Biohydrogen production by dark and photo-fermentation processes

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***Abstract*—The hydrogen can be produced in a biological production process by dark and photo-fermentation of organic substrates. Under anaerobic conditions, hydrogen is produced during conversion of organic substrate into organic acids using fermentative bacteria and during conversion of organic acids into H_2 and CO_2 using photo- fermentative bacteria. This bioprocess has been studied with a number of microorganisms, it is a very complex process and influenced by many factors. In order dark and photo-fermentation process is an important approach for bio-hydrogen production. In this study, different factors haves been examined to enhance biohydrogen production by these organisms, either as a combined or sequential using dark and photo-fermentation process. The effect of each factor on biohydrogen production efficiency is reported. A comparison of hydrogen production efficiency between dark-fermentation, photo-fermentation and two stage processes was investigated.**

Keywords—Biohydrogen; Dark-fermentation; Photo-fermentation; Hydrogen production efficiency

I. INTRODUCTION

The worldwide energy need has been increasing exponentially, the reserves of fossil fuels have been decreasing, and the combustion of fossil fuels has serious negative effects on environment because of CO_2 emission. For these reasons, many researchers have been working on the exploration of new sustainable energy sources that could substitute fossil fuels. Hydrogen is considered as a viable alternative fuel and "energy carrier" of future. Hydrogen gas is clean fuel with no CO_2 emissions and can easily be used in fuel cells for generation of electricity. Besides, hydrogen has a high energy yield of 122 kJ/g, which is 2.75 times greater than hydrocarbon fuels. The major problem in utilization of hydrogen gas as a fuel is its unavailability in nature and the need for inexpensive production methods [1]. There are several means of biohydrogen production, including direct biophotolysis, indirect biophotolysis, photo-fermentation, and dark fermentation [2]. These diverse methods must be improved in order to make biohydrogen an economically viable alternative to other means of hydrogen production. Intensive research studies have already been carried out on the advancement of these processes, such as the enhancement of dark- and/or photo-fermentative H_2 production rates and the development of two stage processes. In the present study we have reported the effects of various factors on hydrogen production by dark and photofermentative bacteria.

II. DARK FERMENTATION PROCESS

Dark-fermentative hydrogen production occurs under anoxic or anaerobic conditions (i.e., in the absence of O_2 as an electron acceptor). The key pathway is the breakdown of carbohydrate rich substrates by bacteria to H_2 and other intermediate products such as volatile fatty acids (VFA's) and alcohols. These substrates have provided building blocks and metabolic energy for the bacterial growth. Biological hydrogen production has been studied in a wide range of organisms belonging to diverse taxonomical groups [3]. Usually anaerobic bacteria such as Clostridia sp., and Enterobacter sp. were used for dark fermentation of carbohydrates to produce hydrogen and VFA (Table 1).

III. PHOTOFERMENTATION PROCESS

Photofermentation is carried out by nonoxygenic photosynthetic bacteria that use sunlight and biomass to produce hydrogen. Purple non-sulfur (PNS) and green sulfur (GS) bacteria such as Rhodobacter spheroids and Chlorobium vibrioforme, respectively, are capable of producing hydrogen gas by using solar energy and reduced compounds. Their photosynthetic systems differ from oxygenic photosynthesis due to their requirement for reduced substrates and their inability to oxidize water [4]. Photosynthetic bacteria have long been studied for their capacity to produce hydrogen through the action of their nitrogenase system [5].

Biological hydrogen production has been studied in a wide range of microorganisms. Table 1 provides a maximum rates and yields of H_2 production from a number of studies, where rate and yield of H_2-production are given as ''units of product volume per culture volume over time'' (ml H_2/lh) and ''amount of H_2 produced (mol) per amount of substrate (glucose or VFAs) (mol)'' (mol H_2/mol).

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TABLE I. BIOLOGICAL HYDROGEN PRODUCTION FROM PURE SUBSTRATES BY DARK AND PHOTO-FERMENTATIVE BACTERIA

Microorganisms	*Substrate*	*Maximum rate of H2 production*	*yields of H2 production (mol H2/mol hexose)*	*References*
Photosynthetic bacteria R. sphaeroides CIP 60.6	Lactate C/N (mM/mM) 50/10 (4500lux, 30°C)	40.02 ml/l.h	-	[5]
R. sphaeroides KD131	Malate (30 mM)	36.1ml/l.h	-	[6]
Fermentative bacteria				
E. coli HD701	Glucose(batch, 30°C)	-	0.376	[7]
Enterobacter cloacae IIT-BT 08	Glucose	20.0 mmol/lh	-	[8]
E. coli ATCC8739	Glucose	-	0.71	[9]

IV. THE FEASIBILITY OF USING TWO-STAGE PROCESS COMBINING DARK AND PHOTO-FEMENTATION

A two-stage process combining dark/photo fermentation was used to increase the overall hydrogen yield from organic substrates and also to reduce the chemical oxygen demand (COD) in the effluent.

