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Tertiary treatment of coke-oven wastewater using suspended and immobilized whole live cells of constructed bacterial–microalgal consortium: modeling and optimization using ANN–GA hybrid methodology

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ABSTRACT

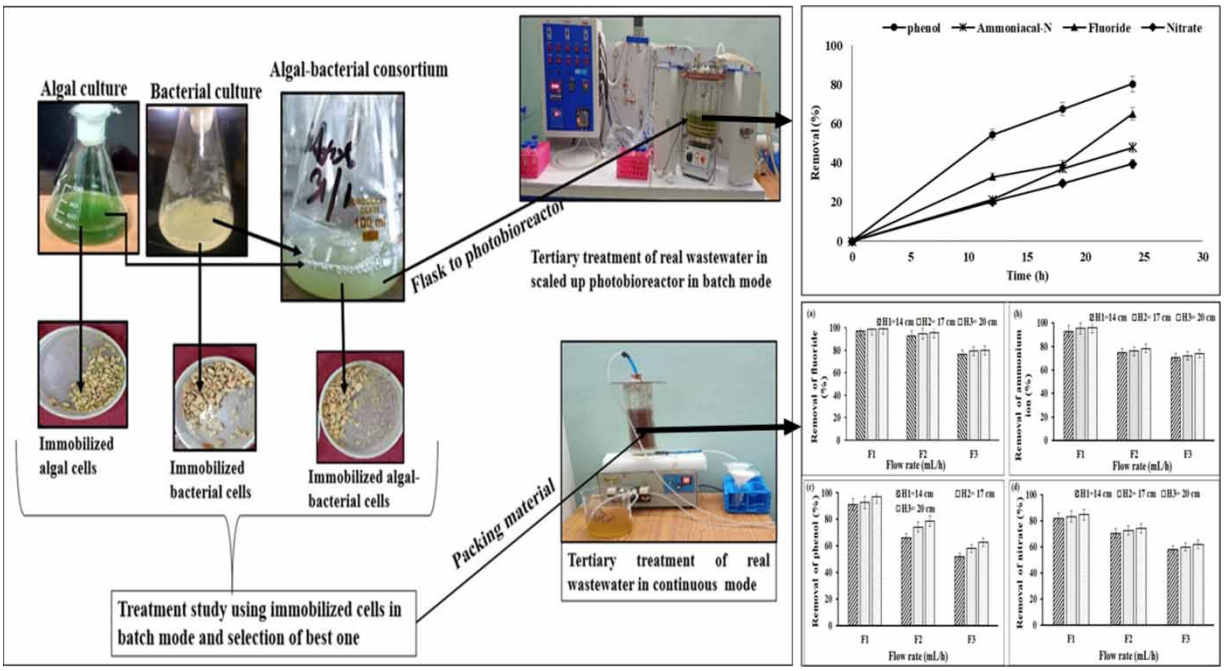
Coke-oven wastewater (CW), containing an array of toxic pollutants above permissible limits even after conventional primary and secondary treatment, needs a tertiary (polishing) step to meet the statutory limit. In the present study, a suitable bacterial–microalgal consortium (Culture C) was constructed using bacterial (Culture B: *Bacillus* sp. NITD 19) and microalgal (Culture A: a consortium of *Chlorella* sp. and *Synechococcus* sp.) cultures at different ratios (v/v) and the potential of these cultures for tertiary treatment of CW was assessed. Culture C4 (Culture B:Culture A = 1:4) with inoculum size: 10% (v/v) was selected for the treatment of wastewater since the maximum growth (3.08 ± 0.57 g/L) and maximum chlorophyll content (4.05 ± 0.66 mg/L) were achieved for such culture in PLE-enriched BG-11 medium. During treatment of real secondary treated coke-oven effluent using Culture C4 in a closed photobioreactor, the removal of phenol (80.32 ± 2.76%), ammonium ions (47.85 ± 1.83%), fluoride (65.0 ± 4.12%), and nitrate (39.45 ± 3.42%) was observed after 24 h. In a packed bed bioreactor containing immobilized C4 culture, the maximum removal was obtained at the lowest flow rate (20 mL/h) and highest column bed height (20 cm). Artificial intelligence-based techniques were used for modeling and optimization of the process.

Key words: Artificial neural network (ANN), bacterial–microalgal consortium, coke-oven wastewater, Genetic Algorithm (GA), immobilization, packed bed reactor, tertiary treatment

HIGHLIGHTS

- Bacterial–microalgal consortium was constructed by varying their ratios (v/v).
- Constructed consortium was used to treat secondary treated coke-oven wastewater.
- Native and constructed bioagents were immobilized individually in an earthen teapot.
- ANN and GA were used for modeling and optimization of the process variables.
- Immobilized constructed consortium was used in a packed bed bioreactor.

GRAPHICAL ABSTRACT



1. INTRODUCTION

India, being the second largest steel-producing country in the world, produces a large amount of wastewater which has direct and indirect effects on the ecosystem. Coke is produced in coke-oven plant (COP), an integral part of the iron and steel industry, by carbonizing coal at a high temperature (900–1,100 °C) in the absence of air. This coke is mainly used in the blast furnace for the production of steel. During the cooling and quenching stage of coke, a large amount of water is used and it is eventually loaded with several pollutants such as dirt, phenols, cyanide, thiocyanate, polyaromatic hydrocarbons, etc. About 4,000 m^3 of fresh water is being utilized for 1,000 tons of coke production, and finally, 1,000 m^3 of wastewater is produced (Rai *et al.* 2020). For by-product slot type COP, again a huge amount of water is used to recover by-products and a part of this water is transformed to wastewater after contamination with several inorganic and organic pollutants such as cyanide, ammonium ions, nitrate, fluoride, sulfide, tar, phenols, etc. (Cavaliere 2019). These two types of wastewater are mixed and termed as coke-oven wastewater (CW). Owing to high loading of pollutants, a thorough treatment of CW is mandatory from a clean environmental point of view. The conventional treatment methods (primary and secondary) are used for the treatment of CW. Several studies performed on the characterization of CW after the conventional treatment methods showed the inability of primary and secondary steps to remove the pollutants such as phenol, ammonium ions, cyanide, fluoride, nitrate, etc., below permissible limits (Biswas *et al.* 2010; Kwiecińska *et al.* 2017). The present study focuses mainly on to achieving the goal of meeting the permissible limits for the said pollutants from secondary treated CW via an environmentally friendly, low-cost method. Though many works have been reported in the literature on the removal of a single pollutant biologically, the studies on the treatment of secondary treated CW consisting of multiple pollutants are sparse.

Positive microbial interactions (symbiosis, mutualism, and commensalism) have been seen in the microbial community. When both species are benefited, the interaction is called as mutualism, while only one organism is benefited and the other is neither benefited nor harmed, the interaction is termed as commensalism. Again, when both species show interaction at a time, either mutually benefited or mutually harmed, termed as symbiosis. These interactions are very important during the survival of the microbial community in harsh conditions as well as betterment in the bioremediation of pollutants. Several studies on the treatment of wastewater (domestic and industrial) were done using the bacterial–microalgal consortium for the effective treatment as compared to their individual action. The advantages of using bacterial–microalgal consortium over single culture are as follows: (i) it makes the system robust for the treatment in harsh conditions, (ii) nutrients uptake specificity spectrum will be increased during the treatment, (iii) co-operative positive interaction increases the removal efficiency,

(iv) bacterial–microalgal consortium helps each other by exchanging O_2 and CO_2 during the treatment process (Jiang *et al.* 2021). Therefore, bacterial–microalgal consortium can be considered as a promising bioagent for the treatment of wastewater.

Literature review showed that *Bacillus* sp., *Synechocystis*, and *Chlorella* genera have immense potential to remediate both organic and inorganic pollutants from industrial wastewater when they were tested individually (Wang *et al.* 2010; Singh *et al.* 2019; Rai *et al.* 2021b, 2021c). Therefore, in the present study, these genera were selected for the treatment of wastewater containing multiple pollutants. Again, immobilized microbial cells have various advantages over the suspended microbial cultures (Park *et al.* 2000; Willaert 2007; Bouabidi *et al.* 2019; Valério *et al.* 2021; Moreira *et al.* 2022). Therefore, immobilized microbial cells can be considered as a potential alternative for the treatment of wastewater. Though several articles were published to treat wastewater using immobilized bacterial–microalgal consortium, as far as known, no articles on the treatment of secondary treated CW using immobilized bacterial–microalgal consortium is reported.

