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# Sand-supported bio-adsorbent column of activated carbon for removal of coliform bacteria and *Escherichia coli* from water

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Abstract New bio-adsorbent carbon materials were synthesized from the leaves and veins of Mucuna pruriens and Manihot esculenta plants, which are locally available in abundance. The synthesized carbons were activated using 0.01N HNO<sub>3</sub>. Surface area of the activated carbons from M. pruriens and M. esculenta plants was found to be quite high, i.e., 918 and 865 m<sup>2</sup>/g, respectively. Scanning electron microscopy analysis of the carbons reflects complex disorganized surface structures of different open pore sizes, shapes and dimensions. These properties of the newly synthesized activated carbons led to the development of a sand-supported carbon column, for its possible use in the removal of coliform bacteria and Escherichia coli (E. Coli) from raw water samples. The removal percentage of E. coli was found to be 100% with both the types of carbon adsorbents, as confirmed from the McCardy most probable number table. Similarly, the removal percentage of coliform bacteria was found to be 99 and 98.7% by M. pruriens and M. esculenta carbon columns, respectively. These activated carbons synthesized from locally available plants possess the characteristics of good lowcost adsorbents which can be easily used for the removal of bacteria from water by adsorption method.

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C. Pongener chubaakum@gmail.com **Keywords** Adsorption · *Manihot esculenta* · *Mucuna pruriens* · Most probable number

### Introduction

In recent years, rampant environmental pollution has led to contamination of water with inorganic, organic as well as biological contaminants. Access to clean, potable water has become a major global challenge, especially in the rural areas of developing countries, and it is in fact alarming that almost 80% of all diseases in developing countries arise from consumption of polluted drinking water. Therefore, water treatment becomes an essential requirement in order to reduce/remove pollutants that are of concern to human health. Among biological pollutants present in water, bacteria such as Escherichia, Citrobacter, Klebsiella, Enterobacter and Serratia are collectively called as coliform bacteria, and among these, Escherichia coli (E. coli) is most common (Guentzel, 1996). Intake of bacteria contaminated water may cause illnesses such as diarrhea, shigellosis, gastroenteritis, salmonellosis, typhoid fever (Cabral, 2010). The presence of *E. coli* in water is very significant as it indicates fecal contamination, and thus increases likelihood of presence of harmful pathogens. Although some E. coli bacteria that live in the intestines of humans and most warm-blooded animals help to maintain the balance of normal intestinal flora (bacteria) against harmful bacteria, many other strains of E. coli causes severe illness like diarrhea, respiratory illness, urinary tract infections, pneumonia, especially among children and elderly population (Goncharuk and Vergolyas 2014). This bacteria can stay alive for prolonged periods in natural aquatic systems (Jiang et al. 2007) and are usually transmitted to humans through contaminated water (Ashbolt



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2004). Therefore, for safe drinking water, removal of bacteria becomes very important. Different processes to reduce/remove bacteria from potable water are available (Ashbolt 2004), which include techniques such as boiling, distillation, reverse osmosis, water filtration, ozonation, use of fiber filters, ceramic filters, UV irradiation, use of water softeners, activated alumina, sediment filter (Mendez et al. 2009). However, it was observed that use of these techniques did not ensure complete removal of pathogens from water (Davis et al. 2009; Hunt et al. 2008). Moreover, these techniques have different requirement like high maintenance, continuous power supply, generation of additional waste/by-products, thereby reducing their cost effectiveness, which makes their utility somewhat limited in certain cases.

Hence, an alternative technique is desired for removal of bacteria from water and for this purpose, adsorption method could be chosen as one of the suitable techniques, because of its porosity and elevated surface area (Akhtar et al. 2005; Jaguaribe et al. 2005; Kobya 2004). Different activation processes such as treatment of carbon with acid/ base further enhance its surface properties thereby increasing its efficiency and selectivity (Chubaakum et al. 2015; Harry and Francisco 2006; Roop and Maneesha 2005). This provides a range of applications of activated carbon in separation and purification methods (Buasri et al. 2013; Ilaboya et al. 2013), and reports are available on synthesis of activated carbon from various plant materials (Aji et al. 2015; Ashoka and Inamdar 2010; Cheenmatchaya and Kungwankunakorn 2014; Chen et al. 2012; Chubaakum et al. 2015; Cobb et al. 2012; Junior et al. 2014; Sivakumar et al. 2012; Tsai et al. 2001; Wartelle and Marshall 2001).

