5. Effect of pretreated, live and dead fungal biomass on the removal of fluoride in aqueous solution and ground water

5.1. Introduction

Research scientists have screened several materials for defluoridation efficiency and number of methods has been developed for the removal of fluoride from drinking water. Most of the methods are difficult and expensive. They need a convinced point of technological ability and can be applied only in developed countries as costly materials and chemicals need to be used. A lot of these methods could not find convenient application due to several drawbacks such as complexity of operational procedure, high capital and operational costs and undesirable changes in other chemical parameters or taste and appearance. The difficulty of fluorosis is linked generally with ground water. Ground water is the normal sources of drinking water supply in villages in India and other developing countries. The defluoridation technique should be economical, easy to operate and suitable to the village people. It must be applied to remove fluoride selectively without negatively disturbing the other chemical characteristics of water.

Now a day’s various techniques have been used for removal of fluoride viz, precipitation (Guo et al., 2013), ion exchange (Tor, 2007), electro dialysis (Malaisamy et al., 2011), electro coagulation (Emamjomeh et al., 2011) and adsorption (Tor et al., 2009). Among the techniques, the adsorption has been prominent process due to its cost effective and efficient adsorptions. Hence, the adsorption method has been widely used for defluoridation. A number of materials was used for removal of fluoride like modified cellulose fiber, polymer, biopolymer composites (Tian et al., 2011), bauxite (Sujana and Anand, 2011), zeolite (Alagumuthu et al., 2010), lateritic soil (Iriel et al., 2018), mixed metal sorbents
Koilraj et al., 2013), biosorbents (Bhatnagar et al., 2011) and natural materials (Wei et al., 2011).

The biological materials have been developed for the removal of organic and inorganic pollutants from water and waste water (Buddo et al., 2008). Various biosorbents have been developed from biological sources viz, chitosan (Jagtap et al., 2011), activated carbon material (Daifullah et al., 2007), cyanobacteria (Biswas et al., 2018), coconut fiber dust (Bhaumik and Mondal, 2015), rice husk ash (Ganvir and Das, 2011), bone char (Muro et al., 2017), fungal biomass (Say et al., 2003) and modified fungal biomass (Pokhrel and Vivaraghavan, 2006) for the removal of fluoride from aqueous medium.

The present investigation has been aimed to achieve the biosorption of fluoride by using biomass of Aspergillus niger (KMF1). The availability of fungal biomass can be obtained from fermentation industries and it is a cheapest and easily available biomass. The fungal biosorbent material was characterized with SEM-EDAX and FTIR. The effect of various environmental parameters such as pH, biosorbent dosage, various fluoride concentrations, agitation time, temperature and coexisting anions on biosorption of fluoride was systematically performed. The batch mode experiments were carried out to identify the equilibrium time, equilibrium adsorbent dosage and equilibrium metal concentration. The experimental data of kinetics and adsorption isotherms were studied using pseudo-first order, pseudo-second order, Langmuir and Freundlich. Thermodynamic study was conducted to identify the optimum temperature for the maximum adsorption of fluoride. Thus, the objectives of this study were framed:

(i) To study the effect of various pH, various biosorbent dosage, various initial fluoride concentration and various concentrations of co-excising anions on the removal of fluoride using biomass of Aspergillus niger KMF1.

(ii) To study the desorption and regeneration of fluoride in aqueous solution.
(iii) To find out significant characteristics of fungal biomass before and after biosorption of fluoride.

(iv) Isotherm parameter, kinetics parameter and thermodynamic parameters on adsorption fluoride from aqueous medium by dead biomass of *Aspergillus niger* KMF1.

(v) Removal of fluoride from fluoride contaminated ground water through column study.

5.2. Materials and Methods

5.2.1. The strain *Aspergillus niger* KMF1

The strain *Aspergillus niger* KMF1 was isolated from the Papppiredypatti block of Dharmapuri district in Tamil Nadu. This strain was maintained as working culture on Sabouraud dextrose agar (SDA) plates under aseptic condition after the incubation at 28 ± 2°C. After 3-4 days of incubation, the plates were stored at 4°C and used as mother culture for further experiments.

5.2.2. Preparation of various pretreated *Aspergillus niger* KMF1 biosorbent and evaluation of their fluoride adsorption capacity

The seven day’s old culture of *Aspergillus niger* (KMF1) spore suspension was transferred under aseptic condition from the agar tubes to sabouraud dextrose broth. The flasks were agitated in a rotary shaker at 120 rpm for seven days at room temperature. After the incubation, the fungal biomass was filtered using a muslin cloth, washed with double distilled water and used as biosorbent for fluoride removal studies. Various pretreated *Aspergillus niger* KMF1 biomass namely, live, dead, CaCO$_3$, CH$_2$O and H$_3$PO$_4$ was made by appropriate methods. The dead fungal biomass was prepared by autoclaved at 15 lbs for 20 minutes. After the autoclave, the fungal biomass was washed with generous amounts of deionized water and used as a biosorbent. For calcium carbonate (CaCO$_3$) treated fungal biomass, about 10 g of calcium carbonate was dissolved in 100 ml of distilled water. Then 15 g of live fungal biomass was transferred into 100 ml of
calcium carbonate and kept in a shaker with 200 rpm for 1 h. In which calcium has a positive charge, so fluoride was bind the cell wall of fungal mycelia to facilitate the sorption from the aqueous solution. For 15% of formaldehyde (CH₂O) treated fungal biomass about 15 ml of formaldehyde was mixed with 85ml of distilled water. Then 15 g of filtered fungal biomass was transferred into 100 ml formaldehyde solution for 30 min (Sudha et al., 2003). For acid treated fungal biomass, about 10 ml of orthophosphoric acid (H₃PO₄) was mixed with 90 ml of double distilled water. Then 15g of filtered fungal biomass transferred into 100 ml of orthophosphoric acid solution for 30 min (Sathishkumar et al., 2008). The harvested mycelium from each pretreatment was used as biosorbents.

