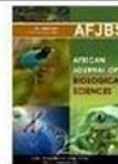


<https://doi.org/10.48047/AFJBS.6.7.2024.1907-1923>



African Journal of Biological Sciences

Journal homepage: <http://www.afjbs.com>



Research Paper

Open Access

Experimental Validation Of Nanotechnology-Enhanced Bio-Filters For Sustainable Wastewater Treatment

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Volume 6, Issue 7, 2024

Received: 29 Mar 2024

Accepted : 22 May 2024

doi: 10.48047/AF5BS.6.7. 2024.

1907-1923

Abstract

In the current scenario, the contamination of aquatic bodies is global issue due to the presence of wide range of toxic contaminants such as; pathogenic microbes, pharmaceuticals, organic contaminants, pesticides and heavy metals. If not treated properly, these contaminants impose a deleterious impact on human health and the environment. Nano-biochar synthesis from waste agricultural biomass having activated nanoparticles may be employed to entrap and adsorb the contaminants such as heavy metals and remove the pathogenic microbes. Therefore, in this study, the synthesis of nano-char from pineapple peels and subsequent integration with charcoal and chicken feather as layer-by-layer deposition system at different ratios. The contaminated water sample filtered was treated through the fabricated filter and treated water collected at different intervals to determine their purification efficiency such as TDS, Conductivity, pH, bacterial count and heavy metal concentration. Results revealed that the nano-composite biofilter consisting of agro-waste, charcoal and chicken feather (4%) possessed high adsorption capacity of heavy metals, prolonged anti-microbial activity. Additionally, the nano-composite biofilter was capable of significantly reducing the TDS, pH and conductivity upto 27.3mg/l, 7.11 and 53 μ S/m respectively in the treated water. Thus, the nano-composite bio-filter may be employed as cost-effective materials for the treatment of waste water at small scale to large scale water treatment plants with simultaneous recycling of the agro-waste to reduce the environmental pollution.

Keywords: Heavy Metal Filter; Pollutants; Charcoal; Nanofillers.

INTRODUCTION

Environmental pollution is one of the major issues needed to be resolved to overcome the ecological crisis and major human health issues [1]. Water is the key source for the life on this planet but the scarcity of water and demand of pure/unpolluted water is increasing day by day [2]. Industrialization results in shortage of land, deforestation, global warming and the major issue is water pollution and the effluent released from the industries are the major source of toxins, xenobiotic, heavy metals, endocrine disrupting molecules and carcinogens in aquatic systems [3,4]. Heavy metals due to their higher density and higher concentration causes hazardous and toxic effects on the living beings, therefore the purification of contaminated water bodies to entrap these toxicants is necessary [5]. The lethal concentration of heavy metals in drinking and ground water causes cellular toxicity, mutation, organ failure, immunosuppression, cancer in animals and retarded growth with reduced metabolic activity in plants [6,7].

There are wide range of water treatment filter system designed for the entrapment and removal of toxic pollutants from contaminated water samples. At commercial level the adequate level of the bio-filter treatment are very costly especially for bulk treatment of industrial effluents and release minor amount of toxic by-products in the treated water. Thus, a lot of research have been going on to develop sustainable low cost materials for filters which are easily operative, requires less maintenance for long term effectiveness [8]. The water filter system was designed to remove heavy metals from the water using bioadsorbent as the potential agent to overcome the above issues. As the scientific community has increasingly recognized natural polymers or their composites as avoiding synthetic adsorbent disadvantages and being the consumer-friendly materials available at low costs, natural polymers are being given more attention in recent years. [9].

In the recent studies the innovative biomaterials at low cost developed from eco-friendly and natural biomass and nowadays used as most promising sources for pollution control applications. Thus, a variety of natural materials are used in waste water treatment facilities to remove pollutants, including ceramics, sands, residues from bauxite refining, biomass char, activated carbons, water hyacinths, etc [10]. One of these successful attributes has been identified as the 'Simanto-gawa system' in Japan, which uses plastic filters, nitrolite, organic matter with high carbon-organic ratio, charcoal-bio, charcoal, and processed limestone as treatment mediums [11,12]. According to a previous study, rocks such as andesite, granite, marble, refuse concrete and refuse cement are extremely effective in treating waste [13]. In spite of this, we should continue to promote small-scale integrated treatment using cheaper treatment mediums.

Several practices and tools are utilized for small to large scale water purification system and remediation of the waste water. However, in market different types of adsorbents are available which uses polymers as a prime material for the adsorbing of heavy metals [14]. The commonly employed polymers include activated carbon, silica gel, activated alumina and ion exchange resins but their efficiency is low and are available at high cost [15]. In the recent studies adsorbents have been used for the heavy metals removal but the synthetic matrix vhas harmful effect on the environment and also the sorption capacity of synthetic adsorbents are lower as compared to bioadsorbents [16].

In the biosorption process several biological products or biomass knock down the concentration of pollutants from the wastewater and reduces the concentration of heavy metals in the wastewater through metabolically mediated or physicochemical pathways of uptake [17]. In last few years, the microbial and plants resources are used potential bioadsorbent for faster heavy metals removal capacity from wastewater, cheaper production of biomass, can extract different heavy metals at a single time and can remove large volume of water pollutants [18,19]. Hence reduces water toxicity more effectively as compared to conventional sewage treatment plants and commercialized treatments.

