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EFFECT OF PHYSICOCHEMICAL FACTORS ON BIOSORPTION OF FLUORIDE BY BACTERIA ISOLATED FROM BARATANG ISLAND – A COMPARATIVE STUDY

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Abstract:
Increased Levels Fluoride in ground water leads to adverse conditions like fluorosis. In spite of availability of several conventional methods of defluorination techniques, Biosorption methods are more feasible and have added advantages. A total of 200 bacterial strains were isolated from Baratang Island soil and subjected to primary screening out of which 16 potential isolates were identified and subjected for secondary screening with two different media, at three different temperatures, pH and incubation periods. At the end of the study 5 potential strains were identified with a maximum of 90 % adsorption. The studies suggest that these strains can be used as a potential microbial species for endemic fluoride defluoridation applications in water after complete evaluation and care.

1. Introduction

Ground water constitutes 97% of global fresh water and is the major preferred source of drinking water in rural as well as urban areas, particularly in the developing countries like India (WHO and UNICEF, 2004). Due to various ecological factors either natural or anthropogenic, the ground water is getting polluted by many natural constituents, of which Fluoride stands first as a pollutant of geogenic origin in many countries of the World (Anwar 2003, Oren *et al.,* 2004, Amina *et al.,* 2004 and Kass *et al.,* 2005).

Fluoride, a natural element which is usually present in various water bodies, can have beneficial as well as detrimental effects on humans depending on its concentration and the total amount ingested. According to the World Health Organization (WHO), the permissible limit of fluoride concentration in drinking water is generally 1.5 mg /L (Song *et al.,* 2019, WHO 2011., Herath, Kawakami and Tafu, 2018., Xia *et al.,* 2019., Chatterjee, Mukherjee and De, 2020).

Fluorosis is playing havoc in more than 25 nations across the World and in many countries and the number of people suffering from fluoride poisoning is staggering. (Taiyuan declarations, 2004). At present, 20 out of 35 States and Union Territories are under fluoride attack. Due to severity of impacts with excess fluoride in ground water, the WHO permissible limit of fluoride in India has been reduced from 1.5 to 1.0 ppm in 1998 (UNICEF, 1999).

Several methods have been developed for fluoride removal from water such as ion exchange, membrane process, electrodialysis, precipitation and coagulation (Ayoob, Gupta and Bhat, 2008., Chen *et al.,* 2010,). The shortcomings of most of these methods are high maintenance and

operational costs, secondary pollution (toxic sludge being created), and complicated steps (Babu *et al.*, 2011., Furukawa , 2013). Among the various techniques available, adsorption method seems to be superior because of its flexibility, cost effectiveness, environmentally friendly, simple design and easy operation (Rajkumar *et al.*, 2019, Mohapatra *et al.*, 2009., Salifu 2017). Therefore, an attempt was done in this study by using bacteria isolated from Baratng Island of Andaman & Nicobar as biosorbents to develop Bioremediation processes for the removal of fluoride from aqueous phase. The services of the native microorganisms in the environment are explored for this purpose.
The study includes the isolation and screening of bacterial strains which are effectively removing the fluoride under laboratory conditions. The effect of condition like different nutrient media, temperature, pH and incubation period were investigated on the Biosorption process.

2. Methodology

2.1 Collection of soil sample:

Soil samples for the present study were collected from **Baratang** island of **Andaman.** Soil samples were collected in sterilized polythene bags with a sterilized spatula and transported under controlled conditions to laboratory for further analysis. A view of soil sampling site is included in Image 2.1.

Image 2.1 Soil sampling site

2.2 Isolation of Microbial populations

Isolation of microbes was done using serial – dilution agar plate procedure and Enrichment culture technique.
The media used for isolation of different strains were:

a) Glycerol Yeast Extract - Actinomycetes.
b) Sabourauds Dextrose Agar -Fungi
c) Nutrient Agar - Bacteria.

