CHAPTER - 1

INTRODUCTION

Issues of Carbon dioxide

The increasing of atmospheric carbon dioxide (CO$_2$) concentration is considered the main cause of global warming and climate change. About 31% of carbon dioxide has been increased in the atmosphere since 1750 (Fig. 1.1) (Sahoo et al., 2012). Various human activities have resulted in the liberation of the GHGs (greenhouse gases) namely carbon dioxide (CO$_2$), methane (CH$_4$), water vapour, chlorofluorocarbons (CFCs), nitrogen oxides (NOx), sulphur oxides (SOx) and many other gases in to the atmosphere. The major cause for the emissions of carbon dioxide (CO$_2$) is due to the combustion of fossil fuels used for electric power generation, transport and industries.

![Graph showing the increasing level of CO$_2$ from year 1750-2010 (Boden et al., 2010)](image)

Fig.1.1: The increasing level of CO$_2$ from year 1750-2010 (Boden et al., 2010)
The burning of fossil fuel is responsible for the emission of greenhouse gases such as Carbon dioxide (76%), Methane (13%), Nitrous oxide (6%) and Fluorocarbons (5%). Among these gases carbon dioxide is the major green house gas to cause climate change and global warming. The major CO₂ emitting industries are thermal power plants, cement, steel, glass and chemical industries etc. The Global warming and climate change is largely driven by the CO₂ and it is estimated that it is responsible for 60% to the phenomenon and continuing in upcoming years (Hall and House, 1995). The increase of high carbon dioxide in the atmosphere will create environmental disastrous including the rise in the earth’s temperature, acidification of ocean, melting of glaciers, rise in the sea level, extreme weather conditions, change in ecosystems and coral reef bleaching etc. (IPCC, 2007; Hill and Ralph 2008). The Thermal Power Stations are releasing the flue gas containing CO₂ and it contains about 4 – 14% and up to 0.022% NOx and SOx (Kumar et al., 2011).

**CO₂ Capture and Sequestration Technologies**

The accumulation of anthropogenic activities for the emission of carbon dioxide in the atmosphere is a reality and a challenge for science and technology today for climate change. The issue of increasing atmospheric CO₂ is necessary to be reduced via biologically and avoid the climate change and global warming. The definition of the carbon dioxide capture and sequestration is a separating of CO₂ from the industrial gases and energy related sources and transport to storage location for a long period isolation (IPCC, 2007). There are conventional methods for the Carbon dioxide capture and Sequestration (CCS) including CO₂ capture by chemical reaction method, CO₂ storage by the injection into deep Ocean and the CO₂ sequestration by biological method using photosynthesis through plantations, food
crops, aquatic photosynthetic micro-organisms and afforestation. Photosynthetic organisms are used for bio-fixation of CO$_2$ to stop or reduce the cause of global warming. Green photosynthetic plants are used for carbon sequestration, but which planting of trees are not cost effective, slow growth and require large amount of land, but photosynthetic algae are seen more feasible solution. The biological method of carbon dioxide capture and sequestration using microalgae is an attractive and proven approach for CO$_2$ fixation and sequestration (Yanayi et al., 1995; Wang et al., 2008).

**Microalgae CO$_2$ fixation**

Atmospheric CO$_2$ concentration was decreased over geologic time scale and O$_2$ concentration has increased through the functions of photosynthetic microorganisms. Therefore the photosynthetic organisms such as eukaryotic microalgae, cyanobacteria, and non-oxygen-evolving photosynthetic bacteria can adapt with the dramatic environmental and climate change. But the microalgae is having its own advantages like rapid multiplication rate, good survival rate in the adverse environmental conditions, cost effective techniques and less labour intensive. The microalgae can be isolated from different sources such as lakes, ponds, rivers, springs, soil, seawater, industrial waste water and sewage water etc.

Microalgae are unicellular or multi-cellular photosynthetic microorganisms that can able to fix CO$_2$ efficiently from different sources, such as from atmosphere, industrial exhaust gases and soluble carbonate salts. Microalgae are fast growing group of unicellular and multi cellular microorganisms which have the ability to fix the CO$_2$ from the atmosphere with high efficiency than that of
terrestrial plants and produce high biomass compared to energy crops. The most frequently used microalgal families are *Chlorophyceae* (green algae), *Cyanophyceae* (blue-green algae), *Bacillariophyceae* (including the diatoms) and *Chrysophyceae* (including golden algae) (Eppley and Dyer, 1965). Therefore, microalgae are the best candidate for CO₂ sequestration when compared to terrestrial land plants.

**Advantages of Microalgal Biomass**

The high CO₂ fixing microalgae contain high growth rate, high biomass production and it is useful for biodiesel production and eco-friendly with commercial usage and low cost. And also the production of microalgae biomass results many value added by products such as glycerol, pigments, food, feed and waste water recycling including biofuel. The advantage of carbon dioxide fixed microalgae biomass is incorporated into lipids and carbohydrates (Sawayama *et al.*, 1999; Lee *et al.*, 2001; Becker 1994; Metzger and Largeau 1999 and Olaizola 2003). Therefore, microalgae can be used for CO₂ capture from power plants, steel industries and cement industries plants as well as transport vehicles exhaust.