The plant materials isolated from the agro-waste such as coconut husk, peels, baggas, algal biomass may be used as bioadsorbents [20]. Pineapple peels are rich source of natural polymers used as effective bioadsorbent for the removal of heavy metals, dye Safrinin-O from the wastewater [21]. In another study, the chemical oxidization of pineapple peels effectively

removed Pb (II) and Cd (II) from waste water [22]. Beside that chicken feather (CF) are also used for the effective removal of the toxic dye, Blue FCF from wastewater and oil from the waste water [23]. Feathers can be used for forming composite biofilter as it is effective in extracting hydrophobic contaminants from wastewater. In our research we are focusing on agricultural waste as the main source for making nano-bioadsorbent as sustainable, eco-friendly, low cost and easily available material. Charcoal is used because it is a good cleaning agent and is effective in grabbing pollutants from the wastewater [24,25].

In the nanotechnology approaches the size reduction of the filter particles or biochar improve the pollutant entrapment efficiency due to higher surface to volume ratio for better adsorption and bioactivity [26,27]. Therefore, in the recent studies nanobiochar and nanofibers (NF) are studied as potential material for the waste water treatment and pollutants removal from soil and contaminated water bodies [28]. The nano-biocomposite of Pineapple peel nano-fiber with keratin protein rich CF and charcoal at different concentration will be fabricated and evaluated as nanochar-fibrous bio-filter for water purification. The synergistic effect of all the constituents may result in a better sorption of the organic and inorganic pollutants, reduces the toxicity and decontaminate water as mentioned in the literature [29]. The selection of the materials is done on the basis of their effectiveness and efficiency so that the efficient adsorbent material designed for low cost biofilter development. In the present study, we focused to evaluate the synergistic effect of the nanomaterials fabricated from different natural biomass and investigated their efficiency for the treatment of waste water. The fabricated nanofibrous composite will be utilized for treatment of municipal and industrial waste water and applicability of the treated water for domestic and agricultural usage. This composite nanochar-fibrous bioadsorbent is highly effective in removing the large volume of heavy metals in less duration without harming the environment and in more economical manner in comparison to other treatments.

MATERIALS AND METHODS

Materials

To prepare cellulosic NFs, pineapple peel and charcoal were collected from kitchen waste, chicken feather from poultry waste and rinsed them distilled water to remove dirt or impurities. For the synthesis of the NFs and nanocomposite as an adsorbent material for bio-filter fabrication all the reagent must be taken are of laboratory grade. However, the characterization will be done by following standard protocols and using analytical grade reagents purchased from Hi Media and Sigma Aldrich Pvt. Ltd.

The waste water to evaluate the efficiency of the fabricated bio-filter system were collected in the month of April, 2022 from the University Sewage treatment plant (STP) and Industrial area, Dehradun, Uttarakhand, India.

Synthesis of Nano-fibers (NF)

For the synthesis of NF, pineapple peels were treated physicochemically by modifying the protocol as given in the literature [30]. In brief the collected pineapple peel were thoroughly washed with water, chopped into fine pieces, dried in an oven at 70°C to remove all the moisture and finally ground to make fine powder. Subsequently, the peel powder was chemically digested in acidic solution consisting of 92.9% acetic acid (CH₃COOH), 0.3% hydrochloric acid (HCl) and 6.8% distilled water in ratio of 1:10 (peel powder: acidic solution) at 115°C for 2 hours. The treated sample was then filtered and thoroughly washed with hot acetic acid (≥98% v/v pure grade) to decolorize and remove lignin content. Decolorized fibers were then air-dried and treated with 4% sodium hydroxide (NaOH) and 24% hydrogen peroxide (H₂O₂) solution in ratio of 1:40 at 50°C for 2 h. Further, the treated fibers were filtered and neutralized by washing with water and dried for acid-hydrolysis. Dried fibers were then soaked in hot concentrated sulphuric acid (H₂SO₄) (≥98% v/v pure grade) solution (55-60°C) surrounded by the ice bags to avoid any type of damage at the time of treatment. There after the sample was stirred on Magnetic Stirrer at 600-650 rpm for 60-90 minutes. After this sample was centrifuged at 4500 rpm for 10 minutes, and the pellet were collected and neutralized to collect the nano-fiber. Finally as a resultant very fine and small sized sample of about 150-200 nm were obtained which are known as Nanocellulose.

Fabrication of bio-composite filter

Multilayered bio-filter system was designed for the entrapment of contaminants present in the wastewater sample by simple non-toxic crosslinking agents as shown below (Table. 1). Nano-fibers-charcoal were cross-linked with 5% citric acid and mixed the sample to make a viscous solution and cast a thick layer. Subsequently a layer of charcoal and keratin protein rich chick feather to make composite layers and used as the Biofilter/ Bioadsorbent to clean up the impurities of polluted water. The fabricated Biofilters contain 4 layers: first of NF, second layer NF-feather, third layer NF-charcoal and last fourth layer of NF-charcoal and feathers. All the layers were arranged from top to bottom. The morphological analysis of the prepared sample was done by using ImageJ software to analyze the FESEM images to measure the fiber size, particle size and interactions.

