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Microalgal sp. biosorption process optimization for removal of fluoride in potable water applying RSM and ANN Biosimulation studies

[*]R. S. Upendra and Pratima Khandelwal

Dept. of Biotechnology, New Horizon College of Engineering, Outer Ring Road, Bellandur Post, Marathahalli, Bangalore -560103, Karnataka, India

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ABSTRACT

Existing fluoride removal systems are not effective due to the high treatment time and operational cost which addresses an urgent need in developing an effective alternative fluoride removal system. Scanty research was reported using Microalgae sp. as a potential natural biosorbent agent in removing fluoride from the ground water and no research was documented on applying RSM and ANN as a mathematical tools to optimize the Biosorption process. With these lacunae, the present study aimed in optimization of the biosorption process of Microalgae SP. for enhancing fluoride removal efficiencies applying Response Surface Methodology (RSM) and Artificial Neural Network (ANN). In the present study, initially four parameters of the Microalgae sp. biosorption process such as pH, contact time, sorbent dosage, and agitation were optimized using CCD-RSM. Further optimized value of the four factors with respect to end fluoride content was compared and validated using ANN methodology. The optimized condition of the present study i.e (7 pH, Three days of contact time, 1.75ml sorbent dosage and 150 rpm agitation speed) was resulted in reduction of fluoride from initial content of 2mg/l to the final content of 0.55mg/l. ANN has reported the error values of (1.0) for the optimized trail of the CCD-RSM design. The present study concluded by providing validated optimized design reporting approx 4 fold (0.55 mg/ml) reduction in fluoride content with respect to initial fluoride content (2.0 mg/ml). The pilot plant (200 L) scale-up studies for the reported optimized design are under investigation.

Keywords: *Microalgae sp.*, Fluoride removal, Grounder water, Response Surface Methodology and Artificial Neural Network.

INTRODUCTION

Safety and portability of the drinking water was depending on content of dissolved salts and minerals which also determines domestic, industrial and commercial utilization of the water [1, 2]. Fluoride is one such component that is present in water essentially. Fluorine is the most abundant element in nature and exists in natural resources like rocks, geochemical deposits and present in foods like sea fish, tea, and cheese [3]. Intentional addition of fluoride in prescribed limits has proven benefits of dental caries prevention [4]. Fluoride is often called a two-edge sword – in small dosages, it has remarkable influence on the dental system by inhibiting dental carries, while in higher dosages more than 1.5 mg/l causes molting of teeth, lesion of endocrine glands, thyroid, liver and other organs [5]. In India most of the rural population depends on ground water sources and growing urban population on municipal sources it is no surprise that fluoride contamination is widespread [6]. 15 states in India are endemic for fluorosis. With 62 million people suffer from dental, skeletal and non-skeletal fluorosis. 6million children below 14 years effected [7, 8].

Table-1: Fluoride dosage ranges and its effects [9, 10]

Sl no	Fluoride Concentration (mg/ml)	Effects
1.	<1.0	Inhibiting dental carries.
2.	≤1.0	Safe limit.
3.	1.0-3. 0	Dental fluorosis (discoloration, mottling, and pitting of teeth). Lesion of endocrine glands, thyroid, liver and other organs**.**
4.	3.0-4.0	Stiffness, Skeletal fluorosis (disease affects the bone and ligaments).
5.	4.0-6.0 and above	Skeletal deformities, paralysis, crippling.

Karnataka with 30-50% of its districts affected with fluoride contamination requires immediate attention [11].

Table-2 List of fluoride affected districts in Karnataka [12].

Slno	District	Village	Fluoride (mg/l)
1.	Gulbarga	Hattiguddur	7.4
2.	Raichur	Gangavathy	5.15
3.	Bellary	Sanavaspur	7.4
4.	Tumkur and Chitradurga	Bommainapalya	3.2
5.	Kolar	Jagampalli	3.4

Fluoride removal is conventionally conducted by Chemical precipitation, Flocculation, Ion exchange, Reverse osmosis, Adsorption etc [14,15]. Most commonly faced problems with these methods are long treatment time, high cost of resins and operational costs, membrane fouling, high energy input, incomplete metal removal, secondary pollution chances, generation of toxic sludge or other waste products [16]. Therefore it is necessary to adopt a method that is environment friendly and cost effective. Data from biological sources or calculations involving biomaterial tends to be generated in large amounts. Analysis therefore becomes difficult. Also chances of error, false positives increases. Statistical tools have provided us the liberty to deal with such huge data.

