

**HYDROTHERMAL CONVERSION OF WASTEWATER GROWN ALGAL
BIOMASS INTO VALUE ADDED PRODUCTS AND INVESTIGATIONS
ON THE EFFECTS OF HEAVY METALS ON PRODUCTS**

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INDIAN INSTITUTE OF TECHNOLOGY DELHI

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ON THE EFFECTS OF HEAVY METALS ON PRODUCTS**

by

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CERTIFICATE

This is to certify that the thesis entitled '**Hydrothermal conversion of wastewater grown algal biomass into value added products and investigations on the effect of heavy metals on products**' being submitted by **Ms. Farah Naaz** 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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Farah Naaz

ABSTRACT

The present study has attempted to address the actual hurdles in the feasibility of algae-based fuels and products. An innovative algal biorefinery concept has been developed where algae is used for the remediation of municipal wastewater during its cultivation in a robust cultivation system, followed by its conversion to high value products in a sustainable manner. To start with, the first step of the study was to select microalgal species having potential of phycoremediation of metal contaminated water and biomass production in different forms of cultivation systems. For this, two different microalgal cultures; 1) *Chlorella pyrenoidosa* (CP) and 2) consortium of *Chlorella pyrenoidosa* and *Phormidium* (PA6) were cultivated in two different modes of cultivation systems i.e., in suspended and attached biofilm systems. The study aimed to highlight the suitability of an algal strain/consortium to different cultivation systems for metal removal and biomass production. The six commonly found heavy metals in wastewater; Cd, Cr, Pb, Cu, Ni and Zn, were used at 1 mg L⁻¹ of concentration. CP performed better in suspension systems with individual metal spiking. However, PA6 performed better in mixed metal spiked media (that was closer to the real wastewaters), especially in attached biofilm systems (>60% removal of all metals) and hence, it was selected for further studies. The next step was to produce the selected microalgae, PA6 in the attached cultivation systems using raw municipal wastewater at pilot scale (100 L) under outdoor natural conditions, followed by the valorisation of harvested algal biomass. The two biomass processing techniques investigated in the study were hydrothermal liquefaction (HTL) and anaerobic digestion (AD) to produce biocrude and biogas, respectively. For both the processing systems, Net Energy Ratios were calculated (from biomass cultivation to the biocrude/biogas production) and compared to find out which of the two energy conversion routes was energetically more favourable. The wastewater grown algae produced 346.59 ± 5 mL g⁻¹ VS_{fed} of cumulative biomethane after AD, and 43 ± 2% (dried biomass basis) of biocrude after HTL, respectively. NER_{HTL} (0.08) was observed to be higher than the NER_{AD} (0.007), proving HTL to be a comparatively better route in terms of energy recovery from the wastewater grown algal biomass.

Further, the Life Cycle Assessments (LCAs) was performed for 3 developed algal biofilms-based conversion systems; 1) wastewater grown algae processed via anaerobic digestion (AD), 2) wastewater grown algae processed via hydrothermal liquefaction (HTL) and 3) synthetic media grown algae processed via HTL. Results showed that HTL of wastewater grown algae had 41.1 %

lesser impact compared to AD of the same biomass and 98% lesser impact than HTL of synthetic media grown algae. The former process also showed positive impact on the eutrophication reduction due to the treatment of wastewater (59-92% nutrients removal) during cultivation. The global warming potential for biocrude production from WWA was 10 times lower than biomethane production from the same biomass and 21 times lower than the biocrude production from synthetic media grown algae. The results of the comparative LCAs proved that HTL of wastewater grown algae is a more sustainable route for valorizing the biomass than AD of wastewater grown algae or HTL of synthetic media grown algae.

The HTL of the biomass (water:biomass ratio of 4:1) at 27 bars (using N₂ as the reaction gas), 230° C for 20 minutes of reaction time produced a biocrude that was rich in specialty chemicals. The HTL biocrude yield was appreciable but it comprised of high nitrogen and oxygen content (that decreased its fuel value) and hence, it required upgradation (deoxygenation and denitrogenation). Furthermore, the GC-MS analysis of the biocrude showed the presence of high value compounds that required enrichment. Efforts were made to improve the quality (upgradation/enrichment) of the HTL products by varying the catalyst (ZSM-5 or KOH)/solvent (methanol or butanol)/reaction gas (CO₂) during the HTL reactions, keeping all other HTL conditions same as that of the optimized reaction. The biocrude yield from ZSM-5 catalyzed HTL was 55% of that obtained by K₂CO₃ catalyzed HTL, but with reduced nitrogen (1.2% of TS) and oxygen (22.3% of TS) content that increased its HHV to 44.5 MJ Kg⁻¹ (1.6 times of K₂CO₃ catalyzed HTL biocrude). The biocrude obtained from HTL with alcoholic solvents comprised of 38% and 52% esters and that with CO₂ as the reaction gas had >65% hydrocarbons in it. On the other hand, the LCMS analysis of aqueous fraction showed the presence of highly valuable pharmaceuticals (minoxidil and ethosuximide), that were quantified to be 18.96 ± 0.24 mg kg⁻¹ and 41.07 ± 0.42 mg kg⁻¹, respectively. Additionally, this calcium and phosphorus rich aqueous fraction was hydrothermally mineralized to produce a carbonated hydroxyapatite (25.5%, dried biomass basis), a bone mineralizer (synthetic bone).

