set of interrelated concepts. The densest and most relevant clusters are the green, red, and blue. The green cluster groups concepts related to bio-H_2 production, highlighting terms such as “hydrogen production,” “fermentation,” “dark fermentation,” “photofermentation,” and “bio-H_2.” This cluster includes the most frequent and central keywords on the map. “Fermentation” refers to the biological decomposition of organic compounds under anaerobic conditions, frequently applied to waste treatment (42).

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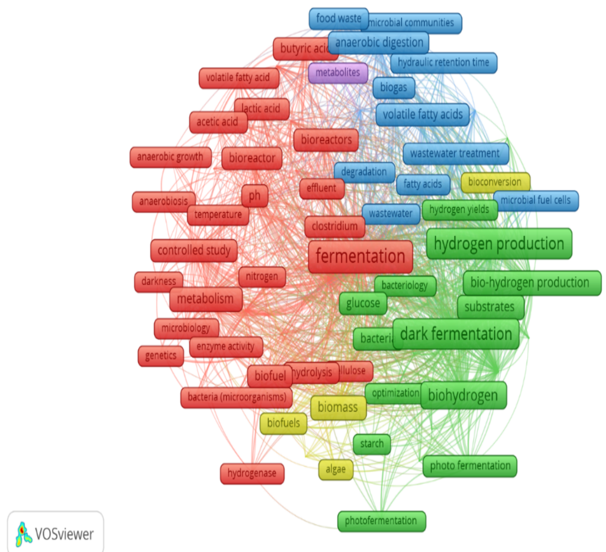

Figure 8. Concept and cluster network map. Source: prepared by the author using VOSviewer 1.6.20 software (19)

As shown in Figure 3, "dark fermentation" is a type of fermentation involving anaerobic microbial processes in the absence of light, typically at temperatures between 25 and 80 °C, using glucose and water to produce acetic acid (although butyric and lactic acids may also be generated which are called volatile fatty acids or VFAs), carbon dioxide, and H_2 via the reaction below. This process can be coupled with "photofermentation", which consumes VFAs in the presence of light to generate H_2 and CO_2, thereby maximizing overall H_2 yield (43). Both reactions are theoretically limited to a maximum production of 4 mol H_2/mol glucose:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

The red cluster focuses on biological parameters and operational conditions typically associated with the microorganisms involved in DF, such as "anaerobiosis," "pH," "temperature," "metabolism," "enzymatic activity," "genetics," "hydrogenase," and "hydrolysis." It also addresses common derivatives and chemical compounds from the DF process, such as "lactic acid," "volatile fatty acids," and "butyric acid." These parameters are critical because H_2 production by DF competes with other metabolic processes when environmental conditions vary. Changes in pH, temperature, or substrate availability can favor alternative metabolic routes in which generated H_2 is consumed in secondary reactions to form VFAs and other by-products, thereby reducing process efficiency and selectivity.

The blue cluster comprises concepts related to the production process itself, such as the influence of different "microbial communities," "food waste," and "wastewater" as feedstocks, as well as "biogas" production, a common by-product in biological reactors that competes with H_2 generation by consuming H_2, CO_2, and acetic acid to form methane (CH_4). This cluster also includes the emergence of "microbial fuel cells," which typically achieve higher yields than DF but require an

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external voltage, a proton exchange membrane, and the presence of electroactive microorganisms. Lastly, the yellow cluster includes relevant terms such as “algae,” as recent publications have explored the potential of certain microalgae and cyanobacteria species to produce H_2 directly via bio-photolysis or indirectly through DF and other hybrid processes (44).

Figure 9 presents the temporal evolution of key concepts, where colors indicate the average year of term appearance and the size of the rectangles represents their frequency. Prior to 2010, terms such as “anaerobiosis,” “darkness,” and “bacteria” were prominent, reflecting an initial focus on basic DF operating conditions. Between 2010 and 2020, new concepts emerged related to process optimization, including “inoculum,” “clostridium,” “glucose,” “pH,” and “pretreatment.” Since 2020, research has shown a transition toward process integration and by-product valorization. Terms such as “metabolites,” “food waste,” and “biofuel cell” have become prominent, indicating a shift toward sustainability and energy efficiency, through the synergy between DF and technologies such as MECs (45,46). These results not only enable the tracing of thematic evolution but also highlight emerging areas with high innovation potential, such as the integration of DF with bio-electrochemical platforms or the valorization of VFAs as intermediates for other bioenergy processes (47).

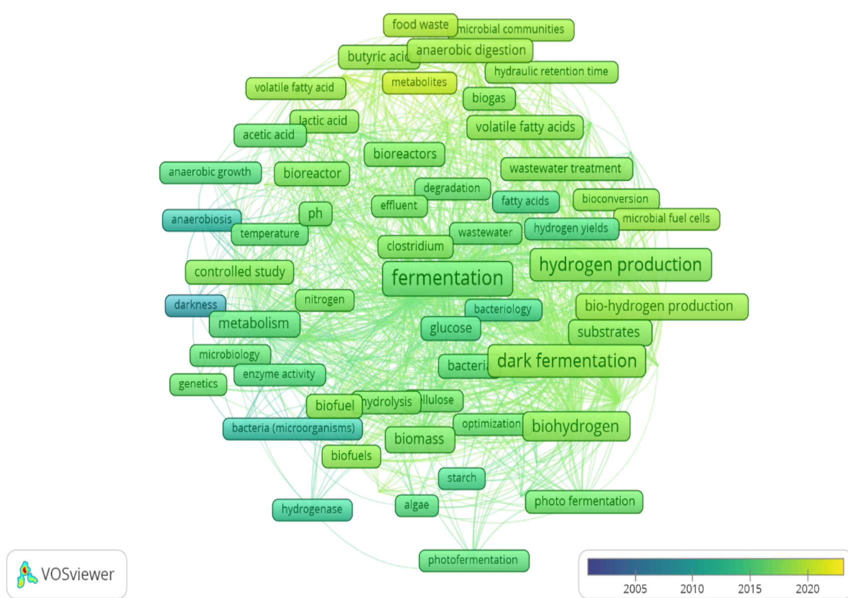

Figure 9. Network map of emerging concepts over time. Source: prepared by the author using VOSviewer 1.6.20 software (19)

Evolution of Technological Language: IPC Codes

In line with the findings obtained from the co-occurrence analysis of scientific literature where key terms such as “DF,” “hydrogen production,” and “bacteria” were identified, a complementary study was carried out in the technological domain through the analysis of patents. For this purpose, the International Patent Classification (IPC) system was employed, which categorizes inventions according to their technical field and offers a standardized framework comparable to the use

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of keywords in scientific articles (48). This methodological alignment enables the integration of scientific and technological language, providing a comprehensive view of field development. The most representative IPC codes were identified in Table 3 and subsequently analyzed in terms of patent citations (CT) and technological domains (TDs), as shown in Figures 10 and 11, respectively. This strategy enabled the identification of predominant technological areas, their temporal evolution, and the instrumental and biotechnological approaches that have gained prominence in the intellectual property protection of this emerging field.

