CHAPTER 1
INTRODUCTION

1.1 Overview: Bio-fuel and bioethanol

Bioethanol, ethyl alcohol derived from biological origin, has drawn renewed attention in view of the energy crisis that is becoming evident more and more in recent years. In the backdrop of ever widening gap between global demand and supply of fuel, and emerging concerns regarding environmental pollution and global warming, ethanol based bio-fuels are gaining core attention in the future energy policies. A number of sources are now being used world-wide to produce bioethanol which mainly includes sugar based plants (Sivakumar et al., 2010). In this connection, date fruits, the staple fruit in the arabian region, which are rich in sugar contents can be considered as a potential source of bioethanol (Etiévant, 1991; Alonso et al., 2010).

The global warming issue is caused by using excessive fossil fuels. Therefore, renewable clean energy and bio resource fuel are required for replacing fossil fuel to reduce the greenhouse gas emission. Another prominent cause is the energy crisis issue and the continuous increase of global petroleum prices has impacts on human life and world politics too (Adinarayana et al., 2005). In order to solve these issues, a renewable energy should be developed and introduced as new feed stocks. Bioethanol is a form of renewable energy that has been produced from common agricultural feedstock such as sugar cane, potato, manioc and maize from the middle of last century (Al-Farsi et al., 2007). From
2007 to 2008, the share of bioethanol, which produced by fermentation process, has been increased from 3.7% to 5.4% (Al-Farsi et al., 2007).

In addition, these clean energy sources have attracted the attention of researchers as alternative blending fuel due to their high octane number. Many researchers showed that blending fuel incurs better results in terms of fuel ratio, engine performance and exhaust emissions. The act of blending (addition of ethanol to gasoline) has two effects on the blended fuel properties: (1) an increase of the octane number, (2) a decrease in the heating value (Cazetta et al., 2007; Chandel et al., 2007). They also reported that the CO and HC emissions decreased by 46.5% and 24.3% from starch-based feed stocks. The best performance and emissions results were obtained for 20% ethanol with 80% gasoline blend.

Despite being environmentally cleaner and renewable in nature, bioethanol based fuels has possible ecological drawbacks as large scale production is land incentive, requires additional energy and may cause pollution. Changes in land use pattern for bioethanol production deviating from food crops may also threat global food security which has become a major issue for debate. In view of this, a second-generation of bioethanol has been on a rise which is derived from agricultural waste such as lignocellulosic materials such as crop residues, grasses, leaves, sawdust, woodchips, sludges, municipal solid waste and livestock manure (Hossain et al., 2009; Staniszewski et al., 2007; Sun and Cheng, 2002; Wen et al., 2004; Zayed and Meyer, 1996).

In connection to increased production and use of bioethanol, research and practices in the field of bio-fuel have also increased giving rise to second and third generation bio-
fuels. To utilize this potential resource efficiently, more research is needed and more efficient sources of bio-fuel need to be discovered. To optimize its contradiction with food production, water resource and deforestation. New feedstock searching is a consequence process of all researchers to enhance the using of bioethanol and suggests the appropriate resources in respect to different geographical region of the world and lead this research forward as well.

Thus, in Middle East, dates are among the most available fruits, hence its waste is viewed as an obvious feedstock for liquid bioethanol, because it is easy to manage and ferment, has high saccharide content and no acidic component. Though, the production of syrup from dates has already been commercially established but innovative studies like bioethanol production by fermentation could bring expansion to new procedure and separation systems at the same time add to the economic production value.

In this backdrop, the current study attempts to examine the potential of rotten date biomass as feedstock for ethanol production.

1.2 Objectives

This study was conducted to achieve the following objectives:

1. To study the produce bioethanol from waste dates via fermentation;

2. To optimize the yeast concentration and selected physical parameters, which may influence the process of bioethanol production;

3. To determine the fuel properties produced from date and potential use in the reduction of greenhouse gases.
In recent years, the highly unstable global energy market, as well as large increases in oil and natural gas prices has led Canada and other countries to assess future fuel developments and explore alternatives to fossil fuels.

A survey of existing literature in the field of bioenergy, source of biofuel, bioethanol procedure and production, with particular attention and significance put on its use as fuel, revealed a wide array of theoretical, analytical and applied approaches. In this discourse, as it would appear, a significant part of the literature addressed the potentiality and feasibility of bioethanol as an alternative solution to world-wide apprehension of energy crisis and attempted to highlight possible commercially viable sources and production procedures of bioethanol (Nigam, 2000; Balat, 2007; Mohan et al., 2008; Behera et al., 2010). Another portion of the literature emphasised of second and third generation of bio-fuel sources (Goh et al., 2010; Tan et al., 2010) underscored by the philosophical and economic debate circling around the issue of food security given the pressure on agricultural land-use and use of food-crop in fuel production (Pimentel, 2001; Pimentel, 2003; Seelke and Yacobucci, 2007). Meanwhile, the environmental benefits as a corollary of replace of fossil fuel with renewable and comparatively clean bioethanol is also found to be well document in many of the scholarly articles (Goldemberg, 2008; Borjesson, 2009; Chandel et al., 2007).

