1.Introduction:

The depletion of the fossil fuel and global warming caused by the emission of greenhouse gases from the combustion of fossil is currently driving researchers in the direction of finding alternative and environmentally friendly fuel. Biofuels are one of the numerous options being considered. Bioethanol is considered as the most promising biofuel to replace gasoline, especially due to its properties. This biofuel is a liquid oxygenated fuel containing 35% oxygen produced from the microbial fermentation of monomeric sugar obtained from carbohydrate sources such as corn, soybeans and sugar cane.

When bioethanol is produced from edible feedstocks such as corn and sugar cane, it is called first generation (1G) bioethanol

Second-generation (2G) bioethanol if the feedstock is a lignocellulose. Examples of these lignocellulose biomass is switch grass, cornstalks, wood, herbaceous crops, waste paper and paper products, agricultural and forestry residues, pulp and paper mill waste, municipal solid waste and food industry waste.

The third generation (3G) bioethanol is obtained when algae are used as the feedstock. Algae bioethanol is gaining traction possibly due to high carbohydrate content and absence of lignin in most available algae.

The fourth-generation (4G) bioethanol is obtained from the modification of *E. coli* gene altercations through the application of metabolic engineering or systems biology strategies.

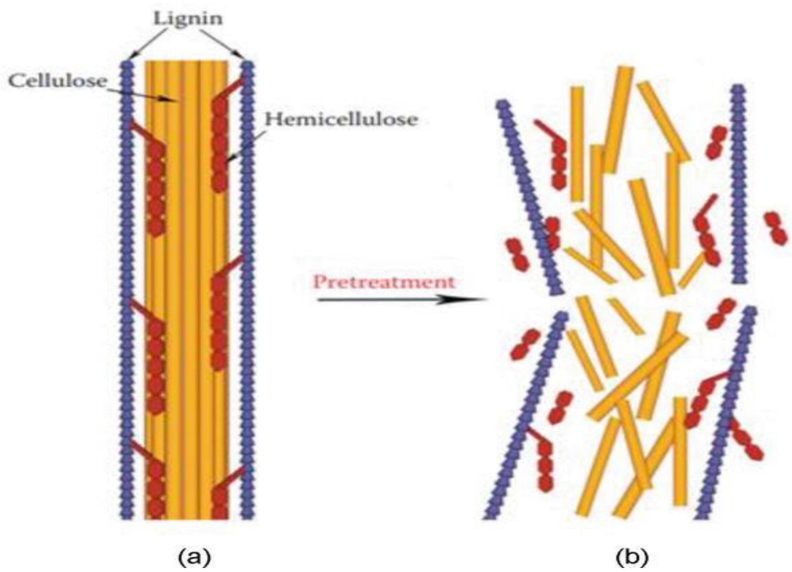
2. Bioethanol production process

The processes involved in the production of bioethanol from different feedstocks include pretreatment, hydrolysis, fermentation and ethanol recovery. These processes are explained below:

2.1 Pretreatment

These may be either traditional or advanced pretreatments. Traditional pretreatments are classified into four categories which include chemical, physical, physicochemical, and biological methods while advanced pretreatment method may be either acid-based fractionation or ionic liquid-based fractionation (ILF).

The pretreatment of lignocellulosic biomass through various methods helps to release cellulose usually embedded in a matrix of polymers consisting of lignin and hemicellulose by disrupting the original structure.



2.1.1 Traditional pretreatments

(1) physical pretreatment- this involves the breaking down of the size of the lignocellulosic biomass and crystallinity by methods such as milling, grinding, irradiation and extrusion.

(2) Chemical pretreatment: these include acid, alkali, oxidative delignification, and organic acid (organosolvation) methods.

(3) Physicochemical: this combines the features of both physical and chemical pretreatments.

2.1.2 Advanced pretreatment methods for lignocellulose

There are two general techniques used in CSLF which include (1) acid-mediated fractionation and (2). Ionic liquid-based fractionation (ILF). These are discussed below:

2.1.2.1 Acid-mediated fractionation

The cellulose solvents such as phospholic acid and organic solvents like acetone or ethanol are usually used at mild operating conditions of 1 atm and 50^{o}C to separate lignocellulosic biomass.

2.1.2.2 Ionic liquid-based fractionation

Ionic liquids (ILs) are salt solutions consisting of significant quantity of organic cations and small/inorganic anions that exists as liquid at relatively low temperatures like room temperature.

