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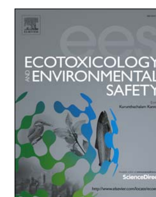

# Evaluation of fluoride bioremediation and production of biomolecules by living cyanobacteria under fluoride stress condition

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ABSTRACT

Application of microalgae for defluoridation has gained interest in recent years. In the present study, bioremediation of fluoride using living cyanobacteria, *Starria zimbabweensis*, collected from wastewater of coke-oven effluent treatment plant, Durgapur, India, has been investigated. Initially, the cyanobacterial strain was grown in BG11 medium at 25 °C, 45 μmol/m$^2$/s irradiation in 18 h: 6 h light:dark cycle in an algal incubator. Samples were withdrawn after 2 days interval and analyzed for its dry biomass and lipid content. Optimum inoculum size of 10% and age of 16th day were assessed based on maximum dry biomass (9.307 ± 0.01 g/L) and lipid (244.05 ± 0.02 mg/L) production. SEM-EDX and FTIR studies of both native and fluoride treated biomass were done to emphasize the changes. During kinetic study of defluoridation, initial fluoride concentration was varied in the range of 10–50 mg/L. Maximum fluoride removal (66.6 ± 0.11%) and dry biomass (18.19 ± 0.12 g/L) were obtained at 10 mg/L fluoride concentration using 10% of 16th day's inoculum. Biomass and lipid content were found to increase 2 and 4 folds, respectively under fluoride stress condition. Furthermore, chlorophyll, carbohydrate and protein content of the biomass were also compared between control and fluoride contaminated conditions. Fatty Acid Methyl Ester (FAME) analysis was done using Gas Chromatography (GC) to compare the lipid profile of native and fluoride loaded strain.

## 1. Introduction

An increase of fluoride level in both groundwater and surface-water due to industrial activity has played a negative impact on our ecosystem. Fluoride ($F^-$) is a naturally occurring strongest oxidizing element, found in air, water and soil. It occurs as a free anion, hydrofluoric acid and different fluoride complexes (Edmunds and Smedley, 1996). Fluoride in drinking water can act as double edged sword (Biswas et al., 2016). While fluoride concentration less than 0.5 mg/L in drinking water has a negative impact on human beings (Fawell et al., 2006), chronic intake of excessive fluoride (> 2.0 mg/L) has been linked to skeletal and dental fluorosis. Fluoride does not affect the growth of aquatic organism at low concentration. The $EC_{50}$ [half maximal effective concentration] for inhibition of bacterial nitrification is 1218 mg/L of fluoride concentration (Fawell et al., 2006). $LC_{50}$ [lethal concentration required to kill 50% of population] (48 h) for aquatic invertebrates ranges from 53 to 304 mg/L (Camargo, 2003). $LC_{50}$ (96 h) for freshwater fish varies from 51 to 460 mg/L (Camargo, 2003). The $EC_{50}$ (96 h) based on growth for freshwater and marine algae are 123 and 81 mg/L, respectively. For the plants, the ability of accumulation and tolerance are varied from species to species (Fawell et al., 2006). Leaves become damaged (7–50%) at higher concentration of fluoride (> 130 mg/L) (Fawell et al., 2006). The major source of fluoride exposure in human beings is through drinking water (Fawell et al., 2006). For individual person, water consumption increases with temperature, humidity and health state (Murray, 1986). In USA, young children consume 1.2 mg/L fluoridated water, which is estimated approximately 0.5 mg $F^-$/day (USEPA, 1996). For those, who drink 1 L per day of same concentration of fluoride, may consume up to 1.2 mg $F^-$/day (USNRC, 1993). Similarly, the standard values are varied based on climatic condition. According to Bureau of Indian Standards (BIS), the desired range of fluoride for drinking water is 0.6–1.2 mg/L, while discharge limit for industrial wastewater is 2 mg/L (BIS, 2012). The fluoride level in uncontaminated fresh surface water is below 1.0 mg/L, although the addition of industrial effluent can increase the concentrations of fluoride to toxic level (Fawell et al., 2006).