2.1 Bio-fuels: bioethanol

Bio-fuels refer to a wide spectrum of fuels that are originated from biomass or biological sources. By definition, bio-fuels are solid, liquid or gaseous sources of energy
that are derived from biological matters such as plant matter and residues such as forestry and agricultural crops and by-products, and municipal wastes (Balat, 2007). Gaining popularity of such biologically originated fuels is underpinned by price hike and crunching reserve of non-renewable traditional fossil fuels, growing energy crisis, and emerging concern over climate change geared by greenhouse gas emission from fossil fuels. In view of this, liquid bio-fuels, such as bioethanol, are considered as alternative sources of energy for transportation and industrial uses. Bio-fuels, despite their higher cost of production, have drawn additional interest given the fact that they are able to reduce greenhouse gas significantly and can burn with higher efficiency. Bioethanol, which is chemically ethyl alcohol derived from biological sources such as sugar cane, potatoes, maize, various fruits, maniocs, and vegetable wastes, are sources of renewable energy (Behera et al. 2010; Hossain et al., 2009; Staniszewski et al., 2007; Sun and Cheng, 2002; Wen et al., 2004; Zayed and Meyer, 1996). Besides being used in alcoholic beverages, this ethanol derivative from biomass is now considered as a renewable fuel that can be used as transport fuel even at its purest form. Moreover, bioethanol can be used in existing technology of motor engines i.e. unmodified petrol-run vehicles with traditional fuel-transmission infrastructure and can easily be used as additives for traditional gasoline (Hansen, 2004). Being blessed with lower carbon emission, bioethanol based fuel system is relatively clean (Balat, 2007) and comparative advantage in terms of greenhouse gas emission could even be higher when replacing non-renewable hydrocarbon fuels. It is recommended to use bioethanol as an alternative fuel or as gasoline additive (Kim and Dale, 2005; Henke et al., 2005) or even required as an ecologically favourable fuel oxygenate (Borjesson, 2009).
2.2 Bioethanol as a source of bioenergy

Bioethanol, ethanol derived from biological sources, is one of the oldest products extracted using biotechnologies (Behera et al. 2010). The use of bioethanol, extracted using traditional biotechnology in the earlier ages, was probably not in the area of energy source, rather was used to prepare alcoholic beverages (Reed, 2002). Nevertheless, development of biotechnological tools and processes are always on the track of inventing newer products, substrates and processes which are cheaper and/or easier to produce (Behera et al. 2010). For such historic uses as beverages, ethanol was derived through fermentation of plant sugars from sugarcane, corn etc. Scientists hypothesized about the production process of ethanol from many other biological sources with an efficiency over thousand times than before (Champagne, 2007). In the course of development, bioethanol has also found its new uses and a number of studies have mentioned it to be one of the possible solutions to the much feared energy crisis. Given the limitations of the non-renewable fossil fuels, Blottnitz and Curran (2007) advocated the crucial role that bioethanol can play as a possible solution to the future need for a sustainable and cheap fuel. In Germany and France the emerging industry of internal combustion engine had been using bioethanol a gasoline additives (Demirbas, 2008a). As a transportation fuel, it was being used in Brazil since 1925, and until early 1900s the use of bioethanol was widespread in US and Europe (Balat, 2007). However, the enthusiasm of bio-fuel ebbed due to its higher cost of production, especially after the World War II when petroleum based fossil fuels became much cheaper. It was until 1970s, when the world saw the oil crisis, popularity of bio-fuel as alternative source of energy gained momentum and since then many countries including Brazil and US are promoting bioethanol usage as transport fuel (Balat, 2007).
The rise of bioethanol as a fuel substitute is a newer contribution and still has a long way to go before capturing an eminent share in the global fuel market. The automobile industry, albeit had changed very little in passing decades, has been evolving in the face of recent technological, social and environmental changes that are forcing the search for new alternatives to both propulsion systems and oil-derived fuels. Bioethanol is able to be used with current engine technology, it is feasible to substitute 10 %, or even 20 % of petrol (gasoline) with ethanol within 2020 (Balat, 2007). Looking back to the history of bio-fuel use, it can be traced back to the mid-1920s when ethanol was widely blended with petrol in almost all industrial countries, except in the USA. In the Scandinavian countries 10-20 % blend was common, and ethanol was mostly produced from paper mill waste (Kadar et al., 2004). In the USA, the combination of raising taxes, a concerted campaign by major oil producers and the availability of cheap petrol effectively killed off ethanol as a major transport fuel in the early part of the 20th century. It was only during the Second World War when ethanol achieved some prominence, particularly in Brazil and the USA due to fuel shortages. However, afterwards, the availability of cheap petrol effectively eclipsed the use of ethanol as fuel for nearly three decades in most countries (Rothman et al., 1983).
Table 2.1: World fuel ethanol production for 2010 and 2011.

<table>
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<th>Continent</th>
<th>Millions of Gallons</th>
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<tr>
<td></td>
<td>2010</td>
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<tr>
<td>North &amp; Central America</td>
<td>13720.99</td>
</tr>
<tr>
<td>South America</td>
<td>7121.76</td>
</tr>
<tr>
<td>Brazil</td>
<td>6921.54</td>
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<td>Europe</td>
<td>1208.58</td>
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<tr>
<td>Asia</td>
<td>785.96</td>
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<td>China</td>
<td>541.55</td>
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<tr>
<td>Canada</td>
<td>356.63</td>
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<td>Australia</td>
<td>66.04</td>
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<td>Africa</td>
<td>43.59</td>
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<td><strong>Total</strong></td>
<td><strong>13720.99</strong></td>
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Source: Renewable Fuels Association RFA (2011)

http://ethanolrfa.org/pages/World-Fuel-Ethanol-Production

The United States and Brazil are the world leaders for bioethanol production, which exploit corn and sugarcane, respectively, and both of them account for about 70% of the world bioethanol production. Renewable Fuels Association in 2007 has listed the USA as the major producer of bioethanol or ethyl alcohol (Table 2.1). Maize, their main crop has been used for this purpose because if compared to other crops with biofuel potential, maize gives more material for bioethanol production where both starch (from their seed) and cellulosic material (from the stover, algae) can be used (Antizar-Ladislao and Turrion-Gomez, 2008).
Bioethanol has potential to replace 353 billion liters of gasoline, which accounts about 32 % of the global gasoline consumption (Balat et al., 2008). The lignin-rich fermentation residue, which is the co-product of bioethanol made from lignocellulosic-based substrate, could be used to generate 458 terra-watt-hours (TWh) of electricity, about 3.6 % of world electricity production (Kim and Dale, 2004).

