Chapter 1 INTRODUCTION

The development and urbanisation of a country are directly and positively associated with energy supply. Presently, fossil fuels fulfil approximately 80% of the total energy demand in the country. However, the problems associated with excessive consumption of fossil fuels have pushed the world in the evolution of alternative sources of clean energy. Biofuels are the fuels generated by thermo-chemical or biochemical processes from renewable substrates. Biofuel sustainability plays a crucial role in future energy generation.

1.1 Biofuel sustainability

The concept of sustainability is "the development that fulfils the needs of the present without compromising the ability of future generation to accomplish their needs". Some common elements of biofuel sustainability are optimal scale, efficiency, equity, socio-economic issues and environmental effects and emissions (Solomon, 2010). The replacement of fossil fuels with biofuels require large scale production. The element optimal scale includes water availability, labour and land requirements, soil degradation, air and water pollution from crop production, biofuel refining and loss of biodiversity (Solomon, 2010). Efficiency is the energy generation from fuel in return to energy consumption in its creation. Element equity considers the geographic distribution of resources and food versus fuel concern (Solomon, 2010). The socio-economic issues include low funding for extended biofuel production, job creation, and health concerns. Burning of traditional fuel generates harmful emissions that have an adverse effect on the human being. The substitution of traditional fuel to biofuel is beneficial to health, especially for women. The criteria to evaluate the environment impact considers greenhouse gas emissions, soil, water, air and ecosystem preservation.

1.2 Generations of biofuels

1.2.1 First-generation of biofuel

The first-generation biofuels are produced from food crops. Bioethanol and biodiesel are the two main types of biofuels used in the transportation sector. Bioethanol is an alternative of gasoline produced by the fermentation process. The substrate for bioethanol production is classified as sucrose-containing biomass (sugarbeet, sweet sorghum sugarcane, etc) and starchy feedstocks such as wheat, corn, barley, rice, etc(Hirani et al., 2018). Biodiesel is the substitute of diesel, generated by transesterification process using edible vegetable oil crops such as soybean, coconut, palm, peanut, rapeseed, etc. First-generation biofuels raise the food vs fuel concern. The amount of crops use to produce 1 terajoule of each of biodiesel and bioethanol can feed 110 and 90 people, respectively (Rulli et al., 2016). Moreover, increased deforestation rate, large CO_2 emissions, loss of habitat and threat to biodiversity were noticed in Malaysia and Indonesia due to replacement of old forest with oil palm plantations (Rulli et al., 2016).

1.2.2 Second generation of biofuel

Second-generation biofuels are produced either by the non-edible crop, specially grown on the land that is not suitable for growing food crops or the non-edible part of ordinary crops. It also includes other substrates such as forest residue, municipal solid waste, industrial effluent and food waste. The chemical properties of the cell wall present in lignocellulosic biomass create hindrance in the biofuel production process. Therefore, biomass complexity is reduced by altering the cell wall composition through different pretreatment techniques to augment their consumption for biofuel production. The problems associated with this generation biofuel are sophisticated downstream processes, land and water use competition and high production cost (Leong et al., 2018).

1.2.3 Third generation of biofuel

Third generation biofuels are produced by using algae as a substrate. Algae is characterised as macroalgae and microalgae according to their size and morphology. Macroalgae are also called seaweeds. These are multicellular algae found in marine habitat. The family of macroalgae includes red, green and brown algae. The blue-green algae (cyanobacteria) are also considered macroalgae (Suganya et al., 2016). Microalgae are the unicellular algae found in fresh or marine habitat. Algae are the promising feedstock as they are devoid of lignin and enriched with polysaccharides. Microalgae can produce special chemicals and nutritional products by using photosynthesis process. It has the ability of oxygenic photosynthesis and hydrogen production. Growth of microalgae can occur in both arable and non-arable land without the need for pesticides or herbicide (Rashid et al., 2013). Microalgae need less space to grow and also they can grow in a natural and artificial environment in an eco-friendly manner (Chen and Wu, 2011). The main limitations associated with this type of biofuel are insufficient biomass production for commercialisation and high production cost and stabilisation up to large scale.

1.2.4 Fourth generation of biofuel

The fourth-generation biofuels are produced by genetically modified biomass to achieve high production yield. The substrates used are engineered microalgae, macroalgae and cyanobacteria. The several improvement mechanisms are used to increase the biofuel production such as lipid and carbohydrate mechanism, improved efficiency of nutrient use, improved photosynthesis efficiency, increased stress tolerance, improved cell disintegration and bioflocculation (Abdullah et al., 2019; Das et al., 2020; Khan and Fu, 2020).

