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Research Article

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Biobutanol production from cellulose rich agricultural waste using *Clostridium species*

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ABSTRACT

Biofuels in particular are receiving climbing attention due to the increase in industrialization, population, global demand for energy driven by fast depletion of conventional fuels. Biobutanol a solvent is a convertible to jet fuel blends with gasoline and also acts as a rich fuel extender. Biobutanol when blended with fuel can be used in internal combustion engines releasing carbon dioxide making environmental friendly biofuel. Further biobutanol is used much than ethanol due to its high calorific value, less volatile, low vapor pressure, relatively low heat of vaporization and is highly explosive over bioethanol. As well biobutanol rather than synthetic butanol is produced using ABE (Acetone Butanol Ethanol) fermentation reducing the use of conventional chemical methods. ABE fermentation was achieved using Clostridium species whose genes help in the conversion of biomass into butanol. In addition to this, yield and productivity of the biofuel can be used to recover high yields biobutanol through metabolic and genetic manipulation and by inculcating efficient pre-treatment technologies. In this article higher yields (0.57- 0.65 %) of biobutanol is produced using lignocellulosic substrate,Moringaolieferaa soft wood whose bark through proper pretreatment and saccharification process yields monosacharideswhich are fermented using Clostridium sp..(MTCC 1349) for the maximum production ofbiobutanol.

Keywords: Additive, Extender, ABE fermentation, Pre- treatment, Saccharification.

INTRODUCTION

Perceiving the escalating diminution, emission of GHG (green house gases) and the issues caused by energy security, alternative energy sources (here biofuels) has got climbing attention universally [1-7].Alternative source drawn from biomass appears to be a significant energy source to mitigate the increasing demand for the fuel energy source and the CO2 emitted during the combustion of this fuel can be recycled in synthesizing biomass making it more eco-friendly [8]. Biobutanol,a versatile four carbon alcohol is convertible to jet fuel blends with gasoline and also acts as a rich fuel extender. Biobutanol when blended with fuel can be used in internal combustion engines releasing carbon dioxide making environmental friendly biofuel. Further biobutanol is used much than ethanol due to its high calorific value, less volatile, low vapor pressure, relatively low heat of vaporization and is highly explosive over bioethanol [9-18].Biobutanol contains 110,000 BTUs per gallon, closer to gasoline's 115,000 BTUs, and is safer to handle with Reid value of 0.33 psi, which is a measure of a fluid's rate of evaporation when compared

to gasoline at 4.5 and ethanol at 2.0 psi [19, 20].An 85% butanol/gasoline blends can be used in unmodified petrol engines and contains 22% oxygen makes it a beneficiary fuel that is cleaner burning than ethanol [21, 22].

Biobutanol rather than synthetic butanol is produced using ABE (Acetone Butanol Ethanol) fermentation reducing the use of conventional chemical methods. ABE fermentation was achieved using *Clostridium species* whose genes help in the conversion of biomass into butanol.

Clostridium strains are saccharolytic butyric acid-producingbacteria which under appropriate operating conditions ferment a starch substrate (pentoses, hexoses,mono-, di- and polysaccharides), glucoses, cellulosic-based materials and other biomass feed stocks. These strains have the ability to secrete profuse enzymes that aid the metabolism of polysaccharides into monosaccharides [21].

