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# Removal of Fluoride from Synthetic Wastewater Using Carbonised Saw Dust and Suspended and Immobilised Culture of *Pseudomonas oleovorans* Strain NITD 20 – A Comparative Study

**Bhaskar Bishayee, Abhilasha Rai, Biswajit Ruj[1] and Susmita Dutta***
Department of Chemical Engineering, National Institute of Technology Durgapur, Durgapur –713209, India
[1]Environmental Engineering Group, CSIR-Central Mechanical Engineering Research Institute, Durgapur – 713209, India
✉ susmita.dutta@che.nitdgp.ac.in

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**Abstract:** In the present research, three agents such as saw dust (carbonised), waste of the timber industry, suspended bacterial cells of *Pseudomonas oleovorans* strain NITD 20 and immobilised whole live bacterial cells were used individually for fluoride removal from synthetic wastewater. Carbonised saw dust was used first as an adsorbent at optimum conditions such as pH 7±0.2, initial fluoride concentration of 15 $mg \cdot L^{-1}$, particle size of 0.10 mm, adsorbent dose of 60 $g \cdot L^{-1}$, contact time of 10 h and stirring speed of 120 rpm, and it showed 79.04±0.196% fluoride removal. In the present study, both suspended and immobilised cells of '*Pseudomonas oleovorans* strain NITD 20' were used for the defluoridation process. Immobilisation was done onto the carbonised saw dust. The maximum removal was observed for suspended cells at 94.5±2.1% and immobilised cells at 98±1.23% in 10 h and 8 h incubation periods, respectively, from 15 $mg \cdot L^{-1}$ fluoride containing synthetic wastewater.

**Key words**: adsorption, bacterial cells, carbonised saw dust, fluoride, immobilisation.

## Introduction

Fluoride is a naturally occurring compound obtained from fluorine, the lightest member of the halogen group and the 13$^{th}$ most plentiful element present on the earth. Several industries such as fertiliser, metallurgical, electronics, semiconductor, ceramics and glass industries produce fluoride containing wastewater (Damtie et al., 2019). The improper disposal of such wastewater into the environment leads to fluoride contamination in groundwater. The permissible level of fluoride in drinking water is 1.5 $mg \cdot L^{-1}$, according to World Health Organization (BIS, 2012).

Several fluoride removal techniques such as adsorption, coagulation-flocculation, and membrane process were applied for the defluorination process (Solanki et al., 2022). Among these techniques, adsorption is found to be very suitable due to its low-cost and easy-to-handle characteristics. The naturally available low-cost adsorbents, as well as waste materials, were applied to investigate their efficiency in the defluoridation process (Bishayee et al., 2020). It can be stated that naturally occurring materials and waste materials have huge prospects to be used in defluoridation process owing to their low-cost or no-

*Corresponding Author

cost nature, ease of availability and moderately high effectiveness as adsorbents for fluoride removal.

The research on biological treatment methods employing living microbial cells is progressing as a developing field for the removal of fluoride from wastewater (Kebede et al., 2016; Maity et al., 2018). In such a process, microorganisms may be used either as suspended culture or in immobilised form. Immobilisation of bacterial cells can improve the efficiency of the fluoride removal process and also have some advantages like (i) bacterial cells, immobilised on the porous adsorbent, enhances fluoride removal efficiency owing to the combined action of adsorption and active bioremediation by live cells and (ii) the methods are low-cost and eco-friendly in nature.

Shanker et al. (2020) observed bioremediation of fluoride using the novel bacterium *Acinetobacter* sp. (GU566361), isolated from potable water, and it showed 57.3% removal efficiency of fluoride after 10 h of incubation. Therefore, the bacterial strain capable of higher removal of fluoride in a short span of time is being looked upon by the present researchers.

