STUDY ON VARIOUS PHYSICO-CHEMICAL AND PHYSIOLOGICAL FACTORS REGULATING CARBON SEQUESTRATION, BIOMASS AND LIPID PRODUCTION IN MICROALGAE

SUMMARY OF THESIS

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Summary

Global warming and climate change have become serious environmental issues, across the globe. This prompts for a quick action to minimize the high concentration of CO_2, (the most important green house gas), from the atmosphere. Although carbon capture and storage methods have been worked upon to a large extent, their cost-effectiveness and long-term environmental safety is also a cause of concern. Bio-sequestration of carbon in organic, inorganic, or gaseous formusing microalgae, the sun-driven sink of carbonhas emerged as an alternative way of converting CO_2into biomass.The fixed form of organic and inorganic carbon, if transformed into gaseous CO_2, may enhance the intensity of global warming. One important strategy to reduce atmospheric CO_2involves carbon fixation by photosynthetic organisms such as plants, algae, and cyanobacteria. In both the natural and engineered systems the amount of carbon fixed in the form of biomass and lipid is highly influenced by nutrient availability and environmental factors. Microalgae appear to be reliable organisms due to multiple benefits from short life cycles to profuse growth rates and high CO_2 fixation capacity as compared to plants. Over several decades microalgae have attained huge impetus from both academia and industry; however, their cultivation and further processing are techno-economically demanding. Microalgae in addition to CO_2 capture can produce biomass, which can further be utilised in generating industrially important products. The cultivation of microalgae for carbon capture and utilizing biomass for extracting valuable products give algal bio-sequestration an advantage over other methods.

Algae have gained tolerance to environmental stress conditions including, high and varying nutrient levels, changing pH conditions, high and low light intensities. It becomes very important to understand and monitor the potential factors that might

affect the algal growth rate and carbon sequestration potential. The algal biomass can be used as feedstock for several valuable products, including biofuel production, food, feed, fertilizer, and pharmaceutical products. Literature reveals that significant work has been doneon carbon sequestration usingmicroalgae. Very few studies are available on the effect of physiological and physico-chemical factors on the photosynthetic apparatus and macromolecule of microalgae. Therefore, the present thesis is devoted to understanding the synergistic interaction among various physiological, physico-chemical factors and nutritional variations to develop a highly productive bio algae system, which is much needed for commercializing biofuel production.

In the present investigation, an attempt has been made to study the ability of *C. vulgaris* to withstand the various environmental stresses and nutritional alterations. The overall stress tolerance ability was exploited for carbon sequestration, biomass production, and improvement in lipid synthesis, which serves as a source of biofuel. The first objective of the work was to isolate a strain of microalgae, which has the potential to sequester more carbon and could yield a better amount of biomass. The carbon sequestration was studied in three different forms of carbon, i.e, organic carbon, inorganic and gaseous forms of carbon. The growthof microalgae under harsh environmental conditions such as varied pH conditions, excess CO_2 supply combined with high light intensity, different spectral quality of light, salinity stress, limited nitrogen nutrition, and phosphorous nutrition, was also optimized concerning photosynthetic efficiency, biomass, total organic carbon content, proline, and lipid content.

The present research work can be concluded as:

- Microalga *C. vulgaris* was grown under varying doses of sodium bicarbonate. The cells of *C. vulgaris* in response to the addition of $NaHCO_3$ (0-25 mM) showed the best growth and maximum concentration of all the biomolecules (protein, lipid, and carbohydrate) at 20 mM, $NaHCO_3$ supplementation.

