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Production of Biobutanol by the utilization of Soybean lignocellulose Waste by using Clostridium Acetobutylicum MTCC 11274

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Abstract: ***Clostridium acetobutylicum MTCC 11274 provided 14.2 gm / l ABE (acetone, butanol and ethanol) for fermentation of diluted sulphuric acid treated soybean straw hydrolysate. The total production of butanol was 8.8 gm / l. There was 39 g / l of sugar hydrolyzed from the stalks. The yield and productivity of ABE was 0.36 & 0.19 gram / L h. The pre-treatment and fermentation of acid resulted in a good amount of butanol and total production of ABE.***
Keywords: Biobutanol, lignocellulose, soybean straw hydrolysate, ABE, Clostridium acetobutylicum.

I. INTRODUCTION

Regular sources of petroleum are declining, and their value is also rising. We are a big cause of global warming as well. With this situation, we need to build a sustainable, eco-friendly and cost-effective alternative biofuel [1]. Butanol is the petroleum's best alternative biofuel. It has properties similar to that of gasoline & stronger than ethanol. It can be combined in any combination with gasoline and can also be used in current internal combustion engines as an individual fuel [2]. Butanol is currently being produced using methods of chemical & fermentation [3]. During fermentation, bacteria convert sugars to solvents. Sugars are converted by ABE fermentation into acetone, butanol & ethanol in a ratio of 3:2:1 [4]. Clostridia strains are fermented with ABE, clostridium acetobutylicum is best from the species tested. This strain is also higher in butanol production than other clostridia species [5].
Fermentation substrate is an important factor that affects the production cost of butanol[6]. The most abundant renewable resource on the earth is lignocellulose, and it has great potential as a fermentation substrate. Hemicelluloses are the second most abundant polysaccharides in nature, representing approximately 20 to 35% of lignocellulosic biomass [7]. Since the cost of substrates has the greatest influence on the price of butanol[8], we have focused our research on using Clostridium acetobutylicum, a strain-producing butanol, to use agricultural residues for the fermentation of butanol. Although agricultural residues such as straws (wheat, rice and soybean) and maize fiber are available economically, these materials must first be subjected to pretreatment and enzymatic hydrolysis in order to produce fermentation hydrolysates[9]. The processes used to make these hydrolysates often produce chemical by-products that inhibit cell growth and fermentation [10]. These blockers include salts, furfural, furfural hydroxymethyl (HMF), acetic, ferulic, glucuronic and r-coumaric acids, and phenolic compounds [11]. There are a number of approaches available to reduce inhibitory effects of hydrolysates on cultures including dilution of the hydrolysate, removal of inhibitors using overliming and/or adsorbent resin/molecular sieve[17], and development of inhibitor tolerant/metabolizing strains [12]. The separated hydrolysis and fermentation (SHF) of soybean straw hydrolysate was carried out by *C. acetobutylicum* to test its potential for butanol production. [13] Since, cost of substrate affects the price of butanol production most significantly [14] the major objective of these studies was to produce butanol from soybean straw hydrolysate. The inhibitory effects of substrate were removed by dilution and filtration approaches.

II. MATERIALS & METHODS

A. Strain and Innoculam Development

Purchased from IMTECH, Chandigarh, India, culture of *Clostridium acetobutylicum* MTCC 11274. Spores of C. MTCC 11274 acetobutylicum was held at 4 ° C in distilled water. The spores are heat-shocked for 2 min at 75 ° C and transferred for spore germination to the growth medium [15]. 10 ml of active-growing cells (from the liquid medium) were inoculated in 100 mL of the P2 medium inoculum growth, prepared in a 125 mL bottle with a screw cap [16]. Glucose, yeast extract and stock solutions (minerals, buffers, and vitamins) are present in the P2 medium [17]. Glucose and yeast extract solution were sterilized at 121 ° C for 15 minutes, followed by cooling to room temperature. At that time, a solution of 100 mL glucose-yeast extract was added to 1 mL of each of the filter sterilized stock solutions. The inoculum was allowed to grow at 37 ° C for about 18 h when it was ready to be inoculated into the medium of production of ABE where soybean straw hydrolysate was used.