Disposal of metal contaminated biomass after bioremediation poses challenges due to non-availability of suitable techniques. Hence, HTL of algal biomass obtained from heavy metal remediation in attached biofilm systems was performed to investigate the effect of metals on HTL products and study their fate. The biocrude yield from HTL of metal laden biomass was comparable to the biocrude yield from HTL of biomass without metal contamination (30%, dried

biomass basis). The heavy metal analysis of the solid and aqueous fraction showed that >70% of the metals had partitioned into the solid fraction whereas <1% were in the aqueous fraction. The aqueous fraction was also rich in nitrate and phosphate which could be reused for algal cultivation. Hence, hydrothermal liquefaction could be a very useful technique for valorization of metal contaminated biomass for a sustainable biorefinery.

Algal cultivation in wastewater and outdoor natural conditions followed by HTL of the harvested biomass to recover in parallel high value biocrude, solids and aqueous fraction, could be a potential biorefinery approach targeting multiple sectors.

सार

वर्तमान अध्ययन ने शैवाल आधारित ईंधन और उत्पादों की व्यवहार्यता में वास्तविक बाधाओं को दूर करने का प्रयास किया है। एक अभिनव अलगल बायोरिफाइनरी अवधारणा विकसित की गई है जहां एक मजबूत खेती प्रणाली (cultivation system) में इसकी खेती (cultivation) के दौरान नगरपालिका अपशिष्ट जल के उपचार के लिए शैवाल का उपयोग किया जाता है, इसके बाद इसे स्थायी रूप से उच्च मूल्य वाले उत्पादों में परिवर्तित किया जाता है। अध्ययन का पहला चरण सूक्ष्म शैवाल प्रजातियों का चयन करना था जिसमें धातु दूषित पानी के phycoremediation और हार्वेस्टिंग (harvesting) प्रणालियों के विभिन्न रूपों में बायोमास उत्पादन की क्षमता हो। इसके लिए दो अलग-अलग माइक्रोएल्ल कल्चर; 1) क्लोरेला पाइरेनोइडोसा/*Chlorella Pyrenoidosa* (सीपी/CP) और 2) क्लोरेला पाइरेनोइडोसा/*Chlorella Pyrenoidosa* और फोर्मिडियम/*Phormidium* (PA6) के कन्सोर्सियम (consortium) की हार्वेस्टिंग दो अलग-अलग तरीकों की हार्वेस्टिंग प्रणालियों में की गई थी यानी तरल माध्यम में शैवाल वृद्धि (suspended) और संलग्न बायोफिल्म सिस्टम (algal biofilm) अध्ययन का उद्देश्य धातु/ हैवी मेटल (heavy metal) हटाने और बायोमास उत्पादन के लिए विभिन्न खेती प्रणालियों के लिए एक शैवाल प्रजाति/संघ की उपयुक्तता को उजागर करना है। अपशिष्ट जल में आमतौर पर पाए जाने वाले छह भारी धातुएं; Cd, Cr, Pb, Cu, Ni और Zn, का उपयोग 1 mg L^{-1} सांद्रता में किया गया। व्यक्तिगत धातु स्पाइकिंग के साथ तरल माध्यम में शैवाल वृद्धि (suspended) प्रणालियों में सीपी/CP ने बेहतर प्रदर्शन किया। हालाँकि, PA6 ने मिश्रित धातु स्पाइकड (spiked) मीडिया (जो वास्तविक अपशिष्ट जल के अधिक करीब था) में बेहतर प्रदर्शन किया, विशेष रूप से संलग्न बायोफिल्म सिस्टम (सभी धातुओं को हटाने का $>60\%$) में और इसलिए, इसे आगे के अध्ययन के लिए चुना गया था। अगला कदम बाहरी प्राकृतिक परिस्थितियों में पायलट पैमाने (100 L) पर नगरपालिका अपशिष्ट जल का उपयोग करके संलग्न खेती प्रणालियों में चयनित माइक्रोएल्लो, PA6 का उत्पादन करना था, इसके बाद हार्वेस्टिड हुए अलगल बायोमास का मूल्यांकन किया गया। अध्ययन में जांच की गई दो बायोमास प्रसंस्करण तकनीकें क्रमशः बायोक्रूड और बायोगैस का उत्पादन करने के लिए हाइड्रोथर्मल द्रवीकरण/ hydrothermal liquefaction (एचटीएल/HTL) और एनारोबिक पाचन/ anaerobic digestion (एडी) थीं। दोनों प्रसंस्करण प्रणालियों के लिए, शुद्ध ऊर्जा अनुपात (Net Energy Ratios, NER) की गणना की गई (बायोमास खेती से बायोक्रूड/बायोगैस उत्पादन तक) और यह पता लगाया गया कि दो ऊर्जा रूपांतरण मार्गों में से कौन सा ऊर्जा रूप से अधिक अनुकूल था। अपशिष्ट जल से उगाए गए शैवाल ने एनारोबिक पाचन (एडी) के बाद संचयी बायोमीथेन के $346.59 \pm 5 \text{ mL g}^{-1} \text{ VS}_{\text{fed}}$ और एचटीएल/HTL के बाद क्रमशः $43 \pm 2\%$ (सूखा बायोमास आधार) का उत्पादन किया। एनईआरएचटीएल/ NER_{HTL} (0.08) को एनईआरएडी/ NER_{AD} (0.007) से अधिक पाया गया, जिससे एचटीएल को अपशिष्ट जल से उगाए गए शैवाल बायोमास से ऊर्जा की recovery के मामले में तुलनात्मक रूप से बेहतर मार्ग साबित हुआ।