2.3 Feedstock: sources of bioethanol

Bioethanol can be produced from any plant material that contains glucose such as sugarcane, corn, sugar beet and other cereals such as maize and burley (Behera et al., 2010). Over the course of development, ethanol has been produced from a variety of feedstocks such as bagasse, miscanthus, sorghum, grain sorghum, switchgrass, reed canary
grass, cord grasses, hemp, kenaf, potatoes, sweet potatoes, cassava, sunflower, fruits, molasses, stover, wheat and Jerusalem artichoke (Behera et al. 2010; Hossain et al., 2009; Staniszewski et al., 2007; Sun and Cheng, 2002; Wen et al., 2004; Zayed and Meyer, 1996).

Smith and Holtzapple (2010) categorised feedstocks for bioethanol mainly into three groups: (1) sucrose-containing feedstocks (e.g. sugar cane, sugar beet, sweet sorghum and fruits), (2) starchy materials (e.g. corn, milo, wheat, rice, potatoes, cassava, sweet potatoes and barley), and (3) lignocellulosic biomass (e.g. wood, straw, and grasses). The limitation of using sugar or starch as a source is that the feedstock is expensive and demanded by other crucial applications such as food (Enguídanos et al., 2002).

The two major global producers, USA and Brazil, use sugar cane or molasses (in Brazil) and starch crops e.g., corn (in USA) as the principal feedstocks. Currently almost 95% of the ethanol produced globally, regardless of mode of uses, comes from sugar crops, including sugar cane, corn, maize and sugar beet (Xiberta and Rosillo-Calle, 2005).

Bioethanol produced from these starchy materials (e.g. corn or sugar cane) are specifically designated as first-generation bioethanol (FGB). Despite the benefit of cheap production cost and environment friendliness, the whole issue of FGBs have now been put on to converse given the fact that all the source crops that are currently used as raw materials are food crops and in future this may pose serious pressure on food supply undermining global food security (Tan et al., 2010). In this backdrop, taking food-fuel supply dilemma under serious consideration, a second-generation bioethanol (SGB) has been on a rise which is derived from agricultural waste such as lignocelluloses (Tan et al., 2010).
A potential source for low-cost ethanol production is to utilize lignocellulosic materials (crop residues, grasses, sawdust, woodchips, sludges, livestock manure). Research involving bioethanol production from lignocellulosic waste materials has included crop residues (Rivers and Emert, 1988; Zayed and Meyer, 1996; Cuzens and Miller, 1997; Kim and Dale, 2004), municipal solid waste (Green et al., 1988; Green and Shelef, 1989; Lark et al., 1997; Mtui and Nakamura, 2005), forest products industry wastes (Duff and Murray, 1996; Kadar et al., 2004), leaf and yard waste (Lissens et al., 2004), municipal sludges (Cheung and Anderson, 1997), as well as a few studies involving dairy and cattle manures (Chen et al., 2003; Wen et al., 2004). Crop residues, grasses, leaves, sawdust, woodchips, sludges, municipal solid waste, livestock manure are among the most potential raw materials (Champagne, 2007). Lignocellulosic biomass is envisaged to provide a significant portion of the raw materials for bioethanol production in the medium and long-term due to its low cost and high availability (Gnansounou et al., 2005). Nevertheless, the whole issue of SGB depends on lowering the production cost down to an economically feasible level which is underscored by technology advancement. The key obstacles associated here are low yield rate and the high cost of the hydrolysis process.

Liimatainen et al. (2004) produced bioethanol from potatoes based on the utilization of waste potatoes. Waste potatoes are produced from 5-20% of crops as by-products in potato cultivation. At present, waste potatoes are used as feedstock only in one plant in Finland. Oy Shaman Spirits Ltd in Tynävä (near Oulu) uses 1.5 million kilograms of waste potatoes/year. The study attempted to develop different analytical methods for bioethanol production from waste potatoes and to study the effect of potato cultivar on bioethanol production. Behera et al. (2010) highlighted that mahula (Madhu calatifolia L.) flowers have proved to be a great promise as an alternative bio-resource for ethanol production.
through fermentation. Mahula is a tree commonly found in the tropical rain forests of Asian Sub-continent (Mohanty et al., 2009). Its flower (edible part is ‘corolla’) is rich in fermentable sugar (Swain et al., 2007), which can be utilized as a carbohydrate source for bioethanol production.

2.3.1 Date as bioethanol feedstock

Dates are the most successful and important subsistence fruit in Saudi Arabia as well as in other arid and semiarid regions of the world (Besbes et al., 2004). The date fruit, composed of a fleshy pericarp and seed, is well known as a staple fruit in the Arab region. It is rich in several nutrients such as N, P, K, Ca, Mg etc. and has a high carbohydrate and fat content and is a vital source of sugar and dietary fibre (Al-Farsi et al., 2007). Currently there is little information relating to the production of bioethanol from dates and apples. Thus this was undertaken to investigate the production of bioethanol fuel from waste dates and apple fruit biomass.

