

# Role of Iron Concentration on Hydrogen Production from Waste Water - A Study in Dairy Waste

Yaseen Nalakath  
Civil Dept. MITS  
MITS, Varikoli Ernakulam, Kerala

Anandu S. Babu  
Civil Dept. MITS  
MITS, Varikoli Ernakulam, Kerala

Cerin Mathew Malikudy  
Civil Dept. MITS  
MITS, Varikoli Ernakulam, Kerala

Arun G. Pai  
Civil Dept. MITS  
MITS, Varikoli Ernakulam, Kerala

Julie Jose  
Civil Dept. MITS  
MITS, Varikoli Ernakulam, Kerala

**Abstract**—Hydrogen has been recognized globally as an energy carrier that fulfills all the environmental quality, energy security and economic competitive demands. It is the lightest of all gases and is odourless, colourless and nontoxic. Hydrogen is important because it offers Earth another fuel source and may free it from using fossil fuels someday. It is currently used as a gas and liquid for many different industries and is often used to provide electricity in the fuel cells of automobiles or in internal combustion engines. In the present study, batch experiments are performed using wastewater to examine the influence of iron concentration on hydrogen production. The batch experiments are conducted in varying iron concentrations by checking the physicochemical characteristics of the wastewater. The experiment is mainly conducted by using dairy waste. Iron concentration plays an essential role in the hydrogen production by anaerobic bacteria as it facilitates activation of hydrogenase bacterial action cofactors and hydrogen evolution.

**Keywords**—Hydrogen, Waste water, Sludge, Hydrogenase, Volatile suspended solids, Iron concentration

## I. INTRODUCTION

The worldwide energy need has been increased exponentially, the reserves of fossil fuels have been decreasing, and the combustion of fossil fuels has serious negative effects on environment because of CO<sub>2</sub> emission. Owing to widespread exploitation of fossil fuels severe environmental problems like global warming and ozone depletion have already been initiated, in turn adding to worldwide economical loss by environmental damage.

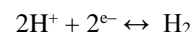
At this juncture, the world is seeking cleaner energy resources to mitigate imminent fuel shortages to provide the energy necessary for future. Hydrogen is considered as the future energy carrier, due to its high energy content and non-polluting nature and also it can be considered as an environmental friendly alternative to fossil fuels and other fuels in automobiles and that which can be produced with less impact on environment. Hydrogen production from the recycling of organic waste is considered as a greener

technology compared to conventional hydrogen production methods.

Hydrogen is considered as a viable alternative fuel and energy carrier of future. Hydrogen gas is clean fuel with no CO<sub>2</sub> 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. Demand on hydrogen is not limited to utilization as a source of energy. Hydrogen gas is a widely used feedstock for the production of chemicals, hydrogenation of fats and oils in food industry, production of electronic devices, processing steel and also for desulfurization and reformulation of gasoline in refineries.

## II. IMPORTANCE OF IRON

The potential use of microorganisms for biological production of hydrogen as a future energy resource makes hydrogen metabolism an emerging field of research. Hydrogenase (H<sub>2</sub>ase) is the name given to the family of enzymes that catalyse the reversible oxidation of hydrogen into its elementary particle constituents, two protons (H<sup>+</sup>) and two electrons:



In the light of this reaction, it is reasonable to postulate the bacterial production of hydrogen as a device for disposal of electrons released in metabolic oxidations through the activity of hydrogenases. These are a heterogeneous group of enzymes with different sizes, subunit compositions, metal contents and cellular localizations. On the basis of metal content of catalytic subunit, H<sub>2</sub>ase can be grouped into two non-homologous classes – those containing only Fe at the active site, called Fe-H<sub>2</sub>ase and those with Ni, Fe and sometimes Se, [Ni-Fe]

H<sub>2</sub>ase and [Ni-Fe-Se] H<sub>2</sub>ase. Initially Fe-H<sub>2</sub>ase was presumed to be present in a limited number of bacteria and anaerobic living protozoa. Subsequently, it was revealed that its distribution in eukaryotes is also quite significant. Genes bearing signatures of Fe-H<sub>2</sub>ase are found not only in prokaryotes and lower eukaryotes, but also in the genome of higher eukaryotes like mammals, although the physiological activity of these proteins is yet to be found out. The presence of the enzyme Fe-H<sub>2</sub>ase in bacteria has been known for over 70 years. The requirement of Fe for its activity was discovered in 1950s.