इसके अलावा, 3 विकसित एल्ल बायोफिल्म-आधारित रूपांतरण प्रणालियों के लिए जीवन चक्र आकलन/Life cycle assessment (एलसीए/LCA) किया गया था; 1) एनारोबिक पाचन (एडी/AD) के माध्यम से संसाधित अपशिष्ट जल/wastewater grown algae (WWA) उगाए गए शैवाल, 2) हाइड्रोथर्मल द्रवीकरण (एचटीएल/HTL) के माध्यम से संसाधित अपशिष्ट जल/WWA उगाए गए शैवाल और 3) एचटीएल/HTL के माध्यम से संसाधित सिंथेटिक मीडिया उगाए गए शैवाल। परिणामों से पता चला कि अपशिष्ट जल से उगाए गए शैवाल के एचटीएल/HTL में उसी बायोमास के एडी/AD की तुलना में 41.1% कम प्रभाव था और सिंथेटिक मीडिया उगाए गए शैवाल के एचटीएल/HTL की तुलना में 98% कम प्रभाव था। पिछली प्रक्रिया ने हार्वेस्टिंग के दौरान अपशिष्ट जल के उपचार ($59-92\%$ पोषक तत्वों को हटाने) के कारण यूट्रोफिकेशन (eutrophication) में कमी पर भी सकारात्मक प्रभाव दिखाया। WWA से बायोक्रूड उत्पादन के लिए ग्लोबल वार्मिंग (global warming) क्षमता उसी बायोमास से बायोमीथेन (biomethane) उत्पादन की तुलना में 10 गुना कम और सिंथेटिक मीडिया से उगाए गए शैवाल से बायोक्रूड (biocrude) उत्पादन से 21 गुना कम थी। तुलनात्मक LCAs के परिणामों ने साबित कर दिया कि अपशिष्ट जल से उगाए गए शैवाल का HTL बायोमास के मूल्यांकन के लिए अपशिष्ट जल से उगाए गए शैवाल या सिंथेटिक

मीडिया से उगाए गए शैवाल के HTL की तुलना में अधिक टिकाऊ पर्यावरण हितैषी (sustainable/eco-friendly) मार्ग है।