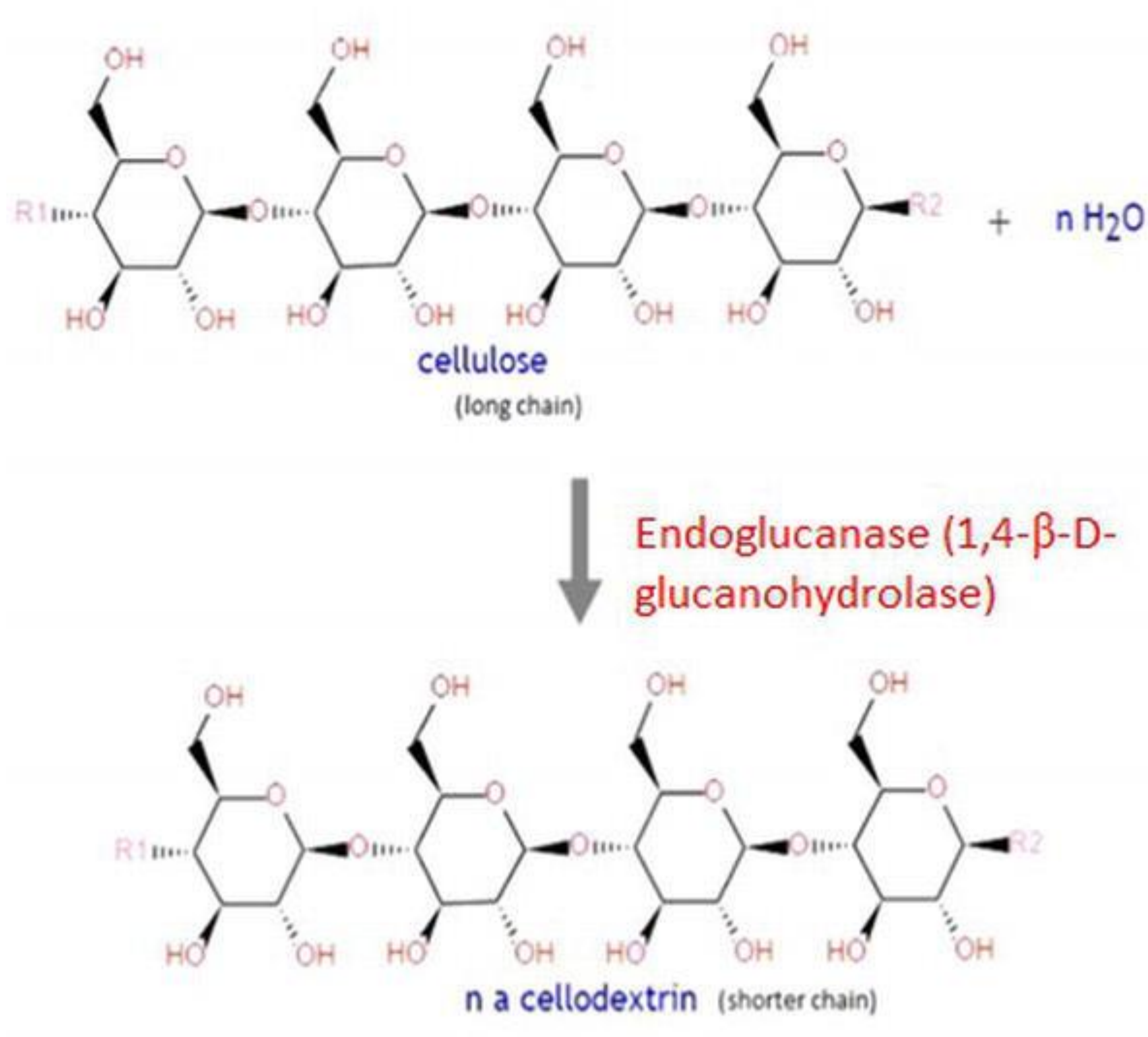
2.2 Hydrolysis

Following the pretreatment of the lignocellulosic biomass is the hydrolysis of polymeric carbohydrate (cellulose and hemicellulose) to produce sugar monomers. This stage is required since enzymes needed in the succeeding stage (fermentation) can only digest sugar monomers.

