

# Evaluating the effectiveness of the banana (*Musa* spp. ABB cv. Kluai Namwa) peel for the removal of fluoride from water

Manisha Poudyal<sup>a</sup>, Sandhya Babel<sup>a\*</sup>

<sup>a</sup> School of Biochemical Engineering and Technology, Sirindhorn International Institute of Technology (SIIT), Thammasat University, Pathum Thani, Thailand

\* Corresponding author: sandhya@siit.tu.ac.th

## Abstract

This study aims to evaluate the effectiveness of *Musa* spp. ABB cv. Kluai Namwa peel powder, an agro-based biomass as an adsorbent for the removal of fluoride from artificially contaminated water. A series of batch adsorption experiments were carried out to determine optimum dose, pH, agitation speed, contact time, initial fluoride concentration and the effect of co-existing ions (F/Cl<sup>-</sup> and F/SO<sub>4</sub><sup>2-</sup>) in binary components system reaction conditions for the maximum defluoridation. Fourier Transform Infrared spectroscopy (FTIR) was employed for functional group analysis and Scanning Electron Microscope (SEM) techniques for understanding the surface morphology. The percentage removal of fluoride increased with the increase in the amount of adsorbent dose, agitation speed and contact time for the tested fluoride solution (10 ppm), whereas the removal followed a downward trend for the increasing fluoride concentration. The defluoridation rate decreased to some extent with the increase in sulphate concentration, but was independent of the presence of chloride ion. The binding of fluoride ions was found to be pH dependent with the optimal sorption occurring at neutral pH. The FTIR study highlighted the absorption bands pertaining to rich organic functional groups like hydroxyl, amine and carbonyl groups. The fluoride adsorption obeyed both Langmuir and Freundlich isotherms at constant adsorbent dose and temperature. The linear relationships with high correlation coefficients ( $R^2$ ) values close to unity showed that the obtained experimental data are statistically significant. So, this agro-based biomass can be recommended to be used as an environment friendly and low cost adsorbent for lowering the fluoride ion concentration to the standard permissible limit.

**Keywords:** Fluoride, Kluai Namwa peel powder, Batch Adsorption, Surface characterization, Adsorption Isotherm

## 1. Introduction

Fluoride (F<sup>-</sup>) is the simplest anion of fluorine and is persistent in all the environmental components of earth, including hydrosphere, biosphere, geosphere and atmosphere. On a global scale, fluoride originates dominantly from the natural processes and trace amount is commonly encountered in water sources. Basically, fluoride is released into water bodies as a result of weathering of fluoride bearing minerals, or leaching from soil into groundwater under favorable dissolution conditions and percolating water pressure. Ingestion of fluoride through food and air is relatively less compared to water. Systemic fluorides such as community water fluoridation and dietary fluoride supplements are often promoted in order to lessen the tooth decay. Fluoride concentration at the optimum level (0.5 - 1 mg/l) in primary drinking water is beneficial for the normal mineralization of bones and teeth, and helps in the effective reduction of dental caries. However, continued consumption of water containing more than 1.5 mg/l fluoride will result in dental fluorosis and severe skeletal fluorosis [1, 2]. Endemic fluorosis has been reported in at least 25 developed and developing countries across the globe.

The WHO guideline value of fluoride in drinking water is 1.5 mg/l, set with a target between 0.8–1.2 mg/l to maximize the benefits and offset harmful effects. In absence of alternative water supplies, the only feasible way to prevent fluorosis is to defluoridate the water by employing the additive and adsorptive methods. Nalgonda technique is an additive method commonly used in domestic level, while this process generates higher quantity of alum sludge compared to electrochemical defluoridation methods. Besides, the

technologies like chemical precipitation, hybrid precipitation-microfiltration, oxidation-reduction, membrane process based on reverse osmosis and nanofiltration, ion-exchange resins, electro-chemical methods based on electrolysis and membrane separation, and adsorption by Activated Alumina (AA), bone char, costly commercial activated carbons and nanoparticles are highly efficient in lowering the fluoride concentration to the permissible level. Yet, their use have been found to be limited due to social, financial, cultural, and environmental factors [3, 4].

Adsorption has been recognized as a highly efficient method for the removal of fluoride from aqueous medium, as it offers economic feasibility and simplicity in design and operation. In addition, agro-based biomass are gaining more attention in the adsorption process since they can meet the demand for safe, eco-friendly, abundant and easily available low cost adsorbents. So, far defluoridation of water by using *Musa* spp. ABB cv. Kluai Namwa (banana) peel has not been reported anywhere in the literature. Banana peel, which represents about 40% of total weight of the fresh fruit [5] is considered as a major horticultural by product produced at great quantities and often discarded as solid waste. The annual world production of bananas in about 120 countries is estimated to be over 68 million tons and in South-East Asia, Thailand ranks third in terms of both area and production after Philippines and Indonesia. Particularly, Kluai Namwa is the most widely disseminated ABB cultivar grown as the favorite cooking banana, and covers almost 70% of all bananas that are cultivated in Thailand [6]. The present investigation is focused on bioadsorption of fluoride in a batch system by Kluai Namwa peel powder as a novel and cost-effective adsorbent. The isotherm studies are also carried out by using Langmuir and Freundlich models to elucidate the sorbent-sorbate interactions.

