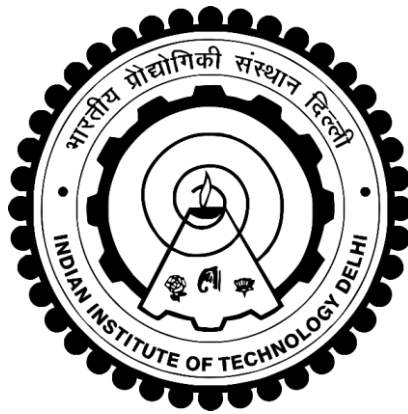


**INVESTIGATING THE IMPACT OF NANO-EMULSIONS AND  
NANOPARTICLES ON MICROALGAE**

**HARSHITA NIGAM**



**CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY**

**INDIAN INSTITUTE OF TECHNOLOGY DELHI**

**JUNE 2023**

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by

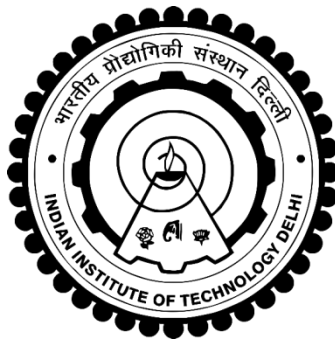
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Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



**Indian Institute of Technology Delhi**  
JUNE 2023

## CERTIFICATE

This is to certify that the thesis entitled “**Investigating the impact of nano-emulsions and nanoparticles on microalgae**”, being submitted by **Ms. Harshita Nigam** to the Indian Institute of Technology Delhi for the award of “**Doctor of Philosophy**” is a record of bonafide research work carried out by her. She has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis. To the best of our knowledge the results contained in this thesis have not been submitted in part or full to any other university or institute for award of any degree or diploma.

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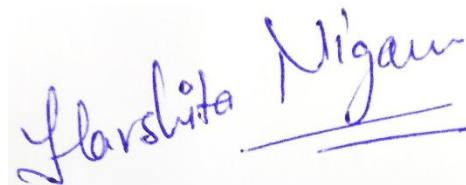
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**Harshita Nigam**

## 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<sup>-1</sup>, 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<sub>2</sub>) 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<sup>-1</sup>) were added to the algal growth media (BG11) to cultivate *Chlorella pyrenoidosa*. The iron

nanoparticles (IONPs) at 200 mg L<sup>-1</sup> promoted growth and displayed the highest biomass yield (1.94 g L<sup>-1</sup>) and 40 % enhancement in chlorophyll-a over the control (0.89 g L<sup>-1</sup>). The enhanced CO<sub>2</sub> bio-fixation rate (~ 28 mg L<sup>-1</sup>d<sup>-1</sup>) as well as nutrient (phosphate and nitrate) uptake rate was revealed in the presence of 200 mg L<sup>-1</sup> 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<sup>-1</sup>) 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<sup>-1</sup>) was observed at 1 - 5000 mg L<sup>-1</sup> of PS NP compared to control (8.34 µg mL<sup>-1</sup>). 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<sup>-1</sup>) of PS NP in terms of nucleic acid degeneration at 1262 cm<sup>-1</sup> (detected by FTIR analysis) and increased extracellular polymeric substances (EPS) secretion of 253 mg g<sup>-1</sup> compared to control (92.2 mg g<sup>-1</sup>). 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<sup>-1</sup> 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<sub>2</sub> supplement for microalgal growth media. Iron nanoparticles supplemented growth media enhance nutrient availability to microalgal cells, resulting in enhanced microalgal growth, biomass, and CO<sub>2</sub> 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.

## सार

इस अध्ययन में सूक्ष्म शैवाल के बायोमास उत्पादन को बढ़ाने के लिए एक प्रभावी पूरक प्रस्तावित किया गया है। अध्ययन ने सूक्ष्म शैवाल की खेती के लिए दो नैनो-प्रणालियों की सिफारिश की: तेल-इन-वॉटर नैनोइमल्शन और नैनोकणों ने जलीय प्रणाली को प्रतिस्थापित किया।

सबसे पहले, सूक्ष्म शैवाल स्ट्रेन क्लोरेला पायरेनोइडोसा के विकास के लिए एक उपन्यास नैनोइमल्शन-आधारित मीडिया का विश्लेषण किया गया है। दो प्रकार के नैनोइमल्शन, सिलिकॉन ऑयल (SE) और पैराफिन ऑयल नैनोइमल्शन (PE) पांच अलग-अलग सांद्रता (1 से 5%) में ब्लू-ग्रीन 11 (BG 11) के साथ मिश्रित MSE और MPE के रूप में नामित मीडिया *Chlorella pyrenoidosa* के विकास के लिए तैयार किए गए हैं।