Saw dust is a waste material available mainly from wood/timber industries. The presence of silica, alumina, iron and other metal oxide components makes it a good and effective adsorbent. The investigations on fluoride removal using native saw dust were carried out by a few researchers (Mann and Mandal 2014; Singh et al., 2019); however, in the present article attempt was made to enhance the fluoride removal of saw dust by two distinct methods such as (i) activation of saw dust by carbonising at a higher temperature and (ii) immobilisation of live whole bacterial cells onto the carbonised saw dust. In point of fact, three agents have been used in the present study to remove fluoride from wastewater. They are (i) carbonised saw dust, (ii) suspended bacterial cells of *Pseudomonas oleovorans* strain NITD 20, and finally (iii) the same live bacterial strain, immobilised onto carbonised saw dust. Thus, a hybrid technique of simultaneous adsorption and bio-removal via the use of immobilised cells has been implemented with an aim to enhance the fluoride removal efficiency from synthetic wastewater and here lies the novelty of the study. A comparative study was performed to assess the fluoride removal efficacy of these three agents under similar conditions.

## Materials and Methods

All the experiments were performed in three sets, and results were represented with Mean±SD values.

### Collection, Culturing and Growth of Bacterial Cells

The bacterial cells *Pseudomonas oleovorans* strain NITD 20 was collected from the present laboratory of the Chemical Engineering Department, National Institute of Technology Durgapur, West Bengal. The stock solution of fluoride (1000 mL of 100 $mg \cdot L^{-1}$) was prepared by dissolving 0.221 g of NaF (Merck, India) in distilled water. The sample was grown on fluoride (2-50 mg/L) containing Luria–Bertani Agar (LBA) medium (HiMedia, India) plates. The plates were placed in a BOD incubator for 14 h. A growth study of the bacterial sample was performed according to Rai et al. (2020).

### Preparation of Adsorbent

Saw dust was collected from the local timber industry at Benachity, Durgapur, Paschim Bardhhaman, West Bengal, India (Latitude 23° 52′ 04′′ N and Longitude 87° 31′ 19′′ E). At first, it was washed several times with distilled water followed by drying in a hot air oven at 80°C for 24 h. The dried saw dust was ground and sieved into different particle sizes (0.1- 0.624 mm). Thereafter, it was carbonised by carbonising in a muffle furnace at 300°C for 1.5 h in a porcelain disk. It was then cooled for 0.5 h in a desiccator.

### Characterisation of Carbonised Saw Dust

Different characterization methods were carried out to study the physico-chemical nature of the native and carbonised saw dust. The Scanning Electron Microscopy (SEM) (Model: SIGMA HD, ZEISS) study was performed for morphological analysis of the adsorbent, while Energy Dispersive X-Ray Spectroscopy (EDS) (Model: Oxford X-MAX 50) analysis was done to get the elemental composition of the native and carbonised saw dust. The surface area, pore volume and pore radius of the adsorbent were measured by Brunauer-Emmett-Teller (BET) analyser (Model: Quanta-chrome Nova-Win, version 11.03).

### Parametric Study to Examine the Effect of Operating Variables on the Removal of Fluoride Using Carbonised Saw Dust in the Batch Contactor

To see the effect of different operating variables on fluoride removal, contact time (0.5-10 h), particle sizes (0.1-0.620 mm), initial fluoride concentration (2-15 $mgL^{-1}$) and adsorbent dose (10-70 $gL^{-1}$) were varied individually during adsorption using carbonised saw dust in the batch study. During the parametric study, when one variable was changed, others were kept constant at some specified values following the protocol of One Factor at a Time (OFAT) analysis (Rai et al., 2020). The pH was kept constant at 7 for making the process more economical. The stirring speed and temperature were also kept constant at 120 rpm and 37±1°C. All experiments were performed in the conical flasks (500 mL), where a specific amount of adsorbent was added to 200 mL of a fluoride solution. After the specific time intervals, the samples were filtered using Whatman filter paper (grade 42). Finally, the filtrate was measured for residual fluoride concentration using Thermo Scientific Orion Ion meter (Model no: Orion Star A214 pH/ ISE meter). Fluoride removal (%) using carbonised saw dust was measured using the following equation:

$$\%\ \text{removal} = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

where $C_i$ is initial concentration in mg·L$^{-1}$; $C_f$ is the final concentration in mg·L$^{-1}$.