Water testing

The effectiveness the fabricated biofilter systems were evaluated at different parameters as mentioned below by following the standard protocols as mentioned in the literature. Simply as the normal household water filters operation, we added 10ml waste water samples as influent (Sewage and industrial) collected from the pollution sites and collect the filtered or passed out water as effluent in the collection vials for physiochemical analysis. It is recommended to test a single filter collected effluent separately stored for each analysis in order to overcome the data error.

(i) **TDS analysis:** The concentration of total dissolved solutes or solids (TDS) in the water sample before and after treatment were calculated according to the standard protocol. Take 5 ml of water sample was passed through the filters and poured in the petri-plate. Thereafter the petri plates were kept in the hot air oven at 40-45°C for 5-10 min for evaporation of the water.

Record the measured weight of the petri-plates before and after sample drying and TDS was calculated by following the formula mentioned below:

$$TDS = \frac{(A-B)}{\text{Sample Volume in ml}} \times 1000$$

Where: A= Weight of dried residue + dish, B= Weight of empty dish, TDS UNIT= mg/L

Table 1: Composition of nanochar fibrous biofilters containing cellulosic nano-fibers, chicken feathers and charcoal crosslinked with citric acid

	Layer	Nanofibrous	Chicken Feathers	Charcoal
Bio-filter 1	Layer 1	2 gm	---	---
	Layer 2		0.2 gm	---
	Layer 3		---	2 gm
	Layer 4		0.2 gm	2 gm
Bio-filter 2	Layer 1	2 gm	---	---
	Layer 2		0.4 gm	---
	Layer 3		---	2 gm
	Layer 4		0.4 gm	2 gm
Bio-filter 3	Layer 1	2 gm	---	---
	Layer 2		0.2 gm	---
	Layer 3		---	2 gm
	Layer 4		0.4 gm	2 gm
Bio-filter 4	Layer 1	2 gm	---	---
	Layer 2		0.4 gm	---
	Layer 3		---	2 gm
	Layer 4		0.2 gm	2 gm

(ii) **pH analysis:** The pH of the water sample was measured by using pH calibrator (Eutech pH meter) or meter before and after filtration. Briefly, all the filtered and un-filtered water samples were equilibrated at room temperate and distilled water was taken as control sample. Recorded the values of the given water samples in triplets.

(iii) **Conductivity:** Conductivity of water samples was measured by Conductivity meter (Eutech CON 700) to determine the free ions concentration in the water sample. Briefly, all the filtered and un-filtered water samples were equilibrated at room temperate and distilled water was taken as control sample. The Standard Unit of Conductivity=S/m, where S stands for Siemens and m is meter. However, we observed the readings in micro levels i.e., conductivity = $\mu\text{S/m}$

Metal adsorption efficiency

The bioadsorption efficiency of the bio-filters for the heavy metals was measured by following standard protocols [31]. Briefly, the collected industrial and sewage water samples filter through fabricated biofilters in the Nitric acid (HNO_3) rinsed HDPE bottles for metal analysis. In order to determination the heavy metals concentrations all collected samples were prepared. In order to determine heavy metal levels in water samples, atomic absorption spectroscopy (Shimadzu) was performed with hollow cathode lamps for Copper (Cu), Cadmium (Cd), Zinc (Zn), and Mercury (Hg) as radiation sources and air acetylene as fuel as the radiation source. Standard operating parameters were set i.e., maximum wavelengths Cu (324.7), Cd (228.8), Zn (213.9) and Hg

(253.6). Subsequently, Filtered and unfiltered Water samples were digested using aqua regia (HNO₃ 67%: HCl 37% = 3:1) and minerals were extracted using a Berghof MWS-2 microwave digester. Typical calibration curves for measuring heavy metal levels in the water were used, along with blank and standard solutions for calibrating the devices separately. All the samples and standards were run in duplicate.

Anti-microbial activity

The anti-microbial effect of the nanochar-fibrous biofilter was estimated by following the standard protocol [32]. The unfiltered and filtered water samples were used as inoculum to measure the number of microbial cells by simply culturing in the nutrient culture broth (Basal growth medium) at 37°C. The water samples were mix with the nutrient broth at ratio of 1:100 (v/v) and thereafter the tubes were incubated at 37°C for 24h. After incubation the absorbance of the controlled nutrient broth and water sample inoculated nutrient broth were recorded at 590nm wavelength to measure the growth rate. Besides that the microbial growth rate was measured by inoculating the water samples on nutrient agar plate and incubating the samples for overnight (24 h) at 37°C. The inoculated petri-plate images captured to check the microbial growth.

Statistical analysis

The statistical analysis were done by ANOVA, all the samples were taken in triplicates (n=3) to calculate the mean± Standard error and $p \leq 0.05$.