In the present study, an attempt has been made to remove total coliform bacteria and E. coli from water by using newly developed carbon-based columns. The work was designed so as to utilize the adsorption capacity of activated carbon for removal of bacteria from water samples. Thus, this paper describes the synthetic details of two new types of activated carbons; a methodology for construction of sand-supported carbon columns and their application in the removal of bacteria from water. This methodology has an additional advantage because it can also remove other water pollutants, including trace elements, thereby making it more versatile (Ngah and Hanafiah 2008). Further, by using abundantly available plant materials as precursors for the synthesis of activated carbons, there is reduction in the cost of starting materials, which makes the technique quite cost-effective.

#### Materials and methods

For this present study, carbon was synthesized from readily available plants Mucuna pruriens and Manihot esculenta which were then activated using 0.01N HNO<sub>3</sub>. Since adsorption efficiency of the activated carbon depends on the carbon's surface properties, different analytical techniques for surface studies such as SEM, surface area analysis were used to understand the surface properties of the activated carbon. For bacterial studies, agar solution, double- and single-strength lactose broth, brilliant green bile salt media were prepared using standard procedures (American Public Health Association 1995). For removal of coliform bacteria and E. coli from water samples, sandsupported carbon columns were constructed using glass chromatography column. The methods of synthesis of carbon, its activation, characterization, column construction, preparation of different solutions for bacterial studies are presented in the following section.

#### Preparation of activated carbon

For the synthesis of activated carbon, the raw materials, i.e., leaves and veins of *M. pruriens* and *M. esculenta* plants, were collected and sun-dried for a few days. The dried materials were crushed manually into smaller pieces and were packed in clean stainless steel containers and put in a Muffle furnace at 700  $\pm$  20 °C for 4 h in a uniform nitrogen flow wherein carbonization was done. The already carbonized materials were then grounded into fine powder with mortar and pestle and washed with distilled water to remove impurities and dried in hot air oven at 110 °C. The dried carbons were then pulverized in the planetary Ball Mill at 600 rpm for around 10 min to obtain uniform size. Once the carbons were prepared, activation was done by the following process.

#### Activation of carbon

Fifteen gram of the prepared carbon was taken in a 500-ml beaker, and 0.01N HNO<sub>3</sub> solution was gradually added until the sample was fully submerged. The mixture was then thoroughly shaken for 3 h in a rotary shaker. Thereafter, it was filtered using Whatman No. 42 filter paper. The carbon was washed with double-distilled water several times for removal of excess of acid to maintain the pH of the carbon between 6.9 and 7 and then dried in an oven at 110 °C. After complete drying, the dried activated carbons were stored in airtight containers for further study.



#### Characterization of synthesized carbon

In order to understand the surface properties of the prepared activated carbon, the following studies were done.

#### Scanning electron microscopy study (SEM)

The surface morphology of the activated carbon plays a vital role in the adsorption of different adsorbates and also provides information about the random distribution of different pores of varying shapes and sizes. Thus, in order to obtain the surface morphological information, SEM images were recorded at different magnification by using SEM-JEOL JMS 6390 LV instrument.

#### Surface area analysis

The surface area of the adsorbent greatly influences the adsorption process and is a valuable indicator of quality and performance of the adsorbent. Thus, measurement of surface area for *M. pruriens* and *M. esculenta* carbons was carried out using surface area analyzer (Smart instrument, SS93/92).

### **Column construction**

In order to assess the efficacy of the prepared activated carbons for bacteria removal, a column was constructed using 100-ml glass column of 2 cm diameter ( $18 \times 300$  mm). The column was first packed with 2 g of sterilized sand, followed by addition of 1 g of activated carbon. Thereafter, 2 g of sand was again added in order to sandwich the carbon between the two sand layers,



Fig. 1 Setup of sand-supported column

thereby preventing the adsorbent from floating (Fig. 1). The total volume occupied by the sand and the carbon adsorbent in the column was  $18 \times 50$  mm. The same experimental procedure was followed for both the synthesized carbons.

