

# Bioaugmentation novel methods for Biohydrogen production

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**Abstract**— Bioaugmentation is the improved treatment by adding actively growing and specialized microbial strains into a system to enhance the ability of the process to respond to any fluctuations or to digest or degrade certain compounds. Whereas Biohydrogen is the production of hydrogen biologically. It occurs most commonly by bacteria, algae and archaea. Biohydrogen is a potential biofuel now-a-days as the non renewable energy resources are depleting, which is obtained from the cultivation of or from waste organic materials. In this paper bioaugmentation novel methods for bio hydrogen production by photo-fermentation bacteria immobilized on agar gel granule and with two different combined bacterial cell cultures (ratios) giving high yield are discussed.

**Index Terms**— Bacteria, Bioaugmentation, Biogas, Biohydrogen, Biofuel

## I. INTRODUCTION

The primary methods of biological hydrogen production are Dark and photo-hydrogen productions. During the dark fermentation process the fermentative bacteria uses organic substances as the energy source and electrons for the purpose of metabolic activities and hydrogen production. In photo-fermentation process, organic substrates are utilized as electron donors and light as source of energy [1, 2]. Dark fermentation hydrogen production with the immobilization technology has been widely studied [3,4]. Under mild conditions, the operations to produce hydrogen from biomass and waste materials are conducted that is why the biological hydrogen production is considered an environmentally friendly process [5, 6].

In order to achieve maximum hydrogen production, bioaugmentation of lactic acid-producing bacteria, *Lactobacillus delbrueckii* ssp. *bulgaricus* TISTR 895 is used in photo-fermentation process from glucose by purple non-sulfur photosynthetic bacteria (PNSB), *Rhodobacter sphaeroides* KCU-PS5. The strain TISTR 895 is a homo-fermentative lactic acid-producing bacteria which produces lactate as its sole end-product [7,8]. In general,

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bacteria were immobilized mainly on carrier such as polyvinyl alcohol [9], polyacrylamide [10], or agar gel [11]. The strain KCU PS5 is able to produce hydrogen from lactate [12].

It was shown that enhancement and stabilization of hydrogen production process can be done by immobilized dark fermentative bacteria. For improving the bio-hydrogen process, there are only few reports about the immobilization of photo-fermentation bacteria [13,14]. When hydrogen is burned as a fuel, water is formed or is used to generate the electricity [15]. Hydrogen is a energy carrier and an valuable alternative fuel. It has high energy content ie., 122 kJ/g which is 2.75 times higher than other hydrocarbon fuels [16].

## II. IMMOBILIZED BACTERIA

The experiment is focused on the factors affecting the photo-hydrogen production. It was shown that immobilized *Rhodospseudomonas faecalis* RLD-53 exhibited the highest hydrogen yield of 3.15 mol H<sub>2</sub>/mol acetate under the following optimal conditions. Inoculum age of 24 h, biomass of 4 mg/ml in agar, agar granule diameter of 2.5 mm, agar concentration of 2%, light intensity of 9000 lux. Immobilized photo-fermentation bacteria will not only enhances the hydrogen production but also increase acid-tolerance capacity even at pH 5.0. The immobilized photo-fermentation bacteria can be applied in the combination of dark and photo-fermentation for hydrogen production with high yield [17].

Table1: Different parameters studied for high hydrogen production by Immobilized bacteria [17].

S.NO	Parameter	Optimum parameter	Hydrogen Production
1	Effect of gel granule size on photo-Hydrogen Production.	2.5 mm of size granule	270 ml/reactor
2	The consumption of acetate under different agar granule size in immobilized photo-hydrogen production	0.018 g acetate/l/h utilization efficiency of substrate	75% - 85% (3.15 mol H <sub>2</sub> /mol acetate)
3	The Effect of Inoculum Age	24 h	246 ml/reactor
4	The Effect of Agar Concentration	1.5% of agar and 2% of agar	254.98 ml/reactor and 249.67 ml/reactor
5	The Effect of Bacterial Concentration in Agar Gel	2 - 4 mg/ml	High
6	pH Tolerant Capacity	6.5 pH	Hydrogen yield of 250 ml and rate of 23.4 ml H <sub>2</sub> /1h
7	Requirement of Light Intensity	7 000 - 9 000 lux	246 ml-H <sub>2</sub> /reactor and 255 ml-H <sub>2</sub> /reactor

