The society we are living in is facing hike in oil prices and increased global warming threats because of intensive use of conventional fossil fuels such as coal and crude oil. In developing countries, 30% of the energy demand is from the transportation sector and 90% of which depends upon fossil fuels (U.S. Energy Information Administration, 2011). The emphasis on the research of alternative and sustainable fuel resources has gained importance due to presumed fear of fossil fuels shortage and the resultant environmental threats from them, particularly in terms of CO₂ emissions (Joshi et al., 2016, Singh et al., 2012). Biofuels have been advocated as a promising alternative to the use of fossil resources in the rapidly growing transportation fuels sector and can be defined as fuels produced from renewable resources such as plant and woody biomass (The European Parliament and the Council of the European Union, 2012, Energy Independence and Security Act, 2007, Hertel et al., 2010, Joshi et al., 2016). In the United States, the Energy Independence and Security Act of 2007 shows the consumption of 35 billion gallons of ethanol-equivalent biofuels by 2022 (Energy Independence and Security Act, 2007). Ethanol is currently being blended with gasoline at 10% (E10) and 85% (E85). Car manufacturers are producing more engines able to run off higher ethanol concentrations. There are many ways to produce ethanol. Bioethanol production from lignocellulosic biomass by using synthesis gas fermentation is a relatively new technology. This technology combines gasification of nearly all the components of biomass into synthesis gas (also called syngas) which mainly consists of (CO, H₂ and CO₂) and fermentation of syngas components into acetic acid, ethanol and other valuable metabolites using homo-acetogenic organisms (Daniell et al., 2012). However, the two separate second generation fuel production technologies are the, a) Fischer-Tropsch (FT) process, where a metal catalyst is used to convert synthesis gas to liquid fuels, and b) biochemical approach where lignocellulosic biomass components are either directly or after initial pretreatment are fermented to liquid fuels (Daniell et al., 2012). These above discussed two approaches are currently the focus of research on second
Introduction

generation biofuel research and development; in Europe the majority of fuel plants utilizes the biochemical approach, while in North America both of these approaches are followed (Gnansounou and Dauriat, 2010). Gas fermentation technology utilizes strategies from both approaches and hence can be considered as a hybrid (Datta et al., 2011, Wilkins and Atiyeh, 2011). Syngas fermentation process have several advantages over chemical catalytic process such as, higher substrate specificity of biocatalysts, lower energy costs, greater resistance to catalyst poisoning and the lack of requirement for a fixed CO:H₂ ratio (Bredwell et al., 1999). However, due to its high available fuel energy value relative to the fossil energy input, ethanol obtained using a cellulosic feedstock appears to be most applicable from an energy standpoint (Wang, 2005). With regards to syngas fermentation, biomass (grasses, woods, agricultural residues, etc.) can be gasified to yield a “syngas” made up of carbon monoxide (CO), carbon dioxide (CO₂), and hydrogen (H₂), with small amounts of impurities (Daniell et al., 2012). The overall stoichiometry for the fermentation of syngas to form ethanol is:

\[
\begin{align*}
6\text{CO} & \quad + \quad 3\text{H}_2\text{O} \quad \rightarrow \quad \text{C}_2\text{H}_5\text{OH} \quad + \quad 4\text{CO}_2 \\
2\text{CO}_2 & \quad + \quad 6\text{H}_2 \quad \rightarrow \quad \text{C}_2\text{H}_5\text{OH} \quad + \quad 3\text{H}_2\text{O}
\end{align*}
\]

One of the main advantages of syngas fermentation is the wide variety of raw materials that can be utilized as a feedstock; such as prairie grasses, wood chips, solid municipal wastes and paper wastes. This process is also well-suited to raw materials such as softwoods that are normally difficult to handle (Dayton and Spath 2003). A second main advantage is that gasification can break down cellulosic, hemicellulosic and lignin bonds that are difficult to break down using fermentative or enzymatic reactions. This provides a greater conversion efficiency of biomass to energy (McKendry 2002). Clostridium ljungdahlii, Butyribacterium methylotrophicum and Clostridium autoethanogenum (Gaddy and Clausen, 1992, Abrini et al. 1994, Bredwell et al. 1999) are known examples of bacteria that have been shown to produce ethanol from syngas. Bioethanol production through acetogenic fermentation of the gaseous substrates (CO, CO₂ and H₂) follows the Wood-Ljungdahl and acetyl-CoA pathways. Clostridium carboxidivorans (means carbon dioxide devouring), a bacteria isolated from an agricultural settling lagoon in Stillwater, Oklahoma, has
been found to produce higher than average rates of ethanol and butanol from CO-rich mixtures (Liou et al., 2005). Syngas fermentation for ethanol production based on the acetyl-CoA metabolic pathway is an emerging area and the process require significant research interventions.

