Applied Energy 156 (2015) 174–184

Contents lists available at ScienceDirect

Applied Energy

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

Hydrogen production from glycerol by *Escherichia coli* and other bacteria: An overview and perspectives

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HIGHLIGHTS

- Glycerol is a perspective carbon containing source to produce H_2 by *Escherichia coli*.
- Metabolic pathways, responsible hydrogenases and dependence of H_2 production on external factors are summarized.
- H_2 production can be improved by glycerol added to glucose containing medium.
- H_2 production biotechnology would be further developed using glycerol as a feedstock.

ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 25 June 2015
Accepted 4 July 2015

Keywords:
Biohydrogen production
Glycerol
Dark fermentation
Hydrogenases
E. coli and other bacteria

ABSTRACT

Hydrogen (H_2) is a clean, effective and renewable fuel which can be produced by different methods including biological ones, namely fermentation and biophotolysis. To improve fermentative H_2 production the strategies, implicating use of by-products, utilization of carbon containing organic wastes and optimization of biotechnology process conditions, are developed. Glycerol, a biodiesel by-product, can serve as a cheap carbon containing source to produce H_2 by *Escherichia coli*. Recent data on metabolic pathways, responsible hydrogenases and dependence of H_2 production on external factors during glycerol fermentation are summarized. The strains are constructed to enhance H_2 yield. The mixed carbon sources (glycerol and glucose) fermentation is a novel approach: glycerol added to glucose containing medium increases H_2 production; different carbon sources comprising wastes can be used. H_2 production from glycerol by different bacteria is overviewed; cultures types, new technologies and optimal conditions, purification of H_2 and developing bioreactors are highlighted. All of these are significant for further developing H_2 production biotechnology from glycerol and perspective for applied energy systems.

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http://dx.doi.org/10.1016/j.apenergy.2015.07.009
0306-2619/

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1. Hydrogen as a perspective fuel and estimation of its global market

Molecular hydrogen (H_2) is a valuable gas as an effective, clean and renewable energy source and as feedstock for chemical, food, pharmaceutical and some other industries, metallurgy, production of electronic devices. H_2 is also used in space exploration, especially in space shuttles. Importantly, high energy (~142 kJ g^{-1}; ~3.5 time higher than oil) is released and only water (H_2O) but not carbon dioxide is formed during H_2 combustion [1,2]:

$$2H_2 + O_2 \rightarrow 2H_2O.$$

H_2 has low radiation level and relatively safe: the explosion limits by volume for H_2 in air of 18.3–59% are much higher than those for gasoline (<3.3%) and natural gas (<14%) [3].

Moreover, H_2 is not available in nature but it can be produced from unlimited sources like water or renewable ones like biomass and carbon containing wastes. Therefore, demand on H_2 production has increased considerably in recent years and H_2 can ultimately replace oil and natural gas.

H_2 is produced in both small-scale and large-scale volumes. World production of H_2 is more than 50 million tons [4] and according to the International Energy Agency estimated to be of ~65 million tons by production capacities in China, USA, European and other countries [5]. H_2 production is increased over the world rapidly (~6–10%); it will decrease costs of H_2 production which is competitive with oil and natural gas [3]. This can improve the efficiency and reliability of energy systems. H_2 is already used in gas stations (>200 stations distributed in North and South America, Europe, Asia and Australia and ~1000 stations will be constructed by 2025) for engines of different transport vehicles (cars, buses and ships are made by different companies like Daimler, Ford, Nissan, Mercedes, BMW etc) and in fuel cells to generate electric power [3,6]. As for growing economy, proposed global energy demand by 2050 will be tripled, and produced fossil fuels cannot satisfy energy market. Thus, very soon H_2 and fuel cells can become one of the main components in global energy economy.

