# BIOREACTOR ENGINEERING AND PROCESS AUTOMATION FOR $CO_2$ BIOFIXATION AND PHB PRODUCTION IN MICROALGAL SYSTEMS

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by

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*Dedicated to my Parents…*

## CERTIFICATE

This is to certify that the thesis entitled "**Bioreactor Engineering and Process Automation for $CO_2$ Biofixation and PHB Production in Microalgal Systems**" being submitted by **Mr. Saptarshi Dey** to the Indian Institute of Technology Delhi for the award of "**Doctor of Philosophy**" is a record of bonafide research work carried out by him. He has worked under my guidance and supervision and has fulfilled the requirements for submission of this thesis. To the best of my 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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## ABSTRACT

Microalgal systems effectively capture $CO_2$ and produce valuable bio-products like biofuels and bioplastics, offering environmental and economic benefits. However, high costs, scalability challenges, and operational inefficiencies hinder commercialization, requiring advanced reactor designs and cost-effective solutions for practical implementation. The present work has systematically addressed challenges associated with microalgal systems by developing and optimizing innovative reactor designs and cultivation strategies along with microalgal valorization pathways for conversion of carbon from sequestered $CO_2$ to value added lipids and algal biopolymers.

We screened an indigenous algal strain PA4(isolated from an STP and previously reported to have high biomass yield and $CO_2$ tolerance) for the natural accumulation of the algal biopolymer Polyhydroxybutyrate (PHB) under complete photoautotrophic conditions. The strain was found to naturally accumulate PHB of around 32 μg $mL^{-1}$ in BG-11 media without any nutrient or light stress. PA4 was taxonomically identified to be the fresh water algae *Poterioochromonas malhamensis.* To scale up $CO_2$ assisted photoautotrophic growth, a novel photobioreactor was conceptualised and designed at a prototype scale of 10 L, aimed at microbubble assisted $CO_2$ sequestration to recover $CO_2$ from low concentration sources (5% v/v) and maximize production of microalgal value added products without any nutrient stress. A venturi microbubble generator was amalgamated with a bubble column and Reynold's number was used to define the hydrodynamic flow regime by media recirculation by co-current liquid-gas flow between the column and a retention tank. At a steady volumetric liquid flow rate $Q_L$, corresponding to laminar flow conditions, gas flow rate $Q_G$ was varied in the range 0.1-1 L•$min^{-1}$ to determine the volumetric mass transfer coefficient $K_La$ and the $CO_2$ mass transfer efficiency. $K_La$ and $Q_G$ values were found to be linearly dependent in the $Q_G$ range 0.1-0.7 L•$min^{-1}$ while $CO_2$ capture efficiencies in the range 86% to 98% were obtained. The

optimized media recirculation sufficed for culture agitation and further aeration for mixing was not required. A correlation coefficient was derived relating change in gas-liquid interfacial area with change in $Q_G$. The resultant system obtained biomass yield of 0.454 $gL^{-1}$ and a carbon content of 38.9% volatile suspended solids (VSS). Furthermore, lipid accumulation of 35.9% on a dry cell weight basis and lipid productivity of 11.64 mg $L^{-1}$ $d^{-1}$ exhibited feasible valorization of sequestered carbon.

Taking into consideration the efficacy of microbubbles in high-rate mass transfer and recirculation as a better agitation and nutrient mixing mode, the carbonation column was scaled up to a 20 L volume. A novel microbubble generator assembly for homogeneous bubble generation was fabricated using resin-based SLA 3D printing. We obtained ideal microbubble profile in the column at various $Q_L$ and $Q_G$ values corresponding to laminar, transient and turbulent flow regimes using high speed imaging technique coupled with MATLAB based computational image processing. The microbubble density in the system was found as high as 90.84% and did not go below 60% under any and all varied conditions of generation or height of imaging. While the smallest bubble size recorded was around 93 µm, the most probable bubble sizes obtained using Gaussian Distribution showed bubble size in the range 482 -702 µm at transient liquid flow regime with $Q_L$ = 10 $Lmin^{-1}$ and $Q_G$ = 0.1 $Lmin^{-1}$ conditions. For feasible scaleup and bulk growth, the microbubble-based carbonation system was integrated with a 200 L raceway pond and structurally and functionally hybridised to maximise $CO_2$ scrubbing from a gas source of 5% concentration and valorise into algal biomass compound fractions. pH feedback-based carbonation loop was integrated between the raceway and carbonation column to keep the culture saturated with bicarbonate in the pH range 7.2 - 8.5. The resultant pilot hybrid photobioreactor system was further tested for sustainable *P. malhamensis* biomass cultivation using modified BG-11 media-carbonation system. We obtained a biomass yield of 0.423 $gL^{-1}$ and $CO_2$ bio-fixation rate of 44.05 $mgL^{-1}d^{-1}$,

comparable to those obtained at 10 L prototype scale. A higher ‘C’ and lower ‘O’ content in biomass from the hybrid system over the prototype indicated the biomass has better quality and suitability towards biofuel feedstock applications.