RESULTS

Fabrication of bio-filters

Nanofibrous composite bio-filter was successfully synthesized in a multi-layered form having different concentration of chicken feathers and charcoal to evaluate the finest combination (Figure 1). The schematic representation for the synthesis of nano-biofilter showed that successful formation of nano-fibers from pineapple peel waste of dimension 200 ± 35 nm, and the after citric acid crosslinking the charcoal particles were entrapped on the nano-fibers. Subsequently, the incorporation of the chick feather results in the bio-composite having fibrous and crystalline structure for better sorption of pollutants from waste water.

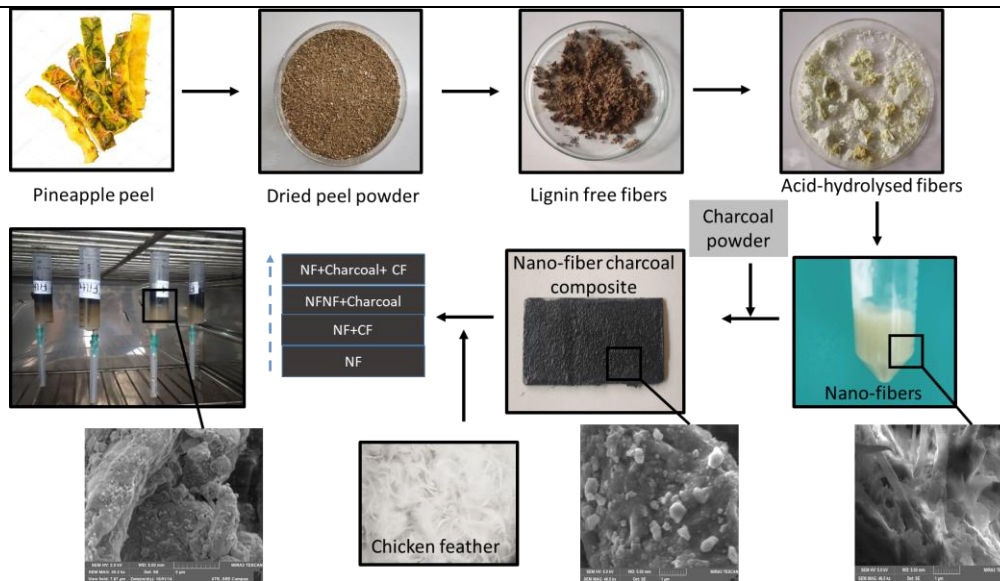


Figure 1: Schematic representation of the designing of nanochar-fibrous bio-filters for the treatment and purification of waste water. Here NF- Nanofiber, CF- Chicken feathers.

TDS, pH and conductivity analysis

After filtration of both industrial and sewage water from the all the four different fabricated Biofilters significant reduction of solute concentration with variable outcome were obtained as shown in Figure 2. However, the maximum sorption of solutes upto 27.3mg/L from the sewage water was observed in the Biofilter 2 as compared to the 3 and 4 with least adsorption efficiency in the Biofilter 1. The composition variation in the Biofilters may affect the presence of free-functional groups for the entrapment of free salts or particles in the water.

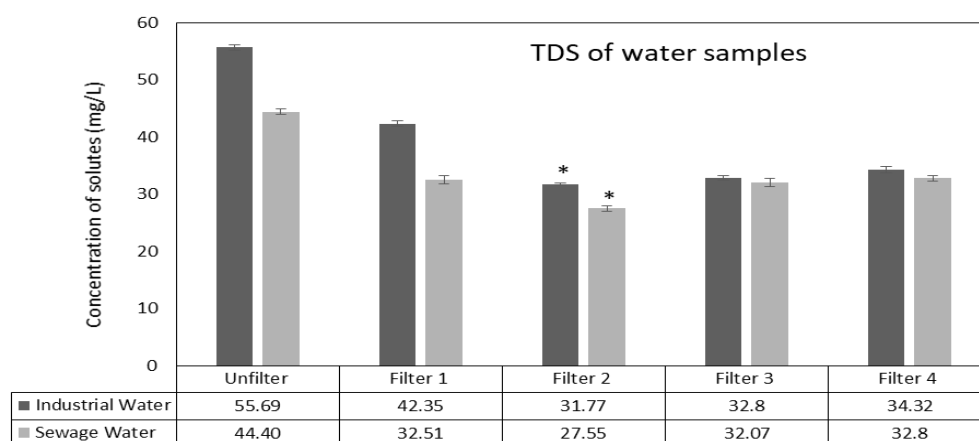


Figure 2: TDS analysis of the industrial and sewage water pre and post-treatment indicate the significant absorbance of the solutes on the filter matrix. Here * indicates the significant difference at $p \leq 0.05$.

The contaminated industrial wastewater was found to be more acidic than the sewage water (Figure 3). The Biofilter 2 showed significant reduction of pH of treated water near to neutral range from alkaline to 7.11 for sewage and acidic to 6.9 for industrial wastewater respectively. Similar outcomes were obtained in the conductivity measurements due to reduction of ions and salts concentration. The order of conductivity reduction efficiency of different Biofilters for the

treated wastewater samples was observed to be Biofilter 2 > Biofilter 3 > Biofilter 4 > Biofilter 1 (Figure 4). The materials for making composite layer are present in higher amount in Biofilter 2 thus the cross-linking occurs smoothly in Biofilter 2 due to which it is more impactful in lowering the conductivity than other Biofilters. Likewise, the higher amount of the composite in the Biofilter 2 provides more surface receptors for the entrapment of the particle form the wastewater.