2.4 Bioethanol production via fermentation

A number of biotechnological processes were employed in the production of bioethanol. Basic procedures involved are hydrolysis, fermentation and distillation. Hydrolysis converts the cellulosic materials of the biomass into sugar while microbial fermentation converts the sugar into alcohol (Balat et al., 2008). Yeasts are the most common microbial agents used for fermentation (Siqueira et al., 2008). Finally, bioethanol is recovered from the extracts through distillation. Fermentation process converts glucose ($C_6H_{12}O_6$) or sugar into alcohol ($C_2H_5OH$) and carbon dioxide ($CO_2$) with the help of microorganisms such as yeast. Theoretically, 0.51 kg of ethanol can be produced from 1 kg
of glucose while emitting 0.49 kg of CO$_2$ (Demirbas, 2008b). The simplified fermentation reaction equation for the carbon sugar, glucose, is:

$$\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CH}_3\text{CH}_2\text{OH} + 2\text{CO}_2 \quad \text{Eq. (1)}$$

Bioethanol can be produced using either free or immobilized cells. Using immobilized cells is advantageous over free cell due to enhanced yield, ease to separate cell mass from the bulk liquid, reduced risk of contamination, better operational stability and cell viability for several cycles of operations (Chandel et al., 2007; Nigam, 2000). Among the different immobilization technologies, entrapment of microbial cells within the polymeric matrices such as agar agar, calcium alginate, gelatin, k-carrageenan, etc. have been studied widely (Adinarayana et al., 2005; Kar and Ray, 2008). Two most suitable carriers for cell immobilization are entrapment in calcium alginate bead (Kar and Ray, 2008) and Agar cubes (Lark et al., 1997), because these techniques are simple, cost effective and nontoxic. Lin and Tanaka (2006) stated that nearly all of the ethanol fermentation technologies begin with removal of large or unsuitable materials, followed by mechanical processing to remove undesirable materials and contaminants. Hydrolysis breaks down the resultants to simpler compounds and depending on the technology, this may include high temperature, acid treatment and/or high pressure. Following the initial hydrolysis phase, the slurried material is then fermented to produce alcohol, which is then purified through distillation and/or filtration to produce the desired fuel-grade quality ethanol.
The advantages of immobilized cells over free cell systems have been extensively reported (Plessas et al., 2007). Cell immobilization can be more effective because cell washout in continuous operation is prevented, and, hence, cell separation and/or recycle are not required for maintaining high cell density in the bioreactor; thus, the bioprocesses can be operated more efficiently (Tzeng et al., 1991). Many researches concerned with immobilized cells have been carried out throughout the world. Particularly, there is an increasing interest in the practical applications of immobilized cells in ethanol production (Kobayashi and Nakamura, 2004) and considerable researches have been performed over the last 20 years into the use of immobilized cell systems for the production of fuel and potable grade ethanol (Bardi et al., 1996).

2.4.1 Yeast fermentation and enzyme hydrolysis

Yeasts are the most commonly used microorganisms for ethanol fermentation. Anaerobic cultivation of *Saccharomyces cerevisiae* generates, besides ethanol, carbon dioxide, glycerol and cell biomass as the most significant byproducts. Carbon dioxide is an inevitable fermentation product, but the off-gas can be sold as a high-quality raw material and is, therefore, more of a logistic problem. Glycerol can be produced as a compatible solute during osmotic stress (Brandberg et al., 2007).

Bai et al. (2008) critically reviewed some ethanol fermentation technologies from sugar and starch feedstocks, particularly those key aspects that have been neglected or misunderstood. Compared with *Saccharomyces cerevisiae*, the ethanol yield and productivity of *Zymomonas mobilis* are higher, because less biomass is produced and a higher metabolic rate of glucose is maintained through its special Entner-Doudoroff
pathway. However, due to its specific substrate spectrum as well as the undesirability of its biomass to be used as animal feed, this species cannot readily replace *S. cerevisiae* in ethanol production. The steady state kinetic models developed for continuous ethanol fermentations show some discrepancies, making them unsuitable for predicting and optimizing the industrial processes (Lin and Tanaka, 2006). The dynamic behavior of the continuous ethanol fermentation under high gravity or very high gravity conditions has been neglected, which needs to be addressed in order to further increase the final ethanol concentration and save the energy consumption. Ethanol is a typical primary metabolite whose production is tightly coupled with the growth of yeast cells, indicating yeast must be produced as a co-product (Sun and Cheng, 2002). Technically, the immobilization of yeast cells by supporting materials, particularly by gel entrapments, is not desirable for ethanol production, because not only is the growth of the yeast cells restrained, but also the slowly growing yeast cells are difficult to be removed from the systems (Bai *et al.*, 2008). Moreover, the additional cost from the consumption of the supporting materials, the potential contamination of some supporting materials to the quality of the co-product animal feed, and the difficulty in the microbial contamination control all make the immobilized yeast cells economically unacceptable (Lin and Tanaka, 2006). In contrast, the self-immobilization of yeast cells through their flocculation can effectively overcome these drawbacks.

A wide range of research can be found that have attempted to explore efficient fermentative organisms, low-cost fermentation substrates, and optimum environmental conditions for fermentation to occur. Cellulose-to-ethanol biotransformation can be conducted by various anaerobic thermophilic bacteria, such as *clostridium thermocellum*.
(Ingram and Doran, 1995), as well as by some filamentous fungi, including *Monilia* sp. (Saddler and Chan 1982), *Neurosporacrassa* (Gong *et al.*, 1981), *Neurospora* sp. (Yamauchi *et al.*, 1989), *Zygosaccharomyces rouxii* (Pastore *et al.*, 1994), *Aspergillus* sp. (Sugawara *et al.*, 1994) and *Paecilomyces* sp. (Gervais and Sarrette, 1990). However, studies on the fermentation process utilizing these microorganisms have shown this process to be very slow (3-12 days) with a poor yield (0.8-60 g/L of ethanol), which most probably is due to the low resistance of microorganisms to higher concentrations of ethyl alcohol. Another disadvantage of this process (particularly in the case of bacterial fermentation) is the production of various by-products, primarily acetic and lactic acids (Herrero and Gomez, 1980).