**CO₂ concentration Mechanisms (CCM)**

Photosynthetic organisms have developed special mechanisms for acclimating and adapting to changes in both CO₂ and O₂ concentrations, such mechanisms are called CO₂-concentrating mechanisms (CCM). During the low availability of CO₂ conditions *Chlamydomonas reinhardtii* and other microalgae can adapt to changes due to the induction of CCM. Algae have evolved carbon concentrating mechanisms (CCMs) during the imbalance between the high demand of inorganic carbon and low ambient CO₂ concentration. There are three major
constituents of CCM namely, i) Active bicarbonate (HCO$_3^-$) uptake transporters, ii) Suite of Carbonic anhydrases (CAs) localized strategically within the cells, iii) Sub-cellular micro-compartment within which most RuBisCO is located (i.e., the pyrenoids within the chloroplasts) (Meyer and Griffiths 2013). The functions of this enzyme to take up inorganic carbon from the external environment into the cells and to elevate the CO$_2$ level around Rubisco. The most studied component of the CCM in _C. reinhardtii_ is the extracellular carbonic anhydrase CAH1 and α-carbonic anhydrase that is encoded by the gene Cah1 (Fukuzawa _et al._, 1990).

**Carbonic anhydrase**

Carbonic anhydrase is an enzyme which catalyses the hydration and dehydration of CO$_2$ and plays a critical role in the physiology. The Carbonic anhydrases (CAs) are zinc-containing metallo-enzyme which is responsible to catalyze the reversible conversion of carbon dioxide to bicarbonate and protons (CO$_2$ + H$_2$O $\leftrightarrow$ HCO$_3^-$ + H$^+$) with very high efficiency. Various one or more CA activity has been reported inside and outside of the cell in depending upon the microalgal strains. Many unicellular microalgae consist of abundant external CA and increase the replacement of high CO$_2$ from bicarbonate. The enzyme Carbonic anhydrase are found in animal cells, plant cells, algal cells, archaeabacteria and eubacteria. They are isoymes and categorized into six families such as α, β, γ, δ, ε, and ζ respectively.

**Bio prospecting of microalgae**

Microalgae are producing high value byproducts such as polyunsaturated fatty acids, biofuels, natural colorants, pigments, vitamin A, minerals, biopolymers,
proteins, therapeutic substances, dietary supplements for human, animals and aquaculture and other bio-compounds (Cervantes et al., 2013; Lopez et al., 2009). Microalgae are consist of proteins (50-70%), lipids (30%), glycerol (40%), carotene (up to 8-14%) and also contains high concentration of vitamins B1, B2, B3, B6, B12, E, K, D, etc., compared to the other plants and animals (Priyadarshani and Rath, 2012). Microalgal compound β-1, 3-glucan is useful as an active immunostimulator, free-radical scavenger and reducing lipids from blood cells. The authors Priyadarshani and Rath (2012) were suggesting that β-1, 3-glucan compound also acts against gastric ulcers, wounds, constipation, and atherosclerosis, hypercholesterolemia and antitumor.

**Biodiesel from Microalgae**

The biodiesel (monoalkyl esters of long-chain fatty acids ) is defined as a Fatty acid methyl esters, it is derived from the Triacylglycerol (TAG), which can be synthesized from the biomass of plants, animals and microalgal cells. Biodiesel is a primary advantages and it is one of the most renewable fuels, non-toxic and biodegradable. The TAGs are consisting of three long chain fatty acids attached to a glycerol backbone. The biofuel productions by plants are very much lower and another alternative source is microalgae have been proposed for the biofuel production (Chisti 2007 and 2008). Algae can able to synthesize TAGs and it is considered as a second generation feedstock for production of biofuel specifically biodiesel.
Algae can be synthesizing fatty acids principally for esterification into glycerol-based membrane lipids, which contains about 5–20% of their DCW. Microalgae can synthesize and accumulate large quantities of neutral lipids and oil. The microalgae as a source of biofuel is not a new but it is now being taken seriously because of the rising price of petroleum and more important thing is emerging concern about global warming that is associated with burning of fossil fuels. Therefore, many researchers suggested that microalgae are very good candidates for fuel production because of their advantages like faster growth compared to other energy crops, higher photosynthetic efficiency and higher biomass production.