बायोमास क्षमता 1% MPE में अधिकतम पाई गई, उसके बाद 1% MSE और नियंत्रण यानी क्रमशः 3.20, 2.75 और 1.03 ग्राम / लीटर। रंगद्रव्य संश्लेषण को नियंत्रण की तुलना में 1% MSE में 76% और 1% MPE में 53% बढ़ाया गया था। नैनोइमल्शन में विकसित सूक्ष्म शैवाल बायोमास लिपिड और कार्बोहाइड्रेट सामग्री में वृद्धि दर्शाता है। फील्ड एमिशन स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोप (FESEM) से प्राप्त माइक्रोग्राफ के अनुसार, माइक्रोएल्गल कोशिकाएं रूपात्मक रूप से बरकरार और आकार में सामान्य थीं। प्रमुख तंत्र जो सूक्ष्म शैवाल वृद्धि और बायोमास उत्पादन का समर्थन करता है, नैनोइमल्शन की बढ़ी हुई कार्बन डाइऑक्साइड (CO<sub>2</sub>) अवशोषण प्रवृत्ति प्रतीत होती है क्योंकि न तो तेल और न ही सर्फैक्टेंट को विकास सब्सट्रेट के रूप में देखा गया था। इसके अलावा, कुछ अतिरिक्त तंत्र, जैसे कि बड़े पैमाने पर स्थानांतरण और प्रकाश की तीव्रता, बढ़े हुए सूक्ष्म शैवाल विकास के लिए भी जिम्मेदार हो सकते हैं।

नैनोइमल्शन मीडिया की व्यावहारिक उपयोगिता की विभिन्न विकास मीडिया और माइक्रोएल्गल स्ट्रेन/कंसोर्टियम (*Chlorella minutissima*, *Synechocystis pevalekii*, PA4 consortium , और *Navicula* sp. ) के साथ इसकी अनुकूलता और इसकी पुनर्नवीनीकरण की क्षमता के संदर्भ में जांच की गई थी। परिणामों ने *Chlorella minutissima*, *Synechocystis pevalekii*, PA4 consortium , और *Navicula* sp. की बायोमास उपज में वृद्धि दिखाई, उनके संबंधित नियंत्रण की तुलना में 26%, 36%, 51% और 50%। 1% MSE की पुनर्चक्रण क्षमता ने नियंत्रण की तुलना में *C. pyrenoidosa* की ~45% बढ़ी हुई वृद्धि दिखाई। इसके अलावा, पर्यावरणीय प्रभावों का विश्लेषण करने के लिए, Sima Pro 9.0 सॉफ्टवेयर द्वारा ReCiPe मिडपॉइंट और एंडपॉइंट विधि का उपयोग करके पारंपरिक विकास मीडिया, यानी, BG 11(SC-1) और 1% MSE (SC-2) में सूक्ष्म शैवाल विकास का तुलनात्मक LCA प्रदर्शन किया गया था। 1 किलो सूक्ष्म शैवाल बायोमास उत्पादन की कार्यात्मक इकाई के साथ "cradle to gate" दृष्टिकोण पर आधारित है। निर्णायक रूप से, 1% MSE (SC-2) में सूक्ष्म शैवाल वृद्धि ने BG 11 विकास मीडिया (99.25 Pt) की तुलना में कम पर्यावरणीय प्रभाव (53.39 Pt) दिखाया। इसलिए, MSE में पर्यावरण के अनुकूल तरीके से पारंपरिक

पोषक तत्वों और संसाधनों की खपत को कम करने की क्षमता है।

आगे के अध्ययन के लिए पॉलीस्टाइरीन (PSNP) और आयरन ऑक्साइड (IONP) नामक दो नैनोकणों का चयन किया गया। *Chlorella pyrenoidosa* के विकास के लिए एल्गल ग्रोथ मीडिया (BG11) में आयरन नैनोपार्टिकल्स (50, 100, 150, और 200 mg L<sup>-1</sup>) के चार अलग-अलग सांद्रण जोड़े गए। 200 mg L<sup>-1</sup> पर आयरन नैनोपार्टिकल्स (IONP) ने विकास को बढ़ावा दिया और उच्चतम बायोमास उपज (1.94 g L<sup>-1</sup>) और नियंत्रण (0.89 g L<sup>-1</sup>) पर क्लोरोफिल-ए में 40% वृद्धि प्रदर्शित की। बढ़ी हुई CO<sub>2</sub> जैव-निर्धारण दर (28 mg L<sup>-1</sup>d<sup>-1</sup>) के साथ-साथ पोषक तत्व (फॉस्फेट और नाइट्रेट) की खपत दर, नियंत्रण की तुलना में 200 mg L<sup>-1</sup> IONPs की उपस्थिति में प्रकट हुई थी। MTT परख ने दिखाया कि IONPs की उपस्थिति में सूक्ष्म शैवाल कोशिकाएं अधिक सक्रिय थीं। Flow Cytometry (FC) विश्लेषण इस बात पर प्रकाश डालता है कि IONPs की उच्च सांद्रता में अधिक जीवित सूक्ष्म शैवाल कोशिकाएं पाई गईं। FESEM-EDX विश्लेषण ने पुष्टि की कि सूक्ष्म शैवाल कोशिकाएं रूपात्मक रूप से अच्छी थीं।

