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BIOLOGICAL HYDROGEN PRODUCTION BY BACTERIA USING PINEAPPLE PEEL

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Abstract: Biohydrogen is a renewable source of energy. It can be produced by using technologies similar as thermochemical, electrolysis, print electrochemical and natural etc. Among these technologies, the natural system (dark fermentation) is considered more sustainable and ecofriendly. Dark turmoil involves anaerobic microbes which degrade carbohydrate rich substrate and produce hydrogen. Lignocellulose biomass is an abundantly available raw material and can be employed as profitable and renewable substrate for biohydrogen product. Although there are numerous hurdles, nonstop advancements in lignocellulose biomass pretreatment technology, microbial fermentation (mixed substrate and co-culture fermentation), the involvement of molecular biology ways, and understanding of colorful factors (pH, T, addition of nanomaterial's) effect on biohydrogen productivity and yield render this technology effective and able to meet unborn energy demands. Farther integration of biohydrogen product technology with other products similar as bio-alcohol, Unpredictable adipose acids and methane have the eventuality to ameliorate the effectiveness and economics of the overall process. In this composition, colorful styles used for lignocellulose biomass pretreatment, technologies in trends to produce and ameliorate biohydrogen product, a coproduction of other energy coffers, and techno- profitable analysis of biohydrogen Product from lignocellulose biomass are reviewed.

Key points: Biohydrogen, Anaerobic and dark fermentation, Energy source, lignincellulose.

1. INTRODUCTION:

Hydrogen is considered as a one of the voluntary Energy and "energy carrier" for the coming days. No emigration of CO2 occurs in hydrogen energy and can fluently be used in energy cells for electricity product. According to the recent reviews on hydrogen it shows that the demand of hydrogen is adding day by day. Hydrogen is used as a reactant in hydrogenation process, as energy in rocket machines, as a O2 scavenger and also as a coolant in electrical generation. The hydrogen demand is adding day by day and because of this increase in demand of hydrogen, development of cost effective and effective hydrogen product technologies has earned the notable attention. In agreement with sustainable development and waste minimization issues

bio-hydrogen gas product from renewable sources, also known as “green technology” has entered considerable attention in recent times. product of clean energy source and effective application of waste accoutrements make natural hydrogen product a new and promising approach to meet the adding energy requirements as a cover for fossil energies. Hydrogen is produced biologically through memoirphotolysis of water using algae and cyanobacteria, photodecomposition of organic composites by photosynthetic bacteria, fermentative hydrogen product from organic composites, and mongrel systems using photosynthetic and fermentative bacteria. Hydrogen is a promising indispensable energy carrier and is also considered to be a clean energy. It only produces water when burned with oxygen and has an energy content2.75 times advanced than hydrocarbon energies. Hydrogen can be produced using natural, chemical, and physical processes. The natural hydrogen product process has gained further interest than chemical and physical processes because it's a sustainable process that consumes lower energy. Biological hydrogen product can be divided into two types, i.e., a phototrophic process and a dark turmoil process. Dark turmoil has advantages over the phototrophic process in terms of its capability to continuously produce hydrogen from a variety of feedstocks without an external input of energy. Pineapple waste is principally composed of residual pulp, peel, and cores. Pineapple waste isn't considered seductive as beast feed because of its high fiber content, high answerable carbohydrate and low protein content. still, the pineapple waste excerpt, i.e., the juice attained after the pineapple waste has been squeezed by a presser, substantially contains sugars and organic acids that can be employed as the substrates in the product of hydrogen and ethanol. Due to its composition, we tried to use the pineapple waste excerpt in hydrogen product using anaerobic mixed societies. In order to achieve maximum hydrogen product, there was a need to give suitable turmoil conditions, especially the environmental factors similar as temperature, pH, nutrient addition, buffer, substrate attention and ferrous iron. The applicable attention of substrate could enhance bacterial growth and exertion. An inordinate substrate attention can beget a figure- up of unpredictable adipose acid (VFAs) in the turmoil broth, leading to a low pH in the system. also, pineapple waste excerpt contains a high Quantum of short chain organic acids, which in turn means that the excerpt would have a low pH and a high acid attention. A high acidic content in the substrate can beget adverse goods on hydrogen product. therefore, the addition of a buffer to offset a drop in pH is demanded. End nutrient and iron are frequently used to enrich microorganisms able of producing hydrogen. The important rudiments contained in the Endo – nutrient similar as Cu2, Co2, Mg2 are essential for microorganism growth and exertion. Iron is the important element for the exertion of hydrogenate, which is an important enzyme for hydrogen product. original pH is a physical factor that also has a great influence on hydrogen Product. In general, the applicable range of original pH for hydrogen product and cell growth is in the ranges of 4.0 –7.0. still, and if enough If the original pH is outside the optimal range buffer capacity isn't present, the hydrogen product process will be inhibited. study the biological hydrogen product by Bacillus sp. Using pineapple peel. Collection of the fruit waste- pineapple peel, Study the natural pretreatment process (Enzymatic system), insulation of hydrogen producing bacteria, Anaerobic fermentation.