## **2. Materials and Methods**

### **2.1. Preparation of Bioadsorbent**

Banana peels were collected from the local fruit market in Thailand and were washed thoroughly with tap water to remove the attached fleshy residues. The peels were dried in sunlight for about 7-8 hours followed by hot air oven at  $120 \pm 2$  °C for 36 hours. It is supposed that higher temperature would expand the surface area of the adsorbents and provides a better interaction between the ligands on the cell wall and the fluoride ions. The dried peels were crushed using mortar and pestle and sieved by 250 BSS, mesh size. Finally, the screened banana peel powder (BP) was stored in sterilized airtight container.

### **2.2. Experimental procedure of adsorption studies**

The stock fluoride solution of 1000 mg/l was prepared by dissolving 2.21 g AR grade anhydrous sodium fluoride in Milli-Q water. Experimental test solution of 10 mg/l was prepared by serial dilution of fresh stock solution. A series of batch adsorption experiments were carried out in 250 ml Erlenmeyer flasks, with 50 ml of test solution at room temperature ( $25 \pm 3$  °C). The flasks were sealed so as to prevent the change in solution volume during the experiments. The optimum reaction conditions for the maximum defluoridation was determined by studying the influence of adsorbent dose, pH, agitation speed, contact time, initial fluoride concentration and effect of co-existing ions in binary components system. After predefined time intervals, samples were filtered with Whatman No. 42 filter paper and the fluoride concentration was measured. The optimum value of each parameters was recorded for the design experiments.

To obtain the FTIR spectra, very small amount of sorbents were ground and mixed with spectral-grade KBr, and then pressed under high vacuum pressure to obtain a transparent pellets. The FTIR data were collected over  $4000$ – $500$   $\text{cm}^{-1}$  at a resolution of  $4$   $\text{cm}^{-1}$ . Moreover, for examining the surface morphology, small fragments of banana peel was mounted on 10 mm aluminum stub with a carbon adhesive and then coated with platinum before observation. The operation was carried out at variable pressure (VP) mode accelerating voltage.

### **2.3. Instrumentation**

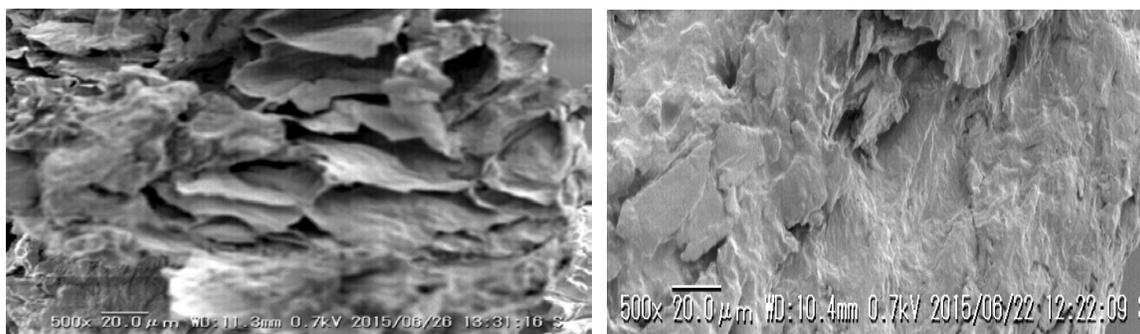
The NB-101 M Orbital shaker (N-Biotek Inc., Gyeonggi-Do, Korea) was used for agitating the samples. The residual fluoride concentration after the adsorption experiments was measured using ExStik FL700

Fluoride meter equipped with ion selective electrode (FLIR Systems, USA). The measurement procedure follows the American Society for Testing and Materials (ASTM) and EPA standard methodology, using total ionic strength adjustment buffer (TISAB) reagents. The pH of the solution was measured using Hanna digital pH meter. The morphological characterization of banana peel before and after fluoride sorption was studied by using 3D real surface view microscope VE-8800 Series (KEYENCE, Japan). FTIR spectra was recorded on Thermo Nicolet 6700 FTIR Spectrometer (Thermo Electron; USA) for the functional groups analysis.