The Actinomycetes and Fungal media were supplemented with Chlorotetracycline to inhibit the growth of bacteria (Cappuccino, 2005 and Aneja, 2003). The isolated Bacterial strains were used for Biosorption studies.
Individual colonies of bacteria which vary in shape and color were picked up using a sterile inoculating wire loop and sub cultured and purified by streaking onto Nutrient agar plates. Plates were incubated at 37^{o}C for 24 hrs to obtain pure colonies (Prescott, Harley and Klein, 2005). The purity of each bacterial isolate was checked under the microscopic examination. The purified isolates were maintained on Nutrient agar slants and kept at 4^{o}C and sub cultured for every 4 weeks.

2.3 Primary screening:

Isolated bacterial strains were screened for their Biosorption activity. In the screening process, the ability of isolated bacteria to grow in **fluoride** media was tested to identify the potential strains for Biosorption process.

Isolated colonies were inoculated in Nutrient Broth medium and incubated for 24 hrs. Fresh Basal Medium having concentration of fluoride (10 mg / L) was prepared. Overnight fresh cultures were transferred as inoculums and incubated at 37^0C for 24hrs. Biosorption activity was measured by SPADNS method (Monica Bhatnagar, 2002). The positively responded strains were subjected to secondary screening.

2.4 Biosorption studies (Secondary screening):

Biosorption process is effective when conditions like Temperature, pH, and Oxygen content permit microbial growth and activity. Hence, optimization study was carried out in order to find the effect of Nutrients, pH, Temperature, Duration of incubation, availability of molecular Oxygen and concentration of fluoride on percent defluoridation (Ramanaiah, Venkata Mohan and Sarma, 2007; Venkata Mohan *et al.*, 2006; Bhtnagar *et al.*, 2002; Bhatnagar and Bhatnagar, 2000 and Sinha *et al.*, 2000).

In optimization studies, the effect of nutrient medium was studied by using four different types of media: 1. Nutrient broth 2. Peptone water. The influence of temperature on the Biosorption process was studied at 3 different temperatures; 10^0C, 37^0C and 60^0C representing Psychrophiles, Mesophiles and Thermophiles respectively.

The Biosorption experiments were conducted at pH 4.0, 7.0 and 10.0 representing Acidophiles, Neutrophiles and Basophiles respectively.

At each temperature and pH the following are the experimental conditions.

- Incubation period: - 24 hrs, 48 hrs and 72 hrs.
- Inoculums volume: - 1mL.
- Fluoride concentration: - 10 mg / L, 20 mg/L and 30 mg / L.
- Aerobic conditions.

3. Results and Discussions

3.1 Isolation of Microbial populations

The number of micro organisms isolated from the soil was calculated using the formula

$$\text{No. of cells / g} = \frac{\text{No. of colonies (average of 3 replicates) X Dilution factor}}{\text{Dry weight of the soil.}}$$

Dilution factor = Reciprocal of the dilution

Organism	Dilution	Dilution factor	Number of colonies/ plate			Average number of Colonies/ dilution
			I	II	III	
Bacteria	10^{-4}	10^4	69	51	63	183 / 3 = 61 X 10^3
	10^{-5}	10^5	61	46	52	159 / 3 = 53 X 10^4
	10^{-6}	10^6	52	43	49	144 / 3 = 48 X 10^5
	10^{-7}	10^7	43	32	39	114 / 3 = 38 X 10^6
Actino-mycetes	10^{-3}	10^3	18	15	15	48 / 3 = 16 X 10^2
	10^{-4}	10^4	11	9	7	27 / 3 = 9 X 10^3
	10^{-5}	10^5	9	5	4	18 / 3 = 6 X 10^4
	10^{-6}	10^6	7	5	3	15 / 3 = 5 X 10^5
Fungi	10^{-2}	10^2	8	6	7	21 / 3 = 7 X 10^1
	10^{-3}	10^3	5	3	4	12 / 3 = 4 X 10^2
	10^{-4}	10^4	5	2	2	9 / 3 = 3 X 10^3
	10^{-5}	10^5	4	2	2	9 / 3 = 3 X 10^4

Table 3.1 Enumeration of Microbial Flora from the soil of Baratang Island.

