INVESTIGATIONS ON MECHANISM, PROCESS OPTIMIZATION AND FEASIBILITY ANALYSIS OF FUNGAL ASSISTED ALGAL FLOCCULATION

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by

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CERTIFICATE

This is to certify that the thesis entitled "**INVESTIGATIONS ON MECHANISM, PROCESS OPTIMIZATION AND FEASIBILITY ANALYSIS OF FUNGAL ASSISTED ALGAL FLOCCULATION**" being submitted by **Mr. Arghya Bhattacharya** 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 our guidance and supervision and has fulfilled the requirements for 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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Arghya Bhattacharya

Abstract

In the present study, a method for rapid flocculation of *Chlorella pyrenoidosa* cells with *Aspergillus fumigatus* pellets was developed. The process could flocculate 99% algal cells within 3 h. In order to identify the critical parameters, apart from the flocculation conditions (different fungal-algal ratios, flocculation temperature and agitation), the effect of cultivation time and various pretreatments (autoclaving, cycloheximide exposure) for *A.fumigatus* was also investigated. Results revealed that 24 h old fungal pellets flocculated at 38 ^{o}C at 1:5 fungal-algal ratio showed the best flocculation efficiency. The cell viability assay showed that a viable and metabolically active fungal pellet is a prerequisite for flocculation. SEM studies confirmed that in addition to viability, an intact, and undamaged hyphae is also required for algal attachment. FTIR of the algae, treated fungi and algal fungal floc indicated sharp change in peak intensity at 1024 cm^{-1} and 1370 cm^{-1} indicating the involvement of specific groups in algal-fungal interaction. Finally, this method was tested on wastewater grown algae and 95% flocculation was achieved within 3.5 h. The algal fungal flocs (1650 μm diameter) could be easily separated from the culture. Hence, this process could serve as an alternative for concentrating microalgal cultures for biofuel production in a cost effective way.

The mechanism study of the flocculation process revealed that neither algae nor fungi had any surface molecules which caused the interaction. Analysis of the fungal spent media showed that there was some extracellular factor which aided in the flocculation process. Microscopic examination of fungal spent media incubated algal cells showed the formation of pilus like structures on the algal cell surface. Biochemical analysis of the fungal spent media indicated that the active molecule might be a sugar like molecule. HR-LC-MS analysis of the fungal spent media led to the identification of N-acetyl-glucosamine (GlcNAc) which had a role in the flocculation

process. Further studies with GlcNAc incubated algal cells confirmed its role in the flocculation process. Limited studies have been reported in the recent time but no workable mathematical model has been developed for the same. In the present study, a mathematical model has been developed for fungal-assisted algal harvesting which shows that the process is not a second order process unlike other flocculation models. The process is also dependent on the radius of the algal cells and fungal pellets. Moreover, the flocculation process is affected by the velocity gradient of the system. The model was validated using different experiments viz. different fungal-algal ratio, variation in rpm, different algal strains, algae grown in different wastewaters and finally in a 10 L photobioreactor. The proposed model is found to be in agreement with the experimental results along with $r^2 > 0.90$ in most of the cases.

The flocculation process was the carried out in a 10 L photobioreactor. The results showed that 85% of the algal cells were harvested within 4 h. The mathematical model developed using batch studies also held true for the flocculation in the photobioreactor. Theoretical calculation of energy requirement for the flocculation process was done and it was calculated to be 1.50 kW h kg^{-1} of algal biomass. The cost of the process could be brought down by suitably integrating with different industrial processes. The developed process could be further extrapolated for harvesting of microalgae for use in food, feed and nutraceutical industries thereby reducing the overall cost of these products.

साराशं

वर्तमान अध्ययन में, *Aspergillus fumigatus* गोले के साथ *Chlorella pyrenoidosa* कोशिकाओं के तेज़ी से फ़्लोक्यूलेशन (flocculation) के लिए एक विधि विकसित की गई थी। प्रक्रिया 3 h के भीतर 99% शैवाल कोशिकाओं को flocculate कर सकता है। महत्वपूर्ण पैरामीटर की पहचान करने के लिए फ्लोक्यूलेशन स्थितियों (विभिन्न कवक:शैवाल अनुपात, फ्लोक्यूलेशन तापमान और आंदोलन) के अलावा, *A.fumigatus* के लिए खेती के समय और विभिन्न प्रत्यारोपण (autoclaving, cycloheximide exposure) का प्रभाव भी जांच किया गया था। नतीजे बताते हैं कि 24 h पुराने कवक गोले 38°C पर 1:5 कवक: शैवाल अनुपात सबसे अच्छा फ्लोक्यूलेशन दक्षता (efficiency) दिखाते हैं। सेल व्यवहार्यता (cell viability) परख ने दिखाया कि एक व्यवहार्य और चयापचय सक्रिय कवक गोल फ्लाक्यूलेशन के लिए एक पूर्व शर्त है। SEM अध्ययनों ने पुष्टि की कि व्यवहार्यता के अलावा, एक अखंड, और अवांछित हाइफ़ा भी शैवाल के लगाव के लिए आवश्यक है। शैवाल के FTIR, कवक और शैवाल कवक फ्लोक ने चरम तीव्रता में तेज अंतर को 1024 cm^{-1} और 1370 cm^{-1} पर दर्शाया है जो शैवाल : कवक परस्पर क्रिया में विशिष्ट समूहों की भागीदारी को दर्शाता है। अंत में, इस विधि का परीक्षण अपशिष्ट जल में उगाए जाने वाले शैवाल पर किया गया था और 95% फ्लोक्यूलेशन 3.5 h के भीतर हासिल किया गया था। शैवाल कवक फॉक्स (1650 माइक्रोन व्यास) आसानी से संस्कृति से अलग किया जा सकता है। इसलिए, यह प्रक्रिया जैव ईंधन उत्पादन के लिए लागत प्रभावी तरीके से सूक्ष्मजीव संस्कृतियों पर ध्यान केंद्रित करने के विकल्प के रूप में कार्य कर सकती है।