इसके अलावा, *Chlorella pyrenoidosa* पर पॉलीस्टाइरीन नैनोसस्पेंशन (PS NP) के सात सांद्रता (1, 10, 100, 200, 500, 1000, और 5000 mg L<sup>-1</sup>) के प्रभाव का इसके विकास, क्लोरोफिल-a संश्लेषण, ऑक्सीडेटिव तनाव, और सेल व्यवहार्यता के संदर्भ में विश्लेषण किया गया था। PS NP की बढ़ती सांद्रता के साथ *Chlorella pyrenoidosa* के विकास पर विरोधी प्रभाव देखा गया है। नियंत्रण (8.34 mg mL<sup>-1</sup>) की तुलना में पीएस एनपी के 1-5000 mg L<sup>-1</sup> पर क्लोरोफिल-a संश्लेषण (8.08 - 3.93 mg mL<sup>-1</sup>) में गिरावट देखी गई। MTT परख और ROS आकलन के परिणामों ने PS NP की विषाक्तता की पुष्टि की, जो कि सूक्ष्म कोशिकाओं में ऑक्सीडेटिव तनाव को प्रेरित करती है। 1262 cm<sup>-1</sup> (FTIR विश्लेषण द्वारा पता लगाया गया) पर न्यूक्लिक एसिड अपघटन के मामले में PS NP की उच्च खुराक (1000 और 5000 mg L<sup>-1</sup>) पर उच्च प्रभाव देखा गया और 253 mg g<sup>-1</sup> के बाह्य कोशिकीय पदार्थ (EPS) साव में वृद्धि हुई है नियंत्रण की तुलना में (92.2 mg g<sup>-1</sup>)। SEM छवियों ने सूक्ष्म-एल्गल सतह पर एकत्रित नैनो-प्लास्टिक को दिखाया, जबकि ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी (TEM) माइक्रोग्राफ ने उच्च सांद्रता (1000 और 5000 mg L<sup>-1</sup> PS NP) पर सेल दीवार में मामूली विरूपण के साथ नैनो-प्लास्टिक के आंतरिककरण का खुलासा किया। विशेष रूप से, उच्च सांद्रता उच्च जोखिम की ओर ले जाती है, जो सेलुलर कार्यक्षमता और उनके चयापचय साव को नकारात्मक रूप से प्रभावित करती है।

माइक्रोएल्गल कोशिकाओं के विकास और बायोमास को बढ़ावा देने के लिए, यह कार्य एक अद्वितीय नैनोइमल्शन-आधारित माइक्रोएल्गल कल्टीवेशन सिस्टम का वर्णन करता है जो माइक्रोएल्गल विकास मीडिया के लिए उपयोगी CO<sub>2</sub> पूरक के रूप में कार्य कर सकता है। IONP सप्लीमेंटेड ग्रोथ मीडिया माइक्रोएल्गल कोशिकाओं के लिए पोषक तत्वों की उपलब्धता को बढ़ाता है, जिसके परिणामस्वरूप माइक्रोएल्गल ग्रोथ, बायोमास और CO<sub>2</sub> बायो-फिक्सेशन क्षमता में वृद्धि होती है।

हालांकि, PS NP ने सूक्ष्म शैवाल कोशिकाओं पर नकारात्मक प्रभाव दिखाया, यह सुझाव देते हुए कि चयनित नैनोकणों की प्रकृति ने भी एक महत्वपूर्ण भूमिका निभाई। संक्षेप में यह शोध, दो अलग-अलग नैनो-प्रणालियों के लिए माइक्रोएल्गल प्रतिक्रिया की एक व्यवस्थित समझ प्रस्तुत करते हैं, जो बेहतर माइक्रोएल्गल विकास और बायोमास उत्पादन के लिए सुरक्षित और टिकाऊ हैं और नैनोटेक्नोलॉजिकल समाधान विकसित करने में सहायता करेगा।

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