Additionally, PHB content in the biomass from both 10 L prototype and 200 L pilot scale were tested using Gas Chromatography. The PHB yield was found to be 5.79% DCW basis at 200 L scale compared to 4.95% DCW basis in 10 L prototype while maintaining 5% $CO_2$ concentration and identical carbonation and recirculation conditions. The results were significantly higher than those obtained for the respective controls.

Our results show that scaleup did not compromise biomass yield or $CO_2$ bio-fixation, but rather enhanced PHB production further. Overall, the best features of the novel 10 L prototype were scaled up successfully through photobioreactor hybridization to further enhance biopolymer production potential in the indigenous algal isolate with only $CO_2$ as the carbon source. The outcomes of the present work show the potential of microbubble-based carbonation and photobioreactor hybridization at enhancing $CO_2$ capture in algal systems and subsequent valorization of inorganic carbon into organic molecules of high commercial value, a much sought after scenario for commercial applications of microalgal CCUS technologies.

## सार

सूक्ष्मशैवाल प्रणालियाँ $CO_2$ को प्रभावी रूप से पकड़ने और जैवईंधन तथा जैवप्लास्टिक जैसे मूल्यवान जैव-उत्पादों के उत्पादन में सक्षम हैं, जो पर्यावरणीय और आर्थिक दृष्टि से लाभकारी हैं। तथापि, उच्च लागत, बड़े पैमाने पर कार्यान्वयन की चुनौतियाँ और संचालन में अक्षमता इनके व्यावसायीकरण में बाधा उत्पन्न करती हैं, जिसके समाधान हेतु उन्नत जैव-रिएक्टर डिज़ाइन और लागत-प्रभावी उपायों की आवश्यकता है। वर्तमान शोधकार्य ने इन चुनौतियों को व्यवस्थित ढंग से संबोधित करते हुए नवीन रिएक्टर डिज़ाइन, संवर्धन रणनीतियों तथा $CO_2$ से प्राप्त कार्बन को मूल्यवर्धित लिपिड और सूक्ष्मशैवाल जैवबहुलकों (biopolymers) में रूपांतरित करने की वैलोरीकरण पथों का विकास और अनुकूलन किया है।

एक स्वदेशी सूक्ष्मशैवाल प्रजाति PA4 (जो एक STP से पृथक की गई थी और पूर्व में उच्च बायोमास उत्पादन और $CO_2$ सहनशीलता हेतु रिपोर्ट की गई थी) को पूर्ण प्रकाश-स्वपोषी (photoautotrophic) अवस्था में Polyhydroxybutyrate (PHB) के स्वाभाविक संचय हेतु स्क्रीन किया गया। यह प्रजाति BG-11 माध्यम में बिना किसी पोषक तत्व या प्रकाश तनाव के लगभग 32 $\mu g\ mL^{-1}$ PHB का स्वाभाविक संचय करती पाई गई। PA4 की टैक्सोनोमिक पहचान *Poterioochromonas malhamensis* नामक ताजे जल की शैवाल के रूप में की गई।

$CO_2$-सहायता प्राप्त प्रकाश-स्वपोषी संवर्धन को स्केल-अप करने हेतु एक नवीन फोटोबायोरिएक्टर को 10 L प्रोटोटाइप स्केल पर डिज़ाइन और परिकल्पित किया गया, जिसका उद्देश्य माइक्रोबबल आधारित $CO_2$ अवशोषण द्वारा 5% (v/v) सांद्रता वाले $CO_2$ स्रोत से कार्बन को पुनर्प्राप्त करना और सूक्ष्मशैवाल के मूल्यवर्धित उत्पादों को अधिकतम करना था। वेंचुरी माइक्रोबबल जनरेटर को बबल कॉलम से जोड़ा गया, और मीडिया पुनर्चक्रण के माध्यम से कॉलम और रिटेंशन टैंक के बीच सहप्रवाह (co-current) तरल-गैस प्रवाह द्वारा हाइड्रोडायनामिक प्रवाह व्यवस्था को परिभाषित करने हेतु Reynold's number का प्रयोग

किया गया। एक स्थिर वॉल्यूमेट्रिक लिक्विड फ्लो दर QL (जो लैमिनर फ्लो की स्थिति को दर्शाती है) पर गैस फ्लो दर $Q_G$ को 0.1–1 $L \cdot min^{-1}$ की सीमा में बदलते हुए वॉल्यूमेट्रिक मास ट्रांसफर गुणांक KLa और $CO_2$ मास ट्रांसफर दक्षता का निर्धारण किया गया। $Q_G$ के 0.1–0.7 $L \cdot min^{-1}$ के रेंज में KLa और $Q_G$ के बीच रैखिक (linear) संबंध पाया गया जबकि 86% से 98% तक $CO_2$ अवशोषण दक्षता प्राप्त हुई। अनुकूलित मीडिया पुनर्चक्रण ने संस्कृति को पर्याप्त घुलनशील $CO_2$ प्रदान किया, जिससे अतिरिक्त वायुकृतन (aeration) की आवश्यकता नहीं रही। गैस-लिक्विड इंटरफेसियल क्षेत्र में $Q_G$ के अनुसार परिवर्तन को दर्शाते हुए सहसंबंध गुणांक (correlation coefficient) प्राप्त किया गया।