The present study was focused on developing anaerobic mixed bacterial culture that have the capability to utilize synthesis gas for ethanol and acetic acid production. Four different types of media including (ATCC 1754 PETC, DSMZ 640, Saxena, 2008 and PBM) were tested for maximum metabolites production using most efficient syngas fermenting microbial consortium TERI SA1. Further, two approaches were simultaneously used for isolation and phylogenetic identification of most efficient syngas fermenting anaerobic consortium TERI SA1 involving 16S rRNA sequencing of culturable bacterial isolates as well as meta-genomic approach (by making a 16S rRNA gene library of total community DNA). Alongwith, comparative study between most efficient syngas fermenting mixed consortium TERI SA1 and individual bacterial isolates ASH051 and ASH052 for analyzing maximum metabolites production efficiency was also performed. Different physiological and operational parameters such as pH, temperature, syngas pressure and agitation speed were optimized for enhanced metabolites production from syngas fermentation. After that fed-batch fermentation study under optimized parameter conditions was also carried out to enhance the final metabolites concentration. In addition, optimization study was carried out in order to investigate the effect of three nitrogen sources on metabolite formation from synthesis gas bioconversion: (yeast extract (0.0–2.0 g/L), ammonium chloride (0.0–1.5 g/L) and corn steep liquor (0.0–10 g/L). Finally, a 10 L horizontal prototype with a working volume of 7.5 L was designed specifically for syngas bioconversion, to further enhance ethanol and acetic acid production. The reactor was designed horizontally to increase the contact area between the syngas components (CO and H2) and the liquid medium inside the reactor. From these bases, it will be possible to design an efficient 100 L prototype for further process scale-up and pilot scale fermentation studies in pilot scale reactor.

1.1 Synthesis gas and its application

(Syn)thesis gas is a mixture of carbon monoxide (CO), carbon dioxide (CO2), hydrogen (H2) and other trace gases produced by partial combustion of carbonaceous
materials including lignocellulosic biomass, organic waste, coal, petroleum, plastics, reformed natural gas and other carbon containing materials via gasification process (Dayton and Spath, 2003, Xu et al., 2011). Gasification process involves heating the material at high temperature (>700 °C), without combustion with a controlled amount of oxygen and or steam (Figure 1.1). The resulting gas mixture is called syngas (from synthetic or synthesis gas) or producer gas and is itself a fuel (McKendry, 2002, Kirkels and Verbong, 2011, Matsumura et al., 2005). Syngas mixture mainly depends on the process of gasification and its design, feedstock and their moisture content (McKendry, 2002). After gasification, the syngas mixture is passed through a series of filters to remove unwanted pollutants such as tar and solid particles (McKendry, 2002, Munasinghe and Khanal, 2010b). Anaerobic microbes such as Clostridium ljungdahlii, Clostridium autoethanogenenum, Clostridium carboxydvorans, Clostridium ragasdalei, Eubacterium limosum etc. are well capable to utilize syngas as a carbon and energy source for alcohols (ethanol or butanol) and organic acids (acetate or butyrate) production (Figure 1.1, Kundiyana et al., 2011, Abrini et al., 1994, Younesi et al., 2005, Rajagopalan et al., 2002).

Figure 1.1: Biomass gasification into syngas and syngas bioconversion to ethanol (http://www.rsc.org/chemistryworld/Issues/2009/April/Thebiofuelfuture.asp)
1.1.1 Advantages of using Syngas

World energy consumption of unsustainable oil, coal and natural gas is estimated to increase up to 44% in coming 20 years (Acevedo et al., 2010). Syngas from biomass feedstock has been identified as a sustainable alternative for growing energy demands and has several advantages such as a higher availability of biomass, no competition with food source and low feedstock cost (Cotter et al., 2009b). Fuel grade ethanol and other valuable platform chemicals production have remarkable growth in the fuel industry by an increasing demand for renewable sources (Coskata, 2011, Kuo, 2011). Production of liquid biofuel from syngas is an emerging concept that can utilize a wide variety of biomass. Main advantages of the conversion of syngas to biofuels are;

a) The production of beneficial product from waste materials which need to be discarded in landfills or oceans.

b) Generation of syngas from plant waste biomass gasification is another promising concept, means that any carbon-based material can be gasified to produce syngas (Filippis et al., 2004).

c) Carbon monoxide and carbon dioxide is released during the conversion of biomass to syngas. Approximately all the carbon in the biomass including the lignin content is converted to syngas (Henstra et al., 2007).

d) This makes the syngas conversion process an efficient energy producer and an environmentally friendly concept for the recycling of waste biomass.

1.2 Different methods of syngas bioconversion

Thermochemical conversion transforms the lignocellulosic feedstock into mixture of carbon monoxide, hydrogen and carbon dioxide (also called syngas) by partial combustion of biomass (McKendry, 2002, Kirkels and Verbong, 2011, Matsumura et al., 2005). These gases can be converted to liquid transportation fuels or chemicals by either chemical catalytic (Figure 1.2, Fischer Tropsch) or microbial method (Griffin and Schultz, 2012).
1.2.1 Chemical catalytic method

Fischer Tropsch (FT) method is employed to convert syngas components (CO, CO₂ and H₂) by using metal catalyst (Dry, 2002). Syngas can be introduced to a catalytic reactor where carbon monoxide and water are combined via a metal catalyzed process to produce ethanol, higher alcohols and liquid fuels (Tijmensen et al., 2002). FT reaction generally produces different length of hydrocarbons, which are an alternative method for the production of conventional diesel, kerosene and gasoline (Dry, 2002, Mirwald and Inderwildi, 2012). FT synthesis includes cobalt, copper, ferrous, aluminum, zinc, molybdenum, nickel, rubidium and ruthenium as metal catalyst (Dry, 2002).

1.2.2 Drawbacks and challenges of chemical catalytic method

Major pitfalls in FT synthesis are the high cost of catalysts, fixed H₂/CO ratio, catalyst poisoning due to toxic gasses, sulphur contaminants together with high operating parameters like temperature and pressure (Griffin and Schultz, 2012, Van steen and Claeys, 2008, Dry, 2002, Datta et al., 2011).
Drawbacks

a) Sulphur contaminants found in the synthesis gas, primarily hydrogen sulphide (H₂S) and carbonyl sulphide (COS) are potent catalyst poisons. These gases must be removed in energy intensive purification steps that add significantly to the product cost (Van steen and Claeys, 2008).

b) Catalytic processing of synthesis gas often requires strict CO/H₂ ratios to maintain a particular product mix, necessitating gas recompression and shift reaction conversion operations (Dry, 2002).

c) The gas-phase reactors operate at high temperature, pressure and operate under extreme condition thus increasing reactor cost as well as potential safety issues (Datta et al., 2011).

d) In addition to these, the product specificity of the catalysts is often poor, resulting in a broad product spectrum (Datta et al., 2011).

