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Microbiology

KEY WORDS:

BIO-HYDROGEN PRODUCTION USING *BACILLUS* SP. FROM SUGARCANE (*SACCHARUM OFFICINARUM*)

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ABSTRACT

The bacterial strains were isolated from cow dung sample. The predominant bacterial strain was identified based on the various biochemical characterstics as *Bacillus* sp. Then the bacterial strain was employed for hydrogen production using water displacement metod. 500 ml of water was displaced at 6.8 pH in 24 hours by hydrogen gas produced by *Bacillus* sp., using sugarcane juice. The more amount of water is being displaced by *Bacillus* sp. at a pH 7.5 (785 ml) in 24 hours compared to the other pH conditions, pH 4.5 (410 ml), 5.5 (520 ml), 6.5 (700 ml), and 8.5 (730 ml) in sugarcane juice. The estimation of water displacement by hydrogen gas was upto 11 days using sweet sorghum juice employing *Bacillus* sp.

INTRODUCTION

A large proportion of the world's energy needs are being covered by fossils fuels. This has resulted in both, the increase in CO_2 concentration in the atmosphere and the rapid depletion of fossil resources. The former is considered the main cause of global warming and associated climate change, whereas the latter will lead to an energy crisis in the near future (Kapdan and Kargi, 2006). The major advantage of energy from hydrogen is the absence of polluting emissions since the utilization of hydrogen, either via combustion or via fuel cells, results in pure water (Claassen *et al*., 1999). *Bacillus* is a genus of gram positive, rod shaped bacteria and a member of the division firmicutes. *Bacillus* species can be obligate aerobes (or) facultative anaerobes and test positive for the enzyme catalase. Ubiquitous in nature, *Bacillus* sp. includes both free-living and pathogenic species .under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods.many *Bacillus thuriengiensis* genome was incorporated incorporated into corn (and cotton) crops. Biohydrogen can be produced by strict and facultative anaerobes (*Clostridia, Micrococci* and *Methanobacteria*, etc.) aerobes (*Alcaligenes* and *Bacillus)* and also by photosynthetic bacteria (Nandi and Sengupta, 1998). H_2-producing bacteria are commonly tolerant to harsher environmental conditions (Kawagoshi *et al*., 2005). Biomass, as a product of photosynthesis, is a versatile non-petroleum renewable source that can be utilized for sustainable production of hydrogen Major resources of biomass include agriculture crops and their waste byproducts, lignocellulosic products such as wood and wood waste, waste from food processing, aquatic plants and algae and effluents produced in the human habitat. (Saha *et al*., 2008). Hence the present study is aimed for Bio-Hydrogen production using *Bacillus* spp. from sweet sorghum and sugarcane juice.

MATERIALS AND METHODS

Collection Of Sample:

The cow dung sample was collected from Agri farm, The Gandhigram Rural University-Deemed to be University Gandhi gram, Dindigul district, Tamilnadu, India.

Isolation And Identification And Mass Multiplication Of Bacillus Species:

Isolation Of Bacillus Species:

1gm of cow dung sample was mixed with 100ml of sterilized distilled water and serially diluted the sample. The serially diluted sample were individually plated on nutrient agar medium and incubated at 28^0 C $\pm$ 2^0 C) for 2 days and the predominant colony was selected for further studies.

Identification Of *Bacillus* Species:

The *Bacillus* sp. was identified upto genus level by cultural, morphological and following biochemical characteristics such as Gram's staining, methyl red test, Voges proskauer test, citrate utilization test, urease test, starch hydrolysis, glucose utilization test, catalase test, nitrate reeducates test, hydrogen sulphide (H_2S) production test in accordance to the Bergy's manual of determinative bacteriology.

Mass Multiplication Of *Bacillus* Species:

For starter culture preparation 100ml of Nutrient broth was prepared. 10ml of mother culture of *Bacillus* sp. was inoculated. Broth was incubated till reach optical density of 0.8 at a wavelength of 600 nm. When the OD is reached 0.8 the starter culture was ready for using further work.

Collection Of Substance:

Sugar cane waste was collected from the cultivation area.

Extraction Of Juice:

Sweet sorghum stalks and sugarcane were grained with sugarcane juice extracting machine approximately 1 lit of juice were collected form sugarcane separately.