### 3. Results and Discussion

#### 3.1. SEM studies of bioadsorbent

Figure 1 (a) and (b) represents the SEM images of the banana peel biomass before and after fluoride adsorption at a resolution of 500 $\times$  and 20  $\mu\text{m}$  particle size. Before adsorption, the adsorbent exhibited irregular and rough porous surface with heterogeneous voids. These characteristics increase the adsorption capacity of the adsorbent and act as reactive adsorption centers for fluoride adsorption. After adsorption, the surface morphology of the adsorbent was significantly changed. The peels appear to have smooth surface as the pores and caves were partially covered by fluoride.



(a) (b)  
**Fig.1** SEM images for banana peel: (a) Before adsorption; (b) After fluoride adsorption

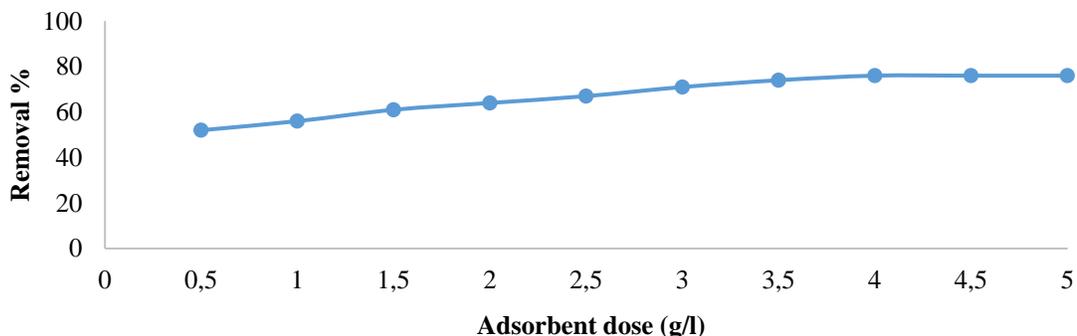
#### 3.2. FTIR analysis of bioadsorbent

FTIR spectra of banana peel displayed a number of peaks indicating the complex nature of adsorbent. The broad absorption band at 3447.7  $\text{cm}^{-1}$  is assigned to O-H stretching of hydroxyl groups of alcohols and phenols. Similarly, the peaks pertaining to 2918.1  $\text{cm}^{-1}$  and 1758.2  $\text{cm}^{-1}$  are due to the C-H stretching of alkane representing aliphatic nature of the adsorbent, and asymmetrical stretching of C=O bond of carboxylic acids or ester. The adsorption peaks appearing at the region of 1636.4-962.6  $\text{cm}^{-1}$  are basically attributed to ester, polysaccharide or protein, and the peak at 1384.2 is due to the stretching vibration of -COO. The absorption bands at around 1758.2 – 1384.2  $\text{cm}^{-1}$  can also be interpreted as the characteristics of C=C in aromatics rings. Peaks at 1043.3 and 1089.9  $\text{cm}^{-1}$  are attributed to Si-O stretching and Si-O bending indicating the presence of silica. Moreover, peaks in the region of lower wave numbers can be attributed to N containing bioligands and N-H deformation of amines respectively [7, 8]. Banana peel (BP) is highly fibrous material mainly composed of polymers such as lignin, hemicellulose and pectins, and thus contain various rich organic functional groups (-OH, -NH<sub>2</sub>, -COOH) playing a major role in fluoride adsorption.

#### 3.3. Optimization of adsorbent dose

The effect of adsorbent dose on the fluoride removal was assessed by varying the amount of BP from 0.5 to 5 g/l in the fluoride solution of 10 mg/l, and the mixture was constantly shaken at 150 rpm for 2 hours. Figure 2 indicates that the adsorption efficiency increased with the increase in adsorbent dose. The number of available adsorption sites is increased with the increase in adsorbent dosage, providing better sorbate-sorbent interaction. The maximum removal of 76 % was attained at the dose of 4 g/l with no further

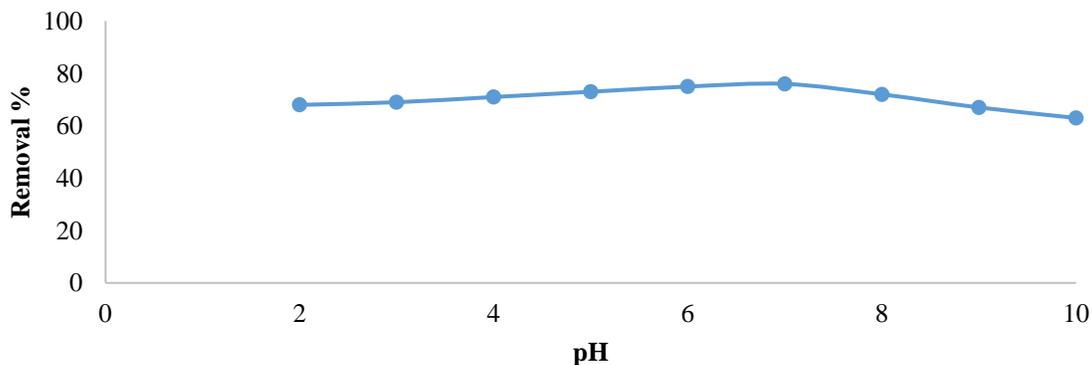
appreciable increase in the removal beyond this dose. The flattening of curve at higher doses of BP was due to the shortage of fluoride ion in solution with respect to higher exchangeable sites on the adsorbent [9]. Besides, the net surface area is reduced due to the overlapping of active sites at higher doses resulting in the marginal increase in fluoride removal. Further experiments were carried out with 4 g/l as an optimum dose.



