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Biobutanol Production from Rice Husk

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***Abstract:** This research aimed to study biobutanol production using Lignocellulosic renewable substrate Rice Husk, carried out using Clostridium acetobutylicum MTCC 11274 via the Separate hydrolysis and fermentation (SHF) process. The simple sugars were exposed using a delignification process with different concentrations of Ammonia solution and some were hydrolyzed using conc. H_2SO_4 after getting treated with ammonia. The sugars were estimated using DNSA Method after which samples were fermented. The concentration of Biobutanol was estimated using GC-MS showing 2.2 gm/L in one of the tested samples.*
***Keywords:** Biobutanol, Rice Husk, Clostridium acetobutylicum, Lignocellulose, Pretreatment.*

I. INTRODUCTION

Modernization and comfortable lifestyles, increasing population, and innovations in technology, along with industrialization, have increased the quantity and variety of waste being generated as well as depletion of natural resources are at its peak [1]. Every day, a million tons of waste are produced around the world. There is a severe crisis for proper waste disposal systems as land is not left due to modernization and making polluting the environment. Waste management is a major economic and environmental issue throughout the world. Among all wastes, a very common type of waste is an agricultural waste [2] There are several applications in which these wastes can be used to generate different kinds of energy [3]. Another problem of fossil fuel usage and the environmental damage it causes can be solved using highly sustainable management [3] This waste could be a good source of producing biofuels to replace the use of fossil fuels.

Biofuels that are produced from feedstock don't come under human utilization. Feedstock is material that is not generally useful for human beings. Though it is a food crop they are no longer useful for consumption. These types of biofuels are known as second-generation fuels or "advanced biofuels" because extraction of fuels from this source is very difficult. This biofuel can be used in existing internal combustion engines, either alone or combined with petroleum-based fuel [4]. One of the most produced wastes is Rice Husk ($\sim 1.5 \times 10^{11}$ kg) [5.] Rice husks contain a combination of cellulose (28.6–43.3%), hemicelluloses (22.0–29.7%), and lignin (19.2–24.4%), along with appreciable amounts of silica and other minor components [6]. Bio-alcohol is a potential transportation fuel that could eventually replace fossil-derived petroleum for a more sustainable future. This fuel can be made by enzymatically hydrolyzing pretreated lignocellulosic biomass and fermenting the generated sugars with appropriate bacterial strains at the same time [7].

Table: I
Comparative Properties of Fuel [9]

S. No.	Properties of fuels	Fuels			
		Butanol	Gasoline	Ethanol	Methanol
1.	Energy density (MJ/L)	29.2	32	19.6	16
2.	Air-fuel ratio	11.2	14.6	9	6.5
3.	Heat of vaporization (MJ/kg)	0.43	0.36	0.92	1.2
4.	Research octane number	96	91–99	129	136
5.	Motor octane number	78	81–89	102	104

Among all bio alcohols, biobutanol is considered one of the most promising renewable biofuels [8]. Butanol is chosen over other fuels due to its property of lower volatility, less hygroscopic (thus does not pick up water), and less corrosive [10]. As it does not absorb water, thus, less susceptible to the separation of biofuel/gasoline blends in the presence of water.

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Due to this property butanol can be added at the refinery (at any concentration) and be transported and delivered through the existing infrastructure (pipelines, storage tanks, filling stations) [11] as shown in table: 1 without causing damage, and ethanol can only be added shortly prior to use and butanol have a lower vapor pressure [10]. However, large-scale industrial production of biobutanol is still restrained by the low efficiency of its production technology for which further research in the future is in great demand [8].

The current research aimed to evaluate Biobutanol production's feasibility in the fermentative process using Rice Husk. Since Biobutanol production costs are high, using this substrate and *Clostridium acetobutylicum* which is most capable of producing butanol and acetone [8] could affect the price significantly.

II. MATERIALS & METHODS

A. Preparation and characterization of Rice Husk

Rice Husk was purchased from a local rice mill during its harvesting season. The sample was washed 2-3 times with normal water and 2 times with distilled water to remove dirt. After washing the sample had been weighed and sun-dried. The sample was ground in the grinder to make a fine powder. The fine powder was sieved from a 100 mesh-sized sieve. The samples were then stored in an airtight bottle prior to the experiments [12].