Artificial neural network (ANN) is an intelligence system that has been developed based on the biological model of a neuron. It is now widely used as an alternative and appealing tool for non-linear process modeling, particularly in cases where phenomenological models are difficult to develop. In the present study, ANN–GA hybrid technology has been used to model and optimize the process variables for the treatment of real and synthetic secondary treated coke-oven effluent (SSTCE) comprising of pollutants like phenol, ammonium ions, cyanide, fluoride, and nitrate above their permissible limits using suspended and immobilized bioagents under the batch mode of operation. The details of the optimization strategy and its importance have been incorporated in Annexure I, Supplementary file. The main objectives of this research article are (i) the construction of a suitable bacterial–microalgal consortium with definite microbial ratio, (ii) selection of suitable nutrient media felicitous for constructed consortium, (iii) removal of multipollutants (phenol, ammonium ions, cyanide, fluoride, and nitrate) from secondary treated CW using the selected consortium in both suspended and immobilized one, (iv) application of ANN–GA hybrid technology to model and optimize the process variables for treatment of SSTCE and real secondary treated CW, and (v) tertiary treatment of real CW in packed bed column. A unique combination of thorough experimentation with a hybrid robust process strategy comprising of ANN model and GA optimization techniques portrays the novelty of this work.

2. MATERIALS AND METHODS

All experiments were performed in three sets and values were shown as mean ± SD. All chemicals were AR-graded and purchased from Merck, India; if not then that is mentioned in the text.

2.1. Microbial culturing and cultivation

The bacterial strain (Culture B: *Bacillus* sp. NITD 19), the green algal-cyanobacterial consortium (Culture A: a consortium of *Chlorella* sp. (green algae), and *Synechococcus* sp. (cyanobacteria)) were used in the present study to remove five pollutants such as cyanide, phenol, ammonium ions, fluoride, and nitrate from SSTCEs. The isolation, identification, and culturing of bacterial strain '*Bacillus* NITD 19' and the consortium of '*Chlorella* sp.' and '*Synechococcus* sp.' were described by Rai *et al.* (2021a) and Rai *et al.* (2021b), respectively.

2.2. Preparation of bacterial–microalgal consortium

The experiments were designed by varying both the culture media and the ratio of bacterial strains (Culture B) and green algal-cyanobacterial consortium (Culture A). The best medium and the ratio were selected based on dry biomass content and chlorophyll content (representative of algal growth). In the present study, the different media as well as different ratios of microbial cells were examined with an aim to increase biomass production. To prepare constructed consortium, the ratio (volume/volume) of culture B and culture A varied as shown in Table 1.

It is well known that the growth of microbial cells depends mainly on the nutrient availability. Four different media were prepared such as (i) a modified minimal basal medium (MBM) (K_2HPO_4: 12.18 g/L, KH_2PO_4: 4.08 g/L, $(NH_4)_2SO_4$: 3.3 g/L, $MgSO_4$: 0.06 g/L, $MnSO_4$:0.00151 g/L, $C_6H_{12}O_6$: 0.5 g/L) (Upendar *et al.* 2017), (ii) BG-11 ($NaNO_3$: 1.5 g/L, K_2HPO_4: 0.04 g/L, $MgSO_4.7H_2O$: 0.075 g/L, $CaCl_2.2H_2O$: 0.036 g/L, Citric Acid: 0.006 g/L, Ferric ammonium citrate: 0.006 g/L, Na_2CO_3: 0.02 g/L, EDTA: 0.001 g/L, Trace metal: 1 mL/L) (Sen *et al.* 2018), (iii) MBM + BG-11 (1:1 v/v), (iv) poultry litter extract (PLE)-enriched BG-11 (BG-11 + PLE) (Rai *et al.* 2021c). These four different growth media MBM, BG-11, MBM + BG-11, and BG-11 + PLE were designated as GM1, GM2, GM3, and GM4, respectively.

Table 1 | Ratio of constructed bacterial–microalgal consortium (at temperature (25 ± 1 °C), light intensity (24,000 lux), photoperiod (16 h/8 h (day/night)) and shaking speed (120 rpm))

Constructed bacterial–microalgal consortium (Culture C)							
Ratio of bacterial (Culture B) microalgal consortium (Culture A)	C1 1:1	C2 1:2	C3 1:3	C4 1:4	C5 2:1	C6 3:1	C7 4:1

All the constructed consortiums (C1–C7) were transferred individually to 50 mL of sterile media from the well-grown laboratory cultures. pH of the medium and microbial concentration were kept constant at 7 and 10%, respectively. The cultures of all constructed consortium (C1–C7) taken in different mediums were incubated in the Algal incubator (LAB-X, Kolkata, West Bengal, India) at a specified temperature (25 ± 1 °C), light intensity (24,000 lux), photoperiod (16 h/8 h (day/night)), and shaking speed (120 rpm). The samples were collected after 10 days of incubation. The sample was centrifuged at 10,000 rpm for 3 min. Pellets were washed with deionized water three times and finally, they were used for measurement of biomass concentration and chlorophyll content. To get dry biomass content, pellets were dried in a hot air oven at an elevated temperature (60 ± 2 °C) overnight and weight was taken.

Chlorophyll content was measured using the solvent-extraction method where methanol (90%) was used as a solvent (Biswas *et al.* 2018; Das *et al.* 2020). For chlorophyll extraction, a specific amount of biomass was mixed with 90% methanol and the mixture was placed in hot water until all green biomass was transferred to the methanol solution. Finally, the absorbance of the green solution was measured at wavelengths 663 and 645 nm. The following formulae have been followed for the calculation of the total Chlorophyll (Biswas *et al.* 2018; Das *et al.* 2020):

$$Total\ chlorophyll = Chlorophyll\ a + Chlorophyll\ b,$$

where

$$Chlorophyll\ a = 12.7\ (OD_{663}) - 2.69\ (OD_{645});$$

$$Chlorophyll\ b = 22.9\ (OD_{645}) - 4.68\ (OD_{663})$$

The suitable bacterial and algal ratio vis-à-vis the most suitable constructed consortium was selected based on the maximum cell dry biomass and maximum chlorophyll content. A preliminary idea of the appropriate medium was also assessed based on the same output variables.

2.3. Tertiary treatment of SSTCE using selected constructed consortium in suspended form in a batch reactor

In the present study phenol, ammonium ions, cyanide, fluoride, and nitrate were considered as model pollutants since they are found to be present in real secondary treated coke-oven effluent, obtained after secondary treatment of CW in the effluent treatment plant. Therefore, SSTCEs were prepared by adding these pollutants at a particular concentration. The volume was made up by adding different media. The initial concentrations of these pollutants in SSTCE were maintained as phenol = 10 mg/L, ammonium ions = 200 mg/L, cyanide = 0.8 mg/L, fluoride = 10 mg/L, and nitrate = 50 mg/L. The types of pollutants and their concentrations in SSTCE were chosen based on the literature review and analysis of real wastewater collected from COP effluent runoff.

pH and temperature are important factors for the removal of pollutants. In the present study, the removal of pollutants was done at pH 7 and temperature 25 ± 1 °C. Rai *et al.* (2021a) tested and found that neutral pH and 25 ± 1 °C temperature are suitable for the removal of ammonium ions, phenol, and cyanide.

To prepare individual standard solutions (1,000 mg/L), sodium fluoride (2.21×10^3 mg), ammonium chloride (3.819×10^3 mg), sodium nitrate (1.517×10^3 mg), phenol (1.0×10^3 mg), were dissolved in 1.0 L of deionized water in separate volumetric flasks. Standard cyanide (Merck, India) solution was used to prepare the cyanide solution. The stipulated concentrations of all the pollutants in SSTCEs were maintained by mixing all the standard solutions proportionately. To get the final volume of SSTCEs, the requisite volume of different culture media (GM1–GM4) was added individually. The solutions, thus prepared, were termed as SSTCE1, SSTCE2, SSTCE3, SSTCE4 where MBM (GM1), BG-11 (GM2), MBM + BG-11 (GM3), and BG-11 + PLE (GM4) media were used, respectively. The final selection of the best medium

was done based on the maximum removal of all the pollutants. Typical values of the pollutants in wastewater from COP effluent and reference of the literature cited for the type of pollutants and their concentrations in SSTCE are shown in Table 2.