1.3 Biogas as biofuel

Biogas is the gaseous fuel produced by a series of steps with the help of several groups of microorganisms by utilising the biodegradable organic matter through anaerobic digestion (AD). It is primarily a mixture of CH_4 and CO_2 (Divya et al., 2015).

1.3.1Biogas composition

The composition of biogas is highly dependent on the substrate used in its production. Carbohydrate, fat and protein content of the substrate significantly affect the biogas composition. The methane content of biogas increases with increasing concentration of protein and fat (Chandra et al., 2012). The anaerobic digestion of carbohydrate, proteins and lipids generate biogas with methane concentration of 50, 68.8 and 69.5 %, respectively (Alves et al., 2009). Beside CO_2 and CH_4 traces of N_2, H_2S, O_2 and various volatile organic compounds are present in biogas according to substrate composition (Li et al., 2019). The biogas production from different options is depicted in Table 1.1.

Table 1.1 Composition of biogas for different options (Khan et al., 2017)

Biogas	CH_4 (%)	CO_2 (%)	N_2 (%)	O_2 (%)	H_2S (PPM)	Benzene (mg/m^3)	Toluene (mg/m^3)
Landfills	45-62	24-40	1-17	1-2.6	15-427	0.6-35.6	1.7-287
Sewage digesters	58-65	33-40	1-8	<1	0-24	0.1-0.3	2.8-11.8
Organic waste digesters	60-70	30-40	1	1-5	10-180	0.1-1.1	3-7

1.3.2 Advantages of biogas technology

A list of several advantages is given below:

1. Biogas is an eco-friendly fuel. Less greenhouse gas emission occurs during its combustion as compared to fossil fuels (Divya et al., 2015).
2. Biogas is produced from no cost or low-cost substrates. A variety of substrates such as organic solid waste, wastewater, lignocellulosic residue, food waste, etc. are used for biogas production (Divya et al., 2015). It simultaneously facilitates waste management and energy generation.
3. Biogas can be produced by using simple reactors and diverse microbial species (Nguyen et al., 2019).
4. There is limited need for downstream processing for separation of product as compared to liquid biofuels such as ethanol, butanol, etc. (Kleerebezem et al., 2015)
5. Besides biogas, anaerobic digestion also produces manure (digestate) enriched in macro and micronutrients (Divya et al., 2015).

1.3.3 Biochemistry and microbiology of Anaerobic digestion

The anaerobic digestion is the degradation of organic matter in absence of oxygen through the four steps of hydrolysis, acedogenesis, acetogenesis and methanogenesis as shown in Figure 1.1.

Hydrolysis

Firstly the complex matter such as carbohydrate, protein and fats are converted into simple sugars, amino acids and long-chain fatty acids by the action of extracellular enzymes produced by hydrolyting bacteria. Hydrolysis is dependent on the biodegradability of the substrate. Higher biodegradable matter needs less time for hydrolysis. The microbial species and enzymes involved are *Streptococcus*, *Bacillus*, *Enterobacteria*, etc. and cellulose, amylase, protease, lipase and xylanase, respectively (Divya et al., 2015).

Acedogenesis

The acedogenesis is the formation of short-chain fatty acids/ volatile fatty acids (VFA), alcohols, CO_2 and hydrogen mediated by acedogenic bacteria such as *Micrococcus*, *Syntrophomonas*, *Clostridium*, *Pseudomonas*, *Flavobacterium*, etc. by secreting the enzymes such as formate hydrogen lyase, acetaldehyde dehydrogenases, acetate kinase, etc.(Divya et al., 2015). The product formation of this phase is determined by the partial pressure of the hydrogen. In favourable condition, the production of acetate, CO_2 and hydrogen takes place at a low partial pressure of hydrogen while the formation of more intermediates such as alcohols, other VFAs (butyric, propionic, etc.) occur at a high partial pressure of hydrogen (Robles et al., 2018).

Acetogenesis

The products (VFAs and alcohol) of acedogenesis phase are converted into acetate, hydrogen and CO_2 in the third phase of anaerobic digestion known as acetogenesis. This phase is mediated by hydrogen-producing acetogenic bacteria such as *Syntrophomonas*, *Syntrophobactor,* etc. and H_2 utilising acetogenic bacteria like *Syntrophospora, Clostridium,* etc. with the help of the enzymes hydrogenases and carbon monoxide dehydrogenases, respectively (Divya et al., 2015). Acetogens can grow autotrophically and heterotrophically by using a wide range of carbon sources, electron donors and acceptors as shown in Table 1.2.

