

In silico design of biomass-to-hydrogen Pathways: A review

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ABSTRACT

Hydrogen production from biomass has emerged as a promising renewable energy solution. However, significant challenges such as thermodynamic inefficiencies, high raw material costs, low hydrogen molar yields, and difficulties in using lignocellulosic feedstocks hinder its large-scale implementation. Conventional methods have not been able to effectively address these issues, which makes modern approaches, such as *in silico* strategies, essential. This review explores the role of computational models like genome-scale metabolic modeling, synthetic biology, and metabolic pathway reconstruction in overcoming these barriers. By utilizing vast genomic databases and advanced computational tools, researchers can optimize microbial systems, improve hydrogen yields, and design more efficient biohydrogen production processes. These *in silico* methods provide a pathway to enhance the efficiency of biomass processing and enable the development of scalable and sustainable hydrogen production technologies. The review highlights recent advancements and discusses the potential of *in silico* approaches to address key technological and economic limitations, paving the way for the future of biohydrogen energy.

1. Introduction

The increasing global demand for hydrogen as a clean energy source has prompted the exploration of alternative production methods beyond traditional fossil fuels. While hydrogen is a promising fuel due to its high energy density and minimal environmental impact, current production methods face significant challenges, such as high costs and substantial carbon emissions [1]. The need for sustainable and cost-effective hydrogen production is driving the search for new methods, particularly through the utilization of renewable resources such as green biomass, including plant residues, agricultural waste, cyanobacteria, and microalgae [2].

Over the past decade, numerous studies have investigated biochemical and thermochemical pathways for biomass-to-hydrogen conversion. These include dark fermentation, photofermentation,

gasification, and pyrolysis, which have been summarized in several review articles. Such reviews have primarily focused on the technological potential and limitations of these experimental approaches, addressing issues such as feedstock variability, process optimization, and energy efficiency [3]. However, despite their contributions, most existing reviews pay little attention to the integration of computational frameworks that could complement experimental research and accelerate the development of scalable solutions. This gap highlights the need for a more comprehensive analysis of advanced modeling approaches in the context of biomass-to-hydrogen production.

To unlock the full potential of biomass as a hydrogen source, genome-scale metabolic models (GEMs), computational fluid dynamics (CFD), and constraint-based modeling (CBM) offer promising solutions [4]. These *in silico* methods enable precise modeling of complex biological and chemical processes involved in hydrogen production, providing a systematic approach to optimizing metabolic networks and

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Abbreviations		IFP	Interstitial Fluid Pressure
ATP	Adenosine Triphosphate	IPCC	Intergovernmental Panel on Climate Change
BBGE	bioinformatics-Based Gene Editing	LCOH	Levelized Cost of Hydrogen
BSCs	biosynthetic compounds	ME	Metabolic Engineering
CBP	consolidated Bioprocessing	ME-Model	Integrated Model of Metabolism and Macromolecular Expression
CCGT	Combined Cycle Gas Turbine	MFA	Metabolic Flux Analysis
CFD	Computational Fluid Dynamics	MLA	Machine Learning Algorithms
CBM	Constraint Based Modeling	NAD ⁺	Nicotinamide Adenine Dinucleotide (Oxidized form)
EPS	extracellular polymeric substances	PEP	Phosphoenolpyruvate
FDA	Food and Drug Administration	SAR	Structure-Activity Relationship
GDP	Gross Domestic Product	SHF	Separate Hydrolysis and Fermentation
GHG	Greenhouse Gas	SMR	Steam Methane Reforming
GSM	Genome-Scale Metabolic Modeling	SSCF	Simultaneous Saccharification and Co-Fermentation
GCs	general compounds	TO	Thermodynamic Optimization

enhancing yields [5] (Table 1).

Genome-scale metabolic reconstructions, for example, allow the exploration of entire metabolic networks, making it possible to predict microbial behavior and optimize conditions for maximum hydrogen production. These models are particularly powerful when applied to

organisms with complex metabolic pathways, such as algae, which require a detailed understanding of compartmentalization and metabolic interactions to improve biohydrogen production [6].

By utilizing GEMs, CFD, and CBM, researchers can not only optimize biomass conversion processes but also predict and model chemical

Table 1
Computational approaches for optimization of hydrogen production from biomass sources.

Strategy	Strain	Characteristics	Substrate	H ₂ yield	Economic feasibility	Advantages	Disadvantages	Ref.
MFA	<i>Escherichia coli</i> (engineered strain)	Mesophilic, genetically engineered for enhanced hydrogenase enzyme activity, facultative anaerobe	Glucose, organic waste	2.11 mol H ₂ /mol glucose	Reduces capital expenditure on reactor design by improving fluid flow, mass transfer, and mixing within bioreactors, thus lowering operational inefficiencies	Optimized bioreactor designs for increased gas yield; Easily scalable - Established genetic modification protocols	Limited native hydrogen production capacity; Requires genetic modifications for higher yields; Sensitive to pH variations	[73]
GSM	<i>Rhodobacter capsulatus</i> JP91	Photosynthetic, capable of anaerobic hydrogen production via nitrogenase enzyme, flexible metabolic pathways	Organic acids, acetate, malate	5,5 mol H ₂ /mol substrate	Maximizes economic viability through targeted metabolic engineering, optimizing the nitrogenase enzyme pathway for higher hydrogen yields	High hydrogen yields in the presence of light; Metabolically versatile; Capable of hydrogen production under anaerobic conditions	Requires continuous light energy, making the process energy-intensive; Sensitive to oxygen exposure, which inhibits nitrogenase activity	[74]
CBM	<i>Clostridium butyricum</i>	Obligate anaerobe, capable of fermentative hydrogen production, models optimized using flux balance analysis (FBA) and metabolic flux analysis (MFA)	Lignocellulosic hydrolysates, glucose	3.2 mol H ₂ /mol glucose	Improves economic feasibility by predicting metabolic bottlenecks and suggesting genetic or process-level modifications to maximize hydrogen yields without extensive laboratory trials	Accurate prediction of flux distributions; Enables targeted strain engineering; Reduces experimental costs and time	Dependent on model quality and completeness; Poor prediction in novel conditions; Requires extensive curation and validation	[75]
MLA	<i>Enterobacter aerogenes</i>	Facultative anaerobe, robust and versatile, engineered for enhanced hydrogenase activity, tolerant to a wide range of substrates	Glucose, glycerol, waste biomass	2,63 mol H ₂ /mol glucose	Predictive modeling through machine learning reduces costs associated with trial-and-error experiments by identifying optimal fermentation conditions, substrate concentrations, and enzyme efficiencies	Capable of high hydrogen production from various substrates; Tolerant to environmental fluctuations; Easily engineered	Dependent on large, high-quality datasets for accurate machine learning predictions; Model accuracy decreases with novel substrates or environmental conditions	[76]
CFD	<i>Chlamydomonas reinhardtii</i>	Photosynthetic microalgae, hydrogen production through hydrogenase activity, optimal under sulfur deprivation	Organic acids, acetate	9.4 mol H ₂ /mol substrate	Computational fluid dynamics enhances economic viability by reducing unwanted by-products, such as organic acids, and channeling more resources towards hydrogen production	Renewable hydrogen production via photosynthesis; Potential for large-scale, open-pond cultivation; Low operational costs in suitable climates	Low hydrogen production under normal conditions; Requires sulfur deprivation or stress conditions to induce hydrogenase activity; Sensitive to light and temperature variations	[77]