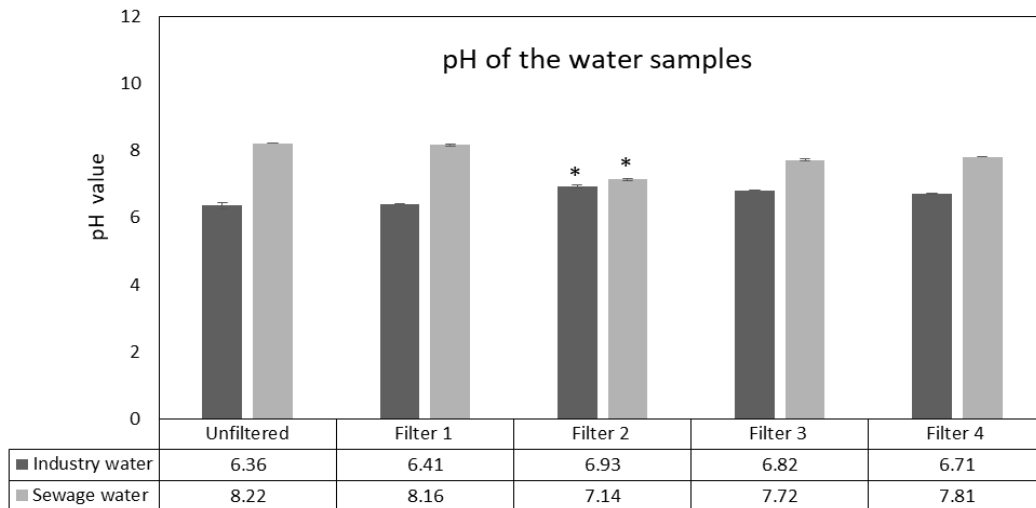


Figure 3: pH analysis of the industrial and sewage water pre and post-treatment indicate the significant change in the ionic balance in water sample passing through the filter matrix. Here * indicates the significant difference at $p \leq 0.05$.

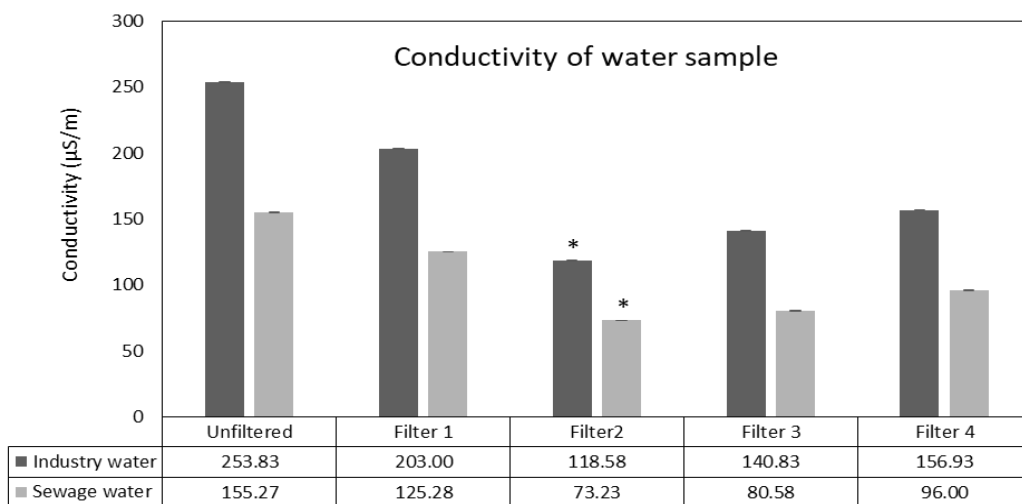


Figure 4: Conductivity measurement of the industrial and sewage water pre and post-treatment indicate the significant change in the free ions balance in water sample passing through the filter matrix. Here * indicates the significant difference at $p \leq 0.05$.

Metal sorption efficiency

The bioadsorption efficiency of different composite Biofilters for Cd and Cu from different wastewater samples is depicted in the Figure. 5,6. It shows that the bioadsorption efficiency of Biofilter 2 in the case of both Cd and Cu is significantly higher as compared to others. In the similar way, the bioadsorption of Zn and Hg from different wastewater samples by Biofilter 2

was significantly higher as compared to other Biofilters (Figure. 7,8). In the Biofilter 2 perfect cross-linking occurred due to the presence of higher quantity of material for the formation of composite thus it showed maximum heavy metal adsorption capacity. Biofilter 1 has the least bioadsorption efficiency for all the heavy metals as compared to others.

