**Ph.D. Topic: INVESTIGATING THE IMPACT OF NANO-EMULSIONS AND NANOPARTICLES ON MICROALGAE**


## Abstract:

In this study, an effective supplement for enhanced biomass production of microalgae has been proposed. The study recommended two nano-systems for microalgae cultivation: oil-in-water nanoemulsions and nanoparticles substituted aqueous system.

Firstly, a novel nanoemulsion-based media has been analyzed for the growth of freshwater microalgae strain *Chlorella pyrenoidosa*. Two types of nanoemulsions, silicone oil (SE) and paraffin oil nanoemulsion (PE) at five different concentrations (1 to 5%) mixed with Blue-green 11 (BG 11) media designated as MSE and MPE have been prepared for the cultivation of *Chlorella pyrenoidosa*. Biomass potential was found maximum in 1% MPE followed by 1% MSE and control i.e, 3.20, 2.75, and 1.03 g $L^{-1}$, respectively. The pigment synthesis was enhanced by 76% in 1% MSE and 53% in 1% MPE compared with the control. The microalgal biomass grown in nanoemulsions shows an increase in lipid and carbohydrate content. According to micrographs obtained from field emission scanning electron microscope (FESEM), microalgal cells were morphologically intact and normal in shape. The key mechanism which supports the enhanced microalgal growth and biomass production appeared to be the enhanced carbon dioxide ($CO_2$) absorption tendency of the nanoemulsion as neither oil nor surfactant was observed as a growth substrate. Besides, some additional mechanisms, such as improved mass transfer and light intensity, could also be responsible for enhanced microalgal growth.

The practical utility of nanoemulsion media was examined in terms of its compatibility with different growth media and microalgal strains/consortium (*Chlorella minutissima*, *Synechocystis pevalekii*, PA4 consortium, and *Navicula* sp.) and its ability to be recycled. Results showed increased biomass yield of *Chlorella minutissima*, *Synechocystis pevalekii*, PA4 consortium , and *Navicula* sp. by 26%, 36%, 51%, and 50% compared with their respective control. The recyclability of 1% MSE showed ~ 45% enhanced growth of *C. pyrenoidosa* compared to the control. Further, to analyze the environmental impacts, comparative LCA of microalgal cultivation in conventional growth media, i.e., BG 11(SC-1) and 1% MSE (SC-2), was performed using ReCiPe midpoint and endpoint method by Sima Pro 9.0 software based on a "cradle-to-gate" approach with the functional unit of 1 kg microalgal biomass production. Conclusively, the microalgal cultivation in the 1% MSE (SC-2) showed the less environmental impact (53.39 Pt)

compared to BG 11 growth media (99.25 Pt). Hence, MSE has the potential to reduce the consumption of conventional nutrients and resources in an eco-friendly manner.

Secondly, two nanoparticles named polystyrene (PS NP) and iron oxide (IONPs) were selected for further study. Four different concentrations of iron nanoparticles (50, 100, 150, and 200 mg $L^{-1}$) were added to the algal growth media (BG11) to cultivate *Chlorella pyrenoidosa*. The iron nanoparticles (IONPs) at 200 mg $L^{-1}$ promoted growth and displayed the highest biomass yield (1.94 g $L^{-1}$ ) and 40 % enhancement in chlorophyll-a over the control (0.89 g $L^{-1}$). The enhanced $CO_2$ bio-fixation rate (~ 28 mg $L^{-1}d^{-1}$) as well as nutrient (phosphate and nitrate) uptake rate was revealed in the presence of 200 mg $L^{-1}$ IONPs compared to the control. MTT assay showed that microalgal cells were metabolically more active in the presence of IONPs. The flow cytometry analysis highlights that more live microalgal cells were found at higher concentrations of IONPs. FESEM-EDX analysis confirmed that the microalgal cells were morphologically intact.

Further, impact of seven concentrations (1, 10, 100, 200, 500, 1000, and 5000 mg $L^{-1}$) of polystyrene nanosuspensions (PS NP) on *Chlorella pyrenoidosa* was analyzed in terms of its growth, chlorophyll-a synthesis, oxidative stress, and cell viability. An antagonistic affect has been observed on the growth of *C. pyrenoidosa* with increasing concentrations of PS NP. The decline in chlorophyll-a synthesis (8.08 - 3.93 µg $mL^{-1}$) was observed at 1 - 5000 mg $L^{-1}$ of PS NP compared to control (8.34 µg $mL^{-1}$ ). The results of the MTT assay and ROS estimation confirmed the toxicity of PS NP that induce oxidative stress in microalgal cells. Higher impacts were observed at higher doses (1000 and 5000 mg $L^{-1}$) of PS NP in terms of nucleic acid degeneration at 1262 $cm^{-1}$ (detected by FTIR analysis) and increased extracellular polymeric substances (EPS) secretion of 253 mg $g^{-1}$ compared to control (92.2 mg $g^{-1}$). SEM images showed aggregated nano-plastics adsorbed on the microalgal surface, whereas Transmission electron microscopy (TEM) micrographs revealed the internalization of nano-plastics with a slight deformation in the cell wall at higher concentrations (1000 and 5000 mg $L^{-1}$ PS NP). Conclusively, higher concentration leads to high exposure risk, negatively impacting cellular functionality and their metabolic secretions.

In order to promote the growth and biomass of microalgal cells, this work describes a unique nanoemulsion-based microalgal cultivation system that may function as a useful $CO_2$ supplement for microalgal growth media. Iron nanoparticles supplemented growth media enhance nutrient availability to microalgal cells, resulting in enhanced microalgal growth, biomass, and $CO_2$ bio-fixation capacity. However, the PS NP showed negative impacts on microalgal cells, suggesting that the nature of selected nanoparticles also played an important role. In the nutshell, the findings present a systematic understanding of microalgal response to two different nano-systems, which will aid in developing safe and sustainable nanotechnological solutions for improved microalgal cultivation and biomass production.