Challenges

a) Low cost controlled preparation of catalysts in a commercially reproducible manner.

b) Efficient dispersion of nanometric catalysts in the slurry reactor processing.

c) Unpredictable behavior of catalysts during their life cycle.

d) Cost and quality of the effective separation of the wax-catalyst by-product mixture.

1.2.3 Why Microbial Method

The bioconversion of synthesis gas into biofuels by microbial means is a promising option for biofuels production (Munasinghe and Khanal, 2010b). The syngas fermentation into ethanol and other platform chemicals has been considerably preferred due to several benefits over the biochemical and FT process (Abubackar et al., 2011, Griffin and Schultz, 2012, Klasson et al., 1992, Hasler and Nussbaumer, 1999), such as;
Introduction

a) Lignin is also get breakdown and utilized alongwith cellulose and hemicellulose;

b) Not required complex pretreatment steps and costly enzymes as in cellulosic enzymatic fermentation;

c) Higher specificity of the microorganisms;

d) Not affected from H₂:CO ratio for bioconversion;

e) Operation of syngas fermentation is aseptic due to generation of syngas through higher gasification process performed at higher temperature;

f) Bioreactor can be operated at ambient conditions; and

g) No risk of metal catalyst poisoning.

1.3 Current scenario about demand and availability of transport fuels

According to International Energy Agency the transportation sector accounts for approximately 60% of the world’s total oil consumption (IEA, 2008). The world’s total demand of oil, natural gas and coal reserves were 168.6 billion tons, 177.4 trillion cubic meters, and 847.5 billion tons respectively by the end of 2007, according to the recently released 2008 BP (British Petroleum Company) Statistical Review of World Energy (BPC, 2008). In 2007, world oil production was 3.90 billion tons, a decrease of 0.2% from the year 2006 (BPC, 2008). South America and Brazil already have policies that mandate at least 22% bio-ethanol on motor fuels and encourage the use of vehicles that use hydrous bio-ethanol (Balat and Balat, 2009). Biofuels have gained considerable attention because of the relative abundance of feedstock in all regions, their easy utilization in combustion engines and compatibility with existing fuel distribution infrastructure (Purcell, 2011). They can also provide a new end market for agricultural commodities, thereby revitalizing rural areas. The first significant large-scale momentum for the production and use of biofuels occurred in Brazil and the United States, as a response to the 1973 oil export embargo imposed by the Arab members of OPEC (Organization of the Petroleum Exporting Countries) against Japan, the United States and Western European countries (Walter, 2006). Currently biofuels are once again the centre of attention for the debate on energy,
Introduction

partially in response to those circumstances that occurred more than 30 years ago, namely high oil prices and oil supply insecurity (Naikwade et al., 2012). In addition to that a strong global consensus currently advocates for reductions in GHG (greenhouse gas) emissions as a crucial step to combat rising global temperatures. Government agencies seeking to control emissions of harmful gases are now promoting biofuels because of their potentially cleaner emissions profile as compared to fossil fuels (Tyner and Taheripour, 2007).

1.3.1 First and second generation transport fuel

First-generation biofuels refers to the fuel produced from food crops such as sugar cane, soya bean and other food-based feedstock (Naik et al., 2010) (Figure 1.3). These biofuels at present are in considerable amount in the market and their production technologies, in spite of ongoing improvements is well established. The most important biofuels of the 1st-generation are bioethanol, biodiesel, and biogas (Elmekaway et al., 2013).

Second-generation fuels are derived from non-edible lignocellulosic biomass (LC) (Nath et al., 2012, Naik et al., 2010). Lignocellulosic biomass is either residues of forest management or food crop production (e.g. corn stalks or rice husks) or whole plant biomass (e.g. grasses or trees grown specifically for biofuel purposes) (Figure 1.3). These, feedstocks also called as cellulosic biomass, are a complex composite material significantly consisting of cellulose, hemicellulose and lignin bound to each other in the plant cell wall (Yang and Wyman, 2006, Pan et al., 2006). The lignocellulosic biomass such as agricultural residues (e.g. corn stover, wheat and barley straws), agri-processing byproducts (e.g. corn fiber, sugarcane bagasse, etc.), and energy crops (e.g. switch grass, poplar, red grass etc.) do not compete with food and feed, and is considered to be renewable feedstock for ethanol production (Nath et al., 2012, Elmekaway et al., 2014, Mussatto and Teixeira, 2010). There is major interest in moving from the current first generation of liquid biofuels to the second generation bio-fuels. Compared to the feedstock for first generation biofuels, lignocellulosic biomass is generally not edible and therefore does not compete directly with food production (Elmekaway et al., 2014, Mussatto and Teixeira, 2010). This can be bred specifically for energy purposes and includes more of the plant waste material, thereby in this way further increasing land use efficiency. These features of
Introduction

Lignocellulosic biomass help in substantial energy requirements and environmental benefits for second-generation biofuels production over the first-generation biofuels (Elmekaway et al., 2014, Mussatto and Teixeira, 2010). As an alternative to the enzymatic or biochemical approach to manufacture cellulosic ethanol, pyrolytic methods could solve some of the logistical and processing problems associated with commercializing non-food ethanol (Daniell et al., 2012).

