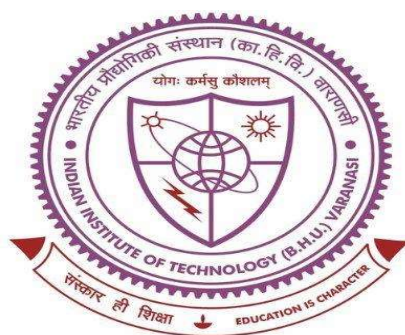


Design and Development of Microalgae based Wastewater treatment process by Machine Learning Tools



**Thesis submitted in partial fulfilment for the
Award of Degree**

DOCTOR OF PHILOSOPHY

By

VISHAL SINGH

**School of Biochemical Engineering
Indian Institute of Technology
(Banaras Hindu University)
Varanasi – 221005
India**

Roll No. 19011005

2023

CERTIFICATE

It is certified that the work contained in the thesis titled " Design and development of microalgae-based wastewater treatment process by machine learning tools" by Vishal Singh has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D. Degree.


07/03/2023
Dr. Vishal Mishra

(Supervisor)

School of Biochemical Engineering
Indian Institute of Technology
Varanasi-221005

Indian Institute of Technology

(Banaras Hindu University)

Varanasi – 221005, India

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Signature of the Student

Vishal
Vishal Singh

Place: Varanasi

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Dr. Vishal Mishra
Assistant Professor
School of Biochemical Engineering
Indian Institute of Technology (B.H.U.)
Varanasi-221005

School of Biochemical Engineering

Indian Institute of Technology

(Banaras Hindu University)

Varanasi – 221005, India


Signature of Head of Department/Coordinator of School

समन्वयक
Coordinator
जैव रासायनिक अभियांत्रिकी स्कूल
School of Biochemical Engg.
भारतीय प्रौद्योगिकी संस्थान
Indian Institute of Technology
(काठिंडीविठो) वाराणसी-221005
(B.H.U.) Varanasi-221005

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Abbreviations

ADP	Adenosine diphosphate
AL	Artificial lights
AM	Amplitude modulation
ARM	Association rule mining
ATP	Adenosine triphosphate
BOD	Biological oxygen demand
BP	Biomass production
CCR	Continuous current reduction
Cd^{2+}	Cadmium ion
CDTe	Cadmium telluride
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Co	Cobalt
CO_2	Carbon Dioxide
COD	Chemical oxygen demand
Conc.	Concentration
$\text{Cr}_2\text{O}_7^{2-}$	Chronate ion
Cr^{3+} & Cr^{6+}	Chromium ions
Cu	Copper
Cu^{2+}	Copper ion
DW	Dry Weight
Fe^{3+}	Ferrous ion
FL	Fluorescent tubes

H ₂ O	Water molecule
H ₂ PO ₄ ⁻	Dihydrogen phosphate
HCO ₃ ⁻	Bicarbonate
Hg ²⁺	Mercury ion
HID	High intensity discharge lamp
HMs	Heavy metal
HPO ₄ ⁻	Hydrogen phosphate
IGP	Insulated glazed photovoltaic
IIL	Initial inoculum level
IIPBRs	Internally illuminated photobioreactors
INC	Initial nitrogen concentration
InGaN	Blue indium gallium nitride
IPC	Initial phosphorus concentration
LDR	Light-dependent resistor
LED	Light emitting diodes
LHC	Light-harvesting complex
LI	Light intensity
Low-e	Low emissivity
MBWT	Microalgae based wastewater treatment
N	Nitrogen
N/P ratio	Nitrogen/Phosphorus ratio
P	Phosphorus
NADP	Nicotinamide adenine dinucleotide phosphate
NER	Net energy ratio
NH ₄ -N ⁺	Ammonium nitrogen

Ni ²⁺	Nickel ion
NO ₃ ⁻ -N	Nitrate nitrogen
NRE	Nitrogen removal efficiency
O ₂	Oxygen
P	Phosphorus
PAR	Photosynthetically active radiation
Pb ²⁺	Lead ion
PBR	Photobioreactor
PCB	Printed circuit board
PC-LED	Phosphor-converted LED
PMMA	Polymethyl methacrylate
PO ₄ ³⁻ -P	Phosphate phosphorus
PPAL	Parallel plate airlift reactor
PPFD	Photosynthetic photon flux density
PRE	Phosphorus removal efficiency
PS	Photosystem
PV	Photovoltaic
PWM	Pulse width modulation
RE	Removal efficiency
RuBisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
Si	Silicon
SMD	Surface mounting device
SVR	Surface to Volume ratio
Temp.	Temperature
TKN	Total kjedahl nitrogen

TN	Total nitrogen
TP	Total phosphorus
WPE	Wall plug efficiency
WW	Wastewater
$Y_{x/ph}$	Biomass yield on light
Zn^{2+}	Zinc ion

