Preamble

Global scenario of fossil fuels consumption and demand for renewable and sustainable energy sources is discussed in detail. Suitability of biobutanol as a substitute for gasoline along with detailed description about the type of biomass that are being considered or used for its production through biological route are also discussed. Based on the available information presented in this, the broad objective were decided. These are presented at the end of this chapter.

1.1 Global fuel scenario

Global energy demand is steadily rising with increase in world population, industrialization and changes in people's lifestyle. At the current rate of consumption, fossil fuels like coal, petroleum and natural gas are likely to get exhausted by 2050 (Demirbas 2010). The energy demand is expected to increase up to 53% by the year 2030. The petroleum consumption is projected to increase from 89.41 million barrels per day in 2012 to 136.8 million barrels per day by 2030 (Noraini et al. 2014; Shahid and Jamal 2011). The increasing global demand of petro-fuels and decreasing availability of crude petroleum have given impetus to global efforts for developing clean, renewable and sustainable energy sources capable of meeting the increasing energy demand and improving global economy and environment (He et al. 2010). In this regard biofuels like biodiesel, biogas, ethanol and butanol are currently receiving considerable attention as possible replacement for the fossil fuels (Kumar and Gayen 2011). According to the Navigant Research, USA, global demand of biofuels for transportation will increase from 32.4 BGPY (billion gallons per year) in 2013 to 51.1 BGPY by 2022 (Navigant Research, 2014). In 2014, the global biofuel production increased by 9.0% i.e. to 127.7 billion liters including all types of biofuels (ethanol, biodiesel and hydro-treated vegetable oil) (Renewable Energy Policy Network for the 21st Century (REN21), Global Status Report, 2015). According to the White Paper on Renewable Energy Sources, published by the European Commission in 1997, bio-renewables utilization in the production of energy is likely to increase to 20% by 2020 (Mascal and Nikitin 2008). These biofuels can be produced using several types of biomass as feedstock such as foody, non-foody and algal biomass. The CO_2 emitted thereby can be reused for fresh biomass growth (Demirbas 2009a; Demirbas 2009c) making the biofuels carbon neutral (Hoekman 2009).

The key advantage of biofuels is the near future energy security due to the abundance of renewable biomass (Demirbas 2009b). Biofuels such as bioethanol and biodiesel have already attracted the attention of researchers, whereas comparatively less emphasis has been focused on biobutanol (Dutta et al. 2014a). According to energy experts biobutanol has the greatest potential as substitute for gasoline as no alteration in the engine is required due to its air-to-fuel ratio and energy content being similar to gasoline (Ranjan and Moholkar 2012). It can also be used as a supplement for diesel and kerosene (Durre 2007; Garcia et al. 2011).

1.2 Biobutanol

A candidate biofuel should have high calorific value, low heat of vaporization, low volatility and should be non-corrosive and less hygroscopic in nature. Butanol is a four-carbon, colorless and flammable alcohol (C_4H_9OH) and is currently produced through the chemical route by the petrochemical units primarily for use as solvent in different industries. Butanol exists in four different isomeric forms viz., n-butanol, sec-butanol, tert-butanol, and iso-butanol differing in structures and physical properties as shown in Table 1.1.

As a biofuel butanol is superior to ethanol due to its lower volatility, vapor pressure, heat of vaporization, corrosiveness, and explosive nature. Important physical properties of butanol are compared with those of ethanol, gasoline and methanol in Table 1.2 (Bharathiraja et al. 2017). It is clear that most of the properties of butanol are comparable to those of gasoline. Blending ability of butanol with gasoline has been found to be better than ethanol, it also enhances the water contamination tolerance and acts as a rich fuel extender (Merola et al. 2012).

Table 1.1: Properties of various isomeric forms of butanol

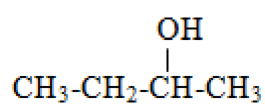
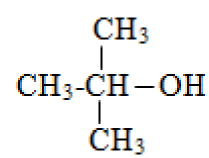
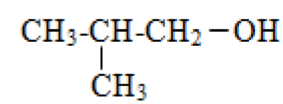
Parameter	n-Butanol	sec-Butanol	tert-Butanol	iso-Butanol
Melting point (ºC)	-89	-114	25.3	-108
Boiling point (ºC)	118	99.5	82.5	108
Vapour pressure (bar)	6.7	16.5	40.7	10.6
Properties	Fermentative product, colorless, specific odor, sparingly miscible in water (7.7 wt% at 20°C)	Sparingly miscible in water (24.5 wt% at 20°C)	Miscible in water	Efficient blending (15%) with petrol on volumetric basis, sparingly miscible in water (8.0 wt% at 20°C)
Chemical Structure	CH_3-CH_2-CH_2-CH_2-OH			

