Renewable and Sustainable Energy Reviews 80 (2017) 1201–1216

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Improving biohydrogen productivity by microbial dark- and photo-fermentations: Novel data and future approaches

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ARTICLE INFO

Keywords:
Hydrogen production
Dark- and photo-fermentation
Bacteria and microalgae
Hydrogenases and nitrogenases
Glycerol
Carbon-containing wastes
Renewable and sustainable energy

ABSTRACT

Hydrogen (H_2) is an effective, environmentally friendly and renewable source of fuel that can be produced during dark- and photo-fermentation by different facultative and obligate anaerobic and purple bacteria and microalgae. This product is known as biohydrogen. It has the advantage of variable yield at low temperature (for mesophiles growing best at moderate temperature) and relatively low production cost, if compared with thermochemical methods. To develop fermentative H_2 production biotechnology using cheap carbonaceous by-products and utilization of organic wastes, the selection or construction of effective bacterial strains and optimization of technology process conditions are required. Here we review recent new data that have been obtained with *Escherichia coli*, *Clostridium beijerinskii, Rhodobacter sphaeroides* and other bacteria. Activities of [Ni-Fe]-hydrogenases of dark-fermentative bacteria and [Mo]-nitrogenase and [Ni-Fe]-hydrogenase of photo-fermentative species have been examined after growth with different carbon sources, using pure cultures, as well as co-culture and mixed-cultures technologies. Importantly, H_2 production from cheap and readily available substrates like crude glycerol or different industrial, agricultural and other carbon-based wastes by bacteria is a sustainable technology. Consequently further approaches and strain-improvement could increase H_2 production in a cost-effective way, and they will lead to both small- and large-scale H_2 production. Moreover, they will provide significant economic and environmental benefits for renewable and sustainable energy supply in the near future.

1. Introduction

Hydrogen (H_2) is an effective, environmentally clean and renewable fuel, because it has three-fold higher energy content (~ 142 kJ g^{-1}) than fossil fuel, such as oil, so its conversion efficiency is high; it generates only water and no toxic by-products and it can be produced from biomass [1,2]. Development of a low-carbon economy is important to decrease green house gas emissions and to avoid climate change, which can be irreversible processes [3]. H_2 can be produced by various methods including dark- and photo-fermentation through the activities of different facultative and obligate anaerobic and purple-sulfur bacteria, as well as by microalgae (Fig. 1). It can also be produced through biophotolysis of water by microalgae and algae as biocatalysts. H_2 production by bacteria, microalgae and algae is known as biohydrogen and this source remains a highly promising one in terms of global energy supply [1]. It has the advantages of high yields at comparatively low temperature (typically between 20 and 45 °C) and has relatively low cost if compared with other methods of production [2] (see Fig. 1). Furthermore, dark-fermentation does not need sunlight, has a significantly higher rate of production and has the added advantage that H_2 can be continuously produced when compared with photo-fermentation.

The European Union's aim is to improve the contribution of H_2 production to 27% of our renewable energy budget, and to reduce green-house gas emissions by > 40% by 2030 (compared to the 1990 level) [4]. These are important goals for the global energy strategy to establish a low-carbon economy and flexible and adaptive power systems. To achieve these goals this requires further development of biohydrogen biotechnology (Fig. 2). Moreover, H_2 is an important part of current and future transport development with the aim to increase use of H_2 vehicles and to build H_2 filling stations. Starting in 2018 Toyota will produce > 3000H_2-powered vehicles per year (e.g. the Toyota Mirai is one of the first vehicles to be sold commercially that uses exclusively a H_2 fuel-cell). A 'H_2 highway' system is planned for European countries these will be served by > 1000H_2-filling stations, which will be constructed by 2023, including > 400 stations in

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http://dx.doi.org/10.1016/j.rser.2017.05.149
Received 12 August 2016; Received in revised form 7 March 2017; Accepted 19 May 2017
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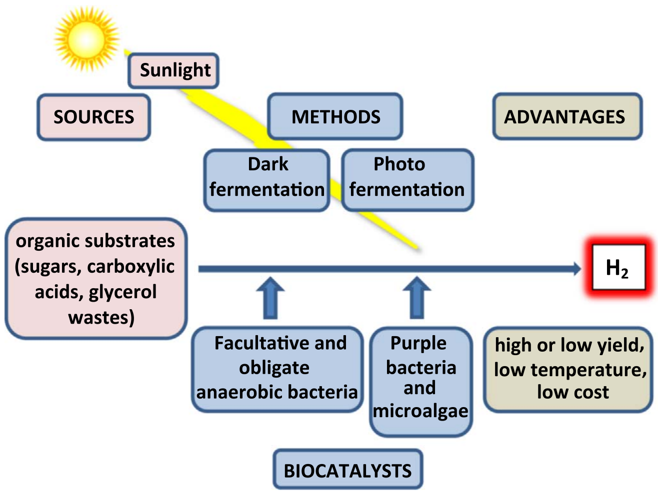

Fig. 1. H_2 production sources and methods of dark- and light-driven fermentations by bacteria as biocatalysts, highlighting their advantages.