Table 3. Top 5 most cited CIP codes in the database

IPC code	Definition
C12M1/00	Apparatus for enzymology or microbiology
C12M1/107	Apparatus for enzymology or microbiology, with means for collecting fermentation gases, such as methane.
C12P3/00	Preparation of inorganic elements or compounds (except CO_2) by biological or enzymatic means
C12P5/02	Preparation of acyclic hydrocarbons
C12R1/01	Microorganisms, bacteria and actinomycetes

Fuente: own elaboration

Figure 10 shows the temporal evolution of the five most cited IPC codes listed in Table 3. Among them, code C12P3/00, corresponding to the preparation of elements or inorganic compounds, except carbon dioxide by biological or enzymatic means, stands out as the most active throughout the entire evaluation period. Notably, it exhibits peaks of activity in 2014 and 2019, with 12 and 8 patent applications, respectively. This trend reflects sustained growth in the development of technologies aimed at H_2 production through low-carbon processes, aligned with increasing pressure for more sustainable energy alternatives (26). Inventions within this code include patent CN111137891 B (49), which proposes the use of nickel-doped magnetic carbon to optimize DF. This material provides essential trace elements for microbial metabolism in fermentation, enhances dehydrogenase activity, accelerates microbial growth, and improves microbial resistance to environmental changes, thereby optimizing nutrient uptake and metabolic rates and maximizing fermentation capacity (49). Similarly, patent CN114410695 A (50) explores the use of hydroxyapatite as an additive to increase H_2 production, induce favorable fermentation pathways (e.g., from ethanol to butyrate), and enhance microbial composition under moderate temperature conditions.

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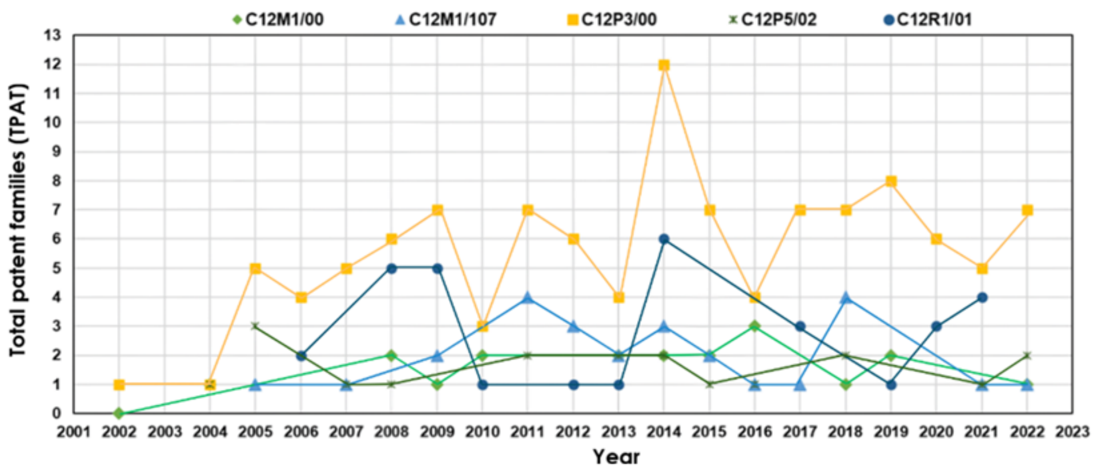

Figure 10. Temporal evolution of the main IPC codes. Source: own elaboration

The second most representative code is C12R1/01, related to the use of microorganisms, bacteria, and actinomycetes, with a significant number of citations in 2014 (CTC = 6), 2009 (CTC = 3), and 2008 (CTC = 3). Its activity peaks in 2008, 2009, and 2014, indicating sustained interest in the engineering of specific microbial consortia to improve process efficiency. Two illustrative patents are IN3532MU2013 A (51)11,2]]},"issued":{"date-parts":[["2015"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} which proposes an integrated DF and PF strategy with optimized nitrogen supplementation, and US2012088266A1 (52) which describes an anaerobic bioreactor system with a thermophilic mixed community capable of achieving high biomass-to-H_2 conversion yields.

The IPC codes C12M1/107, C12M1/00, and C12P5/02 follow in importance under the TPAT indicator and have gained relevance since 2007. This suggests an increasing focus on the design of prototypes and gas collection systems to optimize process performance (29). In particular, C12M1/107, referring to devices for collecting fermentation gases, has been key in developing technologies that reduce the partial pressure of H_2 in reactors, thereby improving the selectivity of the fermentation process. An example is patent WO2017051136 A1, which describes a fermentation system with a membrane reactor and continuous gas extraction via hollow fibers. This design prevents process inhibition due to gas accumulation and enhances overall yield (32).

Figure 11 classifies the IPC codes into technological domains (TDs), allowing protected technologies to be grouped into broader functional categories and facilitating the identification of cross-cutting technological trends. Figure 11 is directly linked to Figure 10, as the most cited IPC codes, such as C12P3/00 and C12R1/01, fall within the dominant domain of Biotechnology, which maintained a high frequency of registrations throughout the analyzed period.

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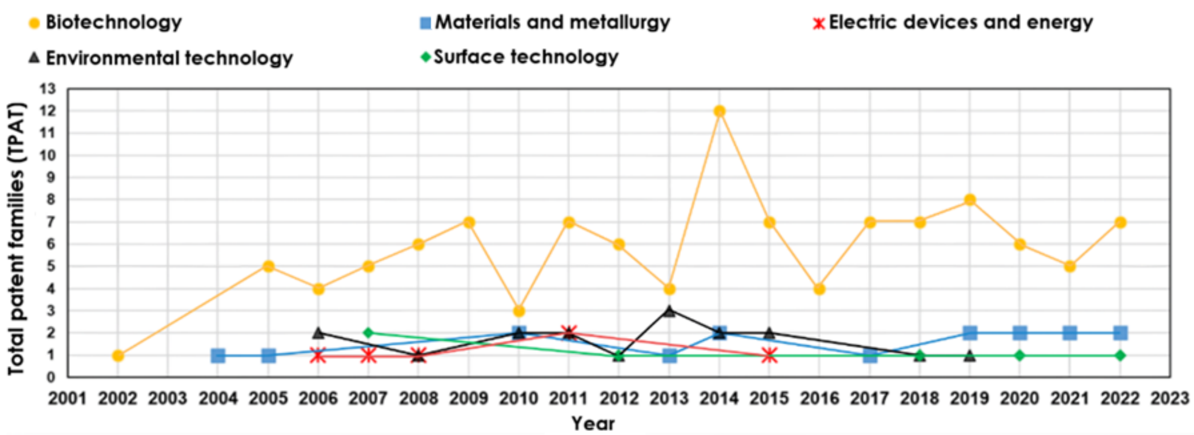

Figure 11. Temporal evolution of the main TD codes. Source: own elaboration

Biotechnology is the leading TD in all evaluated years and includes codes C12P3/00 and C12R1/01 from Table 3 and Figure 10. The most cited patents in this TD address topics such as the improvement of anaerobic bio-H_2 production and the conversion of biomass, including organic waste and hydrocarbon-containing materials (50,53–56). The second most relevant domain is Materials and Metallurgy, directly related to Surface Technology. The most representative IPC codes in this domain are C12P3/00 and C12P5/02, with patents focused on the use of metallic catalytic materials, such as copper, iron, nickel, cobalt, and molybdenum, as well as carbon-derived materials like graphene (57). These catalysts have been applied both in microbial genetic modification (53) and in enhancing fermentative process efficiency (10)clean-burning, and renewability, hydrogen is a fuel believed to be able to change energy structure worldwide. Biohydrogen production technologies effectively utilize waste biomass resources and produce high-purity hydrogen. Improvements have been made in the biohydrogen production process in recent years. However, there is a lack of operational data and sustainability analysis from pilot plants to provide a reference for commercial operations. In this report, based on spectrum coupling, thermal effect, and multiphase flow properties of hydrogen production, continuous pilot-scale biohydrogen production systems (dark and photo-fermentation.