# Preparation of different solutions for bacterial studies

To determine the presence of coliform bacteria, the "most probable number" (MPN) technique was used. For this study, two different reagents, viz, double- and single-strength lactose broth and brilliant green bile salt media, were prepared using standard procedures (American Public Health Association 1995).

# Preparation of double- and single-strength lactose broth

For preparation of double-strength lactose broth, 10 g each of lactose, peptone and beef extract was taken in a 1500-ml glass beaker. Thousand milliliter of distilled water was added into the mixture, and a pH of 6.7 was maintained. (While preparing the lactose broth, the initial pH was found to be 6.6 after addition of 1000 ml of distilled water; this was then diluted with a small amount of water to make the pH of the solution 6.7.) The solution was then autoclaved for 15 min at 120 °C.

For preparation of single-strength lactose broth, 250 ml of the already prepared double-strength lactose broth solution was taken in a 500-ml glass beaker, 250 ml of distilled was added to it, and the solution was autoclaved for 15 min at 120  $^{\circ}$ C.

#### Preparation of brilliant green bile salt media

One gram each of lactose and peptone and 2 g of bile salt along with 1 mg of brilliant green were taken in a 500-ml glass beaker followed by addition of 500-ml distilled water with constant stirring to give brilliant green bile salt media. The pH was maintained at pH 6.7 and was then autoclaved for 15 min at 120  $^{\circ}$ C.

#### Water sample collection

For the present study, raw water sample was collected from stagnant water body. The water body was a natural collection point for waste water from different sources, and thus expected to be contaminated with coliform bacteria. The water samples were collected in 500-ml sterilized stopper bottles. Thereafter, measured amounts of water samples were used for the bacterial tests.



#### Total bacteria colony count

For determination of total bacteria colony count, experiment was carried out under laminar flow environment, wherein 1 ml of the collected sample was taken on a Petri dish and then liquefied readymade agar solution was gently poured over it, followed by incubation for 24 h at 37 °C (Fig. 2). The total bacterial colony count was determined using a digital colony counter (Model: LA660, Hi Media laboratories).

# Total coliform and *E. coli* study by multiple tube fermentation methods

#### Test for coliform bacteria

After the colony count, three sets of five sterilized test tubes each were taken and named as set 1, set 2 and set 3, respectively. Thereafter, 10 ml of water sample was taken in each test tube of set 1 followed by addition of 10 ml of lactose broth double-strength solution. In set 2, 1 ml of water sample was taken in each test tube, followed by addition of 10 ml of single-strength lactose broth solution. In set 3, 0.1 ml of water sample was taken in each tube followed by addition of 10-ml single-strength lactose broth solution.

In the next step, Durham tubes were inverted and gently inserted into each of the test tubes. The bubble formation inside the Durham tubes, which is because of oxidation of lactose present in the solution, confirms bacterial activity. The three sets (i.e., 15 test tubes) were sealed tightly with non-absorbent cotton wool and incubated for 48 h at 37 °C. After completion of incubation, the bacterial activity was studied using Mc Cardy, MPN Table for five tube dilution. The total coliforms were identified by the production of acid and gas from the fermentation of lactose (UNEP/WHO 1996).



Fig. 2 Experimental setup for bacteria count in the laminar flow

#### Test for E. coli

After the presence of coliform bacteria was confirmed, brilliant green bile media test was carried out to study the presence of *E. coli*. For this test, three sets of test tubes, namely set A, B and C containing 5, 5 and 3 test tubes, respectively, were taken and 10 ml of brilliant green bile media reagent was added to each of the test tubes. This was followed by addition of 1 ml each of set 1, set 2 and set 3 solution, respectively, to each test tube of set A, B and C. The newly prepared solutions were then incubated for 48 h at 37  $^{\circ}$ C.

