IMPROVISATION OF TRADITIONAL DYE PRODUCTION PROCESS AND BY-PRODUCTS VALORIZATION OF *INDIGOFERA TINCTORIA* L.

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by

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submitted

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CERTIFICATE

This is to certify that the thesis entitled **"Improvisation of traditional dye production process and by-products valorization of *Indigofera tinctoria* L.",** being submitted by **Ms. Lopa Pattanaik** 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 the award of any degree or diploma.

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by

Lopa Pattanaik

ABSTRACT

The present study emphasized the major limitations in the conventional indigo dye production process, and an attempt has been made to develop a sustainable process by evaluating its economics, improvising the traditional dye production technique, and valorizing the waste based on its characteristics.

Life cycle cost (LCC) analysis was performed to assess the economics of the existing indigo dye production process by considering the small-scale rural indigo industries of India. The system boundary of this study included the cultivation of *Indigofera* biomass, production and processing of indigo dye from the plant biomass, and the cost involved in each step. From LCC analysis, the total cost of natural indigo ($ 48.74/kg) was observed to be five times higher than the commercial synthetic indigo dye ($ 9.1/kg). However, the benefits of natural dye products towards the environment believed to surpass its economic barrier. Further, LCC analysis also estimated the stabilization of the rural economy by the employment of rural farmers and laborers for 1,96,250 man-days/year and 12,50,000 man-days/year, respectively, for an annual dye production of 1000 tons. Utilization of the by-products/bio-wastes generated from these dye industries predicted a cost reduction of approximately 22% of the total cost structure of the natural indigo dye.

The selection of suitable parameter(s) for monitoring the indigo dye formation in the conventional indigo dye production process is crucial to avoid the variability of dye quantity by manual observation. The adequate oxidation required for indigo dye formation was investigated by performing batch experiments based on two assumptions: (i) evaluating the effect of process parameters i.e. dissolved oxygen (DO), oxidation-reduction potential (ORP), and pH on indigo dye formation by installing an aerator system, and (ii) assessing the role of indigo particle size measurement concerning to indigo dye formation irrespective of any

external aeration system. The results indicated that oxidation parameters (DO, ORP, and pH) insignificantly co-related with the indigo dye yield. Whereas the particle size analysis substantiated the indigo dye formation, with a minimum indigo particle agglomeration size ≥ 100 μm.

The potential of two by-products (waste biomass and liquid waste) generated from the indigo dye production process was explored by detailed biomass characterization and nutrient analysis. High mineral content (P = 1513.47 and K = 5672.63 ppm) and C/N ratio (19.66) makes the waste biomass a suitable raw material for composting. Additionally, the ultimate analysis (C = 44.23%, H = 6.62%, N = 2.25%, and O = 37.94%) and lignocellulosic composition (cellulose = 41.15%, hemicellulose = 28.9%) of waste biomass indicated its theoretical potential towards methane (498.94 L/kg VS) and ethanol (281.9 L/ton) production, respectively. Finally, an integrated approach was suggested to enhance the eco-sustainability of the existing dye production process by substituting the current energy and fertilizer need in *Indigofera* biomass cultivation and indigo dye production processes. In addition to that, the phytochemical potential of waste biomass was also explored.

The waste biomass was utilized with microbial inoculum (Jeevamrutha and cow dung) at various ratios to produce stable compost for its possible re-use in *Indigofera* cultivation process. Among seven compost combinations (C1-C7), C4 with 8% Jeevamrutha showed the highest microbial activity, biomass degradation capacity (51%), and plant germination index (GI) >125%. Additionally, the nutrient-enriched liquid waste (chemical oxygen demand (COD) -18887.5 mg/L, nitrate-nitrogen (NO_3^--N)-18.22 mg/L, total ammoniacal nitrogen (TAN)-117.36 mg/L, and total dissolved phosphorus (TDP)- 99.70 mg/L) was treated using various microorganisms (bacteria, algal consortia, and indigenous microorganism) for its re-use in the dye production process. As compared to others, nutrients uptake by indigenous micro-organisms was observed to be much effective and feasible, with a removal efficiency of 79, 82, 86, and 86% for COD, NO_3^--N, TAN, and TDP, respectively. Hence, the incorporation of suitable waste management strategies in the natural indigo dye production process could help in establishing a zero-waste approach.