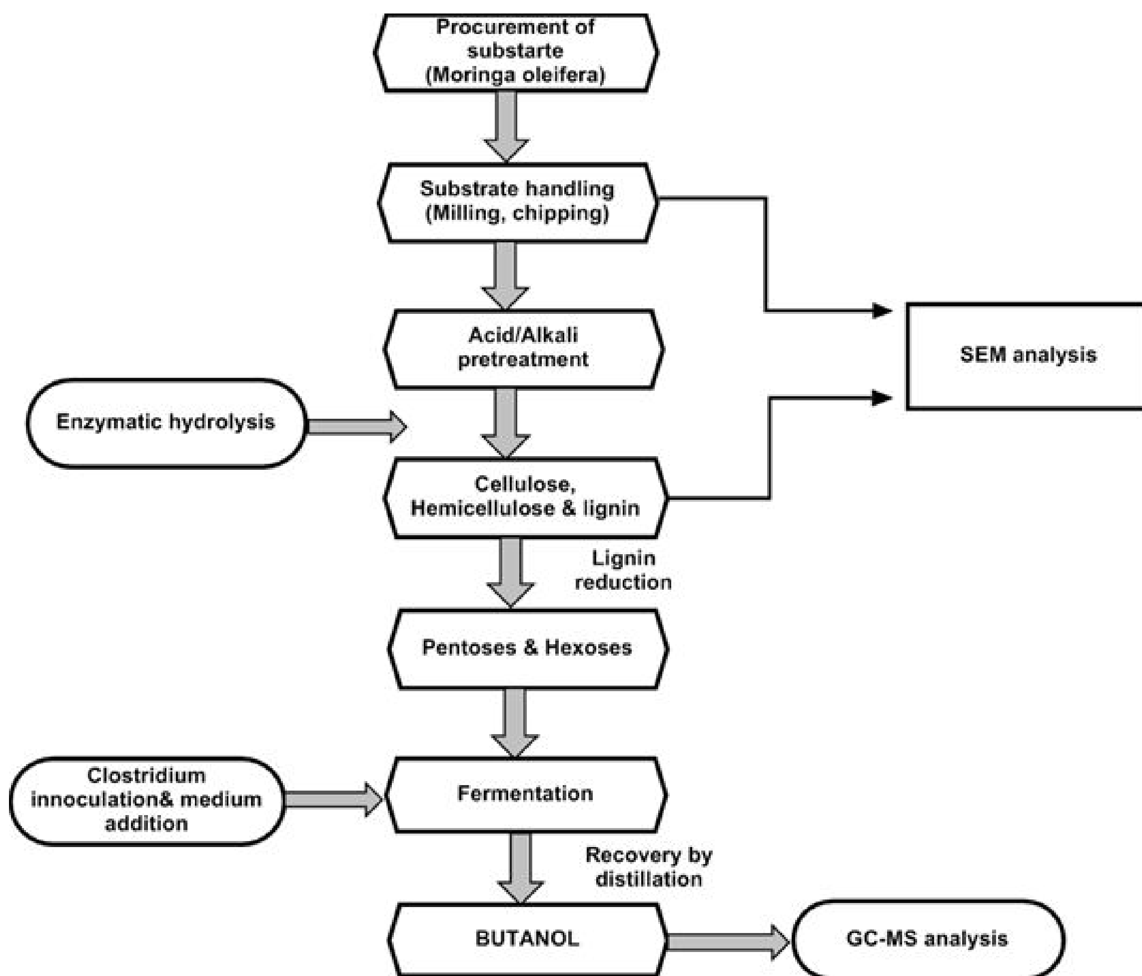


Figure 1: Production pathway of biobutanol using *Moringa oleifera* as raw material.

1.1. Lingnocellulose, a cellulose rich waste

Lignocelluloses are composed of carbohydrate polymers (cellulose,hemicellulose), and an aromatic polymer (lignin). Carbohydrate polymers contain different sugarmonomers (six and five carbon sugars) which are tightly bound to lignin. Lignocellulosicbiomass can be broadly classified into virgin biomass (trees, bushes and grass), waste biomass (corn stover, sugarcane bagasse, straw, saw mill and paper mill discards) and energy crops (switch grass, Elephant grass, popolar, etc). Lignocellulose has evolved to resistdegradation and to confer hydrolytic stability and structural robustness to the cell walls of theplants. For extraction of fermentable sugars, celluloses

andhemicelluloses should be disintegrated from lignin, and then converted into simple monosaccharides by acidhydrolysisor enzymatic conversion. But the recovered sugar solution after hydrolysis contains primarily pentose sugars and the fermentation of thesepentoses is problematic. Only limited numbers of microorganisms that use pentose are knownand the fermentation of pentose sugars at industrial scale is not established yet [23-28].In this article, *Moringaoleifera* (drumstick tree) a lignocellulosic crop is the most widely cultivated species of *moringa*, the only genus in the moringaceae family. The tree is grown mainly in semi-arid, subtropical and tropical region and in sandy soil. It is fast growing, draught resistant and native to India. India is the largest producer of moringa, where Andhra Pradesh leads in the production and area followed by Karnataka and Tamil Nadu. The tree is approximately 10m high which is cut annually to 1m or less and allowed for rejuvenation. This regular pruning of moringa tree results in the accumulation of their stem (soft-wood) which is of no better use hence it can be pretreated and used for biobutanol production using ABE fermentation.