Figure 1.3: Diagrammatic representation of first and second generation biofuels (http://allaboutbiofuels.wix.com/biofuels)

1.3.2 Need of Generating Second Generation Liquid fuels

Current global concern about fossil fuel prices and availability, have increased the interest of many developed and developing countries for energy independence and extensive awareness for the need to reduce greenhouse gas (GHG) emissions (Bryner and Scott, 2006, Balat and Balat, 2009). India is the second largest producer of sugar as the country’s agricultural and rural economy is mainly dependent on the sugarcane farming and related industries (DSDG, 2005, Smith, 2008). The hard currency savings benefit from the use of fuel will be substantial even if one considers blending it with petrol at the rate of 5% or 10% (Balat and Balat, 2009, Gnansounou et al., 2005). Ethanol is mainly produced from the sugarcane molasses and also from variety of renewable agricultural feedstock including grain such as corn, wheat, sorghum, rice and millets (Balat and Balat, 2009, DSDG, 2005, Wang, 2002). The use of ethanol for application such as blending with petrol, production of downstream industrial
chemicals and beverage purpose will offer great feasibility, stability and diversity in the agricultural and energy sector (Balat and Balat, 2009, Gnansounou et al., 2005). Bioethanol outbreaks the seven major national issues: sustainability, global climate change, biodegradability, urban air pollution, carbon sequestration, national security and the farm economy (Balat and Balat, 2009, Gnansounou et al., 2005). Lignocellulosic biomass is proposed to provide a significant portion of the raw materials for biofuels production due to low cost and high availability (Pandey et al., 2015, Balat and Balat, 2009). The fuel prizes are expected to remain high in the coming years due to increasing demand of alcohol. It has become difficult for liquor manufacturing units to purchase good quality alcohol from the open market and run them economically (Dhanuraj and Kumar, 2014). Other well established route to manufacture portable alcohol and fuel ethanol is by using grains as feedstock (Kline et al., 2008). However, considering the increase in demand as a food source and its rising price, the availability and feasibility of using food grain as a feedstock is doubtful. Further expansion of alcohol production from many of these feedstock’s, thus triggers debate on food/feed versus fuel, limiting the use of first generation feed stock for fuel ethanol production (Seelke and Yacobucci, 2007). Thus, for sustainable fuel grade ethanol production, non food feedstock such as lignocellulosic raw material should be used (Singh et al., 2012). Therefore, it shows the urgency for development of second generation biofuel.

1.3.3 Next generation transport fuel

Next generation biofuel production includes the thermo-chemical conversion of biomass to syngas which is further fermented to bioethanol (Figure 1.5, Katiyar et al., 2015, Naik et al., 2010, Munasinghe and Khanal, 2010b). Lignocellulosic biomass feed stocks from agriculture, forests or municipal waste, with the help of partial oxidation is converted to syngas which after removal of contaminants can be readily used as feedstock for microbes (Katiyar et al., 2015, Balat and Balat, 2009). Syngas is a mixture of CO, CO₂, H₂ and other gases. The conversion of syngas to biofuel is followed by two methods; catalytic method and microbial fermentation (Daniell et al., 2012). Break down of lignin content into smaller carbonaceous compound in gasification process (Figure 1.3.3) has a great significance for next generation transport biofuel production (Joshua, 2007, Elmekaway et al., 2013). Hydrogen is a
versatile fuel and can be used for power generation as well as transport fuel. It is considered as clean fuel since water is only the byproduct (U.S Department of Energy, 2010, Liu et al., 2008). Hydrogen can also be produced by biological systems which are environmentally friendly and requires less input than chemical processes (Mohan, 2010, Das and Veziroglu, 2001, Liu et al., 2008) (Figure 1.3.3). Anaerobic bacteria can utilize syngas through specific pathways producing different biofuels and chemicals (Cotter et al., 2009a).

Figure 1.4: Next generation biofuels production from lignocellulosic biomass (http://news.mongabay.com/bioenergy/2007/10/quick-look-at-fourth-generation.html)

1.4 Ethanol as an alternative Fuel

Alternative fuel can be defined as the fuels synthesized from resources other than petroleum. Some are produced domestically, reducing our dependence on imported oil, and some are generated from renewable sources (http://www.fueleconomy.gov/feg/current.shtml, Balat and Balat, 2009). Often, they produce less pollution than gasoline or diesel. Ethanol is produced domestically from corn and other crops and produces less greenhouse gas emissions than conventional fuels (http://www.fueleconomy.gov/feg/current.shtml, Balat and Balat, 2009). Ethanol as a vehicle fuel is not a new concept. Henry Ford and other early automakers suspected it would be the world's primary fuel before gasoline became so readily available (http://www.afdc.energy.gov/fuels/ethanol_fuel_basics.html, Bharathiraja et al., 2014). Todays, researchers are agreed that ethanol can substantially fulfill our nation's petroleum need. In addition, studies have determined that ethanol and other
biofuels can subside 30% or more of the U.S. gasoline demand by 2030 (http://www.afdc.energy.gov/fuels/ethanol_fuel BASICS.html). Ethanol is a renewable fuel made from various plant materials collectively known as "biomass" (Figure 1.4b). More than 95% of U.S. gasoline contains ethanol, typically E10 (10% ethanol, 90% gasoline), which helps to oxygenate the fuel and reduce air pollution (http://beef2live.com/story-ethanol-101-0-108519, Balat and Balat, 2009). Ethanol is also available in the form of E85, or high-level ethanol blends (Figure 1.5). This blended fuel can be utilized in flexible fuel vehicles, which have the feature to run on high ethanol blending level or alone gasoline or any blending composition of both of these. Another blending level, E15, has been tested and approved for use in newer vehicles, and is slowly becoming available (http://beef2live.com/story-ethanol-101-0-108519, Yacobucci and Schnepf, 2007).