- The role of sodium bicarbonate in the regulation of salinity stress-induced alterations in the growth, nutrient status of the cells, photosynthetic performance, and level of cell constituents including lipid in the microalga *C. vulgaris* was studied. The cells of *C. vulgaris*grown in the presence of NaClconcentrations (0-400 mM), with and without bicarbonate (20 mM) showed a higher level of growth, protein, and carbohydrate, suggesting an improved NaCl tolerance of microalga due to the presence of bicarbonate. However, total lipid and proline content showed an increase with the rising concentration of NaCl, particularly in the absence of bicarbonate.
- The addition of $NaHCO_3$ (20 mM) exhibited its antagonistic effect against the adverse effect of salinity on the growth, level of macromolecules except for proline.This was further confirmed by the SEM-EDS analysis of NaCl treated cells, exhibiting morphological variations as well as reduced accumulation of Na and Cl. The chlorophyll fluorescence induction kinetics revealed NaCl induced decline in the photosynthetic performance and quantum yield was mitigated by the presence of $NaHCO_3$, while non-photochemical quenching of chlorophyll fluorescence was enhanced.Since the ambient CO_2 concentration can not be controlled in the open water bodies, we have selected inorganic bicarbonate salt to see its impact on the biomass production. The present work isan effort to manipulate the growthconditions of microalga in the aquatic system by adding $NaHCO_3$, which mitigates the effect of salinity stress on the synthesis of macromolecules as well as photosynthesis.

- The effect of varying pH conditions on algal growth and cell constituents in the *C. vulgaris* was examined. In addition, the interactive effect of bicarbonate and pH conditions on the photochemistry of PSII and cell constituents like protein, carbohydrate, total organic carbon, and lipid. The present findings on microalga *C. vulgaris* showed a pH-dependent increase in the level of protein and carbohydrate content by the addition of bicarbonate, particularly between pH 7.5-8.5. However, the presence of $NaHCO_3$ enhanced the total organic carbon (TOC) and lipid content with increasing pH (6.5 to 10.5). These observations were supported by the FTIR analysis of cell constituents in response to pH as well bicarbonate.
- The photosynthetic parameters like Fv/Fm, Mo, ABS/RC, and ETo/RC showed optimum values at pH 8.5. However, these parameters were negatively influenced by the addition of HCO_3^-, particularly under alkaline pH conditions (pH 8.5-10.5).The values ABS/RC, NPQ, and qE values significantly increased with increasing alkalinity as well as with the addition of HCO_3^-.
- The effect of enhanced alkalinity, as well as bicarbonate, was significantly reversed by the uuncouplers and ptrotonophores, suggesting that the maximum adverse effect of both bicarbonate alkalinity was mediated by the high proton gradient across the membrane.
- Combined role of CO_2 and light intensity in the regulation of photochemistry of PS II, which has been elucidated by studying the photosynthetic performance parameters of microalga under varying light intensities.The present study contradicts the earlier notion of converting the excess light energy and CO_2 into higher biomass. The present findings have revealed that the excess CO_2 does not mitigate the photoinhibitory effect of high light intensity, but the presence

of excess CO_2 under the high light intensity acts synergistically to enhance the photoinhibition.

- Microalga *C. vulgaris* grown under different concentrations of CO_2 as well as light intensity (50-300 µmol photons/m^2/s). The optimum growth of *C. vulgaris* was observed at 5% CO_2 and 150 µmol photons/m$^{2/}$s. The cell constituents like chlorophyll, protein, and carbohydrate in the cells at low light intensities were enhanced in the presence of excess CO_2 (5%) as compared to air-grown cell culture.
- The lipid content showed a light intensity-dependent gradual increaseof up to 300 µmol photons/m$^{2/}$s, which was further increased in the presence of excess CO_2.
- The photosynthetic parameters like Fv/Fm, Fv/Fo, Mo, Vj, ABS/RC, and TRo/RC showed that excess CO_2 supported improved functioning of photosynthetic apparatus under low light (50 µmol photons/m$^{2/}$s) (LL). However, under the high light (HL) condition, the photosynthetic parameters indicated that the primary photochemistry of PSII was negatively influenced by the excess CO_2.
- The results showed a synergistic effect of CO_2 and high light intensity on the photochemistry of PSII as evident from was an overall decline in the photosynthetic performance index (PIabs), non-photochemical quenching (NPQ), and relative fluorescence decrease (Rfd) ratio in the presence of excess CO_2.
- The other objective of the study wasbased on the mechanism of cellular adaptation to nitrogen limiting conditions, which is crucial for achieving higher lipid productivity. It deals with the biomass, cell composition, nutrient status

