5. Summary and Conclusion

The environmental changes, depletion of fossil fuels and their high cost have forced the human generation to search for alternative fuels. All problems related majorly to the energy crisis can only be solved when new alternatives and sustainable energy sources are developed to replace existing crude oil based fuels. Biodiesel is an alternative liquid fuel produced by chemical reaction between a plant oils or animal fats in presence of a catalyst which is eco-friendly and renewable but, the use of edible vegetable oils for fuel production will compete significantly with food uses, and this would result in undesirable increase in food and biodiesel costs. Algal oils were found to be a good alternative for the production of biodiesel rather than vegetable oils. Microalgae hold great promise for producing non edible oil yields in optimized environment condition.

Microalgae are unicellular auto-photosynthetic organisms which are found flourishing in from vast stretches of river, lake, ponds, marshy swamps, hot water springs, salt and brackish water, frosty rivers etc. For biodiesel production these microalgal species are initially raised in large shallow pond, raceways or controlled photobioreactors. The major pros of algal based biofuels are, these fuels are carbon neutral combustion fuels, are inherently renewable, they absorb CO\textsubscript{2} as they grow therefore, utilize both waste CO\textsubscript{2} and waste water as nutrients. They have higher energy per acre as compared to other biofuels and lastly they can be grown on the land unsuitable for other types of agriculture. Hence from this we can conclude that algal based biofuels are promising source which are in latter stages of development.

Microalgae *Chlorella vulgaris* was chosen to study the effect of various culture conditions on the growth, biomass, lipid accumulation and on photosynthetic pigment composition of the alga. The growth conditions which were studied and optimized were variable pH range, variable salinity, varying the concentration of nitrogen (NaNO\textsubscript{3}), phosphorus (K\textsubscript{2}HPO\textsubscript{4}). These conditions were studied and optimized in order to understand that which all factors are suitable stimulants of lipid production. The combined effect of variable salt, nitrogen and phosphorus on lipid productivity was also studied using response surface methodology. Response surface methodology is a statistical tool which saves time, effort and cost of
chemicals by reducing the trail runs of experiments. The optimum condition obtained by RSM model was then used to grow *Chlorella vulgaris* and lipid sample extracted was tranesterified for FAME analysis to understand composition of algal oil.

In response to pH *Chlorella vulgaris* depicted that maximum (statistically significant) growth and biomass yield occurs at control pH i.e 7 and decrease as range move towards alkaline pH (8 and 9) the result concluded that at the higher pH the carbon availability is limited hence growth is suppressed. The growth and biomass were directly proportional to each other. In terms of days both growth and biomass had shown increasing trend from 0 to 21 day after which the declining trend was observed. The lipid accumulation at pH 9 was highest and as the pH was decreased the lipid accumulation also showed decrease. In all range of pH, maximum amount of pigments (chl a, chl b, total chl, carotenoids) were observed in pH 7 on 28th day. Minimum amount of contents was seen in pH 9 for chla and total chl while, pH 8 for chl b and pH 6 for carotenoids.

In variable salinity range it was observed that the control culture (0.00 M) showed maximum growth with 6 fold increase on 21\textsuperscript{st} day and it decreased as salinity in medium was increased. The biomass also showed similar trend as shown by the growth i.e highest biomass was observed in control culture minimum in culture growing in 0.25 M saline medium. These result could be associated with the fact that *Chlorella vulgaris* was unable to adapt at higher saline ranges. The highest lipid yield was observed in culture growing in 0.25 M saline medium with approximate 3 fold increases and lowest was observed in control culture (0.0 M saline medium with respect to zero day on day 28. The yield increased from zero day to 28th day. During the research it was observed that maximum chlorophyll contents were observed on lower salinity.

*Chlorella vulgaris* depicted a profound rise in lipid yield, the culture growing in NaNO\textsubscript{3} deficient medium and culture growing in medium in which NaNO\textsubscript{3} is replaced by NaCl showed significant increase. However, the lower nitrogen range had adverse effect on growth, biomass and photosynthetic pigments.
Similar are the results for phosphorus limitation or deficiency, the highest growth was seen on control culture (0.04 g l\(^{-1}\)) which significantly increased from zero day to day 28\(^{th}\) likewise, biomass yield was also found maximum on control medium which continuously reduced on decreasing concentration of phosphorous and minimum yield was found on medium without phosphorous and replaced by KCl. Whereas, lipid contents increased with the phosphorous concentration decrease. Lipid content in *Chlorella vulgaris* showed significant increase with number of days of incubation. Minimum lipid content was observed in control culture (0.04 g l\(^{-1}\) K\(_2\)HPO\(_4\)). Maximum lipid yield was observed in culture deficient with K\(_2\)HPO\(_4\) and K\(_2\)HPO\(_4\) replaced by KCl.

Therefore, we can conclude that nitrogen and phosphorus limitation also appeared to be suitable stimulant for lipid accumulation. With the effect of nitrogen and phosphorus limitation we can say that lipid accumulation enhanced when growth was restricted due to unavailability of nitrogen and phosphorus.

Maximum amount photosynthetic pigments were recorded on 0.04 g l\(^{-1}\) phosphorous. Phosphorus starvation reduces chlorophyll a and protein content thereby increasing the relative carbohydrate content in algal cells. Phosphate deficiency has been demonstrated to result in accumulation of astaxanthin and an overall reduction in cell growth.

The results to determine lipid productivity as response to combined effect of variable salt, nitrogen and phosphorus using RSM, the highest lipid content of 25 % DCW and lipid productivity of 84.23 mg l\(^{-1}\)d\(^{-1}\) was obtained in *Chlorella vulgaris* under nitrogen, phosphorus deficient and NaCl 0.15 M conditions. BG 11 control culture had minimum lipid content as well as lipid productivity. These results showed the significance of combined stress that increased lipid productivity by 5.25 times and lipid content by 2.70 times as compared to BG11 medium. Response surface methodology showed that for *Chlorella vulgaris*, the most influential factor affecting the lipid yield was nitrogen followed by phosphorus whereas, NaCl did not show significant influence. The limitation of nitrogen can be considered as an efficient environmental pressure to enhance lipid accumulation. The fatty acid
profile of lipid extracted from *Chlorella vulgaris* under optimum condition obtained by RSM showed suitable fatty acid composition which is required for biodiesel production.

The literature states that polyunsaturated fatty acids with four or more double bonds are commonly seen in microalgal lipid. These bonds are susceptible to oxidation during storage and thus reduce the acceptability of microalgal oil for production of biodiesel. In gas chromatography study the major fatty acids found in algal culture were saturated and mono-saturated fatty acids namely, oleic acid, palmitic acid, stearic acid hence showing the oxidative stability of the biodiesel produced from *Chlorella vulgaris*.

Most of the observations of high lipid content come from algal cultures grown under nutrient (especially nitrogen, phosphorous, or silicon) limitation. Lipid content varies in both quantity and quality with varied growth conditions. While high lipid yields can be obtained under nutrient limitation, this is obtained at the expense of reduced biomass yields.

The green fuel is demand of future generation and it is important to focus on algal biofuels as they can benefit humans in number of ways like they don’t compete with food production and the use of bi-products obtained in the process can be utilized for cost reduction. The significance of this research is that the conditions obtained for high lipid productivity can be applied on large scale to obtain high lipid yield which imperative for high biodiesel yield.

The future prospects for this research include genetic engineering which play an important role in enhancing the overall life cycle of microalgae that are used as biofuels feedstock. Genetic modified microalgae that have the capability to grow under high concentration of CO$_2$, able to tolerate other components in flue gases or wastewaters, and could produce high lipid content within their cells may be created.