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B. Soybean Straw Hydrolysate

Soybean straw from Bhopal's local farmer was obtained. The stalks are grown and harvested completely. A mixture grinder was used to ground the straw into fine particles. The milled straw was processed until tests were conducted at room temperature. In a 150 ml screw-capped container, 10 g of soybean straw was distributed and blended well into 10 mL of 1% (v / v) H_2SO_4. The bottle was aluminum foil wrapped and heated for 1 h at 121 ° C. The pH of the pre-treated barley straw was changed to 6.8 using 2 M NaOH after cooling to room temperature. Then the cellulase & ß-glucosidase enzyme solutions are added (6 ml / l of each). It was then incubated for 72 hours at 45 ° C. During hydrolysis, with a rotary shaker, agitation was sustained at 80 rpm. Centrifugation at 1200xg was pursued to remove sediments. The supernatant liquid was filtered through a filter paper of 110 µm and then sterilized by a filter of 0.2 µm. The sterile hydrolysate was prepared for experimental work in a pre-sterilized container at 4 ° C. The maximum soybean hydrolysate sugar was 39 gm / l.

C. Fermentation

Fermentation experiments in 100 ml culture bottle were conducted using 10 ml of soybean straw hydrolysate. To enrich the medium, the component of the P2 medium was added to hydrolysate. Such solutions ' initial pH has been modified to 6.8 using 2 M NaOH. 0.1 gm of yeast extract and 0.1 ml of each stock solution are applied to the container to achieve the same level of nutrient concentration as the P2 medium. To extract oxygen, nitrogen was purged into the tank. It helps to create the organism's anaerobic conditions. Then, 3 mL of actively growing culture inoculated the bottles. Then the bottles are placed in anaerobic container, instead held at 37 ° C for 72 hours in the incubator. After 72 hours samples were taken and centrifuged at 15,000xg. Clear fluid was processed at 18 ° C until ABE or sugars were prepared for analysis [23].

D. Analyses

Gas chromatography (Agilent Technologies) analyzed fermentation products (Acetone, butanol & ethanol) using a packed column [21]. Sugars have been calculated using DNS process, standard curve has been prepared and sample concentration value has been obtained by standard glucose curve. A method of optical density (540 nm) was used to measure cell concentration and is described as dry weight cell concentration [22]. The statistical analysis was performed using an ANOVA method in one way. The significance level was set at 0.05.