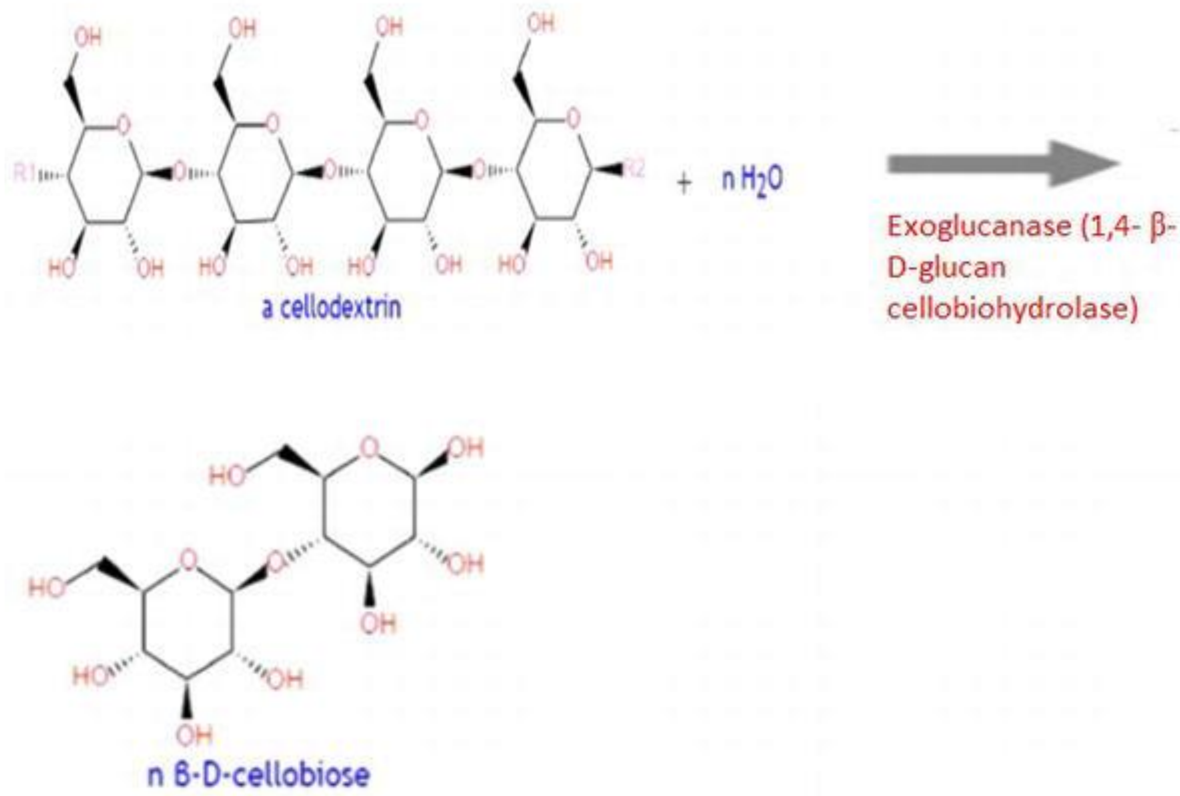
Enzyme-catalyzed hydrolysis uses enzymes to hydrolyze polymeric carbohydrate to sugar monomers under mild operating conditions of temperature 45–50^{o}C and pH 4.8–5.0. This method is efficient and results to high sugar recovery without inhibitor formation and tendency to cause corrosion. The efficacy of the enzyme-catalyzed hydrolysis is affected by factors such as pH, enzyme loading, time, temperature and substrate concentration. The hydrolytic process can be catalyzed by three kinds of cellulase enzymes, name endo-1,4-β-glucanases, cellobiohydrolases and β-glucosidases.

These enzymes are usually very expensive due to high demand from various industries such as paper, textile and food processing industries. The high cost of these enzymes also impacts on the overall cost of production especially as large quantities of enzymes are required. Based on the cost, microorganisms with the potential of secreting cellulolytic enzymes are broadly used in the contemporary times.

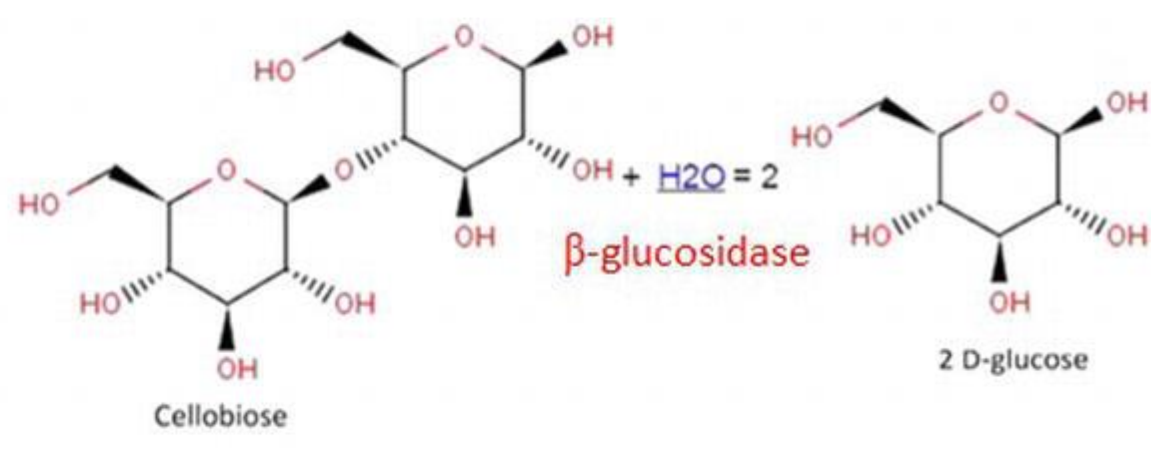
These include *Clostridium*, *cellulomonas*, *Erwinia, Thermonospora, Bacteriodes, Bacillus, Ruminococcus*, *Acetovibrio*, and *Streptomyces*. Others include fungi such as *Trichoderma, Penicillium, Fusarium, Phanerochaete, Humicola, and Schizophillum sp*. The most commonly used microbial enzymes amongst these microorganisms is Trichoderma species. The problems with the microbial enzymes are stability, substrate or product inhibition and catalytic efficiency. Although, with advances in genetic modifications, recombinant DNA techniques and application of various strategies to improve the strains help to increase the quantity of enzymes produced, make them more robust and economically feasible. The efficiency of the cellulose hydrolysis can also be improved by the addition of Polyethylene glycol (PEG) or Tween 20 resulting to increased enzymatic saccharification and reduction in the adsorption of cellulose on lignin.



