Research Article

Synthesis and Assessment of an Antimicrobial Composite Developed Using Silver Nanoparticles and Egg Shell-Activated Charcoal: Application in Water Purification

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ABSTRACT

Aims: As filtration is one of the significant steps in the water purification process, one such filtration material was developed using nitrocellulose filter papers coated with a novel nanocomposite, in the present research, with the aim to evaluate its qualitative antibacterial efficacy and filtration efficiency. **Materials and Methods:** Bio-green synthesis of Silver Nanoparticles (AgNPs) using *Ocimum basilicum* extracts was studied along with different characterization parameters. Egg shell-activated charcoal was developed using standard methods. A nanocomposite containing silver nanoparticles and egg shell charcoal was developed and nomenclatured as SEC_{NC}. The antibacterial activity of SEC_{NC} was determined using the standard well diffusion method. Nitrocellulose filter papers were coated or impregnated with developed SEC_{NC}, and its filtration efficiency and antibacterial efficacy were evaluated. **Findings:** Bio-synthesis of nanoparticles was confirmed from a colourless solution to yellow brown because of the action of surface plasmon vibrations. SEM analysis of AgNPs revealed that the particles were formed as agglomerates with size ranging from 77.17 nm to 84.18 nm. The antibacterial activity of SEC_{NC} ranged from 16 mm to 19 mm for 30 µg/mL concentration. The qualitative antibacterial activity of SEC_{NC}-coated nitrocellulose filters ranged from 29 mm to 31 mm of inhibitory zones. Significant differences in the values for TDS, conductivity, and hardness were found evident between the before and after filtration process. Total plate count results also showed significant difference in CFU/mL between the before and after filtration process. **Conclusion:** The results revealed that water sample was efficiently filtered by the nanocomposite-coated nitrocellulose filters during the study.

Keywords: Egg Shell Charcoal, Filters, Green Synthesis, Nanocomposite, Nitrocellulose, Silver Nanoparticles.

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INTRODUCTION

Nanotechnology recently emerged as a novel significant tool for the development of water purification process.[1] Different filtration material coated with different ions and antimicrobial agents were reported and manufactured till date. Among them, cellulose filters played a crucial role either coated with any metal ions or with any antimicrobial agents to retard the metabolic activities of bacteria or virus.

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Praveena *et al.*, (2016) used these cellulose based filters after impregnating the papers with AgNPs. Earlier, the AgNPs were synthesized by researchers using the standard reduction method which showed filtration efficiency by removing 99% to 100% of *Escherichia coli* in water samples.[2] The authors highlighted that cellulose-impregnated nanoparticles were highly suitable for water filtration technology to retard bacteria and virus present in water.

Indian Ayurveda emphasized about the importance of silver ions as antibacterial metal nanoparticles since 1000 BCE.[1] The application of activated carbon in charcoal form has been used for different ailments because of its structural integrity and porosity.[3]

Sherly Arputha Kiruba *et al*., (2014) in another study, determined the green synthesis of biocidal silver-Activated Charcoal (AC) nanocomposite for disinfecting water. The application of silver and charcoal as a nanocomposite proved to be more potential than using it alone for the filtration process or to retard the microbes in water samples. The researchers also highlighted the efficiency of the developed Ag+AC composite as the filtering agent to remove enteric pathogens such as *Escherichia coli*.[4]

As bamboo charcoal was recently used as a synergistic compound with different metals and polymers as composites, we tried two different types of charcoal from natural source to develop a novel charcoal+metal composite in this study. As a lot of egg shells were dumped in to the environment as waste, the shells were processed in this study to convert as activated charcoal and doped with metal nanoparticles to obtain a novel composite.

Based on the medicinal and pharmacological applications of AgNPs and activated charcoal, green synthesis of silver from *Ocimum basilicum* with egg shell-activated charcoal particles was developed to form a novel nanocomposite. The developed composite was studied in detail aiming

To evaluate antimicrobial properties of developed composite,

To coat or impregnate developed SEC_{NC} onto nitrocellulose based filter papers, and

To determine its qualitative antibacterial efficacy and filtration efficiency.

MATERIALS AND METHODS

Collection and processing of *Ocimum basilicum* leaves

Ocimum basilicum (Basil in Tamil Nadu, India) leaves were procured as dried leaves from a plant nursery house near Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India (Figure 2A). Basil leaves were prewashed in deionised water and kept under shade until completely dried for milling.

