INTRODUCTION

1. Introduction

The increase in global population with the rise in industrial and household energy consumption has led to increased demand for fuels. A case study reveals that world energy consumption may increase by 53% from 2008 to 2035 which may lead to a huge impact on the price of non-renewable energy resources with the advent of their shortage (Li et al., 2014). Studies suggest that the extraction of fossil fuels also affects the biodiversity of our planet (Harfoot et al., 2018). Thus, alternative sources of energy were harnessed to meet the huge demand.

1.1 Bioethanol

Bioethanol is one such alternative source of gasoline, which has gained increased attention in the last few decades. The production of bioethanol from biological sources is expected to increase from 86 billion litres to more than 160 billion litres in 2020 (Saïdane-Bchir et al., 2016). According to World Energy Council, bioethanol is defined as "An alcohol, made by fermenting any biomass with a high content of carbohydrates through a process similar to beer brewing" (Gadonneix et al., 2010). Bioethanol is widely used as global transport biofuel. Currently, bioethanol is blended with ethanol in different ratios such as E10 means 10% of bioethanol is blended with 90% petrol. The use of E85 will need modifications in the vehicle engine. Ethanol serves as an alternative of lead and acts as high octane fuel. Ethanol has also been suggested to act as fuel for biofuel cells and direct ethanol fuel cells.

Bioethanol is produced from hydrolysis of different biomass containing a high concentration of starch and sugars by yeast and bacteria. The overall production of bioethanol can be divided into three major steps

1. Preparation of substrate containing fermentable sugars
2. Fermentation of sugars into ethanol
3. Purification of bioethanol using distillation, rectification and dehydration.

Based on the biomaterial used, bioethanol production has been classified into three generations. With the advancement of research on bioethanol production, the emphasis has been laid on the identification of substrate which is not consumed as a food material. Figure 1.1 shows the brief pathway of ethanol production from glucose where one mol of glucose gives two mol of ethanol and two mol of carbon dioxide through glycolysis.

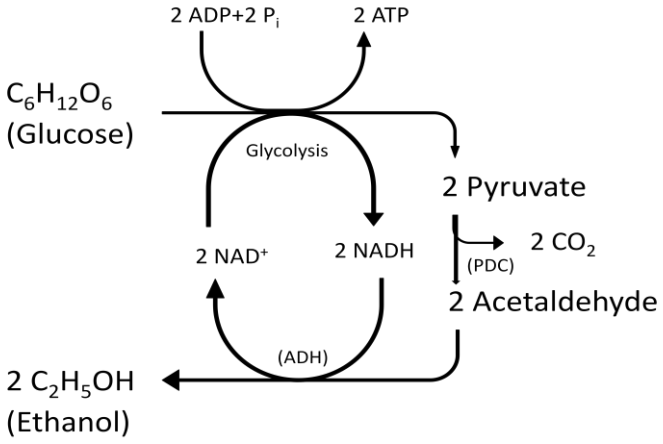

Fig. 1.1: Schematic representation of the ethanol fermentation pathway

1.2 Classification of bioethanol

Based on the substrate utilized for bioethanol production, several generations of bioethanol have been witnessed including the following:

1.2.1 First generation bioethanol

It includes the production of bioethanol using food crops such as barley, sugarcane or corn. The use of food substrate for bioethanol production not only has a huge impact (40-75%) on the cost of production but also is a threat to food supply especially in a developing country like India. Thus a better option was proposed which led to the second generation bioethanol production process.

1.2.2 Second generation bioethanol

Second generation bioethanol was produced using lingo-cellulosic waste. These waste solved the problem of food scarcity. At the same time, the production process was more cumbersome as compared to the first generation bioethanol as the plant cell wall is composed of complex macromolecules. As a result, the cost of bioethanol production using cellulosic waste was much higher as compared to that using corn. The drawbacks of second generation bioethanol include lignin removal issue. Lignin restricts fermentation process. Also, second-generation fuels demand feedstock that needs land in abundance (Um and Kim, 2009; Singh et al., 2011). This economical drawback led to the development of the third generation of bioethanol.