A series of 250 ml conical flask containing 100 ml of constant fluoride concentration with a constant dosage of various pretreated *Aspergillus niger* KMF1 biosorbent of live, dead, CaCO₃, CH₂O and H₃PO₄ was taken in the individual conical flask and biosorption experiment was carried out by temperature at 37°C; agitation at 120 rpm and 15 ppm of fluoride concentrations. Every 10 minutes of time interval, about 5ml of samples were withdrawn from all experiments and the removal of fluoride was analyzed by using UV-Vis spectrophotometer (Model: Cyberlab UV100, USA) at 570 nm according to SPANDS reagent method.

### 5.2.3. Preparation of dead biomass of *Aspergillus niger* KMF1 and characterization

The *Aspergillus niger* KMF1 was screened as a fluoride tolerant fungus. Seven days old culture of KMF1 spore was transferred under aseptic condition to sabouraud dextrose broth and incubated at 30°C ± 2 for seven days and it was shown in Plate 5.1. After the incubation, the biomass was autoclaved at 120°C for 15min and filtered by muslin cloth and well washed with distilled water (Mondal et al., 2017). The prepared dead biomass was used for further experiments. The elemental analysis in dead fungal biomass was performed by using CHNS analyzer
(Elementar vario EL III). It was used to determine the carbon, hydrogen, nitrogen and sulfur content in biomass.

5.2.4. **Effect of various pH on the removal of fluoride in aqueous solution using dead biomass of *Aspergillus niger* KMF1**

The pH is considered to be one of the important parameters which determine the removal of fluoride from the aqueous medium. In the present study, the fluoride adsorption capability of dead biomass of *Aspergillus niger* KMF1 was determined by exposing it to various pH. The pH of the medium was adjusted to 2, 3, 4, 5, 6 and 7 by using 0.1N of HCl and NaOH. A standard solution of 100 ppm was prepared by dissolving 0.022 g of NaF in 1000 mL of distilled water. The respective stock solutions were prepared by mixing appropriate quantities from the standard solution with distilled water. A series of 250 ml conical flask containing 100 ml of aqueous solution with 15 ppm of constant fluoride concentration with varying pH (2, 3, 4, 5, 6 and 7) was prepared. To each flask, about 0.2g of biosorbent was added and biosorption experiment was carried out by the temperature at 30°C and agitation at 120 rpm. Every 10 minutes time interval, about 5ml of samples were withdrawn from all experiments and the removal of fluoride was analyzed by using UV-Vis spectrophotometer at 570 nm according to SPANDS reagent method.

5.2.5. **Effect of various biosorbent dosage on the removal of fluoride in aqueous solution using dead biomass of *Aspergillus niger* KMF1**

The biosorbent dosage is one of the important factors; because of it’s to determine the rate of fluoride removal from the aqueous medium. In the present study, the fluoride adsorption capability of dead biomass of *Aspergillus niger* KMF1 was determined by treating it with different biosorbent dosage. A series of 250 ml conical flask containing 100 ml of constant fluoride solution (15 ppm) with varying biosorbent dosage (0.1 0.2, 0.3, 0.4 and 0.5g) were taken and the biosorption experiment was carried out with temperature at 30°C and agitation at
120 rpm. Periodically, about 5ml of the samples were withdrawn from each experimental flask and adsorption rate of fluoride was analyzed by using UV-Vis spectrophotometer at 570 nm.

**5.2.6. Effect of dead biomass on the removal of various concentrations of fluoride in aqueous solution**

The initial concentration is an important thing that determines the adsorption capability of fluoride from aqueous medium. In the present study, the fluoride adsorption capability of dead biomass of *Aspergillus niger* KMF1 was determined by adopting it to various concentrations of fluoride. A series of 100 ml fluoride solution with 5, 10, 15, 20 and 25 ppm was prepared. To each flask, about 0.2g of biosorbent was added and the biosorption testing was carried out by the temperature at 30°C and agitation at 120 rpm. Every 10 minutes time interval, about 5ml of samples were withdrawn from all flask and adsorption rate of fluoride was analyzed by using UV-Vis spectrophotometer at 570 nm. The fluoride sorption capacity, i.e. amount of fluoride ion sorption by *Aspergillus niger* (KMF1) biomass was calculated using the equation given below equation (1).

\[
q_t = \frac{(C_0 - C_t)}{M} \times V
\]

Where \(q_t\) is the fluoride adsorption capacity of *Aspergillus niger* biosorbent (mg/g), \(C_0\) is initial fluoride concentration (ppm), \(C_t\) is equilibrium fluoride concentration (ppm), \(V\) and \(M\) are volume of solution (L) and weight of the biosorbent used (g).

**5.2.7. Effect of various concentrations of co-existing anions on the removal of fluoride**

In the present study, the fluoride adsorption capability of dead biomass of *Aspergillus niger* KMF1 was determined by exposing it to various concentrations of co-existing anions. A series of 250 ml conical flask containing 100 ml of a constant amount of fluoride (15 ppm), various concentrations (50-200 mg/L) of co-exiting anions (Chloride, sulfate, nitrate and carbonate) and 0.2 g of biosorbent
were taken in the individual conical flask and biosorption experiment was carried out in the temperature at 30°C; agitation at 120 rpm and pH 2. Every 10 minutes time interval, about 5ml of samples were withdrawn from all experimental setup and adsorption rate of fluoride was assessed by using UV-Vis spectrophotometer at 570 nm.