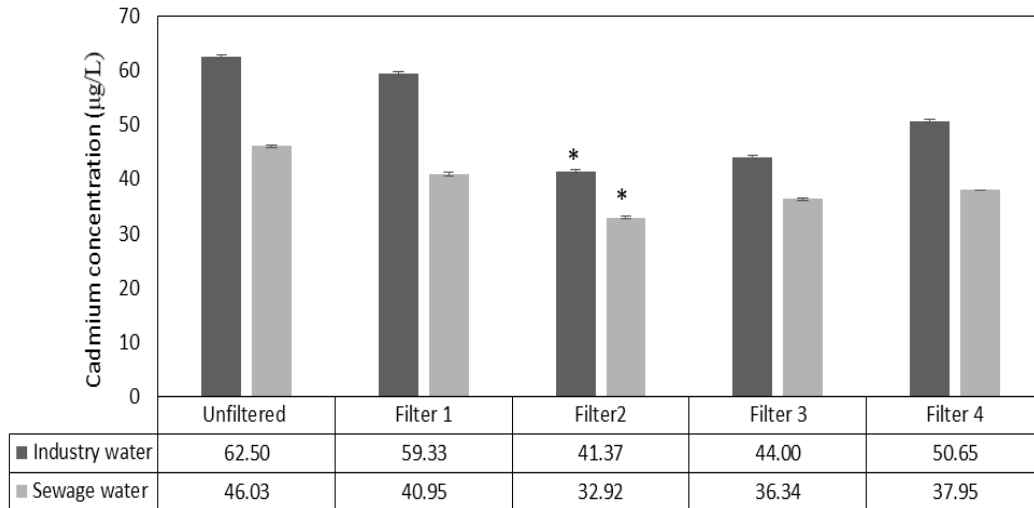


Figure 5: Concentration of cadmium of the industrial and sewage water pre and post-treatment indicate the significant absorbance and effective removal on the filter matrix. Here * indicates the significant difference at $p \leq 0.05$.

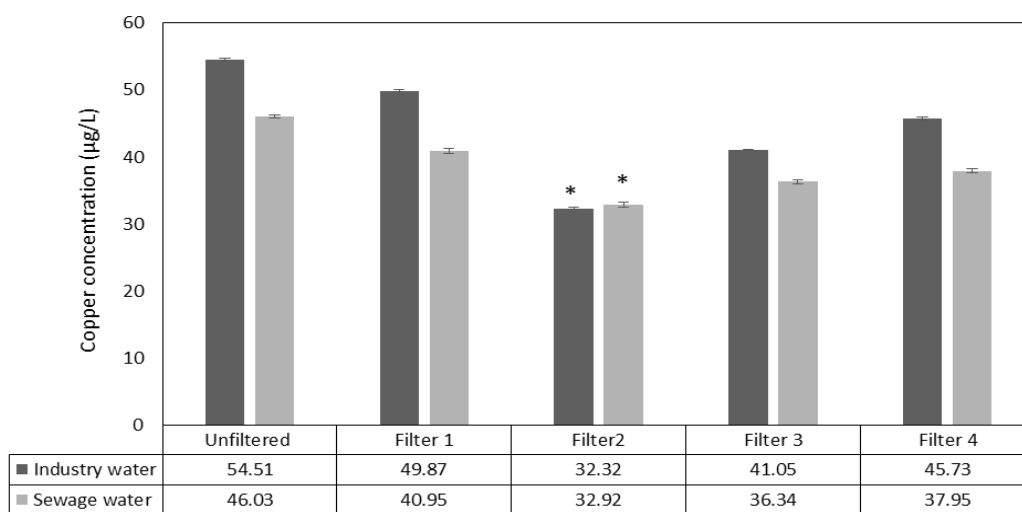


Figure 6: Concentration of copper of the industrial and sewage water pre and post-treatment indicate the significant absorbance and effective removal on the filter matrix. Here * indicates the significant difference at $p \leq 0.05$.

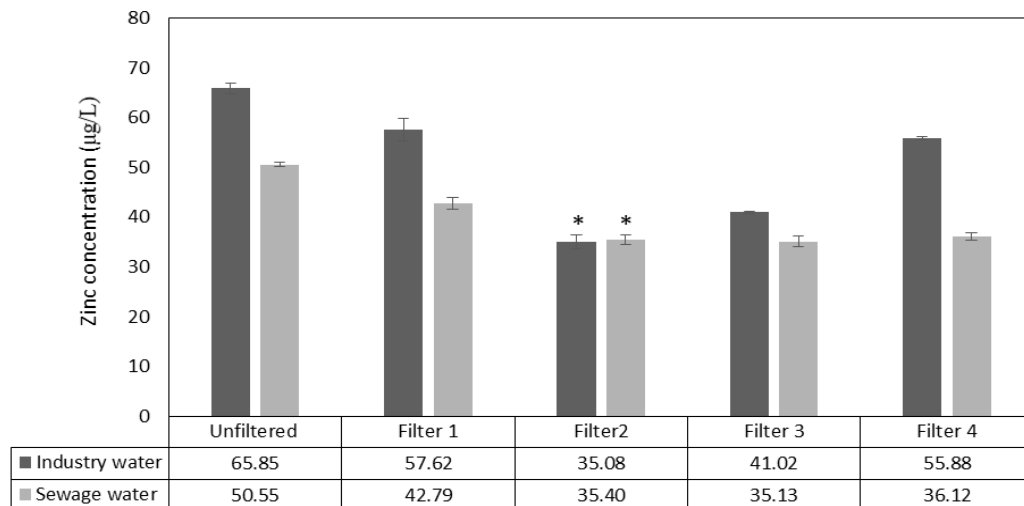


Figure 7: Concentration of Zinc of the industrial and sewage water pre and post-treatment indicate the significant absorbance and effective removal on the filter matrix. Here * indicates the significant difference at $p \leq 0.05$.

