

# Effect of UV-B and salinity stress on biomass and lipid production potential of microalgae: A study on stress induced molecular signaling

## SUMMARY of THESIS

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## Summary

Energy is the fundamental requirement for the existence of lives on the earth. Despite limited supply and increasing demand, fossil fuels remain among the world's cheapest commodities, which continuously dwindling due to overexploitation and anthropogenic interferences. This might have cause a paradigm shift towards a search for renewable source to meet the demand of energy. Replacement of fossil fuels by renewable and more environmentally benign alternatives will occur only if the substitutes can compete sustainable and economically. The renewable sources includes solar energy, agricultural crops (Maize, Sunflower), non-agricultural crops (*Jatropha* sp.) and algae. Microalgae are dominant, photosynthetic, biofuel and largest oxygen producing organisms on the earth indispensable for planet function and ecosystem sustainability. One of these alternatives is based on the oils extracted from algae and commercial-scale pilot facilities to test these are in operation. Over the past few years, microalgae- a third generation biofuel acts as a bridge in the production of bio-energy via engineering of algal metabolism against limited water, land and environmental stress. Off the environmental stressors, salinity and UVR are the major environmental obstacles for the present world and the environment. The high photosynthetic capability of microalgae is not only valuable for lipid production but also beneficial for accumulation of nutrients, carbon sequestration and production of oxygen and balanced N-cycling in the thrust of environment. Mineral deficiencies are often referred to as 'hidden hunger', which can result in severe health problems, and are widespread with up to two billion people worldwide at risk of iodine (I), iron (Fe), zinc (Zn), selenium (Se) calcium (Ca) and magnesium (Mg) deficiencies. However, mineral depravation in soil and water linked with the environmental factor such as excess of salt concentration and high UV-B exposure cause.

Salinity is introduced in the environment by the natural sources, anthropogenic activities and modern agricultural practices. Salinity is becoming an increasing environmental and socioeconomic problem because it leads to the loss of agricultural land at a rate of 0.25 to 0.5 Mha annually around the world. Generally, salt stress is associated with imbalances in ion levels and homeostasis, leading to oxidative stress and ion overload. UV radiation is directly related with ozone depletion caused by various natural as well as anthropogenic activities. UVR develops generation of photochemical smog, photo damage, change in precipitation pattern, coral bleaching, vegetation loss and agricultural escalation and altering ecosystem services etc.

Microalgae grown in the saline habitat and UV-B radiation may result into variety of complications for the living being including osmotic stress, upwelling of nutrients and generation of reactive oxygen species (ROS) which causes physiological alterations, ionic disturbances photobleaching and oxidative damage. However, microalgae have shown tendency to adapt to extreme conditions such as UV-B and salinity stress during the course of evolution. The high photosynthetic attributes of microalgae under various stresses is not only beneficial for lipid production carbon sequestration, accumulation of nutrients, phycoremediation of waste water and environmental cycling of nutrients in the ecosystem.

In the present study, microalgae *C. vulgaris* and *C. humicola* grown under different environmental stress individually and in combination with may NaCl and UV-B exposure exhibit different degree of responses towards their toxicity, tolerance, photosynthetic performance and lipid yield. Algae *C. vulgaris* and *C. humicola* treated with 100 mM NaCl represents distorted cell morphology and increase in cell size by 33.52% (*C. humicola*) and 27.79% (*C. vulgaris*). The Energy Dispersive Spectroscopy data revealed reduction in mineral contents (C, S, Fe, Mg, Si, Mn and

Zn) by 14-54% in both algae after treatment with 100 mM salt. Further, *C. humicola* accumulated high lipid content than *C. vulgaris* in presence of NaCl (100 mM) as evident from the FTIR analysis showing the increased peak length of lipid ( $1740\text{ cm}^{-1}$ ) and increased Lipid/Protein, Lipid/Carbohydrate and Protein/Carbohydrate peak ratio. The activities of antioxidant enzymes viz., superoxide dismutase, catalase and glutathione reductase were increased by 2.5~5 fold in *C. humicola* and *C. vulgaris* as compared to control. The comparative enhanced enzymatic and non enzymatic antioxidants in *C. humicola* make it suitable algae against salt stress.