### Immobilisation of Live Bacterial Cells onto Carbonised Saw Dust

The optimised dose of carbonised saw dust, as analysed during the parametric study on fluoride removal using carbonised saw dust, was autoclaved first. Fresh grown bacterial culture (10% v/v) and 200 mL sterile LB medium were poured into the flask containing sterile carbonised saw dust. Flasks were kept in an incubator for 5 days at specified conditions like pH: 7, temperature: 37±1°C for immobilisation with gentle shaking speed (50 rpm).

### Physico-Chemical Characterisation of Immobilised Bacterial Cells

SEM study was performed to confirm the immobilisation process. A part of the immobilised sample, which was incubated for 5 days for immobilisation, was used for SEM analysis. A proper air-dried sample was fixed with gold for getting good quality surface images.

Extra Polysaccharide (EPS) secretion analysis was also done for the confirmation of the attachment of the bacterial cells onto carbonised saw dust adsorbent (biofilm formation). The EPS estimation method as prescribed by Hazaimeh et al. (2014) was followed. For the EPS evaluation, one reference sample (without bacterial inoculation) was kept along with the bacterial sample.

### Abatement of Fluoride Using Carbonised Saw Dust, Suspended Bacterial Cells, and Immobilised Bacterial Cells From Synthetic Wastewater

The studies to compare the fluoride removal efficacies of three agents such as carbonised saw dust, suspended bacterial cells, and whole bacterial cells immobilised onto the carbonised saw dust, from synthetic fluoride-laden wastewater were performed individually under the similar operating condition as analysed from the parametric study. The values of operating variables were as follows: initial concentration of fluoride: 15 mg·L$^{-1}$, pH 7, temperature: 37±1°C and contact time 10 h. The concentrations of carbonised saw dust, well grown suspended bacterial cells (12-14 h old culture), and immobilised bacterial cells (5 days old) were kept as 60 g·L$^{-1}$, 2% (v/v) and 60 g·L$^{-1}$ (immobilised materials), respectively. While for the first agent, an aqueous solution of fluoride with a definite concentration was used, for the other two bioagents, LB medium supplemented with a stipulated concentration of fluoride was used. Samples were taken from all the setups after every two-hour interval. The samples (carbonised saw dust and immobilised cells) were filtered separately using Whatman paper (grade 42) in the bio safety cabinet. The filtrates from these two setups and samples from the suspended culture were centrifuged at 10000 rpm for 3 min. Finally, the supernatant was analysed for the residual fluoride concentration.

## Results and Discussions

### Growth Study of *Pseudomonas oleovorans* Strain NITD 20

The growth study of bacterial samples is shown in Figure 1. The bacterial growth was observed to have the

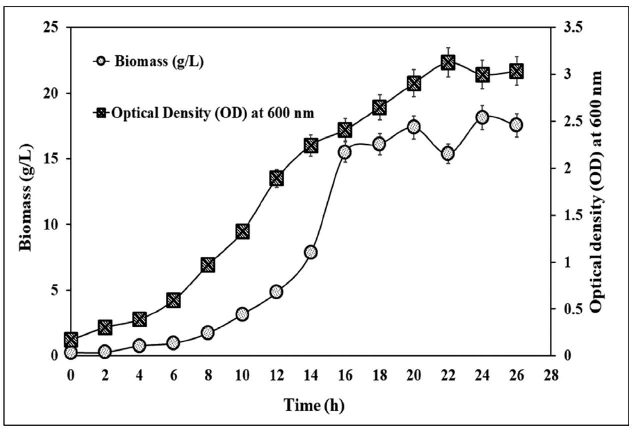


**Figure 1: Growth study of *Pseudomonas oleovorans* strain NITD 20 in terms of biomass (g/L) and optical density (OD) at 600 nm.**

following phases: preparatory phase (0-2 h); exponential phase (2-22 h) and stationary phase (22-26 h) (Rai et al., 2020).