III. RESULTS & DISCUSSIONS

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

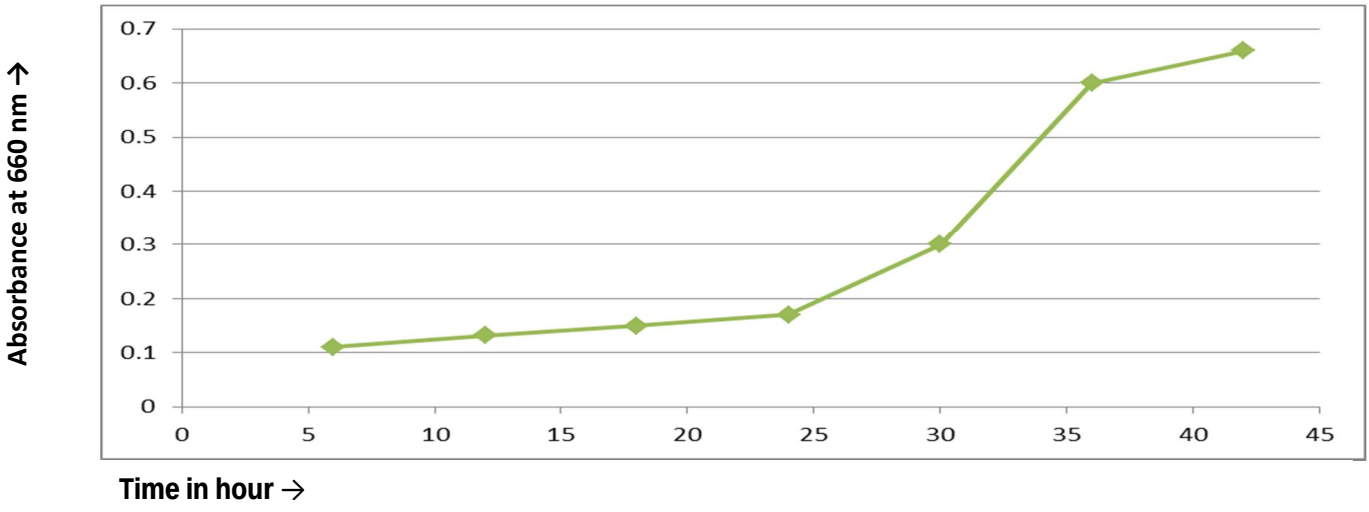


Fig.1 Microbial growth curve

The soybean hydrolysate quantity of total sugars was measured using the DNS method [20]. The maximum soybean straw hydrolysate sugar concentration was 43g / l. The quantity of sugars obtained was lower than the quantity of sugars obtained from rice straw [18].

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From GC chromatograph we calculated the amount of acetone, butanol & ethanol. As an internal norm, propanol was used. The total amount of butanol from hydrolysate treated with acid was 8.8 gm / l. The sum of soybean stalk butanol was lower than hydrolysate rice straw & straw barley hydrolysate [19].

The total amount of ABE obtained from the hydrolysate of soybean stalk was 14.2 gm / l. The ethanol in ABE was 0.5 gm / l ethanol and the acetone was 4.9 gm/l.

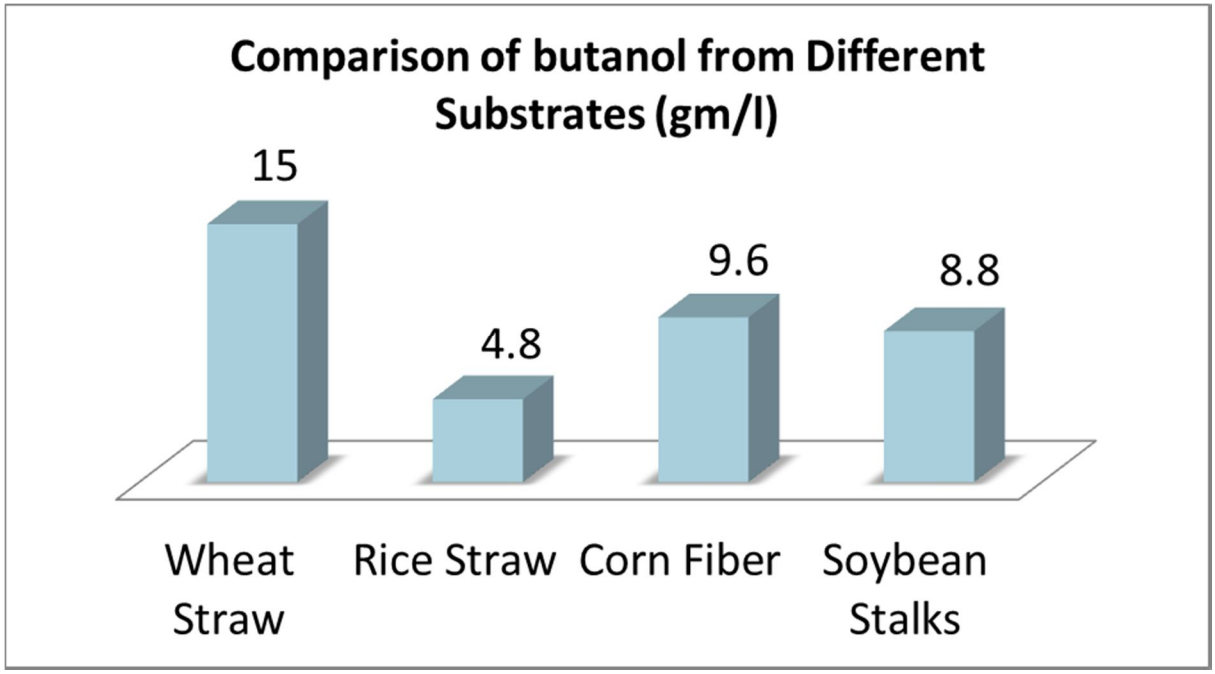


Fig.2 Comparison of butanol obtained from different substrates [24, 25, 26]

IV. CONCLUSION

In this analysis, biobutanol was successfully prepared from sulphuric acid pretreated soybean stalks. The hydrolysis produces maximum sugars of 39 gm / l. The soybean stalks hydrolysate derived biobutanol was 8.8 gm / l. ABE was 14.2 gm / l in total.