Table 1.2: Comparison of properties of different fuels

Properties	Ethanol	Gasoline	Methanol	Butanol
Specific gravity	0.79	0.71	0.79-0.8	0.81
Density at 20°C (g/mL)	0.79	0.7-0.8	0.79	0.81
Energy density (MJ/Kg)	24.84	42.62	20.33	36.05
Melting point (°C)	-114	-57.1 to -56.6	-97.6	-89.3
Boiling point (°C)	78	27-221	64.7	117-118
Auto-ignition temperature (°C)	422	246-280	470	343-345
Flash point (°C)	12.77	13	15.6	25-29
Critical temperature (°C)	239.85	241	239	287
Energy content (BTU/gal)	84,000	115,000	76,000	110,000
Air-fuel ratio	9	14.6	6.5	11.2
Heat of vaporization (MJ/Kg)	0.92	0.36	1.2	0.43
Research octane number	129	91-99	136	96
Motor octane number	102	81-89	104	78
Viscosity (10^{-3} Pa·s)	1.078	0.24-0.32	0.545	2.593

There are several chemical processes based on petrochemical feedstocks that are used industrially for the chemical synthesis of n-butanol. These include: (i) Oxo synthesis, (ii) Crotonaldehyde hydrogenation, and (iii) Reppe synthesis. Among these oxo synthesis (Eq. 1.1–1.3) is the most common route that has been adopted by all major petrochemical industries for butanol production. Oxo process involves hydroformylation of propene followed by hydrogenation of the aldehydes formed that leads to the synthesis of corresponding alcohol (Uyttebroek et al. 2015). BASF (Oxea Group) and Dow Chemicals are the largest producers of n-butanol through the Oxo process (Hahn et al. 2013).

$$CH_3CH - CH_2 + CO + H_2 \rightarrow CH_3CH_2CH_2CHO + (CH_3)_2CHCHO \quad (1.1)$$

$$CH_3CH_2CH_2CHO + H_2 \rightarrow CH_3CH_2CH_2CH_2OH \quad (1.2)$$

$$(CH_3)_2CHCHO + H_2 \rightarrow (CH_3)_2CHCH_2OH \quad (1.3)$$

The main advantage of the chemical route is the involvement of a two-step conversion reaction for butanol production and its major disadvantages are requirement of high temperature and pressure (10-30 MPa, 150-190°C), high cost of catalyst recovery, expensive metal loss, corrosion problem and most importantly environmentally unsafe production process due to the involvement of large quantity of chemicals (Uyttebroek et al. 2015). Production of biobutanol through fermentative route is gaining much interest due to the global environmental concerns. Figure 1.1 shows the general steps involved in the fermentative biobutanol production pathway. Biobutanol was first produced at laboratory scale by Louise Pasteur in 1861 and at industrial scale by ABE (acetone-butanol-ethanol) fermentation in 1912-1914 from cane or beet molasses and cereal grains using Weizmann's organism (*Clostridium acetobutylicum*). ABE fermentation was mainly utilized for the production of acetone that was a component of explosive materials during World War I and butanol, the side products was being utilized as solvent in the rubber and automobile industry (Kumar & Gayen 2011). The major feedstocks used were cane or beet molasses, wheat, rye and maize. Increasing food scarcity and growth of petrochemical industries producing ABE at lower price shifted the attention from fermentative ABE production to chemical route. However, the drawbacks of petrochemical route and depleting oil reserves have again shifted the attention of scientific community towards the fermentative route with a new potential feedstock i.e. lignocellulosic biomass available in abundance. But the cost associated with the processing of lignocellulosic biomass resulted in high ABE cost. Once again research efforts are directed towards the search for a renewable biomass to produce biobutanol with lower processing cost, high product yield and lower environmental impacts.

