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Rural Commercial Development through by Biobutanol Production from Lignocellulosic Material

Dr. Dilip Shukla
Assistant Professor
Chemistry Department
L.B.S. P.G. College ,Gonda upto 2018.
Shri L.B.S. Degree College, Gonda From 2019 till now.

Abstract

A significant research is a need for the synthesis of the alternative energy sources, among the fuels are the most essential thing in a day today life. Researchers are developing the new methodologies for the preparation of fuels from the plant sources using advanced bio-techniques. Fermentation systems in one of such technique which is contributing great impulse to research in the production of butanol from various plant sources. In the present investigation we have extracted butanol by the ABE fermentation using a Lignocellulosic substrate obtained from the non edible plant Pongamia pinnata and we obtained the butanol in good yield i.e., 62.16%, compare to the reported methodologies.

Keywords: *ABE fermentation, Pongamia pinnata, butanol*

INTRODUCTION:

Lignocellulose is the most abundant renewable resource on the planet, and has great potential as a substrate for fermentation. Lignocellulosic biomass comprising forestry, agricultural and agro-industrial wastes are abundant, renewable and inexpensive energy sources. The major constituents of lignocellulose are cellulose, hemicellulose, and lignin, polymers that are closely associated with each other constituting the cellular complex of the vegetal biomass.

Cellulose is a high molecular weight linear homopolymer of repeated units of cellobiose (two anhydrous glucose rings joined via a β-1, 4 glycosidic linkage). Hemicelluloses are the second most abundant polysaccharides in nature, and represent about 20 to 35% of lignocellulosic biomass. Hemicellulose is a linear and branched heterogeneous polymer typically made up of five different sugars - Larabinose, D-galactose, D-glucose, D-mannose, and D-xylose - as well as other components such as acetic, glucuronic, and ferulic acids. Lignin is a very complex molecule constructed of phenylpropane units linked in a large three-dimensional structure. Lignin is closely bound to cellulose and hemicellulose and its function

is to provide rigidity and cohesion to the material cell wall, to confer water impermeability to xylem vessels, and to form a physic–chemical barrier against microbial attack. Due to its molecular configuration, lignins are extremely resistant to chemical and enzymatic degradation. The amounts of carbohydrate polymers and lignin vary from one plant species to another. In addition, the ratios between various constituents in a single plant may also vary with age, stage of growth, and other conditions. However, cellulose is usually the dominant structural polysaccharide of plant cell walls (35–50%), followed by hemicelluloses (20–35%) and lignin (10–25%).

BUTANOL PRODUCING MICROORGANISMS

ABE fermentation process is strictly anaerobic, which means that it must not have any oxygen present. Anaerobic environment is usually maintained by bubbling nitrogen gas. ABE fermentation is typically characterized as a biphasic growth pattern in terms of metabolite production. The first stage is called acidogenesis, during acidogenesis the bacterial culture mainly produces acetic and butyric acids. This occurs during exponential growth of the bacterial culture. The second stage is called solventogenesis. This begins late in the exponential growth phase and continues into the stationary phase of the bacterial culture, where acetone, butanol, and ethanol are the main products. The process produces acetone, butanol and ethanol in a ratio of 3-6-1. ABE fermentation usually uses a strain of bacteria from Clostridium family. Clostridium acetobutylicum is the most well known strain, although Clostridium beijerinckiiis used for this process, with very good results [1].

Estimation of Cellulose

Materials

Acetic/Nitric Reagent: Mix 150 ml of 80% acetic acid and 15 ml of concentrated nitric acid. Anthrone: Dissolve 200 mg anthrone in 100 ml of ice-cold 95% sulphuric acid. Prepare fresh and chill for 2h before use.

Hydrolysis and Detoxification of Hydrolysate

Lignocellulosic husk was hydrolyzed using dilute sulphuric acid. Hydrolysis is carried out in two stages: First stage: 80 gm/l (w/v) of sample is hydrolysed using 0.7% of dilute acid at 180 °C for 10 min and solution is separated and residue is used for second stage.

