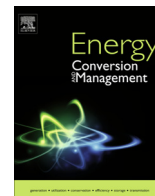




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## A comprehensive overview on light independent fermentative hydrogen production from wastewater feedstock and possible integrative options

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## ABSTRACT

This review focuses on the current developments and new insights in the field of dark fermentation technologies using wastewater as carbon and nutrient source. It has begun with the type of wastewaters (sugar rich, toxic and industrial) employed in the H<sub>2</sub> production and their production performances with pure (or) mixed microbiota as seeding source in the batch reactors. Secondly, well-documented continuous system performances and their failure reasons were examined along with the enhancement possibilities in ways of strategies. A SWOT analysis has been performed to validate the strength and weakness of the continuous systems towards its industrialization and possible scheme of the integration methods have been illustrated. Additionally, an outlook has been provided with enlightening the remedies for its success. Moreover, the practical perspectives of the continuous systems are highlighted and challenges towards scale up are mentioned. Finally, the possible integrative approaches along with continuous systems towards the bioH<sub>2</sub> technologies implementation are enlightened.

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## 1. Introduction

Ever increasing energy demand due to industrialization and technological development threatens the environment with climate change and pollution issues. This intimidating remark has awakened the scientist towards the prospecting of renewable energy sources which are green and sustainable [1,2]. Biofuel production from various organic streams has been put forth as a feasible technology to solve the energy demand in a sustainable manner. Biohydrogen production from wastewater could attract

various benefits such as waste minimization, waste utilization, and also simultaneous energy generation. In the course of the most recent couple of decades, biological hydrogen production has indicated great promise for generating large scale sustainable energy to meet ever increasing global energy demands. Different microorganisms, to be specific microscopic organisms, cyanobacteria, and algae which are capable of producing hydrogen from water, solar energy, and a variety of organic substrates, are investigated and concentrated on in point of interest.

Dark fermentation (DF) is a favorable biotechnological route for hydrogen generation. Compared with other options, the primary advantage of dark fermentation is that the hydrogen production rate (HPR) is orders of magnitude larger than the rates achieved by other microbiological methods [3–6]. Hydrogen production via biological way could be categorized generally 2 types based on the light source that are light dependent (photo fermentation) and light independent (dark fermentation). The later one allures much attention these days, due to the wide applicability and integration possibilities.

*Abbreviations:* DF, dark fermentation; HPR, hydrogen production rate; Hy, hydrogen yield; MEC, microbial electrolysis cells; DFHP, dark fermentative hydrogen production; WW, wastewater; SHPR, specific hydrogen production rate; BHP, biochemical hydrogen potential; EMC, enriched mixed culture; COD, chemical oxygen demand; PCR, polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; So/Xo, substrate to inoculum ratio; v/v, volume per volume; L, liter; mL, milliliter; d, day; h, hour.

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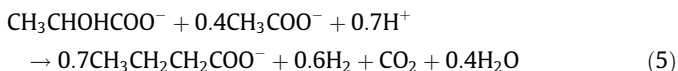
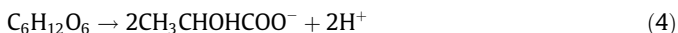
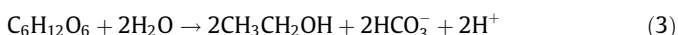
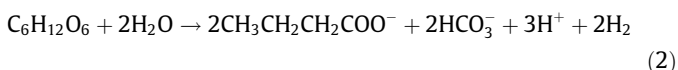
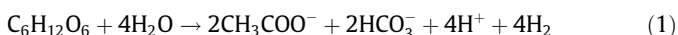
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### 1.1. Biohydrogen production methods

The biological hydrogen production can be achieved by different taxonomic, physiologic types of microorganisms in an anaerobic environment, while the methods are classified as direct or indirect biophotolysis, light-dependent-photo fermentation, light-independent-dark fermentation and microbial electrolysis cell (MEC) (Fig. 1). The dark fermentative otherwise known as light independent hydrogen production is gaining much attention due to the features such as wide organic substrate selection, higher hydrogen production rates (HPR) and direct integration with many other technologies such as bio-electrochemical systems (BES), methanogenesis, and photo-fermentation [1,7].