### 2.4.1.1 Enzymatic hydrolysis

Lin and Tanaka (2006) argued that though acid can be used for hydrolysis, but enzyme perform better for this purpose. There have been several reports about yeasts that could produce extracellular α-amylase and glucoamylase. These include *Candida tsukubaensis* CBS 6389 (Aktinson and Mavituna, 1991), *Filobasidium capsuligenum* (Aktinson and Mavituna, 1991), *Lipomyces kononenkoae* (de Mot and Verachtert, 1985), *Saccharomycopsis bispora* (formerly *Endomycopsis bispora*) (Kelly *et al.*, 1985), *Saccharomycopsis capsularis, Saccharomycopsis ibuligera* (Ebertova, 1966), *Schwanniomyces alluvius* (Gasperik *et al.* 1985), *Schwanniomyces castelli* (Simoes-Mendes, 1984) and *Trichosporon pullulans* (Sills *et al.*, 1984).

### 2.4.2 Batch fermentation

Gunasekaran and Raj (1999) revealed that traditionally, ethanol has been produced in batch fermentation with yeast strains that low tolerance to ethanol concentration. They argued that rather than other ethanogenic microbes (e.g. *Clostridium* sp.) the yeast
Saccharomyces cerevisiae and facultative bacterium Zymomonas mobilis are better candidates for industrial alcohol production. Despite the superiority of the latter over the former one, the study found several limitations of Z. mobilis such as its inability to convert complex carbohydrate polymers like cellulose, hemicellulose, and starch to ethanol; it’s resulting in byproducts such as sorbitol, acetoin, glycerol, and acetic acid; and formation of extracellular levan polymer. Amutha and Gunasekaran (2001) reported that the best strains for ethanol production from saccharified syrups were strains of Z. mobilis and S. diastaticus. Toran-Diaz et al. (1984) investigated the effect of acid-hydrolysed substrate and enzyme-hydrolysed substrate on ethanol production and obtained that ethanol productivity with Z. mobilis grown on Jerusalem artichoke juice was higher than that reported for the yeast Kluyveromyces marxianus by Duvnjak et al. (1981). Further, they observed that the juice of Jerusalem artichoke could be fermented without the addition of any nutrients.

Torres and Baratti (1987) reported that in batch fermentation, sugar concentrations as high as 223 g/L could be fermented to 105 g/L ethanol in 70 h. Results from Gunasekaran and Raj (1999) showed that adaptation of the cells to the higher concentration of sugars in cassava starch hydrolysate (CSH) could help to achieve maximal ethanol concentrations in relatively shorter period of time. With the culture adapted to the concentration of sugars, fermentation was completed in 28 h with a maximum concentration of 80.1 g/L ethanol. In contrast to this, a maximum concentration of alcohol of 78.5 g/L after 40 h of fermentation was obtained with the non-adapted culture.
2.4.3 Fermentation process

Liu and Shen (2008) suggested that are many factors that have influence upon the ethanol yield and fermentation rate in fermentation process, such as fermentation temperature, agitation rate, pH and particles stuffing rate that is defined as a ratio of immobilized yeast particles weight to fermentation solution weight. The immobilization process changes the environmental, physiological and morphological characteristics of cells, along with the catalytic activity (Prasad and Mishra, 1995). The ethanol yield increased from 75.79% to 89.89% while the fermentation temperature was increased from 28 °C to 37 °C (Prasad and Mishra, 1995). The highest yield of ethanol was 89.89% at a fermentation temperature of 37 °C (Prasad and Mishra, 1995). In some degree, ethanol formation is dependent on temperature, and an increase in temperature results in an increased concentration of total ethanol (Etievant, 1991; Mallouchos et al., 2003). In addition, the optimum temperature of free *S. cerevisiae* fermentation was always about 30 °C (Torija et al., 2003). The optimum temperature of immobilized *S. cerevisiae* ethanol fermentation was higher than that of free yeasts. This phenomenon may be due to the reason that the immobilized yeast in fermentation exists heat transfer process from the particle surface to its inside. The maximum yield of ethanol of 85.77% was obtained at pH 5.0 (Torija et al., 2003).

Najafpour et al. (2004) successfully carried out fermentation of sugar by *Saccharomyces cerevisiae*, for production of ethanol in an immobilized cell reactor (ICR) to improve the performance of the fermentation process. The fermentation set-up was comprised of a column packed with beads of immobilized cells. The immobilization of *S. cerevisiae* was simply performed by the enriched cells cultured media harvested at
exponential growth phase. The fixed cell loaded ICR was carried out at initial stage of operation and the cell was entrapped by calcium alginate. The production of ethanol was steady after 24 h of operation. The concentration of ethanol was affected by the media flow rates and residence time distribution from 2 to 7 h (Najafpour et al., 2004). In addition, batch fermentation was carried out with 50 g/L glucose concentration (Najafpour et al., 2004). Subsequently, the ethanol productions and the reactor productivities of batch fermentation and immobilized cells were compared. In batch fermentation, sugar consumption and ethanol production obtained were 99.6% and 12.5% v/v after 27 h while in the ICR, 88.2% and 16.7% v/v were obtained with 6 h retention time (Najafpour et al., 2004). Nearly 5% ethanol production was achieved with high glucose concentration (150 g/L) at 6 h retention time. A yield of 38% was obtained with 150 g/L glucose. The yield was improved approximately 27% on ICR and a 24 h fermentation time was reduced to 7 h (Najafpour et al., 2004). The cell growth rate was based on the Monod rate equation. The kinetic constants ($K_s$ and $\mu_{max}$) of batch fermentation were 2.3 g/L and 0.35 g/L h, respectively. The maximum yield of biomass on substrate and the maximum yield of product on substrate in batch fermentations were 50.8% and 31.2% respectively (Najafpour et al., 2004). Productivity of the ICR were 1.3, 2.3, and 2.8 g/L h for 25, 35, 50 g/L of glucose concentration, respectively (Najafpour et al., 2004). The productivity of ethanol in batch fermentation with 50 g/L glucose was calculated as 0.29 g/L h (Najafpour et al., 2004). Maximum production of ethanol in ICR when compared to batch reactor has shown to increase approximately 10-fold (Najafpour et al., 2004). The performance of the two reactors was compared and a respective rate model was proposed. The present research has shown that high sugar concentration (150 g/L) in the ICR column was successfully converted to ethanol. The achieved results in ICR with high substrate concentration are
promising for scale up operation. The proposed model can be used to design a larger scale ICR column for production of high ethanol concentration.