**Fig.2** Effect of adsorbent dose on adsorption of fluoride

### 3.4. Optimization of pH values

The pH of the test solution was varied from 2-10 by adding drops of 0.1N HCl or NaOH and the experiments were conducted under the aforementioned conditions with the dose of 4 g/l. As seen from Figure 3, the percentage removal of fluoride increased from acidic to neutral pH and then decreased gradually with further increase in pH values above 7. The maximum fluoride removal efficiency was observed 76 % at pH 7. This might be because of the higher coulombic attraction of negatively charged fluoride ion and positively charged  $H^+$  ion along with some neutral charges on the adsorbent surface. Moreover, the reduction of adsorption in the acidic pH range is due the conversion of fluoride into neutral HF, which directly affects the anion exchanging nature, whereas in the alkaline condition, the presence of large number of  $OH^-$  ions causes increased hindrance to diffusion of fluoride ions onto the adsorbent. Similar findings were reported on using *Polyalthia longifolia* leaf powder and activated carbon from the barks of *Vitex negundo* plant for fluoride removal [10, 11]. So, neutral pH was identified as the optimum and was maintained for further sorption studies.

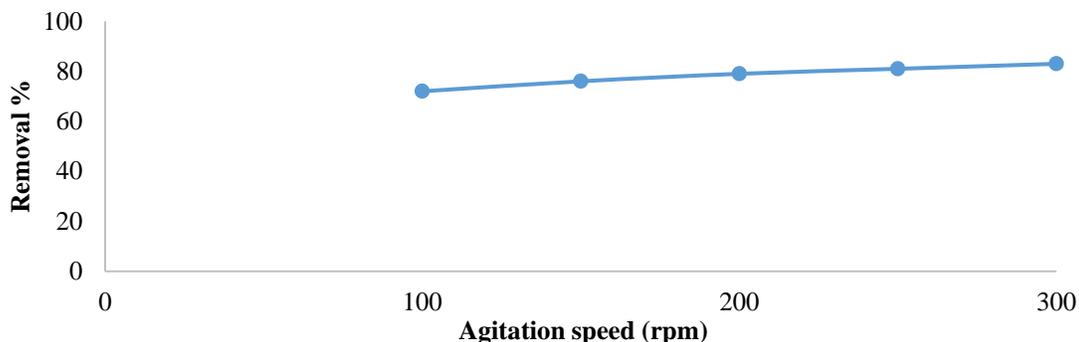


**Fig.3** Effect of pH on adsorption of fluoride

### 3.5. Optimization of agitation speed

Agitation speed plays an important role in the distribution of the solute in the bulk solution and the formation of the external boundary film. So, the influence of agitation speed was monitored under

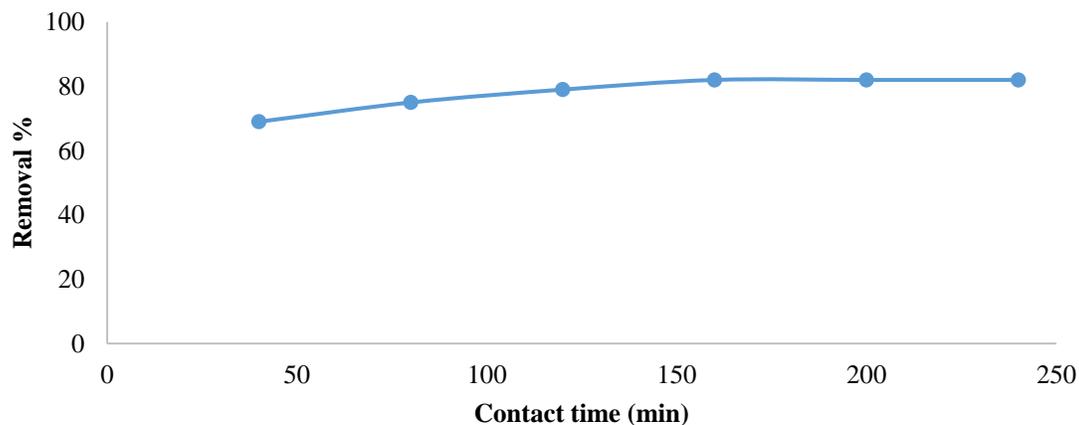
optimized dose of adsorbent and neutral pH for 2 hours. The graphical representation in Figure 4 shows that the rate of adsorption increases with agitation speed and reaches a maximum of 83 % at 300 rpm. The proper contact between the fluoride in solution and the binding sites at higher speed promoted the effective transfer of fluoride ions to the adsorbents sites. At lower speed, the adsorbent tends to accumulate at bottom of the flask, resulting in the burial of various active sites under the above layers of adsorbent. Also, the presence of liquid film thickness around the particles decreases the uptake rate [12]. However, higher speeds need higher energy input and it may also damage the physical structure of adsorbents. So, a 200 rpm agitation speed is sufficient to assure that all the surface binding sites are made readily available for fluoride uptake.