1.2.3 Third generation bioethanol

For third-generation algae was utilized for bioethanol production. There are several benefits of producing bioethanol using algae such as

i) Using algae as a substrate does not pose any threat to the food supply of human communities. Both aquatic, as well as terrestrial algae, may be used for production.

ii) The compositional study shows that algae contain a high amount of sugars and starch.

iii) Algae contain negligible lignin and very low amount of hemicelluloses. As a result, the efficiency of hydrolysis and yields are higher.

iv) Due to the photosynthetic ability of algae the concentration of CO_2 produced from industrial emissions can also be reduced.

v) Algae can be even grown on municipal wastewater.

vi) Use of aquatic algae can reduce the use of land which can be utilized for crop production.

vii) Microalgal cells have higher productivity as well as harvesting cycle as compared to another substrate.

1.3 Algae

Microalgae and macroalgae are the two algal categories on the basis of their morphology and size.

1.3.1 Macroalgae

Macroalgae are also known as seaweeds and are obtained from coastal regions. These algae are multicellular and have a complex structure which resembles the stems, leaves and roots of higher plants. They may belong to different groups on the basis of their pigments such as brown, green or red algae.

1.3.2 Microalgae

Microalgae are mostly photosynthetic, microscopic and unicellular. A major portion of atmospheric oxygen is produced by them. Diatoms, the golden algae and green algae are the major groups of microalgae. Microalgae are a promising substrate for ethanol generation as they show better growth, improved photosynthetic efficacy, and increased carbohydrate

content (Harun et al., 2010). They also combat greenhouse gases and are precious renewable sources when compared to other biomass resources (Harun et al., 2010; John et al., 2011).

1.4 Isolation and cultivation of microalgae

About 30,000 species of microalgae are known with a varied distribution ranging from marine waters, lakes, ponds, rivers and other freshwater sources. Artificially open ponds or bioreactors may be used for microalgal production. In open ponds, a turbine is used for stirring and circulation of the flow stream which also prevents the settling of algae. The cultivation of algae in open ponds is also associated with certain disadvantages such as inefficient utilization of light, losses caused by evaporation as well as large water and land requirements and low productivity of biomass. Also, there are risks of contamination and environmental variations of temperature, pH and CO_2 supply. Thus, the use of closed systems such as photobioreactors (PBRs) has been found to be more efficient for algae cultivation.

1.5 Microalgal biomass production in photo-bioreactors

Many types of PBRs such as Airlift, bubble column, biofilm, flat plate, multistage continuous flow, stirred tank and tubular flow have been effectively employed for the production of algae (Bahadar and Khan, 2013). Maximum biomass production depends upon the favouring conditions for the growth. Significant factors, which affect the growth of microalgae, are light, temperature, pH, nutrients and agitation. Lightpath is a significant parameter in algal production in a photobioreactor. Other factors are the culture volume and total illuminated surface area. Airlift photobioreactors have been widely used in the cultivation of various types of algae due to their simple construction and cost-effective operations. Maintenance cost and contamination risks are low with this kind of photobioreactors.

1.6 Enhancement of microalgae carbohydrate

Amongst microalgae, green algae (Chlorophyta) are a major source of starch which can be utilized for bioethanol production. Most of them contain chloroplasts containing chlorophylls a and b. True starch is stored in chloroplasts which is a storage product and is utilized by the cell as an energy source for its metabolism. The starch granules are visible by electron microscopy around a spherical structure located in chloroplasts known as pyrenoids. *Chlorella, Chlamydomonas, Scendesmus, Tetraselmis* are some of the microalgae known to contain a high quantity of starch and cellulose. Within chloroplasts, carbon dioxide gets converted into glucose which is then polymerized as starch using various biosynthesis enzymes as shown in Fig 1.2.