5.2.8. Desorption and regeneration studies

Desorption and regeneration of biosorbent after the removal of fluoride ion will provide a good knowledge about re-adsorption capacity of biosorbent and it was shown in Plate 5.2. For desorption study, adsorbed 0.2g/100ml fluoride solution with dead fungal biomass of KMF1 was checked with 0.1N of 50 ml of various desorbing agents (HCL, H2SO4, HNO3 and NaOH) at 30°C. All mixtures were allowed to shake for 18 hours. After the desorption process the filtrates were analyzed to determine the fluoride concentration (Deng et al., 2007). The desorption rate was calculated by following equation (2).

\[
\text{Desorption rate} = \frac{\text{Rate of fluoride ions desorption}}{\text{Rate of fluoride ions adsorption}} \times 100
\] ............. (2)

For regeneration study, the biosorbent was introduced again in fresh medium containing fluoride. This process was consecutively repeated for adsorption and desorption cycles.

5.2.9. Instrumental analysis of fluoride uptake by the biomass

The characterization of the biosorbent surface was performed to describe morphology of the biosorbent, as well as the spatial orientation of biosorbent before and after treatment of fluoride. Scanning electron microscopy (SEM) was used to obtain the surface morphology of the biomass before and treatment. Energy dispersive X-ray analysis (EDX) was done using to examine the elements present in the biomass before and after fluoride uptake. The FTIR spectra of the raw and fluoride loaded biosorbent was in order to verify the interaction between the fluoride ions and the functional groups present in the biosorbent.
5.2.10. Removal of fluoride from fluoride contaminated ground water through column study

Column study was undertaken to apply adsorption process flow column setup and it was shown in Plate 5.3. While the flow column provides more contact time, this type of column was superior to provide fluoride adsorption. The volume of the column was determined to get enough empty bed volume to provide sufficient empty bed contact time. The column study was carried out at constant bed heights and constant flow rates on the column of 4 cm internal diameters and 35 cm height of the column. In column, the dead fungal biomass bed height was 8 cm and flow rate was 1ml per minute. This experiment was carried out with water sample obtained from Poothanatham village. The poothanatham village water contains the fluoride level about 14.5 ppm. The fluoride contaminated water sample was passed through the column at 1 ml flow rate per minute. The samples were collected from the outlet of column at every 5 minutes time interval. After the treatment all samples were analyzed for fluoride concentration by using UV-Vis Spectrophotometer at 570 nm.

5.3. Results

5.3.1. The strain Aspergillus niger KMF1

The strain KMF-1 was identified as Aspergillus niger based on the black colony, biseriate and small conidial spores. The macroscopic and microscopic image of the strain KMF1 was shown in Fig. 5.1. Similar morphological characteristics and microscopic observations of the Aspergillus niger were reported by Samson et al. (2010).

5.3.2. Dead biomass of Aspergillus niger KMF1 and its characterization

The plate 5.1 was shown the production of fungal biomass, in which the uniform fungal mycelia beads were produced by Aspergillus niger KMF1. The CHNS analyzer was used to reveal the presence of C, H, N and S. The fundamental, elemental composition of dead fungal biosorbent was depicted in
Table 5.1 and revealed that the biosorbent was cellulosic in nature (Rhangabhashiyam and Selvaraju, 2015). Fungal cellulose has cellulose binding domin (CBD), which promote the adsorption of molecules like ion on the surface of biosorbent (Tomme et al., 1998).

5.3.3. Removal of fluoride in aqueous solution using pretreated biomass of *Aspergillus niger* KMF1

In this study, the effect of various pretreated *Aspergillus niger* KMF1 biosorbents (Live, dead, CaCO₃, CH₂O and H₃PO₄) on the removal of fluoride was studied and the results are presented in Fig.5.2. By using dead biomass, the maximum fluoride adsorption capacity was 94%, followed by live biomass, which achieved about 78% of fluoride adsorption. Dead fungal biomass showed a good adsorption capacity when compared to live biomass due to the autoclaved treatment effectively changed the surface binding sites. By using calcium carbonate (CaCO₃) treated biomass, the maximum fluoride adsorption capacity was 82%, it was higher when compared to live biomass. The calcium carbonates influence the live biomass surface to the positive charge. By using formaldehyde (CH₂O), the fluoride adsorption ability was 83%. By using orthophosphoric acid treated biomass, the rate fluoride adsorption was 88%, because removal of impurities on the surface of fungal biomass. Overall various pretreated fungal biomass, the dead fungal biosorbent was showed maximum fluoride adsorption ability. Hence, the dead fungal biosorbent was selected as an efficient biosorbent for removal of fluoride from aqueous medium and it was used throughout the studies.

5.3.4. Removal of fluoride in aqueous solution at various pH

In this study, the influence of various pH (2-7) on fluoride sorption (15 ppm) by *Aspergillus niger* biosorbent was carried out and the outcome of the study was depicted in Fig. 5.3a&b. The fluoride sorption capacity was gradually decreased when the pH of aqueous medium increased from 2 to 7. The fluoride removal was found higher in the study carried out with pH 2. In the pH4 and 5 for the removal
of fluoride shown almost same as in the aqueous solution with pH 2. The efficient fluoride sorption was observed at pH 2 (92%) due to the protonation functional groups on fungal cell surface, which was assisted the adsorption of negatively charged fluoride ions on surface of biosorbent. The protonation of fungal functional groups provide an overall positive charge on the fungal biomass at lower pH.

**5.3.5. Removal of fluoride in aqueous solution using various biosorbent dosage**

The influence of different dosage (0.1, 0.2, 0.3, 0.4 and 0.5g/100 ml) of biosorbent was studied. The Fig. 5.4a&b was shown the maximum fluoride adsorption (93.85%) which was achieved by using 0.2g of biosorbent. Hence, 0.2g/100ml of dose was chosen as the optimized dose for the further fluoride removal experiment. The fluoride adsorption capacity was increased with increase in biosorbent dose due to the greater bioavailability of fluoride binding sites present on the surface of the biosorbent. The fluoride adsorption capacity was not increased with further increasing of biosorbent dose, due to the aggregation of available fluoride binding sites on the surface fungal biosorbent.