![Figure 1.5: Bioethanol (E-85) petrol pump in Brazil](http://www.fueleconomy.gov/feg/current.shtml)

Ethanol (CH₂CH₂OH) also known as ethyl alcohol, grain alcohol and EtOH is a clear and colorless liquid (http://beef2live.com/story-ethanol-101-0-108519, Balat and Balat, 2009). Ethanol maintains the same structure and chemical formula regardless of the route of its production either from starch and sugar based feedstocks, including corn grain (as it was initially in the United States), sugar cane (initially in Brazil) or from lignocellulosic biomass feedstocks such as wood chips or crop residues (Figure
Introduction

1.6, http://beef2live.com/story-ethanol-101-0-108519, Balat and Balat, 2009). Ethanol has several benefits than gasoline including higher octane number and providing premium blending properties. In addition low octane gasoline is generally blended with 10% ethanol to obtain the standard 87 octane requirement. Therefore, ethanol is the essential component for high level ethanol blends (http://beef2live.com/story-ethanol-101-0-108519, Balat and Balat, 2009). The process of making ethanol to be available as a vehicle fuel undergoes through several systematic steps (http://www.afdc.energy.gov/fuels/ethanol_fuel_basics.html) such as;

- Growing the lignocellulosic biomass feedstocks and their collection and transportation to the ethanol production facility.
- Production of ethanol from biomass feedstocks and their transportation to a blender/fuel supplier.
- Mixing of pure ethanol with different composition of gasoline by the blender/fuel supplier to make it E10, E15 or E85 concentrations, and subsequently distribute it to the fueling stations.

![Lignocellulosic biomass used for ethanol production](http://www.afdc.energy.gov/fuels/ethanol_fuel_basics.html)

**Figure 1.6:** Lignocellulosic biomass used for ethanol production (http://www.afdc.energy.gov/fuels/ethanol_fuel_basics.html)
1.4.1 Ethanol Production by Different countries

The world's highest ethanol fuel producers in 2011 were the United States and Brazil with 13.9 billion U.S. liquid gallons (bg) (52.6 billion liters) and 5.6 bg (21.1 billion liters) respectively, measuring together 87.1% of world’s total production of 22.36 billion US gallons 84.6 billion liters (Licht F.O, 2008 and 2010). Attractive benefits associated with this industry are promoting ethanol industries development in countries such as Germany, Spain, France, Sweden, China, Thailand, Canada, Colombia, India, Australia, and some Central American countries (Table 1.1, Licht F.O, 2008 and 2010). Figure 1.7 shows a dual fuel gas station (ethanol and petroleum) in Sao Paulo, Brazil (http://beef2live.com/story-ethanol-101-0-108519).

Figure 1.7: A dual fuel gas station (ethanol and petroleum) in Sao Paulo, Brazil (http://beef2live.com/story-ethanol-101-0-108519)
Table 1.1: Annual ethanol production Globally (Licht F.O, 2008 and 2010)

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<tbody>
<tr>
<td>1</td>
<td>USA</td>
<td>15,300</td>
<td>14,806</td>
<td>14,300</td>
<td>13,300</td>
<td>13,300</td>
<td>13,900</td>
<td>13,231</td>
<td>10,938</td>
<td>9,235</td>
</tr>
<tr>
<td>2</td>
<td>Brazil</td>
<td>7,295</td>
<td>7,093</td>
<td>6,190</td>
<td>5,577</td>
<td>6,267</td>
<td>5,573</td>
<td>6,921</td>
<td>6,577</td>
<td>6,472</td>
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<tr>
<td>3</td>
<td>EU</td>
<td>1,377</td>
<td>1,387</td>
<td>1,445</td>
<td>1,179</td>
<td>1,371</td>
<td>1,199</td>
<td>1,176</td>
<td>1,039</td>
<td>733</td>
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<td>4</td>
<td>China</td>
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<td>813</td>
<td>635</td>
<td>555</td>
<td>696</td>
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<td>541</td>
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<td>5</td>
<td>Thailand</td>
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<td>6</td>
<td>Canada</td>
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<td>436</td>
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<td>449</td>
<td>523</td>
<td>462</td>
<td>356</td>
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<tr>
<td>7</td>
<td>India</td>
<td>225</td>
<td>211</td>
<td>15</td>
<td>92</td>
<td>66</td>
<td>52</td>
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<tr>
<td>8</td>
<td>Other</td>
<td>490</td>
<td>391</td>
<td>865</td>
<td>752</td>
<td>1,272</td>
<td>247</td>
<td></td>
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<tr>
<td>World Total</td>
<td></td>
<td>26,290</td>
<td>25,471</td>
<td>24,279</td>
<td>21,812</td>
<td>23,429</td>
<td>22,356</td>
<td>22,946</td>
<td>20,159</td>
<td>17,333</td>
</tr>
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</table>

1.4.2 Advantages and Disadvantages of Ethanol as a Fuel

There are both advantages as well as disadvantages associated with the use of ethanol as an alternative fuel to petroleum (www.easychem.com.au). Table 1.2 reveals detailed descriptions about using ethanol as biofuel.
Table 1.2: Advantages and disadvantages of ethanol as a fuel
(www.easychem.com.au)