A total of 200 Bacterial strains were isolated from the soil (10 g) of Baratang Island of Andaman. The bacterial strains were purified by repeated streaking on to Nutrient agar plates and the purified colonies were stored at 4^0C temperature for further use. The isolated 200 strains were subjected to Primary screening.

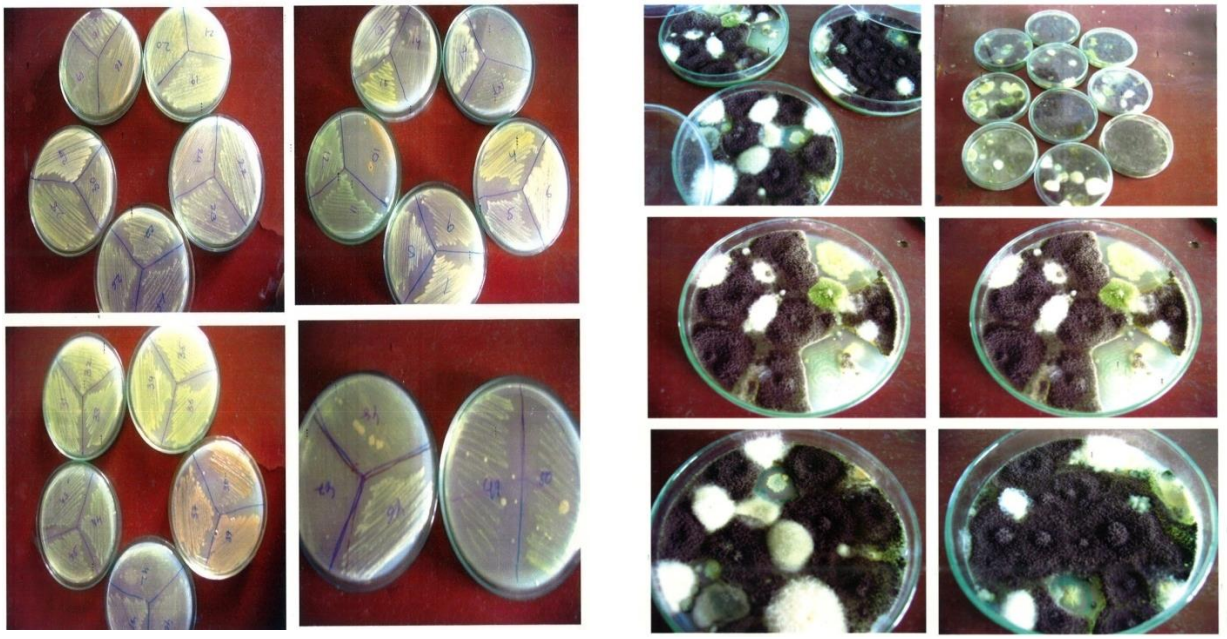

Image 3.1 Few bacterial fungal and Actinomycetes colonies isolated from soil of Baratang Island

3.2 Primary screening

S. No	Strain. No	Vol. of B.M (ml)	*Vol. of NaF^-(ml)	Vol. of Inoculum (ml)	% of Biosorption
1	S_1	2.0	8.0	1.0	90
2	S_2	2.0	8.0	1.0	90
3	S_3	2.0	8.0	1.0	-
4	S_4	2.0	8.0	1.0	-
5	S_5	2.0	8.0	1.0	-
6	S_6	2.0	8.0	1.0	-
7	S_7	2.0	8.0	1.0	-
8	S_8	2.0	8.0	1.0	-
9	S_9	2.0	8.0	1.0	-
10	S_{10}	2.0	8.0	1.0	-

Table 3.2 Primary screening of the first ten bacterial strains.

BM = Basal Medium, pH = 7.0, Temperature = 37^0C, *10mg/L, Incubation period – 24hrs.