Starch accumulation changes with microalgae species and operational conditions. Carbon distribution in cells as carbohydrates and lipids involve light intensity, nutrient starvation, and CO_2 sequestration. Starch molecules are made up of linear as well as branched chains (amylose and amylopectins) which also affect the properties of starch. Many factors affect the starch synthesis as well as degradation including the light intensity, availability of nutrients and other environmental conditions. It was observed that with an increase in mean light intensity, the content of starch also increased (Zachleder and Brányiková, 2014). They also suggested that there is a restrictive temperature at which the accumulation of starch was significantly inhibited.

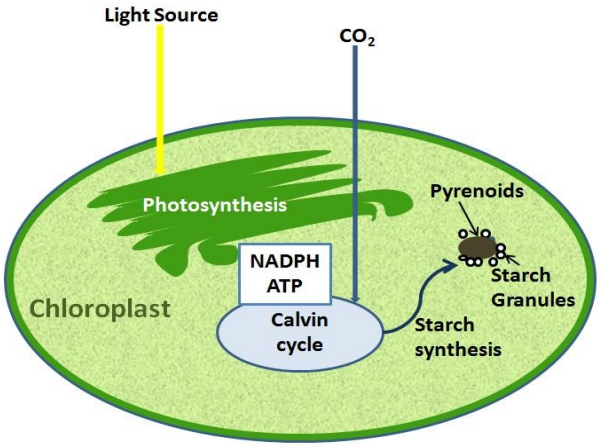

Fig 1.2: Starch synthesis in chloroplasts

The effect of inhibitors which blocked DNA replication was also studied and use of these inhibitors considerably enhanced starch production. Another eukaryotic protein synthesis inhibitor, known as cycloheximide was also found to positively contribute towards starch accumulation in microalgae (Douskova et al., 2008). Limiting the nutrient supply such as phosphorus and nitrogen also led to enhanced starch synthesis (Zachleder and Brányiková, 2014). Sulfur starvation helps in starch enhancement. Starch-rich biomass of microalgae can be obtained through increased light intensity as well as nitrogen deprivation (Markou and Nerantzis, 2013).

1.7 Pre-treatment of biomass

Pre-treatment of microalgae is quite cost-intensive in bioethanol production. Pretreatment procedure should be designed in order to lyse the cell wall and enhance the hydrolysis rate and other fermentation yields. Pre-treatment steps have been classified in Fig 1.3 which includes the pretreatment of both lignocellulosic as well as microalgal biomass.

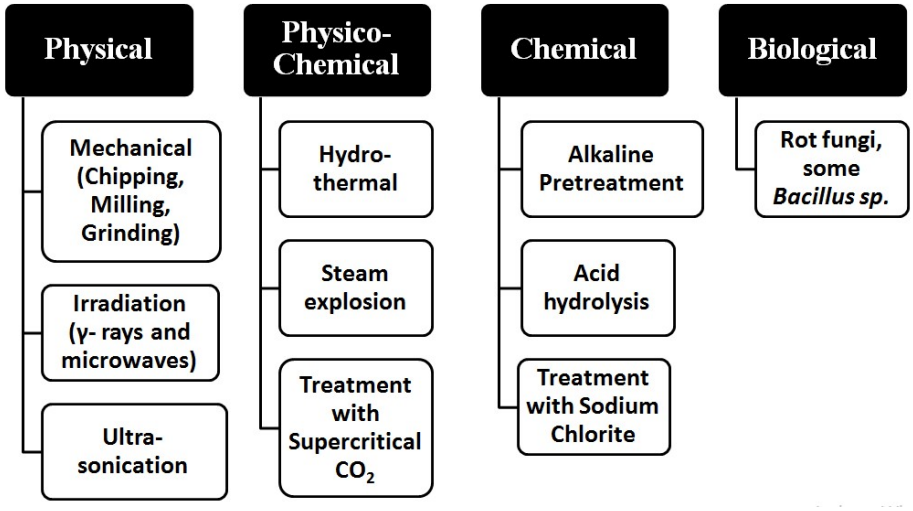

Fig 1.3: Various methods of pretreatment of microalgal biomass (Li et al., 2014)

Among various methods, acid hydrolysis is the most commonly used method for pre-treatment of biomass. Biological pretreatments are mostly used for lignocellulosic biomass. In other cases, enzymes such as α-amylase and glucoamylase may be used for pretreatment of microalgae.