Preparation of plant leaves

The dried leaves were processed to obtain the powder as described by Kaya *et al*., (2008).[5] The dried leaves procured were shade dried further in the laboratory to reduce the remaining moisture content. Further drying was done in an oven at a temperature between 45ºC to 55ºC for 30 min. Dried leaves were milled to powder form, sieved under a 100 µm sieve, and stored in dry room conditions (Figure 2B).[5]

Solvent extraction of *Ocimum basilicum*

Solvent extraction of *Ocimum basilicum* leaves was performed using the standard Soxhlet extraction protocol. The extraction was done in such a way that the ethanol extracts of selected herbs were collected separately. The collected ethanol extracts were stored in brown amber bottles in dark room conditions.[5]

Synthesizing silver nanoparticles using the green method

Silver nitrate was purchased from Sigma-Aldrich, Bangalore, India. All buffer solutions used for the preparation works were made using distilled water. Bio-green synthesis of silver nanoparticles was developed by the method described by Tailor *et al*., (2020).[6] In brief, about 1 mM silver nitrate solution was prepared, as the first step, followed by adding 10 mL of Soxhlet-extracted *Ocimum basilicum* extract. The solution was then heated until colour-changing reaction occurred. In Figure 3, the steps involved in colour change from yellow to brown are presented. The AgNPs synthesized were separated by the process of centrifugation from the reaction mixture and stored.[6]

FESEM analysis of nanoparticles

FESEM analysis of AgNPs was done using the method described by Asha Monica and Senthilkumar, (2020).[7] Microscopic images of different magnification showing the size of nanoparticles were presented separately.[7]

Collection and processing of egg shells

Routinely used egg shells were collected from different sources such as domestic house hold, canteen, hostels, and restaurants. The shell was broken into several pieces (5 mm x 5 mm) and dried in the shade for 3 to 15 days depending on the moisture content present. Dried shells were converted in to charcoal using the modified method described by Thierry Le Blan and Arnaud Vatinel, (2018). Finally, the fine powdered charcoal particles were stored at room temperature for further processes.[8]

FESEM analysis of developed SEC_{NC}

The morphology of SEC_{NC} was studied based on the method described by Amrita Jain *et al*., (2022). The egg shell charcoal indicating its respective morphology were recorded separately.[9]

Development of novel composites using synthesized silver nanoparticles and activated charcoal (silver nanoparticles+egg shell charcoal nanocomposite -SEC_{NC})

Silver nanoparticles and egg shell charcoal were mixed together to develop as a composite in this study. The composite was developed using a method explained by James Nyirenda *et al*., (2022). Initially, silver nanoparticles were placed in a petridish under sterile conditions followed by the addition of charcoal powder mixing slowly with silver nanoparticles in the ratio of 1:3. The developed composite was nomenclature as SEC_{NC}.[10]

Antibacterial activity of SEC_{NC} against water borne pathogens

The standard agar well diffusion method was used to determine the antibacterial activity of SEC_{NC}. Test bacteria were maintained and stored. Sterile Mueller-Hinton Agar (MHA) plates were prepared, allowed to solidify, and kept under freezing conditions. The developed composite was mixed with DMSO solutions separately, and about 20 µl of SEC_{NC} fractions were loaded into their respective wells. In addition, 4 µg/mL of streptomycin as positive control was loaded in a separate well in the same plate to compare the size of inhibitory zones obtained. Test bacteria-inoculated plates were incubated to observe the zone of inhibition after 24 to 48 hr.[4]

Coating or impregnating developed SEC_{NC} onto nitrocellulose-based filter papers or sponges

Coating the developed SEC_{NC} onto nitrocellulose-based filters was done using the method described by Fernandez *et al*., 2016.[11] In brief, about 1 g of SEC_{NC} was added into 10 mL of DMSO solution; the mixture was heated and homogenized in a stirrer. Nitrocellulose membrane filter discs were impregnated in the solution for 20 min; all the discs were further dried at sterile conditions and stored at 4ºC.[11]

Antibacterial efficacy of SEC_{NC}-coated nitrocellulose membrane against water-borne pathogens