Note: MFA, metabolic flux analysis; GSM, genome-scale metabolic modeling; CBM, contrast-based modeling; MLA, machine learning algorithms; CFD, computational fluid dynamics.

reactions at the molecular level [7,8]. These computational tools complement each other by offering distinct insights into metabolic engineering [9]. GEMs provide a comprehensive approach to understanding the full metabolic capacity of an organism, while flux balance analysis (FBA) and CBM focus on optimizing the flow of metabolites through key pathways, identifying bottlenecks, and suggesting potential genetic modifications [10]. CFD, in turn, enables the optimization of bioreactor conditions by simulating fluid dynamics and mass transfer processes, which are crucial for large-scale implementation [11]. An illustrative example is the metabolic engineering of the thermophilic cellulolytic bacterium *Caldicellulosiruptor bescii*, which has been studied for its ability to degrade plant biomass at high temperatures without the need for pretreatment. Using a genome-scale metabolic model of *C. bescii*, researchers identified key pathways for hydrogen and ethanol production, achieving nearly a two-fold increase in yields [12]. This example demonstrates how genome-scale modeling, when combined with other computational techniques, can provide mechanistic insights into metabolic processes, thereby enabling more efficient biofuel production.

Furthermore, recent studies have demonstrated that GEMs, machine learning algorithms, and integrated omics approaches can significantly improve strain design and metabolic pathways for biohydrogen production [13]. For instance, a recent study [14] used deep learning to identify a gene cluster encoding the LDH/EtfAB complex, which may play a role in lactate oxidation metabolism in *Clostridium tyrobutyricum*. Understanding this pathway opens up the possibility of utilizing lactate and acetate as novel substrates for hydrogen production, thereby improving overall yields. These advancements underscore the growing importance of *in silico* frameworks in overcoming the economic and technical barriers of conventional systems, thus advancing the field of biohydrogen production.

The novelty of this review lies in bridging this identified research gap. Unlike previous reviews that have largely emphasized experimental strategies, feedstock analysis, or process-level optimization, this article provides a comprehensive examination of *in silico* approaches, including GEMs, CFD, and CBM, and their integration in biomass-to-hydrogen research. By systematically analyzing how these methods complement each other, we elucidate their unique role in addressing the challenges of large-scale hydrogen production. Specifically, this review highlights how GEMs can reveal organism-wide metabolic capacities, how CBM

and FBA can identify and resolve pathway bottlenecks, and how CFD can optimize reactor design and operation. Taken together, these approaches provide a holistic framework for overcoming both technical and economic barriers, setting this review apart from previous ones [15].

2. Advancements and challenges in biohydrogen production from green biomass

Hydrogen production from green biomass has become increasingly relevant in the context of transitioning to sustainable and environmentally friendly energy sources. Green biomass includes microalgae, cyanobacteria, lignocellulosic materials [16], which are not only widely available but also have significant potential to reduce carbon emissions and enhance energy security (Fig. 1).

2.1. Biochemical pathways for biohydrogen production

Biohydrogen production from green biomass, particularly from microalgae and cyanobacteria, has gained significant attention in the context of sustainable energy solutions [19]. These microorganisms can harness solar energy to produce hydrogen through bio-photolysis, a process that occurs during photosynthesis [20]. The process can be categorized into direct bio-photolysis and indirect bio-photolysis, each with its own advantages and limitations [21]. In direct bio-photolysis, microalgae such as *Chlamydomonas reinhardtii* and cyanobacteria like *Synechocystis* directly convert water and carbon dioxide into hydrogen and oxygen when exposed to light [22,23]. The simplicity of microalgae's nutritional needs and their efficient CO₂ fixation make them ideal for hydrogen production, though the hydrogen yields remain low due to the inefficiency of the enzymatic pathways involved [24]. In contrast, indirect bio-photolysis separates oxygen production from hydrogen generation, allowing for higher yields by preventing oxygen inhibition of hydrogenase enzymes. This method, used by cyanobacteria, first generates oxygen and organic compounds through photosynthesis, followed by hydrogen production in a light-independent phase [25]. While this method circumvents some limitations of direct bio-photolysis, it requires more complex systems and incurs higher capital and operational costs [26].

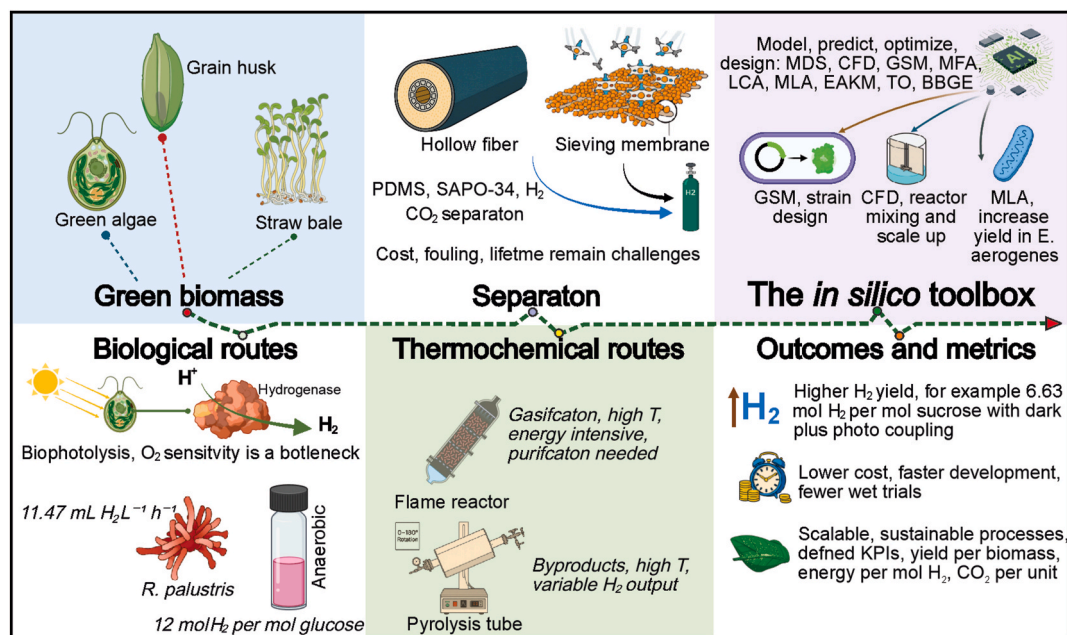


Fig. 1. Green biomass to hydrogen: biological and thermochemical routes enhanced by *in silico* modeling [17,18]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