The selected constructed consortium was added to 90 mL SSTCE solutions (SSTCE1–SSTCE4) separately, taken in 250 mL of conical flasks. Inoculum size was maintained at 10% (v/v). All the flasks were placed in an Algal incubator under specified conditions. The samples were collected after every 1-day interval and centrifuged at 10,000 rpm for 3 min. The supernatant was analyzed for residual pollutants concentrations using standard protocols. Finally, the best medium was selected for the culture of constructed consortium and to be used for making up the volume of SSTCE solution on the basis of maximum removal of pollutants (fluoride, ammonium ions, phenol, nitrate, and cyanide).

In the present study, two types of controls were used: (i) biotic and (ii) abiotic. In biotic control, microbes were inoculated in a suitable medium without any pollutants, and in abiotic control, all pollutants are present in a medium without inoculation of microbes. In biotic control, the growth of the bioagent in a pollutant-free environment (native medium) was assessed and in abiotic control, the fate of the pollutant in a selected medium was examined without any bioagent. The controls were set for each experiment as per the experimental design and the main composition of controls is shown in Table 3.

Ammonium was analyzed following Spectrophotometric-Nessler's reagent method (Jenkins 1982) and during the process, all reagents were made ammonia free. Phenol was analyzed using a modified spectrophotometric method as described by Kang *et al.* (2000). Cyanide, fluoride, and nitrate were measured using Orion™ Cyanide, Fluoride, and Nitrate ion-specific Electrodes (Thermo Fisher Scientific, India).

2.4. Immobilization of whole cells on earthen teapot

In order to increase the removal efficiency of microbes and to decrease the susceptibility toward the toxic effect of the pollutants on the microbes, the synthetic wastewater was treated next with immobilized cultures. The earthen teapot (ETP) was used as a matrix for immobilization of whole live cells of bacteria, green algal-cyanobacterial consortium and selected constructed consortium separately. The ETP is used beside roadside tea-stall. Since its cost is very low, and people do not take tea from used teapots even after washing, simple 'use and throw' principle is followed for these earthen teapots. The disposal of such used earthen teapots, considered market waste, is becoming problematic day by day. Since the usage of plastic cups is prohibited now and there are large numbers of roadside tea-stalls, such used teapots are abundantly available free of cost. In the present work, this waste material was used as a matrix for the immobilization of whole live cells and thus, the utilization of waste material in a benevolent manner is the objective of the current study. The ETP was collected from roadside tea-stalls

Table 2 | Characterization of coke-oven wastewater

Parameters	Real secondary treated coke-oven wastewater (present study)	SSTCE (present study)	Raw coke-oven wastewater (Kwiecińska *et al.* 2017)	Secondary treated coke-oven wastewater (Das *et al.* 2020)	Standard values set by regulatory bodies (CPCB, India)
Cyanide (mg/L)	0.069	0.8	5–20	0.2–4	0.2
Phenol (mg/L)	5.3	10	18–290	2–4	1.0
Ammonium (mg/L)	138.85	200	93–790	75–345	50.0
Nitrate (mg/L)	25.4	50	9.9–18.2	–	
Fluoride (mg/L)	12.34	10	22–72	–	1.5
pH	6.9	7	8.7–9.3	6.5–8.1	5.5–9.0

Table 3 | Experimental design for controls

	Experimental condition			
Controls	pH, IS, IC, etc.	Medium	Bioagent	Pollutants
Biotic	As per experiment	√	√	NIL
Abiotic	As per experiment	√	NIL	√

at Durgapur (23.55°N 87.32°E), West Bengal, India. Collected ETPs were washed thoroughly with tap water and kept at natural conditions for 24 h for drying. Being a soft material, normal air-dried ETPs were ground in a ball mill. The material was then dried in a hot air oven at 100 °C for 2 h. The dried sample was segregated into several fractions based on their size ranges (from 1 to 3 mm) using a sieve shaker. The standard sieves were used for such purposes.

Three types of immobilized microbial cells were used for the treatment of wastewater. They were (i) bacterial cells (Culture B), (ii) consortium of green algal and cyanobacterial cells (Culture A), and (iii) a selected constructed consortium (Culture C4). To prepare the immobilized samples of B, A, and C4, 50 mL of well-grown microbial cultures were added individually to 450 mL of the selected sterile medium taken in 1.0 L of Erlenmeyer flasks under aseptic conditions. A specific amount of autoclaved ETP (5.0 g) of different sizes (1–3 mm) was added separately to each flask. These flasks were placed in algal incubators with a gentle shaking speed of 60 rpm at 25 $\pm$ 1°C. The initial pH of the medium was maintained at its original value. The cultures were kept in the incubators till the biofilm appeared vividly on the ETPs. The appearance of biofilm on the inert surface proved the immobilization process.

2.5. Characterization of biofilm

The attachment of microbial cells to any surface requires some adhesive materials. Extra polysaccharide (EPS) is secreted by the microbial cells to provide the structural support (Vu *et al.* 2009). Therefore, EPS estimation was performed for Culture A, Culture B, and Culture C4 using Alcian dye (Merck, India) to confirm the adhesive material secretion and eventually to get the proof of biofilm formation onto the surface of the solid matrix. EPS is an important structural biomolecule for biofilm formation. Thus, EPS secretion analysis was also done for the confirmation of the attachment of the microbial cells onto earthen teapots. For EPS analysis, 20 mL of culture was mixed with 80 mL of sterile suitable medium in the flask. The mixture was then placed in the incubator for 12 days at 25 $\pm$ 1 °C temperature and 60 rpm shaking speed. One reference sample (abiotic control) was also kept under the same conditions without the inoculation. The samples were taken out from the incubator for every 1-day interval. The grown culture was added with 100 μL of Alcian blue dye (Merck, India), and the mixture was left for 5 min. Finally, the sample was centrifuged at 10,000 rpm for 5 min. The supernatant was analyzed using a spectrophotometer at 606 nm. The amount of EPS produced was calculated from the reduction (%) in the absorbance of dye at OD_{606} nm in the supernatant relative to the control (Hazaimeh *et al.* 2014). A Scanning Electron Microscopy (SEM) study was also performed for the confirmation of the immobilization process. Immobilized cells were dried gently and then gold coating was done for observing the surface morphology of immobilized cells using SEM (Model: SIGMA HD, ZEISS).

2.6. Tertiary treatment of synthetic CW using immobilized cells in a batch reactor

Two factors, such as the size of ETPs (1–3 mm) and types of immobilized whole microbial cells (Cultures A, B, and C4), were varied individually during the treatment of synthetic CW to assess their effects on removal of pollutants. Immobilized cells were added to 100 mL selected SSTCE, and taken in 250 mL conical flasks. The flasks were then placed in an algal growth chamber for 10 days. Samples were collected intermittently and centrifuged. The supernatant was examined for residual concentrations of pollutants (ammonium ions, phenol, fluoride, nitrate, and cyanide).

2.7. Batch studies on tertiary treatment of secondary treated real CW using suspended culture of selected constructed consortium in scaled-up bioreactor

A cylindrical-shaped sophisticated closed photobioreactor made of glass (BOROCILE) was used to treat real wastewater using the suspended culture of a selected constructed consortium. The working volume of the photobioreactor was taken as 2.5 L (where total volume of the reactor was 10 L). The photobioreactor was digitally regulated for pH, temperature, and dissolved oxygen (DO) of the culture medium using the respective probes which were dipped in the solutions to sense the microenvironment of the culture during the treatment process. A stainless-steel jacket attached with white fluorescence light was used to cover the photobioreactor and to provide proper light intensity to the culture for a stipulated time period. The light intensity and photoperiod were maintained automatically at 24,000 lux and 16 h:8 h (day/night), respectively. The photograph of the said photobioreactor is shown in Supplementary material, Figure S1.