**5.3.6. Removal of fluoride in aqueous solution with varying initial fluoride concentrations and contact time**

The effect of initial fluoride concentration on adsorption of fluoride ion from aqueous medium was carried out by varying fluoride concentration (5 to 25 ppm) of fluoride under constant experimental conditions. The Fig. 5.5a was showed the maximum fluoride adsorption achieved at 5 ppm, due to the interaction between fluoride ions with binding sites. Similarly the significant fluoride adsorption was achieved at 15 ppm of fluoride concentrations, which express that efficiency of fungus Aspergillus niger KMF1 biosorbent. The fluoride adsorption experiment was performed with different contact time intervals in the range of 10 to 80 min under constant experimental conditions. The Fig. 5.5b was showed the biosorbent attained saturation point at 50 min. Hence, 50 min equilibrium time was predicted as contact time for throughout the experiments.
5.3.7. Removal of fluoride in aqueous solution using various concentrations of co-excising anions

The effect of co-existing anions on adsorption of fluoride was carried out by using various anionic substances (Cl\(^{-}\), SO\(_{4}\), NO\(_{3}\) and CO\(_{3}\)) in the range between 50 to 200 mg/L with initial fluoride concentration of 15 ppm. The interference experiment was carried out to find out the competence of water existing other anions to fluoride ions on the surface of biosorbent. The Fig. 5.6 was shown that there was no significant change in the adsorption capacity of the fungal biosorbent in the presence of the initial co-existing anions (50 mg/L). The effect of chloride, sulfate and nitrate on the adsorption capacity of biosorbent was minor within all co-existing anions. The carbonate anion on the adsorption capacity of biosorbent was decreased due to the carbonate anion was the greatest competitor to fluoride ion, due to the high columbic repulsive forces. Carbonate decreases the fluoride interaction with the active sites of biosorbent.

5.3.8. Desorption and regeneration studies

The removal of water contaminants by biosorption approach is low cost with regeneration of biosorbents. Also, reusability of biosorbent provides to reduce excess environmental contamination linked with biosorbent disposal. The Fig. 5.7a was showed the percentage of fluoride released after the treatment with various desorption agents, namely, HCL, H\(_{2}\)SO\(_{4}\), NaOH and HNO\(_{3}\). It was observed that the fluorides desorption capacity was higher in alkaline condition than the acid conditions. Out of four desorption agents, NaOH showed as the best desorbing agent as it desorbed about 98.36% of fluoride. The fungal biosorbent was thoroughly washed with distilled water and filtered by Whatman No.1 filter paper and dried at 60°C for the further fluoride sorption experiment. The cyclic regeneration experiment was carried out to find out the loss of fluoride adsorption capacity during each cycle of regeneration. Desorption and regeneration experiments were carried out to four consecutive cycles. The Fig. 5.7b was showed
the regeneration during first cycle was 90%, it was subsequently decreased 87.16% during second cycle and 72.46% was third cycle of regeneration. In which 64.08% of regeneration was achieved at fourth cycle due to decomposition of active sites on the surface of the fungal biosorbent (Khan et al., 2012).

5.3.9. FTIR analysis of dead biosorbent before and after the treatment of fluoride adsorption

The FTIR spectrum is used to identify the different functional groups found on the surface of the dead fungal biosorbent and which are playing a major role for the binding of ions on biosorbent. The FTIR spectra in the range of 4000-500 cm\(^{-1}\) for the dead fungal biosorbent before and after biosorption of fluoride are shown in Fig. 5.8. The FTIR spectrum of dead fungal biosorbent showed the presence of predominant peaks at 3458.42 cm\(^{-1}\) (-OH and -NH stretching), 2924.18 cm\(^{-1}\) (-CH stretching), 1640.08 cm\(^{-1}\) (C=C stretching), 1469.24 cm\(^{-1}\) (CH\(_2\) stretching) 1385.67 cm\(^{-1}\) (CH\(_3\) stretching) and 1120.71 cm\(^{-1}\) (C-N stretching). This was noticed that all efficient functional groups present in dead fungal biosorbent. The Fig. 5.8 was also shown that the intensity of transmittance of peaks is greater in the dead fungal biosorbent loaded with fluoride ion compared to the untreated dead fungal biosorbent. This may be attributed to the attendance of smaller amount free functional groups in the dead fungal biosorbent loaded with fluoride ion. This study provides the support that the functional groups such as -NH\(_2\), CH\(_2\) and -OH were involved in binding the fluoride ion to the dead fungal biosorbent.

5.3.10. SEM-EDAX analysis of fungal biosorbent before and after the treatment of fluoride adsorption

The SEM was used to investigate the surface texture morphology and porosity of fungus biosorbent. The Fig. 5.9 was showed the SEM micrograph and EDAX spectra of Aspergillus niger biosorbent before and after the biosorption of fluoride. Before the biosorption of fluoride, the fungal biosorbent contains several micro porous structure, it can offer a high specific surface area (Fig. 5.9a & c).
After the biosorption of fluoride, the rough surface and impurities were observed on the surface of the biosorbent saturated with fluoride (Fig. 5.9b & d). For EDAX analysis after the biosorption, the fluoride peak was observed in the spectrum, while such a peak could not observe on before the biosorption (Fig. 5.9e & f).

5.3.11. Adsorption isotherm

The biosorption isotherm expresses how the adsorbate molecules dispense between liquid-solid phases, when the adsorption process attains equilibrium. The biosorption equilibrium data was analyzed by Langmuir and Freundlich isotherm.