The domain of Environmental Technology is associated with the development of clean technologies that, when implemented, do not generate secondary effects or disturb environmental balance. These technologies are grounded in sustainability and primarily utilize natural and renewable resources. In this context, biomass is used to produce energy in the form of H_2, resulting in a carbon-neutral process. This domain had a significant presence between 2006 and 2015 but later declined in relevance compared to domains such as biotechnology, materials, and surface technology. The 2023 report from the International Energy Agency (IEA) (40) indicated a shift away from H_2 production based on biomass or waste toward more mature technologies such as electrolysis, which in recent years have received substantial investment due to the declining costs of renewable energy. As a result, biological routes have increasingly been overlooked as "low-carbon solutions" within policy-driven innovation frameworks (58).

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Meanwhile, the domain of Electrical Machinery, Apparatus, and Energy exhibited intermittent records, with activity between 2006 and 2016, followed by a steady decline. An example of common IPC codes within this domain is the H01M8/16 (biochemical or bio-electrochemical fuel cells). This domain encompasses inventions focused on the design of equipment, devices, and process configurations to enhance system efficiency and integration of H_2 with power grids. Notable patents include those by Li et al. (59), which propose devices for the co-production of H_2 and electricity or two-stage systems for H_2 production from waste. However, in the last few years, several publications (60,61) has found that hydrogentopower use cases are energetically inefficient, with overall roundtrip efficiencies typically in the 25–40% range. By comparison, grid-scale Li-ion battery storage commonly achieves 80–90% round-trip efficiency, so purely from an energy-efficiency perspective H_2 is clearly inferior as a short-duration storage medium (62). This is why in recent years, hydrogen-to-power use cases are only considered when they can be justified in specific system roles where duration, transportability, and decarbonization of existing assets matter more than pure round-trip efficiency.

In summary, the results from Figures 10 and 11, integrated with the keyword clusters from Figures 8 and 9, allowed the construction of a conceptual network linking scientific concepts, protected technological areas, and key development parameters. This integrated view reveals that the three fundamental pillars of innovation in bio-H_2 production via DF are: 1) biotechnological processes related to microbial metabolism, 2) functional materials applied as catalysts or supports, and 3) the design and scaling of technological devices.

Exploration () and Exploitation () Indicators

To analyze the dynamics of patent production in DF, exploration (R_t) and exploitation (I_t) indicators were employed. These indicators are based on the use of IPC codes (see Table 3) and allow the identification of the emergence of new codes and the consolidation of specific technological areas. The results of this analysis are presented in Table 4.

Table 4. Technological exploration and exploitation indicators of the top 5 CIPs

Period	Technological exploration (R_t)	Technological exploitation (I_t)
(2008-2012)	33,33%	66,67%
(2013-2017)	10,0%	90,0%
(2017-2022)	0%	100%

Source: own elaboration

Between 2008 and 2012, the R_t indicator suggests a phase of intense technological exploration. This period coincides with a surge in fundamental research aimed at understanding the biochemical mechanisms of DF, identifying efficient microorganisms, and exploring a wide variety of

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lignocellulosic substrates, agro-industrial residues, and wastewater as raw materials. This knowledge expansion enabled the emergence of patents across multiple technological domains. Additionally, new reactor design concepts were developed, including fixed-bed systems, UASB configurations, and hybrid setups incorporating PF. This technical dynamism led to the creation of new IPC codes, reflecting disruptive inventions characterized by a high degree of novelty and conceptual speculation (35,63).

Conversely, the increase in the I_t indicator between 2017 and 2022 indicates a phase of technological consolidation or exploitation. This phenomenon aligns with literature that documents a shift toward refining existing configurations, optimizing operational conditions (pH, temperature, H_2 partial pressure, hydraulic retention time), and integrating unit operations to improve performance indicators such as H_2 production rate and molar yield. During this stage, developments tend to build on already validated knowledge and solutions, reusing the same IPC codes, typical of a transition from an emerging phase toward technological maturity (36,64).

Co-authorship Analysis, Cooperation Networks (RC), Technological Relevance (TR), and Technological Activity (AT)

Figure 12 presents the main normalized patent indicators for the five patent offices with the highest number of registrations (TPAT) worldwide: China (CNIPA), the United States (USPTO), India (IPO), France (INPI), and South Africa (CIPC). Figure 13 shows the distribution of the legal status of patents by country, distinguishing between those that are active/alive and those that are expired/dead.

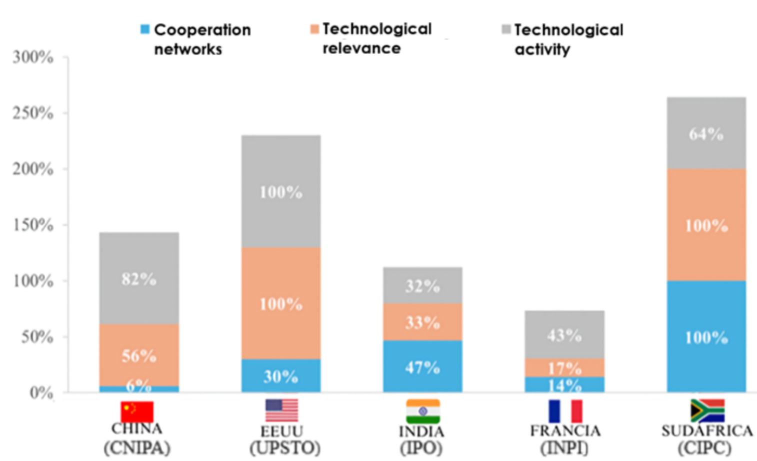

Figure 12. Co-authorship, RC, and VT indicators in the top 5 patent offices. Source: own elaboration

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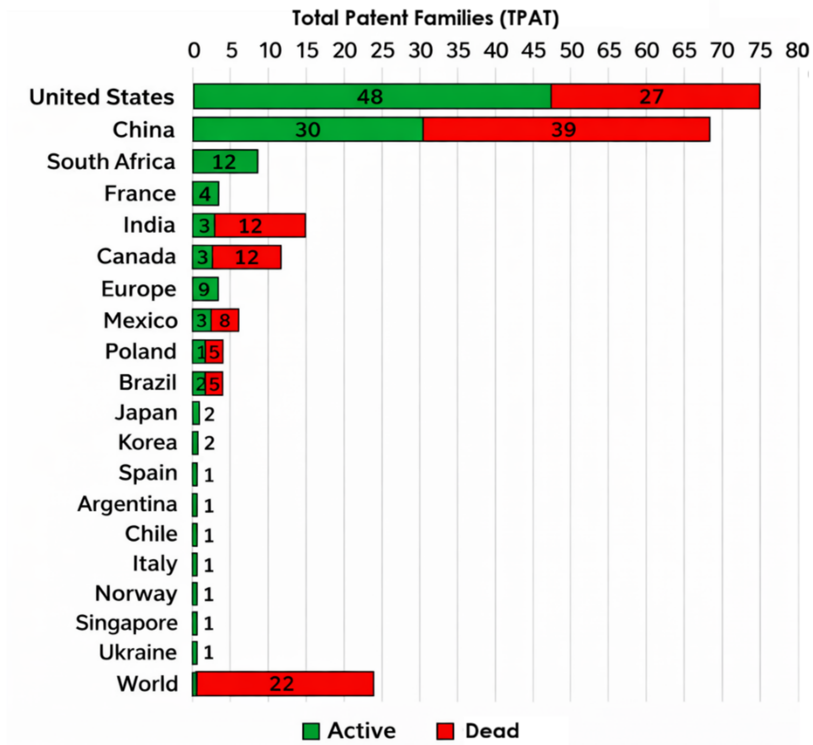

Figure 13. Legal status of patents by jurisdiction. Source: own elaboration

The USPTO (United States) and the CIPC (South Africa) stand out with the highest values in the Technological Relevance Index (VT), indicating a young portfolio with a high proportion of active patents relative to the total number of patents. The USPTO also leads in the Technological Activity Index (AT), which refers to the evolution of patent filings over time, surpassing CNIPA (China), which holds the second position. In terms of the Cooperation Networks (RC) indicator, the CIPC exhibits the highest value, as all its patents correspond to technological developments carried out through international collaboration. In fact, South Africa records more patents developed in cooperation than those produced independently, demonstrating its capacity to attract foreign capital. One example is patent ZA201003407 B, developed collaboratively and still active in the CIPC, which describes a bioreactor with a thermophilic bacterial consortium designed to improve H_2 yield and productivity (52). However, this patent has ceased to be in force in all other jurisdictions where it was filed, remaining active only in South Africa.