B. Bacterial Culture and Maintenance

Clostridium acetobutylicum MTCC 11274 is the microbial strain that was obtained from IMTECH, Chandigarh. Reinforced Clostridial broth (RCM) medium was used as culture growth media in this study. RCM medium contained (gm/L): Peptone, 10; Beef extract, 10; Yeast extract, 3; Dextrose, 5; Agar, 0.5; NaCl, 5; Starch Soluble, 1; Sodium acetate, 3.0; L. Cysteine HCl, 0.5. The medium was adjusted to pH 6.8-7.0 before autoclaving. 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 [13], [14].

C. Pretreatment of Substrate

1 gm of the physically treated sample was soaked in 10 mL Ammonia Solution (25%). Different concentrations of Ammonia by volume (0%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%) were taken and been autoclaved. After heating, the samples were filtered and the filtrate was diluted until pH 7 [5]. Simple sugars were analyzed by the DNSA method, out of which samples treated with 4%, 5%, and 6% ammonia were selected. Then the sample treated with 6% ammonia solution with the highest amount of sugar was selected for further acidic treatment. Different amounts of conc. H_2SO_4 was added to the filtrate obtained from the delignification process to make different concentrations such as (0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%) and were heated in the microwave with 180 watts and 360 watts respectively, out of which sample 2 samples with highest sugar concentration were selected. Afterward, samples were cooled and filtered using 110mm filter paper. The obtained filtrate was neutralized using 10M NaOH until pH 7. Then the sample hydrolysate was stored in the refrigerator for further use [15].

D. Fermentation

Fermentation was carried out in 100 mL culture bottle using 20 mL of Rice Husk hydrolysate. To enrich the medium, the component of the P2 medium was added to the hydrolysate. Such solution's initial pH has been modified to 6.8 using 2M NaOH. The whole culture bottle with P2 media was autoclaved. To extract oxygen, nitrogen was purged into the culture bottle to maintain anaerobic conditions for the microorganisms. Then, 5mL of actively growing culture was inoculated into the bottles. Then the bottles are placed in an anaerobic container, instead held at 37°C for 72 hrs in the incubator. After 72 hrs samples were taken and centrifuged at 15,000 mg. Clear fluid was processed at 18 °C until ABE or sugars were prepared for analysis [16].

E. Analysis

Gas Chromatography (Agilent Technologies) analyzed fermentation products (Acetone, Butanol, and Ethanol) using a packed column [17]. Sugars have been calculated using the DNSA method, a standard curve has been prepared and sample concentration value has been obtained by a standard glucose curve. A method of optical density (540 nm) was used to measure cell concentration and is described as dry weight cell concentration [18].

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III. RESULTS & DISCUSSIONS

A. Microbial Growth

Cell concentration began to rise after 12-16 h. After 48hrs, the stationary phase was hit. Growth almost stopped after 72 hrs. At 24 hrs, the cells were rising actively.