Detoxification

During pretreatment of acid hydrolysis salts, furfural, hydroxymethyl furfural (HMF), acetic, ferulic, glucuronic, p-coumaric acids, and phenolic compounds are produced. These are the microbial inhibitors, they inhibits the growth of clostridium and the productivity of the butanol. Detoxification of the husk hydrolysate was carried out using a modified method (overliming). The pH of the hydrolysate was adjusted to 10.1 with Ca(OH)2 followed by the addition of 1 g/L Na2SO3. The mixture was incubated in water bath for 1 h at 45 °C under 100 rpm agitation. Subsequently, the precipitate (gypsum) was separated by centrifugation at 7,000 g for 10 min. The gypsum formed was discarded.

Inhibitor Removal

During pretreatment and hydrolysis of fiber rich agricultural biomass, compounds such as salts, furfural, hydroxymethyl furfural (HMF), acetic, ferulic, glucuronic, r-coumaric acids, and phenolic compounds are produced. This compound inhibits the growth of organisms and reduces the productivity of butanol. These inhibitors are removed by using organic polymer Amberlite XAD-4 resin, which remove 60–80% furfural and HMF from the hydrolysate. Resins were washed batch wise four times with deionized water to remove residual XAD-4 storage chemicals and pack 60 g of XAD-4 resins in glass column(1.5×51 cm2).At the bottom of glass column glass wool is fixed to avoid the flow of XAD-4 resins from column. Column has filled with XAD-4 resins i.e. 400 ml for 60 gm of resins and inhibitor free sample is collected. Then hydrolysate will be pumped into the column at a flow rate of 8 mL/min and discard the initial diluted solution.

Fermentation

ABE fermentation is most common method for the butanol production from the lignocellulose biomass. Hydrolysate is prepared by acid hydrolysis is used as substrate in the fermentation as a source of carbon for the species which converts the individual sugars into biobutanol. Fermentation sample is inoculated with actively growing culture. During the fermentation sample is taken intermediately for ABE analysis.

Biomass Separations

Growth of organisms produces biomass in the fermentation and this biomass can be removed from the fermentation broth. After batch fermentation of hydrolysate the biomass is separated from fermentation broth. Sample is centrifuged at 10,000 g for 3min. Supernatant obtained after the biomass separation is subjected to extraction [1]

ABE Analysis: The sample collected during the fermentation is sent to Azyme Biosciences Pvt. Ltd. Bangalore, for Gas Chromatographic (GC) analysis.

Recovery: The biobutanol present in the fermentation broth is recovered by Liquid-Liquid extraction method using Olyel alcohol.

RESULTS

Estimation of Cellulose

The amount of cellulose in pongamia husk was estimated using Nitric Reagent Test. The values obtained from spectrophotometer at 630 nm are listed in Table 1. The percentage of Cellulose present in Pongamia husk is found to be 57%.

Hydrolysis and Detoxification

After pretreatment of pongamia husk a clear hydrolysate is obtained, which is detoxified by Ca(OH)2.

Fig 1: Detoxified Clear Hydrolysis.

Inhibitors Removal

Inhibitors which are produced during hydrolysis are removed by treating the hydrolysate with Amberlite XAD-4 resins. Approximately 60 – 80% of inhibitors are removed by this treatment

Fermentation

Figure 1 showing the fermentation of XAD-4 treated hydrolysate in an anaerobic environment which gives visible results for growth of C. acetobutylicum. Intermediately the sample is collected for ABsE analysis.

Fig. 2: Growth of C. acetobutylicum.

Table 1: Values Obtained from Spectrophotometer at 630 nm for Cellulose Estimation.

Volume of stock solution taken (mL)	Amount of cellulose present in this vol (µg)	OD at 630 nm wavelength
0.1	10	0.045
0.2	20	0.076
0.3	30	0.103
0.4	40	0.131
0.5	50	0.162
0.6	60	0.193
0.7	70	0.210
0.8	80	0.256
0.9	90	0.287
1.0	100	0.300

CONCLUSION

The production of biobutanol from lignocellulose material was found to be very economical and cost effective. Further the efficiency of biobutanol production was found to be enhanced as the fermentation process progresses. The results of GC analysis for the samples collected during various time periods of fermentation were showing the yield of extracted butanol as 62.16%.

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