Tyagi and Ghose (1982) studied the rapid fermentation of cane molasses into ethanol in batch, continuous (free-cell and cell-immobilized systems) by a strain of *Saccharomyces cerevisiae* at temperature 30°C and pH 5.0. The maximum productivity of ethanol obtained in immobilized system was 28.6 g/L/h. The cells were immobilized by natural mode on a carrier of natural origin and retention of 0.132 g cells/g carrier was achieved. The immobilized-cell column was operated continuously at steady state over a period of 35 days. Based on the parameter data monitored from the system, mathematical analysis has been made and rate equations proposed, and the values of specific productivity of ethanol and specific growth rate for immobilized cells computed. It has been established that immobilized cells exhibit higher specific rate of ethanol formation compared to free cells but the specific growth rate appears to be comparatively low. The yield of ethanol in the immobilized-cell system is also higher than in the free-cell system.

### 2.4.4 Pretreatment

For fuel ethanol production, pretreatment has been studied as a key step for the effective utilization of lignocellulosic biomass feedstock, due to its recalcitrant nature. Part of the effect of pretreatments is the removal of lignin, a constituent that is known to inhibit saccharification enzymes and fermentative microorganisms (Chang and Holtzapple, 2000). The barley hull is also quite abrasive on processing equipment and makes up a considerable amount of a hulled barley kernel, up to 10–15% of the grain weight. A pretreatment that can reduce the rigidity of this material is therefore desired. Among them, the soaking in
aqueous ammonia (SAA) at low temperature retains the hemicellulose in the solids by minimizing the interaction with hemicelluloses during treatment, which was reported as a feasible approach to increase the fermentation yield and simplify the bioconversion scheme (Kim and Lee, 2007, Kim et al., 2009). Ammonia seems to be a pretreatment reagent with many advantages for an effective delignification as well as swelling of biomass. Furthermore, the retained xylan can usually be hydrolyzed to fermentable pentoses by most commercial cellulase and xylanase mixtures (Kim and Lee, 2005).

2.4.4.1 The pretreatment process

The process of pretreatment has been described earlier (Schell et al., 2007). In summary, the continuous pretreatment system consists of acid and lime (for acid neutralization) supply tanks; a biomass mixer; a high-temperature, high-pressure reactor system; and a flash tank. The pretreatment reactor system is a vertical pulp digestor supplied by SundsDefibrator, Inc. (now Metso Paper USA, Inc. Norcross, GA, USA) and includes the reactor and material feed (plug feeder) and discharge (reciprocating popet values, not shown) systems. The acid and lime delivery systems consist of two fiberglass-reinforced plastic tanks for each system (feeding from one tank at a time) and associated pumps. Acid is diluted to 5–10% (w/w) in the acid tank and lime is mixed with water to approximately 25% (w/w) in the lime tank and continually circulated by a centrifugal pump to prevent settling of lime particulates. Feedstock from the belt conveyor enters a pug mill mixer and is mixed with dilute acid and water. Water is added as needed to adjust the solids concentration in the pretreatment reactor. The wetted feedstock is screw conveyed to a plug feeder that compresses the material into an impermeable plug that is then forced into the pretreatment reactor. Liquid expressed from the material by the plug feeder is pumped
into the pretreatment reactor. The feedstock enters through the side of the reactor and is conveyed to the top by twin screws overflowing a weir and entering the main reactor body. There is no mechanical mixing (e.g., agitator in the reactor) and the material moves by gravity flow to the discharge port at the bottom of the reactor and is directed into the flash tank. Since the consistency of the material is like ‘‘damp sawdust’’, no mixing occurs and hydrolysis of the starch and hemicellulose components are unlikely to reduce the consistency enough at the high solids concentration to promote mixing. A rotating scraper at the bottom of the reactor facilitates movement of material to the discharge port. The reactor is heated by steam to achieve the desired temperature and residence times from 3 to 20 min are achieved by controlling material level in the reactor. The flash tank, which receives the hot pretreated slurry, is a conical screw mixer also used to blend the lime slurry with the pretreated feedstock. Vapor from the flashing mixture exits the top of the tank and is sent to a condenser, while the remaining non-condensable fraction is sent to a scrubber. Pretreated feedstock then exits the bottom of the flash tank and is pumped to the first 9000-l fermentor.

2.5 Use of bioethanol in energy generation

Recently, bioethanol as a fuel is gaining attention around the world in the hardship of price hike and environmental concerns. Governments are announcing commitments in view of bioethanol based fuel usage. International commitments to reduce greenhouse gas emission have also propelled the issue a bit further. The largest programmes in this regard are promoted by the governments of USA, Brazil and a few EU countries and recently US has aimed to increase the usage of bio energy three fold in the next ten years (Demirbas and Balat, 2007; Demirbas, 2008b). Statistics show that global production of bioethanol production has increased considerable in the running decade. Global bio ethanol production
has increased from about 5 billion gallons in 2000 to nearly 18 billion gallons in 2009 (Balat et al., 2008). The World’s Ethanol Production Forecast 2008 – 2012 projected that this production trend will reach about 22.5 billion by 2012. At current situation, US is the world’s biggest producer of bioethanol fuel which shares about 47 % of the global production while Brazil is the world’s largest bioethanol exporter and second largest producer (REN21, 2007). According to Greenenergy International Ltd. (2007), 40 % of Brazil’s traditional petroleum fuel is replaced by bioethanol. However, other large economies of the world such as EU, China, and India along with other advanced developing countries are still to participate in the game. Nevertheless, in view of the emerging developments in international climate talks pushing countries in pursuing renewable energy policies have widely driven the prospects of bioethanol a step forward.