and photosynthetic attributes of unicellular microalga *C. vulgaris* in response to varying C:N ratios. The *C. vulgaris*cells supplemented with glucose, sodium acetate, and sodium citrate (2-20 mM, each) were grown photoheterotrophically for 16 days. The results revealed that microalga initially grew better in the presence of glucose up to 7^{th} day, followed by a gradual decline, perhaps due to depletion of the substrate. The sodium acetate showed its best effect on the *C. vulgaris* up to 16^{th} day as compared to control (without organic substrate).

- Effect of different molar C (Acetate, 4mM):N (0-20 mM) ratio on the growth of microalga revealed maximum growth,Protein, and chlorophyll up to C:N ratio of 1:1. However, carbohydrate content linearly increased with increasing C:N ratio (1:0- 1: 4). The TOC and lipid content increased up to C:N ratio of 1:2 and 1:3.
- The total lipid and lipid/carbohydrate and lipid/protein ratio declined with increasing C:N ratio, indicating that N-straved condition was favorable for lipid synthesis. As evident from the FTIR results, with an increasing level of nitrogen, there is a substantial increase in the protein and carbohydrate content.
- The photosynthetic parameters Fv/Fm, ABS/RC, PIabs, ETo/RC, NPQ, and Rfd showed improvement in the photosynthetic performance with increasing of C:N.The highest values of NPQ and Rfd in absence of nitrogen source indicated the reduced photochemical activity and inhibition of photochemistry of PSII. The C:N molar ratio of 1:2 was found to better to maximum photosystem yield and electron transport. The overall photosynthetic was relatively uninfluenced by the presence of organic carbon when grown photoheterotrophically.

- In other ojective of the study analysis of impact of both mixotrophic (different C/P ratio) cultivation as well as phosphate nutrition on the growth, macromolecular content, and photosynthetic performance of microalga *C. vulgaris* was done.Effect of different molar C (Acetate, 4 mM): P (0-0.5 mM) ratio on the growth of microalga revealed maximum growth, protein, and chlorophyll up to C:P ratio of 40:16. However, carbohydrate content linearly increased up to C:P ratio of 40:4. The TOC and lipid content declined with an increase in C:P ratio. As evident from the FTIR results, with an increasing level of nitrogen, there is a substantial increase in the protein and carbohydrate content, but not in the lipid.
- The photosynthetic parameters Fv/Fm, ABS/RC, PIabs, ETo/RC, NPQ, and Rfd showed improvement in the photosynthetic performance with increasing of C:P ratio.The highest values of NPQ and Rfd were observed in the P-starved starved cells, indicating reduced photochemical activity and inhibition of the photochemistry of PSII.

Thus, this studyoffers proof of the concept of mitigating carbon and simultaneous production of algal biomass, carbohydrates, and proteins for variouspotential applications.Furthermore, the large amount of carbohydrate and lipid accumulationin *C. vulgaris* makes it a promising candidate forthe production of biofuels. Therefore, based on the present study it is suggested that further attempts should be made forlarge-scale cultivation of *C.vulgaris* in outdoor conditions, our findings on pH, salinity tolerance, light intensity and CO_2 supply should be exploited for harvesting better biomass content. The algal culture for CO_2 sequestration/remediation requires a large amount of nutrients, with nitrogenand phosphorus playing a central role.Wastewater discharged from

CO_2-emitting industries maycontain some or all of these. Hence, using *C. vulgaris*, futureattempts can be made to combine the process of removingCO_2 from flue gas and nutrients from wastewater, by whichan economically feasible process can be developed. In addition, the tolerance of this strain should betested against other flue gas compounds such as nitrogen oxides(NOx) and sulfur oxides (SOx).The innovation of microalgal-based CO_2 bio-sequestration isrequired to bring significant advancement in CO_2 bio-mitigationaiming towards solutions for global warming.