Similar pattern was observed when the remaining 190 strains were subjected to screening. Among the 200 bacterial strains that were subjected to primary screening, a total of 16 bacterial strains were identified as potential biosorbents. These strains were designated as S_1, S_2, S_{13}, S_{16}, S_{24}, S_{25}, S_{26}, S_{29}, S_{32}, S_{35}, S_{47}, S_{52}, S_{54}, S_{55}, S_{56} and S_{57} and were subjected to secondary screening.

3.3 Secondary screening

The secondary screening of the designated bacterial strains was carried out in two media under the following conditions pH 4.0, 7.0 & 10.0, temperatures 10^0C, 37^0C & 60^0C and in three successive incubation periods; 24hrs, 48hrs and 72hrs.

After every successive incubation period, the fluoride concentration of the supernatant of the centrifuged media was estimated by SAPDNS method.

pH	4.0			7.0			10.0		
Incubation Period (hrs)	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
Strain Number	**% of Biosorption**								
S_1	-	-	-	-	-	-	-	-	-
S_2	-	-	-	-	-	-	-	-	-
S_{13}	20	50	70	30	60	90	10	20	30
S_{16}	-	-	-	-	-	-	-	-	-
S_{24}	-	-	-	-	-	-	-	-	-
S_{25}	-	-	-	-	-	-	-	-	-
S_{26}	-	-	-	-	-	-	-	-	-
S_{29}	-	-	-	-	-	-	-	-	-
S_{32}	-	-	-	-	-	-	-	-	-
S_{35}	20	50	70	30	60	90	10	20	30
S_{47}	-	-	-	-	-	-	-	-	-
S_{52}	-	-	-	-	-	-	-	-	-
S_{54}	20	50	70	30	60	90	15	30	60
S_{55}	20	50	70	30	60	90	15	30	60
S_{56}	20	50	70	30	60	90	15	30	60
S_{57}	-	-	-	-	-	-	-	-	-

Table 3.3 (a) Biosorption of Fluoride by the designated strains in Nutrient broth medium, at various incubation periods and pH.

Conditions:

Medium : Nutrient broth (2mL).
Incubation temperature : 10^{o}C.
Concentration of Sodium Fluoride : 10mg/L (8mL).
Volume of inoculums : 1.0mL.

pH	4.0			7.0		10.0		
Incubation Period (hrs)	**24**	**48**	**72**	**24**	**48**	**24**	**48**	**72**
Strain Number	**% of Biosorption**							
S_1	20	50	78	40	73	-	-	-
S_2	20	50	80	30	70	-	-	-
S_{13}	25	48	70	65	97	10	30	50
S_{16}	20	50	75	30	70	-	-	-
S_{24}	20	50	77	30	70	-	-	-
S_{25}	20	50	80	35	70	-	-	-
S_{26}	-	-	-	30	60	-	-	-
S_{29}	-	-	-	30	60	-	-	-
S_{32}	-	-	-	30	60	-	-	-
S_{35}	20	50	82	65	95	10	30	50
S_{47}	-	-	15	35	68	-	-	-
S_{52}	-	-	15	30	65	-	-	-
S_{54}	20	50	85	60	92	10	30	50
S_{55}	20	50	85	60	92	10	30	50
S_{56}	20	50	87	60	96	20	50	90
S_{57}	-	-	-	50	75	-	-	-

Table 3.3 (b) Biosorption of Fluoride by the designated strains in Nutrient broth, at various incubation periods and pH.

Conditions:

Medium : Nutrient broth (2mL).
Incubation temperature : 37^{o}C.
Concentration of Sodium Fluoride : 10mg/L (8mL).
Volume of inoculums : 1.0mL.

Incubation period (hrs)	**24**	**48**	**72**
Strain number	**% Biosorption**		
S_1	20	45	68
S_2	25	48	65
S_{13}	30	60	90
S_{16}	20	40	60
S_{24}	20	40	60
S_{25}	25	52	68
S_{26}	20	45	70
S_{29}	20	48	70
S_{32}	20	45	68
S_{35}	30	60	90
S_{47}	18	40	65
S_{52}	20	40	60
S_{54}	30	60	90
S_{55}	30	60	90
S_{56}	33	65	93
S_{57}	20	40	60

Table 3.3 (b1) Biosorption of Fluoride by the designated strains in Nutrient broth medium, at various incubation periods and pH 7.0.