In the next phase of the study, the potential of the selected constructed consortium was investigated for the treatment of secondary treated real CW in scaled-up batch bioreactor. The working culture volume was maintained at 2.5 L in the photobioreactor which was 25 times higher than the volume used in previous studies carried out in Erlenmeyer flasks. Real wastewater was collected from the effluent runoff of the coke-oven secondary treatment plant, and characterized as pH

(7.2), ammonium ions (269.42 mg/L), fluoride (24.25 mg/L), cyanide (0.069 mg/L), nitrate (48.21 mg/L), and phenol (11.6 mg/L). From the characterization study of the collected wastewater, the concentration of cyanide was found below the permissible limit only while all other pollutants were above their stipulated limits and thus, the tertiary treatment of collected real wastewater was needed. The collected wastewater was diluted with the previously selected medium (1:1 v/v), and it was treated with the constructed consortium with an inoculum size of 10% for 24 h. The initial pH was kept similar to real wastewater (6.9–7.2). The sample was collected after 24 h and the efficiency of selected strains was analyzed based on the decrease of concentrations of the pollutants below permissible limits.

2.8. Packed bed reactor with selected immobilized microbial live cells

The packed bed column was made of polyacrylic material in a cylindrical shape. The column height and internal diameter were 24 and 6 cm, respectively. A perforated plate was placed at bottom of the column for the support of the immobilized cells. A single sampling port was placed at bottom of the column for collecting the sample. A pictorial diagram of the packed bed reactor setup is shown in Supplementary material, Figure S2.

The column was initially washed with alcohol twice and exposed to UV light for 20 min in a biosafety cabinet. A specified weight of the inert material (ETP) having an average particle size of 3 mm was washed, dried, and autoclaved. The column was then filled with the sterilized inert material and the requisite bed height was maintained. The bed of inert material was then filled with a sterile medium for supporting the growth of microbial culture during the immobilization process. The suspended culture of a well-grown bioagent, selected from the previous study, was poured in the next stage to get immobilized cell culture. The column was completely packed with sterile cotton and was finally placed in the algal incubator for 12 days. The collected secondary treated CW was diluted with an optimized medium (1:1 v/v) and was fed from the top of the column at a definite flow rate through a peristaltic pump. The performance of the fixed bed reactor was analyzed by varying two parametric factors such as flow rate (20–100 mL/h) and bed height (14–20 cm) in a prescribed manner. The collected sample was centrifuged (10,000 rpm for 3 min), and the supernatant was analyzed for the residual concentrations of the pollutants.

3. MODELING AND OPTIMIZATION

3.1. ANN modeling

ANN modeling is a powerful computer modeling tool that has been utilized to handle a wide range of real-world problems where the first principle-based phenomenology is unknown. In the present study, an ANN model was developed to establish a relationship between the capacity of bioagents (microalgae, bacteria, and constructed bacterial–microalgal consortium) to remove pollutants (phenol, ammonium ions, cyanide, fluoride, and nitrate) from SSTCE4 under the batch mode of operation. The parameters such as the number of days, initial concentrations of pollutants, size of the matrix, and percentage of bacteria were considered as input factors. For suspended culture, the size of the matrix has been considered zero. The developed model was optimized using Genetic Algorithm (GA) methodology to find the optimized values of input parameters for maximum removal of the pollutants. The entire modeling process and optimization were carried out in 'MATLAB R2017A' software Toolbox (The Math Works Inc., USA).

A multi-layered perceptron (MLP), the most extensively used ANN, was utilized in this study because it can approximate (map) any non-linear computable function to an arbitrary degree of precision (Lahiri & Ghanta 2009). The structure of the MLP used in this work is shown in Supplementary material, Figure S3. There are three layers in the network: input, hidden, and output layers. The input layer has the same number of nodes as the number of input parameters (eight) used to create the model. The number of nodes in the hidden layer is adjustable depending on the desired approximation and generalization capabilities of the network model. In the output layer, there is one neuron (percentage removal of pollutant). Five individual ANN models were developed for five pollutants (phenol, ammonium ions, cyanide, fluoride, and nitrate) with the same input parameters for each network.

Total experimental data were divided randomly into training data (70%), validation data (15%), and test data (15%). The development of the model is an iterative process of training data as with every iteration it alters the weights and tries to give the best values of output with minimum acceptable error. After the model was developed, the weights were used in unseen experimental data to check the accuracy of the model.

The performance of the ANN model for any set of experimental data depends on the tuning of meta parameters (number of nodes in the hidden layer and activation function in the input and output layer). The number of nodes in the hidden layer has a significant impact on the accuracy of the model. Very low number of nodes will fail to learn the data and cannot develop a

suitable model, whereas more number of nodes will increase complexity and execution time. Therefore, an optimal number of nodes in the hidden layer is to be evaluated. In this study, the optimal number was automatically evaluated through an algorithm. There are five different types of activation functions (sigmoidal function, tan hyperbolic function, transigmoidal function, linear function, log sigmoidal function). In this work, a GA-based searching tool was developed which evaluated all potential activation functions and number of nodes in the hidden layer and chose the optimal ANN structure automatically.

The constructed ANN model's reliability was predicted statistically using the following three criteria, namely, R^2, RMSE, and performance, the formula for which are listed below:

$$R^2 = \frac{\sum_{i=1}^{N} (y_{experimental_{(i)}} - y_{experimental_{(mean)}})(y_{predicted_{(i)}} - y_{predictedntal_{(mean)}})}{\sqrt{\sum_{i=1}^{N} (y_{experimental_{(i)}} - y_{experimental_{(mean)}})^2} \sqrt{\sum_{i=1}^{N} (y_{predicted_{(i)}} - y_{predictedntal_{(mean)}})^2}} \quad (1)$$

R^2 is the cross-correlation coefficient between input and output variables and for a desired ANN model its value is very close to unity.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} (y_{predicted_{(i)}} - y_{experimental_{(i)}})^2}{N}} \quad (2)$$

$$\text{Performance} = \sqrt{RMSE^2 + (1 - R^2)^2} \quad (3)$$

Generally, the ANN model with high R^2 value and low RMSE is preferred. In this study, a third performance parameter (namely performance) was developed, which combined both R^2 and RMSE. The lower the value of this parameter, the better is the ANN model. In the present study, a GA based search algorithm was employed to determine the best ANN model that corresponds to the lowest value of performance. This algorithm relieves novice users of ANN of the need to employ a time-consuming trial and error approach and automatically evaluates the optimum structure of the ANN model.

The selection of the input parameters for training the ANN is done after a thorough literature review. In this study, the input and output parameters are shown in Table 4. After selecting the input parameters, they are grouped together as input variable

Table 4 | Names and boundaries of input and output parameters used in ANN modeling and optimization

	Minimum	Maximum
Input parameters		
No. of days	0.00	5.00
Size of matrix (mm)	0.00	3.00
Initial concentration of phenol in wastewater (mg/L)	5.28	10.45
Initial concentration of ammonium ions in wastewater (mg/L)	138.84	204.13
Initial concentration of cyanide in wastewater (mg/L)	0.07	0.83
Initial concentration of fluoride in wastewater (mg/L)	10.10	12.34
Initial concentration of nitrate in wastewater (mg/L)	25.4	50.03
Percentage of bacteria	0.00	100.00
Output parameters		
Percent removal of phenol		
Percent removal of ammonium ions		
Percent removal of cyanide		
Percent removal of fluoride		
Percent removal of nitrate		

'x' and fed into the ANN tool. There are five dependent output variables, so five distinct ANN models were built. The functions generated for each ANN model were saved so that calculations with other sets of data (unseen data) can be performed to check the accuracy of the model. The training dataset is used by the ANN model to train the network and develop the model and its fitness and accuracy are checked on the testing and validation dataset.

