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Adsorption of *Escherichia coli* Using Bone Char

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ABSTRACT: The aim of study was providing a novel adsorbent for the removal of *Escherichia coli (E.coli)* as a microbial model from contaminated air especially in hospital units using bone char (BC). The BC was prepared from cattle animal bone by pyrolysis in a furnace at 450°C for 2 h. The characteristics of BC have been determined using scanning electron microscopy (SEM), X-ray diffraction (XRD), Brunauer-Emmett-Teller (BET), pH_{zpc}, apparent density and iodine number. Nebulizer system applied to convert the *E.coli* with different concentration into bioaerosols. The variables included: BC weights (4-10 g), the adsorbent pore size (20-40 mesh) and microbial concentrations (10^3-10^7 CFU/mL). Characteristics of the adsorbent show the ability of the BC to remove *E.coli* from air. The results shows the higher amounts of BC, the more efficiency achieved to purify contaminate air and particles in the range of 20-40 mesh were more practical in removing bioaerosols. An efficient time for removing the more E. coli was 30 minutes. The maximum bacterial efficiency removal achieved was 99.99%. Comparison of removal efficiency with other literature showed that the BC particles were better mineral sorbents than other organic adsorbents and a commercial activated carbon. In this study, we investigated a novel air purification adsorbent and the information obtained in the paper is of fundamental significance for the mineral adsorbents especially bone char in cleaning of indoor bioaerosol. @ JASEM

World Health Organization (WHO) emphasizes that ensuring acceptable indoor air quality is the responsibility of all concerned. People spend 90% of their time in confined places, and thus it should be primary goal to ensure a clean and contaminant free indoor air environment (Chow and Yang 2004). A study done by Environmental Protection Agency concluded that indoor air pollution poses a greater risk than outdoor air pollution (Dinno and Glantz 2007). This indoor air pollution is estimated to be the cause of several health related issues and reduced work productivity among people. Allergies and infection diseases s have increased considerably over the last few decades (Ekhaise et al. 2008). Microbial agents in indoor air are considered a serious health hazard and it has been a major topic of attention in recent years (Rosas 1997). Most of the microbial contamination problems are related to moisture problems in our heating, ventilation and air conditioning (HVAC) systems. Due to the moist conditions prevalent in the ductwork of the HVAC systems, ducts act as perfect breeding grounds for microbes. In addition, the dust accumulated in the HVAC systems may act as nutrients for the growth of microorganisms. The contaminants are then subsequently distributed throughout the building space by the air handling unit (Rui et al. 2008). During the past 10–20 years little progress has been made in addressing the basic problems responsible for the increasing rates of nosocomial infections in many countries (Ghiasvandian 2001). Nosocomial infections increase the cost of healthcare in the countries least able to afford them through increased treatment with expensive medications use of other services. The WHO study found that the highest prevalence of nosocomial infections occurs in ICU and CCU units (Coia 1998). The indoor air environment can potentially place patients at greater risk than the outside environment because enclosed spaces can confine aerosols and allow them to build up to infectious levels. Several studies showed that hospital indoor air contains different types of microbial population (Probhu and Sangeetha 2006). The importance of the estimation of the quantity and types of airborne microorganisms are that these values can be used as an index for the cleanliness of the environment as well as an index they bear in relation to human health and as source of nosocomial infections (Strachan 2009). The source and spread of organisms inside the hospital are important issues especially in operating, ICU and CCU rooms. *E. coli* is a gram negative rod-shaped bacterium that is commonly found in the small intestine of warm-blooded organisms . Most *E. coli* strains are harmless and have some benefits like as vitamin K2 production for their hosts, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls (Morrison et al.1994). The *E.coli* used in the work as a bacterial model. This microorganism was selected in air purification studies based on nonpathogenicity, availability, and high growth in media (Farnsworth and Goyal 2006). Several traditional methods such as HEPA filters, UV ray and disinfection materials most commonly applied for air disinfection, but all of these techniques have their own constraints; the safety of operators, storage problems, cost-effectiveness and the control of their by products are