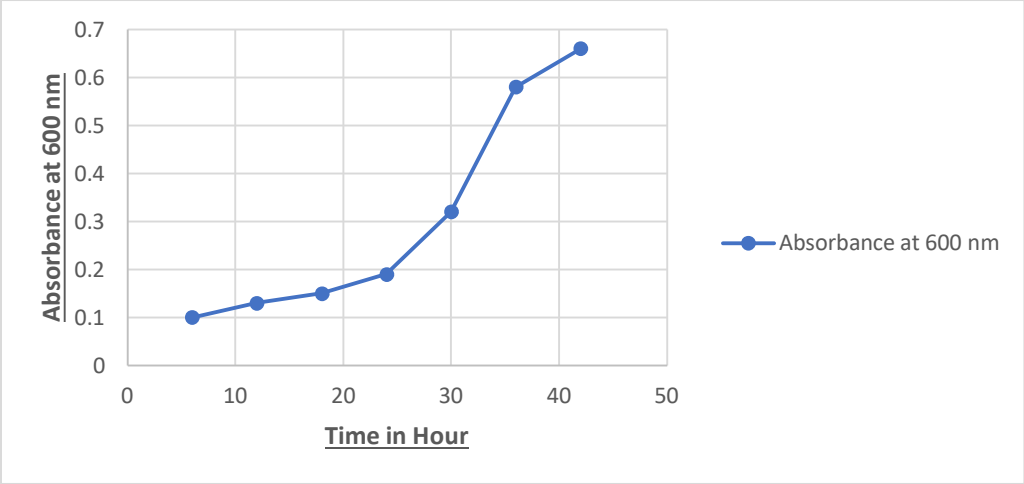


Fig.30. Microbial Growth Curve

The confirmation of the strain Clostridium acetobutylicum is done by two tests: First is the Presence of CO_2 gas in the media. Clostridium produces tiny bubbles of CO_2 in the broth which is a characteristic indication of the strain [19].

B. Glucose concentration in Hydrolysates

Quantitative analysis of sugar concentration was done by DNSA method and measuring the absorbance at 540nm which tests the concentration of sugar present in the delignified substrate with different concentrations of Ammonia and further acid treated substrates. A total of five samples have been screened for the further fermentation process, i.e., delignified sample with 4% ammonia, 5% ammonia, 6% ammonia, delignified with 6% ammonia and further pretreated with 0.5% H_2SO_4 at 180 watts and 360 watts of microwave heat with the glucose concentration 26 gm/L, 28 gm/L, and 32 gm/L, 7 gm/L, and 5 gm/L respectively.

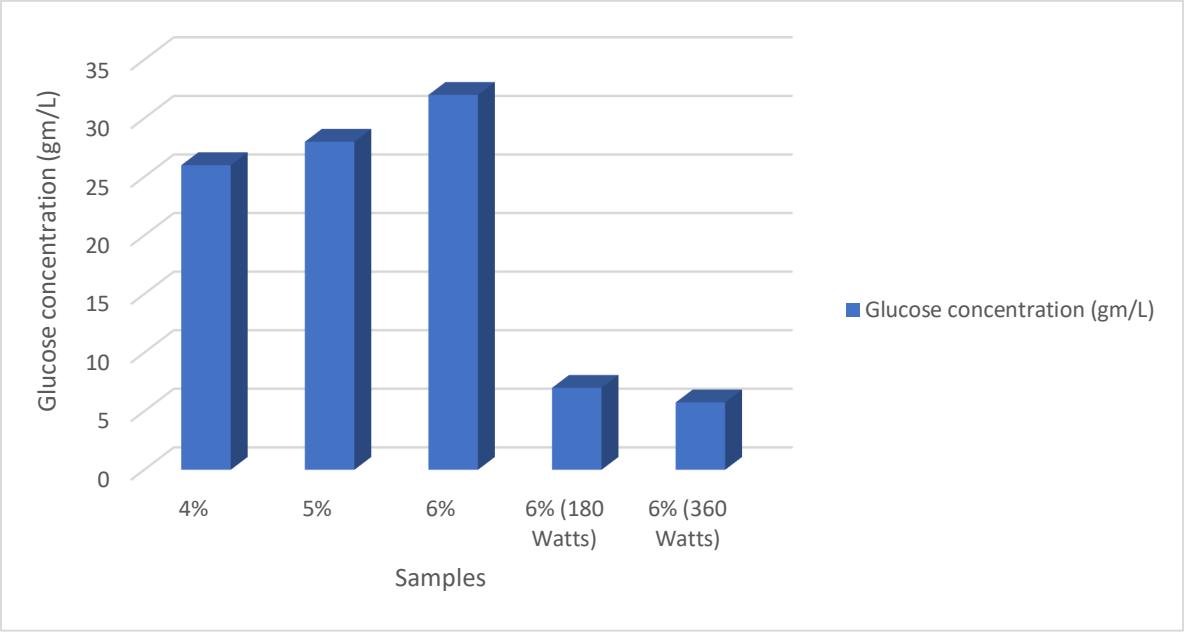


Fig.29. Glucose Concentration (gm/L) in the screened 5 samples

C. Butanol Concentration

The butanol concentration has been analyzed using Gas chromatography. It was observed that delignified sample with 6% of ammonia solution gave the highest production of butanol with a concentration of 2.2 gm/L followed by 4% and 5% ammonia treated samples with 1.6 gm/L and 1.55 gm/L concentration of butanol respectively.