GA is a meta-heuristic-based stochastic optimization technique that uses a combination of natural evolution's 'survival of the fittest' concept and the genetic propagation of features to provide a robust search and optimization process (Lahiri & Ghanta 2010). The benefit of this method is that it intelligently exploits the random search offered by previous data to lead the search into the solution space region with greater performance. In the present study, five distinct ANN models were generated and the optimization of these models was done together using GA. The purpose of doing it is to receive one specific set of optimized values of input parameters which will give maximum percent removal for all five pollutants.

4. RESULTS AND DISCUSSION

4.1. Growth study of constructed bacterial–microalgal consortium in different media

The bacterial–microalgal consortia with different ratios (Cultures C1–C7) were incubated for 10 days in four different mediums (GM1–GM4) and the growth of consortia is depicted in Figure 1. For all the ratios of bacterial and microalgal strains in constructed consortia (Cultures C1–C7), the biomass was measured. The highest amount of biomass (3.08 ± 0.57 g/L) was obtained for Culture C4 (i.e., Culture B:Culture A = 1: 4) in GM4 medium. The order of biomass obtained for Culture C4 in different nutrient medium is as follows: 3.08 ± 0.57 g/L (GM4) > 1.82 ± 0.08 g/L (GM3) > 1.043 ± 0.15 g/L (GM1) > 0.69 ± 0.54 g/L (GM2). However, for pure bacterial culture (Culture B) and for pure green algal–cyanobacterial consortium culture (Culture A), the maximum biomass was obtained as $2.35 \pm .03$ g/L (in GM1) and 0.976 ± 0.01 g/L (in GM4), respectively. Rai *et al.* (2021b) characterized the PLE and found nitrogen and phosphate contents as 59.39 mg/L and 47.33 mg/L, respectively. The N/P ratios of all media were calculated on the basis of the available total N and P in the respective medium. The N/P ratio was obtained as GM1: 0.18:1, GM2: 37.5:1, GM3: 0.26:1, and GM4: 4.05:1. The N:P ratio (37.5:1) was obtained maximum for GM2; however, the growth of Culture B, Culture A, and Culture C were observed minimum. This might be due to the presence of higher nitrogen content in the medium (Rai *et al.* 2020). Devi *et al.* (2013) stated that the nutrients play a key role in their growth, and showed that N + P combination gave higher growth of microalgae. Thus, in the present study, GM4 medium showed the maximum growth for Culture A and Culture C than in any other media. Furthermore, in Culture C4, where the microalgal–bacterial ratio was maximum, the highest growth was observed in GM4. The suitable medium for the growth of bacterial–microalgal consortium was chosen based on the amount of biomass. Since for both Culture A and Culture C4, the highest biomass was obtained in GM4 (BG-11 + PLE), this medium was chosen for further study. The presence of micro/macronutrients (N, P, Ca, Mg, and K) in BG-11 + PLE medium helps the growth of microorganisms (Rai *et al.* 2021b).

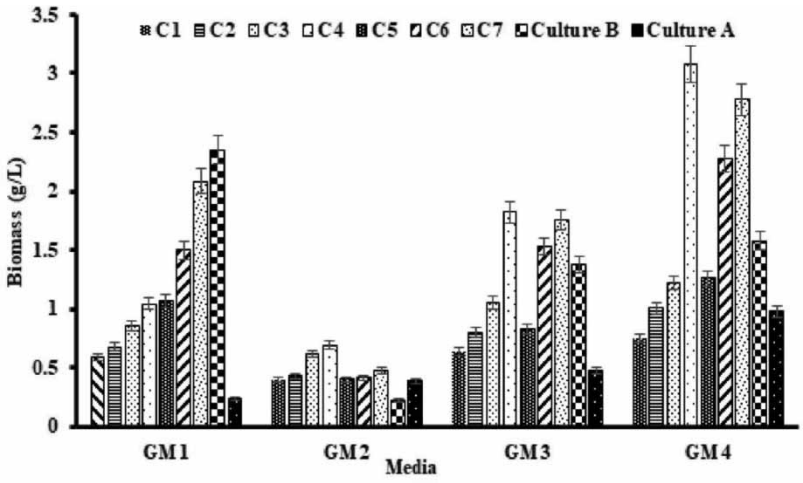


Figure 1 | The growth study of Culture B, Culture A and all constructed bacterial–microalgal consortia (Cultures C1–C7) in different media after 10 days.

In the next phase of the study, the microalgal growth in the consortium was quantified in terms of chlorophyll content (Figure 2). The maximum chlorophyll content (4.05 ± 0.66 mg/L) was obtained with Culture C4 when grown in a GM4 medium. A number of studies showed that the PLE based medium led to higher growth for microalgae (Bhatnagar *et al.* 2011). From the diagram, it is seen that Culture C4 showed the highest chlorophyll contents in all four types of medium. This may be due to the presence of higher microalgal cells than the bacterial cells in the constructed consortium C4. The vigorous growth of both microbes in GM4 is not only because of N and P, but also due to the presence of other cations such as Ca^{+2}, Mg^{+2}, etc. In BG-11 medium solely, the inorganic carbon content is (0.007×10^3) mg/L. The inorganic carbon contents in all the media were found as follows: GM1 – (0.5×10^3) mg/L, GM2 – (0.007×10^3) mg/L, GM3 – (0.507×10^3) mg/L, and GM4 – (0.007×10^3) mg/L. Therefore, it is seen that GM3 has the maximum inorganic carbon content. However, during the growth of bacteria, CO_2 is produced in the medium which helps microalgal growth. On the other hand, during microalgal growth, organic content increases in the medium, and O_2 is produced which facilitates bacterial growth. From the literature, PLE is a suitable medium source for microalgae (Bhatnagar *et al.* 2011). In GM4 medium, the maximum growth of bacterial–microalgal consortium was found because of a combined effect of available nutrients, which supports the growth of both microorganisms. Again both microorganisms support each other. There are several pieces of evidence of the positive interaction of bacterial–microalgal cells, where the presence of bacterial cells supports the growth of the microalgae (Kouzuma & Watanabe 2015; Ramanan *et al.* 2016). On the other hand, the microalgal cells produce organic matter which is being utilized for the growth and energy source by the bacterial cells in the consortium (Ramanan *et al.* 2016).

Since the constructed consortium C4 (Culture B:Culture A = 1: 4) in GM4 medium (BG-11 + PLE) showed best growth in terms of biomass and chlorophyll content, Culture C4 was chosen for further studies.

4.2. Tertiary treatment of SSTCE using bacterial–microalgal consortium in suspended form

The constructed consortium Culture C4 was used next for the tertiary treatment of synthetic secondary treated CW (SSTCE1–SSTCE4). The removal of five model pollutants from SSTCE1 to SSTCE4 using Culture C4 was investigated as shown in (Figure 3(a)–3(d)). The uptake of pollutants was found to vary in different SSTCEs. The bio-removal of the pollutants depends on the concentrations of pollutants, preferences, and uptake capabilities of bioagents. The phenol, ammonium ions, and cyanide were found to be removed fast in all the synthetic wastewater. The concentrations of all pollutants decreased successfully below their permissible limits only in SSTCE4. However, in other synthetic wastewater, only phenol, cyanide, and ammonium ions were removed below the stipulated limits. The maximum removal of pollutants was observed as $95.77 \pm 2.34\%$, $90.76 \pm 3.4\%$, $76.65 \pm 2.31\%$, $92.08 \pm 2.98\%$, and $88.71 \pm 3.37\%$ for phenol, ammonium ions, cyanide, fluoride, and nitrate, respectively in SSTCE4 (Figure 3(d)). Therefore, SSTCE4 was chosen for further studies on the removal of pollutants.