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unlike petroleum, ethanol is a renewable</td>
<td>Ethanol has a lower heat of combustion (per mole, per unit of volume, and per unit of mass) than petroleum</td>
</tr>
<tr>
<td>resource</td>
<td></td>
</tr>
<tr>
<td>Ethanol burns more cleanly in air than</td>
<td>Large amounts of arable land are required to produce the crops required to obtain ethanol, leading to problems such as soil erosion, deforestation, fertilizer run off and salinity</td>
</tr>
<tr>
<td>petroleum, producing less carbon (soot) and</td>
<td></td>
</tr>
<tr>
<td>carbon monoxide</td>
<td></td>
</tr>
<tr>
<td>The use of ethanol as opposed to petroleum</td>
<td>Major environmental problems would arise out of the disposal of waste fermentation liquors.</td>
</tr>
<tr>
<td>could reduce carbon dioxide emissions,</td>
<td></td>
</tr>
<tr>
<td>provided that a renewable energy resource</td>
<td></td>
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<tr>
<td>was used to produce crops required to obtain</td>
<td></td>
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<td>ethanol and to distil fermented ethanol</td>
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<tr>
<td>Ethanol</td>
<td>Typical current engines would require modification to use high concentrations of ethanol</td>
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1.5 Current challenges regarding syngas fermentation to ethanol

Commercial level interest in the synthesis gas fermentation has considerably increased over the past few years. This can be described by an increase in researches in the relevant area and patent filings during this time period, and is further illustrated by the actions of several companies desirable to commercialize this process for the industrial production of transport fuels and platform chemicals (Daniell et al., 2012). Despite the industrial increase in interest in biomass gasification followed by syngas
fermentation, it yet required to achieve at a commercial level (Daniell et al., 2012). The principal challenges associated with industrialization of this technology are:

### 1.5.1 Mass transfer

Major challenge in the commercialization of syngas fermentation is the gas-to-liquid mass transfer limitation as the solubility of synthesis gas components i.e. CO, CO$_2$ and H$_2$ in water is very low (Bredwell et al., 1999). In comparison to oxygen, mass transfer rates of CO and H$_2$ is only 77% and 65% respectively (Bredwell et al., 1999). Due to the low diffusion rate, availability of gases as substrate to the microorganisms is also resulting in low productivity; thereby the bubble diameter in case of mass transfer limitation is playing an important role, leading to the use of microbubble dispersers. In mass transfer limited condition the bubble diameter is inversely proportional to the specific surface area (Abubackar et al., 2011). For the measurement of mass transfer in various reactor configurations volumetric mass transfer coefficient ($k_{La}$) can be used. To overcome the mass transfer limitations there is a need of advancement in the conventional methods of reactor design such as; impeller design, fluid flow pattern, power efficiency, mixing time and baffle design (Munasinghe and Khanal, 2010a, Ungerman and Heindel, 2007). Use of hollow fiber membrane (HFM) and nanoparticle is another innovative approach to enhance the mass transfer rate over conventional reactor configurations (Nerenberg and Rittmann, 2004, Zhu et al., 2008, Klasson et al., 1993). The mass transfer coefficient ($k_L$) for a slightly soluble gaseous substrate can be determined using Eq. 1 (Klasson et al., 1992).

\[
\frac{1}{V_L} \frac{dN_GS}{dt} = \frac{k_{La}}{H} (P_{GS}-P_{LS})
\]

where; $N_GS$ denoted as the molar substrate transferred from the gas phase, $V_L$ (L) is the reactor volume, $P_{GS}$ and $P_{LS}$ (atm.) are the partial pressures of gases in gas and the liquid phase, $H$ (L.atm/mol) is the Henry’s law constant and ‘$a$’ ($m^2/L$) is the gas–liquid interface surface area for unit volume. The difference in the partial pressures of gases is the driving force for mass transfer and thus controls the solubility of the gaseous substrate. High pressure improves the solubility of the gas in aqueous phase. Earlier studies observed mass transfer by using different bioreactor configurations.
The most common strategy for improving the mass transfer in CSTRs is by increasing the agitation speed of impellers (Bredwell et al., 1999, Bouaiifi et al., 2001). By adopting this approach, it is possible to produce micro-bubbles, which is beneficial in increasing the gas–liquid interfacial area for enhancing mass transfer (Bredwell et al., 1999, Bouaiifi et al., 2001). However, the high energy utilization in this process reduces its economic viability at commercial-scale. Subsequently, other bioreactor designs such as trickling bed reactors, air-lift reactors and bubble column reactors can be preferred for an efficient mass transfer (Munasinghe and Khanal, 2010a, Ungerman and Heindel, 2007). The mass transfer rates between stirred-tank and bubble column reactors were compared by Bouaiifi et al. (2001). It was found that the kLa obtained for the bubble column reactor was higher than that of the stirred-tank reactor. Another study of Bredwell and Worden, (1998) tested the hydrodynamic and mass transfer properties of microbubble dispersions in a bubble column reactor and found that the axial mixing of the micro-bubble dispersion was significantly less than the conventional bubble column reactors.

