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Review

Role of chemicals addition in affecting biohydrogen production through photofermentation

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ABSTRACT

Biohydrogen production is considered as one of the most promising alternative processes to produce hydrogen due to its effectiveness, renewability, and environmental friendliness compared to the conventional way, such as thermochemical and electrochemical methods. Biohydrogen production via photofermentation produces hydrogen by photo-decomposing organic matters through the nitrogenase of photosynthetic bacteria. Despite its advantages, high energy requirement during nitrogenase-catalyzed reaction and low biohydrogen production efficiencies in the reality have become the foremost concern in a large scale application of photofermentation. Thus, an improvement of biohydrogen yield and light conversion efficiency is necessary to increase the feasibility of photofermentation. One of the simplest ways to improve photofermentation is by adding suitable chemical enhancer. Various studies have shown that an addition of some chemicals, such as iron, molybdenum, ethylenediaminetetraacetic acid (EDTA), vitamins, buffer solution, and others chemicals could increase the biohydrogen production rates and yields by a significant value. However, an addition of other chemicals, such as nickel ions, diphenylene iodonium, dimethylsulphoxide, and copper ions, might cause an adverse effect on the process. Addition of EDTA, molybdenum, ethanol or yeast may inhibit the photofermentation process, depending on the type of bacteria and substrates used during photofermentation process, as well as the concentration of the added chemicals. Hence, the importance and effects of chemicals addition on photofermentative biohydrogen production were discussed in this review paper.

1. Introduction

In the raising demands of renewable energy resources, hydrogen emerges as a potential replacement for the depleting fossil fuels due to its high heating value and clean combustion product. With water as the primary combustion product, the heating value of hydrogen as a fuel is approximately as high as 3042 calories/m^3 or 143 GJ/tonne [1,2]. Thus, hydrogen is considered to be one of the most promising energy carriers in the future. However, most of the hydrogen production process employs the application of fossil fuel as raw materials, such as natural gas in steam reforming, natural gas in thermal cracking, coal gasification, and partial oxidation of heavier than naphtha hydrocarbons [1]. These processes are known to be energy intensive as they consume fossil fuels and thus become less environmental friendly technology [2].

On the contrary, biological production of hydrogen is commonly operated at lesser energy intensive environment due to the ambient temperature and pressure of the process. Therefore, an application of biohydrogen production is generally more sustainable than thermo- and electrochemical processes. Biohydrogen production could be categorized into several types, namely (i) biophotolysis of water by using algae and cyanobacteria, (ii) photofermentation process by using photosynthetic bacteria, (iii) dark fermentation process by using anaerobic microorganisms and (iv) hybrid systems involving dark and photofermentation process [1,3]. Biophotolysis is a biohydrogen photo-evolution process by algae and cyanobacteria where electrons flow through two photosystems (PSI and PSII) of photosynthesis to the hydrogen-producing enzyme (hydrogenase) via electron carrier (ferredoxin) [1]. Meanwhile, photofermentation produces biohydrogen during decomposition of organic compounds by photosynthetic bacteria via nitrogenase-catalyzed reaction with the help of light energy [1,2]. On the other hand, during dark fermentation, organic compounds were converted to biohydrogen with the action of hydrogenase, volatile fatty acids and carbon dioxide by fermentative bacteria without the presence of light [3]. Furthermore, the hybrid system integrates dark fermentation and photofermentation process in a sequence to enhance biohydrogen production by increasing hydrogen yield and conversion efficiency [1,3]. In the hybrid system, short chain organic acids and

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alcohols produced as the by-products of dark fermentation were reused as the substrates in the following photofermentation process. A hybrid system could also be regarded as a potential option to improve biohydrogen production via a fermentation process.

Photofermentation is known to be one of the most favorable methods due to its high theoretical conversion yield, the absence of oxygen-evolving activity, possibility to use a wide range of light, and ability to utilize waste materials as the organic substrates in the process [1,4]. However, various physicochemical parameters such as C/N ratio, temperature, pH, and light intensity will influence the efficiency of biohydrogen production. Moreover, industrial application of photofermentation has been hindered by the actual low light conversion efficiency and low biohydrogen yield [5]. Inhomogeneous light distribution and polyhydroxybutyrate synthesis were highlighted as the causes of the low efficiency [3]. Thus, an improvement of photofermentation process is necessary to make this method more economically viable in industrial practices. Basak and Das [2] stated that the proposed unit cost of biohydrogen as an energy source was estimated to be US$ 9/GJ with 10% light conversion efficiency.

One of the simplest ways to improve the biohydrogen production yield and rates is through adding chemical enhancer(s) into the fermentation substrate. Various studies showed that addition of iron, molybdenum, ethylenediaminetetraacetic acid (EDTA), vitamins or buffer solutions could help enhance the photo-production of biohydrogen. However, dependent on the substrates, type of bacteria, and the amount of added chemical, addition of these chemicals might also cause an adverse effect on the process. Thus, a thorough study is required before incorporating certain chemicals into photofermentation process. To provide more insights to the significance of chemicals addition, this paper reviews the effects of chemicals addition on photofermentation process with discussions on biohydrogen yield, biohydrogen production rates, and biohydrogen production lag time.

2. Enzymatic system in photofermentative biohydrogen production

Biohydrogen production involves three main biohydrogen producing and consuming enzymes which are responsible to the net biohydrogen evolution. These three enzymes are reversible hydrogenase, membrane-bound uptake hydrogenase, and nitrogenase enzymes [1]. Nonetheless, hydrogenase and nitrogenase are the two main enzymes accountable for the uptake and production of biohydrogen during photofermentation process using anoxygenic photosynthetic bacteria.