In light fermentation, microalgae and cyanobacteria produce hydrogen under anaerobic conditions, utilizing light to decompose volatile solids such as lipids and glycerol with nitrogenase and hydrogenase enzymes [27]. On the other hand, dark fermentation involves the breakdown of macromolecular sugars by anaerobic microorganisms, generating hydrogen and byproducts such as acetate and CO₂ [28]. However, dark fermentation faces several limitations, including reduced efficiency due to the formation of byproducts like volatile fatty acids and alcohols. The high cost of substrates and the low resilience of these systems to contamination make them difficult to scale for large applications [29]. While the combination of light and dark fermentation can improve the hydrogen yield, enhancing the substrate conversion and reducing waste generation, this approach still faces high energy demands and challenges in controlling system parameters such as light intensity and organic compound concentration [30]. For example, research by Ref. [31] used corn straw enzymatic hydrolysate as a substrate, achieving a significant increase in hydrogen production (47.5 mL/g). The high energy input and the complexity of the processes involved remain significant barriers to scaling these technologies [32].

Despite the promising environmental benefits of biohydrogen production from microalgae and cyanobacteria, several challenges remain that hinder its industrial-scale implementation

One key limitation is the low hydrogen yield in the absence of light, which limits productivity in real-world conditions [33]. This is particularly problematic in continuous production systems where light availability may fluctuate. For example, a study [34] demonstrated that the hydrogen production rates of cyanobacteria like *Synechocystis* significantly decreased when exposed to prolonged periods without light, underscoring the dependence of this process on light conditions. A further challenge lies in the oxygen sensitivity of hydrogen-producing enzymes, such as hydrogenases and nitrogenases [35]. These enzymes are essential for catalyzing the production of hydrogen, but they are inhibited by oxygen, which is often present in the natural and industrial environments where biohydrogen is produced [36]. This issue significantly reduces the efficiency of hydrogen production. A study [37] found that the presence of oxygen can lead to the inactivation of hydrogenase enzymes in cyanobacteria, decreasing the overall yield of hydrogen.

Scaling up the biohydrogen production process from laboratory experiments to industrial levels also presents significant difficulties [38]. While small-scale systems have demonstrated the feasibility of biohydrogen production, large-scale bioreactor systems capable of maintaining optimal conditions for microbial growth are required to achieve commercially viable hydrogen yields. According to Ref. [39], bioreactor designs were explored to address the challenges of scaling up, with particular emphasis on the need for systems that can efficiently handle larger volumes while maintaining stable microbial growth and hydrogen production. Another hurdle is contamination resilience. In microbial communities used for biohydrogen production, the introduction of non-target organisms can disrupt the microbial ecosystem, lowering hydrogen yields [40]. For instance, contamination by competitive microorganisms may outcompete the hydrogen-producing strains, leading to a decrease in efficiency. A study [41] highlighted how contamination by *E. coli* in microbial fermentation systems negatively impacted the hydrogen production rate. Therefore, optimizing the microbial communities for increased resilience to contamination is critical to maintaining the stability of the process and ensuring consistent hydrogen output [42]. Efficient hydrogen purification is also a crucial factor for the success of large-scale biohydrogen production. The separation of hydrogen from other gases, such as oxygen and carbon dioxide, requires advanced separation technologies [43].

Addressing these challenges is essential to improving biohydrogen production and achieving its commercial feasibility. *In silico* methods provide valuable solutions to many of these issues. Computational modeling, researchers can enhance enzymatic efficiency by identifying optimal conditions for hydrogenase and nitrogenase activity, optimize

bioreactor designs, helping scale up the process and ensure consistent hydrogen production at larger volumes [44]. Computational tools also enable the design of more resilient microbial communities by simulating different environmental conditions and selecting strains with superior resistance to contamination [45]. By leveraging these computational methods, the development of more efficient and scalable biohydrogen production systems can be accelerated, ultimately paving the way for biohydrogen to become a viable and sustainable energy source [46].

2.2. Thermochemical pathways for biohydrogen production

Lignocellulosic biomass, such as sugarcane bagasse, corn stalks, rice straw, and potato peels, is an abundant and promising feedstock for biohydrogen production [47]. These materials, which consist of cellulose, hemicellulose, and lignin, serve as the structural components of plant cell walls [48]. The challenge in using lignocellulosic biomass for hydrogen production lies in the degradation of these complex polymers into fermentable sugars [47]. Several methods have been explored to unlock the potential of lignocellulosic biomass, but these processes face significant challenges in terms of energy consumption, cost, and efficiency [49]. One method of biohydrogen production from lignocellulosic biomass is gasification, which involves converting the biomass at high temperatures (700–1000 °C) in an oxygen-limited environment. The process produces hydrogen along with carbon monoxide and carbon dioxide. However, gasification requires high energy inputs, and the produced gas mixture often contains impurities that must be purified before use [50]. As noted in Ref. [51], the energy required for gasification is a major limitation, since maintaining high temperatures consumes substantial energy, reducing process sustainability. Moreover, the purification step increases costs and raises environmental concerns due to carbon dioxide emissions. Another thermochemical approach, pyrolysis, involves heating the lignocellulosic material in the absence of oxygen to produce biochar, bio-oil, and gases [52]. While pyrolysis has the advantage of producing biochar, which can be used for soil amendment, it may not always produce sufficient hydrogen. For instance, the pyrolysis of rice husk biomass produced a significant amount of biochar; however, the hydrogen yield was not optimal [53]. Pyrolysis also generates byproducts that require proper disposal, further increasing the overall processing costs [52].

Biological methods, such as fermentation, offer a more environmentally friendly approach to biohydrogen production [54]. However, lignocellulose contains lignin, which is difficult to degrade. To break down lignin, specialized enzymes or chemical treatments are required, making fermentation a complex and expensive process. The fermentation of lignocellulosic biomass by mixed microbial cultures showed promise in producing hydrogen; however, the slow kinetics of the reaction and the need for pretreatment to degrade lignin added to the overall cost and complexity of the process [55]. The heterogeneity of lignocellulosic feedstocks is a significant challenge. Agricultural residues like straw, which often contain high moisture content, require additional steps such as drying before they can be processed. This extra treatment increases both energy and financial costs. In this study, it was highlighted that moisture content in straw can significantly hinder pyrolysis or gasification, requiring pre-drying and additional treatments [56].