Microalgae *C. humicola* and *C. vulgaris* exposed to different durations of UV-B exposure (1/2 h, 1h, 2h, 3h and 4h) revealed that UV-B reduces the photosynthetic performance of photosystem II by altering the light use efficiency and inhibition of RCs as evident from photosynthetic performance index ( $Pi_{ABS}$ ), maximum quantum yield ( $Fv/Fm$ ), net closing rate of reaction centre ( $Mo$ ), Trapping flux ( $TRo/RC$ ) and effective antenna size ( $ABS/RC$ ) as compared to their respective control. Scanning Electron Microscopy (SEM) exhibited variegated structure and increase in cell size which was more pronounced in the case of *C. humicola* after 4h of UV-B exposure. The Energy Dispersive Spectroscopy (EDS) data showed that the content of microelements (C, O, Na, and K) in terms of atomic percent weight was significantly increased in *C. vulgaris* while with *C. humicola*, it was restricted to carbon (C) only. Further, the high antioxidant (ascorbic acid, cysteine and proline) potential and carotenoid/chl a photoprotection response reflected protective response of both algae against UV-B. In the case of lipid, alleviation in carbon allocation in *C. humicola* may be the reason for high lipid yield as compare to *C. vulgaris* after 2h of UV-B exposure. In another study, microalgae *C. vulgaris* and *C. humicola* exposed to different intensity of UV-B exhibited decrease in the level of both chlorophyll and

carotenoids contents with increasing intensities of UV-B. Increase in the chlorophyll content was observed at lower UV-B intensity ( $5 \text{ Wm}^{-2}$ ) in both algae *C. vulgaris* and *C. humicola*, while maximum pigments inhibition was observed at  $15 \text{ Wm}^{-2}$ . The concentration of chlorophyll in algae *C. vulgaris* and *C. humicola* exposed to higher intensity ( $15 \text{ Wm}^{-2}$ ) was decreased ( $0.069 \pm 0.009$  and  $0.058 \pm 0.009 \text{ mg g}^{-1} \text{ fw}$ , respectively) as compared to their respective control ( $0.081 \pm 0.009$  and  $0.079 \pm 0.018 \text{ mg g}^{-1} \text{ fw}$ ). The TBARS content in the algae exposed to different intensities of UV-B showed increase in peroxidative damage as represented by enhanced production of MDA as a result of lipid peroxidation. *C. vulgaris* treated with  $5 \text{ Wm}^{-2}$  intensity showed increase in MDA by 11.21% while at 10, and  $15 \text{ Wm}^{-2}$  of UV-B doses resulted into an increase in MDA by 37.91% and 68.86%, respectively. In the case of *C. humicola* treated with 5, 10 and  $15 \text{ Wm}^{-2}$  of UV-B MDA content was increased by 9.38%, 28.67% and 59.80%, respectively as compared to control.

Microalgae *C. vulgaris* and *C. humicola* treated with NaCl and UV-B were used to study the combined effect of NaCl +UV-B on nutrient status, physiological and biochemical changes in the microalgae *C. humicola* and *C. vulgaris*. The chlorophyll content in algae *C. vulgaris* exposed to UV-B alone for 2h and 4h were severely reduced as evident from significant decrease in chlorophyll by 32.87% and 60.27% respectively as compared to respective control. UV-B exposed algae in the presence of 100 mM salt (NaCl+UV-B) exhibited slight increase in chlorophyll level after 2h UV-B exposure (1.36%) as compared to control (untreated) in microalgae *C. vulgaris*. Further, *C. vulgaris* treated with NaCl (100mM)+UV-B (4h), 60.27% decrease in chlorophyll content was observed. In the case of *C. humicola*, UV-B alone (2h and 4h), resulted into decrease in chlorophyll content from  $0.071 \pm 0.010$  to  $0.053 \pm 0.014$  and  $0.071 \pm 0.010 - 0.028 \pm 0.002 \text{ mg g}^{-1} \text{ fw}$ , respectively. *C. humicola* cells

treated with 100 mM salt and different duration of UV-B (NaCl+UV-B) showed decrease in chlorophyll level i.e., 12.82% (2 h) and 8.45% (4 h) as compared to untreated control.

The present finding concludes that microalgae *C. humicola* was better adapted to NaCl and UV-B stress than *C. vulgaris*. Growing algae in salt affected arable lands and salt lakes/lagoons may be good practices of reclamation of salinity and biomass production by algae which can be utilized for production of biofuel and other value added products in a sustainable manner. Such algae can be helpful for harvesting of algal biomass in salt water and lagoons, which provides a cheap source of lipid and bioenergy in order to fulfill the growing demand of energy in a cost effective and sustainable manner. Overall results explained that low-dose (2h) of UV-B radiation exposure could enhance the photosynthetic performance and lipid yield in microalgae *C. humicola* and *C. vulgaris* due to improved antioxidant status. Further, the transient chlorophyll fluorescence induction study in both algae reflected the closure of photosystem II, diminished reaction centre and reduced flow of electrons in the cells treated with UV-B for 4h. Improved antioxidant status of cells under UV-B exposure for 2h exhibited better accumulation of biomass, lipid yield and synthesis of value added products in both strains in cost effective and sustainable manner. Our investigation have also shown that NaCl (100mM)+UV-B stress can be beneficial for growth and lipid production in algae in area receiving high solar radiation with salinity like marine ecosystem.