A. Dark fermentation

Dark-H_2 fermentation was conducted using strict anaerobe and facultative anaerobe bacteria, giving a theoretical maximum H_2 production yield of 4 mol/mol as illustrated in Eq. (1) [7].

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

B. Photofermentaton

The soluble metabolites resulting from dark fermentation, consisting of VFA's and alcohols, were further used for H_2 production in the subsequent photo fermentation as illustrated in Eq. (2) [7].

$$2CH_3COOH + 4H_2O \rightarrow 8H_2 + 4CO_2 \quad (2)$$

C. Dark and photo fermentations

In fact, combination of the dark and photo fermentation could achieve a theoretically maximum yield of 12 mol H_2/mol hexose Eq. (3) [7].

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \quad (3)$$

Most of the works on combining dark- and photo-fermentation have been operating either as combined or two stages (sequntial) processes for hydrogen production. Biohydrogen production is very complex process and is influenced by many factors such as immobilized or suspended cells system, cell and substrate concentrations, pure or mixtures cultures of hydrogen- producing bacteria, and the effects of physico chemical parameters. The effects of these factors on dark and photo fermentative H_2 production have been reported by many researchers to study and improve the biohydrogen production efficiency. The optimization of biohydrogen-producing methods focused heavily on:

1) Immobilisation approaches: Rates of hydrogen production by photo fermentative bacteria are higher in the case of immobilized cells than that of the suspended cells. Liu et al [10] examined the hydrogen production capability of immobilized R. faecalis RLD-53 using soluble metabolites from ethanol fermentation bacteria Ethanoligenens harbinense B49. Hydrogen yield of 3.15 mol H2/mol acetate, obtained by immobilized R. faecalis RLD-53, raises of about 21% compared to non-immobilized cells. The total hydrogen yield during dark- and photo-fermentation reached its maximum value (6.32 mol H_2/mol glucose). Asadaa et al [11] reported that glucose was converted to hydrogen gas in a yield of 7.1 mol of hydrogen per mole of glucose at a maximum under illuminated conditions using co-immobilized cultures of a lactic acid bacterium, Lactobacillus delbrueckii NBRC13953 and a photosynthetic bacterium, Rhodobacter sphaeroides RV, in agar gels.

2) Effect of substrate and cell concentration: To efficiently reutilize the VFA's byproducts from dark fermentation and raise the H_2 yield in photo-fermentation, the optimal concentrations of by-products and cell concentration, respectively, were investigated. Argun et al [12] studied the effects of the substrate and cell concentration on bio-hydrogen production from ground wheat by combined dark and photo-fermentation and found that the highest cumulative hydrogen (135 ml) and formation rate (3.44 ml H_2 h^{-1}) were obtained with the 20 g L^{-1} wheat powder concentration. However, the highest yield (63.9 ml g^{-1} starch) was obtained with the 2.5 g L^{-1} wheat powder. In variable cell concentration experiments, the highest cumulative hydrogen (118 ml) and yield (156.8 ml

H_2 g^{-1} starch) were obtained with 1.1 g L^{-1} cell concentration yielding an optimal biomass/substrate ratio of 0.22 g cells/g WP. The ammonia concentration and C/N ratio in the effluent of anaerobic fermentation should not be at the inhibitory level for the photosynthetic bacteria [13]. Liu et al [14] studied the effect of diluted ratio of effluents from dark-fermentation processed by C. butyricum, which could be further converted into hydrogen gas by photo-fermentation bacteria R. faecalis RLD-53, to determine suitable range of substrate concentration. Experimental results showed the photo-hydrogen yield decreased when increasing diluted ratio from 1:0.5 to 1:3, and it reached the maximum value of 4368 ml-H_2/l-effluents at the ratio of 1:0.5. The ratio of dark-photo bacteria was at 1:2, the hydrogen yield reached highest value of 4.946 mol-H2/mol-glucose and cumulative hydrogen volume was 5357 ml-H_2/l-culture during the combination process. Additionally, the dark fermentation effluent used as medium to grow Rhodobacter species, it needs compound nutritional added for bacterial metabolism during photo fermentation. Su et al [15] investigated an orthogonal experimental design to optimize the medium composition for hydrogen production from glucose by dark- and photo fermentation, using Clostridium butyricum and Rhodopseudomonas palustris. A dramatic increase of hydrogen yield from 1.59 to 5.48 mol H_2/mol glucose was observed.