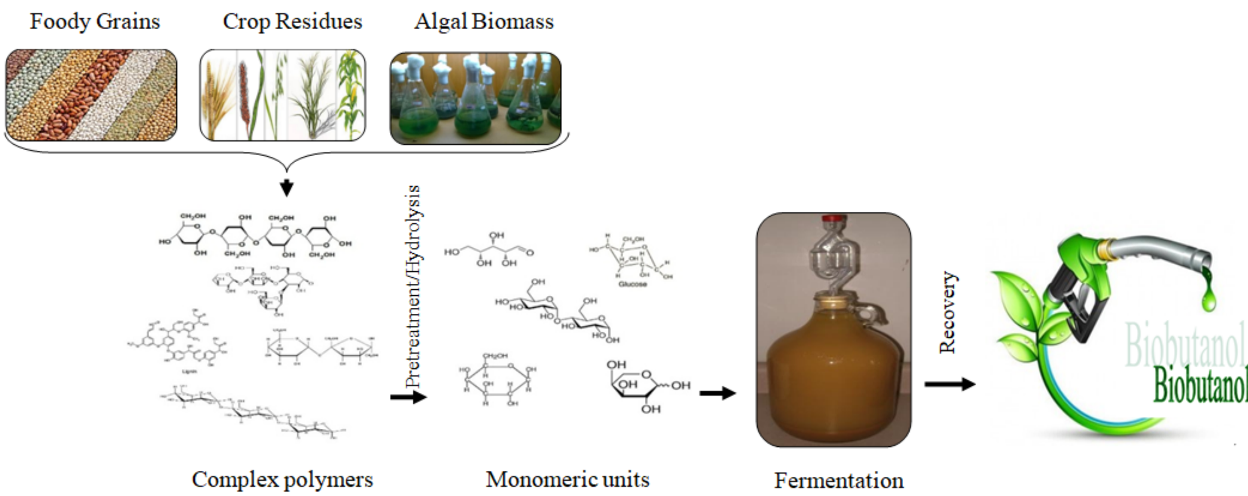


Figure 1.1: Production of biobutanol through fermentation

1.3 Global biobutanol scenario

For an economically viable butanol production process availability of suitable feedstock and separation technique to recover the end-product are the main accountable parameters. Several studies have been carried out to evaluate the economic feasibility of butanol production from corn, wheat straw, whey permeates and molasses (Qureshi and Blaschek 2000; Qureshi and Blaschek 2001a). Presently the cost of butanol produced through petrochemical route ($1.52/kg n-butanol) is lower than the fermentative route ($1.87/kg n-butanol), however, due to certain advantages such as lower feedstock processing cost and environment friendly nature, industries are now moving towards fermentative route of butanol production (Jiang et al. 2015).

Major demand for butanol is in North America, Western Europe and North East Asia including major countries viz., United State, Germany, Japan and China. Global increase in the demand for butanol was recorded as 2.7% per annum during 2005-13. Current annual global demand for butanol is exceeding 1.2 billion gallons and its estimated value is over $6 billion annually (DUBLIN, March 10th, 2017, PRNewswire). During the past few years the market for butanol has grown significantly and is forecast to grow more rapidly during 2014-2019 driven primarily by the Asia-Pacific region countries, with China accounting for more than 1/3rd of the total world demand (MicroMarket Monitor, July 2nd, 2014, PRWEB). In 2013, the Asia-Pacific n-butanol market was valued at $3.0 billion and is expected to reach $4.3 billion by 2018. Presently China is expanding the butanol market and probable demand in the Chinese market is projected to be about 1.64 million tonnes by 2021.

Several industries have started using lignocellulosic biomass for butanol production, but this has proved a costly approach due to the involvement of elaborate biomass pretreatment and detoxification process. The production cost from corn has been estimated as $4.41/gal and from soy molasses as $2.71/gal (Dong et al. 2014). Development of new techniques such as involvement of genetically modified microorganisms with enhanced butanol productivity along with the utilization of algal feedstocks could lead to the price reduction of butanol. Norsker et al. (2011) has estimated the algal biomass production cost as $472/1000 kg or $118/100 kg algal sugar. Recently DuPont and Bio Architecture Lab have started using seaweed biomass for commercial butanol production by investing about $8.8 million for its R&D. Table 1.3 lists some major industries currently engaged in fermentative biobutanol production in developed countries.