Hydrogen Production In Fermentor:

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

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 500 ml fermerntor. 400ml of sugarcane juice with various pH and 10% of inoculum (40ml) was inosculated into the fermentor. After inoculation the fermentor was kept for 24 hours. Gas evolved was pass through 10% NaOH for absorption of CO_2 and other gases

Collection Of Hydrogen:

The gas coming out from 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

Isolation And Identification Of Bacterial Strain:

The predominant bacterial strain was selected and identified based on morphological and biochemical characteristics. The results are shown in table 1.

Table:1 Biochemical Characteristics Of Bacterial Strain

S. No	Biochemical Characteristics												
	Indole test	Methyl Red test	Voges Praskauer Test	Citrate Utilization Test	Catalase Test	Urease Test	Gelatin Hydrolysis Test	Nitrate Reductase Test	Starch Hydrolysis Test	Casein Hydrolysis Test	Glucose Utilization Test	Identification result (Name of the bacterial strain)
1.	+	+	+	+	+	-	+	+	+	-	+	*Bacillus* spp.

Mass Multiplication:

The selected Bacillus species was grown in the appropriate media (Nutrient broth).

Estimation Of Hydrogen Produced By *Bacillus* sp.

Hydrogen produced by *Bacillus* sp.form sweet sorghum juice was allowed to pass into 10% NaoH solution. After absorbtion of other gases by NaOH the remaining H_2 was collected in another bottle.The result of collection of gas is shown in table 2.

Table 2: Maximum Water Displaced By *Bacillus* sp. Using Sugarcane Juice As Substrate

S.No	Substrate	Amount of water displaced
1	Sugarcane	785ml

Hydrogen Production At Various pH:

The results of production of hydrogen gas using Sugarcane juice by *Bacillus* sp. At various pH for 24 hours is given in figure 1.

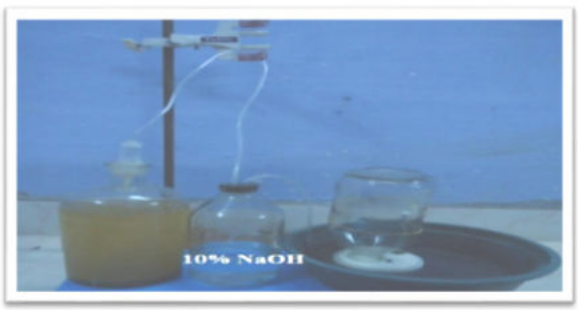

Figure -1 Production Of Hydrogen Gas Using Sugarcane Juice By *Bacillus* sp.

DISCUSSION

Biomass has been used for centuries, currently, biomass ccontributes about 12% of today's world energy supply while in many developing countries of contributes 40-50% energy supply (Demirbas, 2001). Hydrogen is a clean and environmentally friendly fuel, which produces water instead of green house gases when combused. Besides, gasification aims to produce gaseous products further improved by water-gas shift reactions as discussed in the previous section. The gasification process is applicable to biomass having moisture content less than 35%. The effect of initial pH on production of hydrogen using newly *Bacillus* species was investigeted by conducting the fermentation for 48 hours at diffreent pH ranging form 5.0 to 8.0 initial pH of the medium influences the rate of hydrogen production. It was found that the hydrogen production increases with increase in pH up to 7 and then decreases. At lower pH there could be increased formation of acidic meatabolites, which establish the cell's ability to maintain internal pH, resulting in lowering of intracellular level of Adenosine triphospate (ATP) and inhibiting glucose uptake. An optimum pH was found to be 7.0 which results the higher yield of hydrogen (0.12 mol H2 /mol of substrate. (Manikandan *et al*., 2009) The effect of time study on hydrogen production was carried out for 60 hours at room temperature the culture was inoculated 2.0% in a pre cultured broth. The optimum time was found to be 48 hours at which a maximum yield of 0.13 mole H2. In the present the study attempt was made to estimate the hydrogen produced by *Bacillus* sp. Using sugarcane juice as a substrate. And at various pH 4.5, 5.5 ,6.5, 7.5 and 8.5, using water displacement method. The higher amount of water was displaced by *Bacillus* sp. at pH of 7.5 (785 ml) in sugarcane juice as substrate in 24 hours than the other pH of 4.5 (410 ml), pH 5.5 (520 ml), pH 6.5 (700 ml) and pH 8.5(730 ml). Similar kind of study was done by Mankandan et al., 2009. They conclude that the initial pH for hydrogen production by *Bacillus* sp.was 7-7.5. the water displaced was significantly higher in pH range from 7-8.8 .

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