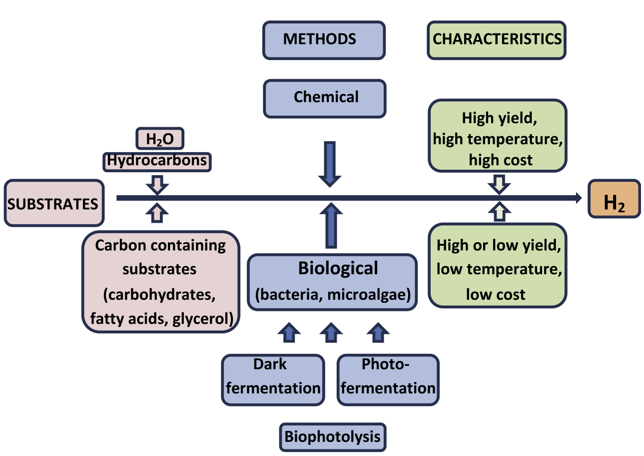

Fig. 1. Comparison of chemical and biological methods for H_2 production with their advantages (high yield, low temperature, low cost) and disadvantages (low yield, high temperature, high cost).

2. Biological methods in global hydrogen production and dark fermentation by *Escherichia coli*

Different methods have been developed for H_2 production. Electrolysis of water, steam reforming of methane or hydrocarbons, gasification of coal, oxidation of oil and natural gas, and auto-thermal processes are well-known and largely used methods for global H_2 production, but not cost-effective due to high temperature requirements (>700 °C), non-friendly method for environment (greenhouse gas emission) and non-renewable one (except electrolysis of water) (Fig. 1) [1,2]. Biological production of H_2 (biohydrogen) includes dark and photo-fermentation of carbon containing substrates, biophotolysis of water by bacteria and microalgae (see Fig. 1). The theoretical maximum yields for H_2 production by these methods can be different according to the well-known reactions for:

$$\text{dark fermentation of sugars (glucose, } C_6H_{12}O_6) - C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2;$$

$$\text{photo-fermentation of organic acids (acetic acid, } C_2H_4O_2) - C_2H_4O_2 + 2H_2O + \text{light} \rightarrow 4H_2 + 2CO_2 \text{ or}$$

$$\text{(succinic acid, } C_4H_6O_4) - C_4H_6O_4 + 4H_2O + \text{light} \rightarrow 7H_2 + 4CO_2;$$

$$\text{biophotolysis of water} - 2H_2O + \text{light} \rightarrow 2H_2 + 6O_2 \text{(direct) or}$$

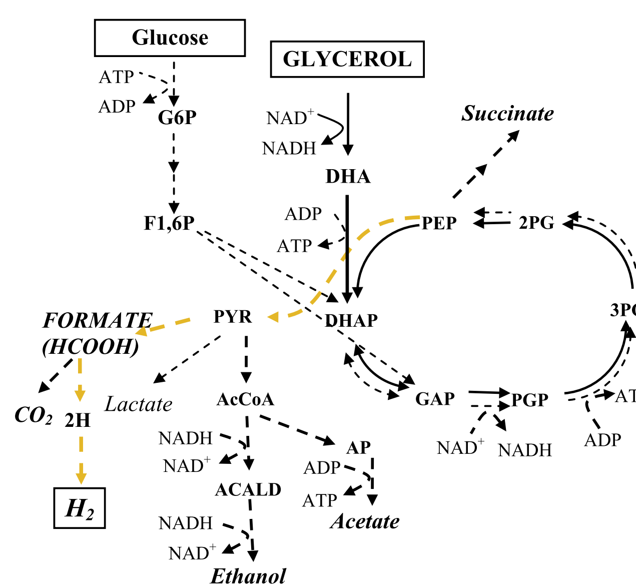

Fig. 2. Combined putative metabolic pathways of glycerol and glucose fermentation in *E. coli*. The pathways are adapted from [2,18,20,23–25]. Linear arrows indicate pathways only for glycerol fermentation, broken arrows indicate pathways only for glucose fermentation, and solid broken arrows indicate pathways for both glucose and glycerol fermentation. The end products are formatted as italics. 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate; AcCoA, acetyl-Coenzyme A; ADP, adenosine diphosphate; ATP, adenosine triphosphate; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate; GAP glyceraldehyde-3-phosphate; NADH, dihydrodiphosphopyridine nucleotide; NAD$^+$, diphosphopyridine nucleotide; PGP, 1,3-diphosphate glycerate; G6P, glucose 6 phosphate; F1,6P, fructose-1,6 diphosphate; AP, Acetyl -phosphate.