In the case of the USPTO, RC reaches 30%, with recent collaborations mostly involving China. One such example is patent US10378028 B2, which proposes a fermentation process under limited oxygen conditions involving interactions between strict anaerobic and facultative bacteria (65). In recent years, U.S. patents have increasingly focused on the valorization of lipid-rich wastes such as fats, oils, and greases for biofuel production (66).

CNIPA (China) has also maintained a steady flow of patents between 2011 and 2022, distributing its technological output across various areas, albeit with a lower level of international cooperation (RC) compared to other offices, reflecting a more domestic market-oriented approach. Among its more recent collaborative patents is CN114058479 A, which describes a fermentation system featuring a dark photosynthetic device and a net-negative carbon balance (59).

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On the other hand, India (IPO) and France (INPI) show lower TPAT indicators compared to China and the United States but have expressed interest in international collaboration to advance through strategic alliances. Notable examples include the Indian patent IN3532MU2013 A on bio-H_2 production through the combination of DF and PF (51)11,2]]},”issued”:{“date-parts”:[[“2015”]]}}}],”schema”:”https://github.com/citation-style-language/schema/raw/master/csl-citation.json”} and the French patent FR3071612 B1, which focuses on monitoring the reaction medium in a DF reactor to prevent ionic strength-related inhibition (67).

Regarding technological relevance (TR), countries with a high proportion of active patents, such as the United States, China, and South Africa, demonstrate emerging technological development and a high level of current interest in the field. These offices concentrate inventions aimed at overcoming key challenges in biological H_2 production, such as process inhibition due to metabolite accumulation, low H_2 production efficiency, and complexity in culture medium preparation (65,68,69). Conversely, offices with a high number of expired patents tend to be those that historically showed a high volume of patenting activity (TPAT) but whose filings are no longer in force or have reached the end of their protection period under current legislation. The United States and China also lead in this regard. The main topics covered by these expired patents include integrated technologies such as H_2 production coupled with PF (70); the use of residual substrates like starch-containing wastewater, cellulose, sludge mixtures, and kitchen waste (33,34,34); the application of biomaterials and nanomaterials (71); and advanced reactor configurations (e.g., bioreactors, sonicated reactors, among others) (54).

Finally, Figure 13 shows Mexico as the leading country in Latin America region with 3 active patents in DF for bio-H_2 production. Latin America is rapidly emerging as a key player in the global green H_2 market, driven by its abundant renewable energy resources, with over 200 projects announced and potential investments exceeding US$120 billion (72). While the region currently relies on gray H_2 for 96% of its production, it is shifting towards green H_2 due to its high potential for low-cost production. Countries such as Mexico, Brazil and Colombia have a strong potential for bio-H_2 alternatives from biomass, but the green H_2 industry is still in its infancy, with only a small fraction of announced projects currently in operation and most of them relying in renewable energy sources, such as solar or wind, to generate H_2 through electrolyzers.

Trend Analysis and Discussion: S-Curve

The technological trend analysis was based on modeling the patent life cycle using the Gompertz function (for scientific publications and patents) and the logistic function (for Technological Domains, TDs), employing Monte Carlo simulations and using the least squares sum as the objective function. The models showed statistically significant fits ($\rho < \alpha$, where $\alpha = 0.05$) and coefficients of determination (R^2) above 90%, validating the use of S-curve models to describe the cumulative evolution of inventions and publications in this field.

The application of the Gompertz model enabled the identification of the phases within the technological life cycle of DF for biohydrogen production, as illustrated by the S-curve in Figure 14: emergence (up to 2007), growth (2007–2012), maturity (from 2012 onward), and saturation forecast (with 90% saturation projected by 2027 and 99% by 2043). Similarly, key indicators were

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estimated with Eqs. 2-4: Technological Maturity Rate (TMR ≈ 83%), Potential Patent Applications (PPA = 22 patents), and Expected Remaining Life (ERL = 33 years). According to this projection, the technology is expected to enter its decline phase around 2055. This behavior indicates an advanced stage of scientific knowledge consolidation regarding DF for H_2 production yet also reveals a disconnection with economic and market feasibility. Despite DF being a technology known since the mid-20th century, its contribution to the global renewable H_2 production market remains limited and insufficient to constitute a paradigm shift. In fact, fermentative H_2 production has been employed for decades, with DF being the primary method for bio-H_2 generation. The presence of hydrogenase enzymes in bacteria, responsible for H_2 production, was demonstrated as early as 1931. Although the basic principles of bacterial fermentation and microbial H_2 production have long been understood and exploited, there has been a significant resurgence of scientific and socioeconomic interest in this area since 2000, resulting in a renewed patent output over time, as indicated in Figure 14.

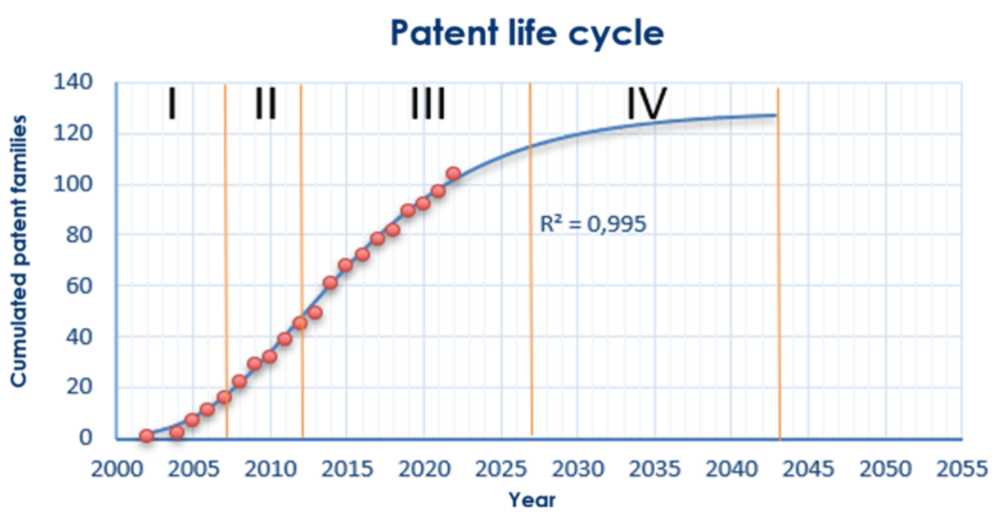

Figure 14. S-curve of the patent life cycle. Source: own elaboration

Table 5 presents a summary of the main types of DF reactors that have generated both scientific knowledge and technical documents such as patents in recent decades. It also highlights the key operational parameters necessary for process implementation, such as reactor volume, substrate type, inoculum source, Hydraulic Retention Time (HRT), temperature, and pH. Finally, the reactors are compared based on criteria essential for process scaling, including substrate consumption (mol H_2/mol substrate) and H_2 production rates (L/day).