EXPERIMENTAL SECTION

1.2. Materials

Soft wood (*Moringaoleifera*) was procured from local farmer from Avadi, Chennai, Tamil Nadu. The wood was cut (approximately 5cm in length and 0.5 cm in diameter) and is dried using hot air at 60 °C for 4 hours. The dried wood pieces are chipped and milled and the overall compositional analysis is made.*Clostridiumsp* (MTCC 1349) was procured from MTCC, Chandigarh. Whose growth medium (Hi media 149) was purchased from Hi media.

1.3. Pre-treatment of soft wood

1.3.1.Sulfuric Acid Pre-treatmet

Sulfuric acid (H2SO4) at concentrations of 0.5, 1, 1.5 and 2% (w/v) was used to pre-treat10 g ground stalks samples at a solid loading of 10% (w/v). The optimal temperature forthe treatment was set to be 90° C and the autoclave temperature was set at 121°C with 15 psi pressure for residence times of 30, 60, and 90 minutes. The collected solids were washed with 750 mL of hot de-ionized water. Using the parts of the solid residues the total residual weight andlignin, carbohydrate and moisture content analyses was determined prior to storing at 4°C for enzymatic hydrolysis. The filtrates from the lignin content analyses were collected and an HPLC carbohydrate analysis similar to that for the initial composition analysis was performed. The reduction in lignin following pretreatment was calculated based on the initial dry-weight of lignin in the untreated sample (LU) and the dry-weight of lignin in the remaining solids after pretreatment (LP). In addition, the percentage of solids recovered was calculated on an oven-dry basis as follows:

$$\% \text{ of solid recovered} = \left(\frac{W2}{W1}\right) * 100$$

Where, W1= dry sample weight of whole biomass before pretreatment (g)
W2= dry sample weight after pretreatment (g)
LU and LP were calculated as follows:

$$LU = \frac{\%LU}{100} * 100$$

Where, %LU = percent acid-insoluble lignin in untreated sample
W = dry sample weight (g)

The percentage of lignin reduction was calculated with the following equation:

$$\% \text{ lignin reduction} = \left(\frac{LU-LP}{LU}\right) * 100$$

Where LP= dry-weight lignin in pretreated sample
LU = dry-weight lignin in untreated whole biomass sample

1.3.2. Sodium Hydroxide Pretreatment

Sodium hydroxide (NaOH) at concentrations of 0.5, 1, 1.5 and 2% (w/v) was used to pretreat 10g ground stalks samples at a solid loading of 10% (w/v). Optimal pretreatmenttemperature were set to be 90° C and the autoclave

temperature was set at 121ºC with 15 psi pressure for residence times of 30, 60, and 90 minutes which were the same as those used for sulfuric acid pretreatment. The analyses performed were also similar to those for sulfuric acid.

1.4. Fermentation

Clostridium sp. (MTCC 1349) was used in the butanol production, 3ml of which was inoculated to the medium (16 to 18hrs culture, room temperature (32 to 37 degree celcius)),. 1L of butanol fermentation medium was takes which contains Beef heart, solids 98 g; Proteose peptone 20 g; Dextrose 2 g; Sodium chloride 5 g; 500ml of hydrolysate which contained the fermentation medium was taken in 3L fermentation vessel on the whole the working volume was taken to be working volume 1.5l, ph 6.5 adjusted using 400g/l NaOH. The vessel was then placed in the anaerobic chamber for 100-168hr.

