CHAPTER I

INTRODUCTION

1.1 World Energy Demand

Increasing population and industrialization has created serious problems of energy requirement. The current scenario of consumption of fuel has led to a situation that will be no oil reserves beyond 2050. Though, oil remains may be in a surplus amount, environmental pollution inclusive of CO_2, emission could be a jeopardizing effect globally, which might lead to climatic change (Yen et al., 2013). The world will need over 1.5 trillion barrels of crude oil to meet demand in the next 25 years alone. Since there is a strong correlation between energy consumption and standard of living and world population growth, higher demand of oil and petroleum based products are gradually increased day by day. The International Energy Agency (IEA) has reported in the reference scenario that world's primary energy need is projected to grow by 55% between 2005 and 2030, at an average annual rate of 1.8% per year. The petroleum reserves are highly concentrated in certain regions of the world, therefore those countries not having these resources are facing energy/foreign exchange crisis, mainly due to import of crude petroleum. It is predicted that 45% of the total energy requirements would be fulfilled by oil and gas which has a vital role in satisfying energy needs of the world (Khan et al., 2009). The petroleum reserves are highly concentrated in certain regions of the world, therefore those countries not having these resources are facing energy/foreign exchange crisis, mainly due to import of crude petroleum (Bisen et al., 2010). Thus a search of new alternative renewable forms of fuel has to be generated against fossil fuel to fulfill our needs.

1.2 India's Energy Demand

China and India are world's leading populated countries in the world and their annual oil consumption rate is 7.5% and 5.5% respectively. If the current situation is being continued that two major populated countries would face serious problems regarding fuel requirement for increased transportation and 45% of oil ought to be

consumed from the total amount of fossil fuel sources in 2030 (Khan et al., 2009). During the year 2005-2006, India has imported 99Mt of crude oil, incumbrating Rs. 171,702 crores on foreign exchange. India, at the end of 2007, produced 37.3 Mt of crude oil which is 1% of world's total crude oil whilst consuming 128.5 Mt which was 3.3% of total world consumption of crude oil. India's transportation fuel requirements are unique in the world. Because India consumes almost five times more diesel than the other countries in the world use gasoline than the diesel fuel. Due to high demand for fossil fuel it is expected that crude oil production will start declining from the beginning of 2012 (Khan et al., 2009).

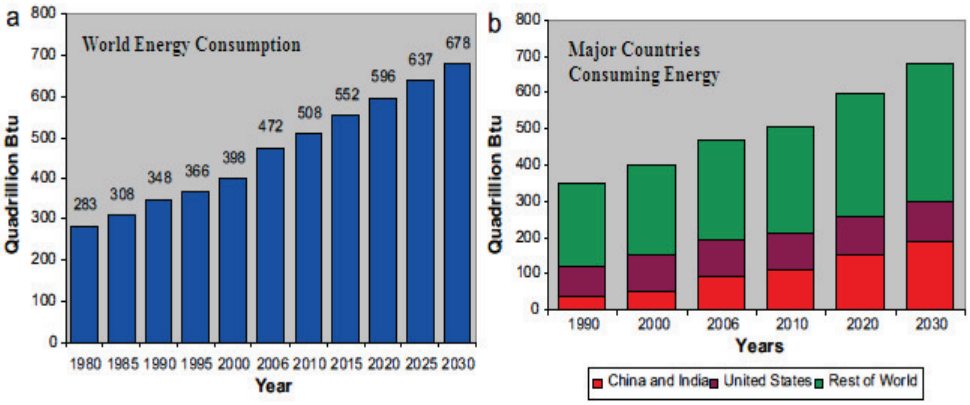

Fig.1 Comparison of energy consumption in past, present and future a) Total World Energy consumption, b) Major Countries consuming Energy

A number of developmental activities are being taken up in India for the production of biofuels, which include a 5% compulsory blend of ethanol in gasoline. These trials are ongoing in various state and the Government of India aims to increase the blends of biofuels with gasoline and diesel to 20% by 2017 (Leduc et al., 2009). India is already entered in the list of biodiesel producing countries. National Biodiesel Mission (NBM) is the biodiesel development plan formed by Planning Commission of the Government of India. NBM had conducted extensive study in the agricultural research centers and started to produce biodiesel from *Jatropha curcas*. The Jatropha nurseries were cultivated on 400,000 hectares (ha) waste land, setting up of seed collection and produce 80,000 Mt/year of biodiesel by transesterification method.