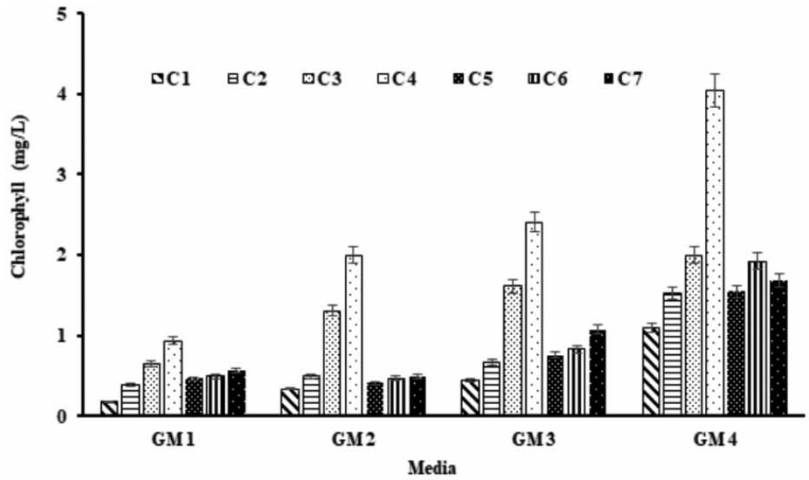


Figure 2 | The chlorophyll content estimation of all constructed bacterial–microalgal consortia (Cultures C1–C7) in different media.

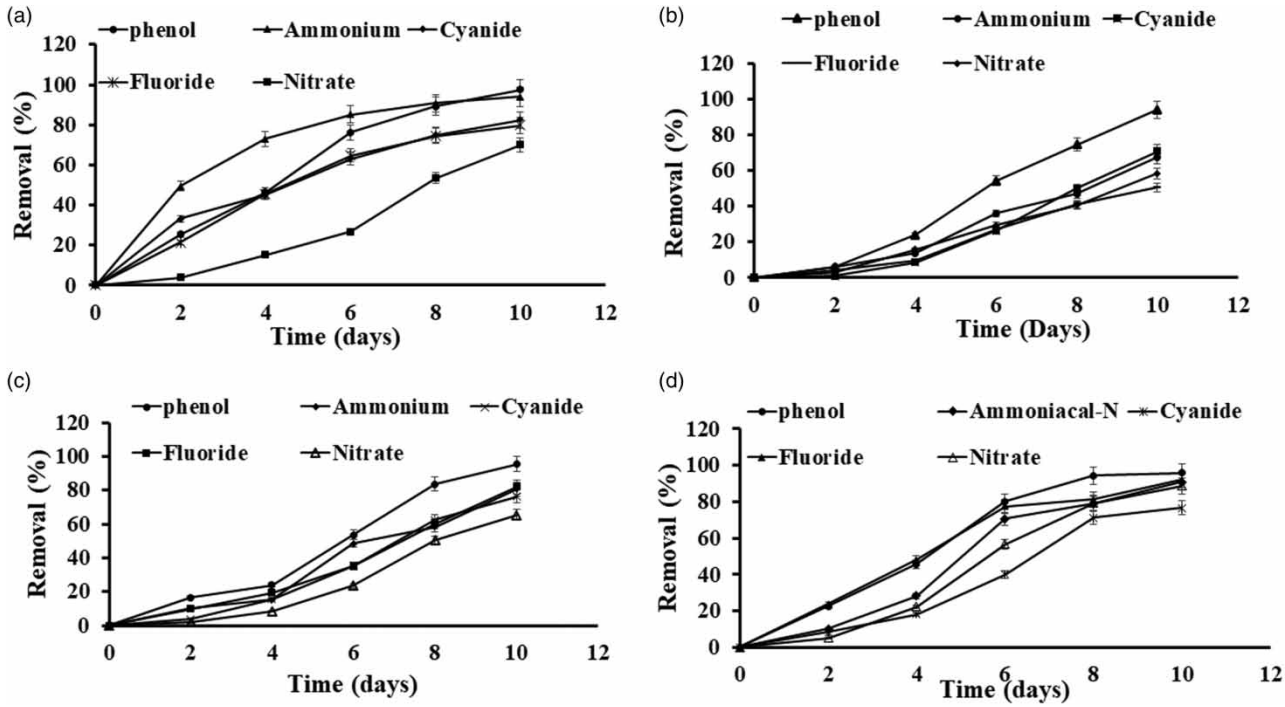


Figure 3 | Removal of phenol, ammonium ions, cyanide, fluoride and nitrate using Culture C4 from (a) SSTCE1, (b) SSTCE2, (c) SSTCE3, and (d) SSTCE4.

4.3. Characterization of immobilized cells

SEM images were studied for both the native matrix (without immobilization) and the matrix after immobilization. SEM images are shown in Supplementary material, Figure S4(a)–S4(d). The rod-shaped bacterial cells were seen onto the solid matrix (Supplementary material, Figure S4(a)) when the pure bacterial culture was immobilized onto ETP. The rod shape of *Bacillus* sp. NITD 19 was already observed by Rai *et al.* (2021a) for suspended culture. Therefore, it can be stated that bacteria retained their shape after immobilization also. SEM image of immobilized microalgal cells (circular and filamentous) is shown in Supplementary material, Figure S4(b). The granular structures were observed for the bacterial–microalgal consortium as seen in Supplementary material, Figure S4(c). All such SEM images confirmed the immobilization of bacteria, algae, and bacterial–microalgal consortium (Culture C4). The SEM image of the native matrix is shown in Supplementary material, Figure S4(d). From the morphological study, the change in surface textures for the immobilized culture from that of the native one specifies the immobilization of microbial strains on the matrix. Zhang *et al.* (2018) showed a granular structure of the immobilized bacterial–microalgal consortium in SEM study. In a previous study, Paliwal *et al.* (2015) showed the SEM images of immobilized bacterial cells onto the corncob for black liquor biodegradation. In another study, Ahmad *et al.* (2018) showed the SEM images of Ca-alginate immobilized microalgal cells for enhancing the bio-sorption of transition metals and Xie *et al.* (2020) performed SEM study of immobilized bacterial–microalgal consortium for affirming their involvement in the sulfamethoxazole degradation. Thus, it can be stated that SEM study is imperative to assess the immobilization of microorganisms onto particular solid matrices and their involvement in the later course of study.

EPS is secreted by the microbial cells and it has several crucial roles like improving the initial attachment of the cells to the matrix, maintaining the mature biofilm structure, protecting the environmental stress, etc. (Czaczyk & Myszka 2007). Czaczyk & Myszka (2007) reviewed the function of EPS on the biofilm formation. The biosynthesis of EPS varies from species to species (Dunne 2002). Adhesion of microbial cells onto a solid matrix was analyzed through the biochemical study by measuring the secretion of EPS as a binder. EPS was estimated for all bioagents and was found as 63.56, 50.33, and 74.6% for bacterial cells, microalgal cells, and constructed consortium of bacterial–microalgal culture (Culture C4), respectively. The higher value of EPS binding for immobilized bacterial–microalgal consortium gives strong evidence of the superior binding capability of the present culture (Culture C4) than that of the other two (Cultures A and B). Both the

SEM study and EPS estimation confirmed the successful immobilization of bioagents onto a solid matrix. The attached microbes were tested for growth in a suitable medium after the treatment studies and growth was observed. Therefore, it can be said that microbial cells remain viable after immobilization and even after the treatment of secondary treated CW.

In some literature, reusability of enzymes was analyzed. Studies on reusability of cells showed no change in their capacity of synthesizing biocatalysts, which is an advantage (Lima *et al.* 2017; Galvao *et al.* 2018; Pinheiro *et al.* 2018).

4.4. Bioremediation of phenol, ammonium ions, nitrate, fluoride, and cyanide from the synthetic coke-oven wastewater (SSTCE4) using immobilized cultures

The whole live immobilized bacterial (Culture B), microalgal (Culture A), and a consortium of bacterial–microalgal cells (Culture C4) onto the different sizes of ETPs (1–3 mm) were used for the treatment of SSTCE4 for 5 days, and removal of phenol, ammonium ions, cyanide, fluoride and nitrate were analyzed. The removal of all the pollutants using immobilized bacterial, a microalgal and constructed consortium of bacterial–microalgal cells (Culture C4) onto the different sizes of ETPs (1–3 mm) are shown in Supplementary material, Figure S5(a)–S5(e).

All the immobilized bioagents were found efficient to treat SSTCE4 solution. While the maximum removal of phenol (99.62%) and ammonium ions (98.72%) was observed with bacterial culture (Culture B) immobilized onto 1 mm of ETP, the maximum removal of cyanide (97.47%), fluoride (97.92%), and nitrate (88.80%) was seen with Culture C4 immobilized onto 1 mm of ETP (Supplementary material, Figure S5(a)–S5(e)).