Dark-fermentative microorganisms are mainly facultative or obligate anaerobic bacteria, which can generate molecular hydrogen during the decomposition of carbohydrate substrates (Fig. 2). The main soluble products are organic acids, including acetic, propionic, and butyric acids, and ethanol [8,9].



### 1.2. Types of wastewaters used in BioH<sub>2</sub> fermentation

Various types of wastewaters have been reported to produce H<sub>2</sub> are tabulated in Table 1, which could be categorized as sugar rich (mainly contains sugars as glucose, sucrose and other carbohydrates), protein-rich (mainly rich in protein and lactose sugars),

toxic (contains many inhibitory compounds) and industrial effluents (mainly discharges from industries).

As various wastewaters are rich in organic content, the exploration of wastewater as substrate for biohydrogen production with concurrent wastewater treatment is an attractive and effective way of tapping clean energy from renewable sources in a sustainable approach. Additionally, it provides dual environmental benefits in the direction of wastewater treatment along with sustainable bioenergy generation. Hydrogen production from industrial wastewaters containing easily hydrolysable carbohydrates, unlike the lignocellulosic counterparts, has gained rapid attention due to the advantages of high organic loading possibilities, low nutrient requirement, mild pretreatment requirement (to remove co-existing hydrogen-consuming bacteria) and positive energy gain. Besides, their readily available soluble organic content and low viscosity aid in the development of continuous system in large scale operations. Higher hydrogen production rates (HPR) and the easy operational procedures of bioH<sub>2</sub> fermentation via light independent pathway make it as striking technology for the sustainable future.

### 1.3. Factors influencing the batch dark fermentative hydrogen production (DFHP)

Dark fermentation via batch mode operation is a complex process wherein inoculum conditioning such as pretreatment, enrichment, substrate types and their pretreatment approach, and environmental parameters optimization such as pH, temperature, and substrate concentration can regulate the metabolic pathway of hydrogen producing bacterial species [5,6]. Thus, careful assessment of each factor is crucial for enhancing the hydrogen production performances. In fact, the optimization of environmental factors and conditions favorable for efficient hydrogen production can be easily performed in a batch mode operation, when compared to the continuous system. Thus, batch mode operation is essential for hydrogen production from organic waste feedstock and further for scale-up of the process. The significant parameters outlined in the above have been further discussed in the following sub-sections.

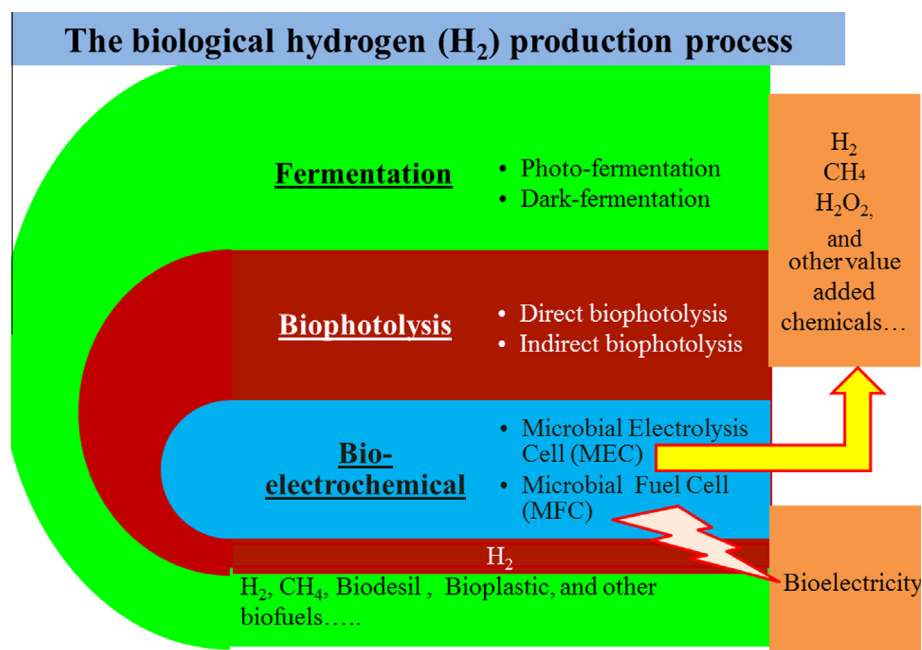


Fig. 1. Schematic representation of the primary biological routes for clean and sustainable H<sub>2</sub> production.

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