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Production of Bio-Hydrogen from Banana Waste by Using Anaerobic Fermentation

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Abstract: ***This research aimed to study the production of biological hydrogen using banana waste as renewable biomass and was carried out by anaerobic fermentation. Clostridium acetobutylicum MTCC 11274 was used as micro-organism for the hydrogen generation. The substrate were hydrolysis by diluted 5% H2SO4 and 5 N NaOH by acidic and alkali pre-treatment method respectively. The volume of gas produced was calculated by the water displacement method.***
Keywords: Biological hydrogen, banana waste, anaerobic fermentation, Clostridium acetobutylicum, water displacement method

I. INTRODUCTION

The climate change and energy shortage problems lead researchers to explore cleaner and more sustainable fuels than the conventional ones [1]. Among various alternative clean fuels, hydrogen is a promising clean fuel that is accepted as an environmentally safe, renewable, and high energy yield (122 kJ g^{-1}) [2]. It is considered as the cleanest available fuel, which is 2.75-fold greater than that of hydrocarbon fuels [3]. The higher heating value of hydrogen is 3042 cals/m^3 (considering water as a product). In combustion, water is the main product, thus, hydrogen is regarded as a clean non-polluting fuel [4]. It can also be generated by applying both sustainable and non-sustainable resources [5-6]. Globally, hydrogen is commercially obtained mostly from the fossil fuel using thermochemical processes [7]. Apart from being used as a clean fuel, Hydrogen is one of the potential energy sources, and it is widely used in several industries such as petroleum and petrochemical industries [8]. It also used for the manufacture of ammonia, methanol production, oil refineries for removal of impurities, and as a fuel in rocket engines [9-10].

Biological hydrogen production is a promising alternative approach for the production of fuel from low cost, renewable, environmentally friendly resources, and non-polluting in nature [11]. Globally, the demand for hydrogen is increasing so the use of organic wastes as a substrate it is a renewable way for the production [12]. As cost of the substrate is a major factor in the economics of bio-hydrogen production, it is necessary to use less expensive and abundant feedstocks to keep the process affordable. Organic waste from industry and agriculture is not only used to generate green energy, but it also aids in bioremediation [13].

Banana is one of the most popular tropical fruits consumed worldwide, and India is the world's largest producer and consumer of banana. Banana waste is edible waste out of which small fractions of the banana peel is used for animal feed and the remaining percentage of banana peel is considered as waste. The use of this organic waste as a substrate source for bio-hydrogen production can be considered a viable gap filling for energy generation. The peel is high in carbohydrates (Table 1) and can be converted into hydrogen and methane.

Table1. Characterization of banana waste sample

Parameter	Weight (%)
Moisture (oven dried)	10.1
Ash (oven dried)	32.26
Total organic carbon	29.64
Cellulose	9.90
Hemicellulose	41.38
Lignin	8.90

The current research work aimed to evaluate the feasibility of hydrogen production in the fermentative process using banana waste. Since, cost of substrate affects the price of bio-hydrogen production most significantly. In this study, *Clostridium acetobutylicum* MTCC 11274 was used as the hydrogen-producing microorganism.

II. MATERIALS & METHODS

A. Preparation and characterization of banana waste

Banana waste were collected from local fruit juice shop. It was cut into small pieces, washed with distilled water to remove external dirt. Wetted banana peels were kept under air for removing the free water from their surface and dried in an oven for 12 hrs. at 110°C. Dried samples were then finely grounded using a mortar and pestle.

The ground banana peels were sieved and graded into small particle sizes (approximately 100 mesh size). The samples were then stored in an airtight bottle prior to the experiments [14].

B. Bacterial Culture and Maintenance

The microbial strain of *Clostridium acetobutylicum* MTCC 11274 was collected from IMTECH, Chandigarh. A synthetic medium, namely Reinforced Clostridial broth (RCM) medium was used in this study.

The composition of RCM medium was as follows (g/L): Peptone, 10; Beef extract, 10; Yeast extract, 3; Dextrose, 5; NaCl, 5; Starch Soluble, 1; L. Cysteine HCl, 0.5; Sodium acetate, 3.0; Agar, 0.5.

The pH of the medium was adjusted to 7.0. RCM medium was then inoculated (10% v/v) by *Clostridium acetobutylicum* MTCC 11274. The strain MTCC 11274 was grown anaerobically in RCM medium at 37°C to be used as an inoculum source in the fermentative hydrogen production [15].