While examining the effect of the size of the immobilized matrix (ETP) on the removal of pollutants, it was observed that 1 mm ETP performs best as an immobilization matrix for all the cultures. It is well known that the smaller the size of the particle, the more will be the surface area. Therefore, smaller particles, owing to having more surface area, may have more immobilized cells to remove the pollutants (Nawi & Salmiah 2012). Again, the Culture C4, immobilized onto 1 mm ETP, was found most efficient for reducing the concentrations of all the model pollutants (phenol (0.23 mg/L), ammonium ions (20.67 mg/L), cyanide (0.021 mg/L), fluoride (0.21 mg/L), and nitrate (5.6 mg/L)) below the permissible limits within 5 days (shown in Supplementary material, Figure S6). Andersson *et al.* (2008) stated that the treatment of wastewater using the attached growth of microbes is more effective than the dispersed one.

4.5. Bioremediation of pollutants from the real wastewater in batch and continuous mode of operation

Real wastewater was subjected to tertiary treatment using Culture C4. The collected wastewater was diluted (50%) with GM4 medium, and was treated under two different modes of operations: (i) batch study in closed photobioreactor using a suspended form of Culture C4, and (ii) continuous study in packed bed column using immobilized form of Culture C4. The removal of phenol, ammonium ions, fluoride, and nitrate was examined in both the cases. In a closed photobioreactor, the removal of phenol ($80.32 \pm 2.76\%$), ammonium ions ($47.85 \pm 1.83\%$), fluoride ($65.0 \pm 4.12\%$), and nitrate ($39.45 \pm 3.42\%$) was observed after 24 h (Figure 4), while pH and DO were found to vary from 6.79 ± 0.5 to 8.05 ± 0.5, and from 25.24 to 42.89%, respectively at 25 ± 2 °C during experimentation. The uptake of pollutants from the surrounding environment (wastewater) causes a change in the pH of the microenvironment. Budgen & Le-Clech (2021) showed an increase in DO during the treatment process.

The column was packed with constructed consortium (Culture C4) immobilized onto the ETP (3 mm). Two parameters such as bed height ($H1 = 14$ cm, $H2 = 17$ cm, and $H3 = 20$ cm) and flow rate ($F1 = 20$ mL/h, $F2 = 50$ mL/h, and $F3 = 100$ mL/h) were tested for the treatment of real wastewater in column study and the removal of major pollutants such as phenol, ammonium ions, fluoride, and nitrate from the real secondary treated CW is shown in (Figure 5(a)–5(d)). The maximum removal of pollutants was observed as 99.43, 96.21, 96.90, and 84.92% for fluoride, ammonium ions, phenol, and nitrate, respectively at the lowest flow rate $F1 = 20$ mL/h and highest column bed height $H3 = 20$ cm. The removal of pollutants was mainly affected by varying flow rates. With an increase in the flow rate from 20 mL/h to 100 mL/h, the removal of fluoride, ammonium ions, phenol and nitrate was found to decrease from 99.42 to 79.91%, from 96.21 to 76.88%, from 96.90 to 62.93% and from 84.92 to 61.99%, respectively, at column bed height $H3 = 20$ cm. The flow rate is inversely related to the residence time of pollutants in the column. Thus, it can be stated that the exposure time of pollutants to the immobilized culture is inversely proportional to the flow rate. Higher the exposure time, more will be the removal of pollutants. The effect of bed height was tested next. It was observed that with an increase in the column bed height from $H1 = 14$ cm to $H3 = 20$ cm,

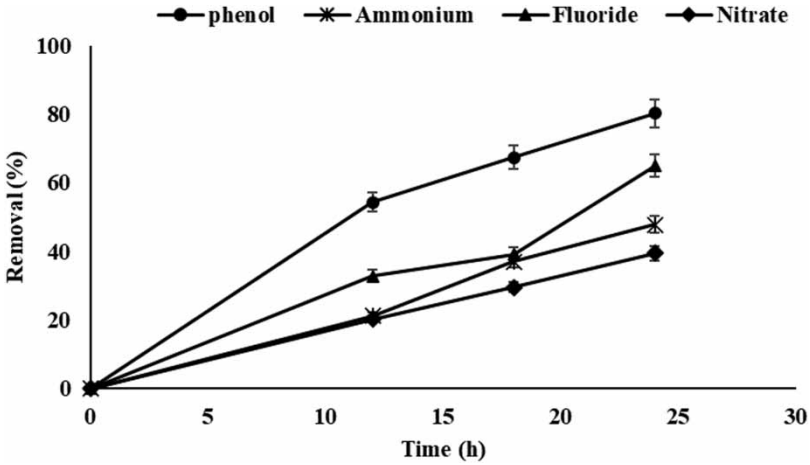


Figure 4 | Treatment of real wastewater using bacterial–microalgal consortium (Culture C4) in photobioreactor.

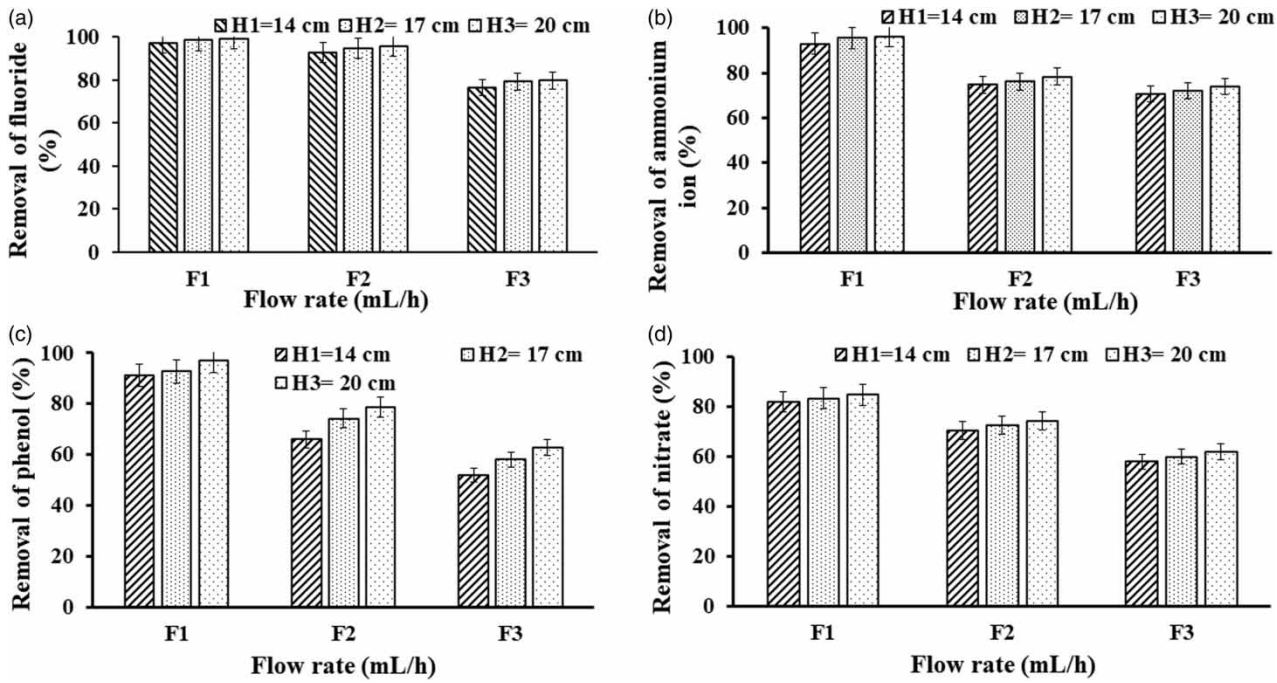


Figure 5 | Bio-removal of (a) fluoride, (b) ammonium ions, (c) phenol, and (d) nitrate from real wastewater at different bed height and flow rate.

the removal of pollutants such as fluoride, ammonium ions, phenol and nitrate increased from 97.26 to 99.42%, from 92.98 to 96.21%, from 91.25 to 96.90% and from 81.97 to 84.92%, respectively. Hence, it can be stated that the lowest flow rate (F1 = 20 mL/h), and highest column bed height (H3 = 20 cm) are suitable for reducing the concentrations of major pollutants below the permissible limits.