## Characterisation of Carbonised Saw Dust

In order to study themorphology of carbonised saw dust, SEM analysis was done. SEM images of native and carbonised saw dust is shown in Figure 2a-b, respectively. The surface structure of this adsorbent was very much rough and uneven with small pores. Carbonised saw dust was found more porous because of surface modification owing to heat treatment. Removal efficiency increases when there is a larger number of adhering sites present on the surface of the adsorbent (Li et al., 2016).

EDS analysis is the other characterisation technique to study the elemental composition of adsorbents. During EDS analysis, a line of spectra (peak) was obtained, which represent the energy (in terms of photons) of each element. The elemental compositions of native and carbonised saw dust are presented in Table 1. Both the saw dust samples consist of silica, alumina, iron oxide, calcium oxide, magnesium oxide, sulphur trioxide, phosphorous and other metal oxides. The presence of alumina, iron oxide and silica compounds enhances the adsorption capability of the adsorbent (Chakraborty et al., 2017).

From the BET analysis, the surface area, pore volume, and pore radius of carbonised saw dust adsorbent was found to be 12.46 $m^2/g$, 0.056 cc/g and 13.84 Å, respectively. Yadav et al. (2013) observed BET surface area, pore volume and average pore diameter for wood saw dust as 11.384 $m^2/g$, 0.789 cc/g and 1.787 nm.

### Parametric Study to Examine the Effect of Operating Variables on the Removal of Fluoride Using Carbonised Saw Dust in the Batch Contactor

*Effect of Contact Time*

The contact time (0.5-10 h) was varied to see its effect on the fluoride removal efficiency of carbonised saw dust in the batch study (Figure 3a). The particle size, dose of adsorbent, initial fluoride concentration, temperature, pH and stirring speed were kept constant at 0.1 mm, 70 $g \cdot L^{-1}$, 15 $mg \cdot L^{-1}$, 37±1°C, 7, and 120 rpm, respectively. Maximum removal (76.46±4.12%) was achieved at a contact time of 10 h. Bishayee et al. (2020) observed the maximum removal at 10 h while carrying out fluoride removal from groundwater using earthen tea pots.

*Effect of Particle Size*

The effect of particle size (0.1-0.624 mm) on fluoride removal efficiency of carbonised saw dust was examined