Table 1.3: Production of biobutanol in developed countries

Companies	Country	Production details
GEVO	Colorado, United State	Isobutanol from glucose using genetically modified yeast
BUTYL FUEL, LLC	Columbus, USA	Biobutanol production with modified and patented Clostridium strain, able to produce high butanol (1.3-1.9 times higher) titer
GREEN BIOLOGICS	Abingdon, UK	Biobutanol production with genetically modified strain (tolerate nearly 4% of butanol concentration)
TETRAVIATE BIOSCIENCE	Chicago, USA	Biobutanol fermentation with mutated and patented *Clostridium beijerinckii*
BUTALACO (bio-based innovations)	Zug, Switzerland	Genetically modified yeast with higher butanol producing property and efficient utilization of C5/C6 sugars
METABOLIC EXPLORER	Clermont-Ferrand, France	Using designed microorganisms for production of butanol from lignocellulosic biomass

Source: Bharathiraja et al. (2017)

1.4 Indian biofuel production scenario

The Ministry of New and Renewable Energy, Government of India, has announced some new policies on biofuels i.e. improved production of second generation biofuels to meet the large fraction of current fuel demand, bioethanol blended gasoline and biodiesel blended diesel supply. In 2003 the Ministry launched a program for 5% ethanol blending in gasoline that increased to 20% by 2017 in the newly proposed policy (National Policy on Biofuels, Government of India). In the first phase of the proposed policy 9 States (Andhra Pradesh, Karnataka, Tamil Nadu, Haryana, Maharashtra, Goa, Punjab, Gujarat, Uttar Pradesh) and 4 Union Territories (Dadra and Nagar Haveli, Pondicherry, Chandigarh, Daman and Diu) were supplied with 5% bioethanol blended petrol while in the second phase this percentage has increased to 10%. According to an article published in “The Hindu” on 27th January 2018 titled “By 2022, biofuel will become a ₹50,000-crore business” currently biodiesel and bioethanol industry is worth ₹6,000 crore and it is expected to increase up to ₹1.25 lakh crore by 2040. Ministry has formulated a number of policies for commercialization of bioethanol and biodiesel together with some new initiatives for biobutanol production from lignocellulosic biomass in the last few years and has sanctioned several new research and development projects to certain institutes for process development.

1.4.1 Indian market in biobutanol production

The compound annual growth rate of Indian butanol market is projected as 10.8% and major industries involved in production are Andhra Petrochemicals Ltd., Somaiya Organic Ltd. and NOCIL (Report N-1155, MicroMarket Monitor, March 2015). Green Biologics Ltd. (Abingdon, UK) is working in collaboration with India-based Laxmi Organics Industries for commercial scale production of biobutanol from sugarcane. Agrosys Products India Pvt. Ltd. and several other industries are at the process development stage for producing butanol on commercial scale.

1.5 Feedstocks for biobutanol production

Selection of suitable feedstock is one of the most important steps governing the economics of the process. On the basis of the type of feedstock, biofuels are broadly categorized as first, second and third generation biofuels. Carbohydrate bearing material such as sugarcane, cereal grains and food industries wastes were utilized as feedstock during early 20th century and are known as the first generation feedstock. With rising world population utilization of these materials as feedstock for the production of biofuels is neither ethical nor economically viable (Zhang et al. 2010). Waste emanating from food industries is an attractive alternative option as feedstock while food versus fuel conflict is the greatest roadblock for the popularization of first generation biobutanol technology (Stoeberl et al. 2011). Inspite of this several countries are producing the common biofuel i.e. ethanol from food crops. United States of America is producing ethanol from corn, Brazil from sugar crops and China from sweet sorghum, cassava and other non-grain crops (REN21, Global Status Report, 2015). Considerable effort has been made globally to adapt a sustainable approach for biofuel production, in this regard lignocellulosic (the second generation feedstock) and algal biomasses (the third generation feedstock) are attracting much attention. In the European Union, straw and other agricultural wastes (rich in lignocelluloses) are the major feedstock for bioethanol production (Raposo et al. 2009). Utilization of lignocellulosic biomass as feedstock is likely to be more sustainable for the Indian subcontinent (Menon and Rao 2012). The greatest impediment for the second generation biobutanol is the release of toxins (Forgione et al. 2008). The detoxification process makes the second generation biobutanol an economically non-viable proposition. Drawbacks associated with the first and second generation feedstock have ultimately increased the interest of researchers in the use of algal biomass for butanol production through biological route (Efremenko et al. 2012; Ellis et al. 2012; Vander Wal et al. 2013). According to the Department of Energy, USA oil producing ability of algae is 100 times more than any other oil producing crop (e.g. soybeans: the leading source of USA biodiesel) and due to high energy content extracted oil can be refined into gasoline, biodiesel, ethanol or jet fuels. Fermentative production of ABE from algae is a promising area but it still needs to be explored to make it economical.