The Langmuir isotherm model assumes that the monolayer exposure of adsorbate (fluoride) on a homogeneous biosorbent surface. The linearized form of the Langmuir isotherm equation was given as (3).

\[
\frac{C_e}{q_e} = \frac{1}{b q_m} + \frac{C_e}{q_m}
\]

(3)

Where \( C_e \) is the equilibrium concentration of fluoride ion in aqueous medium (ppm), \( q_e \) is the amount of fluoride ion adsorbed on the surface of biosorbent (mg/g) and \( b \) is represents the rate constant of adsorption for Langmuir isotherm. The Langmuir isotherm constant \( b \) and maximum fluoride adsorption capacity \( q_m \) were calculated through slope and intercept of the plot (Fig. 5.10a). The correlation parameters obtained by calculation and results were depicted in Table 5.2. Adsorption of fluoride was increased with increasing contact time and equilibrium at 80 min. The obtained all the \( R_L \) values were between 0 and 1 suggesting that biosorption is favorable and low values of \( R_L \) expose the interaction between fluoride ion and biosorbent (Meenakshi and Viswanathan, 2007). Biosorption experimental data were fitted with the Langmuir isotherms. The maximum adsorption of fluoride was 8.29 mg/g for fungal biosorbent. The Langmuir fluoride sorption capacities obtained in the present study compared with previous reports (Table 5.3). The experimental data of fluoride sorption by Aspergillus niger fungal biosorbent found in this is significantly greater than other
biosorbents. Hence, the present study recommends that *Aspergillus niger* (KMF1) is an efficient and low-cost biosorbent for the removal of fluoride from aqueous medium. The Freundlich isotherm indicates that biosorption in heterogeneous mode and exponential distribution sites. The linearized form of freundlich isotherm equation was given as (4).

\[
\log q^2 = \log K + \frac{1}{n} \log C_e 
\]  

Where, \( q_e \) is the amount of fluoride adsorbed on biosorbent mg/g, \( C_e \) is the equilibrium concentration of fluoride ion in aqueous medium (mg/L), \( K \) is representing the rate constant of adsorption for freundlich isotherm. Adsorption intensity \( n \) and freundlich constant \( K \) values are determined by slope and intercepts of plots (Fig. 5.10b).

5.3.12. Adsorption kinetic model

The adsorption kinetic model is important for the determination of the adsorption process capacity. The adsorption of fluoride ion on biosorbent from aqueous medium commonly follows a complex kinetics. The kinetic experimental data of fluoride adsorption on *Aspergillus niger* biomass sorbent was followed by the pseudo first order and pseudo second order. The pseudo first order kinetic model equation (5) was given as (Chiou and Li, 2003).

\[
\ln (q_e - q_t) = ln q_e - k_1t 
\] 

In which, where \( q_e \) and \( q_t \) are amount of fluoride adsorbed on fungal biosorbent (mg/g) at equilibrium and time \( t \) respectively. \( K \) (min\(^{-1}\)) is representing the rate constant of adsorption for pseudo first order. The Fig. 5.11a was showed that plot of \( \ln (q_e - q_t) \) versus \( t \) should give a straight line with slop of the rate constant of adsorption \( (K_1) \) and intercept \( \ln q_e \), it allows determination of adsorption rate constant \( K_1 \) and equilibrium of fluoride adsorption capacity \( (q_e,\text{cal}) \). Over all pseudo first order experimental data may be calculated that the kinetics of fluoride adsorption on *Aspergillus niger* biosorbent was not following the
pseudo first order kinetic. The pseudo second order kinetic model (6) was given as Ho and McKay, 1999.

\[
\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t \quad \text{........................................ (6)}
\]

Where \( K_2 \) is the equilibrium rate constant for pseudo second order (mg/g), the boundary conditions was for \( t = 0, q_t = 0 \) to \( q_t \). The Fig. 5.11b was showed that the co-efficient of determination for the pseudo second order kinetic model \( R^2 \) was found to be higher (0.999) than pseudo first order kinetics. The experimental values of \( k_1, k_2, q_e \) and the correlation coefficient \( (R^2) \) from the linear plots are shown in Table 5.4. The calculated \( q_e \) values were very close to that experimental \( q_e \) values. Hence, the kinetic model may be calculated that the fluoride adsorption on Aspergillus niger biosorbent can be followed by pseudo second order kinetic model than pseudo first order kinetic model. The similar trend has been reported for iron ore and chitosan coated perlite (Vijaya et al., 2010; Kebede et al., 2016).

5.3.13. Thermodynamic parameters of biosorption of fluoride

To investigate the influence of temperature on biosorption of fluoride by using Aspergillus niger biosorbert. The biosorption experiments were carried out at varying temperatures (20, 25, 30 and 35°C). The comparison of fluoride adsorption capacity was with constant initial fluoride concentration of 15 ppm at four different temperatures by constant experimental conditions. The biosorption of fluoride adsorption capacity was increased from 79.49 to 93.32%, when the temperature increased from 20 to 35°C. Biosorption due to motivated by higher temperature, it was indicated that the endothermic mode of biosorption. The nature of adsorption process confirmed based on the determination of changes in thermodynamic parameters such as Gibbs free energy \( (\Delta G^{\circ}) \), enthalpy \( (\Delta H^{\circ}) \) and entropy \( (\Delta S^{\circ}) \). The thermodynamic equations (7 & 8) were given as Karthikeyan and Elango (2008) and Kayira et al. (2014).
\[ k_{ad} = \frac{C_{AE}}{C_e} \]  \hspace{1cm} (7)

\[ \ln K_{ad} = \frac{\Delta S}{R} - \frac{\Delta H}{RT} \]  \hspace{1cm} (8)

Where, \( C_{AE} \) is the concentration of fluoride in solid phase at equilibrium and \( C_e \) is equilibrium concentration. The thermodynamic parameters for the adsorption of fluoride on fungal biosorbent were depicted in Table 5.5. The negative value of \( (\Delta G^o) \) was decrease with gradually increase the temperature at the constant fluoride concentration. This phenomenon was express that the high temperature was more favorable for biosorption of fluoride on fungal biosorbent. The positive value of \( (\Delta S^o) \) was express that more affinity of biosorbent to fluoride ion and express more random less interaction between solid-liquid during the biosorption process (Eren, 2008).