III. BACTERIAL CULTURE RATIO

Most of the studies are focused on bioaugmentation of native microorganisms that are capable of producing hydrogen with the dark-fermentative hydrogen producers. The information on bioaugmentation of purple non-sulfur photosynthetic bacteria with lactic acid-producing bacteria is limited and not studied in depth. The addition of the desired microorganisms or Bioaugmentation of specialized microbial strains into the anaerobic digesters can enhance the performance of microbial community and also increase the hydrogen production. As a method to produce hydrogen, bioaugmentation of *Rhodobacter sphaeroides* KKU-PS5 with *Lactobacillus delbrueckii* ssp. *bulgaricus* TISTR 895 was conducted.

Because of the differences in the optimal conditions, metabolic types and nutritional requirements of the two bacteria cultivation in the same bioreactor was still difficult even though well-characterized microorganisms were used in the fermentation system. Evaluation of the physical and chemical factors affecting hydrogen production of PNSB (Purple Non-Sulfur photosynthetic Bacteria) augmented with LAB (Lactic Acid-producing Bacteria) was conducted using a full factorial design followed by RSM (Response Surface Methodology) with CCD (Central Composite Design) [18].

Table2: Hydrogen Production and hydrogen yield for Bioaugmentation [18].

S.NO	Augmented microorganisms	Native microorganisms	Substrate	Hydrogen Production Rate	Hydrogen Yield
1	<i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> TISTR 895	<i>Rhodobacter sphaeroides</i> KKU-PS5	Glucose (5 g/L)	3.6 ± 0.3 ml H <sub>2</sub> /L h	5.93 ± 0.23 mol H <sub>2</sub> /mol glucose
2	Non-augmentation (strain KKU-PS5 only)	-----	-----	3.2 ± 0.1 ml H <sub>2</sub> /L h	4.38 ± 0.20 mol H <sub>2</sub> /mol glucose
3	Strain TISTR 895 under optimal condition	-----	-----	9.1 ± 0.2 ml H <sub>2</sub> /L h	9.65 ± 0.23 mol H <sub>2</sub> /mol glucose

IV. CONCLUSION

The two Bioaugmentation novel methods analyzed here can be utilized for high biohydrogen production. For immobilized photo-fermentation bacteria, it could promote hydrogen production and the conversion efficiency of substrate by lengthening the time of hydrogen production. It demonstrated that the granule diameter, inoculant age, agar concentration, light intensity and biomass in agar are key factors affecting photo-fermentation hydrogen production and when they are 2.5 mm, 24h, 2%, 4 mg/ml and 7000-9000 lux, the maximum hydrogen yield reached 3.15 mol-H<sub>2</sub>/mol acetate.

The augmentation of lactic acid-producing bacteria in the purple non-sulfur photosynthetic bacteria fermentation system is beneficial to hydrogen production. Here lactate is serving as another substrate for hydrogen production. Cumulative hydrogen production of 3396 ± 66 mL H<sub>2</sub>/L and Hydrogen Production of 9.1 ± 0.2 mL H<sub>2</sub>/L h were obtained

under the optimal conditions i.e., the optimal initial pH, light intensity, and Mo concentration obtained from RSM with CCD of 7.92, 8.37 klux, and 0.44 mg/L, respectively. PNSB augmented with LAB produced hydrogen from glucose with a relatively high hydrogen yield 9.65 ± 0.23 mol H<sub>2</sub>/mol glucose, i.e., 80 % of the theoretical yield. By using appropriate environmental conditions for the cultivation of dark- and photo-fermentative bacteria, bioaugmentation of PNSB with LAB and immobilized photo-fermentation bacteria, an improvement in hydrogen production can be achieved.

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