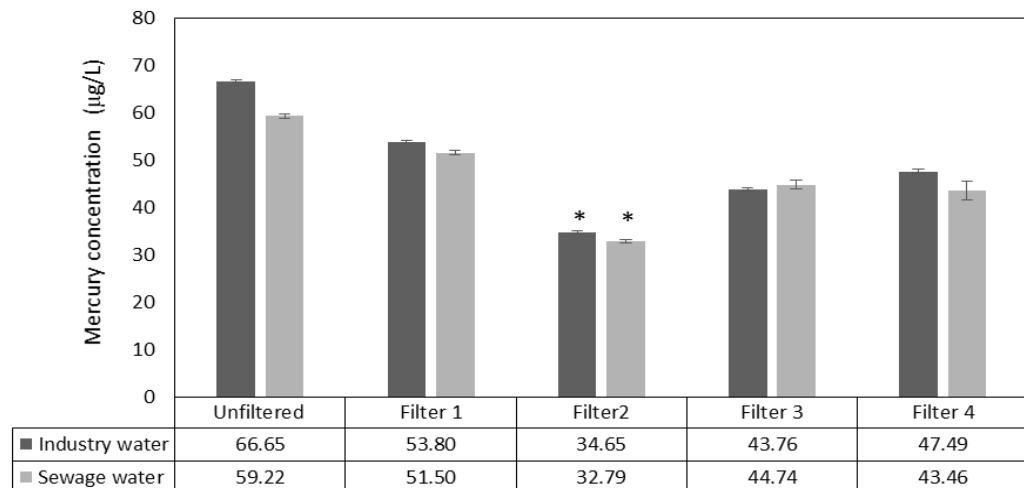


Figure 8: Concentration of Mercury of the industrial and sewage water pre and post-treatment indicate the significant absorbance and effective removal on the filter matrix. Here * indicates the significant difference at $p \leq 0.05$.

Anti-bacterial activity

The water sample collected from the industry and sewage plant shows higher load of the microflora. However, the water filtered through the cellulosic nanochar-fibrous filter results in significant suppression of the bacterial growth. The nano-particles and short peptides present in the bio-filters effectively entrap the bacterial cells and kill them, thereby the number of bacterial cells in the purified water sample become low as compared to the controlled one. Here, the microbial growth shown in biofilters constantly decrease with increasing nano-fiber, charcoal and feather keratin protein concentration in the order Biofilters 2 < Biofilter 3, 4 < Biofilter 1, (Figure 9,10).

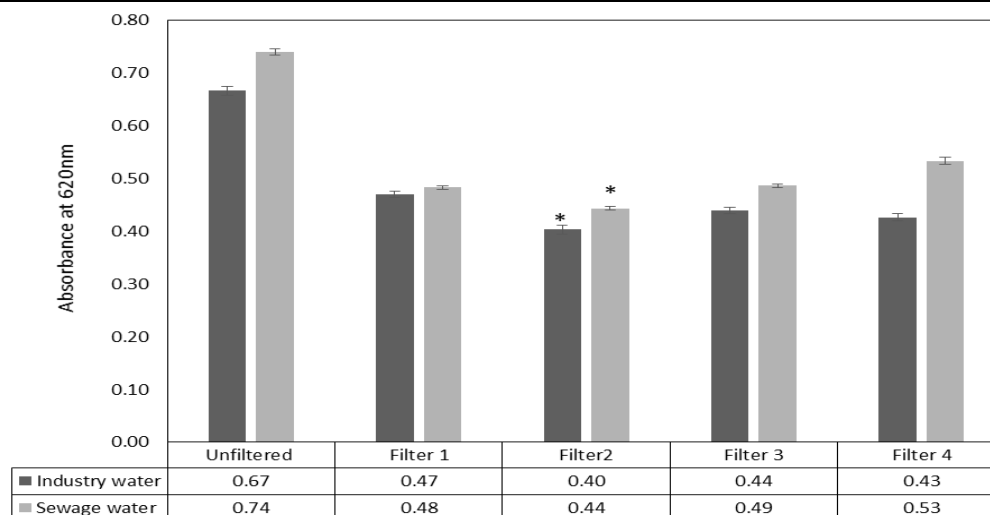


Figure 9: Anti-bacterial activity of the biofilter indicates the significant reduction in bacterial growth in nutrient broth culture and agar medium. Here * indicates the significant difference at $p \leq 0.05$.