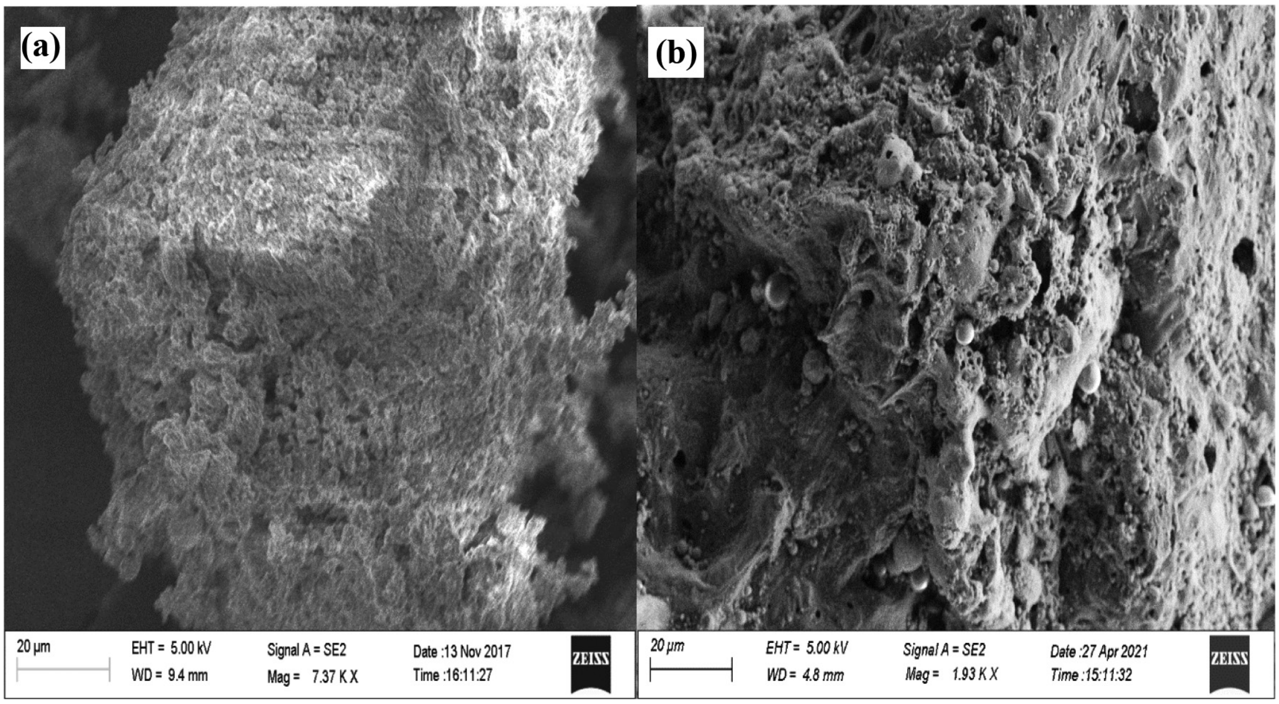


**Figure 2: SEM images of (a) native saw dust and (b) carbonised saw dust.**

next (Figure 3b). The dose of adsorbent, initial fluoride concentration, contact time, temperature, pH and stirring speed was kept constant at 70 g·L$^{-1}$, 15 mg·L$^{-1}$, 10 h, 37±1°C, 7, and 120 rpm, respectively. The removal of fluoride decreased from 76.46±4.12% to 72.5±0.62% with the increase in particle size from 0.1 mm to 0.624 mm. At low particle sizes, the surface area of an adsorbent is more. Hence, removal efficiency increases with decreasing particle sizes (Kebede et al., 2016). The maximum removal efficiency of 76.46±4.12% was obtained at a particle size of 0.10 mm. Mann and Mandal (2014) reported the maximum fluoride removal at a particle size of 90 µm while carrying out fluoride removal from drinking water using saw dust.

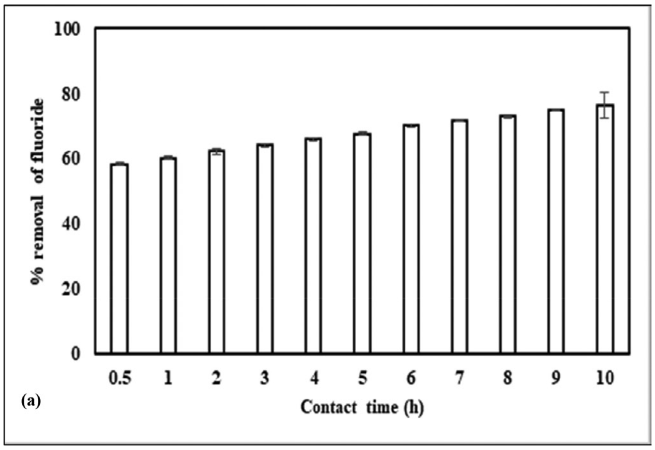


**Figure 3a: Effect of different contact time on fluoride removal using carbonised saw dust (at particle size = 0.1 mm, dose = 70 g·L$^{-1}$, initial concentration = 15 mg·L$^{-1}$, temperature = 37°C, pH = 7, and starring speed = 120 rpm).**