1.5.2 Syngas Quality

Syngas is a mixture of CO, CO\textsubscript{2}, H\textsubscript{2} and traces of other impurities including tars, ash, ethylene, ethane, acetylene and sulfur (SO\textsubscript{x}) and nitrogen (NO\textsubscript{x}) gases (Xu et al., 2011). Syngas utilizing bacteria generally uses CO, CO\textsubscript{2} and H\textsubscript{2} for their growth and production (Daniell et al., 2012, Xu, 2012). Due to other traces gases or impurities the biomass generated syngas sometimes causes problems of culture instability and low carbon conversion efficiency by inhibiting hydrogenase activity (Xu, 2012). Therefore, Syngas should be free from all impurities before being utilized for the microbial fermentation process. Pyrolysis of lignocellulosic biomass also releases tar, which effect microbial activity during syngas fermentation reaction (Xu, 2012). It was observed that cell dormancy and product redistribution were most likely caused by tar present in syngas (Bredwell and Worden, 1998). Several purification methods are currently used for synthesis gas cleaning such as cyclones, different variety of filters (fabric, ceramic and bag filters), rotating particle separators, water scrubbers and wet electrostatic precipitators (Hasler and Nussbaumer, 1999). Using appropriate gasification and preprocessing techniques, impurities from syngas can be reduced to make it available as substrate for bacterial cell. These techniques have also been
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... successfully applied for sugarcane family feedstock to reduce nitrogen and sulfur containing compounds (Turn et al., 2003).

1.5.3 Microbial catalyst

The selection of appropriate microbes for efficient syngas fermentation is a challenging task. Strict mesophilic anaerobes such as *C. ljungdahlii, C. aceticum, Acetobacterium woodii, C. autoethanogenum, and C. carboxydivorans* are frequently being used in syngas fermentation (Klasson et al., 1992, Rajagopalan et al., 2002, Younesi et al., 2005). For the commercialization of process, isolation and identification of anaerobic microorganisms which are more efficient and robust for conversion of syngas into ethanol and other byproducts with higher productivity is another important aspect. In addition, isolation of syngas fermenting thermophiles which can convert syngas components (CO, CO₂ and H₂) into ethanol or butanol would also be an interesting area.

1.5.4 Product Recovery

Ultrasonic atomization, vapor recompression, vapor reuse and vacuum distillation, selective adsorption of water are some of the recovery techniques that have been used for efficient product recovery alongwith reducing the ethanol recovery cost (Sato et al., 2001). Liquid–liquid extraction is a preferably used method for acetic acid recovery. A specific solvent can be used for extracting a considerable amount of pure acetic acid solution. Fockey et al. (2008) suggested a novel extraction/re-extraction technique by utilizing glycerol as solvent to recover ethanol, acetic acid and other biochemicals.

1.5.5 Redirection of metabolic pathway

Metabolic engineering and synthetic biology techniques is promising for the improvement of the gas fermentation process, and the steps for using advanced genetic tools have only recently been taken. Most gas fermentation organisms are not very well characterized, especially when compared to *C. acetobutylicum*, the model organism used in ABE fermentation (Kopke et al., 2011, Durre, 2007, Daniell et al., 2012). The first genome sequence published for syngas fermentation organism was in 2008 (Pierce et al., 2008), and subsequent advances made in the areas of metabolic
engineering and synthetic biology will accelerate further. Syngas fermentation is always associated with acetic acid production, which lowers the culture pH. Redirecting the metabolic pathway towards solvent production by blocking acetic acid production might enhance ethanol production (Abubacker et al., 2011).

1.6 Scale up status and commercialization

Commercial interest in gas fermentation has increased significantly over the past few years. Despite the increase in commercial interest, biomass gasification followed by syngas fermentation has yet to be achieved on a commercial scale (Daniell et al., 2012). One of the major technical challenges associated with commercialisation of syngas fermentation technology is the successful up-scaling of this process from pilot-scale to industrial scale (Kundiyana et al., 2010b). Among the many start-ups and commercial ventures that had taken up commercializing this technology, three companies that were prominently featured included INEOS Bio, Coskata and LanzaTech. The first patent in field of synthesis gas fermentation was filed by J.L. Gaddy (University of Arkansas) in Fayetteville (Gaddy and Clausen, 1992, Gaddy, 1997, Gaddy, 1998). Moreover, the first reports of commercialization came in with the establishment of Bioengineering Resources Inc. (BRI) with a transfer of syngas technology from bench scale to industry (INEOS Bio, 2012a, Klasson et al., 1990). BRI functioned as a pilot unit from the year 2003 and INEOS Bio acquired this technology in 2008 (INEOS, 2012b).

INEOS Bio (http://www.ineosbio.com) was established as a replacement of INEOS in 2008. Their first pilot plant demonstrated a production capacity of 100 gallons ethanol/dry ton of feedstock using patented biocatalyst C. ljungdahlii (Gaddy et al., 2012, Wald, 2012, William, 2012). INEOS Bio started the construction of their first commercial scale plant in 2011, by the name of Indian River BioEnergy Center in Florida (INEOS Bio, 2012c). This plant was planned to produce ethanol from yard, vegetative and household waste, alongwith projected to produce six megawatts (gross) of electricity from unused syngas and recovered heat (INEOS Bio, 2012d). The plant was specifically designed for producing 8 million gallons of ethanol per year and having a utilization capacity of 300 dry tons per day (U.S Department of Energy, 2012). Based on financial status a second commercial plant of INEOS Bio in Teesside, UK was in design (INEOS Bio, 2012e), which was expected it to complete
in 2013 with similar bioethanol and power production rates as in Florida plant (INEOS Bio, 2012a).