Methanogenesis

Methanogenesis is the final step of anaerobic digestion carried out by acetotrophic and hydrogenotrophic methanogens (Khan et al., 2016). A group of methanogens such as *Methanospirillum*, *Methanosarcina*, *Methanobacteria*, *Methanococcus,* etc. secret the enzymes formylmethanofuran dehydrogenase, methyl co-enzyme methyltransferase, etc. and produce the final product methane by consuming the acetate, H_2, CO_2 and methyl alcohol. Production reactions are given below (Robles et al., 2018).

$$4CH_3COOH \rightarrow 4CH_4 + 4CO_2 \quad (1.1)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (1.2)$$

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad (1.3)$$

$$CH_3OH + H_2 \rightarrow CH_4 + H_2O \quad (1.4)$$

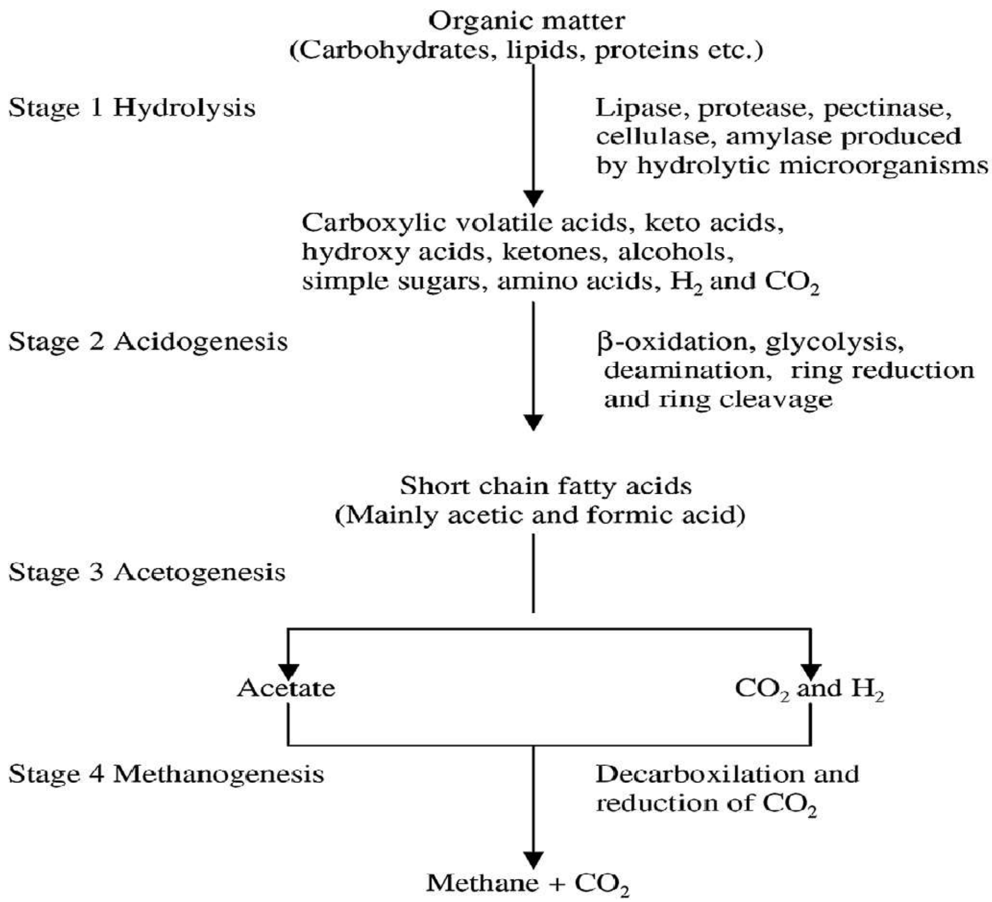

Figure 1.1 Different phases of anaerobic digestion (Diagram is employed from Chen et al., 2015)

Table 1.2 Electron donors and acceptors used by acetogenic bacteria (Schnurer, 2016)

Electron donors	Electron acceptor
CO	CO_2
H_2, Formate	Fumarate
Methyl chloride	Nitrate
Pyruvate	Thiosulfate
Lactate	Dimethylsulfoxide
Glycolate, glyoxylate	Pyruvate
Oxalate	Acetaldehyde
Methoxyacetate and methoxylated aromatics	H^+
Alcohols	
Hexoses, pentoses	
Betaine, acetoin	
Cellobiose	

1.4 Pretreatment methods

The hydrolysis is the rate-determining step of anaerobic digestion. It can be improved by using some pretreatment technique. Pretreatments are generally used due to several advantages such as the increased surface area of the substrate, decreased complexity, increased delignification, increased porosity that leads to better substrate utilisation and better productivity (Abraham et al., 2020). Pretreatments are broadly divided into physical, chemical, biological, and their combinations.

