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Proceeding Paper

Enhanced Biohydrogen Production from Food Waste via Separate Hydrolysis and Fermentation: A Sustainable Approach †

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Abstract: Biohydrogen production from renewable resources holds promise for sustainable energy generation. This study explores the potential of utilizing food waste, a prevalent global environmental issue, as a substrate for efficient biohydrogen production. Two predominant biological methods, dark fermentation and photosynthesis, were evaluated for their feasibility in harnessing carbohydrates from food waste. Dark-photo sequential fermentation emerged as a more practical option. The proposed separate hydrolysis and fermentation approach offers a practical strategy to optimize nutrient conversion and increase biohydrogen yields.

Keywords: biohydrogen; fermentation; green hydrogen; food waste; hydrolysis; renewable energy; sustainable biofuel

1. Introduction

In the context of sustainable food systems and the emerging concept of the circular bioeconomy, the waste generated by the agri-food industry takes on profound significance as a pressing global issue that transcends borders and socioeconomic boundaries. This organic waste, which encompasses both food loss and waste (FLW), and residues and byproducts from the agri-food industry, represents a multifaceted challenge and a crucial component of the broader discourse on environmental sustainability and the circular bioeconomy [1–4]. Within the context of advancing sustainability within the agri-food sector, understanding and addressing these components are of paramount importance.

Food waste refers to the discarding of edible food, is often associated with the end-consumer, and occurs closer to the end of the supply chain due to factors such as spoilage or over-purchasing, thereby posing challenges related, in particular, to consumer behavior and disposal practices [3,5,6]. Food loss pertains to the reduction in the quantity or quality of food in the earlier stages of the food supply chain, from production to distribution. Food loss occurs mainly before the food reaches consumers and can be attributed to inefficiencies in the agricultural sector and the logistical aspects of the supply chain [3,7]. The inefficiencies in food production, distribution, and consumption have led to alarming statistics and estimates. According to the Food and Agriculture Organization of the United Nations (FAO), hunger afflicted 828 million people in 2021, an increase of approximately 46 million from 2020 and 150 million since 2019; it is estimated that 3.1 billion people lack access to a healthy diet [8]. These staggering data not only exacerbate issues of hunger and resource allocation but also contribute significantly to environmental problems, including soil degradation followed by greenhouse gas emissions, as food waste accounts for 8–10% of worldwide greenhouse gas emissions [2,9].

Secondly, residues and byproducts arising from food processing hold a pivotal role in advancing the circular bioeconomy paradigm. These organic materials encompass

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components like marc and pomace, peels, shells, trimmings, and other elements of food products that may not meet the criteria for direct human consumption. When appropriately managed and repurposed, these residues and byproducts can significantly enhance resource efficiency and minimize waste disposal. They become valuable feedstock for circular bioeconomy initiatives, including the production of biofuels, bioplastics, animal feed, and other value-added products [2,4,10,11].

The comprehensive recognition and management of these forms of waste emerged as pivotal imperatives for advancing sustainability goals, mitigating environmental impacts, and unlocking latent potential across diverse applications, notably the domain of biohydrogen (green hydrogen) production [12–14].

In light of these challenges, the quest for sustainable solutions that can address both waste management and renewable energy needs has gained immense importance. Biohydrogen production from renewable resources has emerged as a promising avenue in this context. Hydrogen, as a clean and efficient energy carrier, holds the potential to play a pivotal role in mitigating climate change and reducing dependency on fossil fuels.

The choice of agri-food waste as a substrate for biohydrogen production is particularly intriguing. Food waste is characterized by its high content of starch and protein, making it an economically attractive resource for biofuel production. However, the road to harnessing this potential is fraught with complexity. The challenge lies in converting macromolecules, such as starch and protein, into utilizable carbon sources like glucose and free amino nitrogen (FAN), which are essential for biotechnological processes. This conversion process, known as hydrolysis, often proves to be the rate-limiting step in most bioprocesses.

In this review, a sustainable approach is examined to address the hydrolysis limitation and improve the efficiency of biohydrogen production. This study investigates the utilization of agri-food waste as a substrate, highlighting its dual advantage in mitigating waste disposal challenges and generating alternative energy. Additionally, two prominent biological methods for biohydrogen production, namely dark fermentation and photosynthesis, are thoroughly evaluated.

