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RESEARCH ARTICLE

HYDROGEN PRODUCTION FROM SWEET SORGHUM AND SUGARCANE JUICE USING *Escherichia coli*

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ABSTRACT

In the present study *E .coli* was selected for the fermentative hydrogen production. The *E. coli* strain was isolated from sewage sample and identified based on gram staining and growth on selective media (EMB agar). Isolated E. *coli* was used for hydrogen production using sweet sorghum juice and sugarcane juice. The hydrogen produced by *E. coli* was estimated by water displacement method. The more amount of water was being displaced (420 ml) in 24 h by *E. coli* using sugarcane juice. Whereas 390 ml of water was displaced where the sweet sorghum juice used as a substrate. More amount of water was displaced (420 ml) by *E. coli* using sugarcane juice at the pH of 6.5 when compared with pH 4.5 (315 ml), pH 5.5(390ml),pH 6.5 (420 ml), pH 7.5 (400 ml) and atpH 8.5 (320 ml). The result revealed that the *E. coli* can produce more amount of hydrogen using sugarcane juice as substrate at the pH range from 5.5 (390 ml) to 6.5(420 ml).

INTRODUCTION

Hydrogen is a clean and environmentally friendly fuel. It not only has a high energy content of 122kJ/g which is about 2.27 times greater than hydrocarbon fuels. (Kapdan *et al*., 2006). It is considered to be the cleanest energy carrier because of the combustion by product is only water and it does not produce a greenhouse gas (Das *et al.,* 2001). It can be used as a raw material for various industrial applications. Although hydrogen is the most abundant element in the universe, where it appears naturally on the earth's crust it is bound with other elements such as carbon and oxygen instead of being in its molecular "H_2" form. Molecular hydrogen is produced for various uses, and this can be done in various ways, (Lipman, 2004), Hydrogen has several desirable characteristics; it has high conversion efficiency, it is recyclable and non- polluting, these characters make hydrogen the fuel of future. Furthermore hydrogen could be directly used to produce electricity through fuel cells (Benemann *etal.,* 1996). An important future application of hydrogen could be as alternative for fossil fuels once the oil deposits are depleted. Most of this hydrogen gas is currently made from synthesis gas that comes either from the reformations of natural gas or from the gasification of coal, these processes are costly and environmentally problematic. Therefore, society must develop economical, continuous, environmentally friendly methods of production. Biological production of hydrogen as an end product or byproduct of the metabolism of biological organisms has been proposed as one means of producing needed hydrogen (Zaborsky, 1997). Biohydrogen production can be either photosynthetic or non-photosynthetic. Photosynthetic hydrogen production is carried out by Algae (Cyanobacteria) and photosynthetic bacteria. (Kumazava *et al.*, 1981). Non photosynthetic or fermentative productions carried out by facultative anaerobes and obligate anaerobes. (Kim *et al.,* 1999 and Lee *et al.,* 2002). Works on biohydrogen production have been in progress for more than 3 decades demonstrated that a nitrogen fixing *Cyanobacterium, Anabaenacylindria* produces hydrogen and oxygen gas simultaneously in an argon atmosphere for several hours. There is a practice of combined use of photosynthetic and anaerobic bacteria for the conversion of organic acids to hydrogen. (Khanal, 2004).