The present study explores Biosorption a rather new technique to remove fluoride using *Microalgae sp.* by providing optimal conditions which were achieved applying CCD-RSM and ANN statistical methodologies. Initially *Microalgae spp* was cultured in laboratory on specific media (BBM) under controlled growth conditions. Further the purity of the fully grown culture was studied microbiologically with the standard methods such as Morphological and Microscopic observation. Further the fluoride biosorption abilities of the pure microalgae culture were optimized using CCD-RSM and ANN statistical methodologies. Four parameters i.e pH, contact time, sorbent dosage, and agitation were selected and optimized using Design Expert software version 9.0.4.1, design Type Central Composite and a design model Quadratic (CCD-RSM). Further optimized value of the four factors with respect to end fluoride content was compared and validated using ANN methodology. The optimized condition of the present study i.e (7 pH, Three days of contact time, 1.75ml sorbent dosage and 150 rpm agitation speed) was resulted in reduction of fluoride from initial content of 2mg/l to the end content of 0.55mg/l. ANN has reported the error values of (1.0) for the optimized trail of the CCD-RSM design. The present study concludes by providing validated optimized design reporting approx 4 fold (0.55 mg/ml) reduction in fluoride content with respect to initial fluoride content (2.0 mg/ml). The final fluoride content falls below the permissible limits therefore giving a chance to adopt as a method of fluoride removal for potable drinking water.

OBJECTIVES AND HYPOTHESIS OF THE STUDY

1. Removal of fluoride from potable drinking water using *Microalgae* as natural bio-adsorbents.
2. Optimization of the fluoride removal process by applying RSM and ANN design.

EXPERIMENTAL SECTION

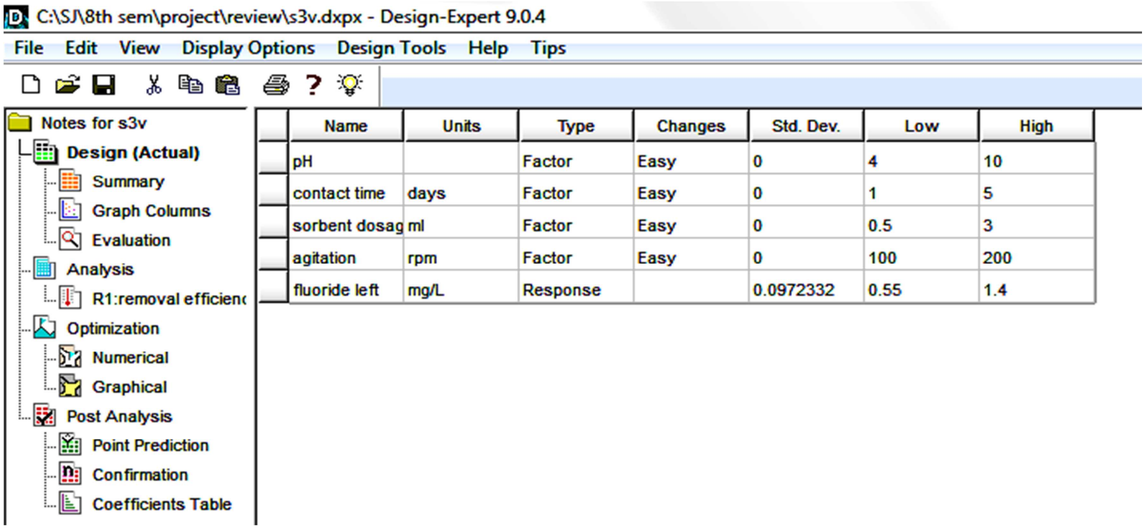
All the chemicals and reagents used in this study were of analytical grade.

Culture and Screening of *Microalgae sp.*: Microalgae spp was cultured on Bolds' Basal media plates [17] and incubated at room temperature under aseptic conditions. After 15 days of incubation fully grown microalgae colonies were screened for their culture purity applying standard microbiological methods such as morphological and microscopic both light and scanning microscopic screening studies. Screened pure cultures were preserved at 4^0C for further biosorption studies [18, 19].