The central aim of this study is to advocate for the implementation of a separate hydrolysis and fermentation approach as a strategic solution to optimize nutrient conversion and increase biohydrogen yields from agri-food waste. This approach employs pretreatment techniques to enhance the conversion of complex organic substrates into nutrient-rich solutions, ultimately accelerating the biohydrogen production process.

2. Agri-Food Waste as a Resource

Agri-food waste is a global environmental challenge that warrants attention due to its sheer scale and potential for resource recovery. Understanding the magnitude of this issue is crucial in appreciating the significance of utilizing food waste as a valuable resource (low-cost feedstock) for biohydrogen production.

One of the key reasons agri-food waste holds promise as a resource for biohydrogen production is its composition. Food waste is rich in carbohydrates, particularly starch and proteins. Starch is a polysaccharide composed of glucose units and is a prevalent component in many food items such as bread, rice, potatoes, and pasta. Proteins, on the other hand, are composed of amino acids and are abundant in various food sources like meat, dairy, and legumes. These carbohydrates and proteins serve as valuable feedstock for biofuel production, as they can be converted into biohydrogen through microbial processes.

Despite the promise of food waste as a resource, its complex nature poses a challenge. Starch and proteins are macromolecules that need to be broken down into simpler, utilizable forms for biohydrogen production. Starch needs to be enzymatically hydrolyzed into glucose, which can then be fermented by hydrogen-producing microorganisms. Proteins, rich in amino acids, require enzymatic or microbial degradation to yield FAN, which is a crucial nutrient for the growth and activity of hydrogen-producing microorganisms. The conversion of these complex substrates into simpler forms is often a rate-limiting step in biohydrogen production processes. The challenge lies in efficiently converting these complex substrates into glucose (or another accessible carbon sources) and free amino nitrogen to facilitate biohydrogen production.

3. Separate Hydrolysis and Fermentation Approach

The separate hydrolysis and fermentation (SHF) approach is a strategic bioprocessing concept that plays a pivotal role in improving the conversion efficiency of complex substrates found in agri-food waste into valuable nutrient-rich solutions and, subsequently, in enhancing biohydrogen production. This approach involves distinct steps in the production process, each optimized for its specific function. In the SHF approach, the overall biohydrogen production process is divided into two separate stages: hydrolysis and fermentation. The hydrolysis stage focuses on breaking down complex macromolecules, such as starch and protein, into simpler components, such as glucose and FAN. This stage is carried out using enzymatic or microbial methods that are tailored to the specific substrate composition. Once the complex substrates are converted into utilizable forms, they are then fed into the fermentation stage, where specialized hydrogen-producing microorganisms (often anaerobic bacteria) are employed to produce biohydrogen from these simpler substrates.

Pretreatment techniques are a crucial component of the SHF approach as they prepare food waste for efficient hydrolysis [15]. Pretreatment methods can include mechanical, chemical, or thermal processes that disrupt the physical and chemical structure of agri-food waste, making it more amenable to enzymatic or microbial action. For instance, mechanical pretreatment can involve grinding or shredding to reduce particle size, while chemical pretreatment may use acids, bases, or enzymes to weaken the substrate's structural integrity. These pretreatment techniques not only aid in breaking down complex substrates but also help release valuable nutrients locked within agri-food waste.

The SHF approach offers several notable advantages for biohydrogen production from food waste: (1) enhanced hydrolysis efficiency, (2) flexibility and control, (3) improved overall biohydrogen production rates, and (4) nutrient-rich solutions, further enhancing biohydrogen production rates.

4. Optimization of Operating Conditions

The success of the SHF approach in enhancing biohydrogen production from agri-food waste relies heavily on the optimization of operating conditions, particularly during the pretreatment stage. These conditions can be tailored to maximize conversion efficiency and address the challenges associated with the complexity of agri-food waste substrates. Operating conditions encompass various factors that can be adjusted to achieve optimal conversion efficiency during pretreatment. These factors include temperature, pH, residence time, and the choice of enzymes or microorganisms [16].

Temperature: adjusting the temperature can significantly impact enzymatic or microbial activity during pretreatment. Higher temperatures may accelerate reactions but must be within the range suitable for the specific enzymes or microorganisms used.

Different pH levels influence the activity of enzymes and microorganisms. Different enzymes have optimal pH ranges, and adjusting the pH to match these ranges can enhance their effectiveness.