### III. LITERATURE REVIEW

Osuagwu Chiemiwo Godday & Agamuthu Pariatamby: Study was about Bio-Hydrogen Production from Food Waste through Anaerobic Fermentation. Food waste was used as the substrate and the Acclimatized food waste substrate enhanced bio-hydrogen production by 90% - 100%.

Upadhyay et al. (2015) conducted a study about the Production of bio-hydrogen gas from wastewater by anaerobic fermentation process. The major criteria for the selection of waste materials to be used in biohydrogen production are the availability, cost, carbohydrate content and biodegradability. Simple sugars such as glucose, sucrose and lactose are readily biodegradable and preferred substrates for hydrogen production. However, pure carbohydrate sources are expensive raw materials for hydrogen production.

R Mullai et al. (2015) conducted a study based on the role of iron concentration in confectionery waste water. Compared to other metal ions, iron has the benefits of high protection during handling as it scarcely reacts with water at low temperature, is easily recoverable, and is efficiently excavated. Iron concentration plays an essential role in the hydrogen production by anaerobic bacteria as it facilitates activation of hydrogenase, bacterial enzyme cofactors and hydrogen evolution.

Maleek et al. (2010) conducted a study based on the Influence of Metal ions on hydrogen production by photosynthetic bacteria grown in Escherichia coli pre-fermented cheese whey. Addition of N<sub>2</sub> gas at intervals activates H<sub>2</sub> production. The addition of Molybdenum, Manganese and iron plays a crucial role in accelerating the rate of hydrogen production.

NanqiRen et al. (2012) conducted a study based on the effects of metal ions and L-cysteine on *hydA* gene expression and hydrogen production by *Clostridium beijerinckii* RZF-1108. Iron is a key component of the cytochrome and iron-sulphur proteins involved in electron transport and probably iron might enhance hydrogen production via the stimulation of enzyme activity such as hydrogenase which contains [Fe-S] clusters in its active site. Iron ions may influence the synthesis of ferredoxin which is related to hydrogen production in suitable concentration ranges.

Yusoff et al. (2008) conducted a study based on Trace metal effect on Hydrogen production using *C. acetobutylicum*. Bio-hydrogen production requires essential micronutrients for bacterial metabolism during fermentation. Sodium, magnesium, zinc and iron are all important trace metals affecting hydrogen production. Among them iron is an important nutrient element to form hydrogenase or other enzymes which almost all biohydrogen production needs fundamentally. The results shows that increasing the iron concentration affect inversely on hydrogen production if used more than the proper concentration and was better for bacterial biomass suggested that the effect was related on the enzymes responsible for hydrogen evolution.

SikShin et al. (2015) conducted a study about the Feasibility of bio-hydrogen production by anaerobic co-digestion of food waste and sewage sludge. Food waste showed higher specific hydrogen production potential than sewage sludge. Carbohydrates are the preferred substrate for fermentative hydrogen producing bacteria. Volatile solids concentration and mixing ratios of waste water and sludge plays a crucial role in hydrogen production. The mixing ratios of food waste to sewage sludge were designed to be 100:0, 80:20, 60:40, 40:60, 20:80, and 0:100 on VS basis; however, the experiments at 20:80, and 0:100 for VS 3.0%, and 40:60, 20:80, and 0:100 for VS 5.0% could not be conducted due to low VS concentration of sewage sludge. The addition of sewage sludge on food waste up to 13– 19% could enhance hydrogen production potential due to balanced carbohydrate/protein ratio.

Mameri et al. (2014) conducted a study based on Dark fermentative hydrogen production rate from glucose using facultative anaerobe bacteria *E. coli*. Temperature has significant influence on the hydrogen production rate. The effect of temperature ranging from 25°C to 40°C on fermentative hydrogen production was investigated in batch tests and the results shows that the hydrogen production rate increases with increasing temperature from 25 °C to 35°C, however, it decreases with further increasing temperature from 35°C to 40°C. The possible reason for the development in bioprocess efficiency with increasing temperature from 25°C to 35°C is that the ability of hydrogen-producing bacteria to degrade substrate and produce hydrogen increased with increasing temperatures. Lower hydrogen production rate is observed at the temperatures higher than 45°C because some essential enzymes and proteins associated with cell growth or hydrogen production, such as hydrogenase, may be inactivated by an increase in denaturation rate of the enzymes when the temperature gets too high.