The main source of hydrogen during a biological fermentative process is carbohydrates, which are very common in plant tissues either in the form of oligosaccharides or their polymers, cellulose hemicelluloses and starch. Thus the biomass of certain plants with high content in carbohydrates could be considered as very promising substrate for biohydrogen production. The maximum hydrogen yield is 4 mole hydrogen per mole of glucose utilized (Nandi and Sengupta, 1998). Many substrates have been used for fermentative hydrogen production; glucose, sucrose and starch have been the most widely used. In recent years, however, a few studies have begun to use organic wastes as a substrate for hydrogen production (Kapdan and Kargi, 2006). Unused lignocellulosic waste biomass from forestry, agriculture, and municipal sources is a potential feedstock for the synthesis of biofuels like hydrogen, which could replace fossil fuels and reduce greenhouse gas emissions (Das and Veziroglu, 2001; Hallenbeck, 2005 and Levin *et al*., 2007). Sweet sorghum (*Sorghum bicolor*) is an annual plant of tropical origin well adapted to sub-tropical and temperate regions and highly productive in biomass. Sweet sorghum stalks are rich in sugars, mainly in sucrose that's amount up to 55% of dry matter and in glucose 13.2% of dry matter. They also contain cellulose (12.4%) and hemicelluloses 10.2% (Billa *et al.,* 1997). Sweet sorghum biomass is rich in readily fermentable sugars and thus can be said to be an excellent raw material for hydrogen production. Out of many new crops that are investigated as potential raw material for energy and industry, sweet sorghum seems to be the most promising one (Dalianis *et al.,* 1996). Although sorghum has been thoroughly investigated as an energy crop for bio ethanol and methane production (Richards *et al,* 1991). It has not

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been used so far as a potential source for hydrogen production. Since both soluble and complex carbohydrates can be utilized either in a single step or separately after extraction process. Extraction is achieved by water at 30^0C. After extractions process the liquid fraction (sorghum extracts) rich in sucrose and a solid fraction contain cellulose and hemicelluloses are obtained. (Mamma *et al.,* 1996). Hence the present study is aimed to produce hydrogen using sweet sorghum and sugarcane as a substrate.

MATERIALS AND METHODS

(i) Collection of sample

The sample was collected in and around Gandhigram Rural Institute (Deemed University), Gandhigram, Dindigul.

(ii) Culture isolation

MacConkey's broth was prepared and 9 ml of broth was transferred in to the 20 ml test tubes. Durham's tubes were dropped upside down into the test tubes. Test tubes were sterilized using autoclave at 121^0C for 15 minutes under the pressure 15 lbs. 1 ml of sewage sample was inoculated into the broth. Then the tubes were incubated at 37^0C for 24 hours.

(iii) Plating of *Escherichia coli*

The MacConkey's broth containing tubes showing positive results were selected for the isolation of *E.coli*.One loopful of culture from the tubes were streaked on MacConkey's plates. After streaking plates were incubated at 37°C for 24 hours.

(iv) Mass multiplication of *Escherichia coli*

For starter preparation 100ml of MacConkey's broth was prepared. 10ml of mother culture of *E.coli* was inoculated. Broth was incubated till to reach optical density of 0.8 at a wavelength of 600nm. The optical density was measured using spectrophotometer. When the OD is reached 0.8 the starter culture was ready for using further work.

(v) Collection of substrate

Young and healthy sweet sorghum stalks and sugarcane were collected from the cultivation area.

(vi) Extraction of juice

Sweet sorghum stalks and sugarcane were grained with sugarcane juice extracting machine approximately 1 liter of juice were collected from sweet sorghum stalks and sugarcane.

(vii) Hydrogen production using fermentor

A batch experiment was carried out in a fermentor. The working volume of fermentor is 500ml. 10% of starter culture was inoculated into the fermentor containing 400 ml of sweet sorghum juice and 400ml of sugarcane juice separately. Initial glucose level should be of 5gm per liter and initial pH 6.8.After inoculation the fermentor was left for one week.Biogas evolved was allowed to pass through 10% NaOH solution for absorption of CO_2.

(viii) Hydrogen production at various pH level

Hydrogen production at various pH (4.5, 5.5, 6.5, 7.5, and 8.5) by using sugarcane juice was carried out in 500ml fermentor. 400 ml of sugarcane juice with various pH and 10% of inoculum (40 ml) was inoculated into the fermentor. After inoculation the fermentor was kept for 24 hours. Gas evolved was passing through 10% NaOH for absorption of CO_2.

(ix) Collection of Hydrogen

The gas coming out from the NaOH bottle was collected in another bottle by water displacement method. The gas volumes were measured by reading from the cylinder scale in the bottle.