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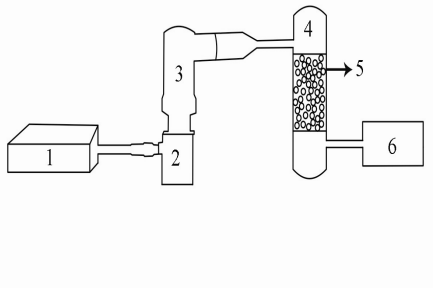
difficult obstacles (Grinshpun and Mainelis 2005). The preparing of the innovative, safe, inexpensive and effective processes for removal of bacteria from air flows is necessary (Chow and Yang 2004). Although different types of adsorbent have been used for contaminants removal, however, scientists have been devoted the cheap materials that might represent a low-cost and readily available material as an absorbent (Rezaee et al. 2009). The mineral sorbents like BC have proved they are more usable in air bacteria removal in comparison with organic adsorbents (Li et al. 2010). Every year in many countries, a large number of domestic animals are slaughtered for meat and these animals have a large amount of bone waste that can be used as feedstock or a fuel for energy generation. Therefore, using these wastes for producing BC sorbent and other products provides a safe and useful disposal route which has benefits for environment (Choy and McKay 2005). Several researches have been done using different adsorbents to remove bacteria from air and water but it is the first time that the BC has been used as a mineral adsorbent for microbial contaminant air purification. The aim of this present study is feasibility of BC for *E.coli* removal from air.

MATERIAL AND METHODS

Preparation and characteristics of the adsorbent: The leg bone from sheep rinsed in water and boiled for 4 h to remove fate and residual protein pieces. The boiled BC was dried in 110°C overnight. Pyrolises of bone was performed in an electrical rectangular furnace that was externally heated at 450°C for 4.5 h. The solid yield from the pyrolysis step was transport to a desiccator and cooled to room temperature. The pyrolyzed bones were crushed and pulverized using standard sieves with the range of 20- 40 mesh. The specific surface area in BC structure was determined via N_2 gas adsorption according to BET method. The solid structures were analyzed using X-ray diffraction (Philips) and scanning electronic microscopy (XL30 Philips model) techniques. The pH_{zpc} of the BC was determined using the batch equilibrium technique with1:1000 and 1:80 solid to liquid ratios in 0.1 (M) KNO_3 solution. Sodium chloride was employed as an inert electrolyte. The initial pH value of the KNO_3 solution was adjusted range from 2 to 12 by adding 0.1 (M) HNO_3 or KOH. The solutions were allowed to equilibrate for 24 h in an isothermal shaker (orbital shaker. OS. 625, IRAN) at 25 ± 1°C. The suspensions were filtered through filter paper, and the pH values were measured again using an ion pH meter (SENWAY 3505, UK) (ASTM D2972-88 2003). A blank test without BC was also made in order to eliminate the influence of interferences (Smiciklas et al. 2000). The iodine number (mg iodine/g BC) was determined by using a 0.1N standardized iodine solution; the titrant was 0.1N sodium thiosulfate (ASTMD 4607 1999).

Preparation of bacterial solution: E. coli (ATCC: 25922) was cultivated in nutrient broth overnight at 37°C. The bacterium was solved in 0.5 McFarland solutions with sterile loop until O.D was reached to 0.08 -0.1 at 620 nm by spectrophotometer (Unico 2100 SUV-VIS, USA) which implies that the cell count reached a minimum of 10^8 CFU/mL. The samples stored at 4°C for 3–4 days (Wand and Vacca 2007). The McFarland tubes were used for preparation of the bacterial solution. 0.5 mℓ of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2.2H_2O$) was added to 99.5 mL of 0.18 M H_2SO_4 (1% v/v) with constant stirring. The McFarland tubes slowly mixed to ensure that it is evenly suspended. Using matched cuvettes with a 1 cm light path and water as a blank standard, the absorbance measured in a spectrophotometer at a wavelength of 625 nm. The standard distributed into screw cap tubes of the same size and volume as those used to prepare the test inoculum. The tubes were seal tightly to prevent loss by evaporation and protected from light at room temperature. Standards

Fig 1: Schematic of the experimental system: (1) air source (2) nebulizer (3) microbial solution storage (4) adsorbent column (5) adsorbent (BC) (6) sampling



Reactor set-up: The system set-up was contained a glassware column (8 $_{cm}$ length, 1.2 $_{cm}$ diameter) with two inflow and outflow ports at 2 and 7 $_{cm}$ distance from the bottom. Bacterial solution was placed into a 12 mL plastic storage of nebulizer (2700 L/min, 50 w, Germany) to convert it in bioaerosol. Tygon lab tubes were used for connections (1/4" ID, 3/8" OD, 1/16" Wall thickness, 25 psi at 70°F Max psi) (Fig.1). All the equipment was sterilized by 70% alcohol, 5% HCl, UV light and autoclave (121°C bar pressure) prior and after usage. The system was started with

switching on the nebulizer. Serial dilution of the concentrated original culture used for nebulization (10^3,10^4,10^5, 10^6 and 10^7 times diluted in the PBS). A rotameter (SKC, USA) was used to regulate bacterial aerosol flow that was usually below 1000 mL/min for eliminating humidity interference. In 5 minutes intervals such as 5, 10, 15, 20, 25 and 30 minutes several samples were taken. The tygon tube which was connected to outflow of adsorbent column held over the EMB agar for 5 minutes. Then, the plates were put in an incubator for 24 h at 37°C. After their growth, the colony forming units (CFU) were counted. Finally, it was compared with initial bacterial aerosol concentration.