Ferreira et al. (2014) conducted a study based on Hydrogen production by dark fermentation and suggested that in order to enhance H<sub>2</sub> production, culture conditions such as the substrate concentration, pH, and temperature need to be considered. Dhanasekar et al. (2014) conducted a study based on the effect of aeration on microbial production of hydrogen from maize stalk hydrolysate. Enhanced production of hydrogen is obtained under micro aerobic condition when compared to strict

anaerobic and partial aerobic condition and also the yield under micro-aeration conditions was found to be almost fivefold higher than the other two conditions. But in the project experiments are carried out under strict anaerobic condition by sparging nitrogen in the reactor since the micro aeration condition is difficult to set up also the Hydrogen production under strict anaerobic condition was increased to 1.83 times using microbial electrolysis cell which is a technology related to microbial fuel cells.

Wan et al. (2009) conducted a study based on the Factors influencing fermentative hydrogen production. The factors influencing fermentative hydrogen production are inoculum, substrate, reactor type, nitrogen, phosphate, metal ion, temperature and  $p^H$ . Nitrogen is a very important component for proteins, nucleic acids and enzymes that are of great significance to the growth of hydrogen-producing bacteria, it is one of the most essential nutrients needed for the growth of hydrogen-producing bacteria. Thus, an appropriate level of nitrogen addition is beneficial to the growth of hydrogen producing bacteria and to fermentative hydrogen production accordingly.

#### IV. METHODOLOGY

##### A. Experimental Setup

A batch reactor is a vessel used to mix chemicals under tightly controlled conditions. It is distinguished from a continuous reactor by its cyclic use, mixing one batch at a time, as opposed to the constant reaction carried out in a continuous reactor. In this project Batch hydrogen production experiments are carried out in a 250-mL Erlenmeyer flask which acted as batch reactor. Working volume was considered as 100ml. The flask was tightly closed using a rubber cork with two outlets, one for sample collection and the other for hydrogen gas. The sample collection outlet was tightly closed using a clamp in order to prevent the entry of air from outside.

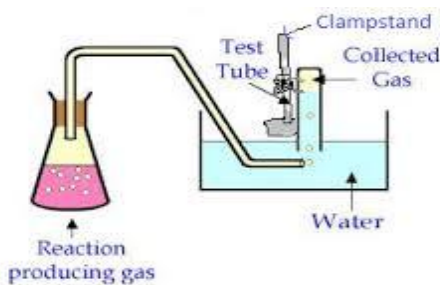


Fig. 1 Experimental set up for water displacement method

The water displacement method is the process of measuring the volume of an irregularly shaped object by immersing it in water. This method served as the basis for the principle developed by the Greek philosopher Archimedes. The principle states that a body immersed in a fluid is buoyed up by a force equal to the weight of the displaced fluid.

The principle applies to both floating and submerged bodies and to all fluids, i.e., liquids and gases. The obtained gas is collected in an inverted jar either graduated or not. If it is graduated the amount of gas can be easily calculated and if it is not suitable methods are adopted to calculate the amount of obtained gas.

##### B. Sample Collected

Sample was collected from a nearby dairy unit. The taken sample includes both waste water and sludge. Waste water is collected from the sedimentation tank which comes just after the skimming tank. The sample without oil and grease are collected since their presence may affect the bacterial action which helps in efficient hydrogen production. Sludge was collected from the bottom of settling tank and it was used in the experiment as it contain large amount of biomass including bacteria which facilitates biological reactions.