3) Hydrogen- producing bacteria: Hydrogen gas production by the light fermentation of the dark fermentation effluent of the VFA's by-products solution, either as a combined or sequential process, was realized by using pure and mixtures cultures of dark and light fermentation bacteria of different strains. A great number of studies used a sequential bio H_2 production stages, only limited number of combined fermentation (co-culture) studies was reported in literature where pure cultures of dark and light fermentation bacteria were used widely compared to mixtures cultures for hydrogen production. Clostridia have been the most extensively used, by many studies, compared to the facultative aerobes bacteria for the first stage dark fermentation, while for the second stage of sequential or combined dark and photo-fermentation process, Rhodobacter species (photosynthetic bacteria) are the main appropriate organisms. Chen et al [16] investigated a two-stage process combining dark and photo fermentation using pure cultures of Clostridium pasteurianum CH_4, , and Rhodopseudomonas palustris WP3-5, respectively, and found that the total hydrogen yield increased from 3.80 (dark fermentation) to 10.02 mol H_2/mol sucrose (dark/photo fermentation). Argun et al [17] reported that a mixture of the three Rhodobacter cultures was used in the succeeding photo fermentation stage to determine the optimum TVFA and NH_4–N concentrations yielding the highest rate and extent of hydrogen formation compared to the pure culture. Yokoi et al [18] obtained higher hydrogen production yield (7.0 mol/mol glucose) by sequential two step fermentation of a mixed fermentative culture of "C. butyricum and Enterobacter aerogene" and photo fermentative culture of Rhodobacter sp. M-19 compared to single stage dark fermentation (2.4 mol/mol glucose) of sweet potato starch residue. Pure cultures of Clostridium beijerinkii (DSMZ-791) and Rhodobacter sphaeroides-RV were used as seed cultures in combined fermentation. The highest hydrogen yield was 90 ml g-1 starch [19].

4) Effect of physico-chimical parameters: The physico chemical parameters are the most essential parameters in bio H2 production [5, 20]. These parameters affect the cell growth, efficiency and yield of hydrogen production and metabolic pathway of microorganism during hydrogen production by an integrated dark and photo-fermentation process. Nath and Das [20] studied the effect of light intensity and initial pH and demonstrated that increased light intensity resulted in an increase in the total volume of hydrogen evolved and also hydrogen production rate. The pH study revealed that cumulative hydrogen production was maximum at initial medium pH of 7.0 _ 0.2. Biomass yield was also high at the vicinity of pH 7.0 and it decreased as the pH increased from 7.0 to 8.0. The overall yield of hydrogen in two-stage process, using Enterobacter cloacae DM 11 strain in dark fermentation and Rhodobacter sphareoides strain O.U.001 in a column photobioreactor, considering glucose as a preliminary substrate was found to be higher compared to a single stage process. Liu et al [14] studied the effect light intensity and light/dark cycle on bio-hydrogen production by the combination of Clostridium butyricum and R. faecalis RLD-53. During the combination process, maximum total hydrogen yield was 5.374 mol-H_2/mol-glucose.

V. COMPARATIVE STUDY BETWEEN THREE MAIN TYPES OF HYDROGEN PRODUCTION TECHNOLOGIE

The microbial production of hydrogen by fermentation can be broadly classified into two main categories; one is light independent and the other is light-dependent. The light independent fermentation processes, commonly known as dark fermentation, employ both obligate as well as facultative anaerobic bacteria for the production of H_2 from a variety of potentially utilizable substrates, including refuse and waste products. It generally gives a high rate of H_2 evolution and does not rely on the availability of light sources. In contrast, in photo-fermentation, small-chain organic acids are used by photosynthetic bacteria as electron donors for the production of H_2 at the expense of light energy [21].

A comparison of three main type of H_2 production technologies are demonstrated in Table.2. Each approach has positive and negative characteristics and each has serious technical barrier that need to be overcome before it could become practical.

VI. CONCLUSION

This paper, describes the main approach utilized to optimize and to explain the effect of each factors affecting the hydrogen production efficiency by dark and photo fermentation

TABLE II. ADVANTAGES AND DISADVANTAGES OF DIFFERENT HYDROGEN PRODUCTION PROCESSES

Process	***Advantages***	***Disadvantages***	***References***
Photo-fermentation	Has the ability to fix N_2 from atmosphere. A wide spectral light energy can be used by these bacteria. Can use different organic wastes.	O_2 has an inhibitory effect on nitrogenase. Light conversion efficiency is very low, only 1–5%.	[22]
Dark fermentation	It can produce H_2 all day long without light A variety of carbon sources can be used as substrates. It produces valuable metabolites such as butyric, lactic and acetic acids as by products. It is anaerobic process, so there is no O_2 limitation problem.	O_2 is a strong inhibitor of hydrogenase Relatively lower achievable yields of H_2. As yields increase H_2 fermentation becomes thermodynamically unfavorable. Product gas mixture contains CO_2 which has to be separated.	[22]
Dark and photofermentation	The complete conversion of organic compounds, usually organic acids, to hydrogen: Reduce the chemical oxygen demand (COD) in the effluent. Enhance the overall yield of H_2 in two-stage process compared to a single stage process.	The major problems in the photofermentation: Inhibitions caused by high concentrations of VFAs and NH_4-N Severe control of physico- chemical conditions. The medium used to grow Rhodobacter species was dark fermentation effluent, it needs complex nutritional added.	This paper

process. The three biohydrogen production processes dark-fermentation, photo-fermentation, two-stage (sequential) and/or combined process were discussed and compared on the basis of hydrogen production efficiency. The advantages and disadvantages of each approach have discussed.

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