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Table 5. Most common bioreactors used in DF processes

Bioreactor (Volume)	Substrate	Inoculum	Operating conditions	Yield (mol H_2/ mol substrate)	Production rate (H_2 in L/day)	Ref
Continuous stirred tank reactor - CSTR (2 L)	Glycerol	Activated sludge from the wastewater treatment plant	HRT: 12 h, 37 °C, pH 5.5	0.58	-	(73)
Continuous stirred tank reactor - CSTR (2.5 L)	Fruit and vegetable waste	Thermotoga maritima DSM 3109	HRT: 24 días, 80 °C, pH 7	3.46	-	(74)
Continuous stirred tank reactor - CSTR (6 L)	Cassava starch and buffalo dung wastewater	Clostridiumsp., Megasphaera sp., and Chloroflexi sp.	HRT: 60 h, 30 °C, pH 5.5	0.165	0.84	(75)
Anaerobic Sequencing Batch Reactor - ASBR (1.3 L)	Organic solids	Sludge from an anaerobic digester in a brewery wastewater treatment plant	HRT: 4.6 a 27 h, 35 °C, pH 5.5, 150 rpm	0.568	1.86	(76)
Anaerobic Sequencing Batch Reactor - ASBR (6 L)	Cassava starch wastewater	Mixed cultures of naturally fermented wastewater and thermally treated anaerobic sludge	HRT: 4 h 30 °C, pH 6, 300 rpm	1.787	0.72	(77)
Upflow Anaerobic Sludge Batch reactor – UASB (6 L)	Wastewater from cardboard factories	Escherichia fergusonii and Enterobacter hormaechei	HRT: 9.6 h, 35 °C, pH 5	1.22	9.38	(78)
Upflow Anaerobic Sludge Batch reactor – UASB (20 L)	Palm oil factory effluents	Seeding sludge	HRT: 6 h, 55 °C, pH 5.5–5.8	2.45	11.75	(79)

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Upflow Anaerobic Sludge Batch reactor – UASB (1 L)	Low concentration wastewater from beverage factory	Anaerobic activated sludge from a municipal wastewater treatment plant	HRT: 3.9–11.4 h, 35 °C, pH 5–6.5	0.172	0.12	(80)

Source: own elaboration

According to Kazimierowicz et al. (81), DF-based H_2 generation presents several fundamental limitations for upscaling. For instance, Table 5 shows that, at industrial scale, mixed cultures (e.g., sludge) are typically preferred due to their practicality in terms of control, operation, and cost, as well as their ability to degrade a broader range of feedstocks. However, H_2 production from mixed cultures is relatively low because of the presence of H_2-consuming microorganisms (such as methanogenic archaea), impurities, and associated gases. Selecting appropriate substrate pretreatment strategies can help mitigate the impact of these factors. Another critical consideration is the HRT, which, at industrial scale, is ideally minimized; however, Table 5 shows retention times of up to 24 days for reactor volumes as small as 2.5 L. Currently, most research and patents have focused on improving production yield and rate by adjusting reactor operating conditions. However, the majority of reactors studied have been designed at laboratory scale and for pure cultures. While these systems have shown promising results, they often fail to consider the technical feasibility of large-scale production, which would involve larger reactor volumes and mixed cultures (82).

In summary, the quantitative S-curve analysis suggests that although DF appears to have reached the maturity phase within its patent life cycle, this trend does not reflect a true state of technological saturation. Rather, DF remains in an experimental and optimization stage, where its perceived maturity is more a consequence of low registration patent density than consolidated development. Although DF is a well-established technology supported by existing infrastructure, its future deployment in H_2 production will depend on overcoming inherent performance and scalability limitations. Moreover, given that the global green H_2 ecosystem remains in an early phase and is generated mostly by solar and wind energy through electrolysis, bio-H_2 continues to be a predominantly an experimental technology, largely confined to universities, research institutes, and R&D departments. Consequently, its transition to sustainable commercial applications will demand disruptive advances in biological catalysis, reactor design, and metabolic optimization, supported by multidisciplinary R&D strategies and public policies geared toward industrial deployment. In this way, DF could emerge as a frontier technology with high potential, whose consolidation will depend on the articulation of scientific innovation, technological scalability, and energy governance.

Limitations of the Study

This study presents several inherent limitations related to patent analysis in emerging technologies such as DF. Patents from the years 2024 and 2025 were not included due to the approximate 18-month delay between the filing and publication of patent applications, which restricts access to recent and complete information. Moreover, the limited number of available patents may have

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influenced the coefficient of determination (R^2) values obtained in the S-curve model, without necessarily reflecting an actual state of technological maturity. Additionally, potential biases may arise from the classification of patents, lack of standardized terminology, and the exclusion of confidential or untranslated data, which could affect the global representativeness of the analysis. Nonetheless, the authors consider that this study provides significant value, as it constitutes the first systematic patent analysis focused on a biological route for H_2 production that is emerging as a cost-effective alternative, potentially well-suited for integration into the existing energy infrastructure of various countries, with particularly relevant potential for the Latin American region.

Conclusions

Hydrogen (H_2) production mediated by microorganisms, particularly through DF, is positioned as a key technology in the transition toward a low-carbon economy. Its main strengths lie in the versatility of usable substrates and the possibility of operating under relatively simple conditions, making it a viable alternative to conventional energy- and carbon-intensive production methods.

The technological life cycle analysis revealed that DF has progressed through the emergence and growth phases and currently stands in a stage of maturity from both scientific and patent perspectives. However, the stagnation observed in indicators such as the total number of patents (TPAT) and the low rate of technological cross-citation (CTC) suggests a slowdown in exploratory innovation, likely attributable to unresolved technical limitations, such as low H_2 conversion efficiency, challenges in industrial-scale implementation and policy-driven decisions related to the development goals of the countries with the most patents in the field.

Furthermore, analysis through IPC classification showed that patented developments in DF have been predominantly concentrated in the technological domain of biotechnology, particularly in areas related to microorganism modification (C12R1/01) and fermentation process optimization (C12P3/00). This indicates that innovative efforts have largely focused on improving microbiological and operational conditions, while aspects such as reactor engineering, energy integration, or the use of advanced catalysts remain relatively underexplored.

The gap between the increase in scientific publications and the relatively small number of active patents also points to a disconnect between academic research and technology transfer, reflecting barriers to the commercial implementation of DF in the global H_2 market. In this landscape, China and the United States stand out as the leading producers of both scientific publications and patents, underscoring their central role in shaping the future trajectory of this technology.

Currently, DF finds itself at a critical juncture. To reach its full potential as a sustainable and economically viable H_2 production technology, it is essential to foster a second wave of innovation focused on technological exploitation, including: i) the design and standardization of robust bioreactors; ii) integration with other biological or electrochemical processes to enhance performance; iii) comprehensive sustainability assessment through Life Cycle Analysis (LCA) and Techno-Economic Assessment (TEA); and iv) validation in relevant pilot-scale environments; (v) business use case for bio-H_2 production.

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Ultimately, coordinated action among academic institutions, the industrial sector, and regulatory bodies is necessary to bridge the gaps between science, technology, and market. Only through a convergent innovation strategy will it be possible to overcome current challenges and position DF as a technological pillar in the future hydrogen-based energy system.