Preface

According to "The United Nations World Water Development Report, 2021: Valuing Water, " global freshwater usage has increased by six times in the last 100 years. More than 80% of wastewater is released into the water streams without proper treatment. Conventional wastewater treatment strategies such as the activated sludge process etc., have significantly impacted wastewater treatment on a large scale. However, these treatment processes have disadvantages such as high energy demand during the aeration process, disposal of secondary sludge, and emission of CO₂. Thus, they are not economically viable for a longer period and large-scale. There is a need to develop a sustainable wastewater treatment process to recover waste as a valuable resource, thus strengthening the circular economy. The biological wastewater treatment process mediated by microalgae is a sustainable approach for treating wastewater with simultaneous recovery of nutrients in the form of microalgae biomass. Microalgae growth in the wastewater is highly influenced by various parameters and environmental conditions such as CO₂ content in the inlet air, temperature, initial inoculum level, pH, light intensity, photoperiod, nutrient concentration in wastewater, and much more, together termed as predictor variables. These variables show mutual interaction with each other and cooperatively affect the treatment process. Therefore, in order to enhance the treatment capability and biomass productivity of microalgae, it becomes essential to optimize these variables and provide their right combination. However, it is practically impossible to test all combinations of variables in a single study. Machine learning algorithms can quickly analyze an extensive dataset and determine the best combinations of predictor variables as desired. Machine learning algorithms play a vital role in analyzing large datasets by detecting significant patterns in the data in a minimal time. Machine learning algorithms can quickly process large dataset and detect different patterns of predictor variables for increasing nutrient

removal capability of microalgae, including nitrogen and phosphorus, simultaneously increasing biomass productivity. Moreover, these algorithms can even generate new information from already reported data which is expected from new experiments. Patterns detected from these algorithms can assist in constructing high throughput experimental designs and assist in carrying out efficient wastewater treatment at larger scale.

In the present analysis, decision tree algorithm was used to determine the effects and the best combination of predictor variables, including microalgae class, pre-cultivation stage deciding factors and operating variables, resulting in high biomass productivity and wastewater treatment capability. Decision tree analysis detected 10 different combinations of predictor variables leading to high nitrogen removal efficiency, 10 combinations for high phosphorus removal efficiency and 8 combinations for increased biomass production. These combinations were tested on recently published experimental findings and nearly 80% accuracy was obtained. The results obtained through machine learning analysis can be used in constructing high throughput experimental designs, which may assist in carrying out the efficient wastewater treatment at large scale. In the continuation of the previous analysis, the decision tree was used to analyse the dataset of class *Trebouxiophyceae* and *Chlorophyceae*. Various combinations of cultivation parameters were determined to enhance their performance in wastewater treatment. Nine combinations of cultivation parameters leading to high biomass production and eleven combinations each for high nitrogen removal efficiency and high phosphorus removal efficiency for class *Trebouxiophyceae* were detected by decision tree models. Similarly, eleven combinations for high biomass production, nine for high nitrogen removal efficiency, and eight for high phosphorus removal efficiency were detected for class *Chlorophyceae*. The results obtained through decision tree analysis can provide the optimum conditions of cultivation parameters, saving time in designing new experiments for treating wastewater at a large scale.

In the next objective, one of the data mining tools, association rule mining has been used to find specific conditions of 11 cultivation parameters for enhancing microalgae growth in wastewater. General rules derived from association rule mining showed that biomass productivity and nutrient removal efficiency can be increased by keeping CO₂ content between 0.53-2.53%, light intensity in the range of 200-1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, initial inoculum level from 0.2-0.4 g/L and N/P ratio nearly 15:1 to 50:1. This extracted information can be used to design future experimental runs and will help in implementation of the process at a large scale without wet laboratory experiments. In the subsequent objective, the models obtained from machine learning analysis were verified both computationally and experimentally. Computational validation of the machine learning models was done by testing the rules extracted from them on some recently reported experimental results. The models were able to correctly classify 8 data points out of 11 for nitrogen removal efficiency, 9 data points out of 11 for phosphorus removal efficiency, and 6 data points out of 8 for biomass production. In experimental verification, models were experimentally verified by using two newly isolated strains that predicted a 10% error after experimental verification.

In the last objective, cost-benefit analysis was performed for the application of LEDs for microalgae cultivation. This objective aims to enlist the applications of light-emitting diodes in microalgal cultivation with reference to internally illuminated photobioreactors coupled with evaluation of the cost and energy balance of the artificial lights. The calculation shows that the electrical energy cost incurred during the application of light-emitting diodes for microalgae cultivation is approximately USD 15.19 kg⁻¹ DW. The collective fraction of electrical energy transformed into chemical energy (microalgae biomass) is around 6-8%. The cost of the light-emitting diodes can be decreased by the application of Arduino-based automated control system to control the power supply to LEDs, photovoltaic powered photobioreactors and additional light. These techniques of input cost reduction have been also

explored deeply in the present study. As estimated, they can reduce the cost of light-emitting diodes by 50%.