The antibacterial activity of SEC_{NC}-coated nitrocellulose membrane was evaluated against the test organisms using the method described by Fernandez *et al*., 2016.[11] In brief, sterile MHA plates were prepared; about 6 mm diameter of SEC_{NC}-coated nitrocellulose membrane was placed over the MHA surface proceeded with test cultures. The plain membrane was placed in the same plate along with the coated membrane to compare the size of inhibitory zones obtained.[11]

Investigating the filtration efficiency of developed nanocomposites for potable water samples

Collection of water samples

The potable water sample was collected from various corporation water discharge points which was artifically inoculated with laboratory grown food and water-borne pathogens. The samples were collected in sterile 100 mL screw cap containers and brought to the laboratory for further studies.[12]

Apparatus and filtration procedure

Customized filtration apparatus was developed to determine the filtration efficiency of SEC_{NC}-coated nitrocellulose membranes separately. The procedure was performed as described by Awwal Musa *et al*., 2020.[12] Different parameters such as temperature, turbidity, TDS, odour, conductivity, pH, acidity, alkalinity, hardness, and DO were determined to test the quality of the test water sample.

Determination of the total plate count

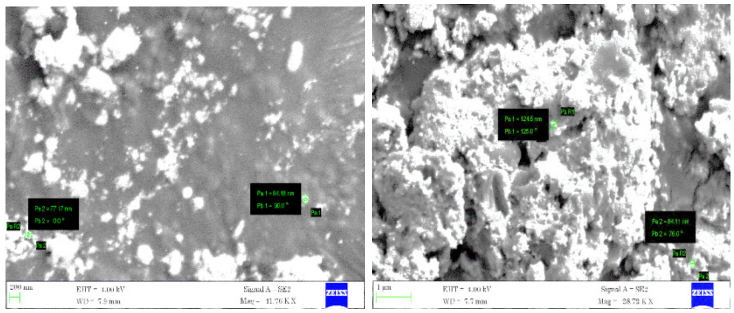
The samples before and after filtration were serially diluted and pour plated onto total plate count agar media. The plates were incubated for 24 hr to 48 hr at 37ºC. After this period, the number of colonies obtained for samples before and after filtration was counted using colony counter and the difference among the processes was recorded as Colony Forming Units (CFUs). The experiment was performed for all collected water samples filtered using SEC_{NC}-coated nitrocellulose membranes.[12]

RESULTS

Green synthesis of silver nanoparticles

Green synthesis of silver nanoparticles was done by the reduction of silver nitrate after exposing to the extracts of plant leaves; this was confirmed from the colour

Figure 1: Synthesis of silver nanoparticles.Synthesis confirmed by colourless to yellowish brown colour solution.



2a: Magnification 1X. 2b: Magnification 2X.

Figure 2: Scanning electron microscopic analysis of silver nanoparticles.

change of colourless to yellowish brown presented in Figure 1.

FESEM of AgNPs

FESEM analysis of AgNPs revealed that the particles were formed as agglomerates (Figure 2a, b). In Figure 2a and b, small agglomerates containing nanoparticles with size ranging from 77.17 nm to 84.18 nm was found evident.

FESEM analysis of developed nanocomposite (SEC_{NC})

FESEM analysis of SEC_{NC} determined the surface morphology and size distribution of silver nanoparticles deposited on the egg shell charcoal surface, respectively. As shown in Figure 3a and 3b, ultrafine and disaggregated silver nanoparticles were homogeneously distributed on the surface of SEC_{NC}.