RESULT AND DISCUSSION

1. Isolation of *Escherichia coli*

The *E. coli* was isolated from sewage sample. The results showed that the MacConkey's broth color changed from purple to yellow and the formation of green metallic sheen confirms the *E.coli.*

2. Identification of *E.coli*

The *E.coli* strain was identified based on the gram staining, growth on selective media (Eosin Methylene Blue Agar) and biochemical tests the results are given in Table 1. The identified bacterial culture was mass multiplied in nutrient broth for further study.

Table 1. Characteristics of *E. coli*

S No	Identification test	Color change Observed	Result
1	Gram staining	Pink and Rod shaped	Gram negative
2	Streaking on EMB plates	Green metallic sheen	Positive

3. Estimation of hydrogen produced by *E.coli* using water displacement method

The total gas produced by *E.coli* from sweet sorghum juice and sugarcane juice was allowed to pass through 10% NaOH solution. After absorption of other gases the remaining hydrogen gas was collected in another bottle the results are given inn Table 2 and Figure 1.

Table 2. Hydrogen production using sugarcane juice and sweet sorghum juice as substrate

S No	Substrate	Amount of water displaced
1	Sugar cane juice	420 ml
2	Sweet sorghum juice	390 ml

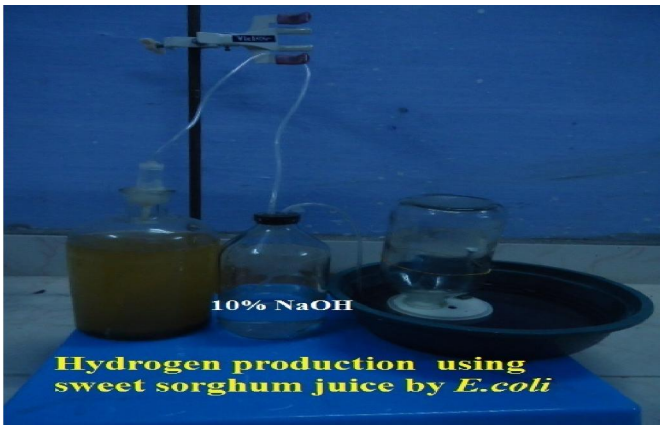

Figure 1. Collection of Hydrogen using water displacement method

4. Hydrogen production at various pH level

The results of production of hydrogen gasby *E.coli* using sugarcane juice at various pH (4.5, 5.5, 6.5, 7.5, and 8.5) at 24 h is given in Figure 2.