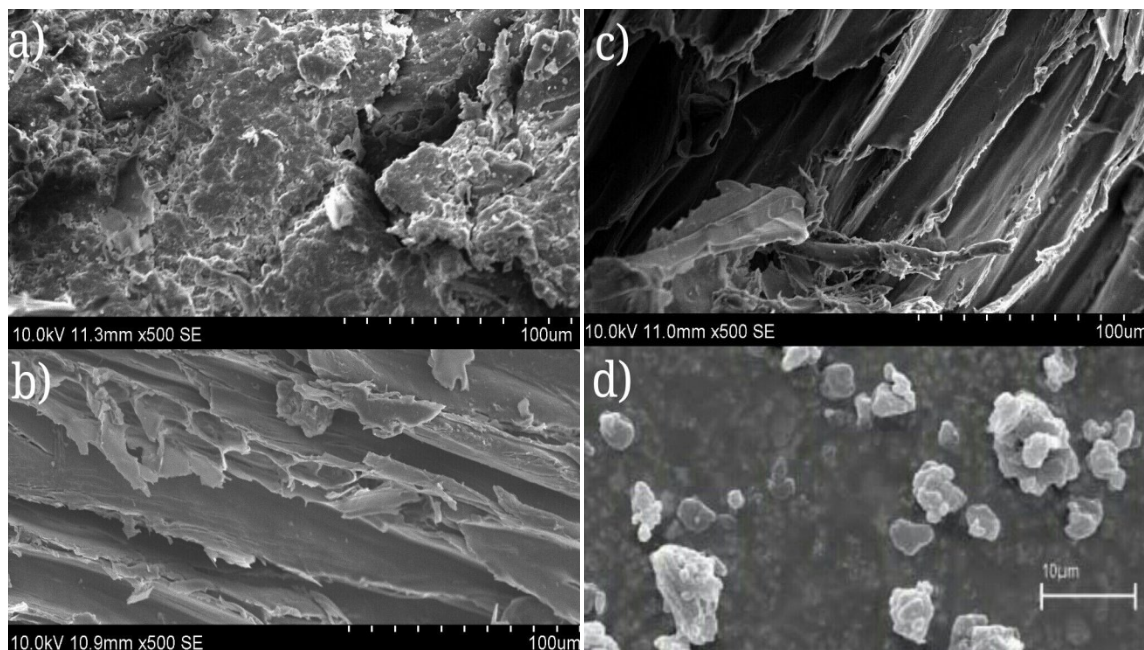
RESULT AND DISCUSSION

1.5. Pre-treatment

1.5.1.SEM analysis

Cellulose possesses unique structure that varies depending on its sources [29].Narrow down of vivid lignocellulosic biomass into its utilizable cellulose structure after pretreatment has been studied under SEM. SEM is usually chosen for examining the microstructure of cellulose due to the examination with less cumbersome sample preparation[29]. The SEM images of softwood before and after pretreatment are depicted in Figure 2. The recovery of cellulose from soft wood after pretreatment shows an overall increase and there also seemed to be a marginal increase in reduction percentage of lignin after pretreatment. This relative increase in the concentration of cellulose and lignin after pretreatment could be due to removal of hemicelluloses [30].

Figure 2: SEM analysis of *Moringaoleifera*before and after pretreatment a) Before; b) Acid treatment; c) Alkali treatment; d) Enzymatic treatment

