

## Abstract

Microalgae bioremediation offers a sustainable approach for waste effluent treatment and nutrient recovery. However, with thousands of unexplored microalgal species, finding robust strains for efficient waste effluent treatment is crucial. Optimal growth conditions, including CO<sub>2</sub> content, temperature, pH, light intensity, photoperiod and nutrient concentrations (Chemical oxygen demand (COD), NH<sub>3</sub>-N, PO<sub>4</sub>-P, N/P ratio), significantly influence microalgae's performance in waste effluent treatment. By using advanced techniques like RSM (response Surface Methodology), ANN (Artificial Neural Network), and GA (genetic algorithm), these variables can be optimized to enhance biomass productivity and nutrient removal efficiency. Modeling and simulation play a crucial role in advancing microalgae-based wastewater treatment by providing valuable insights, optimizing parameters, and enabling efficient and sustainable processes. The co-cultivation of microalgae for effluents treatment presents a viable strategy to enhance assimilation efficiency, prevent culture crashes, and boost biomass productivity. The dual flocculation-coagulation (DFC) harvesting approach is essential for efficient harvesting of microalgae biomass, which can be further utilized for valuable bioproducts and biofuels. Alum and Chitosan combinedly can be used as DFC agent for enhanced biomass harvesting. The use of ANN-GA optimization can help achieve high flocculation efficiency by optimizing input parameters like, pH, Dosage, mixing time (min.), settling time (min.), leading to an improved bioremediation process. The current study looked at the potential of *Diplosphaera mucosa VSPA*, a less studied isolated microalgae species, for cleaning carpet and textile effluent. To assess the potential of *D. mucosa VSPA*, its growth and bioremediation effectiveness were compared to that of a well-known strain, *Chlorella pyrenoidosa*. *D. mucosa VSPA* outperformed *C. pyrenoidosa* in both effluents, with the greatest biomass concentrations reaching 4.26±0.2 g/L in carpet effluent and 3.98±0.2 g/L in textile effluent. *D. mucosa VSPA* also removed 94.0± 2 % of ammonium nitrogen, 71.6±0.8 % of

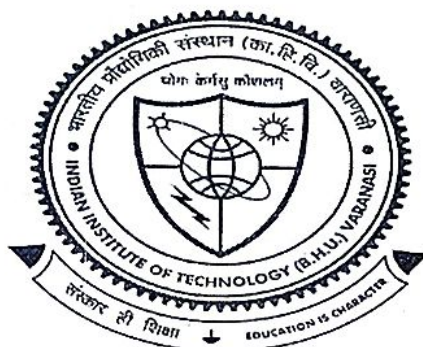
phosphate phosphorus, and  $90.9 \pm 1.2$  % of COD requirement from carpet effluent, which was almost 10% higher than *C. pyrenoidosa*. Both species also eliminated more than  $65 \pm 0.6$  % of the colour from both effluents, satisfying regulatory requirements. Photobiotreatment (PhBT) and the Gompertz model were utilised to mimic the development and removal patterns of microalgae in a photobioreactor. On the basis of the coefficient of regression and the second-order Akaike information criterion (AIC) test, the simulation outcomes indicated that photobiotreatment was the better-fitting model. Studies in modelling can aid in enhancing the performance and scalability of the photobioreactor. The next objective focused on enhancing the biomass yield and wastewater treatment efficiency of *D. mucosa* VSPA by optimizing five key input parameters (pH, temperature, light intensity, wastewater percentage, and N/P ratio). Two competitive techniques, RSM and ANN, were utilized to create predictive models using experimental data obtained through central composite design. The ANN models, constructed using MATLAB and Python, demonstrated higher accuracy and outperformed RSM models. Subsequently, both RSM and ANN models were combined with a genetic algorithm (GA) to identify the optimal input parameter values leading to higher biomass productivity. The hybridized ANN-GA approach, conducted in Python, yielded optimization results with reduced error, specifying the optimal parameter values as 7.78 pH, 28.8 °C temperature, 105.20  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  light intensity, 93.10 wastewater % (COD) and 23.5 N/P ratio. Furthermore, in the subsequent objective, the bioremediation efficiency of the newly isolated microalgal strain, *D. mucosa* VSPA, was assessed for its ability to treat petroleum effluent in a lab-scale simulated raceway bioreactor. Its performance was compared with that of the well-known species, *C. pyrenoidosa*. Notably, *D. mucosa* VSPA exhibited robust growth in the petroleum effluent, achieving high final biomass and lipid concentrations of  $6.93 \pm 0.8$  g/L and  $2.72 \pm 0.3$  g/L, respectively. The treatment efficiency, assessed through the removal of ammonium nitrogen, phosphate phosphorus, and COD exceeded  $90.1 \pm 2\%$ . Control experiments confirmed that