सार

वर्तमान अध्ययन में पारंपरिक इंडिगो डाई उत्पादन प्रक्रिया में प्रमुख सीमाओं पर जोर दिया गया है और इसके अर्थशास्त्र मूल्यांकन, पारंपरिक डाई उत्पादन तकनीक को सुधारने और विशेषताओं पर आधारित अपशिष्ट पदार्थ का मूल्यसिथरीकरण करने के लिए एक स्थायी प्रक्रिया विकसित करने का प्रयास किया गया है ।

भारत के लघु उद्योग इंडिगो उद्योगों पर विचार करके मौजूदा इंडिगो डाई उत्पादन प्रक्रिया के अर्थशास्त्र का आकलन करने के लिए जीवन चक्र लागत विश्लेषण [Life cycle cost (LCC)] किया गया था । इस अध्ययन की प्रणाली सीमा में इंडिगोफेरा (*Indigofera*) बायोमास उत्पादन, पौधों से इंडिगो डाई का उत्पादन और प्रसंस्करण तथा प्रत्येक चरण की लागत विश्लेषण का शामिल था । जीवन चक्र लागत के विश्लेषण से प्राकृतिक इंडिगो की कुल लागत ($ 48.74/kg) व्यावसायिक सिंथेटिक इंडिगो डाई ($ 9.1/kg) से पांच गुना अधिक देखी गई। हालांकि, पर्यावरण के प्रति प्राकृतिक डाई उत्पादों के लाभों ने इसके आर्थिक अवरोध को पार करने का विश्वास किया। इसके अलावा, जीवन चक्र लागत विश्लेषण ने 1000 टन के वार्षिक डाई उत्पादन के लिए ग्रामीण किसानों और मजदूरों के रोजगार (क्रमशः 1,96,250 मानव दिवस प्रति वर्ष और 12,50,000 मानव-दिन प्रति वर्ष) के माध्यम से ग्रामीण अर्थव्यवस्था के स्थिरीकरण का अनुमान लगाया। इन डाई उद्योगों से उत्पन्न उप-उत्पादों तथा जैव-कचरे के उपयोग ने प्राकृतिक इंडिगो डाई की कुल लागत संरचना के लगभग 22% की लागत में कमी की भविष्यवाणी की।

मैनुअल अवलोकन द्वारा डाई की परिवर्तनशीलता से बचने के लिए पारंपरिक इंडिगो डाई उत्पादन प्रक्रिया से इंडिगो डाई के गठन की निगरानी के लिए उपयुक्त मापदंडों का चयन महत्वपूर्ण है । बैच प्रयोग करके दो मान्यताओं के आधार पर इंडिगो डाई के गठन के लिए आवश्यक पर्याप्त ऑक्सीकरण की जांच की गई थी: (i) प्रक्रिया के प्रभाव मापदंडों का मूल्यांकन करना यानी विघटित ऑक्सीजन (DO), ऑक्सीकरण-न्यूनीकरण क्षमता (ORP), और एक जलवाहक प्रणाली स्थापित करके इंडिगो के गठन पर पीएच (pH) का प्रभाव, (ii) किसी भी बाहरी वातन प्रणाली की परवाह किए बिना इंडिगो डाई के गठन से संबंधित इंडिगो कण आकार माप की भूमिका का आकलन । परिणामों से संकेत मिलता है कि इंडिगो डाई की उपज ऑक्सीकरण मापदंडों (DO, ORP, and

pH) के साथ निरर्थकता से सह-संबंधित है । जबकि कण आकार विश्लेषण (न्यूनतम इंडिगो कण ढेर का आकार ≥ 100 μm) ने इंडिगो डाई गठन की पुष्टि की ।

इंडिगो डाई उत्पादन प्रक्रिया से उत्पन्न दो उप-उत्पादों (अपशिष्ट बायोमास और तरल अपशिष्ट) की क्षमता को विस्तृत बायोमास लक्षण वर्णन और पोषक तत्व विश्लेषण द्वारा पता लगाया गया था । उच्च खनिज सामग्री (P = 1513.47 and K = 5672.63 ppm) और C/N अनुपात (19.66) अपशिष्ट बायोमास खाद के लिए एक उपयुक्त कच्चा माल बनाता है। इसके अतिरिक्त, अपशिष्ट बायोमास के अंतिम विश्लेषण (C = 44.23%, H = 6.62%, N = 2.25%, and O = 37.94%) और लिग्नोसेलुलोसिक संरचना (सेलुलोज = 41.15%, हेमिसेलुलोज = 28.9%) ने इसकी सैद्धांतिक मीथेन (498.94 L/kg VS) और इथेनॉल (281.9 L/ton) उत्पादन क्षमता की ओर संकेत किया। अंत में मौजूदा डाई उत्पादन की पर्यावरण-स्थिरता को बढ़ाने के लिए प्रतिस्थापन द्वारा इंडिगोफेरा बायोमास की खेती में वर्तमान ऊर्जा और उर्वरक की जरूरत के अनुसार प्रक्रिया और इंडिगो डाई उत्पादन प्रक्रियाओं के लिए एकीकृत दृष्टिकोण का सुझाव दिया गया था । इसके अलावा अपशिष्ट बायोमास की फाइटोकेमिकल क्षमता तलाश भी की गई।