1.8 Saccharification/ hydrolysis of carbohydrate/starch

Saccharification is a step of bioethanol production where the complex sugars are converted to simple monosaccharides which can be easily fermented by the ethanol producing microorganism. Both chemical and enzymatic hydrolysis methods have been employed for bioethanol production using microalgae. Cellulase and hemicellulase produced by *Trichoderma sp*. have been employed for saccharification stage. Li et al., have compared the values of sugar yield by both enzymatic and chemical hydrolysis from different algal biomass (Li et al., 2014). Shukla et al., studied the saccharification efficiency of whole algal biomass using the following equation (Shukla et al., 2016):

$$Percentage\ of\ saccharification = \frac{Sugar\ released\ during\ hydrolysis}{Total\ carbohydrate\ present\ in\ substrate} \times 100$$

1.9 Fermentation of microalgae carbohydrate

The simple sugars converted after saccharification step is fermented into bioethanol by microorganisms. Selection of microorganism is done on the basis of the yield of bioethanol obtained as well as tolerance to high sugar concentration. *Saccharomyces cerevisiae* (yeast) and *Zymomonas mobilis* are commonly employed for fermentation of microalgal sugar. The fermentation procedure can also be varied to study the effect of different modes such as batch, fed-batch and continuous on ethanol yield and productivity.

Some high yielding recombinant strains of bioethanol producers have also been developed by engineering Gram-negative bacteria such as *Escherichia coli* (Dien et al., 2003). Other process parameters can be manipulated to obtain higher volumetric production including the techniques of photobioreactors operations and environmental factors.

Bioethanol production involves the growth of biomass which is then pretreated to extract the complex sugars, these complex sugars are then hydrolysed in simple carbohydrates, which are then fermented into ethanol. Crude ethanol is then purified using distillation steps. Fig 1.4 shows the schematic diagram of the complete bioethanol production process from microalgal biomass. Different strategies can be adopted to optimize each step of the production process. Bioethanol can be produced by two fermentation processes i.e. SSF and SHF. One such strategy is combining the saccharification and fermentation step together which is known as Simultaneous saccharification and fermentation (SSF). The separate hydrolysis and

fermentation (SHF) is a process where saccharification and fermentation reactions take place in different reactors.

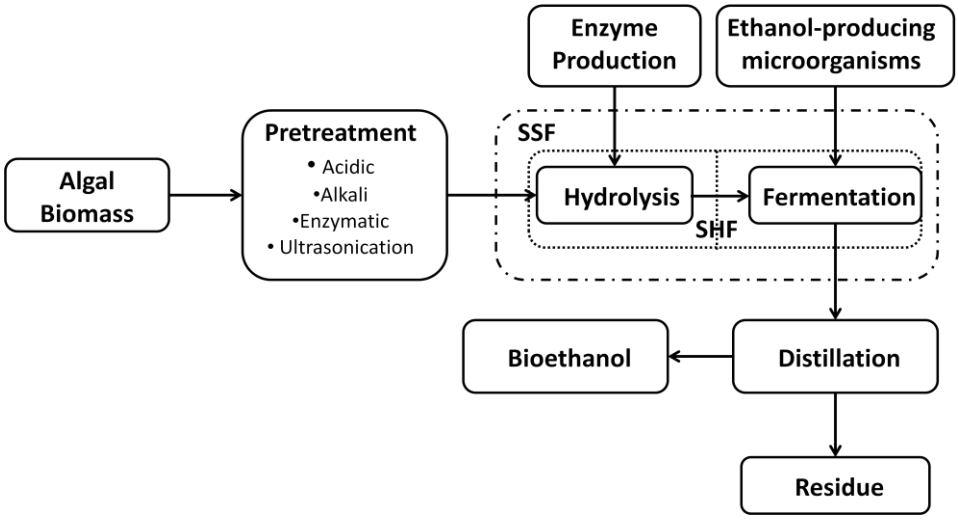

Fig1.4: Production process of Bioethanol (Li et al., 2014)