2.Materials And Method:

2.1. Collection of sample:

Pineapple peel was collected and local shop. Pineapple peel was cutted into small piece, dried and stored at room tempecture.

2.2. Pretreatnment:

Webbing of celluolose degrading bacteria on cmc (carboxyl methylcellulose) agar was done grounded on the standard protocol. Cmc agar in which cellulose is used as energy, was prepared from cellulose 2g, MgSO40.25 g, agar 15g, KH2PO40.5 g, gelatin 2g and congored0.2 g in 1L of distiled water at pH 7 ± 2. The result was autoclaved to prepare sterile media. culturing was done by spreading0.5 mL of fluid on sperate plate containing cmc agar from each serially diluted solution the culture was incubated at 37 ℃ for 5 days. The bacterial colony showing zone of concurrence on cmc was considered as cellulose demeaning bacteria. Hydrolysis of cmc and periphery of clear zone was used descry celluloytic exertion. Hydrolytic value of cellulose demeaning bacteria was expressed as the rate of clear zone periphery to clear zone periphery. Numerical value is attained by dividing clear zone periphery by colony periphery. The cellulose demeaning bacterial enzyme is used to degrade the cellulose and lignicellulose bond in the pineapple peel it convert simple sugar and sugar convert into Energy, primarily ethanol and hydrogen product like bounce. The supernatant and cellulosic bullets were collected by centrifugation 12,000 rpm for 15 mins. The supernatant was sludge was collected for sugar assay by DNS system. The composition of pineapple peel and the supernatant, including cellulose, hemicellulose, lignin and determined.

2.3. Enzymatic hydrolysis:

Treatnment of cellulose material with cellulose degrading degrading bacterial enzyme. The 1% of bacterial enzyme is added to the fermentation medium and kept for 1-2 weeks, for break down the cellulose in the pineapple peel.

2.4. Isolation of hydrogen producing microorganism:

The soil sample is collected from the field and 1gm of soil is taken and dissolve into 10Ml of distilled water. The soil sample is heated in the water bath for 10mins and allowed to cool. Hydrogen producing sample was isolated from soil using serial dilution technique. the sample is inoculated into the nutrient broth at 37℃ for 24hrs. gram staining and biochemical test is performed to identify the microorganism then inoculated into the fermentation medium.

2.5. Anaerobic fermentation:

Expernmental setup:

The hydrogen production experenment was carried out in 250mL of conical flask with a 150mL of working volume and 1% of inoculum is add and the 10 gm of glucose and sucrose is add into the fermentation medium. Then using rubber cork is taken and cover the conical flask and one end of tube is taken and inverted into the conical flask and another end of tube is inverted into the beaker. The beaker containing 20% of KOH the test tube full of water is taken and downward position in the beaker.