Conditions:

Medium : Nutrient broth (2mL).
Incubation temperature : 37ºC.
Concentration of Sodium Fluoride : 20mg/L (8mL).
Volume of inoculums : 1.0mL.

pH	**4.0**			**7.0**			**10.0**		
Incubation Period (hrs)	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
Strain Number	**% of Biosorption**								
S_1	-	-	-	-	-	-	-	-	-
S_2	-	-	-	-	-	-	-	-	-
S_{13}	-	-	30	-	40	70	-	-	-
S_{16}	-	-	30	-	-	-	-	-	-
S_{24}	-	-	-	-	-	-	-	-	-
S_{25}	-	-	-	-	-	-	-	-	-
S_{26}	-	-	-	-	30	50	-	-	-
S_{29}	-	-	-	-	-	-	-	-	-
S_{32}	-	-	-	-	-	-	-	-	-
S_{35}	-	-	30	-	40	70	-	-	30
S_{47}	-	-	-	-	-	-	-	-	-
S_{52}	-	-	-	-	-	-	-	-	-
S_{54}	-	-	30	-	40	70	-	-	-
S_{55}	-	-	30	-	40	70	-	-	-
S_{56}	-	30	50	-	45	80	-	-	30
S_{57}	-	-	30	-	30	30	-	-	-

Table 3.3 (c) Biosorption of Fluoride by the designated strains in Nutrient broth medium, at various incubation periods and pH.

Conditions:

Medium : Nutrient Broth (2mL).
Incubation temperature : 60ºC.
Concentration of Sodium Fluoride : 10 mg/L (8mL).
Volume of inoculums : 1.0 mL.

Among the 16 strains, five designated bacterial stains S_{13}, S_{35}, S_{54}, S_{55} and S_{56} have exhibited 70% sorption of Fluoride at 10^0C temperature, in 72 hrs of incubation period and 4.0 pH. At pH 7.0 all the five strains exhibited 90% sorption while at pH 10.0 they differed a lot. Strains S_{13} & S_{35} recorded 30% sorption and strains S_{54}, S_{55} & S_{56} showed 60% sorption after 72 hrs of incubation. At 37° C temperature and pH 4.0 S_{56} recorded the highest sorption (87%) after 72hrs of incubation. Under neutral conditions (pH 7.0) the designated strains S_{13}, S_{54}, S_{55} and S_{56} exhibited complete sorption after 48 hrs of incubation. Since the strains were exhibiting maximum sorption (100%) in 10 mg/L of Fluoride concentration, the Biosorption was carried out at 20 mg / L of fluoride concentration. The results of the study indicated that the bacterial strains S_{13}, S_{35}, S_{54} & S_{55} reported 90% sorption while S_{56} reported 93% sorption after 72 hrs of incubation (table 3.3 b1). At pH 10.0, strain S_{56} showed 90% sorption after 72 hrs of incubation.

At 60^0C temperature and 4.0pH, S_{56} recorded 50% sorption and at pH 7.0 the same strain showed 80% sorption after 72 hrs of incubation. The sorption was very poor at pH 10.0 and only S_{35} and S_{56} strains recorded 30% sorption after 72 hrs of incubation.