5.3.14. Optimized conditions in batch mode studies

In batch mode process the dead biomass was used for the adsorption process to examine the optimum conditions for the removal of fluoride (Table 5.6). The maximum fluoride adsorption was achieved in 50 minutes of contact time with pH2, initial fluoride concentration of 15 ppm, adsorbent dosages of 0.2g/100 ml and the temperature < 30°C.

5.3.15. Fluoride removal from ground water through column approach

The developed dead fungal biosorbent was used in the present investigation to test the removal of fluoride in contaminated ground water collected from Poothanatham village, Pappireddypatti block of Dharmapuri district. In this field the fluoride seems to be 14.3 ppm in ground water. The column study was carried out by the constant bed height of biosorbent and constant flow rate of ground water collected. Glass column was approximately 350 mm in length and 40 mm in diameter. About 15g dead fungal of *Aspergillus niger* KMF1 biomass was packed in a column under aseptic conditions and fixed respectively in the stand. Through
column, the ground water was passed at a flow rate of 5 ml per minute. Every 5 minutes of time interval, the sample was withdrawn from the column and residue fluoride was analyzed by SPADNS reagent method and the results were presented in Fig. 5.12. The treated ground water contains fluoride concentration below the permissible limit (1.5 ppm) recommended by WHO. There was a significant reduction in other water parameters observed along with fluoride ion were depicted in Table 5.7, it indicates that the biosorbent of \textit{Aspergillus niger} KMF1 can be used as an efficient defluoridating agent.

**5.3.16. Mechanism of fluoride adsorption by dead biomass of KMF1**

The Fig. 5.13 was illustrating the mechanism of fluoride adsorption by dead biosorbent of \textit{Aspergillus niger} (KMF1). The kinetic studies explore that the efficient fluoride adsorption rate and the short adsorption equilibrium time showed that the density of fluoride adsorption active sites found on the dead fungal biosorbent. The SEM images revealed that the irregular surface and porous nature of biosorbent and EDAX analysis confirmed that fluoride adsorption on the dead fungal biosorbent by specific fluoride peak. The FTIR spectra confirmed that the presence of hydroxyl group (OH\textsuperscript{-}) and carboxyl group (COOH\textsuperscript{-}) on the dead fungal biosorbent. Efficient fluoride adsorption was achieved at pH 2 due to the protonation of these OH\textsuperscript{-} groups and COOH\textsuperscript{-} groups by ion exchange and electrostatic attraction.

**5.4. Discussion**

The adsorption of fluoride by fungal biomass is depends on the factor like physical and chemical influences from the environment e.g. pH, temperature, biosorbent dosage, initial fluoride concentration and the presence of co-existing anions. For that, these dependable factors for adsorption of fluoride was considered in the given bioremediation process. A wide range of biomaterials available in nature has been employed as biosorbent for the preferred removal of environmental pollutants. All kinds of microbial, plant and animal biomass and
their derivative products have established with great interest in a variety of ways (Volesky, 2003; Masri et al., 2010). However, in recent years interest has been driven towards the fungal biomass and industrial waste biomaterials. Additionally, a number of groups of biological resources, particularly bacteria, cyanobacteria, algae, yeast, fungi, and lichens have been studied with a great attention for the removal of fluoride, because of their good performance, low cost and availability in large quantities (Volesky, 2007).

5.4.1. Effect of various environmental parameters on the removal of fluoride using dead biomass of *Aspergillus niger* KMF1

The biosorption process is depends on the solution pH, which affect the fluoride adsorption capacity. The presence of active functional groups and their ionic characters contribute the efficient biosorption (Venkata Mohan et al., 2003). The fungal cell wall has high amount of polysaccharides namely cellulose, glucan, pectin and chitin associated with glycoprotein and other components (Macaskie and Dean, 1985; Bowman and Stephen, 2006). These components on the fungal cell surfaces containing more functional groups (such as amino, thiol, carboxyl, phosphate groups) and the biosorption process depends on the protonation and unprotonation of fungal cell wall and its functional groups (Ilhami et al., 2005; Ramanaiah et al., 2007).

The fluoride sorption ability was gradually decreased when the pH of aqueous medium increased from 2 to 7. The efficient fluoride sorption was observed at pH 2 (92%) due to the protonation of functional groups on the fungal cell surface. The previous study reported that decrease in the amount of fluoride removal showed when increase in the pH of the solution. This was investigated as maximum removal of fluoride 74.25% at pH 2 and a minimum 40% at pH 8 (Dave and Machhar, 2015).