Kalam and Masjuki (2002) concluded that there are significant benefits in diverting excess bagasse to ethanol production as opposed to the current practice of open-field burning. Scenario 2 leads to a decrease in carbon monoxide, hydrocarbons, SO$_x$, NO$_x$, particulates, carbon dioxide, methane and fossil fuel consumption. Chemical oxygen demand (from ethanol raw material production) is significantly higher. Non-methane hydrocarbons are from ethanol production. Lime, ammonia & sulphuric acid occur only in Scenario 2. Electricity credits result in negative CO$_2$ and CH$_4$ emissions and lower solid waste. Kaltschmitt et al. (1997) shows some clear ecological advantages of bioethanol over fossil fuels, such as conserving fossil energy sources and reducing global warming potential, but bioethanol also has some definite disadvantages; in particular N$_2$O show no discernible change.
Behera et al. (2010) also voiced the growing need and attempt to look for new, clean and cheap sources for bioethanol. Both first and second generation bioethanol are renewable energy sources. The use of crop residues and other biomass for bio-fuels, however, also raised concerns about environmental problems - serious destruction of vital soil resources (Pimentel, 2003). Preliminary research using residual and waste biomass materials as lignocellulosic feedstocks for ethanol production has shown great promise to date. Further research in this area will result in the development of an innovative waste management approach that uses agricultural, municipal and industrial residues and waste materials as a renewable resource for the extraction of a delignified biomass, and its conversion to bioethanol. Despite the large potential that residual and waste biomass can offer to meet Canada’s future energy needs, there are significant hurdles that must be overcome before the largescale use of residual and waste biomass as an energy resource becomes economically and technologically viable. Further research is critical to investigate its application beyond the laboratory-scale and to develop the necessary biotechnologies (Champagne, 2007).

While considering efficiency of the feed stocks, Gnansounou et al. (2005) focused on several issues such as chemical composition of the biomass, cultivation practices, availability of land and land use practices, use of resources, energy balance, emission of greenhouse gases, acidifying gases and ozone depletion gases, absorption of minerals to water and soil, injection of pesticides, soil erosion, contribution to biodiversity and landscape value losses, farm-gate price of the biomass, logistic cost (transport and storage of the biomass), direct economic value of the feedstocks taking into account the coproducts, creation or maintain of employment, and water requirements and water availability.
2.5.1 Ethanol blend

Kim and Dale (2004) estimated that the potential for ethanol production is equivalent to about 32 per cent of the total gasoline consumption worldwide, when used in E85 (85 per cent ethanol in gasoline) for a mid-size passenger vehicle. Such a substitution immediately addresses the issue of reducing our use of non-renewable resources (fossil fuels) and the attendant impacts on climate change, especially carbon dioxide and the resulting greenhouse effect, but it does not always address the notion of overall improvement. For instance, it is well understood that the conversion of biomass to bio-energy requires additional energy inputs, most often provided in some form of fossil fuel. The life cycle energy balance of a bio-fuel compared to conventional fossil fuel should be positive, but depending on the processing choices, the cumulative fossil energy demand might, at times, only be marginally lower or even higher than that of liquid fossil fuels (von Blottnitz et al., 2002; Pimentel, 2003). Also, ethanol in gasoline may result in decreased urban air quality, and be associated with substantive risks to water resources and biodiversity (Niven, 2005). Ethanol-blended gasolines have the potential to contribute significantly to these emissions reductions. Ethanol is an alternative fuel derived from biologically renewable resources and can be employed to replace octane enhancers such as methylcyclopentadienyl manganese tricarbonyl (MMT) and aromatic hydrocarbons such as benzene or oxygenates such as methyl tertiary butyl ether (MTBE).

Ethanol can be used directly as a fuel, but most often it is blended with gasoline to yield gasohol (Staniszewski et al., 2007). The Brazilian National Bio-Fuel Program, initiated in 1975, stimulated the substitution of gasoline for sugarcane alcohol for
automobile use, and intensified the use of a mixture of ethanol and gasoline as fuel for common cars (Soccol et al., 2005). Anhydrous ethanol is added to gasoline at a 20–26% proportion in volume (Cortez et al., 2003). Today, about 3 million automobiles run on 100% alcohol, and about 60% of all new motor vehicles produced in Brazil are “flex”, i.e. they can run on any mixture of alcohol/gasoline, as well as on 100% alcohol (Grad, 2006). A worldwide interest in the utilization of bioethanol as energy source has stimulated studies on the cost and efficiency of industrial processes for ethanol production. Intense research has been carried out for obtaining efficient fermentative organisms, low cost fermentation substrates, and optimal environmental conditions for fermentation to occur (Cysewski and Wilke, 1978).