In silico methods can play a crucial role in overcoming these barriers. GEMs can be used to simulate microbial metabolism on a genome wide scale, predicting how microorganisms will degrade lignocellulosic biomass and produce hydrogen. For example [57], used GEMs to optimize *Escherichia coli* for biohydrogen production from glucose. These models can be adapted to lignocellulose degradation, improving the efficiency of hydrogen production by optimizing metabolic pathways. In addition to GEMs, metabolic pathway optimization can be employed to design microorganisms with enhanced capabilities for hydrogen production. For instance, Omer et al. (2025) used computational tools to improve the hydrogen production potential of *C. acetobutylicum*, a

bacterium known for its ability to degrade lignocellulosic biomass. By simulating and optimizing the microbial pathways, they were able to enhance hydrogen yield [58]. While lignocellulosic biomass offers significant potential for biohydrogen production, the challenges of pretreatment, slow biological reactions, and feedstock heterogeneity remain major obstacles. *In silico* methods hold promise in addressing these issues by improving the efficiency of microbial systems, reducing pretreatment costs, and enhancing overall hydrogen yield. These approaches can make biohydrogen production from lignocellulosic biomass more economically viable and environmentally sustainable [59].

3. *In silico* strategies in biohydrogen production: metabolic modeling and process optimization

In silico strategies based on computational modeling, simulations, and optimization represent a key tool for enhancing the efficiency and economic feasibility of hydrogen production from biomass [60]. These approaches allow for the exploration of various scenarios without the need for costly and time-consuming experiments and can significantly accelerate the development of new technologies [61]. The key *in silico* methods include CFD, genome-scale metabolic modeling (GSM), CBM, metabolic flux analysis (MFA) (Fig. 2).

These methods not only enable the study of molecular mechanisms underlying microbial metabolism but also facilitate the development of new approaches for engineering microbial cell factories [64].

3.1. Genome-scale metabolic modeling (GSM)

GSMs are large-scale stoichiometric models used to describe metabolic pathways through stoichiometric coefficients and mass balances of participating metabolites. These models are simulated using numerical optimization techniques [65]. The primary objective of GSMs is to provide a comprehensive understanding of biochemical conversions within a living cell or organism, including transport and non-enzymatic reactions [66]. Due to their ease of implementation and high predictive power, GSMs are valuable tools for metabolic engineering and strain development [67]. These models also serve as a means to generate and prioritize hypotheses, complementing experimental research and

guiding further experimentation [68]. Additionally, GSMs can offer mechanistic explanations for experimental results [69]. Genome-scale metabolic modeling is a rational approach used to enhance the production of secondary metabolites. It predicts global metabolic flux distributions under specific genetic and environmental conditions. Also known as large-scale stoichiometric modeling, GSMs describe metabolic pathways experimentally and theoretically, using numerical optimization of stoichiometric coefficients and mass balances [70]. These models are often assumed to be in a pseudo-steady state, addressing issues such as predicting the most efficient pathway for maximizing target bio-products, optimal precursor supply, and intracellular redox balance. GSMs can also predict the effects of gene knockouts and gene over-expression [71]. Due to their predictive capabilities, GSMs are instrumental in gene manipulation for metabolic engineering aimed at increasing secondary metabolite production. Various software programs are available for the rapid reconstruction of draft genome-scale models for different species [70]. GSMs can also identify the requirements of various supplements and biosynthetic compounds (BSCs) for high yield of extracellular polymeric substances (EPS). While many general compounds (GCs) have been detected using these models, incorporating biosynthetic reactions into metabolic models remains a challenge. To fully assess the production potential of EPS using these models, more information on the biosynthetic reactions is required [71]. For example, the production of cyanobacterial EPS is influenced by carbon fixation rates and light utilization efficiency, which are intrinsic properties of individual strains [72]. Several studies have aimed to modify genes responsible for higher CO₂ fixation and light utilization efficiency [73, 74].

GSMs are considered an essential tool in systems biology, integrating metabolic reactions in the form of mass balance equations [75]. Additionally, genomic, transcriptomic, proteomic, and cellular composition data are incorporated into model construction [76]. Protocols for building genome-scale metabolic models have been outlined [77]. These models have been employed for microbial production in *Escherichia coli* [78], yeast [79], and mammalian cells [80]. In microalgae, the first genome-scale model was established for *Chlorella pyrenoidosa* [81]. These models can reduce processing costs by optimizing nutrient supply and enhancing lipid productivity. For instance, the genome-scale model iCZ946 for *Chlorella vulgaris* includes 946 genes, 2294 reactions, 1770

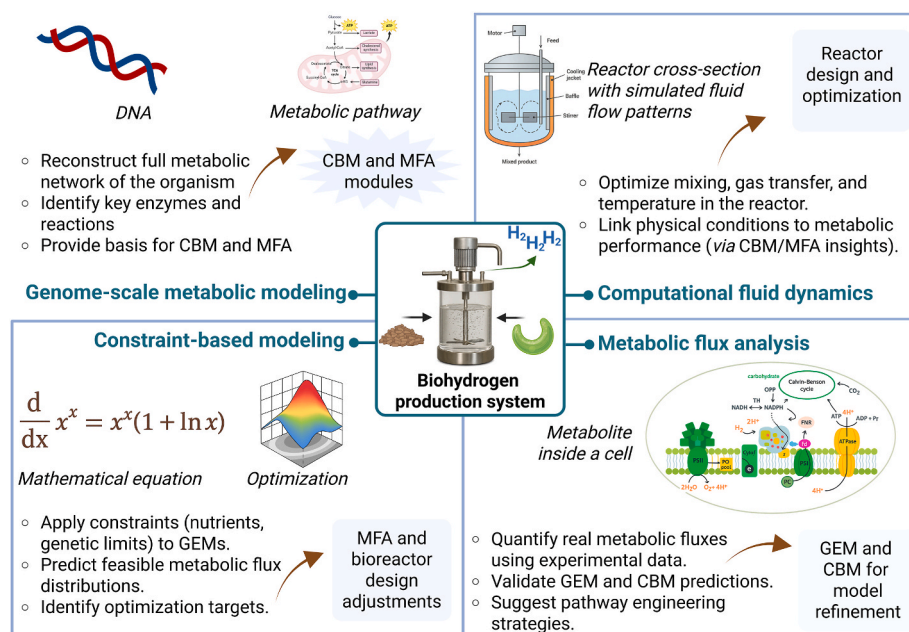


Fig. 2. Key *in silico* methods for enhancing hydrogen production from biomass [58,62,63]. Note: CFD – Computational Fluid Dynamics, GSM – Genome-Scale Metabolic Modeling, MFA – Metabolic Flux Analysis, CBM – Constraint-based modeling. Created in BioRender. Agreement number: BY28MJE336 LJHE.

metabolites, and 5 cellular compartments [82]. This model-driven approach can save 18 % nitrate, while double biomass yield and increasing FA methyl ester yield by 61 %, which improves microalgal lipid production economics [83].