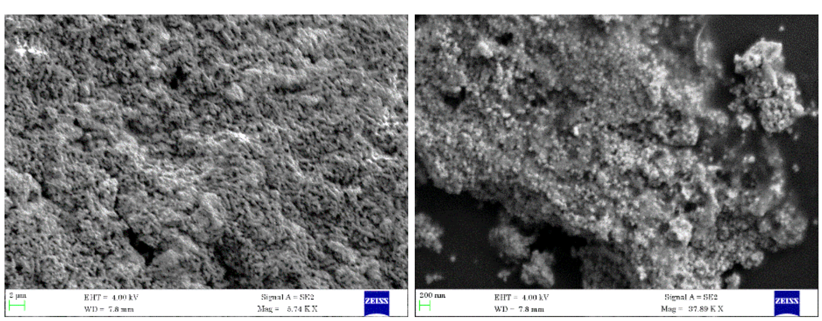
Antibacterial activity of SEC_{NC}

In Table 1, the antibacterial activity of SEC_{NC} against test bacteria was presented. During the analysis, no activity was noticed for lower concentrations. For 30 µg/mL concentration, about 13 mm to 16 mm zones were obtained averse to the test bacteria. *Escherichia coli* and *Salmonella* sp. showed 13 mm inhibitory zone, *Klebsiella* sp. exhibited maximum of 16 mm zone. *Enterobacter* sp. and *Shigella* sp. expressed inhibitory zones of 15 mm and 14 mm, respectively. For higher concentrations (40 µg/mL), *Enterobacter* sp. and Shigella sp. exhibited 18 mm; *Escherichia coli, Klebsiella* sp., and *Salmonella* sp. showed 17 mm, 19 mm, and 16 mm of activity, respectively. Standard streptomycin showed slightly more activity roving from 19 mm to 23 mm averse to all organisms. The zones when compared to the composites, it was found highly significant in restricting the growth of each test bacteria (Figure 4).

Antibacterial efficacy of SEC_{NC}-coated nitrocellulose membrane against water-borne pathogens

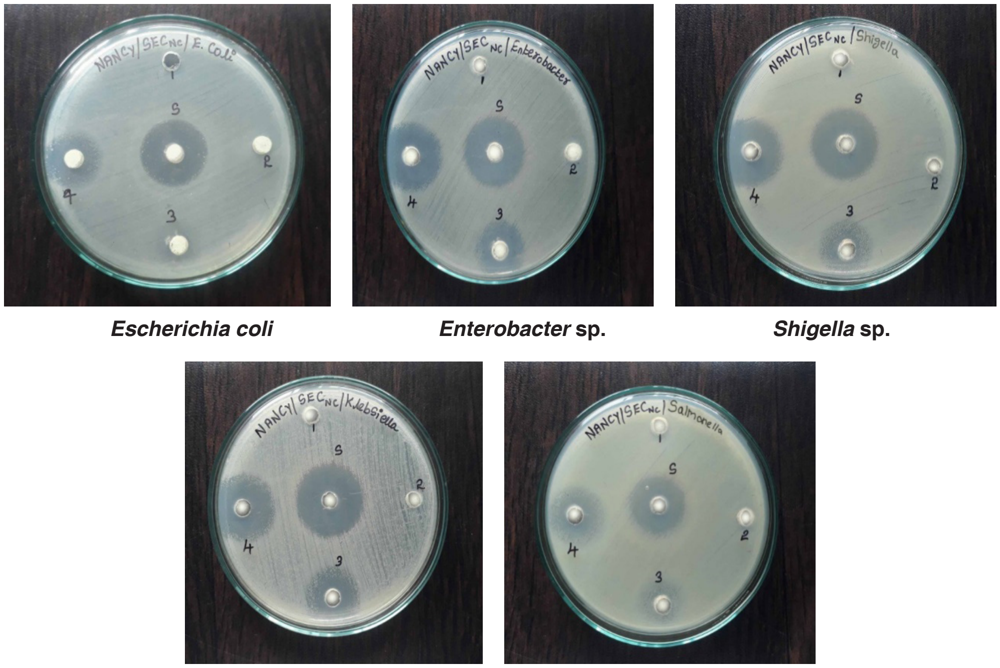
Antibacterial activity of SEC_{NC}-coated nitrocellulose membrane against test bacteria was presented in Table 2. During the analysis, all the test bacteria were inhibited with significant inhibitory zone size ranging from 29 mm to 31 mm for SEC_{NC}-coated nitrocellulose membrane. Maximum inhibitory zone of 31.9±0.57 mm was found evident against a food and water-borne pathogen, *Enterobacter* sp, followed by 30.9±0.57 mm against *Escherichia coli* and 30.3±1.05 mm against *Salmonella* sp. Other two food and water-borne pathogens, *Klebsiella* sp. and *Shigella* sp., showed 29.6±0.75 mm and 29.9±0.57 mm, respectively (Figure 5).

Testing the quality of water



3A: Magnification 1X 3B: Magnification 2X

Figure 3: FESEM analysis of egg shell charcoal.