There is a drawback to the generation of inhibitors in acid treatment that has been reported by [20] and [21]. It can be inferred that the acid treatment with 180 watts and 360 watts of heat lowers the amount of butanol to 1.2 gm/L and 0.6 gm/L respectively in acid-treated samples possibly because of the generation of inhibitors in higher amounts which retarded the production of metabolites in microbes. Among all the pretreatments, alkali treatment with 6% liquid ammonia has given the highest amount (2.2 gm/L) of biobutanol solvent which shows a better substrate than bagasse [5].

The result shows that further acid treatment is not suitable for butanol production may be due to the generation of inhibitors and if compared on the basis of heat, high-temperature treatment put a negative effect on the sample as the butanol concentration in the sample treated under 360 Watts of heat was found to be even less than that of the sample treated under 180 Watts of heat as 360 watts of heat lowered the butanol concentration by approximately 4 folds when compared with the sample that produced the highest amount of butanol.

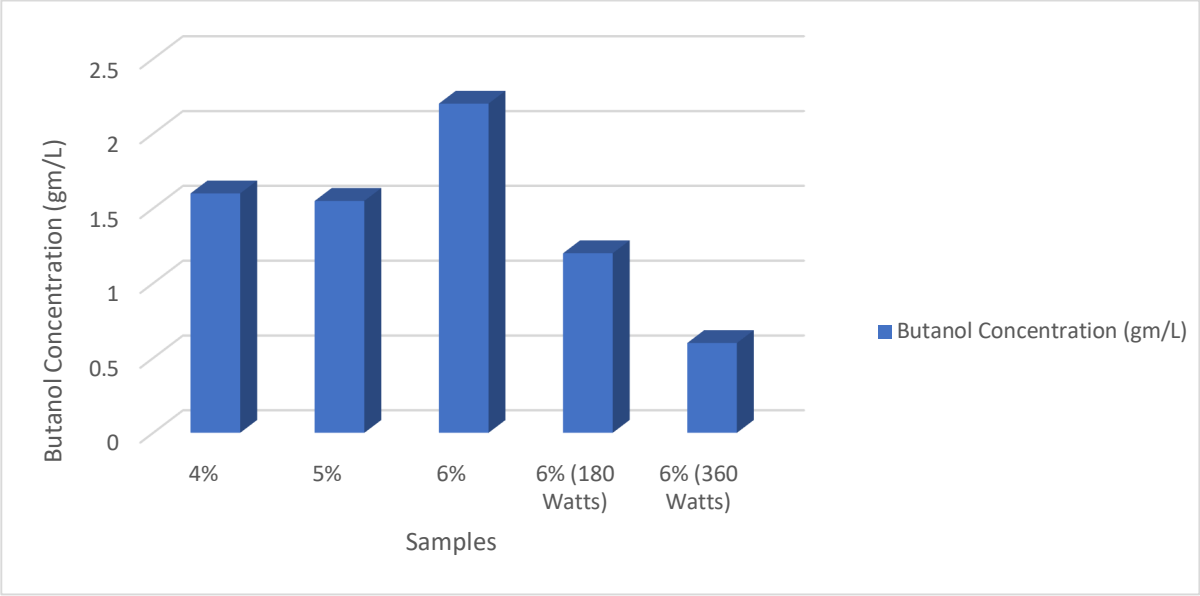


Fig.2. Butanol Concentration (gm/L) in the screened 5 samples

IV. CONCLUSION

