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Review Article

Integrated dark- and photo-fermentation: Recent advances and provisions for improvement

Pankaj K. Rai [a,*], S.P. Singh [b]

[a] Department of Biotechnology and Bioinformatics Centre, Barkatullah University, Bhopal, 462026, M.P., India
[b] Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, 221005, U.P., India

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ABSTRACT

Biohydrogen production from waste materials has been recognized as the promising step towards bioremediation and green energy generation. A large number of microorganisms and diverse range of waste materials have been widely studied for H_2 production. However, H_2 production efficiencies vary depending upon the type of organism, type and composition of waste, physiological conditions, and the reactor used. Some research groups suggest integrated dark- and photo-fermentation processes for optimum utilization of the substrate and higher H_2 yield per mol of substrate. Integration of these two processes increases the H_2 yield, and seems suitable for commercial H_2 production. Review and evaluation of the published data suggest that integrated system holds greater promise for commercial H_2 production. The present review deals with the possibilities of enhancing H_2 yield by integrating dark- and photo-fermentation for commercial use.

Contents

* Corresponding author.
E-mail address: pankajraibhu@gmail.com (P.K. Rai).
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Introduction

Presently, most of the energy demand is met by non-renewable fossil fuels such as wood, coal, petroleum, and natural gas. The rampant consumption of these resources may lead to their depletion in the near future exposing us to a critical challenge of exploring the new and environment friendly energy source. To address the environmental protection and energy crisis issues simultaneously, the emerging biofuel technologies able to utilize biological wastes as feedstock or integrate with the wastewater treatment processes, are fast emerging [1]. Hydrogen with the highest conversion efficiency, with an energy yield of 122 kJ/g and no emission of greenhouse gas (GHG) on combustion, is considered the promising energy carrier and may be, the ultimate biofuel in the long-term [2,3]. Hydrogen (H_2), produced biologically from renewable sources (biomass, waste water, organic wastes) is called "biohydrogen". Waste and/or wastewater containing organic pollutants serve as carbon and energy source for the microbes used for biohydrogen production. It can be produced through two main approaches, photosynthetic (photo-autotrophic and photo-heterotrophic) and fermentative. Biohydrogen production involves direct photolysis, indirect photolysis, photo-fermentation or dark fermentation [4]. Each and every process has some advantages and disadvantages (Table 1). Biological hydrogen production strategies may collectively, contribute to large scale H_2 production as microorganisms produce H_2 from the readily available, renewable feedstock, making the biological process effectively competitive with chemical approaches such as reforming and gasification. Suitable feeds for biohydrogen generation process can be found in agricultural residues [5,6], food wastes [7–9] and effluents from industrial processes such as sugar refining [10] olive processing [11], and cheese production [12–16]. Hence, microbes could effectively be deployed for wastes remediation along with H_2 production, and thus offer dual economic benefit of energy generation and savings in the cost of waste disposal. Integrated systems for biological H_2 production, including dark- and photo-fermentation have attracted considerable attention over the last few years. Recent researches have started to integrate the process of anaerobic waste treatment (dark-fermentation) with light-dependent H_2 production (photo-fermentation) using the fermentative effluents from the former as the substrate for photosynthetic purple non-sulphur PNS bacteria in the latter. A combination of different microorganisms with various capabilities, their individual strengths may be exploited and the weaknesses overcome. This review deals with the progress made in integrated dark- and photo-fermentative biohydrogen production, their mechanisms and strategies for integration along with the provisions for improving the H_2 yield.

Dark-fermentation

Dark-fermentative H_2 production by strictly anaerobic or facultative anaerobic bacteria under anaerobic conditions is considered as the practical approach for H_2 production [17,18]. The process utilizes pure sugars as well as organic wastes as substrates for biohydrogen production, making the process cost-effective and environment friendly. In dark-fermentation, the bacteria grow on organic substrates degrade them through oxidations to increase their biomass and metabolic energy. To maintain the electrical neutrality of the cell, the electrons generated during oxidation of the substrates under anaerobic conditions, are disposed by reductions of protons to H_2 [17,19]. H_2 production by dark-fermentation generally proceeds at a high rate, and is independent of light. Dark-fermentative H_2 production from different organic wastes as the carbon source ends up predominantly in the production of acetic and butyric acid along with other volatile fatty acids (VFAs) together with H_2 [2] as:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2\uparrow + 4H_2\uparrow \quad (1)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2COOH + 2CO_2\uparrow + 2H_2\uparrow \quad (2)$$

The hydrogen yield from glucose is affected by the fermentative pathways adopted and the end products formed. Theoretically, a maximum of 4 mol of H_2 yield is possible from one mol of glucose with acetic acid as the end product while with butyrate, the H_2 yield is lowered to 2 mol H_2/mol glucose [10]. However, practically till today the maximum theoretical H_2 yield is not achieved by any process/method owing to the formation of VFAs and other byproducts during dark-fermentation [20].

A variety of studies have been carried out on dark-fermentation by anaerobic fermentative bacteria like *Clostridia* [21–23], *Enterobacters* [13,15,16,24–27] and *Escherichia coli* [28,29]. Many authors reported H_2 production from mixed microflora obtained from different sources like cow dung [30], and heat pretreated anaerobic sludge [31]. The amount of H_2 produced during dark-fermentation is majorly governed by the type of organism, metabolic route adopted, type of substrate used along with the end products formed.

During dark-fermentation, the accumulation of byproducts (alcohols and acids) beyond a certain threshold inhibits bacterial growth, thus inhibiting H_2 production to end up with lesser H_2 yield. Also, during dark-fermentation, the substrate is not completely utilized, leaving behind a large volume of effluent from the reactors loaded with high concentrations of VFAs [15,16,32]. For making the process more efficient in substrate utilization and environmental protection, a second stage is necessary to recover the energy from the effluent and its bioremediation. Although, the merits of dark-fermentation such as high H_2 production rate, input of low energy, easy handling and sustainability, are demonstrated on lab scale, this technology needs further improvements to go for commercial H_2 production based on organic wastes in terms of cost, efficiency and reliability.

Photo-fermentation

Photo-heterotrophic PNS bacteria are capable of producing H_2 and CO_2 using organic acids as substrate in the presence of light under anaerobic conditions. Gest and Kamen [33] first