Recently, the automated algorithm software pipeline (RAPS) was developed, enabling the creation of genome-scale metabolic networks for algae within an hour [84]. RAPS-based models capture more genes and are more predictive of experimentally determined growth rates, marking a new paradigm for high-quality metabolic modeling. Furthermore, RAPS facilitates the creation of highly predictive models with greater accuracy [84]. GSMs have been developed for various prokaryotic and eukaryotic organisms. Based on the genome of *Chlamydomonas reinhardtii*, the AlgaGEM model integrates 866 unique open reading frames, 1862 metabolites, and 2249 gene-enzyme-reaction associations, along with 1725 unique reactions [85]. This model accounts for compartmentalization within the cytoplasm, mitochondria, plastids, and microbodies, using data from *Arabidopsis thaliana* to enhance model accuracy. AlgaGEM accurately represents the primary metabolism of *Chlamydomonas*, simulating critical processes like photorespiration and phosphoglycolate catabolism. The model has been validated through simulations under autotrophic, heterotrophic, and mixotrophic conditions, showing that disruption of cyclic electron flow increases hydrogen production, consistent with experimental findings from high-producing mutants [86]. AlgaGEM serves as a comprehensive framework for the *in silico* functional analysis of algal metabolism. By simulating metabolic behaviors and predicting the effects of genetic modifications, such as disrupting cyclic electron flow, AlgaGEM aids in targeting specific pathways for optimization. This model enables significant improvements in biohydrogen production efficiency by providing insights into key metabolic processes [85]. Furthermore, metabolic pathway reconstruction, including the addition of new reactions, enhances the efficiency of target product synthesis. A quantitative *in silico* study of pyruvate metabolism in *Carboxydothermus hydrogenoformans* Z-2901 highlighted the importance of enzyme functions for optimizing energy production (ATP and NAD⁺), demonstrating how metabolic modeling can be used to optimize growth rates and hydrogen synthesis [87].

3.2. Computational fluid dynamics (CFD)

CFD is an advanced simulation technique essential for optimizing hydrogen production from biomass. By modeling the behavior of fluids and gases within bioreactors, CFD allows for the analysis of reaction kinetics, heat and mass transfer, and flow patterns. These factors are critical for maximizing production efficiency [88]. In biohydrogen production, CFD can optimize reactor designs, improve gas distribution, and reduce energy losses, ultimately enhancing hydrogen yields [89]. This *in silico* approach provides valuable insights into system dynamics, offering a cost-effective way to fine-tune production processes and accelerate the scaling of biohydrogen production technologies [88]. CFD has been used extensively in studying the pyrolysis kinetics of lignocellulosic biomass. A study focused on agave bagasse simulated the multi-step process of pyrolysis, including drying, devolatilization, tar cracking, and subsequent char gasification reactivity (both homogeneous and heterogeneous reactions). The pyrolysis process, simulated across temperatures ranging from 100 to 1000 °C, examined the release of non-condensable gases (H₂, CH₄, CO, and CO₂) and the evolution of char, analyzing its composition in terms of carbon, hydrogen, oxygen, and ash content. The simulation results indicated that char produced at temperatures above 700 °C was primarily composed of carbon and ash, with values exceeding 72.85 wt% and 12.39 wt%, respectively, confirming the experimental data. The subsequent oxidation process, both isothermal and non-isothermal, demonstrated how varying the heating rate and temperature affected the reactivity rate of chars, with the rate increasing from 0.0080 min⁻¹ at 850 °C to 0.0129 min⁻¹ at 1000 °C. These findings were consistent with experimental results, emphasizing the accuracy of the CFD models for predicting gas and char evolution

during pyrolysis [90]. Additionally, CFD coupled with the discrete element method (CFD-DEM) was employed to study biomass gasification in a three-dimensional fluidized bed. The simulation included heat transfer, as well as both homogeneous and heterogeneous chemical reactions. The effects of operating parameters, such as equivalence ratio (ER), steam-to-biomass mass flow ratio (S/B), and gasification temperature, were analyzed. The results showed that increasing ER and S/B while decreasing gasification temperature led to a decrease in methane and carbon monoxide concentrations in the product gas. The mole fraction of hydrogen in the product gas was directly influenced by changes in the gasification temperature and S/B. As gasification temperature and ER increased, the carbon conversion efficiency improved, whereas at higher S/B values, the efficiency initially increased but then decreased. These insights into gasification conditions are crucial for optimizing the production of hydrogen and other valuable gases during biomass conversion [91].

CFD has also been integrated into metabolic modeling studies. For example, a detailed *in silico* study of pyruvate metabolism in *C. hydrogenoformans* Z-2901 revealed that regulating the concentration of phosphoenolpyruvate (PEP) could significantly alter energy fluxes, including ATP and NAD⁺ synthesis. By optimizing kinetic parameters and metabolite concentrations, this modeling approach demonstrated that introducing specific enzyme functions could enhance hydrogen production by increasing ATP synthesis rates. The optimized model suggested that a PEP concentration of 5 mM maximized ATP and NAD⁺ production, which directly influenced hydrogen synthesis. This model underscores the power of combining CFD with metabolic engineering to improve biohydrogen production by optimizing metabolic pathways [92]. Through CFD simulations, critical operational parameters and reaction kinetics can be fine-tuned to enhance hydrogen production from biomass [93]. By leveraging this *in silico* technique, researchers gain valuable insights into both the physical and biochemical processes that govern biomass conversion, facilitating the development of more efficient and scalable biohydrogen production systems [92].

3.3. Constraint-based modeling (CBM)

CBM is a powerful approach used to optimize metabolic networks, particularly in the context of biohydrogen production from biomass [94]. This approach includes methodologies like FBA and MFA, which enable the detailed modeling of metabolic pathways, the identification of bottlenecks, and the optimization of metabolic fluxes to enhance production efficiency [95]. FBA is a key tool within CBM that uses mathematical models to predict the flow of metabolites under various constraints and growth conditions, such as autotrophic, heterotrophic, or mixotrophic environments [96]. FBA helps identify limiting metabolic pathways and provides insights into how metabolic networks can be optimized to increase hydrogen production. The technique assumes that the system is in a steady-state condition, allowing the identification of optimal flux distributions that maximize the objective function, typically biomass production rate. It uses linear programming to determine the optimal flux vector that satisfies constraints, such as the irreversibility of certain reactions and the capacity limits of metabolic pathways [97]. FBA has been instrumental in guiding genetic modifications aimed at enhancing hydrogen production, particularly in photofermentative processes and in strict anaerobes, highlighting its versatility in biohydrogen research [98].

MFA, on the other hand, offers a more detailed, quantitative approach by measuring actual flux distributions within metabolic networks. MFA utilizes experimental data, such as stable isotope labeling, to determine the fluxes of metabolites and gain insights into the biochemical limitations of metabolic networks [99]. This method allows researchers to identify potential points of intervention to optimize hydrogen production. MFA has been applied to a variety of systems, such as analyzing hydrogen photoproduction in *Synechocystis* sp. PCC6803, where it was used to determine an upper limit for hydrogen

production and identify a wide margin for improvement. Furthermore, MFA was used to define a feasible operating space for hydrogen production that avoids oxygen inhibition, a critical factor in the cost-effectiveness of hydrogen production [100]. Additionally, MFA based on FBA can guide genetic modifications for optimizing hydrogen production, mainly through the disruption of endogenous genes rather than introducing novel cellular activities [101].

