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Prospective Trends in Biotechnology for Biofuel

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***Abstract*—The development of clean alternative biofuel, including biohydrogen, is of great significant interest. One method for biohydrogen is dark and light fermentation when hydrogenases are responsible enzymes for H_2 metabolism. A further development of biotechnology for H_2 production can be directed in the following ways at least: construction or selection of appropriate effective bacterial strains (mutants) and cultures; creation of mixed cultures; application of different carbon sources (sugars, alcohols and organic acids) and their mixtures for co-fermentation as well as organic wastes (glycerol and lignocellulose waste); control of external technology factors (pH, redox potential, substrate concentration), addition of different compounds and their mixtures, especially some heavy metals (Ni, Fe, Mo, Mg, Cu, etc). Biohydrogen has of interest when was producing with bioethanol. It can also be applied for enhanced production of biomethane.**

Keywords—biotechnology for biofuel, hydrogen energy, biomethane, fermentation, bacteria

I. Introduction

Nowadays the energy supply mainly depends on fossil fuels, though significant efforts are being made to use fuels produced from renewable feedstock, as these have less greenhouse gas emissions during both fuel production and using [1-3]. However, due to the current energy crisis and rising concern over climate change, as well as some limitation with fossil fuels in future, the development of clean alternative biofuel is of great significant interest. This is important goal for the global energy strategy to establish a low-carbon economy and flexible and adaptive power systems.

There is also one important argument in favor of biofuels from carbon-containing by-products and waste: this makes it possible to produce decentralized power plants, where power units can be located in close proximity to carbon resources [4]. In addition, the formation of various contaminants, including volatile fatty acids, can be significantly reduced or prevented if the production site is next to the power plant.

II. Hydrogen as Perspective Energy Source

Hydrogen is an effective, environmentally friendly and renewable energy source, since it has 3 times higher energy content (~ 142 kJ g-1) than fossil fuels such as oil, therefore its conversion efficiency is high; it generates only water and does not contain toxic by-products and can be obtained from biomass [2-4]. Biohydrogen has the advantages of relatively high yield at low temperature (for mesophiles growing best at moderate temperatures), cheap and readily available substrates, including organic waste, and low cost compared to thermochemical methods [2-4]. All this is important for a sustainable economy.

One method is the production of H_2 by bacteria (biohydrogen) that perform dark – mixed acids (*Escherichia coli*, *Clostridium bejerinckii*) and light fermentation (*Rhodobacter sphaeroides*). These bacteria use hydrogenases (Hyd) and other enzymes for H_2 metabolism and multiple Hyd enzymes have been revealed with *E. coli* and other bacteria [2-7]; their mechanisms of action are well known for the repeated use of fermentation revisited [8] in the economy. It can also be produced through biophotolysis of water by microalgae and algae as biocatalysts [9].

III. Development of Biotechnology for Hydrogen Production

The aim of current development is to improve the contribution of H_2 production to renewable energy budget. The research is rapidly increased for last decades, and steps from lab-scale to large-scale production are putting forward to H_2 production [2-4].

Important experimental evidences have been obtained; they have well ground significance for H_2 production by bacteria. Actually, by genetic engineering and selection approaches, an improved *E. coli* strain with H_2 production rate of 0.68 mmol H_2 L^{-1} h^{-1} in by-product and new substrate of fermentation - glycerol medium has been obtained; this was 20-fold increased H_2 production compared with wild type [10], and this is not a limitation. Then, sole and mixed culture of dark- and light- fermented bacteria for enhanced H_2 production from lignocellulose waste (distillers' grains, brewery waste) [11-12] provides novel strategic approach for an inexpensive energy generation, as well as to resolve the problem of waste utilization. A 3-7-fold increase can be obtained with *E. coli* upon glycerol and formate, lactose or xylose mixture vs. glycerol, formate, lactose or xylose fermentation [13-16]. Prolong production of H_2 can be also detected during more than 220 h [15].

Moreover, responsible Hyd enzymes of *E. coli* have been revealed with sole and mixed carbon sources, including organic wastes, and for different technology conditions (Table I); these might open a new way to apply appropriate mutant strains for enhanced H_2 production.

All these approaches can not only enhance H_2 production but also decrease in cost of production.

IV. Further Strategy for Hydrogen Production Development

A further development of biotechnology for H_2 production can be directed in the following ways at least (Fig. 1):

- construction or selection of appropriate effective bacterial strains (mutants) and cultures [4, 10]; creation of mixed cultures [11];

- application of different carbon sources (sugars, alcohols and organic acids) and their mixtures for co-fermentation as well as organic wastes (glycerol and lignocellulose waste) [12-18];

- control of external technology factors (pH, redox potential, substrate concentration), addition of different compounds and their mixtures, especially heavy metals (Ni, Fe, Mo, Mg, Cu, etc) [19-22].

In addition, two-step H_2 production with dark and light fermentation when end products of dark mixed acids fermentation (organic acids) can serve as substrates for light fermentation (Fig. 2) is perspective approach to enhance of H_2 production too [11].