C. Pretreatment of Substrate

The sample was subjected to an acid hydrolysis and an alkali hydrolysis with four different concentrations 0.5%, 1.0%, 1.5%, 2.0% respectively. 10 g of banana waste powder (BWP) were mixed in diluted 5% H_2SO_4 and 5 N NaOH (1% w/v) to make a total volume of 100 mL in a 250 mL Schott Duran bottle.

The mixture were then transferred into shake flasks to shake rigorously until no BWP particles were stuck on the bottom of the bottles.

The suspension of BWP were then autoclaved at 121°C for 15 min and 15 lbs. Subsequently, the mixture solution was cooled to the room temperature and filtered using the Whatman filter paper to obtain a clear filtrate. The filtrate obtained was neutralized (pH 7.0) with diluted alkali and acid solution. The sample obtained was kept at 4°C as a substrate for the fermentative hydrogen production [16].

D. Fermentation

Batch test for hydrogen production was done in serum bottles of 125 mL with a working volume of 100 mL of BWP hydrolyzate. It was mixed with 10 mL of concentrated Tryptone-yeast extract-acetate (TYA) medium. The mixture was then transferred into a 250 mL-Schott Duran bottle.

The bottle was then sterilized by using an autoclave. The fermentation reactors were fitted with rubber stoppers and crimped using crimper.

The initial pH of the culture medium was adjusted by 1N NaOH and 1N HCl. BWP hydrolyzate of 0.5% and 1.0% concentrations of acidic pretreatment and 1.0% and 1.5% concentrations of alkali pretreatment were then inoculated (10% v/v) by *Clostridium Acetobutylicum* MTCC 11274.

Anaerobic conditions inside the bottle were provided by purging 100% Nitrogen gas to the void space of the bottle for 10 min. The culture bottles were connected to other two bottles via pipe and kept inside for incubation at 37°C. Gas analysis was done after every 24 hours.

E. Analysis

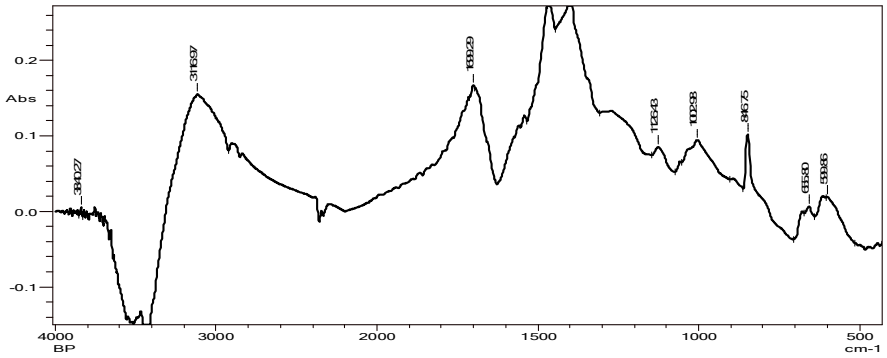
The gas produced was collected through a Syringe and the gas mixture generated from the bottle was passed through 5M NaOH solution to absorb CO_2 gas.

The evolved gas was then transferred to an inverted measuring cylinder containing acidic water with a pH value of 2.0 ± 0.2 to prevent hydrogen gas from dissolving in the water. The volume of cumulative gas produced was calculated by the water displacement method [17].

III. RESULTS & DISCUSSIONS

A. Characterization of banana waste particles

To understand the nature of the functional groups present in banana waste particles, FT-IR Spectra was performed and results were shown in Fig 1.



Wavelength (cm^{-1})

Fig 1: FT-IR spectra of banana waste particles

PEAK	FTIR FREQUENCY	BONDS	FUNCTIONAL GROUPS	PEAK INTENSITY
1463.97	1450-1600	C=O	CARBONYL	Strong
1699.29	1665-1760	C-H	ALKANES	Strong
3116.97	3000-3200	O-H	ALCOHOL	Strong, broad

Table 2: Frequency results of FT-IR spectra of banana waste

Table 3: Hydrogen production during the interval of 24 hrs.

Time (hrs)	Hydrogen gas volume (mL)
0.0	0.0
12	0.0
24	0.0
48	2.0
72	8.0
96	12
120	25
144	36
168	47
192	58
216	65
240	69
264	73
288	76
312	76
336	77
360	77.5
384	78
408	78
432	78
456	78

480	78

The hydrogen gas production began to rise from 48 hours and the volume was gradually increased simultaneously with the increase in time duration. After 384 hours the volume of hydrogen gas was constant and volume was almost stopped in 480 hours. The volume of hydrogen gas produced after 480 hours was 78 mL.