Biodiesel production has not yet commercialized in India and only two firms such as Naturol Bioenergy Limited (NBL) and Southern Online Biotechnologies have started the biodiesel production plant Andhra Pradesh State. Naturol Bioenergy Limited (NBL), a joint venture with the Austrian biodiesel firm Energea Gmbh and the investment firm Fe Clean Energy (USA), has planned to install a 300 tonnes/day (t/d)/(90,000 tonnes/year) (t/y) biodiesel plant in Kakinada, Andhra Pradesh. The State Government allocated 120,000 ha of land for Jatropha cultivation to the firm but cultivation has not yet begun or is in initial stage. The farmers were demanding that the market set the oilseed price, but NBL wants the government to fix a price to reduce its risks in production. Southern Online Biotechnologies has a 30 t/d (9000 t/y) project, which would require about 9500 t/y of oil. It was expected to get about 6000 t/y through cultivation of *Jatropha* and *Pongamia pinnata* oilseeds on wasteland, and plans to make up the balance through animal fats, but the cultivation of these two cultivar have been failed (Khan et al., 2009). Thus in India research should be focused and initiated on alternative to fossil fuel which is more necessary for us than any other country. Therefore, biodiesel is obviously the only alternative option to fulfill the demands.

1.3 Conventional and Alternative Renewable Energy Sources

The basic sources of this energy consists of oil (36%), natural gas (24%), coal (28%), nuclear (6%), and renewable energy such as hydro, wind, and solar (about 7%) (Bajhaiya et al., 2010; Subramaniam et al., 2010; Demirbas and Faith Demirbas, 2010). Generally the conventional sources are referred to as non-renewable sources of energy, which are being used since a long time. These sources of energy are being used extensively in such a way that their known reserves have been depleted to a great extent. But major sectors are mainly depending on non-renewable energy sources such as fossil fuels and their major disadvantage of using petroleum based fuel is atmospheric pollution. Petroleum diesel combustion is also a major source of green house gas (GHG). Apart from these emissions, petroleum diesel combustion is also a major source of other air contaminants including NOx, SOx, COs, particulate matter and volatile organic compounds, which are adversely affecting the environment and causing air pollution resulting in human health problems (Bajhaiya et al., 2010; Subramaniam et al., 2010;

Kozlovska et al., 2012). Coal is one of the most important sources of energy and is being used for various proposes such as heating of housed, as fuel for boilers and steam engines and for generation of electricity by thermal plants. Coal has also become a precious source of production of chemical of industrial importance coal is and will continue to be the mainstay of power generation in India. It constitutes about 70% of total commercial energy consumed in the country. Like coal, petroleum is also derived from plants and also from dead animals that lived in remote past. Natural gas has also been produced in the Earth's curst by the similar process as petroleum and this is also a combustible fuel.

Renewable energy is promising substitute for non-renewable sources due to its clean and ecofriendly in nature. The renewable energy sources are available free of cost, are pollution-free and inexhaustible. Man has used these sources for many centuries in propelling ships, driving windmills for grinding corn and pumping water, etc. Because of the poor technologies then existing, the cost of harnessing energy from these sources was quite high. However, renewable energy sources like hydro, solar, wind and nuclear will not meet the rising demand for road, rail and air transport because of political, economical and practical issues (Demirbas and Faith Demirbas, 2011). Also because of uncertainty of period of availability and the difficulty of transporting this form of energy, to the place of its use are some of the factors which came in the way of its adoption or development.

1.4 Need of Alternative Fuel

The energy sources are grouped into renewable and non-renewable and energy source from the non-renewable is limited. Since the drastic increase in the burgeoning population have created serious problem of energy requirement, there is a current worldwide interest in finding new alternative fuel. Over 80% of the energy we use comes from fossil fuels such as petroleum, coal and natural gas (Demirbas and Faith Demirbas, 2011). About 98% of carbon emissions result from fossil fuel combustion (Balat, 2011). Over consumption of hydrocarbons leading to accumulation of green house gases (GHG) in atmosphere resulted in Global Warming (GW) (Su et al., 2007; Mata et al., 2010; Xin et al., 2011; Ashokkumar and Rengasamy, 2012). The transportation sector worldwide is

almost entirely dependent on petroleum-derived fuels and one-fifth of global CO_2 emissions are created by this sector, which accounts for some 60% of global oil consumption. Around the world, there were about 806 million cars and light trucks on the road. These numbers are predicted to increase to 1.3 billion by 2030 and to over 2 billion vehicles by 2050. GHG contributes not only resulted in global warming (GW) but also to create other impacts on the environment and human life. For example oceans absorb approximately one-third of the CO_2 emitted each year by human activities and as its levels increase in the atmosphere, the amount dissolved in oceans will also increase turning the water pH gradually to more acidic. This pH decrease may cause the quick loss of coral reefs and of marine ecosystem biodiversity with huge implications in ocean life and consequently in earth life. As GW is a problem affecting different aspects of human life and the global environment, not only a single but a host of solutions is needed to address it. One side of the problem concerns the reduction of crude oil reserves and difficulties in their extraction and processing, leading to an increase of its cost (Mata et al., 2010). To find clean and renewable energy sources ranks as one of the most challenging problems facing mankind in the medium to long term. One of the alternatives for liquid fossil fuels which is gaining popularity in recent years is biodiesel. Biodiesel fuel is a direct replacement for conventional diesel fuel in vehicle engines, which is obtained by the transesterification of vegetable oil and animal fat with monohydric alcohols (Kulkarni and Dalai, 2006; Costa and de Morais, 2011).