इंडिगोफ़ेरा की खेती की प्रक्रिया में इसके पुन: उपयोग के लिए स्थिर खाद का उत्पादन करने के लिए अपशिष्ट बायोमास को माइक्रोबियल इनोकुलम (जीवामृत और गाय के गोबर) विभिन्न अनुपात के साथ उपयोग किया गया था । सात खाद संयोजनों (C1-C7) में से , C4 (8% जीवामृत) में सबसे अधिक सूक्ष्मजीव गतिविधि, बायोमास क्षरण क्षमता (51%) और संयंत्र अंकुरण सूचकांक (GI) >125% दिखा । इसके अतिरिक्त, पोषक तत्वों से समृद्ध तरल अपशिष्ट [रासायनिक ऑक्सीजन की मांग (COD)- 18887.5 mg/L, नाइट्रेट-नाइट्रोजन ($NO3^-$-N)-18.22 mg/L, कुल अमोनियाक नाइट्रोजन (TAN)-117.36 mg/L, और कुल घुलित फास्फोरस (TDP)-99.70 mg/L)] को डाई उत्पादन प्रक्रिया में इसके पुन: उपयोग के लिए विभिन्न सूक्ष्मजीवों (बैक्टीरिया, अल्गल कंसोर्टिया, और स्वदेशी सूक्ष्मजीव) का उपयोग करके उपचारित किया गया था। । दूसरों की तुलना में, स्वदेशी सूक्ष्म जीवों द्वारा पोषक तत्वों की निष्कासन दक्षता (COD, $NO3^-$-N, TAN, TDP क्रमशः 79, 82, 86, 86%) अधिक प्रभावी और व्यवहार्य देखी गई । इसलिए, उपयुक्त अपशिष्ट प्रबंधन रणनीतियों का समावेश प्राकृतिक इंडिगो डाई उत्पादन प्रक्रिया एक शून्य-अपशिष्ट दृष्टिकोण स्थापित करने में मदद कर सकती है।

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LIST OF SYMBOLS

Symbol	Description
t	Time
min.	Minute
h	Hour
L	Litter
kg	Kilogram
mg	Miligram
KJ	Kilojoule
MJ	Megajoule
A	Maximum degradation rate
k	Rate constant

LIST OF ABBREVIATIONS

Syntax	**Description**
LCC	Life cycle cost analysis
LCA	Life cycle analysis
APHA	American public health association
DO	Dissolved oxygen
ORP	Oxidation reduction potential
LPM	Litre per minute
NMP	N-methyl pyrrolidone
BHT	Butylated hydroxy toluene
OFAT	One-factor-at-a-time
ADB	After dye extracted biomass
BDB	Before dye extracted biomass
TADB	Tamil Nadu after dye extracted biomass
TBDB	Tamil Nadu before dye extracted biomass
IADB	IIT after dye extracted biomass
IBDB	IIT before dye extracted biomass
ASTM	American society for testing and materials
HHV	Higher heating value
ICP-MS	Inductively coupled plasma–mass spectrometry
TGA	Thermo-gravimetric analysis
TG-DTG	Thermogravimetry-derivative thermogravimetry
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray diffraction
CI	Crystallinity index
NDF	Neutral detergent fibre
ADF	Acid detergent fibre
TMP	Theoretical methane potential
VS	Volatile solids
TEP	Theoretical ethanol potential
WPE	Waste plant extracts

FC	Folin-Ciocalteu
GAE	Gallic acid equivalent
QCTE	Quercetin equivalent
AE	Ascorbic acid
DPPH	2,2-diphenyl-1- picryl-hydrazyl
ABTS	2,2-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt
EDTA	Ethylenediaminetetraacetic acid
NBT	Nitroblue tetrazolium
PB	Phosphate buffer
GA	Gallic acid
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxy toluene
ETA	Ethyl acetate
ACT	Acetone
MET	Methanol
WAT	Water
DSW	Dye extracted solid waste
DLW	Dye extracted liquid waste
JA	Jeevamrutha
CD	Cow dung
FW	Fermented water
EC	Electrical conductivity
TN	Total nitrogen
TC	Total carbon
TOM	Total organic matter
TOC	Total organic carbon
GI	Germination index
VI	Vigour index
RMSE	Root mean square error
NA	Nutrient agar
OD	Optical density
PBS	Phosphate buffered saline

AC	Algal consortia
Chl-a	Chlorophyll a
TDS	Total dissolved solids
COD	Chemical oxygen demand
TAN	Total ammoniacal nitrogen
NH3-N	Ammoniacal nitrogen
PO4-P	Phosphate phosphorous
NO3-N	Nitrate nitrogen
TDP	Total dissolved phosphorus
ANOVA	Analysis of variance
VFAs	Volatile fatty acids
TN	Total nitrogen
DRSI	Discharge and reuse standards for irrigation
DRSIS	Discharge and reuse standards inland surface waters