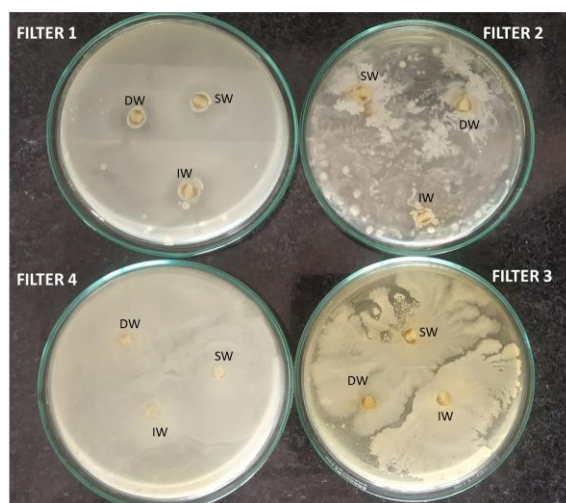


Figure 10: Water sample inoculated in the nutrient agar medium showed the variable growth in bio-filters with least number of bacterial colonies in the bio-filter 2 indicates significant role in entrapment of microbial cells. Here, DW- distilled water, IW- Industrial water, SW-sewage.

DISCUSSION

The development sustainable materials from the bioresources, recycling waste for the development of eco-friendly material is the prime need of today world. In the current scenario multiple-disciplinary scientific approaches have been done by researchers for the designing of nano-system for waste water treatment. Likewise in the present study, Naghdi et al., showed the pinewood nanochar of particle size 60nm significantly adsorbed carbamazepine (CBZ) particles at very low concentration ≤ 20 ppb [33]. Besides that, the Chicken Feather Fiber (CFF) is a potential waste material being utilized for the synthesis of composites for different environmental applications [34]. Reddy et al., developed a cost-effective biodegradable semi-

synthetic polypropylene-jute composite consisting of chicken feather and jute composite [35]. Likewise, in the previous studies agro-waste has been used for the waste water treatment and showed significant removal of pollutants from contaminated water samples [36]. In order to prepare biochar for effective removal of pollutants the particle size reduction results in nano-biochar formation that may be potentially used as highly value bio-catalyst and bioadsorbent [30]. Thus, the nanochar-fibrous multi-layered bio-filter could be potentially used as adsorbent matrix for pollutant and their removal.

In a study a dual membrane hybrid system consists of microfiltration–granular activated carbon (MF–GAC) with nanofibrous filter system was found to effectively remove the dissolved organic carbon (DOC) and upto 90% of inorganic waste [37]. Subsequently, in other study the polyamide nano-membrane was used for the effective removal of the dye from wastewater showed the increase in the pH value ~ 6.4–7.1 with reduction in the conductivity of each dye solution [38]. The sugarcane industry biomass-based membrane filter successfully removed upto 98.5% dye particles form water and be a low-cost adsorbent material with a great adsorption capacity value [39].

Sun et al., chemically digested chicken feather with 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) and potentially removed the Cr(VI) ions upto 87.7% from contaminated water sample at concentration >80 ppm [40]. In a study fish waste synthesized charcoal removed the trace amount of dissolved mercury from the aqueous solutions [41]. Graphitic flaky nanobiochar (NBC) treated water samples revealed the great barrier coefficient for the effective adsorption and removal of Cr(VI), and Cd(II) [42]. Liu et al., evaluated the applicability of nano-biochar for the effective removal Cd from contaminated soil, thereby increases the soil fertility and plant growth [43]. The nanobiochar synthesized from the wheat straw biomass removed upto 127.4 mg/g Hg(II) from the aqueous solution [44]. The Black tea wastes and water hyacinth based biochar containing nanoparticle efficiently removed the heavy metals including Cu, Cd, Zn and Hg from wastewater [45]. Hosseini et al., reported that the semi-synthetic composite of polyaniline and bird feathers remove upto 87.36 % chromium as compared to the charcoal treated water samples and thus as effective filter for heavy metal removal [46].

Nanoparticles or NFs and their derivatives used as nanobiocides to overcome the problem of bio-fouling simply by inhibiting the microbial growth of the pathogens and decontamination of water sample [47]. The biochar of micro to nanometer size range has potential to inhibit the growth of the pathogenic microbes in contaminated water sample and potentially used for the decontamination of sample [48]. The enzyme encapsulated laccase chitosan-nanobiochar matrix showed upto 35% of binding efficiency and exhibited antimicrobial activity against both gram positive and negative bacteria [49].

Thus, the fabricated biofilter should be sustainable and ecofriendly material at low cost. The synergistic effect of the nanochar fibrous matrix with activated charcoal and chick feather keratin protein effectively entrap the pollutants and have toxic effect on microbes in the water samples. Hence, the study outcome indicates the potential applicability for the waste-water treatment as sustainable eco-friendly material at cheap-cost. Although in future studies, other parameters also have to studied by pilot study to evaluate this bio-filter system for large treatment of waste water systems.

CONCLUSION

The above study concludes that the composite nanochar-fibrous Biofilter fabricated by combining agrowaste, charcoal and chicken feather showed highest adsorption capacity of contaminants present in polluted water by simple set up. In our work 4 different com-posite Biofilters were fabricated and compared for their adsorption capacity. In all the four biofilters, the Biofilter 2 showed the maximum adsorption capacity due to the presence of charcoal and chicken feather in higher quantity as compared to other biofilters. Thus, we can conclude that this biofilter system could be used as potential water purification and recycling system for domestic usage. In future, the large-scale set-up can be utilized for the bulk water purification system and to overcome global problem of bioremediation of the water pollution.

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