बायोमास का एचटीएल/HTL (पानी: बायोमास::4:1) 27 बार (नाइट्रोजन/Nitrogen का उपयोग प्रतिक्रिया गैस के रूप में), 230° C (तापमान), 20 minutes (प्रतिक्रिया समय) के लिए एक बायोक्रूड/biocrude का उत्पादन किया जो विशेष रसायनों में समृद्ध था। एचटीएल/HTL बायोक्रूड/biocrude की उपज प्रशंसनीय थी लेकिन इसमें उच्च नाइट्रोजन और ऑक्सीजन सामग्री शामिल थी (जिससे इसके ईंधन मूल्य में कमी आई) और इसलिए, इसे अपग्रेडेशन/upgradation (डीऑक्सीजनेशन और डिनाइट्रोजनेशन) की आवश्यकता थी। इसके अलावा, बायोक्रूड के GC-MS विश्लेषण ने उच्च मूल्य वाले यौगिकों की उपस्थिति को दिखाया जिन्हें संवर्धन की आवश्यकता थी। अन्य सभी एचटीएल/HTL शर्तों को ध्यान में रखते हुए एचटीएल प्रतिक्रियाओं के दौरान उत्प्रेरक (जेडएसएम-5/ZSM-5 या केओएच/KOH)/विलायक/solvent (मेथनॉल या ब्यूटेनॉल)/रिएक्शन गैस (CO₂) को बदलकर एचटीएल उत्पादों की गुणवत्ता (उन्नयन/संवर्धन) में सुधार करने के प्रयास किए गए थे। अनुकूलित प्रतिक्रिया के समान ZSM-5 उत्प्रेरित HTL से बायोक्रूड की उपज K₂CO₃ उत्प्रेरित HTL द्वारा प्राप्त 55% थी, लेकिन कम नाइट्रोजन (TS का 1.2%) और ऑक्सीजन (TS का 22.3%) सामग्री के साथ, जिसने इसके HHV को बढ़ाकर 44.5 MJ Kg⁻¹ कर दिया (K₂CO₃ उत्प्रेरित HTL बायोक्रूड का 1.6 गुना)। अल्कोहलिक सॉल्वेंट्स के साथ एचटीएल से प्राप्त बायोक्रूड में 38% और 52% एस्टर शामिल थे और प्रतिक्रिया गैस के रूप में CO₂ के साथ इसमें > 65% हाइड्रोकार्बन थे। दूसरी ओर, जलीय अंश के एलसीएमएस विश्लेषण ने अत्यधिक मूल्यवान फार्मास्यूटिकल्स (मिनोक्सिडिल और एथोसक्सिमाइड) की उपस्थिति को दिखाया, जिनकी मात्रा क्रमशः 18.96 ± 0.24 mg kg⁻¹ और 41.07 ± 0.42 mg kg⁻¹ थी। इसके अतिरिक्त, इस कैल्शियम और फास्फोरस से भरपूर जलीय अंश को कार्बोनेटेड हाइड्रॉक्सीपैटाइट/carbonated hydroxyapatite (25.5 सूखा बायोमास आधार), a bone mineralizer (सिंथेटिक हड्डी) का उत्पादन करने के लिए हाइड्रोथर्मल रूप से खनिज किया गया था।

बायोरेमेडिएशन के बाद धातु/heavy metal दूषित बायोमास का निपटान/disposal उपयुक्त तकनीकों की अनुपलब्धता के कारण चुनौतियों का सामना करता है। इसलिए, एचटीएल उत्पादों पर धातुओं के प्रभाव की जांच करने और उनके भाग्य/fate का अध्ययन करने के लिए संलग्न बायोफिल्म सिस्टम में भारी धातु/heavy metal उपचार से प्राप्त एलाल बायोमास का एचटीएल किया गया था। धातु से लदी बायोमास के एचटीएल से बायोक्रूड उपज धातु संदूषण के बिना बायोमास के एचटीएल से बायोक्रूड उपज के बराबर थी (30%, सूखा बायोमास आधार)। ठोस (solids) और जलीय अंश (aqueous fraction) के भारी धातु विश्लेषण से पता चला है कि > 70% धातुएँ ठोस अंश में विभाजित हो गई थीं जबकि < 1% जलीय अंश में थीं। जलीय अंश नाइट्रेट और फॉस्फेट में भी समृद्ध था जिसे कार्ब की खेती के लिए पुनः उपयोग किया जा सकता था। इसलिए, स्थायी बायोरिफाइनरी के लिए धातु दूषित बायोमास के मूल्यांकन के लिए हाइड्रोथर्मल द्रवीकरण एक बहुत ही उपयोगी तकनीक हो सकती है।

अपशिष्ट जल और बाहरी प्राकृतिक परिस्थितियों में शैवाल की हार्वेस्टिंग, इसके बाद हार्वेस्टिड हुए बायोमास के एचटीएल के समानांतर उच्च मूल्य वाले बायोक्रूड, ठोस और जलीय अंश में पुनर्प्राप्त करने के लिए, कई क्षेत्रों को लक्षित करने वाला एक संभावित बायोरिफाइनरी दृष्टिकोण हो सकता है।

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