Escherichia coli **_Enterobacter_ sp.** **_Shigella_ sp.**

Klebsiella sp. **_Salmonella_ sp.**

Figure 4: Antibacterial activity of SEC_{NC} against test bacteria.

Table 1: Antibacterial activity of SEC_{NC} against test bacteria.

Sl. No.	Test Bacteria	Zone of Inhibition				
		NC	10µg/mL	20µg/mL	30µg/mL	S
1	*Escherichia coli*	0	0	13	17	22
2	*Enterobacter* sp.	0	0	15	18	22
3	*Shigella* sp.	0	0	14	18	21
4	*Klebsiella* sp.	0	0	16	19	23
5	*Salmonella* sp.	0	0	13	16	19

Table 2: Antibacterial activity of SECNC-coated nitrocellulose membrane test bacteria.

Sl. No.	Test Bacteria	Zone of Inhibition	
		SEC_{NC} coated nitrocellulose	Uncoated nitrocellulose
1	*Escherichia coli*	30.9±0.57	0
2	*Enterobacter* sp.	31.9±0.57	0
3	*Klebsiella* sp.	29.6±0.75	0
4	*Salmonella* sp.	30.3±1.05	0
5	*Shigella* sp.	29.9±0.57	0

The quality of water was tested; the results are presented in Table 3. Except temperature, pH, and dissolved oxygen, significant difference was found evident for other parameters. Most significantly, turbidity was reduced from 125.8 NTU to 5.25 NTU emphasizing the filtration efficiency of developed SEC_{NC}-coated nitrocellulose membrane filters. Acidity was reduced from 32.5 mg/L to 1.8 mg/L; alkalinity was slightly increased from 2.8 mg/L to 4.6 mg/L. Most significantly, hardness was reduced from 185.6 mg/L to 96.3 mg/L; TDS was also reduced form 168 mg/L to 74 mg/L.

Table 3: Water quality parameters before and after filtration using nanocomposite-coated nitrocellulose filters.

Sl. No.	Parameters	Units	Before filtration	After filtration
1	Temperature	ºC	21.3	21.3
2	Turbidity	NTU	125.80	5.25
3	Total Dissolved Solids (TDS)	mg/L	168	74
4	Odour	-	Slightly odour	No odour
5	Conductivity	µS/cm	254	364
6	pH	-	6.8	7.1
7	Acidity	mg/L	32.5	1.8
8	Alkalinity	mg/L	2.8	4.6
9	Hardness	mg/L	185.6	96.3
10	Dissolved oxygen	mg/L	3.1	3.9

Determination of the total plate count

The total plate count of each test organisms before and after the filtration process was enumerated as per microbiological standards, and the number of colony-forming units/mL is presented in Table 4. The difference in the number of colonies prior and subsequent the filtration process for each bacterium

Table 4: Total plate count of test bacteria before and after filtration.

Filtration of water sample	No of colonies forming units per mL of sample (CFU/mL)				
	Escherichia coli	*Enterobacter* sp	*Klebsiella* sp	*Salmonella* sp	*Shigella* sp
Before	$5.4X10^2$	$6.5X10^2$	$4.8X10^2$	$4.6X10^2$	$5.8X10^2$
After	$1.6X10^2$	$1.1X10^2$	$1.8X10^2$	$1.4X10^2$	$1.2X10^2$

was presented in Figure 6. A significant difference in CFU/mL of test bacteria was evident from the images presented. The results revealed that water sample was efficiently filtered by the nanocomposite-coated nitrocellulose filters during the study.