RSM experimental design and biosorption studies: A four factor Central Composite Design (CCD) of RSM was generated with the design-expert 9.0.4.1 software [5,20,21]. The model applied was CCD and a second order polynomial response equation gives the final fluoride content in treated water samples. pH, contact time, sorbent dosage and agitation (rpm) were the selected four principle input variables, the factor levels were coded as −1 (low), 0 (central point) and 1 (high). Adsorption studies were carried out as a batch experiments based on RSM CCD trails

(Table 3), with in the 250 ml conical flask. The solution was then filtered and the residual fluoride ion concentration was estimated.

Table-3. A four factor Central Composite Design (CCD) of RSM trails



Name	Units	Type	Changes	Std. Dev.	Low	High
pH		Factor	Easy	0	4	10
contact time	days	Factor	Easy	0	1	5
sorbent dosag	ml	Factor	Easy	0	0.5	3
agitation	rpm	Factor	Easy	0	100	200
fluoride left	mg/L	Response		0.0972332	0.55	1.4

Quantification of fluoride by UV spectrometric analysis: Treated and prepared water samples were analyzed qualitatively by measuring the absorbance for the presence of fluoride at wavelength 520 nm in three replicates using pure fluoride (Merck) as a standard in UV/Visible spectrophotometer. (Shimadzu, Model no UV-2450 and Software UV-probe 2.21). A calibration curve was prepared from the plot of absorbance against concentration of standard solutions. The concentrations of the sample solutions were determined from the plot [22].

Validation of *Microalgae* Biosorption studies by ANN: In this study, Neural Network MATLAB R 2011a mathematical software was used for simulation. The same experimental data of RSM design was employed in designing the artificial neural network [23,24]. The input variables were pH-7, contact time-3 days, sorbent dosage-1.75 ml and agitation-150rpm. The optimum adsorption capacity was used as a target. The data were randomly divided into three groups, 70% in the training set, 15% in the validation set and 15% in the test set. Trainlm is a training function selected and it is a network training function that updates weight and bias values according to the Levenberg-Marquardt algorithm. All variables and response were normalized between 0 and 1 for the reduction of network error and higher homogeneous results.

RESULTS

Culture and Screening of *Microalgae sp*.: Identified pure cultures of microalgae spp were stored at 4^oC for the further fluoride biosorption optimization studies

RSM experimental design and biosorption studies: *Microalgae sp* fluoride absorption studies were performed based on CCD-RSM trails and the end content of the fluoride in the treated samples were estimated using UV Spectrophotometer.

Quantification of fluoride by UV spectrometric analysis: The quantitative end fluoride content of treated and prepared water sample of each run of RSM CCD trails were discussed in the Table 5. The ANOVA results for Response Surface Quadratic model was discussed in the Table 5. The Model F-value of 11.76 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case D, BC, BD, A^2, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. Relative effects of different process parameters on final fluoride content of the optimized trails were shown in the fig.1.

Table 4. The ANOVA results for Response Surface Quadratic model

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob> F	
Model	1.56	14	0.11	11.76	< 0.0001	Significant
A-pH	6.05E-03	1	6.05E-03	0.64	0.4362	
B-contact time	0.011	1	0.011	1.14	0.303	
C-sorbent dosage	8.89E-05	1	8.89E-05	9.40E-03	0.924	
D-agitation	0.23	1	0.23	24.45	0.0002	
AB	0.016	1	0.016	1.72	0.2095	
AC	3.06E-04	1	3.06E-04	0.032	0.8596	
AD	0.015	1	0.015	1.59	0.227	
BC	0.08	1	0.08	8.44	0.0109	
BD	0.15	1	0.15	16.29	0.0011	
CD	0.013	1	0.013	1.34	0.2654	
A^2	0.059	1	0.059	6.24	0.0246	
B^2	0.055	1	0.055	5.83	0.029	
C^2	0.041	1	0.041	4.34	0.0547	
D^2	4.40E-05	1	4.40E-05	4.66E-03	0.9465	
Residual	0.14	15	9.45E-03			
Lack of Fit	0.14	10	0.014			In significant
Pure Error	0	5	0			
Cor Total	1.7	29				