**Fig.4** Effect of agitation speed on adsorption

### 3.6. Optimization of contact time

The variation in percentage removal of fluoride was studied as a function of contact time from 40 to 240 minutes with the dose of 4 g/l and neutral pH at 200 rpm agitation speed. Figure 5 shows that the efficiency of banana peel increases with the duration of exposure to fluoridated water over the first 160 minutes, thereafter the curve lapses flat denoting the attainment of equilibrium. The obtained result is consistent with several other studies [13, 14]. The removal rate in the early part of process was due to the larger available surface area of the adsorbents in the beginning. Later on, the binding sites becomes saturated, as the active sites in the system can adsorb only one ion in a monolayer. Eventually, a repulsive force is developed between the solute molecules on the solid and bulk phases causing a decrease in adsorption efficiency [15]. So, from this analysis, 160 minutes is chosen as the optimum contact time for effective removal of fluoride.



**Fig.5** Effect of contact time on adsorption

### 3.7. Effect of initial fluoride concentration

The effect of initial fluoride concentration was examined by varying the fluoride concentration from 5 to 40 mg/l under optimized experimental conditions as shown in Figure 6. The results indicate that the fluoride removal was maximum at 5 mg/l concentration which then decreased continuously to 52 % for 40 mg/l fluoride concentration. This is because at low concentrations, there is an active interaction of fluoride ions with the available binding sites facilitating higher adsorption efficiency. On the contrary, the fluoride ions/adsorbent ratio is supposed to increase at higher concentrations resulting in the faster saturation of higher energy sites. Because of this, there will be the lack of binding sites and the competition between the fluoride ions for the remaining low energy sorption sites causes a decrease in removal efficiency [16].

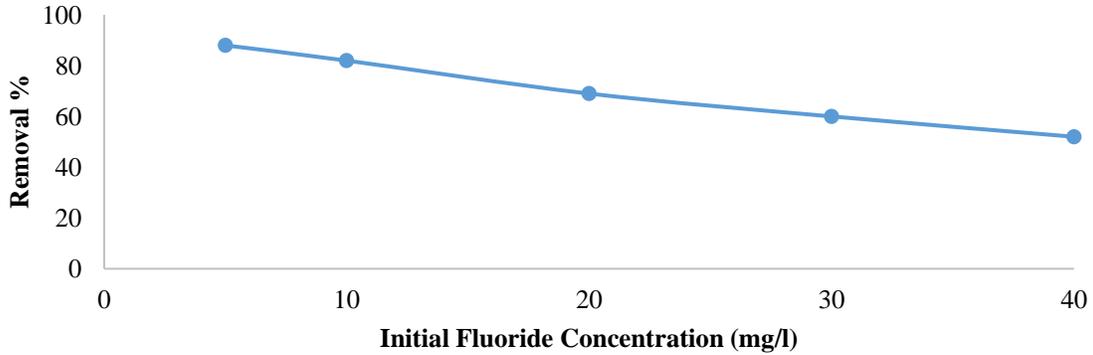
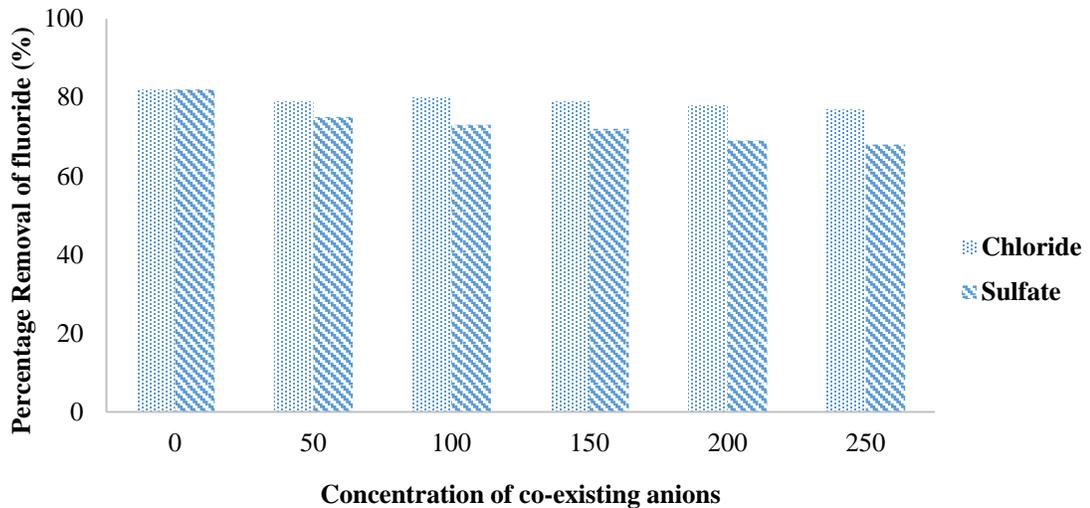