3. Result and discussion:

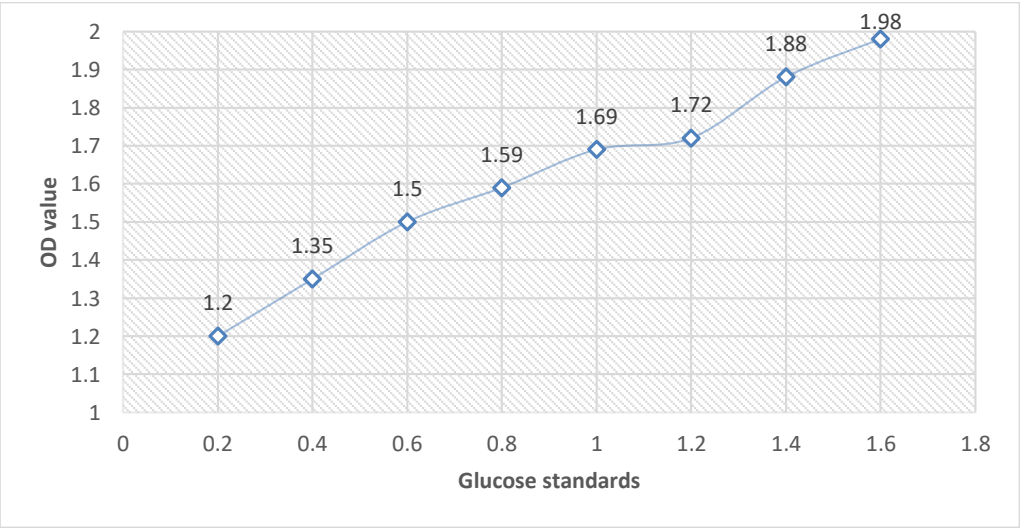
3.1. Sample:

The pineapple peel was collected from the local shop and cut into small piece, dried, and stored at refirged. Glucose and sucrose are the important components present in pineapple peel and glucose is the main carbon source.

3.2. Enzymatic method:

The cellulose degrading bacteria was isolated from the soil using cmc (carboxyl methyl cellulose). The zone was observed in the medium. The isolated and maximum amount of cellulose showed highest enzyme activity. Cmc agar allows us to identify and isolates with cellulase activity on soluble cellulose such as cmc thus representing and beta – glucosidase activity. Cellulose enzyme can break the sugar bond in pineapple peel and have high level of sugar is present in the fermentation medium. The amount of sugar is estimated every day. The estimation of sugar is calculated in DNSA method. The estimation of sugar was present after enzymatic method have 1.73g/L.

TUBES	OD Value
Tube 1	1.60
Tube 2	1.65
Tube 3	1.69
Tube 4	1.72
Tube 5	1.75
Tube 6	1.79
Tube 7	1.88
Tube 8	1.98
Sample	1.73



3.3. ISOLATION OF HYDROGEN PRODUCING BACTERIA:

The soil was taken and heated in the tube because organism can produce the spores. The spores was isolated and inoculated into the nutrient broth. The spore forming bacteria can ability to produce the hydrogen. The hydrogen production organism was grow in nutrient broth at 37℃ for 24hrs. After the incubation gram staining is performed. Gram positive organism and rod shaped bacteria was seen. gram positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain crystal violet to strengthen the bonds of the stain with the cell membrane. Spore staining also done, due to the highly resistant nature of endospores, they are not easily penetrated by strains. This it is necessary to steam the stain into an endospore. The schaeffer- fulton method is the most commonly used endospore staining technique, which uses malachite green as the primary stain. Once the endospore has absorbed the stain it is resistent to decolorization but the vegetative cell is easily decolorized with water leaving the vegetative cells colourless. Finally the vegetative cells are counterstained with safarain to aid in their visualization. When viewed under a microscope the endospores appear green while the vegetative cells are red or pink in spore staining vegetative spore are seen. And finally biochemical test also done for conform the *Bacillus sp*.