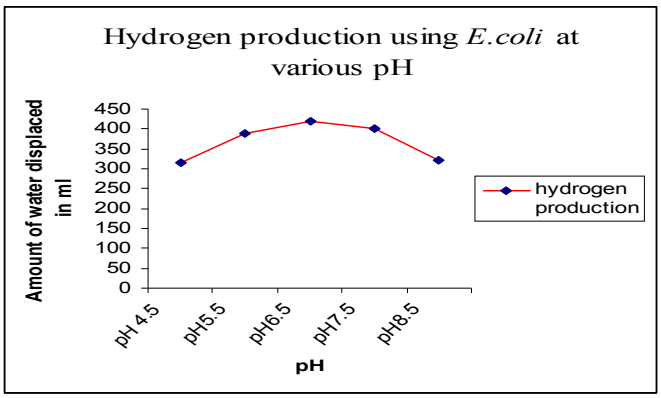

Figure 2. Hydrogen production using *E.coli* at various pH level

DISCUSSION

Hydrogen is considered to be an ideal source of energy for the future because it is easily converted to electricity by fuel cells does not evolve the greenhouse gas carbon dioxide in combustion and is cleanly combustible. Among the many process of hydrogen production microbial hydrogen synthesis is gaining momentum because it is energy saving process (Nandi and Sengupta, 1998). Biohydrogen production from renewable substrates is a promising element in the sustainable hydrogen economy. Very little work has been done on biohydrogen production from renewable substrates using defined or complex microbial consortia. Though raw sewage sludge is abundant in several nutrients, the low hydrogen yield from it suggests the need for nutrient and seed formulation so as to augment its exploitation as substrate for hydrogen production. Different pretreatment techniques coupled with optimal dilution and supplementation is an attempt in this direction. Co-digestion, whole cell immobilization and process optimization should prospectively help in attaining the critical yield value that can upgrade the process for commercial exploitation. (Richards *et al.,*1991). In the present study the attempt was made to estimate the hydrogen produced by *E.coli* using sugarcane juice as a substrate at various pH 5.5, 6.5, 7.5, using water displacement method. The higher amount of water was displaced by *E.coli* at pH of 6.5 (420 ml), compare with pH range of 4.5 (315 ml), 5.5 (390 ml) to 7.5 (400 ml), 8.5 (320 ml) at 24 hour. Sweet sorghum is an annual plant rich in readily fermentable sugar and consider as an excellent biomass for hydrogen production. The highest hydrogen yield obtained from sorghum extract fermented with mixed microbial culture in batch fermentation was 0.86 mol H_2.In the present study hydrogen was produced from sweet sorghum juice and sugarcane juice using *E .coli*. Totally 420 ml of water was displaced by hydrogen when sugarcane as substrate and 390 ml of water was displaced by *E.coli* using sweet sorghum juice.

REFERENCES

Benemann JR. “Hydrogen biotechnology: progress and prospects”. NatureBiotechnology, 1996; 14: 1101-1103.

Billa E, Koullas DP, Monties B and Koukios EG.Structure and composition of sweet sorghum stalk components, Industrial Crops and Products, 1997; 6:297-302.

Dalianis C, Panoutsou C, Dercas N.Sweet and fiber sorghum, two promising biomass crops, In: First European Seminar on Sorghum for Energy and Industry, Toulouse, France, 1-3 April,1996; 173-176.

Das D and TN Veziroglu. Hydrogen production by biological processes: a survey of literature. Int. J. Hydrogen Energy, 2001; 26: 13-28.

Jung GY, kim JR, Jung HO, Park J-Y, Park S. A new chemoheterotrophic bacterium catalyzing water-gas shift reaction. Biotechnology Letter, 1999; 21: 869-873.

Kapdan IK and Kargi F.Bio-hydrogen Production from Waste Materials, Enzyme and Microbial Technology, 2006; 38 (5): 569-582.

Kapdan IK, Kargi F. Bio-hydrogen production from waste materials. Int. J. Hydro. Energy, 2006; 38: 569-582.

Khanal. Biological Hydrogen production: Effects of PH and intermediate products, International Journal of Hydrogen Energy, 2004; 29: 1123 – 1131,

Kumazawa S and A Mitsui. Characterization and optimization of hydrogen photo production by a salt water blue-green alga, Oscillatoria sp. Miami BG7. I. Enhancement through limiting the supply of nitrogen nutrients. Int. J. Hydrogen Energy, 1981; 6: 339-348.

Lee Woon-Jae; Lee Yong-Kuk. "Internal Gas Pressure Characteristics Generated during Coal Carbonization in a Coke Oven". Energy & Fuels, 2001; 15 (3): 618.

Lipman TE. “What Will Power the Hydrogen Economy? Present and Future Sources of Hydrogen Energy,” Institute of Transportation Studies, University of California - Davis, UCD-ITS-RR-04-10, July. 2004; 231-236.

Mamma D, Koullas D, Fountoukidis G, Kekos D, Macris BJ, Koukios E. Bioethanol from sweet sorghum: Simultaneous saccharification and fermentation of carbohydrates by a mixed microbial culture, Process biochemistry,1996;31(4): 377-381.

Nanadi R, Sengupta S. Microbial production of hydrogen: an overview. Crit Rev Microbiol, 1998; 24: 61-8.

Nandi R, Sengupta S, Involvement of anaerobic reductase in the spontaneous lysis of formate by immobilized cells of E coli. Enzyme Microbiol Technol, 1996; 19: 20-25.

Richards BK, Cummings RJ, Jewell WJ. High rate low solids methane fermentation of sorghum, corn and cellulose, Biomass and Bioenergy, 1991;1(5): 249-260.

Zaborsky OR. (Ed.), *Biohydrogen* (Plenum Press, New York.), 1997.

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