2.Enzymatic Hydrolysis

1. Add 1 gram of untreated sugarcane bagasse (NSB) and 10 ml of citrate buffer to the conical flask named NSB+BUFFER and it serves as blank.
2. Add 1 gram of untreated sugarcane bagasse and 10 ml of enzyme solution (1.5 mg/ml) to the conical flask named NSB+ENZYME.
3. Add 1 gram of Pre-treated sugarcane bagasse (PSB) and 10 ml of citrate buffer to the conical flask named PSB+BUFFER.
4. Add 1 gram of Pre-treated sugarcane bagasse and 10 ml of enzyme solution to the conical flask named PSB+ENZYME
5. Incubate conical flasks in shaking water bath at 55 °C for 24 hours at 120 rpm.
6. Boil the conical flasks at 100 °C for 5 minutes for enzyme inactivation.
7. After this, transfer the liquid content of the conical flasks to the microcentrifuge tubes.
8. Now centrifuge these microcentrifuge tubes for 5 minutes at 10,000 rpm. Collect the supernatant for enzymatic assay.
9. Perform DNS assay for estimation of the amount of reducing sugar content and calculate the yield. (Same as that of previous experiment).

DNS Assay:

1. To 0.1 ml of collected supernatant add 0.9 ml of distilled water for all the samples.
2. Add 1 ml of DNS reagent to each tube.
3. Keep it in boiling water bath for 15 minutes.
4. Cool it down and check OD at 540 nm.

S.No.	Amount of pre-treated biomass (g)	Enzyme Concentration (mg/mL)	OD at 540 nm (DNS method)	Sugar (glucose) released (mg/mL)	Sugar (glucose) released (µmol)	Enzyme activity (IU/mL of enzyme)

Results

Conclusion

The yield of reducing sugar obtained for alkali pre-treatment is_____g/g of sugarcane bagasse and enzymatic pre-treatment is_____g/g of sugarcane bagasse.

throughout the alkali pre-treatment process which causes cleavage of the intermolecular ester linkages between hemicelluloses and lignin. This results in solubilisation of lignin and hemicellulose fragments in the alkali solution and brings the cellulose in the interaction of enzymes. Also, alkali pre-treatment changes the lignocellulosic structure via cellulose swelling that leads to reduction in crystallinity and degree of polymerization thereby increasing internal surface area. In addition, removal of acetyl groups and uronic acid substitutions in hemicelluloses during alkali pre-treatment also increases the accessibility of the carbohydrates to enzymatic hydrolysis (Ncibi et al., 2017)

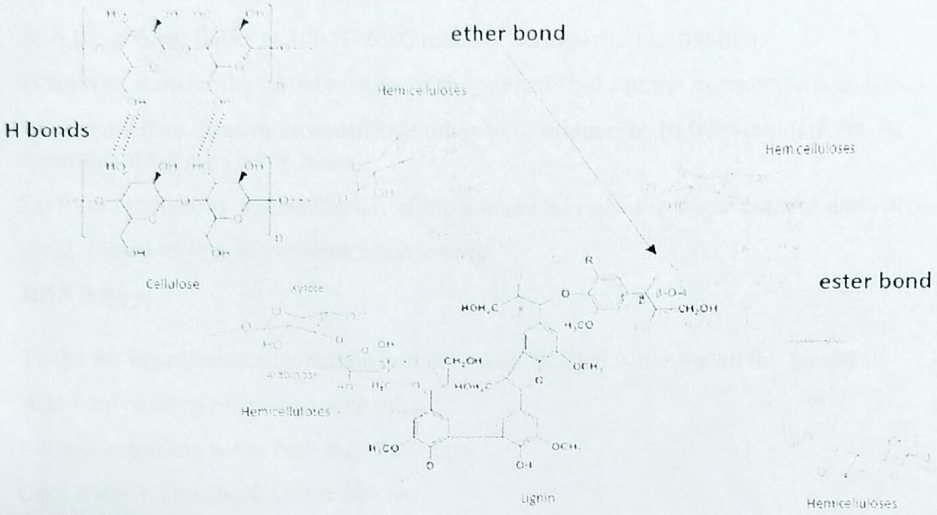

Figure 1. Alkali-labile linkages between lignocellulose components

Procedure

1. Pre-treatment of lignocellulosic biomass (Sugarcane bagasse)

1. Take 100 mL of 1% (w/v) NaOH in a conical flask.
2. Then add 10 g of Sugarcane bagasse to the solution.
3. Autoclave the flask for 30 minutes.
4. After autoclaving filter the solution using filter paper/ muslin cloth.
5. Separate the solid biomass after filtration.
6. Wash the solid biomass with water in order to remove unreacted NaOH.
7. Dry the biomass for overnight at 60 °C.

5. Pre-treatment and enzymatic hydrolysis of lignocellulosic biomass

Aim

To perform the pre-treatment and enzymatic hydrolysis of the lignocellulosic biomass (sugarcane bagasse).

Introduction

Biomass resources are readily accessible around the world as residual wastes and agricultural biomass. The most important and abundant renewable biomass resources include crop residues, such as corn straw, wheat straw, sugarcane bagasse and rice straw. Sugarcane bagasse is composed of cellulose, hemicellulose, lignin, extractives, and several inorganic materials. Pre-treatment is a technique for cellulose conversion processes, and is essential to change the structure of cellulosic biomass to make cellulose more available to the enzymes that convert the carbohydrate polymers into fermentable sugars. Different types of pre-treatment methods of sugarcane bagasse are necessary for the successful conversion of sugarcane bagasse to ethanol. Each pre-treatment method has a specific effect on the cellulose, hemicellulose, and lignin fraction. During the pre-treatment process the compact structure of lignocellulosic is disrupted and cellulose fibre is exposed. Pre-treatment of the lignocellulosic material is carried out to overcome recalcitrance through the combination of chemical and structural changes to the lignin and carbohydrate.

Enzymatic hydrolysis is the process in which cellulases are added to hydrolyse pre-treated lignocellulosic biomass into fermentable sugars. The process involves several key steps: (1) transfer of enzymes from the bulk aqueous phase to the surface of the cellulose, (2) adsorption of the enzymes and formation of enzyme-substrate complexes, (3) hydrolysis of the cellulose, (4) transfer of the hydrolysis products from the surface of the cellulosic particles to the bulk aqueous phase, and (5) hydrolysis of cellodextrins and cellobiose to glucose in the aqueous phase. The overall rate of the process is influenced by the structural features of lignocellulosic biomass and the composition and source of the cellulases. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because it is usually conducted at mild conditions and does not cause corrosion problems. Both bacteria and fungi can produce cellulases for hydrolysis of lignocellulosic materials.

Principle

Alkali pre-treatment is a widely studied chemical pre-treatment method which is based on the solubilization of lignin in the alkali solution. The various alkaline reagents used commonly for alkali pre-treatment are the hydroxides of sodium, potassium, calcium and ammonium. Among these sodium hydroxide was found to be the most effective. A saponification reaction takes place