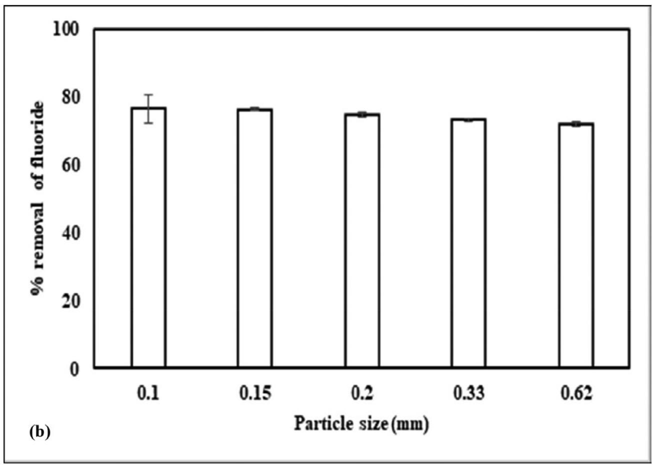


**Figure 3b: Effect of different particle sizes on fluoride removal using carbonised saw dust (at dose = 70 g·L$^{-1}$, initial concentration = 15 mg·L$^{-1}$, contact time = 10 h, temperature 37°C, pH = 7, and starring speed = 120 rpm).**

**Effect of Concentration**

The effect of initial concentration (2-15 mg·L$^{-1}$) on fluoride removal efficiency was investigated using carbonised saw dust (Figure 3c). The particle size, dose of adsorbent, contact time, temperature, pH and stirring speed were kept constant at 0.1 mm, 70 g·L$^{-1}$, 10 h, 37±1°C, 7, and 120 rpm, respectively. The Fluoride removal increased from 58.6±0.74% to 76.46±4.12% with the increase in initial fluoride concentration from 2 mg·L$^{-1}$ to 15 mg·L$^{-1}$. Since adsorption is a mass transfer operation, it depends on driving force, measured as the difference in concentration at bulk and that on the surface of the adsorbent. The higher the driving force, the higher will be the adsorption if the process is mass transfer driven. Maximum removal (76.46±4.12%) was achieved at an initial concentration of 15 mg·L$^{-1}$. Singh et al. (2019) observed maximum removal at an initial concentration of 5 mg·L$^{-1}$ while investigating the efficacy of saw dust in the adsorptive removal of fluoride.

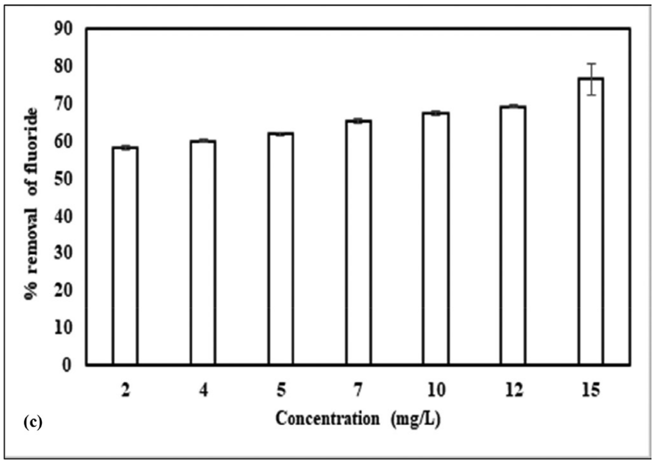


**Figure 3c: Effect of different concentrations on fluoride removal using carbonised saw dust (at particle size = 0.1 mm, dose = 70 g·L$^{-1}$, contact time = 10 h, temperature = 37°C, pH = 7, and starring speed = 120 rpm).**