DISCUSSION

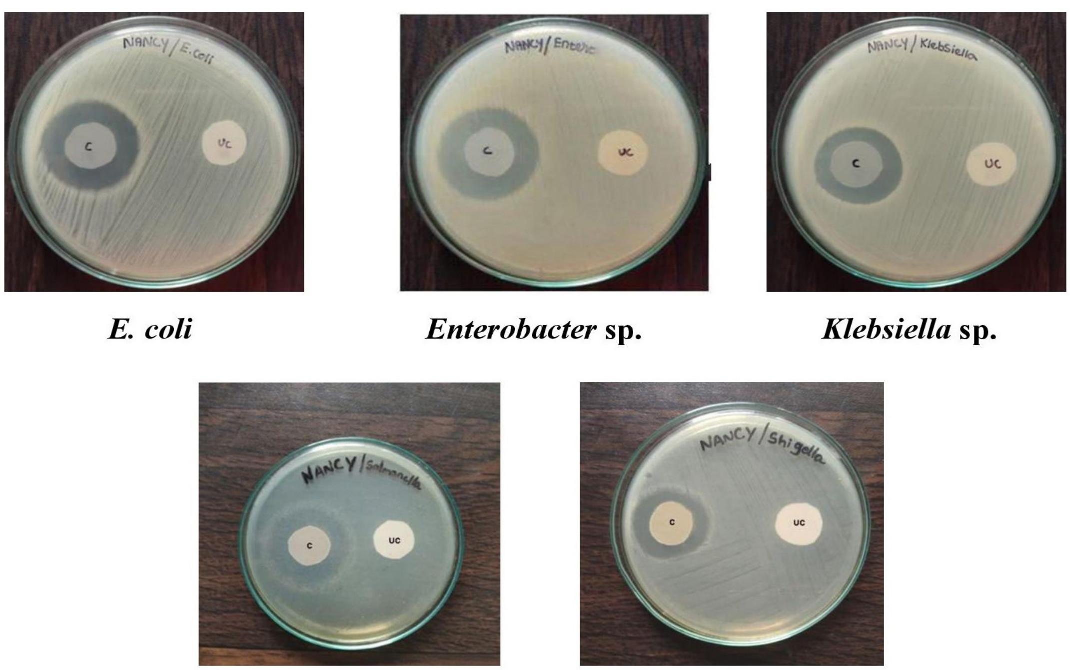
The yellowish brown colour indicated the formation of AgNPs; this was mainly due to the excitation of surface plasmon vibration that happened within 2 hr of reaction time. Formation indicated that silver ions were converted to elemental silver of size ranging as a nanoparticle. As it was reported that natural antioxidants have strong reducing ability, the antioxidants in basil extracts had attributed for reducing $AgNO_3$ (silver-nitrate) to AgNPs.

As shown in Figure 3a and Figure 3b, ultrafine and disaggregated silver nanoparticles were homogeneously distributed on the surface of SEC_{NC}. From the images, it is clear that the nanoparticles were uniformly dispersed and found adsorbed into the porous structures of SEC_{NC}, and hence, they were found homogenous and slightly rough. Similar morphological structures were observed by Ghaedia *et al*., (2012). The researchers used activated carbon+silver nanoparticles to remove synthetic direct yellow dyes.[13]

The antibacterial activity of SEC_{NC} against test bacteria was observed. During the analysis, no inhibitory zones were obtained for lower concentrations, but for higher concentrations, good inhibitory zones were obtained. This indicated the synergistic inhibitory activity of AgNPs and egg charcoal nanocomposite against the bacteria.

The antibacterial activity of SEC_{NC}-coated nitrocellulose membrane against test bacteria also revealed significant results. In Figure 5, inhibitory zones ranging from 29 mm to 31 mm were found significant to retard the bacteria. These results could play a significant role in water filters for effective filtration efficiencies.

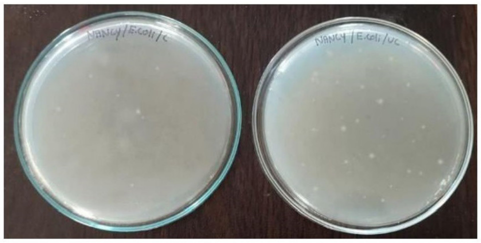
The literature survey also showed similar studies supportive to the present investigation. In a study, Yang *et al*.,[14] developed bamboo charcoal-polyoxometalate composite and determined the antibacterial activity adverse to *Escherichia coli, Bacillus subtilis,* methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. About 9.4 mm to 27 mm of zones



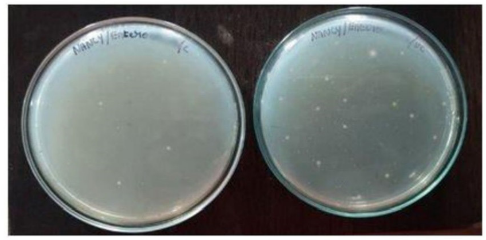
E. coli ***Enterobacter* sp.** ***Klebsiella* sp.**

***Salmonella* sp.** ***Shigella* sp.**

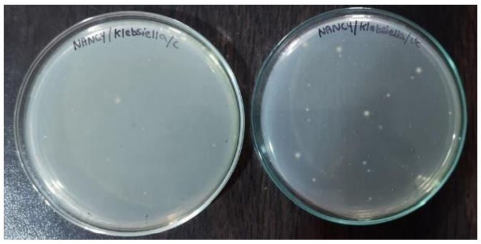
Figure 5: Antibacterial activity of SEC_{NC}-coated nitrocellulose membrane test bacteria.