In order to know the effect of flow rate and bed height on the bio-removal of pollutants, a two-way ANOVA was performed using 'Microsoft Excel 2013'. After the statistical analysis, flow rate was found significant ($P\ value < 1.53 \times 10^{-6}$). The interaction of bed height and flow rate was also found statistically significant (the F value (0.019) obtained was much lower than the $F\ critical$ value (2.72)) for the bio-removal of pollutants.

Table 5 | Partial representative input and output experimental values taken for ANN modeling and GA optimization

Serial No.	No. of days	Size of matrix (mm)	Initial phenol conc. (mg/L)	Initial ammonium ions conc. (mg/L)	Initial cyanide conc. (mg/L)	Initial fluoride conc. (mg/L)	Initial nitrate conc. (mg/L)	Percentage bacteria	% Removal of phenol	% Removal of ammonium	% Removal of cyanide	% Removal of fluoride	% Removal of nitrate
1	0.00	1.00	5.28	138.84	0.07	12.34	25.4	100.00	0.00	0.00	1	0.00	1.00
2	4.00	2.00	10.45	204.13	0.83	10.10	50.03	100.00	88.85	88.99	2	4.00	2.00
3	5.00	3.00	10.45	204.13	0.83	10.10	50.03	100.00	96.65	95.95	3	5.00	3.00
4	5.00	2.00	10.45	204.13	0.83	10.10	50.03	0.00	66.54	73.42	4	5.00	2.00
5	0.00	3.00	10.45	204.13	0.83	10.10	50.03	0.00	0.00	0.00	5	0.00	3.00
6	1.00	3.00	10.45	204.13	0.83	10.10	50.03	0.00	2.22	11.52	6	1.00	3.00
7	0.00	1.00	5.28	138.84	0.07	12.34	25.4	20.00	0.00	0.00	7	0.00	1.00
8	1.00	1.00	10.45	204.13	0.83	10.10	50.03	20.00	51.30	40.89	8	1.00	1.00
9	2.00	1.00	10.45	204.13	0.83	10.10	50.03	20.00	62.45	61.14	9	2.00	1.00
10	5.00	3.00	10.45	204.13	0.83	10.10	50.03	20.00	96.28	76.96	10	5.00	3.00

4.6. ANN modeling for treatment of SSTCE4 using immobilized and suspended bioagents in the batch study

The five distinct ANN models for the percentage removal of pollutants (phenol, ammonium ions, cyanide, fluoride, and nitrate) are successfully developed. Some of the representative data used for training the ANN model are shown in Table 5.

The values of R^2, RMSE, and performance are given in Table 6. From Table 5, it is concluded that the five ANN models that are developed have successfully captured the non-linear relationship between the input parameters and the output dependent variable. The values of R^2 (0.98–0.99) are very high for all the five models. The RMSE error and percentage error are also very low for all the five models. Graphs have been plotted between experimental data and predicted data as shown in Supplementary material, Figure (S7(a)–S7(e)). It is observed that curves for both the experimental and predicted data are almost overlapping each other. This shows that the developed ANN model through rigorous training has excellent prediction capability.

4.7. Optimization using GA for treatment of SSTCE4 using immobilized and suspended bioagents in batch study

The equations of the developed ANN models for the percent removal of the pollutants were optimized using GA. The five ANN models for five pollutants were optimized together such that the values received for the input parameters were able to maximize the removal for all the five pollutants. The objective function was taken as the average percent removal of all five pollutants for a given set of input parameters. GA was used to maximize these objective function values by changing the input parameters within their lower and upper bound. For optimizing the developed ANN models, the upper and lower limit of the input parameters were fed into the optimization tool in 'MATLAB R2017a'.

As the initial concentration of pollutants in wastewater are not in our control so the minimum and maximum values are kept constant during GA execution (vide Table 4). The values of optimized input parameters for synthetic wastewater are shown in Table 7 and the values of optimized input parameters for real wastewater are shown in Table 8. The optimized input values were fed to the ANN models to check the value of the percent removal of each pollutant. It was discovered that for each pollutant, the percent removal was maximized to the point where the pollutant concentration in actual

Table 6 | ANN modeling result

ANN result	% Removal of phenol	% removal of ammonium ions	% removal of cyanide	% removal of fluoride	% removal of nitrate
Best R^2	0.98	0.98	0.99	0.99	0.99
Best RMSE	1.94	1.28	0.86	0.79	0.45
Best Percentage Error	3.73	1.52	1.34	1.43	1.00
Best performance coefficient	1.94	1.28	0.86	0.79	0.46

Table 7 | Best solution of optimization using GA for synthetic wastewater

No. of days	Size of matrix (mm)	Percentage bacteria	Removal of phenol (%)	Removal of ammonium ions (%)	Removal of cyanide (%)	Removal of fluoride (%)	Removal of nitrate (%)
4.56	1.19	43.52	99.99	92.74	99.99	99.60	89.97

Table 8 | Best solution of optimization using GA for real wastewater

No. of days	Size of matrix (mm)	Percentage bacteria	Removal of phenol (%)	Removal of ammonium ions (%)	Removal of cyanide (%)	Removal of fluoride (%)	Removal of nitrate (%)
4.56	1.19	43.52	99.98	90.35	99.99	98.05	88.15

Table 9 | Final removal of contaminants for synthetic wastewater after optimization against their permissible limits

Contaminant	Initial concentration in synthetic wastewater (mg/L)	Final concentration after removal (mg/L)	Permissible concentration as per law (mg/L)	Whether discharge concentration within permissible limit (mg/L)
Phenol	10.45	0.001	1.00	Yes
Ammonium	204.13	14.82	50.00	Yes
Cyanide	0.83	0.00	0.200	Yes
Fluoride	10.10	0.04	1.500	Yes
Nitrate	50.03	5.02	10.00	Yes

Table 10 | Final removal of contaminants for real wastewater after optimization against their permissible limits

Contaminant	Initial concentration in real wastewater (mg/L)	Final concentration after removal (mg/L)	Permissible concentration as per law (mg/L)	Whether discharge concentration within permissible limit (mg/L)
Phenol	5.28	0.001	1.00	Yes
Ammonium	138.85	13.39	50.00	Yes
Cyanide	0.07	0.00	0.20	Yes
Fluoride	12.34	0.24	1.50	Yes
Nitrate	25.40	3.01	10.00	Yes

wastewater was substantially lower than the allowed limit. The values of percent removal for the five pollutants using the optimized input parameters for both synthetic and real wastewater are shown in Tables 9 and 10, respectively.

5. CONCLUSION

The bacterial–microalgal consortium was artificially constructed by adding both cultures (bacterial culture: *Bacillus* sp. NITD 19 and microalgal culture: consortium of green algae (*Chlorella* sp.) and cyanobacteria (*Synechococcus* sp.)) for the removal of major pollutants such as fluoride, ammonium ions, cyanide, phenol, and nitrate from the synthetic and real secondary treated CW. To make the treatment process more efficient, the attached whole live cells of all three cultures were used for the treatment of wastewater and their removal capabilities were analyzed. ETP, the market waste material, was used as the matrix. The immobilized constructed bacterial–microalgal consortium was found effective to remove all the pollutants from SSTCE below the permissible limit in 5 days. The real wastewater was treated with the selected consortium in batch mode and continuous mode using suspension form and immobilized form respectively. The packed bed reactor was found effective to reduce all the major pollutants below the stipulated values.

In this study, an efficient data-driven ANN model is developed which is reliable and has high prediction accuracy (High R^2 and low *RMSE*). An efficient ANN algorithm is developed in this study which automatically chose the best ANN architecture and thus, relieves the novice user to choose these ANN meta parameters. A GA is then applied over this ANN model to maximize the removal percentage of pollutants from industrial wastewater. GA optimizes the input space and finds the optimum process parameters which will maximize the pollutants removal (both synthetic and real) and bring down the contamination level below the permissible range. The experimentation, ANN modeling and GA optimization techniques used in the present study are generic and can be extended to any other similar environmental studies.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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