CrediT authorship contribution statement

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References

1. Nunez C. National Geographic [Internet]. 2023 [cited 2025 Nov 2]. Qué son los combustibles fósiles. Available from: https://www.nationalgeographicla.com/medio-ambiente/2023/12/que-son-los-combustibles-fosiles

2. Raturi S, Singh P, Jha SK, Karn SK. Biohydrogen: Opportunities and challenges as an alternative energy resource. J Appl Nat Sci. 2022 Jun 18;14(2):692-701.

https://doi.org/10.31018/jans.v14i2.3480

3. Sarangi PK, Nanda S. Biohydrogen Production Through Dark Fermentation. Chem Eng Technol. 2020 Apr;43(4):601-12.

https://doi.org/10.1002/ceat.201900452

4. Mohtasham J. Review Article-Renewable Energies. Energy Procedia. 2015 Aug;74:1289-97.

https://doi.org/10.1016/j.egypro.2015.07.774

5. Neha, Joon R. Renewable Energy Sources: A Review. J Phys Conf Ser. 2021 Aug 1;1979(1):012023.

https://doi.org/10.1088/1742-6596/1979/1/012023

6. Mishra P, Krishnan S, Rana S, Singh L, Sakinah M, Ab Wahid Z. Outlook of fermentative hydrogen production techniques: An overview of dark, photo and integrated dark-photo fermentative approach to biomass. Energy Strategy Rev. 2019 Apr;24:27-37.

https://doi.org/10.1016/j.esr.2019.01.001

7. Hossain Bhuiyan MM, Siddique Z. Hydrogen as an alternative fuel: A comprehensive review of challenges and opportunities in production, storage, and transportation. Int J Hydrog Energy. 2025 Feb;102:1026-44.

https://doi.org/10.1016/j.ijhydene.2025.01.033

doi: 10.25100/iyc.v28i1.14858

8. Osman AI, Deka TJ, Baruah DC, Rooney DW. Critical challenges in biohydrogen production processes from the organic feedstocks. Biomass Convers Biorefinery. 2023 Jul;13(10):8383-401.

https://doi.org/10.1007/s13399-020-00965-x

9. D' Silva TC, Khan SA, Kumar S, Kumar D, Isha A, Deb S, et al. Biohydrogen production through dark fermentation from waste biomass: Current status and future perspectives on biorefinery development. Fuel. 2023 Oct;350:128842.

https://doi.org/10.1016/j.fuel.2023.128842

10. Zhang Q, Jiao Y, He C, Ruan R, Hu J, Ren J, et al. Biological fermentation pilot-scale systems and evaluation for commercial viability towards sustainable biohydrogen production. Nat Commun. 2024 May 28;15(1):4539.

https://doi.org/10.1038/s41467-024-48790-4

11. Fuentes-Casullo L. Producción de hidrógeno por fermentación oscura y producción de electricidad a partir de suero de queso y otros subproductos industriales [PhD] [Internet]. Universidad de la República (Uruguay). Facultad de Ciencias.; 2020 [cited 2025 Nov 2]. Available from: https://hdl.handle.net/20.500.12008/27666

12. Xiaohu D, Xiaona A, Ying X. Method for stepwise recycling kitchen waste biomass energy based on component metabolism rate [Internet]. CN115572741A, 2023. Available from: https://worldwide.espacenet.com/patent/search/family/084585155/publication/CN115572741A?q=pn%3DCN115572741A%3F

13. Zhisheng Y, Hao W, Bobo W, Hongxun Z, Jie Y. Weak current enhanced hydrogen production process with agricultural wastes as fermentation substrate [Internet]. CN115074394A, 2022. Available from: https://patents.google.com/patent/CN115074394A/en?oq=CN115074394+A

14. 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. Appl Energy. 2015 Apr;144:73-95.

https://doi.org/10.1016/j.apenergy.2015.01.045

15. Fang H, Li C, Zhang T. Acidophilic biohydrogen production from rice slurry. Int J Hydrog Energy. 2006 May;31(6):683-92.

https://doi.org/10.1016/j.ijhydene.2005.07.005

16. Ramprakash B, Lindblad P, Eaton-Rye JJ, Incharoensakdi A. Current strategies and future perspectives in biological hydrogen production: A review. Renew Sustain Energy Rev. 2022 Oct;168:112773.

https://doi.org/10.1016/j.rser.2022.112773

17. Questel. questel.com [Internet]. 2026 [cited 2025 Nov 2]. Orbit Intelligence: IP Intelligence Software. Available from: https://www.questel.com/patent/ip-intelligence-software/orbit-intelligence/

18. WIPO. wipo.int [Internet]. 2025 [cited 2025 Nov 2]. WIPO Pearl - WIPO's Multilingual Terminology Portal. Available from: https://www.wipo.int/en/web/wipo-pearl/

19. Leiden University. VOSviewer [Internet]. Leiden University; 2024 [cited 2025 Nov 2]. Available from: https://www.vosviewer.com/download

20. Galvez C. Análisis de co-palabras aplicado a los artículos muy citados en Biblioteconomía y Ciencias de la Información (2007-2017). Transinformação [Internet]. 2018;30(3).

https://doi.org/10.1590/2318-08892018000300001

21. Donthu N, Kumar S, Mukherjee D, Pandey N, Lim WM. How to conduct a bibliometric analysis: An overview and guidelines. J Bus Res. 2021 Sep;133:285-96.

https://doi.org/10.1016/j.jbusres.2021.04.070

22. Angel-Ospina AC, Machuca-Martínez F. Ozonización catalítica en el tratamiento de Contaminantes de Preocupación Emergente en aguas residuales: Un análisis bibliométrico. Ing Compet. 2021 Dec 29;24(1).

https://doi.org/10.25100/iyc.v24i1.11603

23. Ampah JD, Jin C, Rizwanul Fattah IM, Appiah-Otoo I, Afrane S, Geng Z, et al. Investigating the evolutionary trends and key enablers of hydrogen production technologies: A patent-life cycle and econometric analysis. Int J Hydrog Energy. 2023 Dec;48(96):37674-707.

https://doi.org/10.1016/j.ijhydene.2022.07.258

24. PHE. Loglet Lab [Internet]. Rockefeller University; 2025 [cited 2025 Nov 2]. Available from: https://www.logletlab.com/

25. Yoon J, Park Y, Kim M, Lee J, Lee D. Tracing evolving trends in printed electronics using patent information. J Nanoparticle Res. 2014 Jul;16(7):2471.

https://doi.org/10.1007/s11051-014-2471-6

26. Chen YH, Chen CY, Lee SC. Technology forecasting and patent strategy of hydrogen energy and fuel cell technologies. Int J Hydrog Energy. 2011 Jun;36(12):6957-69.

https://doi.org/10.1016/j.ijhydene.2011.03.063

27. Blanco Londoño SA, Rodríguez Chaparro T. Producción de biohidrógeno a partir de residuos mediante fermentación oscura: una revisión crítica (1993-2011. Ingeniare Rev Chil Ing. 2012 Dec;20(3):398-411.

https://doi.org/10.4067/S0718-33052012000300014

28. Hosseini SE, Abdul Wahid M, Jamil MM, Azli AAM, Misbah MF. A review on biomass-based hydrogen production for renewable energy supply: Biomass-based hydrogen production for renewable energy supply. Int J Energy Res. 2015 Oct 10;39(12):1597-615.

https://doi.org/10.1002/er.3381

29. Sillero L, Sganzerla WG, Forster-Carneiro T, Solera R, Perez M. A bibliometric analysis of the hydrogen production from dark fermentation. Int J Hydrog Energy. 2022 Jul;47(64):27397-420. https://doi.org/10.1016/j.ijhydene.2022.06.083