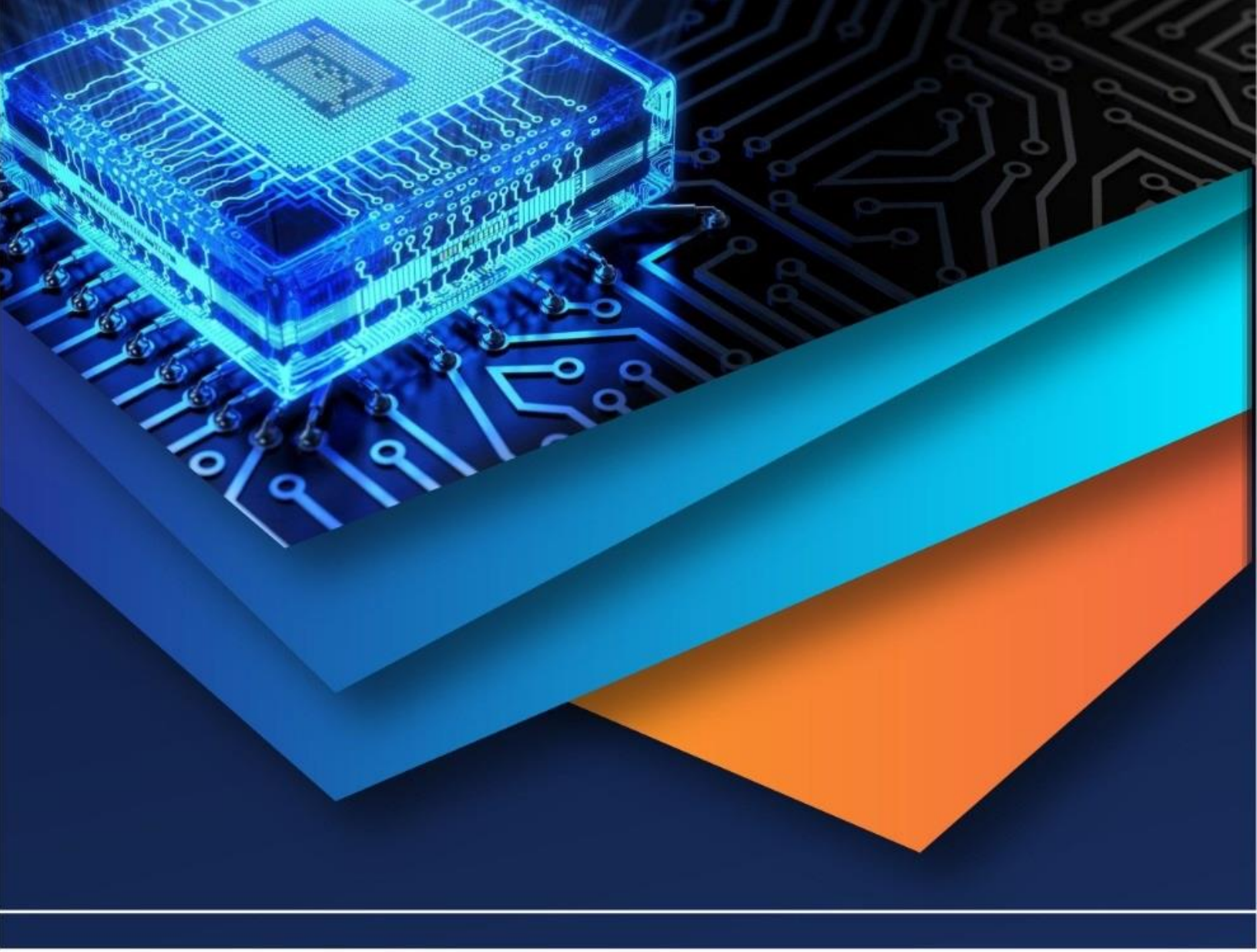
In this study, three pretreatments were done to gain the objective of sugar hydrolysis and solvent production from Clostridium acetobutylicum and Rice Husk. As Rice Husk has higher production and less utilization thus can be utilized to produce butanol which will control the use of fossil fuels and their depletion. The use of Rice Husk waste can lessen the production cost of biobutanol due to the low cost of raw material. All five samples were subjected to ammonia pretreatment and batch fermentation for 72 hrs. After analysis, all five fermentation broths were observed with a considerable amount of biobutanol and the 6% ammonia hydrolysis produced a maximum sugar concentration of 32 gm /L and the concentration of biobutanol derived from it was 2.2 gm/L. Other than substrate and microorganisms, this process revealed its dependence on other factors like temperature, pH, anaerobic conditions, inoculum amount, etc. which could be optimized which is a future prospect of this research and in order to overcome the problems and limitations of ABE fermentation and butanol production, research is being focused on renewable agricultural residues as feedstock for biobutanol.

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