1.5.1 Algal biomass as feedstock

The algal biomass has started attracting the interest of researchers due to its ubiquitous availability, negligible land area requirement for mass scale cultivation, non-toxic nature, large carbohydrate content and lipid storage capacity for obtaining different biofuels- biobutanol, bioethanol, biodiesel, biohydrogen, etc (Daroch et al. 2013). Algal species are photoautotrophic and unicellular or multicellular organisms with higher energy conversion efficiency than land plants. They possess large CO_2 sequestration property thereby reducing the green-house effect with resultant large biomass production that can be used for different purposes (Ullah et al. 2015). These can also be used to treat contaminated sites thereby resulting in an increase in the net energy ratio and thus leading to large biomass generation (Graham et al. 2009). Different storage components of macroalgae, microalgae and cyanobacteria have great prospect for the production of biofuels. Among these microalgae and cyanobacteria are of great interest due to their short doubling time, efficient CO_2 fixation, large carbohydrate storage capability, and lower lignin content. Availability of large fermentable sugar from cyanobacteria makes it more suitable for bioalcohols production compared to lignocellulosic biomass (Abed et al. 2009; Ho et al. 2012; Kushwaha et al. 2014).

1.5.2 Cyanobacteria as sustainable source of energy

Cyanobacteria are oxygenic photosynthetic prokaryotes found almost in every habitat on the Earth (Garcia-Pichel and Pringault 2001). These are also termed as blue-green algae because of the presence of photosynthetic pigments and account for 20–30% of Earth’s photosynthetic productivity and exist in various unicellular and filamentous forms. Among various types of algal biomass cyanobacterial population have been found to be more promising for biofuels production due to their simple nutrient requirements, ability to grow in areas unfit for conventional agriculture, larger biomass production and rich source of various storage compounds (lipid, carbohydrate, proteins etc.) (Machado and Atsumi 2012). At commercial scale foody and lignocellulosic biomasses are commonly used for the production of various bioalcohols, however, several drawbacks associated with this feedstock have led the researchers to shift their attention to cyanobacterial biomass (Markou et al. 2013). Additional advantage of utilizing cyanobacterial biomass as feedstock for biobutanol fermentation is no or little release of toxins during pre-processing (pretreatment/hydrolysis) that decrease the efficiency of fermentative bacteria and reduce the end-product titer marginally. Nearly complete utilization of available carbohydrate from the cyanobacterial biomass to bioalcohols makes it an ideal feedstock for the commercial scale production, however improvement in the carbohydrate concentration for industrial feasibility needs to be examined (Silva and Bertucco 2016). Cyanobacterial biomass has already been utilized for the production of biodiesel and bioethanol but its exploitation for biobutanol fermentation has not been explored much.

Growth of cyanobacteria and storage of different components within its cells depend on various factors such as the availability of various nutrient components, media pH, incubation temperature, light intensity and shaking (Carvalho et al. 2009; Chen et al. 2013a; Gonzalez-Fernandez and Ballesteros 2012). Optimum level of light intensity is an important parameter for cyanobacterial growth as it controls the concentration of different chemical constituents of biomass and over illumination may cause cell damage and photo-inhibition within the cell (Tadros et al. 1993; Zhang et al. 2015). Macronutrients such as nitrogen and phosphorus and micronutrients such as iron and molybdenum are known to play a major role in regulating the metabolism and ultimately the growth of cyanobacteria (Kushwaha et al. 2014; Wang et al. 2010). A lower concentration can limit the growth by increasing the lag phase while a higher concentration may damage the cell membrane, lower down the photosynthesis efficiency and inhibit the growth (Miazek et al. 2015; Shuler and Kargi 2002).

The yield of the biobutanol through fermentative production depends on the pretreatment strategy. As cyanobacterial biomass contains negligible amount of lignin (≤ 1.5%), the thermal treatment together with some chemical treatment is the simplest approach to solubilise it and to release sugar from this complex polymeric structure and improve the effectiveness of further processes (Chen et al. 2013a; Daroch et al. 2013; John et al. 2011; Ververis et al. 2007). Despite various advantages of utilizing cyanobacteria as feedstock for biobutanol production, it is worth mentioning that the technology still needs improvement to get better biomass yield and enhanced carbohydrate accumulation for increased biobutanol productivity.

1.6 Broad objective

In view of the above mentioned existing limitations of production of biobutanol from conventional feedstocks, following broad objectives have been finalized for the present work: (i) Evaluation of the efficacy of locally collected cyanobacterial strains for production of biobutanol through fermentative route, (ii) Process optimization, and (iii) Efficient recovery of biobutanol.