microalgae growth was the primary factor contributing to the significant removal of pollutants from the petroleum effluent. Several growth models, including Gompertz, Logistic, Stannard, Richard, and Schnute, were utilized to simulate the experimental data, successfully validating their accuracy with  $R^2$  values surpassing 0.90. Developing an appropriate model could streamline the scale-up process and reduce the efforts required in the future. In the next objective, the research aimed to assess the efficiency of two microalgae strains, *D. mucosa VSPA*, and *Scenedesmus obliquus*, in co-cultivation mode for treating textile effluent. The co-cultivation was conducted in mixotrophic and heterotrophic modes, with monoculture cultivation serving as a control. The mixotrophic co-cultivation mode demonstrated the highest biomass concentration of  $6.14 \pm 0.03$  g/L in textile effluent, exhibiting 1.5 - 2 times higher biomass productivity compared to mono-cultivation. Moreover, the mixotrophic co-cultivation mode displayed enhanced substrate removal efficiency, achieving more than  $90 \pm 2.2$  % removal of ammonium-nitrogen, COD, and  $80 \pm 1.4$  % removal of phosphate-phosphorus in the textile effluent. Microscopic and flow cytometry analysis indicated a dominant presence of *D. mucosa VSPA* culture (65%) and a lower presence of *S. Obliquus* culture (34%) in the final biomass concentration. The experimental results were validated and simulated using various growth and substrate removal models, ultimately confirming that the co-cultivation strategy outperformed mono-cultivation for treating high-strength industrial effluent. Next study, aims to optimize the efficiency of dual flocculation-coagulation (DFC) harvesting for the enhanced harvesting of *D. mucosa VSPA* through a multi-input optimization approach. By integrating artificial neural networks (ANN) and genetic algorithms (GA), the study seeks to uncover the optimal combination of input parameters to maximize the flocculation process. ANOVA analysis revealed that all input parameters ACD (Alum-Chitosan dosage), pH, mixing time, settling time significantly affect flocculation efficiency. ANN models generated via Python displayed superior prediction performance than others (MSE-0.76, RMSE-0.87). After that, all

models were hybridised with GA to generate a global optimal solution leading to highest flocculation efficiency ( $99.1 \pm 0.4$  %), and results were experimentally verified. All models generated less than 2% error with minimum error generated by the ANN-GA approach performed in Python (0.2). The optimized value for Alum dosage, Chitosan dosage, pH, mixing time, settling time are 16mg/L, 10.2mg/L, 8.91, 15.41 min. and 30.2 min. respectively. In summary, this comprehensive research effort encapsulates the multifaceted exploration of microalgae bioremediation, showcasing its potential for sustainable waste effluent treatment and nutrient recovery. The integration of advanced techniques, co-cultivation strategies, modeling, and optimization methodologies has significantly contributed to enhancing the efficiency and scalability of microalgae-based wastewater treatment processes, with tangible results and findings contributing to the ever-evolving landscape of environmental sustainability.

## EXTENDED ABSTRACT

# “Process Design, optimization, and evaluation of Microalgal Bioremediation and Value addition”



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## **Extended Abstract**

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effluent. *D. mucosa* VSPA also removed  $94.0 \pm 2$  % of ammonium nitrogen,  $71.6 \pm 0.8$  % of phosphate phosphorus, and  $90.9 \pm 1.2$  % of COD requirement from carpet effluent, which was almost 10% higher than *C. pyrenoidosa*. Both species also eliminated more than  $65 \pm 0.6$  % of the colour from both effluents, satisfying regulatory requirements. Photobiotreatment (PhBT) and the Gompertz model were utilised to mimic the development and removal patterns of microalgae in a photobioreactor. On the basis of the coefficient of regression and the second-order Akaike information criterion (AIC) test, the simulation outcomes indicated that photobiotreatment was the better-fitting model. Studies in modelling can aid in enhancing the performance and scalability of the photobioreactor. The next objective focused on enhancing the biomass yield and wastewater treatment efficiency of *D. mucosa* VSPA by optimizing five key input parameters (pH, temperature, light intensity, wastewater percentage, and N/P ratio). Two competitive techniques, RSM and ANN, were utilized to create predictive models using experimental data obtained through central composite design. The ANN models, constructed using MATLAB and Python, demonstrated higher accuracy and outperformed RSM models. Subsequently, both RSM and ANN models were combined with a genetic algorithm (GA) to identify the optimal input parameter values leading to higher biomass productivity. The hybridized ANN-GA approach, conducted in Python, yielded optimization results with reduced error, specifying the optimal parameter values as 7.8 pH, 28.8 °C temperature, 105.20  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  light intensity, 93.10 wastewater % (COD) and 23.5 N/P ratio. Furthermore, in the subsequent objective, the bioremediation efficiency of the newly isolated microalgal strain, *D. mucosa* VSPA, was assessed for its ability to treat petroleum effluent in a lab-scale simulated raceway bioreactor. Its performance was compared with that of the well-known species, *C. pyrenoidosa*. Notably, *D. mucosa* VSPA exhibited robust growth in the petroleum effluent, achieving high final biomass and lipid concentrations of  $6.93 \pm 0.8$  g/L and  $2.72 \pm 0.3$  g/L, respectively. The treatment efficiency, assessed through the removal of ammonium nitrogen,

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