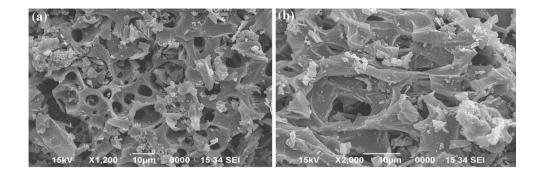
### Test for removal of coliform bacteria and E. coli by sandsupported carbon column

In a typical procedure, 100 ml of water sample was slowly poured into the carbon column and then allowed to stand for 30 min. Thereafter, the water sample was allowed to flow, maintaining the flow rate of the water sample at 25 ml/3.4 min and this water sample was collected in a sterilized bottle. Bacterial activity studies were done following the same experimental procedure that was used for untreated water using double- and single-strength lactose broth solution. Brilliant green bile media test was also done using the same procedure as that for untreated water wherein three sets of test tubes were taken, viz., set A, B and C test tubes, set containing 2,1 and 1 test tubes, respectively. Then 10 ml of brilliant green bile media was taken in all the three sets followed by addition of 1 ml each of set 1, set 2 and set 3 solution, respectively, in set A, set B and set C and was incubated for 48 h at 37 °C.

In order to ascertain whether the removal of coliform and *E. coli* bacteria was possible by sand only, similar tests were conducted by passing the water sample through the column without carbon and both the results were compared.

# **Results and discussion**

The newly synthesized activated carbons were characterized in order to understand their surface properties. SEM micrographs of the activated carbon give a clear picture of the porosity of an adsorbent and also show complex disorganized surface structures of different open pore sizes, shapes and dimensions. The SEM micrographs were taken at different magnification in the range of 1200 and 2000 resolutions. Figure 3a, b shows the SEM images of *M. pruriens* and *M. esculenta* at different magnifications. Slant flakes of different sizes were observed, and their surfaces appeared to be rough and smooth. The rough surface micrographs showed a distinct roughness with oval Fig. 3 a SEM images of Mucuna pruriens carbon. b SEM images of Manihot esculenta carbon



patterns. Within each oval section, the presence of the macropores was clearly noticeable. The surface corrugation was clearly visible from the SEM images which might have been due to HNO<sub>3</sub> treatment. This showed that HNO<sub>3</sub> activation was effective to create well-developed pores distribution on the surface of the precursor, thus leading to large surface area and porous structure of the activated carbon.

The surface area of activated carbon synthesized from M. pruriens was found to be 918  $m^2/g$ , whereas for the carbon synthesized from M. esculenta, the surface area was found to be 865  $m^2/g$ .

# Study on removal of coliform bacteria and E. coli from water samples

To study the removal of E. coli, initially total bacteria count was estimated in order to confirm the activity of bacteria in the water sample under investigation. Once the presence of bacteria was confirmed, the presence of coliform bacteria was analyzed. From the experiments conducted for total bacteria count of the water sample, total bacteria colony was estimated as 200 + colonies and the results are given Table 1. Further when the experiment for the presence of coliform bacteria was conducted, it was observed that among the three experimental sets, all the five (five) test tube tested positive for bacterial activity for both set 1 and set 2, whereas for set 3, only three out of five were found to be positive. The details of the results are given in Table 2. The MPN values obtained for coliform bacteria analysis were (5,5,3) corresponding to 900 bacterial colonies in 100 ml of water sample (Table 2). Similarly, the experiment was further conducted to study the presence of E. coli bacteria using brilliant green bile media test whereby the activity of E. coli was determined by Mc Cardy, MPN Table. The results gave MPN/100 ml value of (5,5,1) corresponding to 350 colonies of E. coli. and are given in Table 3.

Once the collected water samples were passed through sand-supported carbon columns, it was found that there was a considerable reduction in total bacteria colony in the water samples. The bacteria counts reduced from 200 + counts/ml to about 20 + counts/ml when the water was allowed to seep through M. pruriens sand-supported activated carbon column. Similarly, a reduction from 200 + counts/ml to 30 + counts/ml was observed with M. esculenta activated carbon samples (Table 1).