In this study, the maximum fluoride adsorption of 93.85% was achieved by using 0.2g of biosorbent. Hence, 0.2g/100ml of dose was chosen as the optimized
dose for the further fluoride removal experiment. The similar result has been reported for adsorption of fluoride by Neem leaf powder (Rajan et al., 2015). The maximum fluoride adsorption was achieved at 5 ppm, due to their interaction between fluoride ions and the binding sites. The significant fluoride adsorption was achieved at 15 ppm of fluoride concentrations, which express that efficiency of Aspergillus niger biosorbent. The fluoride adsorption capacity was strictly decreased due to the lack of available active sites for high initial fluoride concentrations (Yadav et al., 2013). A similar result has been reported in regards to adsorption of fluoride by sugarcane charcoal (Viswanathan et al., 2009; Mondal et al., 2013; Rafique et al., 2013). The fluoride adsorption experiment was performed with different contact time from 10 to 80 min under constant experimental conditions. The biosorbent attained saturation point at 50 min. Hence, 50 min equilibrium time was predicted as contact time for throughout the experiments. The previous study reported that the maximum fluoride adsorption was achieved at 240 min (Amin et al., 2015).

5.4.2. Characterization of dead fungal biosorbent

The FTIR spectrum was shown the intensity of transmittance of peaks is greater in the dead fungal biosorbent loaded with fluoride ion compared to the untreated dead fungal biosorbent. This may be attributed to the attendance of smaller amount free functional groups in the dead fungal biosorbent loaded with fluoride ion. The previous study reported that presence of -OH stretching, C-H stretching of alkane, C-H and C=O stretching of carboxylic acid or ester, COO-anion stretching, OH bending, C-O stretching of ester enhance the ion binding abilities. Among these, carboxylic and hydroxyl groups played a major role in the removal of fluoride ions (Bhaumik et al., 2014). In this study, before the biosorption of fluoride, the fungal biosorbent contains several micro porous structures and it can offer a high specific surface area. After the biosorption of fluoride, the rough surface and impurities were observed on the surface of the
biosorbent saturated with fluoride. The previous study was reported that adsorption of fluoride ion might be due to the presence of pores and functional active groups present on the biosorbent (Suneetha et al., 2015).

5.4.3. Adsorption isotherm, kinetic and thermodynamics studies

In this study, biosorption experimental data were fitted with the langmuir isotherms. The langmuir model based on the hypothesis that all the active sites are equal and self-regulating indicates the monolayer adsorption method for fluoride onto the consistency adsorbent surface (Ramdani et al., 2010). The values of langmuir model, freundlich model constants were calculated from the intercept and slope of the respective plots. Comparing the values of correlation coefficient (R²) of the langmuir linear model were showed higher efficiency on fluoride removal than that of freundlich model and the correlation coefficients (R²) of two kinds of linear model were higher than those of nonlinear model. All the information disguised that the experimental data can good fit with the langmuir linear model better, which recommended that the homogeneous distribution of active sites on the surface of adsorbent and fluoride adsorption take place in a monolayer adsorption mode during the course of adsorption (Nie et al., 2012). In this study, the calculated qₑ values were very close to that experimental qₑ values. Hence, the kinetic model may be calculated that the fluoride adsorption on Aspergillus niger biosorbent can be fitted with pseudo second order kinetic model than pseudo first order kinetic model. The biosorption of fluoride adsorption capacity was increased from 79.49 to 93.32%, when the temperature increased from 20 to 35°C. Biosorption was motivated by the high temperature and it was indicated that the endothermic mode of biosorption. The negative values of ΔG⁰ noticed that the adsorption of fluoride onto adsorbents was a spontaneous process while the negative ΔH⁰ values disguised the process of adsorption was exothermic. The positive value of ΔS⁰ recommended increased randomness at the solid/liquid interface during adsorption of fluoride (Sujana et al., 2013).
5.5. Conclusion

In this study, dead biomass of *Aspergillus niger* (KMF-1) was prepared and applied for fluoride removal with various environmental parameters. The fluoride adsorption capacity of dead fungal biomass is highly pH dependent. Hence, the best fluoride adsorption rate could be observed at pH 2.0. Also the adsorption capacity of dead fungal biomass was temperature dependent, to increase the temperature, influences the rate of fluoride adsorption. The rate of fluoride adsorption capacity was fast and the equilibrium was observed at 50 min. From the study, the maximum (92%) fluoride adsorption was noted by the dead biomass of *Aspergillus niger* (KMF1) under the optimal condition. The CHNS, FTIR and SEM-EDAX instruments were used to investigate the adsorption features of dead fungal biomass. The experimental equilibrium data was well fitted with freundlich isotherm model. The kinetic adsorption of experimental data could be well fitted with pseudo second order kinetic model. Desorption experiment was indicated significantly by 0.1M of NaOH. For regeneration experiment, the maximum fluoride adsorption capacity (90%) was observed in first cycle of experiment. Hence, the *Aspergillus niger* (KMF1) dead biomass was recommended for removing fluoride from aqueous medium with reusability.
Table 5.1. CHNS analysis of fundamental elemental composition of dead fungal biosorbent

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Aspergillus niger (KMF1) dead biosorbent</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>45.26 %</td>
</tr>
<tr>
<td>H</td>
<td>7.04 %</td>
</tr>
<tr>
<td>N</td>
<td>4.32 %</td>
</tr>
<tr>
<td>S</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Table 5.2. The Langmuir and Freundlich sorption constants for biosorption of fluoride

<table>
<thead>
<tr>
<th>F⁻ (ppm)</th>
<th>Langmuir isotherm</th>
<th>Freundlich isotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q₀ (mg g⁻¹)</td>
<td>B (L mg⁻¹)</td>
</tr>
<tr>
<td>5</td>
<td>8.29</td>
<td>1.68</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.3. Comparison of biosorption capacity of *Aspergillus niger* (KMF-1) fungal biomass for fluoride ion with other reported biosorbents