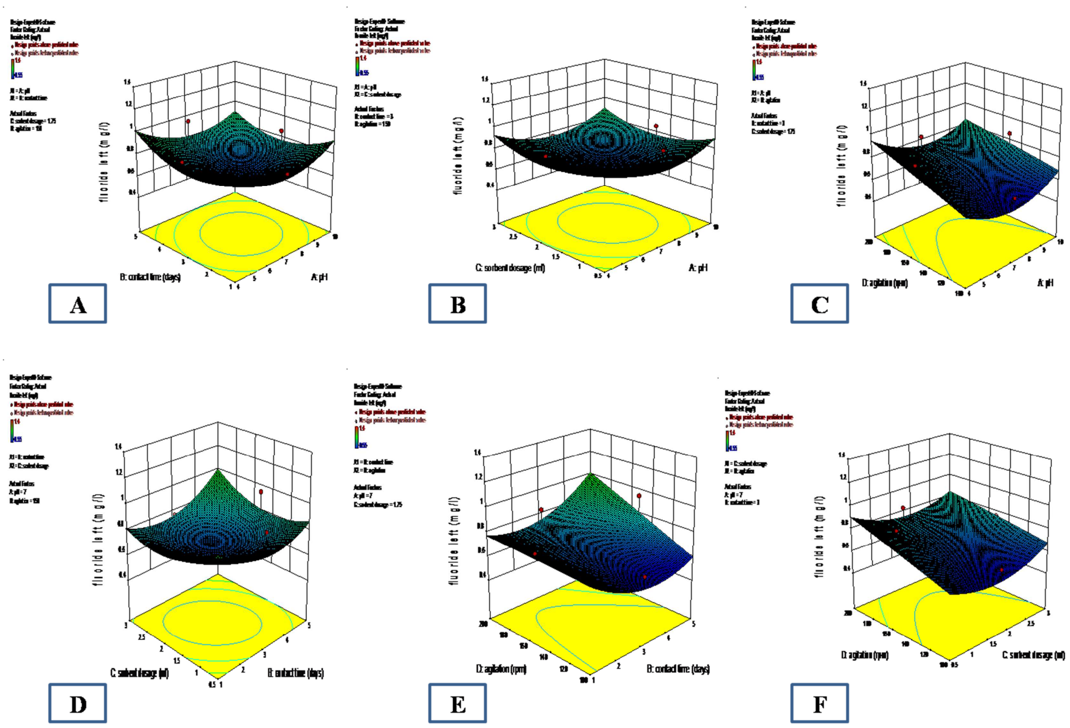


Fig 1. Response surface 3D plots exhibiting relative effects of different process parameters on end fluoride content of the optimized trails

A) 3D graph of pH vs Contact time B). 3D graph of pH vs Sorbent dosage. C) 3D graph of pH vs Agitation D) 3D graph of Contact time vs Sorbent dosage. E) 3D graph of Contact time vs Agitation. F) 3D graph of Sorbent dosage vs Agitation.

Validation of Microalgae Biosorption studies by ANN: The optimal architecture of ANN model in this case has three-layer ANN, with tangent sigmoid transfer function (tansig) at hidden layer with 11 neurons and linear transfer function (purelin) at output layer. A regression analysis between ANN outputs and the experimental data was carried out. This ANN model indicated a precise and effective prediction of the experimental data with a correlation coefficient of 0.999, 0.95355, 0.99609 and 0.98107 for training, validation, testing and all data, respectively. (Fig. 2) The simulated value of end fluoride content as predicted by Feed forward model (0.4250 mg/l) of ANN was in close agreement with the experimental values (0.55 mg/l) and accurately matching with the predicted value of central composite design of RSM.