pH	**4.0**			**7.0**			**10.0**		
Incubation Period (hrs)	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
Strain Number	**% of Biosorption**								
S_1	-	-	-	-	-	-	-	-	-
S_2	-	-	-	18	40	60	-	-	-
S_{13}	10	30	50	30	55	82	15	40	58
S_{16}	10	30	50	30	50	70	15	40	55
S_{24}	-	-	-	25	58	79	-	-	-
S_{25}	-	-	-	-	-	-	-	-	-
S_{26}	10	30	50	20	50	75	10	25	45
S_{29}	10	30	50	20	40	60	10	25	45
S_{32}	10	30	50	20	40	60	10	28	50
S_{35}	20	35	60	35	65	90	20	45	68
S_{47}	10	20	40	20	40	60	10	25	42
S_{52}	-	-	-	-	-	-	-	-	-
S_{54}	20	40	60	28	58	82	23	45	65
S_{55}	20	40	60	28	58	82	23	45	65
S_{56}	25	55	72	35	68	93	25	54	80
S_{57}	-	-	-	-	-	-	-	-	-

Table 3.4 (a) Biosorption of Fluoride by the designated strains in Peptone water medium, at various incubation periods and pH.

Conditions:

Medium : Peptone water (2mL).
Incubation temperature : 10^0C.
Concentration of Sodium Fluoride : 10mg / L (8mL).
Volume of inoculums : 1.0mL.

pH	**4.0**			**7.0**		**10.0**		
Incubation Period (hrs)	**24**	**48**	**72**	**24**	**48**	**24**	**48**	**72**
Strain Number	**% of Biosorption**							
S_1	15	43	78	20	50	-	-	-
S_2	15	40	60	50	90	-	-	-
S_{13}	20	45	64	50	90	15	40	63
S_{16}	20	50	65	50	90	10	25	50
S_{24}	20	50	67	50	90	13	30	60
S_{25}	-	-	-	50	90	-	-	-

S_{26}	10	20	40	50	90	-	-	-
S_{29}	10	20	40	50	90	-	-	-
S_{32}	15	40	50	50	90	-	-	-
S_{35}	38	50	86	50	90	20	40	60
S_{47}	-	-	-	50	90	-	-	-
S_{52}	25	53	70	50	90	-	-	-
S_{54}	40	74	85	50	89	28	52	74
S_{55}	40	74	85	50	89	28	52	74
S_{56}	43	76	87	50	85	30	58	75
S_{57}	10	20	40	50	90	10	25	50

Table 3.4 (b) Biosorption of Fluoride by the designated strains in Peptone water medium, at various incubation periods and pH.

Conditions:

Medium : Peptone water (2mL).
Temperature of Incubation : 37^0C.
Concentration of Sodium Fluoride : 10mg / L, (8mL).
Volume of inoculums : 1.0mL.

Incubation period (hrs)	**24h**	**48**	**72**
Strain number	**% Biosorption**		
S_1	25	50	90
S_2	25	50	90
S_{13}	25	50	80
S_{16}	25	50	90
S_{24}	25	50	90
S_{25}	25	50	90
S_{26}	25	50	90
S_{29}	25	50	90
S_{32}	25	50	90
S_{35}	33	68	93
S_{47}	25	50	90
S_{52}	25	50	90
S_{54}	33	68	93
S_{55}	33	68	93
S_{56}	30	60	90
S_{57}	25	50	90

Table 3.4 (b1) Biosorption of Fluoride by the designated strains in Peptone water medium, at various incubation periods and pH – 7.0.

Conditions:

Medium : Peptone water (2mL).
Temperature of Incubation : 37^0C.
Concentration of Sodium Fluoride : 20mg / L, (8mL).
Volume of inoculums : 1.0mL.

pH	**4.0**			**7.0**			**10.0**		
Incubation Period (hrs)	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
Strain Number	**% of Biosorption**								
S_1	-	-	-	-	-	-	-	-	-
S_2	-	-	-	-	-	-	-	-	-
S_{13}	30	62	80	35	70	90	20	40	60
S_{16}	25	52	75	40	60	80	20	40	60
S_{24}	10	30	55	20	40	60	-	-	-
S_{25}	10	30	55	20	40	60	-	-	-
S_{26}	20	45	70	30	57	75	20	40	60

S_{29}	20	40	60	40	60	80	20	40	60
S_{32}	18	40	65	40	60	80	20	40	60
S_{35}	23	50	72	40	60	80	28	57	75
S_{47}	-	-	-	40	60	80	-	-	-
S_{52}	20	40	60	40	60	80	-	-	-
S_{54}	25	55	77	38	64	90	20	40	60
S_{55}	25	55	77	38	64	90	20	40	60
S_{56}	30	57	75	40	60	88	30	55	72
S_{57}	24	50	70	28	58	75	20	40	60

Table 3.4 (c) Biosorption of Fluoride by the designated strains in Peptone water medium, at various incubation periods and pH.