Physical pretreatment includes mechanical treatment such as milling, grinding, etc. to reduce particle size, extrusion that provides shear stress to disintegrate the substrate by the rise in temperature and pressure, the thermal treatment that includes hydrothermal treatment in the form of steam explosion (200-220 ℃) and hot water treatment (140-220 ℃) which provide auto-hydrolysis of biomass, microwave irradiation and ultrasound treatment that alters the internal structure of biomass (Yu et al., 2019; Duque et al., 2017).

Chemical treatment is done by loading of alkali (NaOH, $Ca(OH)_2$, ammonia, etc.) acid (H_2SO_4, HCl, SO_2, CH_3COOH,etc.), organic solvents (ethanol, methanol, etc.), hydrogen peroxide, ozone, NMMO, etc. to the substrate (Behera et al., 2014). The alkali treatment extensively dissolves lignin whereas the acid treatments directed to dissolve hemicellulose and cellulose (Behera et al., 2014).

Biological treatment is done by employing microorganisms such as white rot fungi (*Phanerochaetechrysosporium,* etc.) to degrade the lignin and reduce the complexity of the substrate (Wan and Li, 2012). The biological treatments are considered as environment-friendly and cost-effective treatment (Wan and Li, 2012). The combination of physical and chemical treatments such as thermo-acid (Hesami et al., 2015), microwave-alkali or acid (Zhu et al., 2016), hydrothermal-alkali (Chandra et al., 2012), a chemical with ultrasound treatments (You et al., 2019) etc. are effectively used to disorganise the cell wall and improve the biodegradability of the substrate.

1.5 Locally available biomass in Varanasi

Varanasi also is known as Banaras, spiritual capital of India, is a district located in the east of the Uttar Pradesh, India. The population of Varanasi was 3.67 million in the census of 2011. It is quite famous having industries of silk, carpets and craft. Besides, the city is also dependent on agricultural products.

1.5.1 Agricultural residue

The main crops grown in Varanasi district can be divided into four sections. These are commercial, fibres, foodgrains and oilseeds. Sugarcane is the only crop grown under commercial category. Sanhamp is the most grown fibre in the district. Foodgrains include cereals such as rice, wheat, corn, sorghum, millet, rye and pulses. Groundnut, linseed, rapeseed, mustard, sesamum and sunflower are grown under the category oilseeds. The productivity of wheat, paddy, maize, barley is 28.03, 20.20, 14.70 and 18.49 Quintal/hectare in the district. The various crops produce a large amount of residue that can be used as a substrate for biofuel production. The variety of trees such as Sagwan, Sissoo, Mango, etc. are extensively used for furniture manufacturing in Varanasi. As a result, a large amount of sawdust from the processing of woods of different trees is produced that can also be processed for biofuel production.

1.5.2 Other wastes

Sewage can also be used as a substrate for biofuel production. Different sewage treatment plants are located in Bhagwanpur, Dinapur and Diesel Locomotive Works (DLW). The amount of municipal solid waste (MSW) generated in Varanasi was 500 ton/d in 2015-16 according to the central pollution control board. There are various dumping sites such as Banaras Hindu University (BHU) campus, Bypass Vishwasundri road, Ramna dumping ground, etc.presentin Varanasi. The MSW can be collected from these dumping sites and used for biofuel production.

1.6 Origin of the problem

A large amount of waste generation and energy demand is the problem of a growing world. Biofuel production from organic waste is a magnificent way to mitigate the above-mentioned problems. Biofuel production includes biodiesel through transesterification, bioethanol, biomethanol, biobutanol, biohydrogen production from the fermentation, biogas production from anaerobic digestion and bio-oil, biochar and syn-gas from pyrolysis. The limitation of biodiesel production is the fuel vs. food dilemma as they formed from animal and vegetable fats. The fermentation is carried out by microorganism to produce biofuel. Special kind of species is used to generate the particular type of biofuel such as *Clostridium butylicum,* (Elsharnouby et al., 2013)for biohydrogen, *Saccharomyces cerevisiae* for bioethanol production (Azhar et al., 2017), etc. The maintenance of a special type of microbial culture is slightly difficult due to contamination of other microbial species (Beckner et al., 2011). It requires proper handling of the culture. The expensive bioreactors can add the cost to the process. The other limitation associated with liquid fuels is the steps involved in the downstream processing for their separation and purification (Patrascu et al., 2018). Pyrolysis is the thermo-chemical conversion of biomass into biofuel. It requires an associated energy source and special equipment (Canabarro et al., 2013). Therefore, biogas seems the most attractive option over other biofuels due to the associated benefits of technology. Hence, this work was decided and the objectives were deduced based on the literature survey.