Fig.6 Effect of initial fluoride concentration on adsorption

### 3.8. Effect of Chloride and Sulphate on Fluoride Adsorption

To assess the suitability of BP for the adsorption of fluoride, defluoridation studies were conducted in the presence of chloride and sulphate ions at neutral pH with the obtained optimized conditions. The initial concentration of fluoride was 10 mg/l in all experiments, whereas concentrations of chloride and sulphate varied from 50 to 250 mg/l. It can be inferred from Figure 7 that there was no remarkable influence on the fluoride removal in presence of monovalent chloride ions, while the presence of divalent sulfate at higher concentrations resulted in the decrease of fluoride removal. This is because chloride ion is basically low affinity ligand and their adsorption mechanism is via formation of weaker bonds with the active sites at the outer-sphere complexation. Sulfate ions, on the other hand is partially inner and outer sphere complex forming species [17]. Besides, there might be the competition between fluoride and sulphate ions for the sorption sites, as the high coulombic repulsive forces might reduce the probability of fluoride interactions with the binding sites [18]. Similar results were reported by other researchers [19, 20].



**Fig.7** Effect of initial fluoride concentration on adsorption

### 3.9. Adsorption Isotherm Models

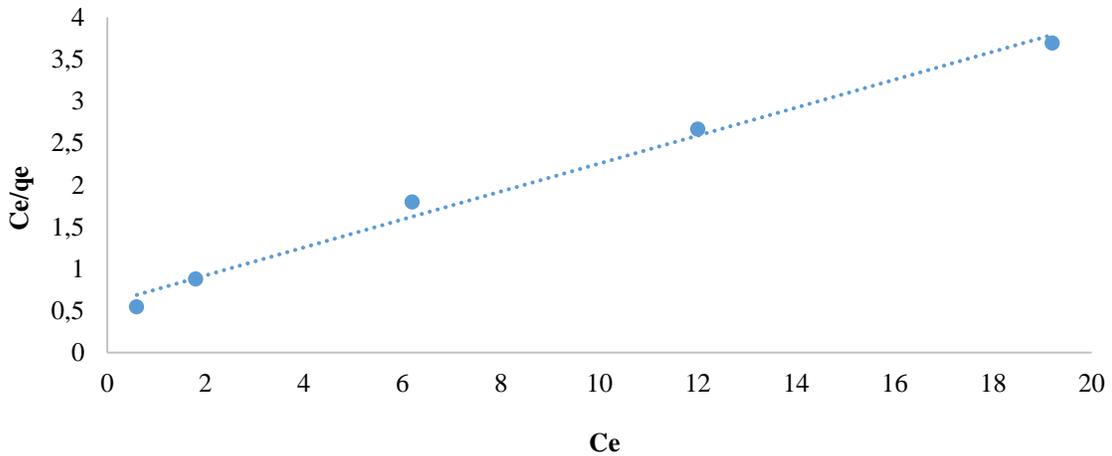
The adsorption isotherms are important for design purposes, as they deal with the specific relationship between equilibrium amount of fluoride on the adsorbent and the solute concentration in solution at constant temperature and pH. The obtained equilibrium data were analyzed with Langmuir and Freundlich isotherm equations.

#### 3.9.1. Langmuir Isotherm Model

Langmuir isotherm model assumes monolayer formation on the adsorbent surface having energetically equal homogeneous sites, with no lateral interaction and steric hindrance between the adsorbed molecules, even on adjacent sites [21]. The linearized form (Type I) is expressed as;

$$\frac{C_e}{q_e} = \frac{1}{Q_o b} + \frac{C_e}{Q_o} \quad (1)$$

Where,  $q_e$  is amount adsorbed per unit weight of adsorbent (mg/g),  $C_e$  is equilibrium concentration (mg/l), and  $Q_o$  and  $b$  are Langmuir constants related to the measures of maximum adsorption capacity (mg/g) and adsorption affinity coefficient (l/mg). The constants  $Q_o$  and  $b$  is calculated from intercept and slope of the graph plotted between  $C_e/q_e$  Vs  $C_e$  as shown in Figure 8. The adsorption isotherms parameters along with the correlation coefficients are presented in Table 1.