IV. CONCLUSION

The current work revealed that hydrogen production is a very sensitive process, as it strongly depends on multiple factors like composition of the substrate, temperature, pH of the culture medium and inoculum amount. It was inferred from this experiment that banana waste is the possible substrate for the generation of bio-hydrogen and could be used as an inexpensive energy source. In this paper, the feasibility of hydrogen production from banana waste by anaerobic fermentative process was analyzed and considerable volume of hydrogen gas was obtained.

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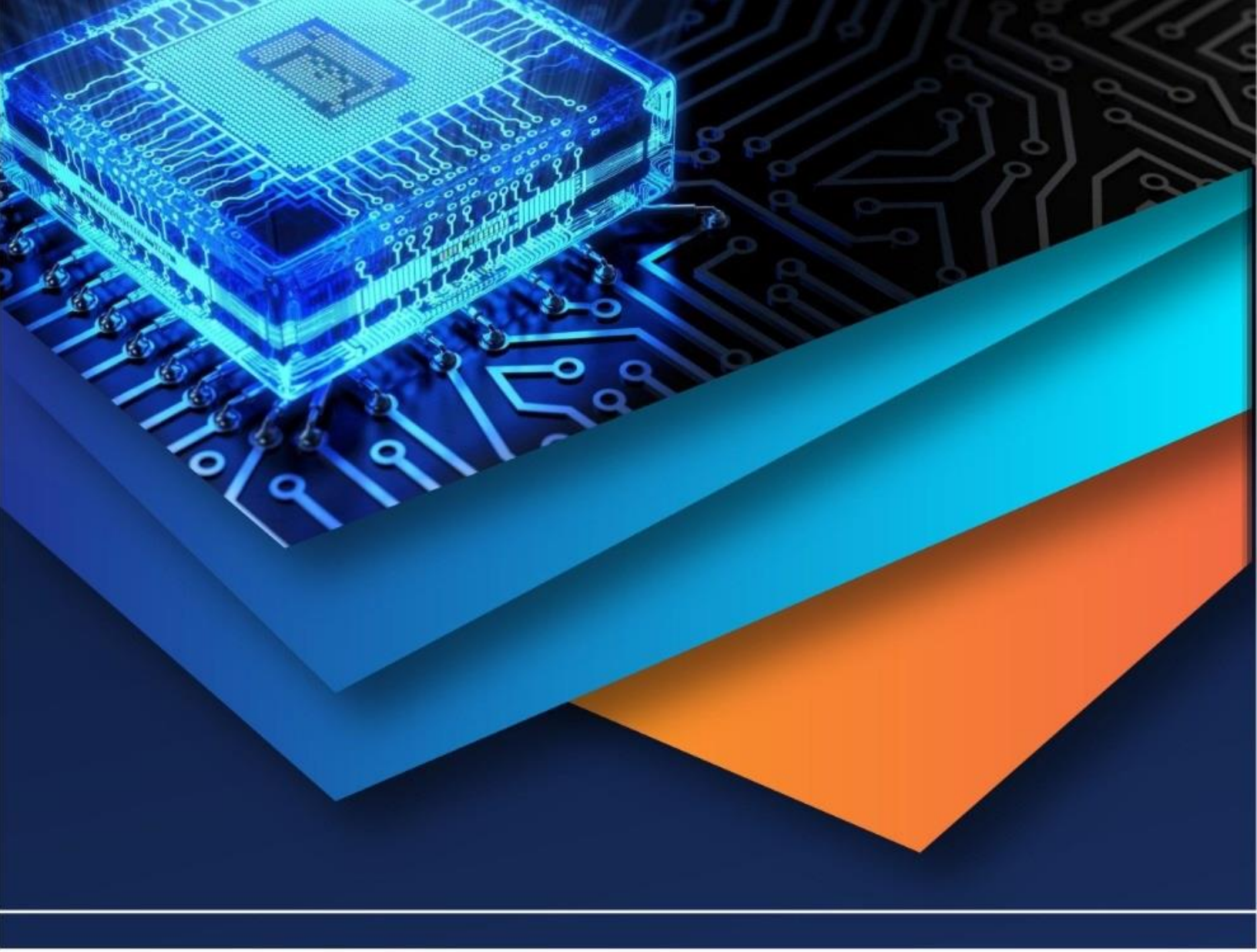

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