*Effect of Adsorbent Dose*

The effect of adsorbent dose (10-70 g·L$^{-1}$) on fluoride removal efficiency of carbonised saw dust was studied finally (Figure 3d). The particle size, initial fluoride concentration, contact time, temperature, pH and stirring speed were kept constant at 0.1 mm, 15 mg·L$^{-1}$, 10 h, 37±1°C, 7, and 120 rpm, respectively. It is seen that fluoride removal increased from 69.3±0.85% to 79.04±0.196% with an increase in adsorbent doses from 10 g·L$^{-1}$ to 60 g·L$^{-1}$. This happens mainly because of the availability of more active sites at a higher adsorbent dose (Bishayee et al., 2020). Further increase in dose

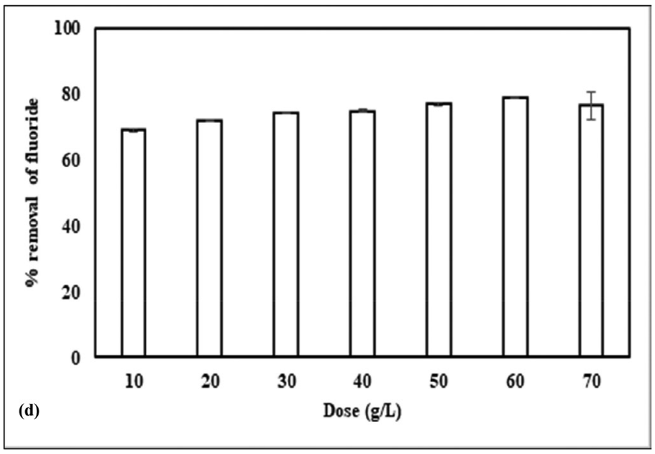


**Figure 3d: Effect of different adsorbent doses on fluoride removal using carbonised saw dust (at particle size = 0.1 mm, initial concentration = 15 mg·L$^{-1}$, contact time = 10 h, temperature = 25°C, pH = 7, and starring speed = 120 rpm).**

from 60 g·L$^{-1}$to 70 g·L$^{-1}$, a decrease in removal from 79.04±0.196% to 76.46±4.12% was observed. This may be due to the crowding of a large amount of adsorbent in a specific volume of solution and thus, resulting in the decrease of the available active site for fluoride adsorption (Nasrullah et al., 2015). Therefore, 60 g·L$^{-1}$ was chosen as a suitable dose for further studies. Kofa et al. (2017) also observed a similar value for the removal of fluoride using fired clay pots.

## Physico-Chemical Characterisation of Immobilised Bacterial Cells

The morphology of immobilised bacterial cells is shown in Figure 4. From the SEM image, it is clear that rod shaped bacteria are attached to the surface of carbonised saw dust. Biofilm formation is related to the production of Extra Polysaccharide (EPS) (Hazaimeh et al., 2014).

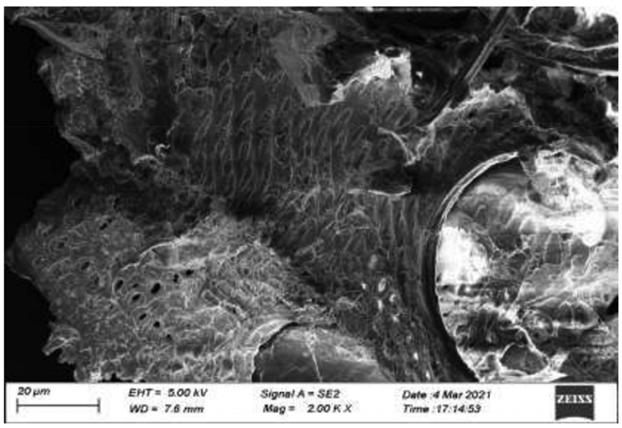


**Figure 4: SEM image for bacterial immobilised cells onto carbonised saw dust.**

Therefore, an EPS production study was also performed to get the idea of adhesive material secretion for binding the bacterial cells onto saw dust. After 5 days of immobilisation of bacterial cells, EPS production was observed as 57±1%. A similar value of EPS (56.7 %) was reported by Hazaimeh et al. (2014) during the immobilisation of bacterial cells onto the carrier.