##### C. Experiment conducted

- Before and after conducting the experiment, test for  $p^H$  and solids were done.
- The  $p^H$  of the sample should be within the range of 6-7 and test temperature should be 25°C-35°C.
- Heated the sample for 2 hours at 110°C for inactivate the methanogens and enhancing hydrogen producing bacteria.
- Mix waste water and sludge in different proportions with different iron concentrations
- Observe the evolution of hydrogen for 3 days

#### V. RESULT AND DISCUSSIONS

The experiment is conducted in various iron concentrations of sludge and waste water like 50:50, 60:40, 70:30, 80:20, and 90:10. The following are the values obtained for solids and  $p^H$  before and after conducting the experiment and the corresponding evolution of hydrogen for each mix proportions of sludge and waste water.

##### A. 50% WASTE WATER AND 50% SLUDGE

TABLE I. BEFORE TESTING

Sl.no	Parameter	Obtained value
1	pH	6.9
2	Total solids	550 mg/l
3	Total fixed solids	50 mg/l
4	Filterable solids	150 mg/l
5	Non filterable solids	1000mg/l
6	Volatile solids	500 mg/l

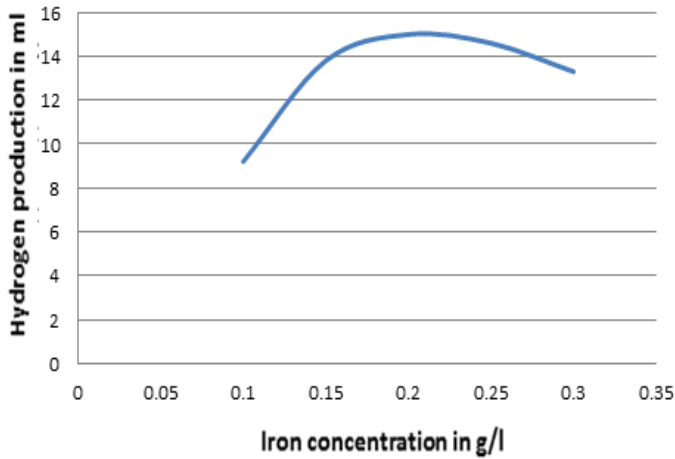


Fig. 2 Hydrogen production

TABLE II. AMOUNT OF HYDROGEN PRODUCED IN VARIOUS IRON CONCENTRATION

Iron Concentration in g/l	0.1	0.15	0.2	0.25	0.3
Hydrogen production in ml	9.2	13.8	15	14.6	13.3

TABLE III. AFTER TESTING

Sl.no	Parameter	Obtained value
1	pH	6.7
2	Total solids	250 mg/l
3	Total fixed solids	50 mg/l
4	Filterable solids	100 mg/l
5	Non filterable solids	700 mg/l
6	Volatile solids	200 mg/l

**B. 10% WASTE WATER AND 90% SLUDGE**

TABLE IV. BEFORE TESTING

Sl.no	Parameter	Obtained value
1	pH	6.8
2	Total solids	650 mg/l
3	Total fixed solids	100 mg/l
4	Filterable solids	350 mg/l
5	Non filterable solids	1200 mg/l
6	Volatile solids	550 mg/l

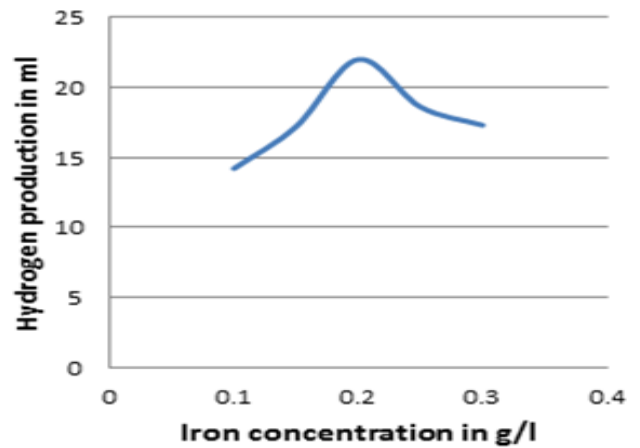


Fig. 3 Hydrogen production

TABLE V. AMOUNT OF HYDROGEN PRODUCED IN VARIOUS IRON CONCENTRATION

Iron Concentration in g/l	0.1	0.15	0.2	0.25	0.3
Hydrogen production in ml	14.2	17.2	22	18.6	17.3

TABLE V. AFTER TESTING

Sl.no	Parameter	Obtained value
1	pH	6.8
2	Total solids	800 mg/l
3	Total fixed solids	100 mg/l
4	Filterable solids	450 mg/l
5	Non filterable solids	750 mg/l
6	Volatile solids	700 mg/l

**VI. CONCLUSIONS**

- Maximum hydrogen production is obtained at  
 - .2 g/l of iron concentration  
 - 90% sludge and 10% waste water
- There is no change in total fixed solids before and after experiment
- There is a reduction in the value of solids after the experiment.