The combination of FBA and MFA, both as part of the CBM framework, allows for the fine-tuning of metabolic networks to overcome bottlenecks in hydrogen production. These methods enable researchers to optimize microbial strains for more efficient biohydrogen production under a range of environmental conditions [102]. By applying CBM techniques, metabolic engineering can target specific metabolic pathways for optimization, enhancing hydrogen yields and making biohydrogen production more sustainable. CBM techniques do not rely on detailed kinetic parameters, which are often absent in many metabolic models. Instead, these models focus on stoichiometric data, such as reaction coefficients, biomass composition, and ATP requirements. This makes CBM particularly useful in the absence of comprehensive kinetic data for metabolic reactions. The lack of unique solutions in CBM due to the greater number of variables than equations means that these approaches search for physiologically relevant solutions that maximize biomass production or hydrogen yield. Such optimization provides a robust framework for understanding cellular metabolism, predicting cellular behavior, and guiding experimental designs [103]. Another powerful application of CBM is the integration of **machine learning algorithms (MLA)** to predict and analyze metabolic data, enhancing predictions for hydrogen yield. For example, MLA was employed to analyze data from *Enterobacter aerogenes*, leading to an increased hydrogen yield of 3.2 mol H₂ per mol of glucose [104]. This integration

of machine learning with metabolic modeling further enhances the precision and applicability of CBM in optimizing biohydrogen production.

In summary, CBM, through techniques such as FBA and MFA, plays a crucial role in optimizing metabolic networks for biohydrogen production. These methods enable the identification of metabolic bottlenecks, guide genetic modifications, and provide insights into the optimization of metabolic pathways, making them indispensable tools in metabolic engineering for sustainable bioenergy solutions. These achievements highlight the potential of *in silico* approaches for the development and optimization of microbial hydrogen production processes. The combined use of these strategies offers the opportunity to enhance the efficiency of existing methods, making them a crucial direction in biotechnological research [105].

4. Evaluating the impact and future prospects of *in silico* approaches in biohydrogen production

Many of the challenges mentioned earlier, such as high costs, scalability issues, and process inefficiencies, can be effectively addressed using *in silico* approaches. *In silico* approaches have revolutionized biohydrogen production by providing powerful tools for optimizing and accelerating the process. These computational methods not only reduce the time and costs associated with traditional experimental methods but also offer enhanced predictive capabilities and precision in modeling metabolic pathways and reactor designs [106]. As biohydrogen production technologies advance, *in silico* approaches continue to offer unique advantages that will play a significant role in the future of sustainable energy (Fig. 3).

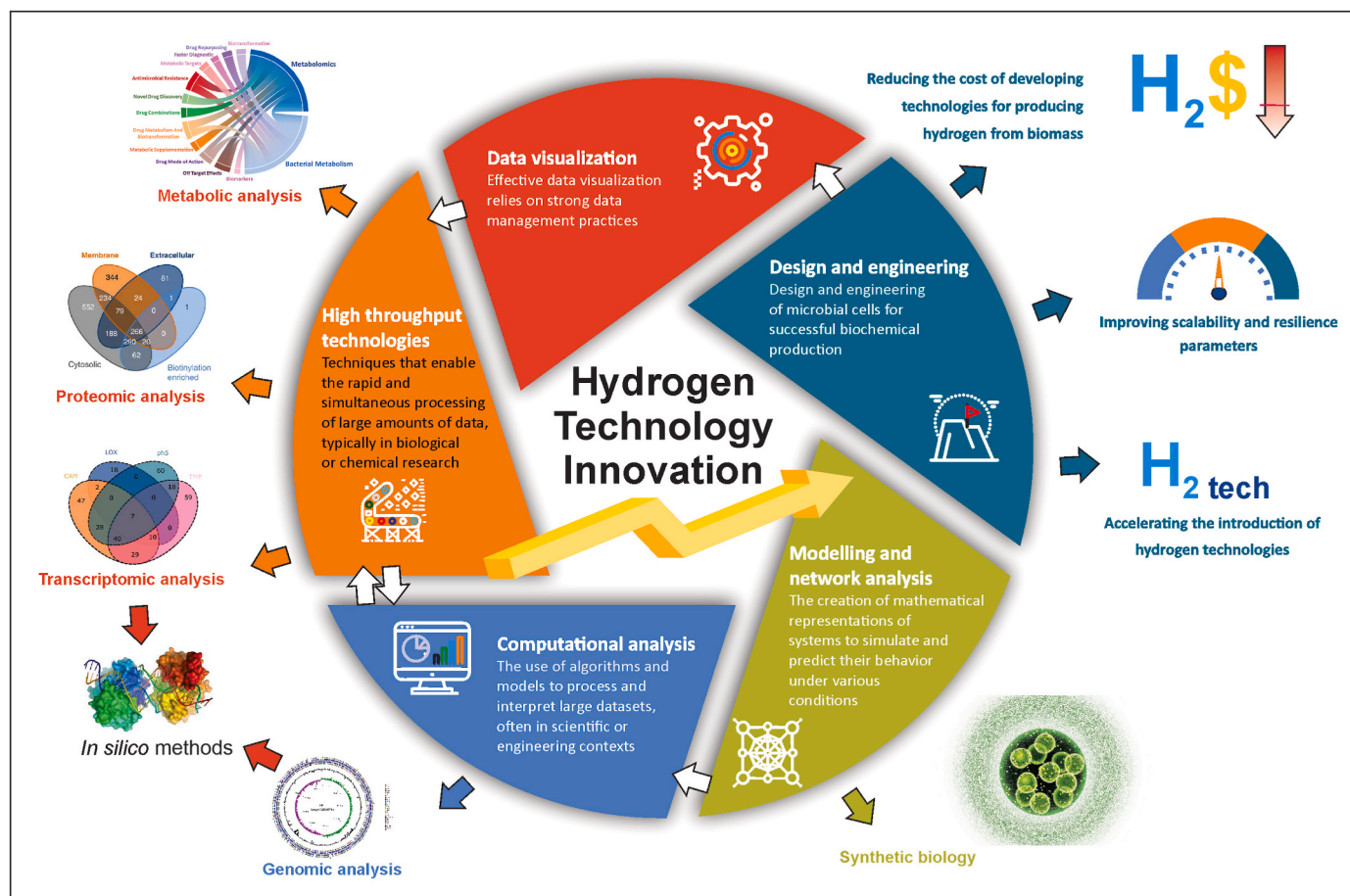


Fig. 3. Advantages and prospects of *in silico* approaches in hydrogen production [94,95].