BIOCHEMICAL TEST	*Bacillus sp*
Indole	-
Methyl red (MR)	-
Vogues proskauer(VP)	+
Citrate	+
Oxidase	+
Motility	+
Catalase	+
Spore	+

3.4. Anaerobic Fermentation:

The conical flask contain pineapple peel and 1% of *Bacillus sp* culture was added. Glucose and Sucrose sugar was added into the fermentation medium. Before added the culture and sugar pH is adjust to ±7 in the medium. The enzyme can breakdown the sugar. The *Bacillus sp* is the facultative anaerobes. The fermentation medium was kept in anaerobic fermentation. The *Bacillus sp* have the ability to produce the hydrogen in anaerobic fermentation because the organism can adjuct its metabolism and respiration depending upon the availability of oxygen. During fermentation some other gas also produce KOH can utilize the gas finally the hydrogen can be filtered in the test tube. The glucose added medium was incubated at 37℃ for 24hrs the hydrogen producing bacteria can produce 6.5mL of hydrogen/100mL. The sucrose added medium was incubated at 37℃ for 24hrs the hydrogen producing bacteria can produce 4mL of hydrogen/100mL.

4. CONCLUSION:

The pineapple peel have the high amount of cellulosed and sugar production and enzymatic hydrolysis. The enzyme hydrolysis was utilized as fermentation medium. Then added the bacillus sp culture along with glucose and sucrose will be added in the fermentation medium. pH ±7 at 37°Cfor 24hrs incubated. The glucose added medium have 6.5mL of hydrogen/100ML production during fermentation, in this medium 60% of hydrogen will be produced. The 20gm of sugar could produce 0.06 hydrogen per ml. The sucrose added medium have 4mL of hydrogen/100ML production during fermentation, in this medium 40% of hydrogen will be produced. The 20gm of sugar could produce 0.04 hydrogen per ml. The hydrogen can be used as alternative source or fuel.

5. REFERENCE:

1. Baseman, J. Hydrogen biotechnology: Progress and prospects. *Nat. Biotechnology*. **1996**, 14, 1101–103.
2. Cheong DY, Hansen CL (2006) Abiogenesis characteristics of natural, mixed anaerobes converting carbohydrate-rich synthetic wastewater to hydrogen. Process Bioche41 (8):1736–1745.
3. Chen, W.H.; Chen, S.Y.; Khanal, S.K.; Sung, S. Kinetic study of biological hydrogen production by anaerobic fermentation. *Int. J. Hydro. Energy* **2006**, *31*, 2170–2178.
4. Coria, R.T.P.; McCabe, P.; Magadha's, M.M.A.; Macedon, G.R.; Shetty, K. Production of phenolic antioxidants by the solid-state bioconversion of pineapple waste mixed with soy flour using *Rhizopus oligosporus*. *Process Brioche.* **2004**, *39*, 2167–2172.
5. Das D, Veziroglu TN. Hydrogen production by biological processes: a survey of literature. Into J Hydrogen Energy 2001; 26:13–28.
6. Fang HHP, Li CL, Zhang T (2006). Acidophilic Biohydrogen production from rice slurry. Into J Hydrogen Energy 31(6):683–692.
7. Fan YT, Zhang YH, Zhang SF, Hour HW, Ran BZ. Efficient conversion of wheat straw wastes into biohydrogen gas by cow dung compost. Bio resource Techno 2006; 97:500–5.
8. Garret GM, Brenner DJ, Krieg NR, Staley JT...Berge's manual of systematic bacteriology. The Proteobacteria, Part A, Introductory. Vole Two, New York; Springer. 2005.
9. Kawagoshi Y, Hino N, Fujimoto A, Macao M, Fujita Y, Sugimura S, Furukawa K (2005) Effect of inoculum conditioning on hydrogen fermentation and pH effect on bacterial community relevant to hydrogen production. J Basic Boeing 100(5):524–530.
10. Momirlan, M.; Veziroglu, T.N. Current status of hydrogen energy. *Renew. Sustain. Energy Rev*.**2002**, *6*, 141–179.