Table1: The Bone char characteristics

Properties	Range	Unit
pH_{zpc}	8.5	–
BET-surface area	130.75	m^2/g
Apparent density	0.768	g/cm^{-3}
Pore volume	8.8	cm^3/g
Iodine number	15.8	mg /g
Size distribution	20-40	mesh

RESULT AND DISCUSSION

Bone char characteristics: The characteristics of the adsorbent are presented in Table 1. The pH_{zpc} of an adsorbent is a very important property that determines the pH at which the adsorbent surface has net electrical neutrality. At this value, the acidic or basic functional groups no longer contribute to the pH of the solution. Experiments showed that the pH_{zpc} of the BC was about 8.5. It has been reported that at any pH below pH_{zpc} the surface charge is positive whereas at pH level above pH_{zpc} the surface charge is negative based on the value found for pH_{zpc}, it can be deduced that the BC surface charge is positive as the solution pH is less than 8.5. The positive charge on the surface of the BC may enhance the removal of *E. coli* via adsorption. The pH_{ZPC} is higher than which reported by Jiang et al. (2007). The differences may be related to the adsorbent characteristics because they used clay for the adsorbent while we used BC. Specific surface area was determined with the BET method. The specific surface area of BC particles was 130.75 m^2 /g.

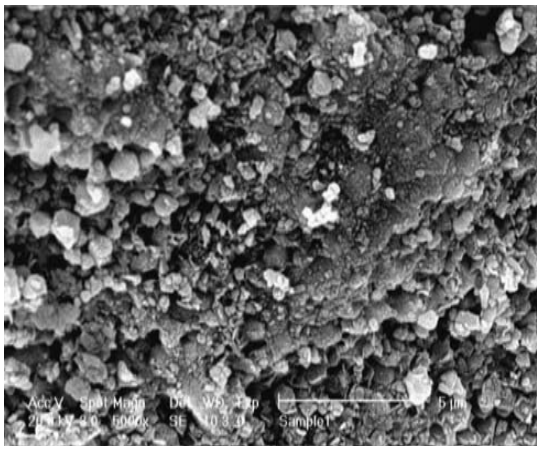


Fig 2: Scanning electron micrograph of bone char particles with 5000X magnifications