**ACKNOWLEDGMENT**

The authors would like to express an acknowledgement to Mary Lissy P N, Head of Civil Engineering Department of Muthoot Institute of Technology and Science, Yaseen Nalakath, Assistant Professor of Muthoot Institute of Technology and Science and management of Muthoot Institute of Technology and Science, Varikoli, Ernakulam for providing the facilities in the environmental engineering laboratory to accomplish this study. The author also wishes to acknowledge cooperation given by laboratory technician from Faculty of Civil Engineering, to complete this study.

---

## REFERENCES

- [1] Mullai et al. "Role of iron concentration in confectionery waste water" Journal of Environmental Engineering, ASCE 2015
- [2] Upadhyay et al. "Production of Bio-Hydrogen Gas from Wastewater by Anaerobic Fermentation Process" International Journal of Chemical Studies 2015
- [3] SikShin et al. "Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge" International Journal of Hydrogen Energy 2015
- [4] Dhanasekar et al. "Effect of aeration on microbial production of hydrogen from maize stalk hydrolysate" International Journal of Chemical Studies 2014
- [5] Mameri et al. "Dark fermentative hydrogen production rate from glucose using facultative anaerobe bacteria E coli" International Journal of Hydrogen energy 2014
- [6] Ferreira et al. "Hydrogen production by dark fermentation" International Journal of Chemical Studies 2014
- [7] Pariatamy et al. "Bio-hydrogen production from food waste through anaerobic fermentation" International Journal of Hydrogen Energy 2014
- [8] Mullai et al. "Artificial neural network (ANN) modelling for hydrogen production in a continuous anaerobic sludge blanket filter (ASBF)" International Journal of Applied Sciences 2013
- [9] Ana Raquel Ribas Cordeiro "Biological hydrogen production using organic waste and specific bacterial species" International Journal of Chemical Studies 2013
- [10] NanqiRen et al. "Effects of metal ions and L-cysteine on hydA gene expression and hydrogen production by Clostridium beijerinckii RZF-1108." Journal of Biological sciences 2012
- [11] Tyagi et al. "Fermentative hydrogen production – An alternative clean energy source" International Journal of Hydrogen Energy 2012
- [12] Maleek et al. "Influence of Metal ions on hydrogen production by photosynthetic bacteria grown in Escherichia coli pre-fermented cheese whey" Journal of Environmental Protection 2010
- [13] Wan et al. "Factors influencing fermentative hydrogen production" International Journal of Hydrogen Energy 2009
- [14] Thirumavalavan et al. "Microbial production of hydrogen from sugarcane bagasse using bacillus sp. Bagasse extract is used as a carbon source" International Journal of ChemTech Research 2009
- [15] Yusoff et al. "Trace metal effect on Hydrogen production using C.acetobutylicum." Journal of Biological sciences 2008
- [16] Zhang yu et al. "Hydrogen characteristics of the organic matter of municipal solid wastes by anaerobic mixed culture fermentation" International Journal of Hydrogen Energy 2008
- [17] Xuemei Liu et al. "Recent advances in fermentative biohydrogen production" International Journal of Hydrogen Energy 2007
- [18] JianquanShen et al. "Effect of ferrous iron concentration on anaerobic bio-hydrogen production from soluble starch" International Journal of Hydrogen Energy 2006
- [19] Veziroglu et al. "Role of Fe-hydrogenase in biological hydrogen production" International Journal of Hydrogen Energy 2006
- [20] Hang-SikShin et al. "Feasibility of bio-hydrogen production by anaerobic co-digestion of food waste and sewage sludge Food waste" International Journal of Hydrogen Energy 2004