प्रणाली से 0.454 $gL^{-1}$ बायोमास उपज और 38.9% VSS कार्बन सामग्री प्राप्त हुई। इसके अतिरिक्त, 35.9% (DCW आधार पर) लिपिड संचयन और 11.64 mg $L^{-1}$ $d^{-1}$ की लिपिड उत्पादकता प्राप्त हुई, जो सजीव कार्बन का उपयुक्त वैलोरीकरण दर्शाती है।

माइक्रोबबल्स की उच्च-दर मास ट्रांसफर क्षमता और पुनर्चक्रण की दक्षता को देखते हुए, कार्बोनेशन कॉलम को 20 L वॉल्यूम तक स्केल-अप किया गया। एक नवीन माइक्रोबबल जनरेटर असेंबली SLA 3D प्रिंटिंग तकनीक द्वारा निर्मित की गई। विभिन्न $Q_L$ और $Q_G$ मानों के तहत लैमिनर, ट्रांज़िएंट और टर्बुलेंट प्रवाह दशाओं में हाई-स्पीड इमेजिंग तकनीक और MATLAB आधारित छवि विश्लेषण द्वारा आदर्श माइक्रोबबल प्रोफ़ाइल प्राप्त की गई। प्रणाली में माइक्रोबबल घनत्व अधिकतम 90.84% और न्यूनतम 60% पाया गया। सबसे छोटा बबल आकार ~93 μm रिकॉर्ड किया गया जबकि Gaussian वितरण के अनुसार सबसे संभावित बबल आकार 482–702 μm के बीच थे ($Q_L$ = 10 $Lmin^{-1}$ और $Q_G$ = 0.1 $Lmin^{-1}$ पर)।

स्केलेबल और थोक संवर्धन के लिए माइक्रोबबल आधारित कार्बोनेशन प्रणाली को 200 L रेसवे तालाब से एकीकृत कर, 5% सांद्रता वाले गैस स्रोत से $CO_2$ स्क्रबिंग को अधिकतम करने हेतु संरचनात्मक और कार्यात्मक रूप से संकरित (hybridized) किया गया। pH फीडबैक आधारित कार्बोनेशन लूप को रेसवे और कार्बोनेशन कॉलम के बीच जोड़ा गया ताकि संस्कृति को pH 7.2–8.5 के बीच बाइकार्बोनेट संतृप्ति में रखा जा सके।

संकर प्रणाली में संशोधित BG-11 और कार्बोनेशन प्रणाली द्वारा P. malhamensis का स्थायी संवर्धन परीक्षण किया गया। इसमें 0.423 $gL^{-1}$ बायोमास उपज और 44.05 $mgL^{-1}d^{-1}$ की $CO_2$ जैव-स्थिरीकरण दर प्राप्त हुई, जो कि 10 L प्रोटोटाइप से तुलनीय थी। संकर प्रणाली की बायोमास में उच्च 'C' और निम्न 'O' सामग्री, उसकी गुणवत्ता और जैवईंधन अनुप्रयोगों के लिए उपयुक्तता दर्शाती है।
इसके अतिरिक्त, 10 L प्रोटोटाइप और 200 L संकर प्रणाली दोनों में बायोमास से PHB की मात्रात्मकता गैस क्रोमैटोग्राफी द्वारा की गई। 5% $CO_2$ सांद्रता और समान कार्बोनेशन तथा पुनर्चक्रण स्थितियों के तहत, PHB उपज क्रमशः 4.95% (10 L) और 5.79% (200 L) DCW आधार पर प्राप्त हुई, जो कि नियंत्रण समूहों की तुलना में उल्लेखनीय रूप से अधिक थी।

परिणाम दर्शाते हैं कि स्केल-अप से बायोमास उपज या $CO_2$ जैवस्थिरीकरण में कोई समझौता नहीं हुआ, बल्कि PHB उत्पादन में और वृद्धि हुई। समग्र रूप से, 10 L प्रोटोटाइप की सर्वोत्तम विशेषताओं को संकर फोटोबायोरिएक्टर द्वारा सफलतापूर्वक स्केल-अप किया गया, जिससे स्वदेशी शैवाल में केवल $CO_2$ को कार्बन स्रोत के रूप में उपयोग कर जैवबहुलक उत्पादन की क्षमता बढ़ाई जा सकी। यह कार्य दर्शाता है कि माइक्रोबबल आधारित कार्बोनेशन और फोटोबायोरिएक्टर संकरण $CO_2$ पकड़ने और अकार्बनिक कार्बन को उच्च मूल्यवर्धित जैव-उत्पादों में बदलने की दिशा में सूक्ष्मशैवाल CCUS प्रौद्योगिकियों के लिए एक अत्यधिक संभावित समाधान प्रस्तुत करता है।

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