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Hydrofluoric acid [HF] is used as reactant in several industries such as phosphatic fertilizer industry, coal washing, semiconductor or metals manufacturing unit, etc. The rinse water of such industries contains fluoride beyond permissible limit (100 to 10,000 mg/L) leading to toxic effect on the ecosystem (USNRC, 1993). Sodium fluoride [NaF] is used in the different industries such as in the re-smelting of aluminum, pickling of stainless steel, manufacturing of coated papers including insecticides and wood preservatives (Fawell et al., 2006). Cryolite [$Na_3AlF_6$] is used for the production of aluminium and pestiside (USEPA, 1996). Rock phosphate [$3Ca_3(PO_4)_2{\cdot}CaF_2$] is used for phosphate fertilizer industry (USEPA, 1996). Random disposal of industrial wastewater into the environment affects both soil and groundwater quality (Das and Das, 2003; Huang and Liu, 1999). The proper treatment of wastewater has become one of the major environmental challenges, faced by industrial world today (Haikel, 1986; Kurosaki, 1998).

Different conventional defluoridation techniques, like coagulation-precipitation (Kurosaki, 1998; Huang and Liu, 1999), electro-dialysis (Lahnid et al., 2008), nano-filtration (Liu et al., 2007), ion-exchange (Meenakshi and Viswanathan, 2007), adsorption (Singh et al., 2016), etc., are being used already. However, these methods for wastewater treatment have some drawbacks like high cost, energy consumption, post-treatment secondary pollutants and inefficiency of removing all pollutants present in wastewater (Gentili and Fick, 2016). The advantages of biological treatment are operational simplicity and less sludge production. Micro-organisms and plants have the capability of developing resistance to different pollutants through bioaccumulation, biotransformation and biosorption (Chouhan et al., 2012). The little usage of bacterial sludge produced during biological removal of pollutants is one of the major drawbacks of such process. On the other hand, one of the major limitations of phytoremediation is the long-term dedication, as the process is dependent on a plant's ability to grow under non-ideal conditions (Wang et al., 2002). Hence, phycoremediation can be one of the most promising platform for removal of pollutants from wastewater (Cho et al., 2011; Hu et al., 2000; Kushwaha et al., 2014; Sindelar et al., 2015).

Algae can grow in domestic, municipal and industrial wastewater rapidly using the pollutants as their nutrient and thus, mitigation of pollutants occurs automatically in an eco-friendly manner (Mahapatra et al., 2014). The algal biomass is rich in hydrocarbons, polysaccharides and lipid and thus, can be utilized as the feed-stock for the production of different bio-fuels, including bio-diesel, bio-ethanol, etc (Oswald and Gotaas, 1957; Ramachandra et al., 2013). The biofuels, on the other hand, help to reduce the consumption of fossil fuels (Bhatnagar et al., 2010; Craggs et al., 2012; Dean et al., 2010; Gentili and Fick, 2016). While mostly physico-chemical and bacteriological methods (Chouhan et al., 2012) have been used to remove fluoride from wastewater, very few works on Bioremediation of fluoride using microalgae have been reported in literature (Ali, 2004; Bhatnagar and Bhatnagar, 2000, 2004; Pitre et al., 2014). However, a complete study to show the variation of chlorophyll, carbohydrate, protein and lipid content during defluoridation is limited. Thus, the assessment of capacity of fluoride removal and generation of macromolecules of microalgal species is the thrust of the present study (Gupta, 2005).

In the present work, *Starria zimbabweensis*, a living cyanobacterial strain (blue-green algae) isolated from industrial effluent treatment plant, has been used to treat fluoride laden simulated and real wastewater. The production of biofuel precursor from the resultant biomass has been assessed.

## 2. Materials and methods

All chemicals unless otherwise stated were obtained from Merck (India). To avoid undesired reaction between fluoride and glass material, PVC flasks (250 mL) were used in all the experiments. Volume of solution was maintained as 100 mL.