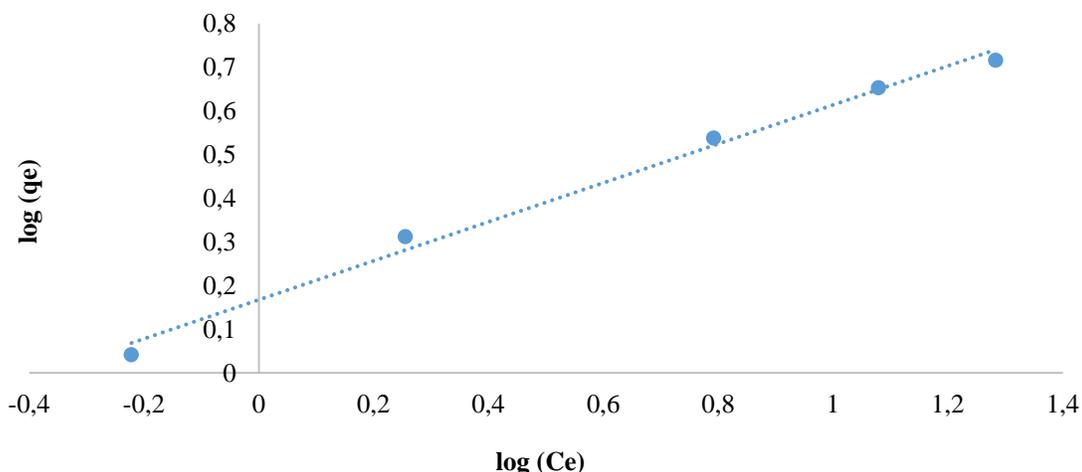
**Fig.8** Langmuir isotherm model for adsorption of fluoride on to banana peel powder (Process Conditions: Adsorbent dose: 4 g/l, Neutral pH, Agitation speed: 200 rpm, Contact time: 160 minutes)

#### 3.9.2. Freundlich Isotherm

The Freundlich isotherm is based on multilayer adsorption state and describes adsorption on heterogeneous surface with a non-uniform distribution of heat of sorption [22]. The logarithm form of Freundlich isotherm is given as,

$$\log q_e = \log K + \frac{1}{n} \log C_e \quad (2)$$

where,  $q_e$  is the amount of fluoride adsorbed per unit weight of adsorbent (mg/g),  $C_e$  is the equilibrium solute concentration in solution (mg/l),  $K$  is the Freundlich adsorption coefficient denoting the adsorption capacity, and  $n$  represents the adsorption intensity. The empirical constants,  $K$  and  $\frac{1}{n}$  were obtained from the linear correlations between the values of  $\log q_e$  and  $\log C_e$  as shown in Figure 9 and are listed in Table 1.



**Fig.9** Freundlich isotherm model for adsorption of fluoride on to banana peel powder (Process Conditions: Adsorbent dose: 4 g/l, Neutral pH, Agitation speed: 200 rpm, Contact time: 160 minutes)

**Table 1.** Parameters of Langmuir and Freundlich isotherms for adsorption of fluoride on Banana Peel

Langmuir Constants			Freundlich Constants			
Q <sub>o</sub>	b	R <sup>2</sup>	K	N	1/n	R <sup>2</sup>
5.99	0.283	0.990	1.46	2.23	0.44	0.991

The observed linear relationships for both models are statistically significant as evidenced by the R<sup>2</sup> values close to unity. As can be seen from Table 1, the calculated value of adsorption intensity (n) between 1 to 10, indicates the favorable conditions for adsorption due to increase in the bond energies with the increase in surface density. So, the smaller values of 1/n implies strong interaction between the adsorbents and fluoride ions. Data pertaining to the adsorbent fitted reasonably well to both the isotherms indicating physicochemical adsorption process.

#### 4. Conclusions

The potentiality of easily available and inexpensive agro-based waste has been investigated for the treatment of fluoride contaminated water. The removal efficiency of the banana peel powder was found to increase with the increasing dose of the biomass and exposure time with constant agitation speed (200 rpm), whereas the removal rate decreased with the increase in initial fluoride concentration. At low fluoride concentration, the adsorbent exhibited high selectivity for fluoride ions with coexisting chloride and sulphate ions in a binary system. The main advantage of using these types of agro-based adsorbents is that they work optimally at pH 6 and 7, which reduces the cost of post defluoridation pH adjustment. When the initial fluoride concentration is maintained at 5 mg/l, the removal efficiency of the adsorbent is 88 % at optimum conditions, and the fluoride concentration of the treated water is below the regulated standard. Furthermore, the biosorbent was characterized by FTIR spectroscopy and scanning electron microscopy (SEM) techniques to support the obtained results. Both Langmuir and Freundlich isotherm models fitted well to the experimental data and are statistically significant. Finally, the use banana peel powder can be employed as eco-friendly and cost-effective adsorbent adsorbent for the defluoridation of water.