30. Gadhamshetty V, Arudchelvam Y, Nirmalakhandan N, Johnson DC. Modeling dark fermentation for biohydrogen production: ADM1-based model vs. Gompertz model. Int J Hydrog Energy. 2010 Jan;35(2):479-90.

https://doi.org/10.1016/j.ijhydene.2009.11.007

31. Pradhan N, Dipasquale L, D'Ippolito G, Panico A, Lens P, Esposito G, et al. Hydrogen Production by the Thermophilic Bacterium Thermotoga neapolitana. Int J Mol Sci. 2015 Jun 4;16(6):12578-600.

https://doi.org/10.3390/ijms160612578

32. Ernst B, Clion V, Dumas C. Dispositif de production d'hydrogène [Internet]. WO2017051136A1, 2017 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/WO2017051136A1/fr

doi: 10.25100/iyc.v28i1.14858

33. Li R, Liu X, Ji M, Wu Y, Zhang J. Method for coproducing hydrogen gas and methane by sludge-kitchen waste mixed fermentation [Internet]. CN104372030A, 2015 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/CN104372030A/en

34. Su H, Cheng J, Zhou J, Cen K, Lin H. Method used for producing hydrogen by taking cellulose as raw material [Internet]. CN105713925A, 2016. Available from: https://patents.google.com/patent/CN105713925A/zh

35. Cox M, Nondorf L, Cox S. Anaerobic production of hydrogen and other chemical products [Internet]. US8501463B2, 2013. Available from: https://patents.google.com/patent/US8501463B2/en

36. Fanhe Q, Qingtao Q, Zhiwen Q. A method for producing hydrogen through fermentation using crop straw as raw material [Internet]. CN107012195B, 2022 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/CN107012195B/zh

37. Poege F, Harhoff D, Gaessler F, Baruffaldi S. Science quality and the value of inventions. Sci Adv. 2019 Dec 6;5(12):eaay7323.

https://doi.org/10.1126/sciadv.aay7323

38. REN21. Renewables 2025 Global Status Report Collection - Bioenergy [Internet]. World Bioenergy Association; 2025 [cited 2025 Nov 2]. Report No. Available from: https://www.ren21.net/gsr-2025/technologies/bioenergy/

39. Bilgili M, Tumse S, Nar S. Comprehensive Overview on the Present State and Evolution of Global Warming, Climate Change, Greenhouse Gasses and Renewable Energy. Arab J Sci Eng. 2024 Nov;49(11):14503-31.

https://doi.org/10.1007/s13369-024-09390-y

40. IEA. Energy Technology Perspectives 2023 [Internet]. Paris: IEA; 2023 [cited 2025 Nov 2]. Report No. Available from: https://www.iea.org/reports/energy-technology-perspectives-2023

41. Olivo C, Lebedeva I, Chu CY, Lin CY, Wu SY. A patent analysis on advanced biohydrogen technology development and commercialisation: Scope and competitiveness. Int J Hydrog Energy. 2011 Oct;36(21):14103-10.

https://doi.org/10.1016/j.ijhydene.2011.04.100

42. Słupek E, Kucharska K, Gębicki J. Alternative methods for dark fermentation course analysis. SN Appl Sci. 2019 May;1(5):469.

https://doi.org/10.1007/s42452-019-0488-2

43. Ahmad A, K R, Hasan SW, Show PL, Banat F. Biohydrogen production through dark fermentation: Recent trends and advances in transition to a circular bioeconomy. Int J Hydrog Energy. 2024 Jan;52:335-57.

https://doi.org/10.1016/j.ijhydene.2023.05.161

44. Jaramillo A, Satta A, Pinto F, Faraloni C, Zittelli GC, Silva Benavides AM, et al. Outlook on Synthetic Biology-Driven Hydrogen Production: Lessons from Algal Photosynthesis Applied to Cyanobacteria. Energy Fuels. 2025 Mar 20;39(11):4987-5006.

https://doi.org/10.1021/acs.energyfuels.4c04772

45. Estrada-Arriaga EB, Hernández-Romano J, Mijaylova-Nacheva P, Gutiérrez-Macías T, Morales-Morales C. Assessment of a novel single-stage integrated dark fermentation-microbial fuel cell system coupled to proton-exchange membrane fuel cell to generate bio-hydrogen and recover electricity from wastewater. Biomass Bioenergy. 2021 Apr;147:106016.

https://doi.org/10.1016/j.biombioe.2021.106016

doi: 10.25100/iyc.v28i1.14858

46. Julkipli J, Babel S, Bilyaminu AM, Rene ER. Hydrogen and biodiesel production from food waste: a review. Environ Chem Lett. 2024 Apr;22(2):585-607.

https://doi.org/10.1007/s10311-023-01674-3

47. Akhlaghi N, Najafpour-Darzi G. A comprehensive review on biological hydrogen production. Int J Hydrog Energy. 2020 Sep;45(43):22492-512.

https://doi.org/10.1016/j.ijhydene.2020.06.182

48. Arsad SR, Ker PJ, Hannan MA, Tang SGH, R S N, Chau CF, et al. Patent landscape review of hydrogen production methods: Assessing technological updates and innovations. Int J Hydrog Energy. 2024 Jan;50:447-72.

https://doi.org/10.1016/j.ijhydene.2023.09.085

49. Jishi Z, Wenqian Z, Mengchen Y, Lihua Z. Preparation of Nickel Nanoparticles Doped with Activated Carbon and Their Hydrogen Uptake at Room Temperature [Internet]. CN111137891B, 2021 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/CN111137891B/zh

50. Lihua Z, Haoe M, Lu W, Jinlong Z, Weifang D, Maojuan M. Hydroxyapatite as well as preparation method and application thereof in dark fermentation hydrogen production [Internet]. CN114410695A, 2022 [cited 2025 Nov 2]. Available from: https://eureka.patsnap.com/patent-CN114410695A

51. Lal B, Subudhi S, Ravindra H, Tompala A, Rao P, Choudary N, et al. Process for sequential bio-hydrogen production through integration of dark fermentation process with photo fermentation process [Internet]. IN2013MU03532A, 2015 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/IN2013MU03532A/Scottsdale-AZ-Condo-Rentals.html

52. Myles-Gray V. Bioreactor process for production of hydrogen from biomass [Internet]. ZA201003407B, 2011 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/ZA201003407B/en

53. Debryun R, Dodson J, Pfeiffer R, Szaloczi E, Ulrich G, Weber J. Generation of Hydrogen from Hydrocarbon Bearing Materials [Internet]. US20070248531A1, 2007. Available from: https://patents.google.com/patent/US20070248531A1/en

54. Elbeshbishy E. Enhancement of Biohydrogen and Biomethane Production from Wastes Using Ultrasonication.

55. Lehoux R, Hafez H, Sehdev R, Salt D. Conversion of biomass, organic waste and carbon dioxide into synthetic hydrocarbons [Internet]. US9816035B2, 2017 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/US9816035B2/en

56. Medoff M. Method for quenching an irradiated biomass [Internet]. US9187769B1, 2015 [cited 2025 Nov 2]. Available from: https://www.patbase.com/legal/public/index.php?id=43952875&lang=cn&page=reassignments

57. Yan M, Yu Y, Li L, Wang L, Liu Z, Liu H. Method for improving hydrogen production performance of dark fermentation by utilizing nitrogen doped graphene [Internet]. CN115992186B, 2023. Available from: https://patents.google.com/patent/CN115992186B/zh

58. Crawford H. hlk-ip [Internet]. 2023 [cited 2025 Nov 2]. Hydrogen Patents - Innovations and Trends. Available from: https://www.hlk-ip.com/news-and-insights/hydrogen-patents-innovations-and-trends/