फ़्लोक्यूलेशन प्रक्रिया के तंत्र अध्ययन से पता चला कि न तो शैवाल और न ही कवक के पास सतह के अणु थे जो पारस्परिक क्रिया का कारण बनते थे। कवक प्रयुक्त मीडिया (media) के विश्लेषण से पता चला कि कुछ बाह्य कोशिकाएं थीं जो फ़्लोक्यूलेशन प्रक्रिया में सहायता करती थीं। कवक प्रयुक्त

किए गए मीडिया (media) की माइक्रोस्कोपिक परीक्षा में शैवाल कोशिकाओं से उगाए गए कोशिकाएं रोआँ (pilus) की संरचना को शैवाल सेल सतह पर संरचनाओं की तरह दिखाती हैं। कवक व्यय मीडिया (media) के जैव रासायनिक विश्लेषण से संकेत मिलता है कि सक्रिय अणु चीनी (sugar) की तरह अणु हो सकता है। कवक प्रयुक्त मीडिया के HR-LC-MS विश्लेषण ने N-acetyl glucosamine (GlcNAc) की पहचान की जिसके कारण फ्लोक्यूलेशन प्रक्रिया में भूमिका निभाई गई। GlcNAc इनक्यूबेट हुए शैवाल कोशिकाओं के साथ आगे के अध्ययन फ़्लोक्यूलेशन प्रक्रिया में अपनी भूमिका की पुष्टि की। हाल के दिनों में सीमित अध्ययनों की सूचना मिली है लेकिन इसके लिए कोई व्यावहारिक गणितीय मॉडल विकसित नहीं किया गया है। वर्तमान अध्ययन में, कवक:सहायक शैवाल कटाई के लिए एक गणितीय मॉडल विकसित किया गया है जो दिखाता है कि प्रक्रिया अन्य फ्लोक्यूलेशन मॉडल के विपरीत दूसरी ऑर्डर प्रक्रिया नहीं है। यह प्रक्रिया शैवाल कोशिकाओं और कवक गोले कि त्रिज्या पर भी निर्भर है। इसके अलावा, फ्लोक्यूलेशन प्रक्रिया प्रणाली के वेग ढाल (velocity gradient) से प्रभावित होती है। मॉडल को विभिन्न प्रयोगों का उपयोग करके मान्य किया गया था जैसे कि। विभिन्न कवक:शैवाल अनुपात, आरपीएम (RPM) में भिन्नता, विभिन्न शैवाल उपभेदों विभिन्न अपशिष्ट जल में उगाए जाने वाले शैवाल और अंततः 10 L फोटोबायरेक्टर में। प्रस्तावित मॉडल ज्यादातर मामलों में $r^2 > 0.90$ के साथ प्रयोगात्मक परिणामों के साथ समझौते में पाया जाता है।

फ़्लोक्यूलेशन प्रक्रिया 10 L फोटोबायरेक्टर में की गई थी। नतीजे बताते हैं कि 85% शैवाल कोशिकाओं को 4 h के भीतर हटाया गया था। बैच अध्ययनों का उपयोग करके विकसित गणितीय मॉडल फोटोबायरेक्टर में फ़्लोक्यूलेशन के लिए भी सच रहा। फ़्लोक्यूलेशन प्रक्रिया के लिए ऊर्जा आवश्यकता की सैद्धांतिक गणना की गई थी और इसे शैवाल बायोमास के $1.50\ kW\ h\ kg^{-1}$ रूप में गणना की गई थी। विभिन्न औद्योगिक प्रक्रियाओं के साथ उपयुक्त रूप से एकीकृत करके प्रक्रिया की

लागत को कम किया जा सकता है। खाद्य पदार्थए खाद्य और न्यूट्रास्यूटिकल उद्योगों में उपयोग के लिए सूक्ष्मजीव की कटाई के लिए विकसित प्रक्रिया को आगे बढ़ाया जा सकता है जिससे इन उत्पादों की कुल लागत कम हो जाती है।

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