4.1. Cost reduction in technology development

These methods substantially reduce costs associated with experimental research and process optimization [107]. Conventional catalyst design, reactor engineering, and biomass conversion require numerous costly and time-consuming laboratory experiments [108]. Computational modeling simulates and evaluates multiple scenarios virtually, reducing physical experiments [109]. This lowers direct expenditures and accelerates technology development. This reduces the number of required experiments, which, in turn, lowers research costs and accelerates the development process. Identifying efficient microorganisms with selectively high cellulolytic and hemicellulolytic activities and understanding their growth dynamics during lignocellulose degradation can directly enhance biohydrogen production and indirectly reduce the need for costly pretreatment technologies [110]. Enzymatic pretreatment plays a crucial role in improving the efficiency of biomass conversion processes, such as pyrolysis and gasification. These processes rely on the effective breakdown of complex lignocellulosic structures to enhance the production of hydrogen. Various enzymes contribute to this process by hydrolyzing cellulose, hemicellulose, and lignin, thereby optimizing feedstock quality and facilitating better thermal conversion outcomes [111].

EcXyl43, derived from *Enterobacter* sp., has been characterized based on its structural and functional properties. Molecular modeling has revealed a three-dimensional structure that includes a conserved N-terminal catalytic domain and an auxiliary C-terminal domain. EcXyl43 demonstrated remarkable efficiency and thermal stability under optimal conditions. The β -xylosidase enzyme EcXyl43 from *Enterobacter* sp. Exhibits remarkable thermal stability and efficiency, proving particularly beneficial for prolonged pyrolysis reactions [112]. Cellulases hydrolyze cellulose into glucose, which serves as the primary feedstock for pyrolysis and gasification. Notable cellulases within *Trichoderma reesei* have demonstrated high efficiency in breaking down biomass, making it suitable for subsequent processing [113]. Hemicellulases degrade hemicellulose into fermentable sugars, such as xylose and arabinose [114]. Lignin-degrading enzymes, such as laccases and peroxidases (MnP and LiP), from *Phanerochaete chrysosporium* facilitate lignin breakdown, reducing its inhibitory effects on biohydrogen production by removing structural barriers within the biomass [115]. Esterases and cutinases assist in breaking down ester linkages within lignocellulose, improving overall biomass liquefaction and enhancing heat transfer efficiency during thermochemical processing [116]. These enzymes are often integrated into microbial fermentation processes to enhance biohydrogen production, creating synergy between biological and thermochemical methods. Enzymatic hydrolysis produces a more homogeneous feedstock by breaking down complex biomass components, thereby reducing carbon deposition and improving gas yields. Optimized enzymatic degradation of cellulose and hemicellulose ensures a more efficient conversion of biomass-to-hydrogen, thereby increasing the overall process efficiency. Enzymatic pretreatment methods lower energy input requirements, reduce operational costs, and make biomass conversion more economically viable than traditional chemical pretreatment methods. The integration of enzymatic pretreatment into pyrolysis and gasification processes is an effective strategy for enhancing hydrogen production. With continued advancements in enzyme engineering and process optimization, these approaches hold significant potential for developing sustainable and cost-effective bioenergy solutions [117]. This enzyme can serve as an effective supplement to hemicellulases in long-term pyrolysis reactions, proving particularly beneficial in separate hydrolysis and fermentation (SHF) processes compared to simultaneous saccharification and co-fermentation (SSCF) or consolidated bioprocessing (CBP). Sequential combustion in a hydrogen production system using steam methane reforming (SMR) aims to reduce capital and operating costs by utilizing a unified carbon capture system for both the hydrogen production process and combined cycle gas turbine (CCGT) power plant. This innovative approach can be widely implemented in

various post-combustion CO₂ capture methods [118].

A biomass-based hydrogen production system integrated with an organic Rankine cycle was developed and analyzed to predict hydrogen output and power generation across a range of operating conditions [119]. A modified equilibrium model yielded favorable results for the composition of the produced syngas compared with the experimental data [120]. System optimization was performed using a genetic algorithm informed by response surface modeling. The preferred optimal solution was determined using linear programming methods, achieving a hydrogen yield of 39.31 mol/kg and an output power of 3558.08 kW within a multi-objective preference analysis framework [121]. Recent advances in fungal secretome research, facilitated by improved computational and experimental secretomics techniques, have led to significant breakthroughs in the understanding of industrially relevant fungi. In addition, novel enzymatic mechanisms and interactions have been discovered that contribute to the degradation of renewable polysaccharide-rich resources, such as cellulose. These discoveries are particularly valuable for reducing the costs associated with biomass pretreatment, as optimized fungal enzymes enable more efficient and selective hydrolysis of complex plant polymers. By integrating *in silico* enzyme design and screening tools, the production of cost-effective and high-performance enzyme cocktails becomes feasible, thereby enhancing the overall economic viability of biohydrogen production from lignocellulosic feedstocks [53].

Studies conducted in Cologne (Germany) and Houston (USA) have analyzed the cost of renewable hydrogen supply for various industrial sectors. In Cologne, the minimum delivery cost was estimated at € 9.4/kg for small volumes transported from northern Egypt and € 7.6/kg for large-scale demand using pipelines from southern France. In Houston, similar figures are € 8.6/kg for gaseous hydrogen delivered by truck from the Gulf of Mexico region and € 7.6/kg for large supplies from Southern California [122]. The study also highlights regions with the most promising potential for low-cost hydrogen, where production costs range from € 6.7–€ 7.8/kg. Variables such as the year of infrastructure development, type of electrolyzer used, and level of hydrogen demand significantly impact the overall costs. By 2050, the delivery cost is expected to decrease to € 7.8/kg in Cologne and € 6.8/kg in Houston owing to technological advancements and process optimization [123]. During the development of catalysts for hydrogen production from biomass, researchers can simulate the properties of dozens or even hundreds of different catalysts in a virtual environment and predict their efficiency and stability under various conditions. Once the most promising candidates are selected, they can be tested under real-world conditions. This approach helps avoid costs associated with materials and processes that may not yield the expected results [124].

In silico technologies can be applied at various stages of development, from theoretical design to industrial operation [124]. This enables the avoidance of errors that may arise in the absence of the preliminary calculations. In the field of hydrogen production, this includes modeling the interactions between biomass and catalysts, assessing energy losses in the reactor, and predicting various system operation scenarios [125].

4.2. Improvement of scalability and sustainability parameters

Scalability remains one of the most critical challenges in transitioning hydrogen production from controlled laboratory environments to industrial-scale systems [126]. While processes such as pyrolysis, gasification, and biofermentation demonstrate promising efficiencies in small-scale trials, they often suffer from a decline in performance when scaled up because of the complex interactions between physical, chemical, and biological parameters [127]. In traditional settings, this challenge arises from increasing process complexities, such as heat transfer, reagent distribution, and the maintenance of stable reactor conditions. This is where *in silico* approaches reveal their true value. These include limitations in heat and mass transfer, uneven substrate distribution, and difficulties in maintaining consistent operating

conditions within large bioreactors [128].