## Abatement of Fluoride Using Carbonised Saw Dust, Suspended Bacterial Cells, and Immobilised Bacterial Cells From Synthetic Wastewater

To ascertain the best agent, a comparative study was performed by contacting each of the agents with fluoride solution under similar conditions for 10 h (Figure 5). Figure 5 shows that suspended bacterial cells and immobilised bacterial cells were more promising agents as compared to carbonised saw dust for meeting the permissible limit (1.5 mg·L$^{-1}$) of fluoride within 10 h. However, immobilised bacterial cells were found to be most efficient to meet the permissible limit within 8 h while suspended cells achieved the permissible limit in 10 h. Upon immobilisation of carbonised saw dust, the bioagent has access to a larger surface area to remove the contaminants as compared to suspended cells (Sharma et al., 2019). The maximum removal of fluoride was observed as 79.04±0.196%, 94.5±2.1% and 98±1.23% for carbonised saw dust, suspended bacterial cells, and immobilised bacterial cells, respectively (Figure 5). The enhanced removal of fluoride with immobilised cells might be due to the combined effect of adsorption by carbonised saw dust and bioremoval by its surface bound live bacterial cells. Mohammad and Kumar (2019) also used the immobilised *Actinobacter* on the surface of sweet lemon peel (dose 14 g·L$^{-1}$)

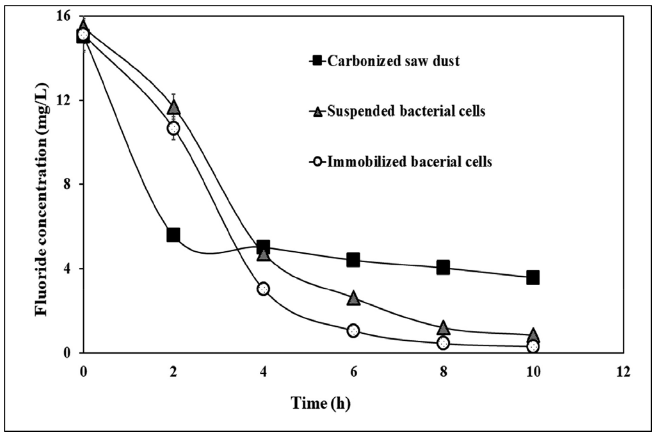


**Figure 5: Variation of fluoride concentration with time using carbonised saw dust, suspended bacterial cells, and immobilised bacterial cells onto carbonised saw dust.**

for the removal of fluoride from the initial fluoride concentration of 20 $mg \cdot L^{-1}$ and obtained 94.47 % removal in 87 h.

## Conclusion

In the present study, a comparative study was performed to ascertain the best removing agent between carbonised saw dust and suspended and immobilised culture of *Pseudomonas oleovorans* strain NITD 20 in the removal of fluoride from synthetic wastewater. EDS analysis showed the presence of more amount of $SiO_2$ and $Al_2O_3$ in carbonised saw dust than its native form which is an advantageous point when the adsorption of fluoride is concerned. All the said agents were contacted with fluoride solution in batch contactor under the most suitable condition as analyzed from a parametric study of fluoride removal using carbonised saw dust as a sole agent. The highest removal of fluoride (98±1.23%) in less time (8 h) in the case of immobilised bacterial samples may be due to the combined action of adsorption onto the porous matrix of carbonised saw dust and effective bioremoval of live bacterial cells. Therefore, it can be stated that the employment of immobilised bacterial cells in the removal of fluoride is not only eco-friendly but easy to use as well. The application of such a system in the treatment of real wastewater in continuous mode is seen as the scope of future work.

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