**Coal-based thermal power stations in the world**

About 124,287 MW of electricity have been installed by the year 2006, in which Thermal energy contributes about 66%, Hydro energy with 26%, Nuclear energy engulfs 3% and 5% was renewable energy. Coal-based thermal power station alone contributes about 85% of thermal energy production (World Energy Outlook, 2007). About 1200 Mt of CO\textsubscript{2} emission was estimated between the year 2007 and 2008, in which Coal-combustion alone contributes about 800 Mt (67%) of CO\textsubscript{2} emission (World Energy Outlook, 2007). Due to huge demand of coal for energy production accelerates the need for technologies to mitigate greenhouse gases liberated from coal combustion. Eventhough many conventional technologies are there to reduce air pollutants but which are associated with acid rain which addresses the negative effect of greenhouse gases. Comparatively, coal combustion emits more CO\textsubscript{2} than any other mode of thermal energy production including petroleum or natural gas. The flue gas liberated due to coal-based thermal energy production contributes about more than 7% of total CO\textsubscript{2} emissions in the world (Li et al., 2008).
Among the coal-based thermal power stations present around the world, China, United States and India are top three contributors with more number of coal-based thermal power stations (Fig. 1.2). The top three countries alone contributed to about 76% of coal-based thermal power production in comparison with other countries among the world (Fig. 1.3) (List of coal-based thermal power stations, Wikipedia).

![Fig. 1.2: Coal-based thermal power production among the world](image)

![Fig. 1.3: The top three contributors of coal-based thermal power production with other countries](image)
Status of thermal power stations in India

The Coal based thermal power plants occupies about 84% of power production in India followed by Gas based thermal power stations (15%) and diesel based thermal power station (1%) as of 2011 (Fig. 1.4). About 2.1 lakh mega watt capacity production of power was granted to 267 thermal power plants by the environment clearance (Union Ministry of Environment of Forests) between 2007 and 2011. Among the 267 thermal power plants 200 (74.90%) are coal based power stations (Fig. 1.5). In India the power capacity was granted about 91,500 MW in addition to the present installed capacity during the five year between 2007 and 2011 which was doubled the capacity of power production already installed in our country. The annual installation is increasing at an average of 7,500 MW for every five year plan. According to the number of coal based thermal power stations in India, the State Tamil Nadu placed on top with 27 coal-based thermal power stations (Fig. 1.6) [Thermal power plants report (CSE), 2011].

![Fig. 1.4: Percentage contribution of Coal based thermal power stations in India [Thermal power plants report (CSE), 2011].](image)
Fig. 1.5: The pie diagram shows the contribution of coal based thermal power stations in India [Thermal power plants report (CSE), 2011].

Fig. 1.6: Number of Coal based and other thermal power stations in India [Thermal power plants report (CSE), 2011].
Therefore, three coal-based thermal power stations were selected from three States including Tamil Nadu, Maharashtra and West Bengal. The selection was based on the presence of more number of coal-based thermal power stations in the states Tamil Nadu and Maharashtra. The State West Bengal was selected due to percentage of contribution of coal-based thermal power stations towards the power production rather than other mode of power production (Fig. 1.4).

**Kolaghat Thermal Power Station** is a major thermal power station in West Bengal. It is located at Mecheda, approx. 55 km from Kolkata in the Purba Medinipur district. The power plant is operated by West Bengal Power Development Corporation Limited. The power plant has six units of 210 MW each for a total capacity of **1260 MW**. The units were commissioned in two stages during the period of 1984 to 1995.

**The Kolaghat Thermal** Power Plant (KTPP) (22°10' N and 88°00' E) is situated on the right bank of the Rupnarayan river in the district of Purba Medinipur, West Bengal (Map 1). It is located 80 km south west of Kolkata and 50 km northwest of Haldia, one of the major industrial regions of South Bengal. The KTPP has a total installed capacity of 1260 MW with six units, generating about 7500-8000 metric tonnes of fly ash every day by consuming a total of 18000 tonnes of coal (*Source: KTPP Office, 2009*).

**Vallur Thermal Power Station** is a power plant located in Athipattu village, Vallur in Thiruvallur district, North Chennai, India. The power plant is operated by NTPC Tamil Nadu Energy Company Limited, a joint venture between NTPC Limited and TANGEDCO and has three units with 500 MW each.
The present research study focused on the isolation of potent waste water microalgal strains from three different thermal power stations Kolaghat Thermal Power Station, West Bengal; Vallur Thermal Power Station, Tamil Nadu and Khaparkheda Thermal Power Station, Maharashtra, India for CO$_2$ capture and sequestration from the flue gas emission of Thermal power stations using NaHCO$_3$ as carbon source. At the same point of view, the obtained microalgal biomasses were evaluated for the production of different kinds of fatty acid methyl esters towards biodiesel production.
OBJECTIVES

➢ Collection and Physio-Chemical analysis of waste water samples in and around the selected three Thermal Power Stations (Vallur, Kolaghat and Khaperkhada), in India.

➢ Isolation and Molecular Identification of microalgae from the collected waste water samples.

➢ Evaluation of \( \text{CO}_2 \) fixation rate in microalgae under various concentrations of \( \text{NaHCO}_3 \) with respective controls.

➢ Extraction and Transesterification of Fatty acid methyl esters from the micro algal biomass.

➢ Analysis of Fatty acid methyl esters (FAME) by GC–MS and FT-IR of selected Microalgae.

➢ Determination of the molecular weight of Carbonic anhydrase enzyme present in the selected microalgal strains by SDS-PAGE.

➢ Determination and comparative study of the carbonic anhydrase gene from selected microalgae