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CHAPTER 1
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The world petroleum resources are being consumed at a rapid rate. Increase in the world population and industrialisation is further adding to this consumption and price rise. Along with these, the concerns about global warming, instability and issues on national security have led to resurgence in the development of alternative energy sources that can displace fossil transportation fuel. (Alper and Stephanopoulos, 2009; Gupta et al., 2009). The alternative transportation fuels that are currently being investigated include biodiesel, bio-alcohols, biohydrogen and synthetic petroleum hydrocarbons (Alper and Stephanopoulos, 2009). Among them, the biofuel is expected to be most widely used around the globe is ethanol.

Ethanol as biofuel has several advantages such as low toxicity, biodegradable nature, safer alternative to methyl tertiary butyl ether (MTBE), generation of fewer airborne pollutants than petroleum fuel and easy integration in the existing vehicle. Ethanol in vehicles can be either blended with the gasoline as a fuel extender and octane enhancing agent or can be used as a fuel in internal combustion engines (McCarthy and Tiemann, 1998). Currently most biofuel in the form of ethanol is derived from starch or sugars, however the continuous use of these feedstock will cause food versus fuel competition.

Lignocellulosic biomass represents an abundant carbon-neutral renewable resource for the production of Bioethanol (Ragauskas et al., 2006). Lignocelluloses are mainly comprised of cellulose, hemicellulose, and lignin (Kuhad et al., 1997) and have evolved complex structural and chemical mechanisms for resisting assault on its structural sugars from the microbial and/or chemical degradation (Himmel et al., 2007). Several technologies have been developed during the last 80 years that enable
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this conversion process to occur. The bioconversion process mainly consist of three major steps i.e., pretreatment, hydrolysis and fermentation. The purpose of the pretreatment is to remove lignin and hemicellulose fraction, reduce cellulose crystallinity and increase the porosity of the materials.

The pretreated material is subjected to the enzymatic hydrolysis and the resultant hydrolysates are fermented to ethanol using fermenting microbes (Kuhad et al., 2011a). Chemical hydrolysis has long been recognized as an efficient pretreatment for removing the hemicellulosic and lignin fraction from the lignocellulosic substrate to enhance its biological conversion to sugars and subsequently to ethanol. The chemical treatment resulted in the generation of toxic byproducts such as furfural, hydroxymethyl furfural (HMF) and phenolics, which significantly affect the metabolic activity of the microbes during fermentation (Palmqvist and Hahn-Hägerdal, 2000; Chandel et al., 2007). Therefore, to make the hydrolysates amenable to fermenting microbes, detoxification of acid hydrolysate is required (Palmqvist and Hahn-Hägerdal, 2000; Saha et al., 2005; Chandel et al., 2007; Gupta et al., 2009; Kuhad et al., 2011a; Gupta et al., 2011). In the literature various physical, chemical and biological detoxification methods are described, but a cost- effective detoxification strategy is yet to be worked out.

The pretreated cellulose-rich biomass is then hydrolysed with a consortia of cellulolytic enzymes i.e., endocellulase, exocellulase and β-glucosidase. These enzymes act synergistically and hydrolysed the cellulosic polymer to simple sugars (glucose) (Zhang et al., 2006; Kuhad et al., 2011a). Enzymatic hydrolysis has demonstrated better results for the subsequent fermentation because no degradation components of glucose are formed, however, the process is costlier (Sanchez and Cardona, 2008). Various improvements have been attempted in the last three decades that would lower the
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effective enzyme cost, including enzyme reusage, higher enzyme production or using
genetic engineered systems (Zhang et al., 2006; Kuhad et al., 2011a); but still there is a
need to search for more competent solutions.

Since the lignocellulose contain both hexose and pentose sugar, the efficient
fermentation of both the sugars has become a pre-requisite for the cost-effective
production of bioethanol. A variety of microorganisms are known to ferment hexose
sugars, but the microbes for pentose fermentation are limited in number. The most
common microbes tried for pentose fermentation are Candida shehatae, Pichia stipitis
and Pachysolen tannophilus (Abbi et al., 1996a, b; Kuhad et al., 2011a). However, none
of these yeasts have been found to be very promising. Intensive efforts have been made
towards utilization of all the sugars present in hydrolysates by employing both
genetic manipulation as well as process improvement approaches.

A number of microorganisms are capable of producing extracellular cellulase
enzyme among which Cellulomonas fimi is a widely used candidate for cellulase enzyme
production. Cellulomonas fimi produces at least six β-1,4-glucanases, of which four
(CenA, CenB, CenC, and CenD) are endoglucanases and two (CbhA and CbhB) appear
to be cellubiohydrolases that are the functional equivalents of T. reesei CbhI and CbhII.
The preferential attack of cellulose at the ends of glucan chains by C. fimi
cellubiohydrolases CbhA and CbhB is strongly suggested by hydrolysis experiments
using cellooligosaccharides (Gilkes et al., 1997; Meinke et al., 1993; Shen et al.,1995a;
Shen et al., 1995b). Earlier studies have indicated that CenA attacks susceptible linkages
in soluble Carboxymethyl Cellulose (CMC) in a relatively nonprocessive manner i.e., the
enzyme dissociates from the substrate after each hydrolytic event. While CenB and
CenD attack CMC in a similar way, C. fimi CenC seems to act in a more processive
fashion (Moser et al., 1989; Tomme et al., 1996). Therefore, CenC activity was analyzed
in order to determine if the enzyme behaves in a similarly processive manner on cellulose.

Previous determinations of molecular size during hydrolysis have shown that the choice of substrate is an important consideration (Kleman et al., 1994).

Currently, most commercial cellulases are produced from *Phanerochaete* sp, *Aspergillus* sp and *Trichoderma* reesei (Adney et al., 2003; Barnett et al., 1991) usually used to describe a mixture of cellulolytic enzymes whose synergistic action is required for effective breakdown of substrate to its monomeric units.

The action of cellulases involves the concerted action of

(i) endoglucanases (endo-1, 4-β-glucanases, EGs), which can hydrolyze internal bonds preferably in cellulose amorphous regions releasing new terminal ends, which randomly attacks the internal, β1,4-linkages

(ii) cellobiohydrolase (exo-1, 4β-glucanases, Cbhs), which acts on the existing or endoglucanase generated chain ends. Both enzymes can degrade amorphous cellulose but, with some exceptions, Cbhs are the only enzymes that efficiently degrade crystalline cellulose. Cbhs and EGs release cellobiose molecules which cleaves off cellobiose units from the non-reducing ends of the glucan, and

(iii) β 3-glucosidase, which hydrolyzes cellobiose to glucose.

Over all, use of biomass to produce bioethanol holds much promise for providing a renewable, indigenously produced liquid energy source that can be a viable alternative to petroleum-based fuels. However, there is a great scope of improvement in each and every step of the processing steps including selection of feedstock, pretreatment, hydrolysis and fermentation.
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Keeping in view the potential and sustainable usefulness of bioethanol and all the given facts of cellulosic ethanol production, the present investigation was carried out to alternative sources of bioenergy, with the following objectives:

1. To use potential availability of some agricultural residues in India like rice straw, rice husk and wheat straw as raw materials.
2. To achieve high yields of fermentable sugar from lignocellulosic components through hydrolysis using microorganisms.
3. To determine the effect of sugar yield using individual as well as consortium of different microorganisms (Cellulomonas fimii, Trichoderma reesei, Phanerochaete chrysosporium and Aspergillus awamori).
4. To achieve higher yields of ethanol at economic level using fungi Saccharomyces cerevisiae (fungus) and Zymomonas mobilis (bacteria).