1.5 Overview of Biodiesel Production

Currently the overall process of production of biodiesel is higher than the petroleum products because the cost of substrate accounts for about 75% of the total production (Ahmad et al., 2011). Hence selecting the suitable feedstock is important for reducing production cost. Table 1 shows different feed stocks for the production of biodiesel in various countries.

Table 1 Various Feedstock for the Biodiesel production in different Countries (Adopted from Ahmad et al., 2011)

Country	Feedstock
USA	Soybean
Europe/EU	Rapeseed, Sunflower
Western Canada	Canola oil
Africa	Jatropha
India	Jatropha
Malaysia/Indonesia	Palm oil
Philippines	Coconut
China	Waste cooking oil
Spain	Linseed oil
Greece	Cottonseed

Generally biodiesel is produced by transesterification method using acid, alkali and enzymatic transesterification process. Predominant drawback of the acid and alkali transesterification processes are energy input, elimination of salt, difficulty in recycling glycerol, soap formation and need waste water treatment (Ognjanovic et al., 2009; Bisen et al., 2010; Jeon and Yeom 2010; Kawakami et al., 2011; Rodrigues and Ayub, 2011; Yoshida et al., 2012).

Recently, research has been oriented towards production of biodiesel by enzymatic synthesis. During enzymatic transesterification, soap does not formed when the oil contains high FFA, waste water treatment is not required, high production yield under milder conditions, ecofriendly process and produces high quality of biodiesel. Although the cost of enzymes is high, it can be cost effective by repeated use of lipase enzymes by immobilization technique (Salum at al., 2010; Gumbyte et al., 2011; Gharat and Rathod et al., 2013).

Most of lipases are sensitive and easily inactivated by short chain alcohols such as ethanol and methanol during enzymatic transesterification (Shimada et al., 1999;

Sivaramakrishnan and Muthukumar, 2012). In this research work marine microalgae oil are used for biodiesel production by alternative method of non alcoholic enzymatic route with methyl acetate used as acyl acceptor.

1.6 Objective

The main purpose of this work was to produce biodiesel from two marine microalgae *Chlorella salina* and *Nannochloropsis oculata* using immobilised lipase using interesterfication. This research has several aims to achieve its desired output and the following objectives have been undertaken to:

- To optimize the growth conditions of marine microalgae
- To study the effect of nitrogen on growth of microalgae and enhance the intracellular lipid accumulation at under nitrogen depleted condition
- To harvest microalgae using chemical flocculants and find out flocculation efficiency of different chemicals
- To study the effect of light, darkness and temperature on enhancing flocculation efficiency of microalgae
- To harvest microalgae using bioflocculant, marine bacterium *Bacillus subtilis* and to evaluate various parameters which enhance the bioflocculation using Response Surface Methodology (RSM)
- To study the various lipid extraction techniques to extract intracellular oil from microalgae by conventional methods
- To evaluate the various pretreatment processes for enhancing intracellular oil recovery on microalgae which are grown under nitrogen repleted and depleted conditions

- To isolate the lipase producing novel strain from marine sediment using Tween20 agar and identify the species using morphological, biochemical characterization and confirmed by gene sequence using 16S rRNA

- To immobilise the crude lipase extracted from marine bacteria using sodium alginate for interesterification process

- To evaluate and optimise the various parameters involved in enzymatic interesterification of marine microalgae oil

- To analyse the fatty acid compositions of biodiesel produced from microalgae using Gas Chromatography

1.7 Organization of the Thesis

There are five chapters in this thesis and each chapter gives important information of the thesis and organized as follows;

Chapter 1: Introduction and Background of biodiesel and its importance

Chapter 2: Review of Literature- Provides detailed survey of significance of microalgae, lipid content and biomass productivity of various microalgal species, cultivation, harvesting of microalgae and different lipid extraction methods, various sources of biodiesel, various methods of biodiesel production, various catalysts employed for biodiesel, factors affecting biodiesel yield and uses of algae.

Chapter 3: This chapter demonstrates the materials and methods involved in cultivation of selected marine microalgae, growth parameters, harvesting by chemical flocculants. Optimization of bioflocculation using RSM, procedures for extracting of oil using different lipid extraction methods, increase lipid content by nitrogen depletion and various pretreatment processes for enhance oil recovery. It also contains isolation of lipase producing marine bacteria, analysis of its biochemical characterization and construction of phylogenetic tree to confirm the bacterial strain. Lipase production, immobilization and analysis of various parameters affecting biodiesel yield.

Chapter 4: Describe in details of results and discussions for each topics. The optimum conditions are identified for better microalgae growth, effect of parameters affecting harvesting of marine microalgae was reported in detail. Kinetic modeling was done for cell growth and product formation and reported. Complete details of parameters affecting immobilised lipase interesterification reaction and biodiesel yield. Fatty acid composition of biodiesel obtained from two marine microalgae was reported.

Chapter 5: Conclusion: Summarizes the all experiments carried out and fulfill the objective of the research. Specify the future research to be carried out in marine microalgal biodiesel productions.