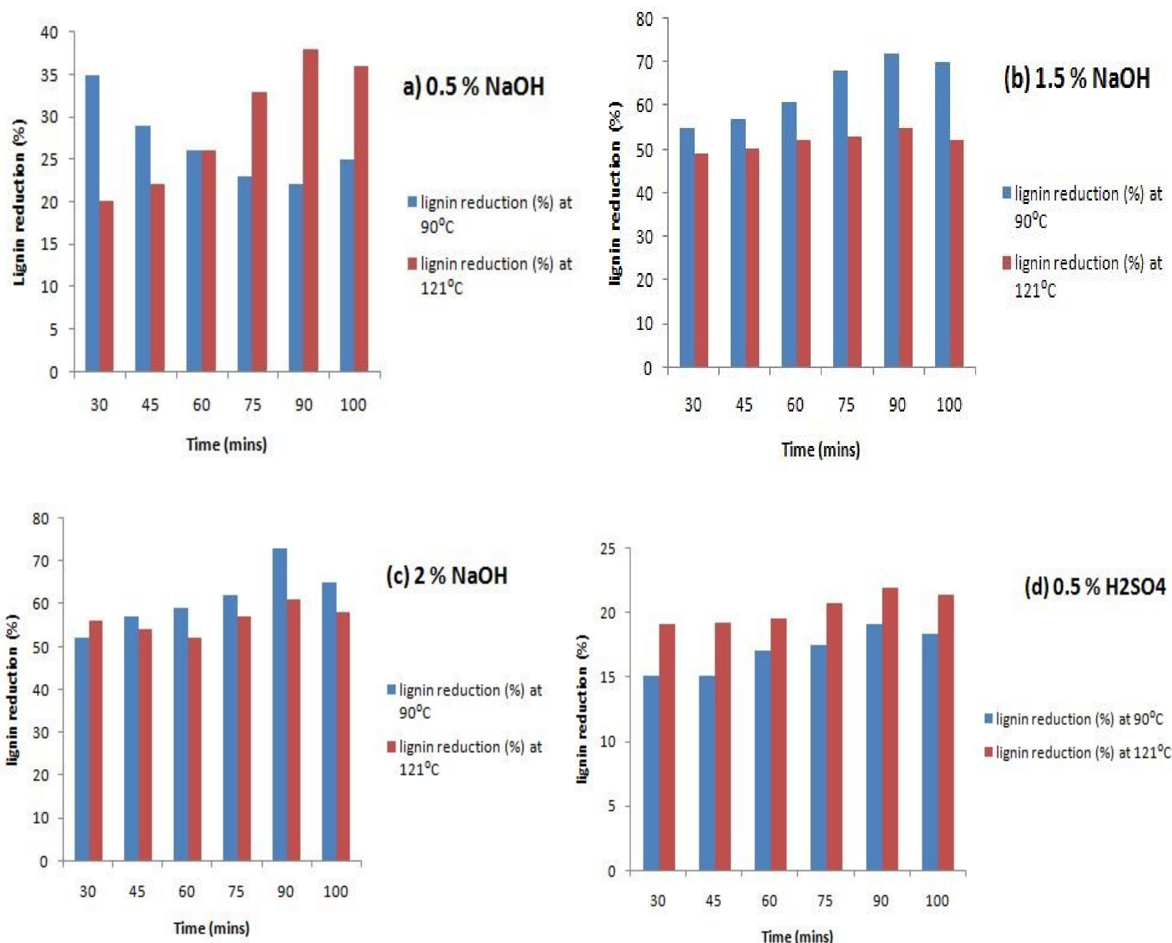


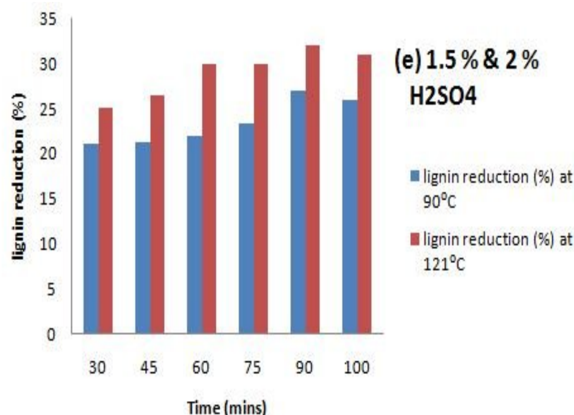
1.5.2. Cellulose estimatiom and lignin reduction

For 0.5% NaOH the lignin reduction percentage after alkali treatment at 30 mins/90oC was 35%, at 60mins/90oC was 26% and at 90mins/90oC was 22 %. For 1.5% of NaOH at 30 mins/90oC lignin reduction was 55%, at 60mins/90oC was 61% and at 90mins/90oC was 72%. For 2% of NaOHat 30 mins/90oC was 52%, at 60mins/90oC was 59% and at 90mins/90oC was 73%. During autoclaving temperature the lignin reduction percentage were found to be 20%, 26% and 38% respectively for 30, 60 and 90mins for 0.5NaOH; in case of 1.5% NaOH 49%, 52%, 55% were recorded for 30, 60 and 90mins respectively and in case of 2% NaOH 56%, 52%, 61% for the same condition.

The amount of lignin from acid pretreatment(H2SO4) ranged from 15% (30 min, 90°C) to 19% (30 min, 121°C/15psi), 17% (60min,90oC) to 19.5% (60 min, 121°C/15psi), 19% (90min, 90oC) to 22% (90 min,121°C/15psi) for 0.5% H2SO4 and for 1.5 and 2% H2SO4 21% (30 min, 90°C) to 25% (30 min, 121°C/15psi), 22% (60min,90oC) to 30% (60 min, 121°C/15psi), 27% (90min, 90oC) to 32%(90 min,121°C/15psi), with changes in concentration, temperature and time causing the most significant changes in the lignin contents.the optimized concentration of lignin reduction was depicted in Figure 3. The cellulose content after enzymatic hydrolysis were estimated for acid and alkali pretreatment and it was observed that sulfuric acid at 2%, 60mins, 15psi had maximum of 43% compared to rest and in the case of sodium hydroxide the same condition gave maximum cellulose i.e. 47% .