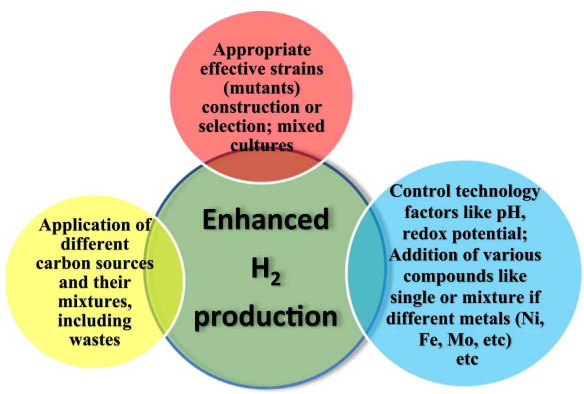

Fig. 2. Main approaches adopted to enhance H_2 production by bacteria.

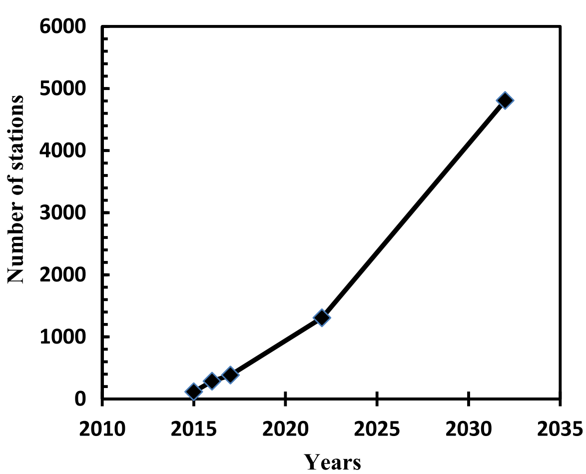

Fig. 3. H_2-filling stations numbers biulded in 2015–2016 and planned for 2017–2032 [6].

Germany alone [5]. Generally a rapid increase in the number of H_2-filling stations (Fig. 3) and their distribution worldwide is planned [6]. In addition, H_2 will be explored as an alternative fuel source in airplanes and space shuttles. As with hybrid technology, which is now widely adopted, we see an even more rapid acceleration in the development and acceptance of H_2 vehicles.

There is also one important argument in favor of biohydrogen from carbon-containing by-products and wastes: it makes feasible to manufacture decentralized power stations, where the generating units can be located in the proximity of carbonaceous resources. This H_2 can also provide security and reduce operating expenses for its transportation. Furthermore, the generation of various contaminants, including volatile fatty acids, can be drastically reduced or avoided if the production site is close to the power station.

H_2 is also a significant feedstock for the chemical, food, pharmaceutical and some other industries, and is important in metallurgy and the production of electronic devices [7]. These are the main fields of H_2 application.

To develop fermentative H_2 production biotechnology using carbonaceous by-products and organic wastes, studies to select effective H_2-producing heterotrophic bacteria or the genetic modification of already characterized strains for the optimization of this technological process have been initiated. These approaches could increase H_2 production in inexpensive ways (Fig. 2). They will benefit both small- and large-scale H_2 production capabilities globally and provide significant benefits for renewable and sustainable energy supply in the near future.

Although there have been several excellent reviews on microbial biohydrogen production published within the last few years [1,2,7–19], this is a rapidly-expanding field and there have been many exciting new recent findings in this subject, particularly from patents (Fig. 4) and, thus, an updated review is required. This review highlights new findings in the field and developments in H_2 production biotechnology (see Fig. 2) focusing on biohydrogen from organic by-products like glycerol, their mixtures with different organic compounds like sugars and organic acids contained in organic wastes and various carbon-based wastes like distillers grains (DG) and brewery wastes by bacterial fermentation processes. The findings are with *Escherichia coli*, *Clostridium beijerinskii*, *Rhodobacter sphaeroides* and other fermenting bacteria; pure, co- and mixed bacterial cultures are considered and compared; some novel ideas concerning basic and applied research on biohydrogen production like general physiology, genetics and biochemistry of responsible enzymes – hydrogenases (Hyd), interaction between these enzymes and hydrogen cycling through the membrane in the cells and the stimulatory and inhibitory effects of different heavy metals and their mixtures are also presented and discussed. They will be useful to draw novel approaches (see Fig. 2) for enhanced H_2 production by bacteria.

2. Cheap organic substrates and advantages for H_2 production

Future economically viable alternatives for the development of H_2 production biotechnology in both the small- and large-scale require the

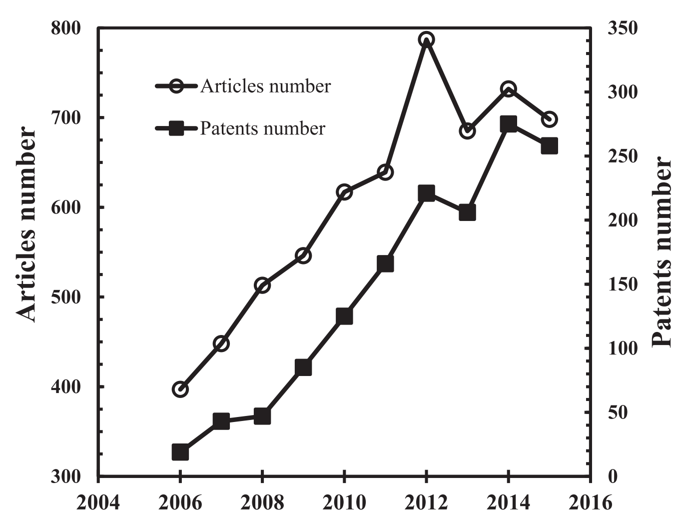

Fig. 4. Number of articles published in international peer-reviewed journals and of patents between 2006 and 2015 by Scopus. Titles on H_2 production by bacteria were searched.