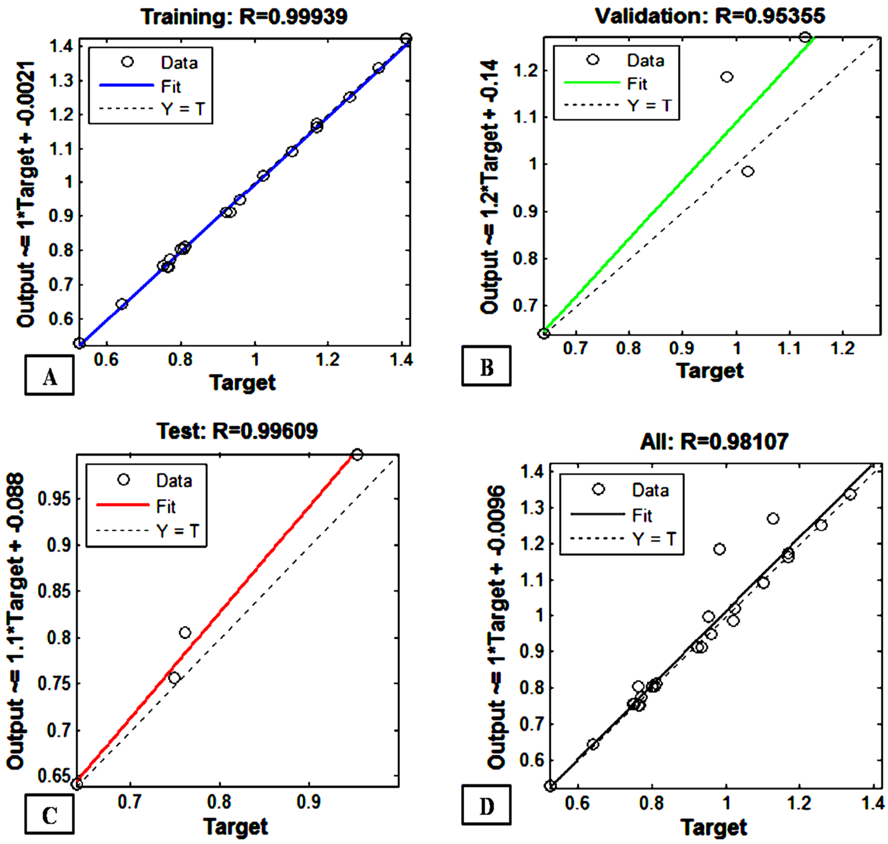


Fig 2: ANN regression plots showing training, validation, target and all regression values

Table 5. A four factor CCD-RSM trails with fluoride removal efficiencies

Factor1	Factor2	Factor3	Factor4	Final Fluoride content (mg/l)			Error
A: pH	B: Contact time(days)	C:Sorbent dosage (ml)	D:Agitation (rpm)	RSM Actual Value	RSM Predicted	ANN Predicted	
10	1	3	100	1.02	1.02	1.02	0
4	5	3	200	1.4	1.41	1.522316936	-0.1223
7	3	1.75	150	0.55	0.64	0.55	0
4	5	0.5	200	1.31	1.34	1.31	0
10	1	0.5	100	1.1	1.10	1.1	0
10	3	1.75	150	0.86	0.77	0.86	0
10	5	0.5	200	1.13	1.17	1.293213	-0.1632
7	3	1.75	100	0.62	0.52	0.62	0
10	1	3	200	0.94	0.94	0.94	0
4	1	3	200	0.96	0.96	0.96	0
4	1	0.5	200	1.17	1.17	1.17	0
10	5	0.5	100	0.72	0.75	0.72	0
7	5	1.75	150	0.95	0.81	0.823384	-0.126516
4	1	0.5	100	0.98	1.02	1.116721422	-0.862379
7	3	1.75	150	0.55	0.64	0.55	0
10	1	0.5	200	1.11	1.13	1.109999921	0
4	5	3	100	0.97	0.98	0.970000005	0
4	3	1.75	150	0.9	0.81	0.900000003	0
7	3	1.75	200	0.83	0.75	0.830000056	0
7	3	1.75	150	0.55	0.64	0.55	0
7	3	1.75	150	0.55	0.64	0.55	0
7	3	0.5	150	0.95	0.77	0.873748974	0.076251
7	3	1.75	150	0.55	0.64	0.55	0
7	3	1.75	150	0.55	0.64	0.55	0
4	5	0.5	100	0.78	0.80	0.877943264	-0.0979
10	5	3	100	0.94	0.95	0.939999958	0
7	3	3	150	0.76	0.76	0.742746618	0.0173
10	5	3	200	1.27	1.26	1.269999862	0
4	1	3	100	0.95	0.93	0.950000003	0
7	1	1.75	150	0.8	0.76	0.799999967	0

DISCUSSION

In the present study, attempts were made to optimize the Fluoride removal efficiencies of Microalgal Biosorption process for potable water using CCD-RSM and validated the optimized trail with ANN. Initially the culture purity of the selected *Microalgae sp.* was studied microbiologically. Further the fluoride biosorption abilities of the pure microalgae culture was optimized using CCD-RSM and ANN statistical methodologies considering four process parameters i.e pH, contact time, sorbent dosage, and agitation. Optimized value of the four factors with respect to end fluoride content was compared and validated using ANN methodology. The study reported that 7 pH, Three days of contact time, 1.75ml sorbent dosage and 150 rpm agitation speed were found to be the optimum parameters which resulted in reduction of fluoride from initial content of 2mg/l to the end content of 0.55mg/l. ANN has reported the error values of (1.0) for the optimized trail of the CCD-RSM design.