#### Acknowledgements

Authors are thankful to Sirindhorn International Institute of Technology, Thammasat University for supporting the budget and laboratory for this research.

#### References

1. Soni, R. and S. Modi, *A Review on Effects of Fluoride on Human Health in Rajasthan*. International Journal of Innovative Research and Development, 2013. **2**(4): p. 638-646.
2. AbuZeid, K. and L. Elhatow. *Impact of fluoride content in drinking water*. in *Arab Water Healthy Conference Egypt: Cairo*. 2007.
3. Ingle, N., et al., *Defluoridation techniques: Which one to choose*. Vol. 1. 2014. 1-4.
4. Mohapatra, M., et al., *Review of fluoride removal from drinking water*. Journal of environmental management, 2009. **91**(1): p. 67-77.
5. Anhwange, B., T. Ugye, and T. Nyiaatagher, *Chemical composition of Musa sapientum (banana) peels*. Journal of Food Technology, 2008. **6**(6): p. 263-266.
6. Molina, A., et al. *Advancing banana and plantain R&D in Asia and the Pacific-Vol. 13*. in *Proceedings of the 3rd BAPNET Steering Committee meeting held in Guangzhou, China*. 2004.
7. Memon, J.R., et al., *Banana peel: a green and economical sorbent for Cr (III) removal*. Pak J Anal Environ Chem, 2008. **9**(1): p. 20-25.
8. Mahmoud, M., et al., *Evaluation of Egyptian banana peel (Musa sp.) as a green sorbent for groundwater treatment*. Int. J. Eng. Technol, 2014. **4**: p. 648-659.
9. Manna, S., et al., *Defluoridation of aqueous solution using alkali-steam treated water hyacinth and elephant grass*. Journal of the Taiwan Institute of Chemical Engineers, 2014.
10. Bharalia, R.K. and K.G. Bhattacharyab, *KINETIC AND THERMODYNAMIC STUDIES ON FLUORIDE BIOSORPTION BY DEVDARU (POLYALTHIA LONGIFOLIA) LEAF POWDER*.
11. Suneetha, M., B.S. Sundar, and K. Ravindhranath, *Removal of fluoride from polluted waters using active carbon derived from barks of Vitex negundo plant*. Journal of Analytical Science and Technology, 2015. **6**(1): p. 1-19.
12. Anwar, J., et al., *Removal of Pb (II) and Cd (II) from water by adsorption on peels of banana*. Bioresource Technology, 2010. **101**(6): p. 1752-1755.
13. Zazouli, M.A., et al., *ADSORPTION OF FLUORIDE FROM AQUEOUS SOLUTION BY MODIFIED AZOLLA FILICULOIDES*. Adsorption, 2014.
14. Gandhi, N., et al., *Removal of fluoride from water and waste water by using low cost adsorbents*. Int J Chem Tech Res, 2012. **4**: p. 1646-1653.
15. Srivastava, V.C., et al., *Adsorptive removal of phenol by bagasse fly ash and activated carbon: equilibrium, kinetics and thermodynamics*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2006. **272**(1): p. 89-104.
16. Chakrabarty, S. and H. Sarma, *Defluoridation of contaminated drinking water using neem charcoal adsorbent: kinetics and equilibrium studies*. Int J ChemTech Res, 2012. **4**(2): p. 511-516.
17. Onyango, M.S., et al., *Adsorption equilibrium modeling and solution chemistry dependence of fluoride removal from water by trivalent-cation-exchanged zeolite F-9*. Journal of Colloid and Interface Science, 2004. **279**(2): p. 341-350.
18. Rajkumar, S., et al., *Low-cost fluoride adsorbents prepared from a renewable biowaste: Syntheses, characterization and modeling studies*. Arabian Journal of Chemistry.
19. Cai, H., et al., *Enhanced removal of fluoride by tea waste supported hydrous aluminium oxide nanoparticles: anionic polyacrylamide mediated aluminium assembly and adsorption mechanism*. RSC Advances, 2015. **5**(37): p. 29266-29275.
20. Kumar, N.P., N.S. Kumar, and A. Krishnaiah, *Defluoridation of water using tamarind (Tamarindus indica) fruit cover: Kinetics and equilibrium studies*. Journal of the Chilean Chemical Society, 2012. **57**(3): p. 1224-1231.
21. Foo, K. and B. Hameed, *Insights into the modeling of adsorption isotherm systems*. Chemical Engineering Journal, 2010. **156**(1): p. 2-10.
22. Liu, L.-e., et al., *Equilibrium, kinetic, and thermodynamic studies of lead (II) biosorption on sesame leaf*. BioResources, 2012. **7**(3): p. 3555-3572.