In silico strategies offer powerful tools to address these scale-up bottlenecks. CFD, for example, enables the simulation of mixing patterns, temperature gradients, and gas-liquid interactions in reactors of various sizes, helping engineers optimize reactor geometry and operating conditions before physical construction [129]. A notable example is the design of large-scale bioreactors for fermentation, where ensuring a uniform distribution of nutrients and gases among microorganisms is crucial [62]. Simulations allow the testing of a wide range of parameters, such as feed rates, oxygen concentration, and other critical factors, to determine the optimal operating conditions. They also help identify potential scaling issues, such as the formation of zones with insufficient or excessive substrate concentrations, which can reduce the overall process efficiency [130]. *In silico* approaches also play a key role in enhancing the sustainability of production systems. For instance, in the development of catalysts for pyrolysis or gasification, it is essential to consider the performance of the catalyst over long operational periods [131]. Computational models can predict catalyst degradation, their responses to external influences, and interactions with biomass [132]. This enables the preemptive development of more stable and durable materials, significantly extending the equipment lifespan and reducing maintenance costs. The integration of *in silico* strategies not only facilitates efficient process scalability but also contributes to the creation of more resilient and cost-effective hydrogen production systems. By minimizing trial-and-error experimentation and enabling the simulation of complex interactions at scale, *in silico* methods substantially enhance the scalability and sustainability of hydrogen production systems [133]. Quantitative metrics, such as increased yield per unit biomass, reduced energy input per mole of hydrogen, and lower CO₂ emissions per functional unit, can serve as critical benchmarks for evaluating the real-world impact of these strategies [134]. The integration of such computational approaches is essential for transforming hydrogen from a niche solution into a globally competitive and environmentally sustainable energy resource, helping industries overcome traditional scale-up challenges while ensuring long-term operational sustainability and positioning hydrogen as a viable and competitive renewable energy source [135].

4.3. The impact of *in silico* approaches on accelerating the deployment of hydrogen technologies

A key advantage of applying *in silico* approaches is their ability to significantly accelerate the integration of new technologies [136]. While traditional development methods require several years to progress through all stages from laboratory research to commercialization, computational methods allow for a substantial reduction in this timeline. A notable example is the design of bioreactors for hydrogen production from microalgae [137]. Modeling helps predict system behavior under real-world conditions, enabling a swift transition from conceptual design to industrial implementation in a shorter time frame. The application of *in silico* methods facilitates more optimized experimentation and prototyping, significantly reducing the time and resources required for research and development. By simulating various conditions and materials, researchers can precisely determine the optimal pathways and configurations without incurring the substantial costs associated with physical testing. This cost reduction is crucial for emerging technologies, as it allows companies to allocate their financial resources more efficiently [138].

In silico optimization contributes to increased production yields by fine-tuning the process parameters and optimizing the reaction environment. For instance, computational modeling enables the prediction of performance outcomes for various catalysts and biomass feedstocks, directly influencing hydrogen production efficiency [139]. This optimization not only enhances the profitability of hydrogen production but also reduces the processing time, thereby lowering operational expenses [140]. Ultimately, these improvements strengthen the competitiveness

of hydrogen compared to traditional fuels, making it a more attractive alternative in the energy market than before. Such advancements in hydrogen efficiency are crucial for broader acceptance of hydrogen as a sustainable energy source [141]. Modeling allows for the rapid identification of weaknesses in project design, optimization of technological processes, and enhancement of economic performance, which are essential for attracting investments and partnerships. This is particularly relevant for emerging and ambitious technologies, such as hydrogen energy, where rapid deployment is necessary to compete with conventional energy sources [139]. The use of computational methods accelerates not only the development phase but also the subsequent adaptation of technologies to different regions and conditions. *In silico* approaches can preemptively model how gasification or pyrolysis processes perform under varying climatic conditions and with different types of biomasses, enabling more precise adaptation of technologies to specific needs [133].

In silico strategies significantly accelerate the development and implementation of hydrogen technologies by reducing development costs, improving scalability, and ensuring system sustainability. They provide unique opportunities for optimizing hydrogen production processes, thereby enabling faster and more efficient industrial adoption. The application of computational methods makes it possible to create more sustainable and cost-effective solutions that will play a key role in the transition to sustainable energy [141].

5. Conclusion

Over the past decade, computational *in silico* techniques have become essential tools for enhancing biohydrogen production from biomass, providing innovative solutions to overcome challenges in efficiency, scalability, and cost-effectiveness. This review has highlighted the latest advancements in metabolic modeling, CFD simulations, CBM methods, such as FBA and MFA. These techniques significantly expand the capabilities of traditional approaches, providing new tools for more accurate and rapid analysis of metabolic networks and the development of optimized biochemical processes.

In silico strategies offer substantial advantages, including cost reduction, accelerated technology development, and improved predictive accuracy. These methods not only predict possible pathways for enhancing hydrogen production but also reduce the number of experimental tests, speeding up the development process. Modeling biological and chemical processes in a virtual environment enables the optimization of parameters such as metabolite fluxes, reaction conditions, and scaling processes to industrial levels without the need for costly laboratory studies.

A key area of focus is the application of *in silico* approaches in biotechnology and microbial engineering, including the genetic modification of strains to enhance hydrogen production capabilities. New advances in synthetic biology, such as using hydrogenases from non-photosynthetic microorganisms and integrating them with photosystems, open new horizons for sustainable and efficient hydrogen production. These approaches, supported by *in silico* models, allow the creation of microorganisms that can perform efficiently under oxygen-limited conditions, addressing the oxygen inhibition challenge that photosynthetic microorganisms face.

In silico research opens new opportunities for further improvements in bioenergy technologies. These methods are not only a powerful tool for scientific research but also a critical component for developing practical solutions that can significantly enhance the sustainability and competitiveness of hydrogen as a renewable energy source. Moving forward, with the advancement of computational technologies and model refinement, we can expect even greater strides in optimizing and scaling hydrogen production processes, which will ultimately help accelerate the transition to sustainable energy solutions and reduce reliance on fossil fuels.

CRediT authorship contribution statement

Ardak B. Kakimova: Writing – review & editing, Methodology, Investigation. **Assemgul K. Sadvakasova:** Validation, Formal analysis, Data curation. **Bekzhan D. Kossalbayev:** Writing – original draft, Supervision, Project administration, Conceptualization. **Elena V. Zadneprovskaya:** Resources, Investigation. **Tao Xu:** Visualization, Software, Data curation. **Dilnaz Zaletova:** Visualization, Validation, Formal analysis. **Suleyman I. Allakhverdiev:** Writing – review & editing, Supervision, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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