Pitre et al. [25] examined the sorption efficiencies of aluminium (Al) and fluoride (F) by four species of green algae (i.e. *Chlamydomonas reinhardtii*, *Pseudokirchneriella sub capitata*, *Chlorella vulgaris*, and *Scenedesmus obliquus*) and reported that, the all four screened green algae were found to be less efficient in the removal of aluminium (Al) and fluoride (F); the present study provides a validated optimized design reporting approx 4 fold (0.55 mg/ml) reduction in fluoride content with respect to initial fluoride content (2.0 mg/ml). Shahjee et al. [26] investigated the efficiency of Bleaching Powder for the removal of excess fluoride in the aqueous solution under batch adsorption process and concluded that the bleaching powder is a good adsorbent with the removal efficiencies of fluoride up to 5ppm concentration. Mondal et al. [27] studied the removal of fluoride using Tea ash as adsorbent through batch studies. The authors reported that the adsorbent was efficient for the uptake of fluoride at pH 6 and contact time 180 minutes. Ramesh et al. [28] investigated the adsorption potential of bottom ash for defluoridation of drinking water using batch and continuous fixed bed column studies and reported maximum efficiency of 83.2 %. Kaushik et al. [29] studied removal of fluoride from groundwater using broken concrete cubes as the adsorbing media in batch adsorption study and found to remove about 80% fluoride at 120 minutes of contact time with adsorbent dose of 6mg/100ml at pH 7.0. Notably, present investigation made use of natural occurring microalgae for biosorption process and reported significant removal of fluoride well within the range (0.55 mg/ml) of WHO permissible fluoride limits (1.0 mg/ml).

Alagumuthu et al. [30] investigated the removal of fluoride from the water using *Cynodon dactylon* as adsorbent under batch adsorption process and achieved maximum removal of fluoride (83.77 %), which was close to the range of the present investigation report of approx 4 fold (0.55 mg/ml) reduction in fluoride content with respect to initial fluoride content (2.0 mg/ml). Satish et al. [31] screened the fluoride removal abilities of various treated natural adsorbents such as Mangrove plant leaf powder (MPLP), Almond tree bark powder (ATBP), Pineapple peel powder (PPP), Chiku leaf powder (CLP), Toor plant leaf powder (TPLP) and Coconut coir pith (CCP) and reported that the percentage removal of fluoride at pH 2 was found to be high, which intern difficult to maintain for the large treatment volumes and the present investigation reduce the burden of maintaining the low pH condition by providing the biosorption optimum pH condition at pH (7.0) for the enhanced removal of fluoride with the end content 0.55 mg/ml. Considering these references, it was concluded in the present study, which explored natural biosorption abilities of *Microalgae sp.* to remove fluoride by providing optimal conditions, which were achieved applying CCD-RSM and ANN statistical methodologies.

CONCLUSION

In the present investigation, *Microalgae sp. was* used as natural biosorbent agent and the process parameters such as pH, contact time, sorbent dosage and agitation were optimized using Central Composite Design (CCD) of Response Surface Methodology (RSM). The optimized values of RSM with respect to the final fluoride content (0.55 mg/l) after treatment process was validated using feed forward model of Artificial Neural Network (ANN). ANN predicted value (0.55 mg/l) was 100 % matching with the optimized experimental value of RSM design (0.55 mg/l) and the error was found to be (0). In conclusion, an optimized process was developed for the removal of excess fluoride from the portable water using *Microalgae sp.* biosorption process. Final concentration of 0.55 mg/l of fluoride was achieved which is well below the WHO permissible fluoride limits (1.0 mg/ml).

The Pilot plant (200 L) scale up studies for the reported optimal design is under the investigations.

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