Coskata, Inc. (http://www.coskata.com) got its establishment in 2006 in Warrenville, IL, USA used syngas fermentation technology and bacterial strains \(C. \text{ragsdalei}\) and \(C. \text{carboxidivorans}\) obtained from Oklahoma State University and the University of Oklahoma (William, 2012). Coskata used and also discussed the identification and development of their patented ethanol producing strain “\(\text{Clostridium coskatii}\)” (Zahn and Saxena, 2011). Coskata started its syngas fermentation technology demonstration plant in Madison, PA in October 2009 (William, 2012). They utilized synthesis gas produced from woody biomass and municipal solid waste through a plasma gasification process designed and fabricated by Westinghouse Plasma Corporation (Coskata Inc. Coskata, 2012). Coskata then demonstrated the construction of bioethanol producing commercial plant in Alabama from the bioconversion of wood chips and waste with a production capacity of 16 million gallons of ethanol per year, which was planned to be scaled upto 78 million gallons, (William, 2012). This plant was supposed to use an indirect biomass gasifier in place of the Westinghouse Plasma Corporation gasification technology used in demonstration plant, (William, 2012). They also reported an expected yield of 100 gallons of ethanol/bone dry ton of softwood, with planned cost up to USD$1.50 per gallon (William, 2012). Moreover, in July, 2012 the company changed its strategy and reported the construction of a commercial plant which was utilizing reformed natural gas as the sole feedstock for platform chemicals production (Lane, 2012).

LanzaTech (http://www.lanzatech.co.nz) started its plant with the focus on the use of synthesis gas and CO-rich gases to produce ethanol and 2, 3-butanediol. They established their first plant in Auckland, New Zealand in 2005 and used the already patented strain of \(C. \text{autoethanogenum}\) (Heijstra et al., 2012). LanzaTech worked on the distinct concept of producing biochemicals such as 2,3-butanediol and butanol alongwith traditional fuels like ethanol by syngas fermentation (Kopke et al., 2011, Koepke and Liew, 2012). LanzaTech also worked in collaboration with nylon producing company INVISTA for biosynthesis of butadiene from CO (Lanzatech, 2012a). LanzaTech currently in process of running a pilot-scale plant at the BlueScope Steel facility in New Zealand since 2008 and have also established another pre-commercial unit for producing 100,000 gallon ethanol per year from steel mill off
gases in partnership with BaoSteel in Shanghai, China (Lanzatech, 2012b, Lanzatech, 2012c). In addition, construction of one more steel mill-based plant has begun with Shougang Group, for developing a demonstration plant located at a Shougang steel mill (Lanzatech, 2012d). Finally, LanzaTech have established the foreclosed Fuels plant in Soperton, Georgia for the purpose of generating biomass derived synthesis gas from woody biomass (Herndon, 2012).

Kiverdi Inc. (http://www.kiverdi.com/#recycle-1-2-1) has created a bioprocess and commercialized its technology that uses a special class of chemoautotrophic microbes called as nature's "Super Charged Carbon Recyclers" which can transform carbon dioxide and other gases into protein, high-value oils, and bio-based products, that can be used in a variety of consumer and industrial applications. It was founded in 2008 and is based in Berkeley, California. Kiverdi, Inc. offers oil-based products and chemicals for detergents, biomaterials, and fuel additives. The company provides consumer chemicals including fatty acids, fatty alcohols, and triacylglycerides used in bar and liquid soaps, shampoos, skin creams, and detergents. Additionally, it offers biomaterials used in synthesis of bioplastics,nylons, and other polymers. The company also develops drop-in fuels used in vehicle and jet engines. The Kiverdi technology can target a range of bio-based compounds. For example, limonene is a compound found in citrus fruits, which is commonly used as a biodegradable, non-toxic cleaning formulation ingredient or as a solvent. Kiverdi's technology can produce a limonene alternative, which isn't reliant on weather conditions or geographic location, and can be manufactured in a much more cost effective way. Moreover, modern agriculture is also one of the largest polluters, emitting more greenhouse gases than our cars, trucks, planes and trains combined. Kiverdi's protein-rich meal can provide solutions. It has 50% greater protein content than soy meal and amino acid profile similar to an animal protein. Further, Kiverdi's technology recycles carbon dioxide into oils with properties similar to plant-based oils and derivatives, creating sustainable alternatives. This includes palm oil (PALM+), but it also includes higher-value oils such as macadamia oil (http://www.kiverdi.com/#recycle-1-2-1).
1.7 Separation of volatile fatty acids and ethanol from the product stream

Volatile fatty acids, along with other chemicals, are utilized in many industries as reactants, solvents and also as catalysts according to their requirement. Fermentation broth from reactor is in dilute form, from which the acids need to be recovered. However, many technologies have been used for this purpose and acids can be separated from the stream and can be converted into other value-added products like esters. Membrane processes and biological processes are being researched academically and practiced industrially. These technologies have their own benefits and limitations with respect to conversion, energy consumption etc. These techniques are not beneficial in all the cases and hence an alternate process technology is required like reactive separation (Talnikar and Mahajan, 2014). Reactive separation (RS) is beneficial especially when the acid needs to be further converted into other useful chemicals, due to additional usefulness or because of lack of alternative or due to cost considerations alone.

Conventional process technologies are dependent on reactor configuration followed by the subsequent separation technique. However, this technology sometimes suffers from lesser conversion, difficulties in separation etc. Hence, to overcome these issues, reactive separation is advantageous over other processes in terms of the recovery of the useful compounds. Reactive distillation (RD), reactive extraction (RE) and reactive chromatography (RC) are some other separation technologies that can be useful for acid recovery in an economically feasible way (Talnikar and Mahajan, 2014).

Alcohol substantially free of water is prepared by fermenting a fermentable biomass feedstock in a fermentation unit, thereby forming an aqueous fermentation liquor containing alcohol; extracting said aqueous fermentation liquor with an organic solvent containing an extractant for said alcohol, thereby forming an alcohol-organic solvent extract phase and an aqueous raffinate; contacting said alcohol-organic solvent phase with a carrier gas thereby separating said alcohol from said alcohol-organic solvent phase and forming an alcohol laden solvent vapor; and separating alcohol substantially free of water from said carrier gas (Daniel W Tedder, 1985).