TABLE I. RESPONSIBLE HYD ENZYMES WITH SOLE AND MIXED CARBON SOURCES AND FOR DIFFERENT pH

Sole or mixed carbon	Responsible Hyd enzymes		References
	pH 7.5	*pH 5.5*	
Glucose (2g L^{-1}) and glycerol (10 g L^{-1})	Hyd-3	Hyd-3, Hyd-4 mainly, and Hyd-2 in some extent	[13]
Glucose (2 g L^{-1}) and formate (10 mM)	Hyd-3 and Hyd-4	Hyd-3	[14]
Glycerol (10 g L^{-1}) and formate (10 mM)	Hyd-3, Hyd-4 mainly, and Hyd-2 in some extent	Hyd-3	[14]
Glycerol ((10 g L^{-1}) and lactose (1 g L^{-1})	Hyd-3, Hyd-4 mainly, and Hyd-2 in some extent	Hyd-3 and Hyd-4	[15]
Glycerol ((10 g L^{-1}) and xylose (4 g L^{-1})	Hyd-3 and Hyd-4	Hyd-3 and Hyd-4	[16]
Brewery waste	Hyd-3 and Hyd-4	Hyd-3 and Hyd-4	[11-12]

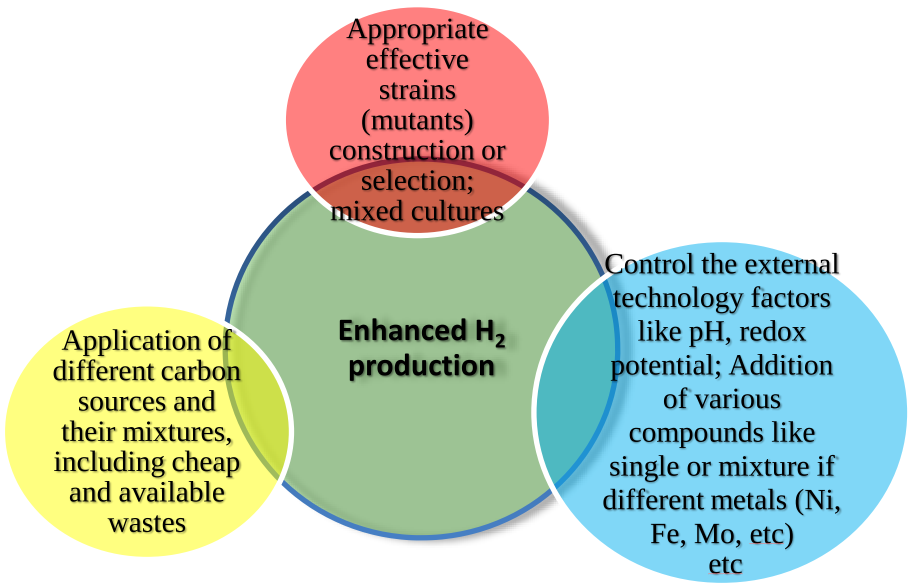

Fig. 1. Directions to enhanced H_2 production by bacteria (*E. coli*).

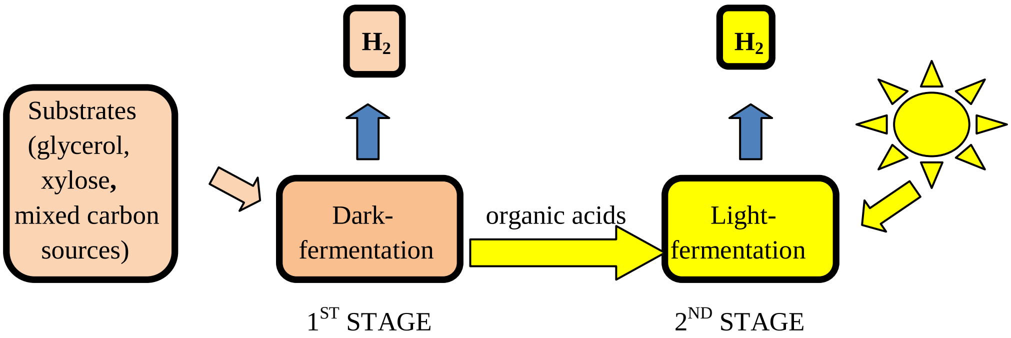

Fig. 2. A scheme for two consecutive stages H_2 production with dark- and light-fermentation.

V. Hydrogen and the Other Biofuels Production

It is interesting that some bacteria can produce hydrogen with bioethanol from by-products and organic wastes [10, 23]. It would be effective to use biohydrogen to improve production of other types of biofuel, especially biomethane. Bioconversion of CO_2 evolved during biogas production from biomass with biohydrogen to biomethane based on the activity of hydrogenotrophic methanogens are being researched; effectivity and limitations are determined [24-25]. It was found that the addition of H_2 to reactors treating food waste showed great potential for enhanced CH_4 yield and biogas upgrade [26]. This creates the possibility to optimize hydrogenotrophic methanogenesis towards obtaining biogas also of the right quality. Moreover, combination of H_2 and CH_4 systems enhanced the energy production efficiency from wastes [27].

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