2.5.2 Engine emission

With increasing gap between the energy requirement of the industrialized world and inability to replenish such needs from the limited sources of energy like fossil fuels, increasing levels of greenhouse pollution from the combustion of fossil fuels in turn aggravate the perils of global warming and energy crisis (Mohan et al., 2008). Motor vehicles account for a significant portion of urban air pollution in much of the developing world. According to Goldemberg (2008), motor vehicles account for more than 70% of global carbon monoxide (CO) emissions and 19% of global carbon dioxide (CO₂) emissions. CO₂ emissions from a gallon of gasoline are about 8 kg. There are 700 million light duty vehicles, automobiles, light trucks, SUVs and minivans, on roadways around the world. These numbers are projected to increase to 1.3 billion by 2030, and to over 2 billion vehicles by 2050, with most of the increase coming in developing countries (Hansen, 2004). This growth will affect the stability of ecosystems and global climate as well as
global oil reserves. The world’s total proven oil, natural gas and coal reserves are respectively, 168.6 billion tons, 177.4 trillion cubic meters, and 847.5 billion tons by the end of 2007, according to the recently released 2008 BP Statistical Review of World Energy (British Petroleum Company, 2008). With current consumption trends, the reserves-to-production (R/P) ratio of world proven reserves of oil is lower than that of world proven reserves of natural gas and coal — 41.6 years versus 60.3 and 133 years (British Petroleum Company, 2008), respectively. In 2007, world oil production was 3.90 billion tons, a decrease of 0.2% from the previous year (British Petroleum Company, 2008). According to International Energy Agency statistics (International Energy Agency IEA, 2008), the transportation sector accounts for about 60% of the world’s total oil consumption. Interest in the use of bio-fuels worldwide has grown strongly in recent years due to the limited oil reserves, concerns about climate change from greenhouse gas emissions and the desire to promote domestic rural economies.

2.5.3 Bioethanol for electricity production

The term bio-fuels can refer to fuels for direct combustion for electricity production, but is generally used for liquid fuels in transportation sector (Balat, 2007). The use of bio-fuels can contribute to the mitigation of greenhouse gas emissions, provide a clean and therefore sustainable energy source, and increase the agricultural income for rural poor in developing countries. Today, bio-fuels are predominantly produced from biomass resources. Biomass appears to be an attractive feedstock for three main reasons (Cadenas and Cabezudo, 1998; Hammond et al., 2009): (1) it is a renewable resource that could be sustainably developed in the future, (2) it appears to have formidably positive environmental properties resulting in no net releases of carbon dioxide and very low sulfur
content, and (3) it appears to have significant economic potential provided that fossil fuel prices increase in the future. Bio-fuels are liquid or gaseous fuels made from plant matter and residues, such as agricultural crops, municipal wastes and agricultural and forestry by-products.

Liquid bio-fuels can be used as an alternative fuel for transport, as can other alternatives such as liquid natural gas (LNG), compressed natural gas (CNG), liquefied petroleum gas (LPG) and hydrogen. Bio-fuels could significantly reduce the emissions from the road-transport sector if they were widely adopted. They have been shown to reduce carbon emissions, and may help to increase energy security. There are many different types of bio-fuels, which are produced from various crops and via different processes. Bio-fuels can be classified broadly as bio-diesel and bioethanol, and then subdivided into conventional or advanced fuels (Hammond et al., 2009). This paper summarizes policy and regulatory drivers for bioethanol fuel in the major producing countries, describes usage trends and projections, development of biomass feedstocks, and improved conversion technologies.

2.6 Environmental implication of bioethanol

Bio-based systems have several possible ecological drawbacks. Agricultural production of biomass is relatively land intensive, and there is a risk of pollutants entering water sources from fertilisers and pesticides that are applied to the land to enhance plant growth. One focused on ethanol alone and presents generally unfavourable recommendations (Niven, 2005). The other review looked at biofuels more generally and presented more favourable result for ethanol but cautioned with respect to some of its environmental impacts (Quirin, 2004).

<table>
<thead>
<tr>
<th>Annual CO₂ emission (%)</th>
<th>Percentage of global (100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>23.30</td>
</tr>
<tr>
<td>USA</td>
<td>19.91</td>
</tr>
<tr>
<td>India</td>
<td>5.5</td>
</tr>
<tr>
<td>Russia</td>
<td>5</td>
</tr>
<tr>
<td>Japan</td>
<td>4.28</td>
</tr>
<tr>
<td>Germany</td>
<td>2.69</td>
</tr>
<tr>
<td>Canada</td>
<td>1.9</td>
</tr>
<tr>
<td>UK</td>
<td>1.84</td>
</tr>
<tr>
<td>Australia</td>
<td>1.28</td>
</tr>
<tr>
<td>Malaysia</td>
<td>1.0</td>
</tr>
<tr>
<td>Lebanon</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Figure 2.2: Air toxic emission from on road mobile source.

It must be noted that a number of studies that looked specifically at the North American corn-to-ethanol route, were very critical as to its environmental sustainability (Pimentel 2003; Patzek, 2004). Whilst the issue of sustainability is complicated, one that encompasses human and environmental health as well as societal needs, it is clear that our
efforts to identify solutions should be broad in scope to avoid shifting problems from one place to another (Curran, 2004). Whilst this type of analysis is often inspired by the controversial results of Pimentel on ethanol from corn in the United States (Pimentel, 2001), the bulk of the studies report moderate to strong fossil fuel substitution effects for bioethanol systems. It must be noted that no additional land is needed when by-products (e.g., molasses) or lignocellulosic residue are used as feedstock for fermentation. For ethanol made from a waste product taken to carry no environmental burden, a fossil energy replacement can also be determined on a per hectare basis. Results will differ on a case-by-case basis, depending on how efficiently wastes and by-products are already used, and how the industrial systems are configured. For ethanol from lignocellulosic feedstocks, the contribution to fossil energy replacement is of a similar magnitude to that of the starch crops. With scientific evidence now increasingly mounting that climate is changing, and that this can be attributed to the large-scale use of fossil fuels, the potential of bio-fuels to deliver transportation energy in a carbon-neutral way is receiving increasing attention.

Thus, renewable clean energy and bio-resources fuel are required to be used together with fossil fuel to reduce CO, NO and CO₂ emissions (Costa and Sodre, 2010). Another prominent and related issue is the energy crisis and the continuous increase of global petroleum prices which had a great impact on the transportation and electricity costs worldwide. In order to solve these issues, renewable energy should be introduced and developed as new feedstock.