Hydrolysis of long chain cellulose to a shorter chain cellulose (cellodextrin)



Hydrolysis of cellodextrin to cellobiose catalyzed by exoglucanase (1,4- β-D-glucan cellobiohydrolase)

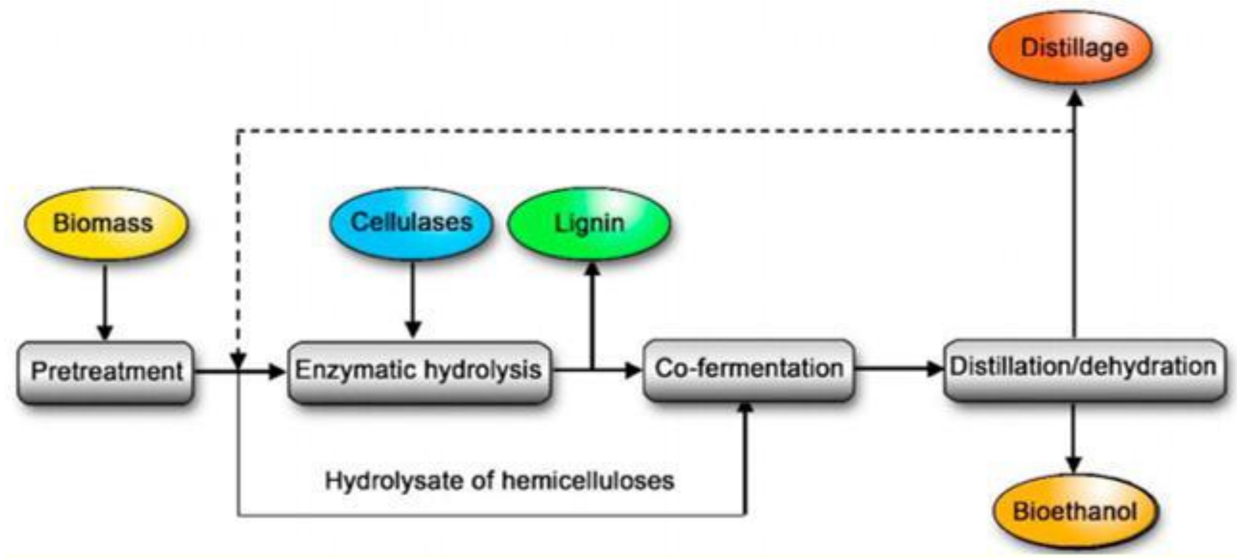


Hydrolysis of cellobiose to 2 D-glucose catalyzed by β-glucosidase

2.3 Fermentation processes

This is a biological process that involves the conversion of the monomeric units of sugars obtained from the hydrolysis step into ethanol, acids and gases using microorganisms such as yeast, fungi or bacteria

Saccharomyces cerevisiae converts glucose, mannose or fructose which can be obtained from the hydrolysis of cellulose to ethanol while xylan from the hydrolysis of hemicellulose can be converted to xylose.



Separate hydrolysis and co-fermentation (SHCF)

2.4 Ethanol recovery

The fermentation of monomeric sugars is usually followed by ethanol recovery from the fermentation broth. Usually, the water content of the broth is reduced to approximately 0.5% by volume enabling the formation of anhydrous ethanol with a minimum of 99.5% by volume. This operation is constrained by the azeotropic nature of ethanol-water solution and can be carried out based on the principle of distillation (i.e. leveraging the difference in boiling point of the components of the solution).