2.1. Classification and function of hydrogenase

Hydrogenase enzyme is found to be responsible for the initial uptake and evolution of biohydrogen during photofermentation by using photosynthetic bacteria. This reversible reaction can be represented by a simple redox reaction as shown in Eq. (1) [4]. The direction of this reaction depends on the redox potential of the components that is able to interact with hydrogenase [6].

$$\text{Hydrogenase: } H_2 \leftrightarrow 2H^+ + 2e^- \quad (1)$$

Various types of hydrogenase (Fig. 1) exist in the microbial system but [NiFe]-hydrogenase (for the bacterial system), [FeFe]-hydrogenase (for green microalgae) and [Fe]-hydrogenase are the mainly known hydrogenase enzymes classified by the metal atoms on their active sites [6,7].

On the other hand, [NiFe]-hydrogenase constitutes the largest number of hydrogenase in purple bacteria and it can either produce or consume biohydrogen, depending on the subunits present in the enzyme. The core of [NiFe]-hydrogenase enzyme consists of α, β heterodimer [6]. α-subunit contains bimetallic active site with a larger size than β-subunit [6]. The small β-subunit consists of Fe-S clusters which act to transfer electrons between the active site and electron acceptor/donor binding site [6]. With a full sequence analysis, [NiFe]-hydrogenase can be further classified into four different groups. Group I [NiFe]-hydrogenases plays their role in electron transport chain, transferring electrons to the quinone pool with the involvement of cytochromes or heme-containing proteins [7]. This enzyme structure is characterized by a long signal peptide at the N terminus of their small subunit and located in the periplasmic side [6]. In purple bacteria, the most common group 1 [NiFe]-hydrogenase found is the biohydrogen-consuming membrane-bound *hup*SL hydrogenase [7]. Group II [NiFe]-hydrogenase functions to detect the presence of hydrogen in the environment, and stimulate the cellular reactions controlling the synthesis of hydrogenases [6]. Group II hydrogenase does not contain a signal peptide at its N terminus and typically located in the cytoplasmic side [6].Group III [NiFe]-hydrogenase is also known as a bidirectional heteromultimeric cytoplasmic enzyme that works reversibly and can re-oxidize the cofactors, namely NAD or NADP, use NADPH as an electron donor, and provide reducing equivalents for heterodisulfide reductase [6]. Lastly, Group IV [NiFe]-hydrogenase consists of multimeric enzymes with at least six subunits, biohydrogen producing, energy conserving, membrane-associated hydrogenases [6,7]. The main role of Group IV [NiFe]-hydrogenase is to reduce protons from water to discharge the excessive reductive equivalents produced from anaerobic oxidation or organic compounds [6,7]. Evolution of biohydrogen by Group IV occurred from reduced ferredoxin during the oxidation of carbonyl group of acetate to carbon dioxide [6]. During the photofermentation, an illumination of broth triggers [NiFe]-hydrogenase to initiate a short surge of biohydrogen evolution and then was typically followed by a biohydrogen uptake.

[FeFe]-hydrogenase possesses a monomeric structure and contains only a catalytic subunit with two iron groups located in the active center [6,7]. In purple bacteria, no [FeFe]-hydrogenases have been yet identified, and it was agreed that purple bacteria could not synthesize [FeFe]-hydrogenase enzyme [7]. However, in *Rhodopseudomonas palustris and Rhodopsirillum rubrum,* the *hydC* genetic expression was found in their genomes and was annotated as a structural [FeFe]-hydrogenase [7]. Further investigation revealed that the [FeFe]-hydrogenase genes in *R. palustris* and *R. rubrum* act as the biohydrogen consuming hydrogenase in their effort to maintain the redox state of the cells [7]. On the other hand, [Fe]-hydrogenase was found in methanogenic bacteria, which acts to catalyze the reduction of carbonate with biohydrogen to methane [7]. In contrary to [FeFe]- and [NiFe]- hydrogenases, [Fe]-hydrogenase is not redox active and it does not catalyze the reversible redox reaction (Eq. (1)) [6]. Similar to [FeFe]- hydrogenase, this enzyme has not also been identified in purple bacteria system [7].

2.2. Classification and function of nitrogenase

On the other hand, nitrogenase is considered as one of the main enzymes accountable for the production of biohydrogen under an anaerobic environment in a photofermentation process by using photosynthetic bacteria. Nitrogenase is essentially a metalloprotein complex that is responsible for biological fixation of molecular nitrogen, and it is commonly found in both archaea and bacteria [6,7]. During the nitrogen fixation process, nitrogenase can produce biohydrogen while maintaining redox balance. The biohydrogen production process is classified as an irreversible and energy-intensive reaction. In the absence of nitrogen, nitrogenase catalyzes the production of biohydrogen while consuming four ATP per mole of biohydrogen produced [6]. An inhibition of this enzyme activity is strongly influenced by the presence of ammonia, oxygen, or low C/N ratio [4,7]. Thus, ammonium removal and anaerobic condition are necessary during a biohydrogen production via photofermentation.

The typical structure of nitrogenase consists of reductase subunit and dinitrogenase complex [8]. The reductase subunit contains a Fe-S protein, whereas the dinitrogenase complex consists of a Mo-Fe-S protein (*nifD* and *nifK* genes). In detail, nitrogenase consists of three metal-