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Review Article

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

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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 H₂ production. However, H₂ 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 H₂ yield per mol of substrate. Integration of these two processes increases the H₂ yield, and seems suitable for commercial H₂ production. Review and evaluation of the published data suggest that integrated system holds greater promise for commercial H₂ production. The present review deals with the possibilities of enhancing H₂ yield by integrating dark- and photo-fermentation for commercial use.

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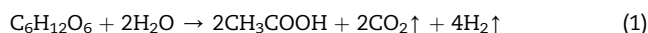
Introduction

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 (H₂), 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 H₂ production as microorganisms produce H₂ 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 H₂ production, and thus offer dual economic benefit of energy generation and savings in the cost of waste disposal. Integrated systems for biological H₂ 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 H₂ 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 H₂ yield.

Dark-fermentation

Dark-fermentative H₂ production by strictly anaerobic or facultative anaerobic bacteria under anaerobic conditions is considered as the practical approach for H₂ 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 H₂ [17,19]. H₂ production by dark-fermentation generally proceeds at a high rate, and is independent of light. Dark-fermentative H₂ 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 H₂ [2] as:



The hydrogen yield from glucose is affected by the fermentative pathways adopted and the end products formed. Theoretically, a maximum of 4 mol of H₂ yield is possible from one mol of glucose with acetic acid as the end product while with butyrate, the H₂ yield is lowered to 2 mol H₂/mol glucose [10]. However, practically till today the maximum theoretical H₂ 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 H₂ production from mixed microflora obtained from different sources like cow dung [30], and heat pretreated anaerobic sludge [31]. The amount of H₂ 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 H₂ production to end up with lesser H₂ 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 H₂ production rate, input of low energy, easy handling and sustainability, are demonstrated on lab scale, this technology needs further improvements to go for commercial H₂ production based on organic wastes in terms of cost, efficiency and reliability.

Photo-fermentation

Photo-heterotrophic PNS bacteria are capable of producing H₂ and CO₂ using organic acids as substrate in the presence of light under anaerobic conditions. Gest and Kamen [33] first

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