59. Li Y, Zhang Z, Jing Y, Zhang H, Yue J, Jiang D, et al. Système et procédé de fermentation biologique pour la coproduction d’hydrogène et d’alcanes avec émission négative de carbone [Internet]. WO2023087972A1, 2023 [cited 2025 Nov 2]. Available from: https://patents.google.com/

doi: 10.25100/iyc.v28i1.14858

patent/WO2023087972A1/fr

60. Escamilla A, Sánchez D, García-Rodríguez L. Assessment of power-to-power renewable energy storage based on the smart integration of hydrogen and micro gas turbine technologies. Int J Hydrog Energy. 2022 May;47(40):17505-25.

https://doi.org/10.1016/j.ijhydene.2022.03.238

61. Haoxin D, Qiyuan D, Chaojie L, Nian L, Zhang W, Hu M, et al. A comprehensive review on renewable power-to-green hydrogen-to-power systems: Green hydrogen production, transportation, storage, re-electrification and safety. Appl Energy. 2025 Jul;390:125821.

https://doi.org/10.1016/j.apenergy.2025.125821

62. Sustainability Directory. energy.sustainability-directory [Internet]. 2025 [cited 2025 Nov 2]. What Is the "Round-Trip Efficiency" of a Hydrogen Energy Storage System? Available from: https://energy.sustainability-directory.com/learn/what-is-the-round-trip-efficiency-of-a-hydrogen-energy-storage-system/

63. Saady NMC. Homoacetogenesis during hydrogen production by mixed cultures dark fermentation: Unresolved challenge. Int J Hydrog Energy. 2013 Oct;38(30):13172-91.

https://doi.org/10.1016/j.ijhydene.2013.07.122

64. De Gioannis G, Friargiu M, Massi E, Muntoni A, Polettini A, Pomi R, et al. Biohydrogen production from dark fermentation of cheese whey: Influence of pH. Int J Hydrog Energy. 2014 Dec;39(36):20930-41.

https://doi.org/10.1016/j.ijhydene.2014.10.046

65. Xing D, Yu N, Li W, Zhen L, Yang Y. System for hydrogen production under limited aerobic conditions [Internet]. US10378028B2, 2019. Available from: https://patents.google.com/patent/US10378028B2/en

66. Abomohra AEF, Elsayed M, Esakkimuthu S, El-Sheekh M, Hanelt D. Potential of fat, oil and grease (FOG) for biodiesel production: A critical review on the recent progress and future perspectives. Prog Energy Combust Sci. 2020 Nov;81:100868.

https://doi.org/10.1016/j.pecs.2020.100868

67. Trably E, Paillet F, Escudie R, Bernet N, Couhert C. Procédé de contrôle d'un réacteur de fermentation sombre [Internet]. WO2019058073A1, 2017. Available from: https://patents.google.com/patent/WO2019058073A1/fr

68. Hafez H. Method and system for production of hydrogen, methane, volatile fatty acids, and alcohols from organic material [Internet]. CA2919263C, 2021 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/CA2919263C/de

69. Pachapur V, Sarma S, Maiti S. Process for hydrogen production from glycerol [Internet]. US10801043B2, 2019 [cited 2025 Nov 2]. Available from: https://patents.google.com/patent/US10801043B2/en

70. Tang J, Fu P, Zhao Q, Zhao R, Yu P, Ren Q, et al. Dark fermentation-light fermentation coupled biological hydrogen preparing process [Internet]. CN106520842A, 2017. Available from: https://patents.google.com/patent/CN106520842A/zh

71. City Z, Lihua Z, Chuanfang F. A kind of Nanoscale Iron carries charcoal and its preparation and the application in dark fermentation and hydrogen production process [Internet]. CN107858379A, 2018. Available from: https://patents.google.com/patent/CN107858379A/en?oq=CN107858379A\

doi: 10.25100/iyc.v28i1.14858

72. Fonseca P. knowablemagazine.org [Internet]. 2023 [cited 2025 Nov 2]. The rise of green hydrogen in Latin America. Available from: https://knowablemagazine.org/content/article/technology/2023/green-hydrogen-latin-america

https://doi.org/10.1146/knowable-020723-2

73. Silva-Illanes F, Tapia-Venegas E, Schiappacasse MC, Trably E, Ruiz-Filippi G. Impact of hydraulic retention time (HRT) and pH on dark fermentative hydrogen production from glycerol. Energy. 2017 Dec;141:358-67

https://doi.org/10.1016/j.energy.2017.09.073

74. Saidi R, Liebgott PP, Gannoun H, Ben Gaida L, Miladi B, Hamdi M, et al. Biohydrogen production from hyperthermophilic anaerobic digestion of fruit and vegetable wastes in seawater: Simplification of the culture medium of Thermotoga maritima. Waste Manag. 2018 Jan;71:474-84.

https://doi.org/10.1016/j.wasman.2017.09.042

75. Wadjeam P, Reungsang A, Imai T, Plangklang P. Co-digestion of cassava starch wastewater with buffalo dung for bio-hydrogen production. Int J Hydrog Energy. 2019 Jun;44(29):14694-706

https://doi.org/10.1016/j.ijhydene.2019.04.138

76. Santiago SG, Morgan-Sagastume JM, Monroy O, Moreno-Andrade I. Biohydrogen production from organic solid waste in a sequencing batch reactor: An optimization of the hydraulic and solids retention time. Int J Hydrog Energy. 2020 Sep;45(47):25681-8.

https://doi.org/10.1016/j.ijhydene.2019.11.224

77. Andreani CL, Tonello TU, Mari AG, Leite LCC, Campaña HD, Lopes DD, et al. Impact of operational conditions on development of the hydrogen-producing microbial consortium in an AnSBBR from cassava wastewater rich in lactic acid. Int J Hydrog Energy. 2019 Jan;44(3):1474-82.

https://doi.org/10.1016/j.ijhydene.2018.11.155

78. Farghaly A, Tawfik A, Danial A. Inoculation of paperboard mill sludge versus mixed culture bacteria for hydrogen production from paperboard mill wastewater. Environ Sci Pollut Res. 2016 Feb;23(4):3834-46.

https://doi.org/10.1007/s11356-015-5652-7

79. Mahmod SS, Azahar AM, Tan JP, Jahim JM, Abdul PM, Mastar MS, et al. Operation performance of up-flow anaerobic sludge blanket (UASB) bioreactor for biohydrogen production by self-granulated sludge using pre-treated palm oil mill effluent (POME) as carbon source. Renew Energy. 2019 Apr;134:1262-72.

https://doi.org/10.1016/j.renene.2018.09.062

80. Lay CH, Vo TP, Lin PY, Abdul PM, Liu CM, Lin CY. Anaerobic hydrogen and methane production from low-strength beverage wastewater. Int J Hydrog Energy. 2019 May;44(28):14351-61.

https://doi.org/10.1016/j.ijhydene.2019.03.165

81. Kazimierowicz J, Dębowski M, Zieli□ski M. Biohythane Production in Hydrogen-Oriented Dark Fermentation of Aerobic Granular Sludge (AGS) Pretreated with Solidified Carbon Dioxide (SCO2). Int J Mol Sci. 2023 Feb 23;24(5):4442.

https://doi.org/10.3390/ijms24054442

82. Anjum S, Aslam S, Hussain N, Bilal M, Boczkaj G, Smułek W, et al. Bioreactors and biophoton-driven biohydrogen production strategies. Int J Hydrog Energy. 2023 Jun;48(55):21176-88.

https://doi.org/10.1016/j.ijhydene.2023.01.363

doi: 10.25100/iyc.v28i1.14858