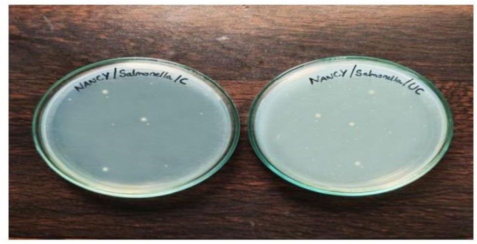
Escherichia coli



Enterobacter



***Klebsiella* sp.**



***Salmonella* sp.**

Shigella *sp.*

Figure 6: Total plate count before and after filtration.

were recorded to developed composites. Attained values were found almost similar to the composites developed in our current study as the zone size was ranging from 13 mm to 19 mm for SEC_{NC}.

The mode of action of charcoal on the bacterial metabolism was well studied and cited by many researchers. By burning materials such as coconut shell, coal, sawdust, wood, bone char, petroleum coke, etc., fine black powder is formed resulting in activated charcoal. Like silver, charcoal also has their own potential to retard the growth and arrest the metabolism of susceptible bacteria. By the process of activating the charcoal, its surface area, internal structure, and the pore size have been modified. The pore size of the carbon atom present in the activated charcoal is reduced because of this activation process exhibiting an increased surface area. This activated charcoal is more porous; hence, it has good absorption properties. The major advantage of activated charcoal is it does not cause any harm to humans as it does not contain any toxins.[15] The microorganisms which come in contact due to contact with skin, sweat, or wounds, an effective antimicrobial property against microbes such as *Staphylococcus aureus, Micrococcus luteus, Escherichia coli, and Candida* species was exhibited. Klepp *et al.*, (2016) reported that charcoal finished fabric could adsorb malodour-causing bacteria and its metabolic by-products which come in contact with the skin surface. Charcoal finished fabric shall be used to prevent the adherence of odour-causing bacteria.[16] Thierry Le Blan and Arnaud Vatinel, (2018) stated that the activated charcoal has a good antimicrobial property and also controls odour while the fabric is finished with charcoal.[17]

Different water quality parameters when tested prior and subsequent filtration processes revealed that the water was efficiently filtered by the nanocomposite-coated nitrocellulose filters. Significant differences in the values for TDS, conductivity, and hardness were found evident between the before and after filtration process. Optimizing the filtration conditions would increase the filtration efficiency. This shall be studied in our future research work.

SUMMARY AND CONCLUSION

The filtration efficiency of a novel composite SEC_{NC}-coated nitrocellulose filters was investigated in this study. The findings showed significant results toward the filtration efficiency of the coated filters. The qualitative antibacterial activity of SEC_{NC}-coated nitrocellulose filters ranged from 29 mm to 31 mm of inhibitory zones. Significant differences in the values for TDS, conductivity, and hardness were found evident between the prior and subsequent filtration process. Total plate count results also showed significant difference in CFU/mL between the before and after filtration process. The present findings revealed that the water sample was efficiently filtered by the SEC_{NC}-coated nitrocellulose. Activated charcoal from egg shell shall is also considered to be used along with other types of materials to improve filtration efficiency because the activated charcoal has the ability to reduce the odour, turbidity, and bacterial numbers and improves water quality. In addition, it is recommended that further studies are needed to evaluate the potentiality of activated charcoal for removing other contaminants such as heavy metals, toxic salts, and compounds present in waste waters.

CONFLICT OF INTEREST

The authors declare no conflict of interest in the present research.

ABBREVIATIONS

FESEM: Fourier emission scanning electron microscopy; **SEC_{NC}:** Nanocomposite containing silver nanoparticles and egg shell charcoal; **TDS:** Total dissolved solids; **DO:** Dissolved oxygen.

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