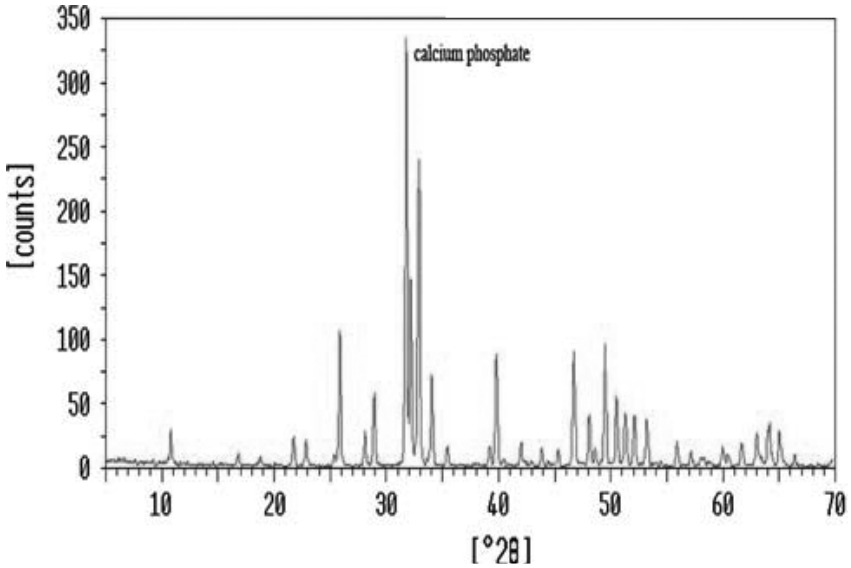


Fig 3: X-ray diffraction of bone char particles

This result is lower than report of Yazi Liu and Shaogui (2007) for activated carbon which was 850.640 m^2 g^1 BET surface area and higher than results of Jiang et al. (2007) which was 101.6 m^2 g^{-1}. The appearance of pore structure of the BC surface using the SEM is shown in figure 2. Although the thermal process causes the transformation of calcium and phosphate ions to apatite in bones, the pore structure of bone is still retained. Some carbon from the bone distributes on bone char to form a carbon surface during calcinations. The X-ray diffraction tests reveal that the BC is a mixed adsorbent composed of basic tricalcium phosphate and amorphous carbon (Figure 3). After the thermal process, calcium and phosphate ions in the bones rearrange to form a hydroxyapatite structure. The re-formation of calcium and phosphate compounds can be proved by powder XRD patterns, which was performed by the Philips X-ray powder diffractometer (Cheung *et al.* 2002). Structurally, the calcium phosphate is in the hydroxyapatite form. The amorphous carbon fraction is distributed throughout the whole of the entire hydroxyapatite structure but most of the carbon exists as a highly active thin film that covers the porous hydroxyapatite surface. Diffracted beams have been used in the range $10° < 2\theta < 70°$.

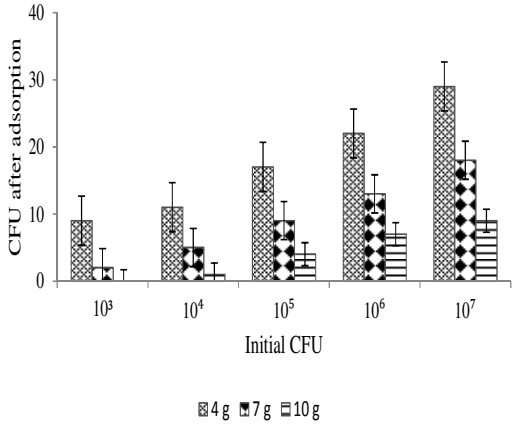


Fig 4: Effect of amounts of BC on adsorption of *E. coli* by the BC 20-40 mesh

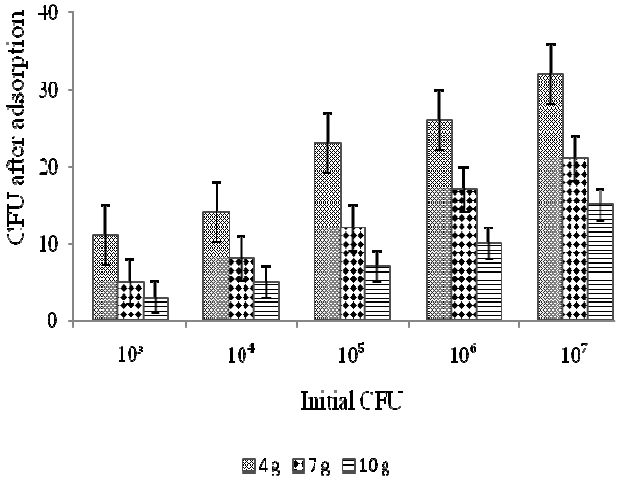


Fig 5: Effect of amounts of BC on adsorption of *E. coli* by the BC >40 mesh

The E. coli adsorption experiments: The reactor was examined with different concentrations of the *E.coli* (10^3- 10^7CFU/10mL). After passing the bacterium through the adsorbent, outflow was conducted over selective media (EMB agar) in 5, 10, 15, 20, 25 and 30 minutes. All experiments were conducted in three replicates. According to the result, when we used the higher amounts of BC, the more efficiency achieved to purify contaminate air (Figure 4). The results shows particles in the range of 20-40 mesh were more practical in removing bioaerosols in contrast to the >40 mesh particles (Figures 4 and 5). Rivera and Bautist (2001) reported 87.8% *E. coli* removal by activated carbon while we achieved 99.99% *E. coli* removal by the BC. It indicates that the BC as a mineral adsorbent provides more applicable sorbent-bed. The regeneration of adsorbent is also an important aspect of air purification. For this research regeneration BC was been carried out by thermal process that was regenerated and rendered *E. coli* free by heating at 200°C for 30 min. Bone char as a mineral adsorbent for bioaerosol has high efficiency in comparison with organic sorbents. The results presented here indicate that a BC is a suitable and effective adsorbent for the removal of *E.coli* from air. The adsorbent has several advantages such as being inexpensive, easy access to materials and needless to any activation.

A significant effort towards developing this laboratory exercise was devoted with the goal of: -Obtaining high efficiency for removal of *E.coli* from air; -Replacing mineral adsorbents with organic ones in microbial air purification; -Reducing cost in purchasing adsorbents and -Regenerating the adsorbent by simple thermal process

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