### 2.1. Collection, isolation and culturing conditions

Initially, the algal bloom was collected from wastewater of coke-oven effluent treatment plant (Latitude: 23°50′92″N, Longitude: 87°32′10″E), Durgapur, India. The postulation for selection of the collection site of the test-strain is that the coke-oven wastewater contains different anionic pollutants namely cyanide, sulphate, nitrate, sulphide, etc. The algal strain that grows in such wastewater may have resistance to such anionic pollutants and have bio-remedial capacity. Initially, the algal biomass was grown in diluted wastewater and then transferred to BG11 medium at 25 ± 2 °C, 45 ± 2 μmol/m$^2$/s irradiation in 18 h: 6 h of light:dark cycle in an algal incubator (as the control condition). Light irradiation and photoperiod in the present study was chosen based on some published literatures (Grinten et al., 2005; Sen et al., 2017). A consortium of cyanobacterium and diatom were used to grow under a wide range of temperatures (7–25 °C) and irradiation of light (5–200 μmol/m$^2$/s) and better growth conditions were: Temperature = 25 °C and light irradiation = 40 μmol/m$^2$/s under light:dark photo period of 16 h: 8 h (Grinten et al., 2005). Isolation was carried out on BG11 agar plates, by streaking method supplemented with higher concentrations of fluoride (Andersen and Kawachi, 2005). Two antibiotics (Penicilin and Streptomycin) and one fungicide (Amphotericin) were added at the time of plate-solidification in the bio-safety cabinet. The dose of antibiotics and fungicide were followed according to the standard protocol to obtain pure isolate (Andersen and Kawachi, 2005). The appeared colonies on BG11 agar plates were transferred to fresh autoclaved BG11 media for further growth. The following process was continued until pure algal strain was obtained. The isolated test strain was sent to Yaazh Xenomics, Tamil Nadu, India, for molecular identification.

The composition of BG11 used was as follows (g/L of deionized water): 1.5 $NaNO_3$, 0.04 $K_2HPO_4$, 0.075 $MgSO_4{\cdot}7H_2O$, 0.036 $CaCl_2{\cdot}2H_2O$, 0.006 Citric acid, 0.006 Ferric ammonium citrate, 0.001 EDTA, 0.02 $Na_2CO_3$ and 1 mL of trace metal solution (2.86$H_3BO_3$, 1.81 $MnCl_2{\cdot}4H_2O$, 0.222 $ZnSO_4{\cdot}7H_2O$, 0.39 $NaMoO_4{\cdot}2H_2O$, 0.079 $CuSO_4{\cdot}5H_2O$, 49.4 $Co(NO_3)_2{\cdot}6H_2O$). pH of BG11 was adjusted to 7.2 using 0.4(N) HCl and 0.1(N) NaOH solution. The test strain was grown in 100 mL of autoclaved BG11 medium in 250 mL flasks for 18 days and sample was collected after 2 days of interval. Biomass was harvested by centrifugation (eltek, TC 8100 F, India) at 5000 rpm for 15 min. The pallet was washed twice and dried at 60 °C (hot air oven) for 12 h. The lipid content of sample strain was determined using standard Bligh-Dyer method (Bligh and Dyer, 1959).

### 2.2. FESEM-EDX and FTIR analysis of collected strain

Field Emission Scanning Electron Microscopic (FESEM) images of living algal strain, with and without fluoride, were taken using FESEM (HITACHI–S-3000N, Japan) to represent their surface morphology at preferred magnification. Sample was taken on cover-slip, air dried and coated with gold-palladium coating for 30 min. Then coated samples were analyzed at working distance of 8.2 mm. Energy Dispersive X-ray study (EDX) was performed to get elemental information of the samples using the same instrument. FTIR (Fourier transform infrared spectroscopy) analyses of both untreated and treated algal mass were done using FTIR Spectrometer (Perkin-Elmer 2000, Malaysia) to characterize the functional groups responsible for fluoride binding. FTIR study was also done with the supernatant obtained after sonication. Both control and fluoride loaded living algal biomass were disrupted separately using ultra-sonicator (MISONIX XL-125, India) at a resonance of 10 kHz for 5 min and the suspension was centrifuged to separate the supernatant for analysis. The range of spectra was over 4000–800 cm$^{-1}$ and data was analyzed using Origin 8 Pro. The spectrogram represented the external and internal compositions of algal biomass.

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