When the water sample was passed through the M. pruriens carbon column, the MPN value, which was initially (5,5,3) corresponding to 900 bacterial colonies of coliform bacteria, reduced to (2,1,1) corresponding to 9 bacterial colonies. Similarly for M. esculenta carbon column, the initial value reduced to (2,2,1) corresponding to 12 bacterial colonies (Table 4). However, when the activity for E. coli was estimated by Mc Cardy, MPN Table, after passing through the column, the activity gave MPN/100 ml value of (0,0,0) signifying 100% removal of E. coli by the carbon made of *M. pruriens* activated carbon (Table 5). Interestingly, the activity for *E. coli* in the water samples after passing through *M. esculenta* carbon column was also found to be (0,0,0), signifying the fact that total E. coli removal was possible by both these carbon columns. In order to confirm that the removal of bacteria from water samples was by carbon samples only, the same experi-

Table 1	Showing total
bacterial	count/1 ml

No.	Sample	Total count/1 ml
1.	Untreated water	200+
2.	Treated water with sand	200+
3.	Treated water with sand-supported Manihot esculenta activated carbon	30+
4.	Treated water with sand-supported Mucuna pruriens activated carbon	20+



No.	Set	No. of test tubes	Sample (ml)	Double-strength lactose broth (ml)	Single-strength lactose broth (ml)	MPN/100 ml
1	1	5	10	10	-	(5,5,3) corresponding to 900 bacterial
2	2	5	1	-	10	colonies
3	3	5	0.1	-	10	

Table 2 Bacterial colonial studies for untreated water sample

Table 3 E. coli colonial count of untreated water sample

No.	Set	No. of test tube/s	Sample from set 1,2,3 (ml)	Brilliant green bile media	MPN/100 ml value
1	А	5	1	10	(5,5,1) corresponding to 350 colonies of <i>E. coli</i>
2	В	5	1	10	
3	С	3	1	10	

Table 4 Bacterial colonial studies of treated water sample with sand-supported Mucuna pruriens and Manihot esculenta

No.	Set	No. of test tubes	Sample (ml)	Double-strength lactose broth (ml)	Single-strength lactose broth (ml)	MPN/100 ml
1	1	5	10	10	_	(2,1,1) and (2,2,1) for Mucuna pruriens and Manihot esculenta
2	2	5	1	-	10	carbon corresponding to 9 and 12 bacterial colonies, respectively
3	3	5	0.1	-	10	

Table 5 E. coli colonial count of treated water sample with sand-supported Mucuna pruriens and Manihot esculenta

No.	Set	No. of test tubes for <i>Mucuna</i> pruriens and <i>Manihot</i> esculenta carbon respectively	(ml)	Brilliant green bile media	MPN/100 ml value
1	А	2 2	1	10	(0,0,0) and $(0,0,0)$ for both the carbon indicating 100% removal of
2	В	1 2	1	10	E. coli
3	С	1 1	1	10	

mental procedure using only sand column was conducted, the results of which are shown in Table 1. These results signify the fact that both the synthesized activated carbons are excellent adsorbents which could completely remove *E. coli* from water sample. It was also observed that 99 and 98.7% of coliform bacteria could be removed from the raw water samples by using *M. pruriens* and *M. esculenta*  carbon columns, respectively. This removal/reduction is attributed to the presence of numerous pores of different sizes and also to the large surface area of the synthesized bio-adsorbents which provide sufficient active sites that can easily trap the bacteria, thus preventing the passage of bacteria into the filtrate, making the water sample bacteria free.



#### Conclusion

A sand-supported bio-adsorbent column was developed using activated carbons synthesized from the leaves and veins of *M. pruriens* and *M. esculenta* plants, which are locally available in abundance. These sand-supported activated carbon columns have been successfully used for complete removal of *E. coli* bacteria as well as 98-99% removal of coliform bacteria from water samples. This reduction/removal of bacteria due to adsorption onto activated carbon is attributed to its high surface area and the presence of numerous pores of different shapes and sizes which trap the bacteria within the adsorbent. The novelty about these bio-adsorbents is that the raw materials used are readily available almost throughout the year, inexpensive and user-friendly. Ongoing experiments using these synthesized activated carbons have already revealed that they have the capacity for removal of other water pollutants including trace elements. Another advantage of this technique is that no energy is required, thereby making the process more easily applicable. This method can be effectively practiced by using bamboo/hollow tubes of appropriate diameter in rural areas where access to treated public water supply is scarce and modern water purification systems are rare.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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