<table>
<thead>
<tr>
<th>Biosorbents</th>
<th>Biosorption capacity (mg/g)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eichhoria crassipes</em></td>
<td>4.4</td>
<td>Sinha <em>et al.</em> (2003)</td>
</tr>
<tr>
<td>Rice husk</td>
<td>0.820</td>
<td>Vivek Vardhan and Karthikeyan (2011)</td>
</tr>
<tr>
<td>Activated alumina</td>
<td>1.78</td>
<td>Ghorai and Pant (2005)</td>
</tr>
<tr>
<td>Spirogyra 101</td>
<td>1.272</td>
<td>Mohen <em>et al.</em> (2007)</td>
</tr>
<tr>
<td><em>Pleurotus ostreatus</em> 1804</td>
<td>1.272</td>
<td>Ramanaiah <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>Hydrous ferric oxide</td>
<td>16.5</td>
<td>Dey <em>et al.</em> (2004)</td>
</tr>
<tr>
<td>Moringa indica based activated carbon</td>
<td>0.23</td>
<td>Karthikeyan and Ilango (2007)</td>
</tr>
<tr>
<td>Ca-treated Chlorococcum humicola</td>
<td>4.5</td>
<td>Bhatnagar <em>et al.</em> (1991)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> KMF-1</td>
<td>8.29</td>
<td>Present work</td>
</tr>
</tbody>
</table>
**Table 5.4.** The parameters and correlation co-efficient of pseudo first and second order kinetics model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pseudo 1&lt;sup&gt;st&lt;/sup&gt; order kinetic model</th>
<th>Pseudo 2&lt;sup&gt;nd&lt;/sup&gt; order kinetic model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$qe$ (exp) (mg/g⁻¹) $k_1$ (min⁻¹) $qe$ (cal) (mg/g⁻¹) $R^2$</td>
<td>$K_2$ (g/mg⁻¹/min⁻¹) $qe$ (cal) (mg/g⁻¹) $R^2$</td>
</tr>
<tr>
<td>Initial F⁻ conc (ppm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.35 0.05 1.12 0.93</td>
<td>0.07 2.49 0.99</td>
</tr>
<tr>
<td>10</td>
<td>4.61 0.05 2.27 0.99</td>
<td>0.04 4.87 0.99</td>
</tr>
<tr>
<td>15</td>
<td>6.80 0.04 3.05 0.97</td>
<td>0.03 6.95 0.99</td>
</tr>
<tr>
<td>20</td>
<td>6.63 0.06 1.82 0.99</td>
<td>0.07 6.75 0.99</td>
</tr>
</tbody>
</table>

**Table 5.5.** Thermodynamic parameters for the adsorption of fluoride on fungal biosorbent

<table>
<thead>
<tr>
<th>F⁻ concentration (ppm)</th>
<th>Temperature (K)</th>
<th>$-ΔG^°$ (kJ mol⁻¹)</th>
<th>$ΔS^°$ (kJ mol⁻¹K⁻¹)</th>
<th>$ΔH^°$ (KJ mol⁻¹)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>293</td>
<td>-3.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>298</td>
<td>-3.7</td>
<td>68.66</td>
<td>10.01</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>303</td>
<td>-3.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>308</td>
<td>-6.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5.6.** Optimized conditions in batch mode process

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>Biosorbent dosage</td>
<td>2.0g/100 ml</td>
</tr>
<tr>
<td>3</td>
<td>Initial fluoride concentration</td>
<td>15 ppm</td>
</tr>
<tr>
<td>4</td>
<td>Temperature</td>
<td>&lt; 30°C</td>
</tr>
<tr>
<td>5</td>
<td>Contact time</td>
<td>50 minutes</td>
</tr>
</tbody>
</table>
Table 5.7. Physico-chemical parameters of before and after the treatment of field fluoride contaminated water

<table>
<thead>
<tr>
<th>Water quality parameters</th>
<th>Treatment</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>F(^-) (ppm)</td>
<td>14.03</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Cl(^-) (ppm)</td>
<td>975</td>
<td>856</td>
<td></td>
</tr>
<tr>
<td>Na(^+) (ppm)</td>
<td>119</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>8.45</td>
<td>7.84</td>
<td></td>
</tr>
<tr>
<td>Total Hardness (ppm)</td>
<td>982</td>
<td>905</td>
<td></td>
</tr>
<tr>
<td>Total dissolved solids (ppm)</td>
<td>1039</td>
<td>959</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 5.1. The strain *Aspergillus niger* (KMF1)

Fig. 5.2. Effect of various pretreated *Aspergillus niger* KMF1 biosorbent on the removal of fluoride
Fig. 5.3. Effect of various pH (2, 3, 4, 5, 6 and 7) on the removal of fluoride

Fig. 5.4. Effect of various biosorbent dosage (0.1, 0.2, 0.3, 0.4 and 0.5g) on the removal of fluoride
Fig. 5.5. Removal of fluoride in aqueous solution with varying concentrations of initial fluoride and contact time

Fig. 5.6. Effect of various concentrations of co-existing anions on the removal of fluoride
Fig. 5.7. Desorption and regeneration studies

Fig. 5.8. FTIR analysis of dead biosorbent before and after the treatment of fluoride adsorption
Fig. 5.9. SEM-EDAX analysis of fungal biosorbent before and after the treatment of fluoride adsorption
Fig. 5.10. Isotherm studies for the adsorption of fluoride ion by dead fungal biosorbent
(a) Langmuir plot and (b) Freundlich plot

Fig. 5.11. Kinetics studies for fluoride adsorption by dead fungal biosorbent
(a) Pseudo first order and (b) Pseudo second order
Fig. 5.12. Adsorption of fluoride from contaminated ground water through column setup

Fig. 5.13. Illustrating the mechanism of fluoride adsorption by dead biosorbent of *Aspergillus niger* (KMF-1)
Plate 5.1. Preparation of dead biomass of *Aspergillus niger* (KMF1)
Plate 5.2. Desorption and regeneration studies
Plate 5.3. Adsorption of fluoride from contaminated ground water through column setup