It was inferred from this experiment that soybean stalk is the possible substrate for the development of butanol. Soybean stalks were successfully fermented into butanol with the pre-treatment of H_2SO_4.

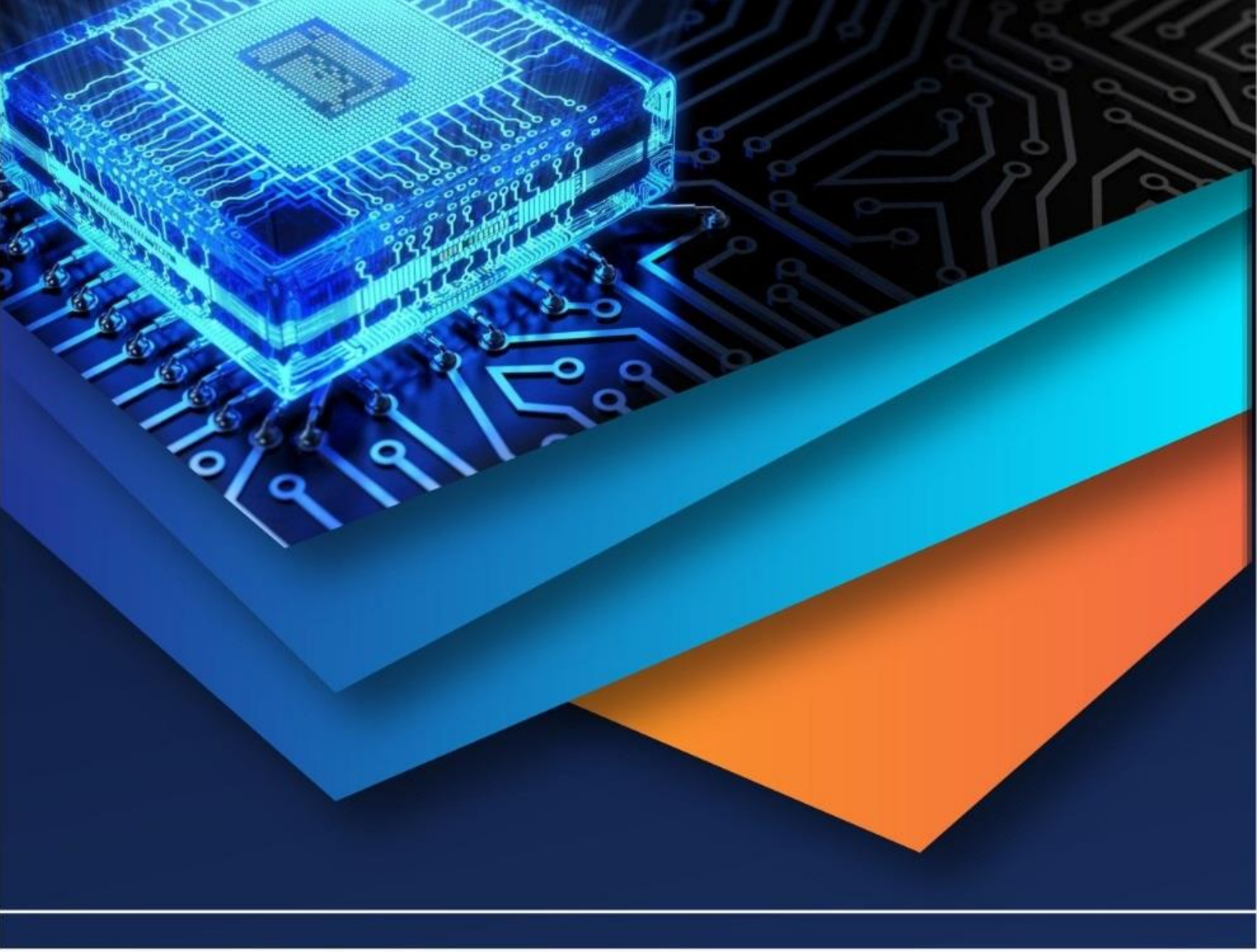
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