Conditions:

Medium : Peptone water (2mL).
Temperature of Incubation : 60^0C.
Concentration of Sodium Fluoride : 10mg / L (8mL).
Volume of inoculums : 1.0mL.

Among the 16 designated bacterial strains, S_{56} exhibited 72% sorption of Fluoride at 10^{o}C temperature and pH 4.0 after 72 hrs of incubation whereas S_{35} and S_{56} strains recorded 90% & 93% of sorption followed by $S_{13,}$ S_{54} & S_{55} showing 82% of sorption at pH 7.0 after 72 hrs of incubation. At pH 10.0, 80% of sorption was reported by S_{56} strain after 72 hrs of incubation. At 37^{o}C incubation temperature, bacterial strains S_{35},$S_{54,}$ S_{55} and S_{56} exhibited 85 - 87% sorption at pH 4.0 after 72 hrs of incubation while at pH 7.0 all the strains recorded 90% sorption after 48 hrs of incubation. Since nearly 90% sorption is reported by all the strains at 37^0C temperature and pH 7.0, sorption studies were carried with increased fluoride concentration (20 mg / L). The studies indicate that strains S_{35}, S_{54} and S_{55} have shown 93% sorption after 72 hrs of incubation (table 3.4 b1). The bacterial strains S_{54} S_{55} and S_{56} also recorded 75% sorption at pH 10.0. At 60^0C incubation temperature, pH 4.0 the designated bacterial strains $S_{13,}$ $S_{35,}$ S_{54}, S_{55} and S_{56} have shown 72 - 80% sorption after 72 hrs of incubation period. The same strains have also exhibited 88 – 90 % sorption at pH 7.0 after 72 hrs of incubation period. At pH 10.0, strain S_{35} and S_{56} reported 75% and 72% of sorption respectively at 72 hrs.

4. Conclusions

A total of 200 Bacterial colonies, 36 Actinomycetes colonies and 17 fungal colonies were isolated from the study soils of the Baratang Island of Andaman Nicobar Island. The primary screening of the 200 bacterial colonies isolated from the study soils revealed that 16 out of 200 bacterial colonies showed more affinity towards Biosorption of Fluoride. The16 strains were Isolated, separated and designated as S_1, S_2, S_{13}, S_{16}, S_{24}, S_{25}, S_{26}, S_{29}, S_{32}, S_{35}, S_{47}, S_{52}, S_{54}, S_{55},S_{56} and S_{57}. These 16 bacterial strains were subjected to Biosorption of Fluoride in 2 different media; Nutrient broth, Peptone water, at different pH 4.0, 7.0 and 10.0 under various Incubation periods; 24 hrs, 48 hrs and 72 hrs.

Basing studies the performance of Biosorption in the 2 different media followed the following order at 37^0C incubation temperature and 7.0 pH.

Nutrient broth > Peptone water

The performance of Biosorption in the 3 pH levels followed the following order in all the 2 different media at 37^0C incubation temperature.

pH 7.0 > pH 4.0 > pH 10.0

The performance of Biosorption at the three incubation temperatures followed the following order in the 2 different media and at 3 pH conditions.

37^0C > 60^0 C > 10^0 C.

Among the 16 designated bacterial strains, 5 strains designated as $S_{13,}$ $S_{35,}$ $S_{54,}$ S_{55} and S_{56} have shown the potential for Biosorption of Fluoride.
The results of the study suggest that the bacterial strains can applied for removal fluoride after complete analysis.

5. References

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