Figure 3: Optimized lignin reduction with various concentrations of NaOH and H_2SO_4

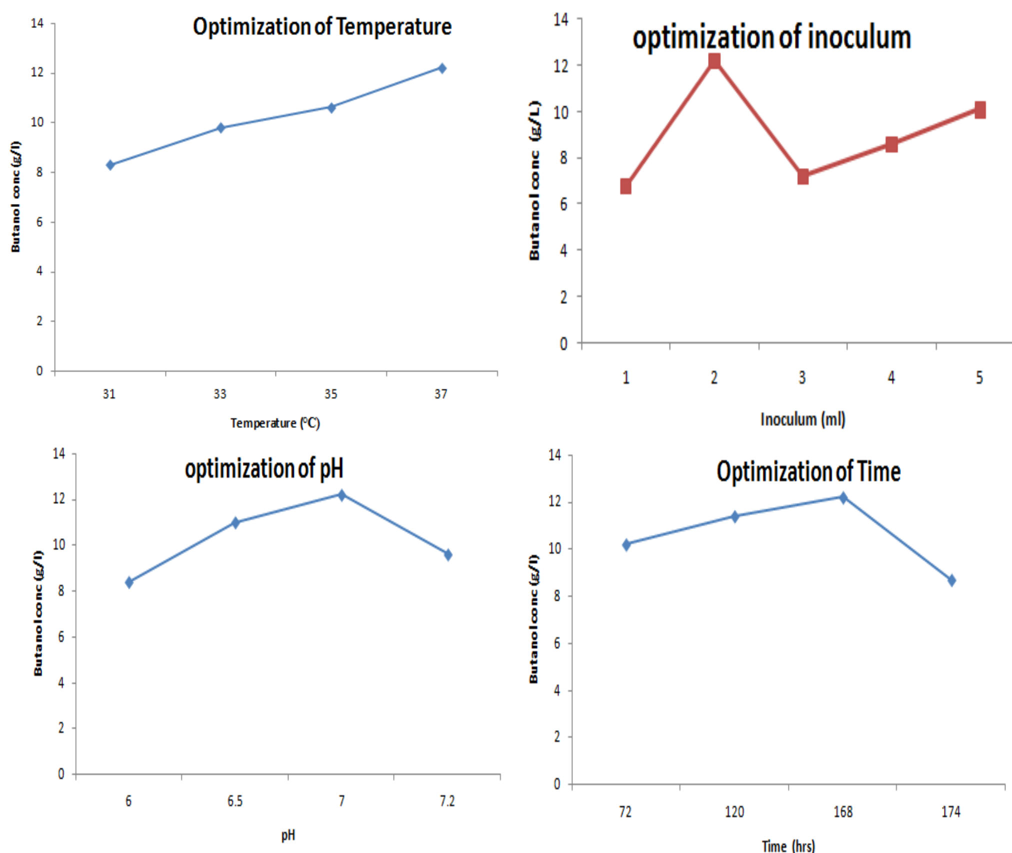




1.6. Butanol production from *Moringaoleifera*

From the enzymatic pretreatment for acid and alkali pretreatment of *Moringaoleifera*, it was found that alkali pretreatment yields the maximum of 47% cellulose when compared to acidpretreatment. Also the growth of *Clostridium sp.* found to increase rapidly after 48 hours with the sugar that was obtained after alkali pretreatment. Further optimization of time, pH, temperature and inoculums was studied which is depicted in the Figure 4. And the overall ABE concentration was found to be 20g/l in which, Acetone 1.8 g/l,Butanol 12.8 g/l and Ethanol 6 g/l. The productivity and yield was obtained as 2.85g/l and 0.57% respectively. The inhibitor of the butanol production was either removed by overliming process or by improving the pretreatment process.

Figure 3: Optimaization of time, inoculum, pH and temperature



CONCLUSION

In this study, *Moringaoleifera* a lignocellulosic waste was used as raw material for biobutanol production by Clostridium sp. (MTCC 1349). In order to select the better method for producing available sugar for Clostridium sp. (MTCC 1349), alkali pretreatment and enzymatic hydrolysis were studied. The optimal concentration of cellulose was obtained from alkali treatment with maximum of 47% and maximum yield of ABE was found to be 0.57%.

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