Residence Time: The duration for which agri-food waste is subjected to pretreatment conditions can be optimized. Longer residence times may lead to more thorough substrate breakdown, but there is a balance to be struck to avoid excessive energy consumption.

Enzymes or microorganisms: the choice of enzymes or microbial strains used in the pretreatment can be tailored to target specific substrates within agri-food waste more effectively. Biohydrogen can be biotechnologically produced through various methods, including direct photolysis, indirect photolysis, photo-fermentation (PF), dark fermentation (DF), and dark-photo sequential fermentation (DF-PF) [16]. Among these approaches, DF, PF, and DF-PF have garnered attention for their distinct advantages, but all have some limitations [17,18]. DF stands out for its ability to produce hydrogen efficiently under ambient pressure and at higher rates compared to photosynthetic methods. It operates under mild reaction conditions, making it versatile and capable of utilizing different types of agri-food waste as feedstock. DF is considered environmentally friendly and holds promise for commercial hydrogen production [15]. PF is notable for its capacity to convert lignocellulosic biomass into biohydrogen. It harnesses a wide spectrum of light, enhancing its efficiency in utilizing solar energy. PF generates effluent, which can be managed and treated. It boasts higher substrate conversion efficiency, reduced pollution emissions, and the flexibility to use various carbon sources compared to alternative methods [19]. DF-PF emerges as a method with the potential to yield substantial biohydrogen output while remaining physically effective and cost-effective [20]. It has been identified as the most efficient process in terms of substrate-to-hydrogen conversion, positioning it as a great candidate for commercial biohydrogen production [17] and sustainable resource management [20].

In an advanced analysis, 26 data envelopment analysis models were examined, encompassing a total of 55 biohydrogen production experiments of the three aforementioned biotechnological groups (DF, PF and DF-PF) to assess the efficiency of biohydrogen yield. The results obtained from this analysis indicate that the average yield efficiencies are as follows: DF stands at 0.2844 and PF at 0.3460, while DF-PF leads with an efficiency score of 0.7040. Among the various combinations of biotechnological processes, the most efficient overall combination is observed in DF-PF, specifically involving *Rhodobacter capsulatus* B10/*Rhodobacter capsulatus*, with the overall highest yield efficiency, followed by *Clostridium butyricum* CGS5/*Rhodopseudomonas palutris* WP3-5, and *Clostridium pasteurianum*/*Rhodopseudomonas palutris* WP3-5 [18].

5. The Perspective Role of Computational Approaches in Advancing Biohydrogen Production

The synergy of computational approaches and genomics tools: traditionally, the identification of microorganisms capable of producing hydrogen involved labor-intensive wet-lab experiments that were costly, time-consuming, and often limited in scope. However, computational biology and genomics tools have introduced a paradigm shift in this area. Researchers can now leverage advanced bioinformatics and genomic analysis to explore the vast genetic diversity of microorganisms, ranging from archaea to algae, with the goal of identifying those with the highest potential for biohydrogen production [21]. By analyzing the genetic makeup of microorganisms, scientists can gain insights into the metabolic pathways responsible for hydrogen production. The key genetic markers and enzymes associated with hydrogen generation can be pinpointed. Moreover, bioengineering plays a pivotal role in improving the hydrogen-producing capabilities of microorganisms. By manipulating the genomes of these organisms, researchers can enhance their efficiency and yield of hydrogen gas. This approach may not only accelerate the development of high-efficiency hydrogen-producing consortia but also can enable the creation of custom-designed microorganisms tailored to the biohydrogen production process.

Advances in biohydrogen process modeling: the design and optimization of biohydrogen production processes traditionally rely on empirical models and experimentation. However, recent advancements in computational techniques have ushered in a new era [22]. Empirical models, including statistical approaches and experimental design methodologies, have provided valuable insights into process optimization. These models help identify the key factors influencing biohydrogen production and guide experimental efforts. Moreover, advanced techniques like artificial neural networks may extend the current modeling capabilities, allowing scientists to capture complex relationships between process variables. For semiempirical modeling, biokinetic models, often coupled with ideal reactor assumptions, have proven effective. These models describe the biological kinetics of hydrogen-producing microorganisms. They range from unstructured to structured approaches, providing valuable tools for predicting biohydrogen production rates and optimizing reactor conditions.

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