Journal of the Nigerian Association of Mathematical Physics
Volume28, No. 1, (*November, 2014*), pp 177 – 182

Effects of Tortuosity on the Migration of Bacteria in Saturated Porous Media

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Abstract

There are few research works that experimentally determines the tortuosity of the porous media and investigate its impact on the transport of bacteria as this is microscopic parameter that influences the hydrodynamics characteristics of porous media. This study examined the effect of tortuosity on the migration of Escherichia coli (a rod, negative strained bacteriaused as indicator for fecal contamination of groundwater) in saturated porous media.Electrical and downward flow methods were used for tortuosity determination and filtration respectively. The findings show that as tortuosity increases, fraction of bacteria retained increases with increasing depth and decreasing porosity. It was also concluded that tortuosity is a function of depth and porosity and that any increment in the tortousity of the sample aids filtration and adsorption which considerably decreases the rate of migration of bacteria. These findings would add to the existing literature on the influence of physical parameters on the migration and retention of bacteria in sand media which has its application in various areas of environmental science and engineering.

Keywords: Bacteria, Tortuosity, Migration, Saturated, Porous media

1.0 Introduction

The tortuosity is defined as the ratio of the average length of all particle path lines passing through a given cross-section during a unit time period to the width of thesample.It was realized that elongation of streamlines not only affects the hydraulic discharge, but also mediates other types of transport phenomena in the porous medium. It has been known since long that flow through a porous medium depends on many factors such as porosity, tortuosity, granule shape and size distribution, saturation, Reynolds number, etc. For proper understanding of transport of microscopic particles like bacteria and viruses in porous media, it is essential to depart from simple macroscopic physical quantities with a limited media-particles interaction and focus on basic areas that examine the parameter that microscopically describes the flow pattern of aquifer and tortuosity is considered in this work because it measured the effect of pore morphology on the bacterial migration and its attenuation. Also, it determined the influence of hydrodynamic, chemical interactions and surface interaction between particles and the media.

Few research examined the effects of this path length on migration of bacteria using chemotaxis; Barton and Ford [1] studied the effects of path length in their work using silica sand in presence of chemical attractant, they observed that chemotaxis does not enhance bacterial penetration. Duffy et al. [2] used random walk algorithm specifically designed to model bacterial migration in a constructed porous matrix to investigate the effect of the obstacle on the effective random motility coefficient of bacteria and to compare the results with experimental measurements. They observed that tortuosity increases with decreasing obstacle diameter. Their work was based on theoretical expression for the tortuosity on analogies to Knudsen diffusion, which suggested that the tortuosity increased in proportion to the particle diameter; however, tortousity is not well defined in terms ofeasily measured properties of the porous media [1] as this would result in shortcomings from their prediction. Former studies on tortuosity did not examine its effect on bacteria migration attenuation and on porosity as this is medium parameter that determines either directly or indirectly the relation between the hydraulic parameters and the particles transport.

The purpose of this research work is to investigate the effects of tortousity on the transport and attenuation of migration of bacteria through sand media using Escherichia coli. The tortuosities were determined in an independent experiment using electrical method at different depths for samples of varying porosity. This enabled us to examine the relationship between tortuosity, porosity and depth. The former were then compared with the bacteria recovery at different depths for various porous media.Escherichia coli is a gram-negative, faculatively anaerobic, straight, rod shaped bacterium, measuring approximately 2.6μm long and 1.1 – 1.5 μm wide occurring singly or in pair [3,4]and is a taxonomically well-

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defined member of the family Enterobacteriaceae. E coli are considered to be specifically of fecal origin [5]. Three general clinical syndromes that can result from infection with this pathogenic type are enteric/diarrhoeal disease, urinary tract infections, and sepsis/meningitis [6].

2.0 Materials and Method

Sand samples were collected from the river bed with polythene bags, washed with deionised water in order to remove fine organic materials that were in the process of decaying as a result of the work of soil micro organism. The sample was then boiled in 1M hydrochloric acid for 2 hrs and latter treated with 1M of NaOH to remove metallic oxide coating on the sand and equilibrate the pH respectively. The resulting sand sample was washed again in deionised water two to three times; sundried and stony pebbles were removed [7]. These were sieved using different mesh sizes to get different soil textures and were labeled A, B, C, D, and E respectively.

Determination of tortuosity

Apparatus used to determine the tortuosity of the media consist of calibrated Pyrex glass 1.0 m long, 2.79 x 10^{-4} m^2 diameter. Sheet of copper plates mounted onto the cork were used as electrode at the opposite faces, one to cover the bottom side tightly that no water were allow to drain, while the other electrode were attached to stick that can be inserted through the second opening (Figure 1). The cylinder was filled with water up to 10.00cm and the other electrode was made to rest tightly on the fluid. A steady D.C supply voltage of 4.50V was fed and the current (I_{H2O}) flows were measured with multimeter. The cylinder was empty and then filled with saturated sand up to length of 10.00cm and were tightened. The same steady voltage were supplied and the current ($I_{sand+H2O}$) were read and recorded. Ohm's law was used to determine the resistance of conducting fluid (water) and that of mixture (sand and conducting fluid). This was repeated for length 20.00, 30.00, 40.00 and 50.00cm respectively and their corresponding current and resistance were recorded and determined. The tortuosity of the samples was determined using equationbelow [8]

$$`Ʈ' = \frac{R(sand+water)}{R(water)} \text{ x } ϕ$$

Where R = resistance in Ohms (Ω), ϕ = Porosity (dimensionless) and Ʈ is the tortuosity of the media (dimensionless)

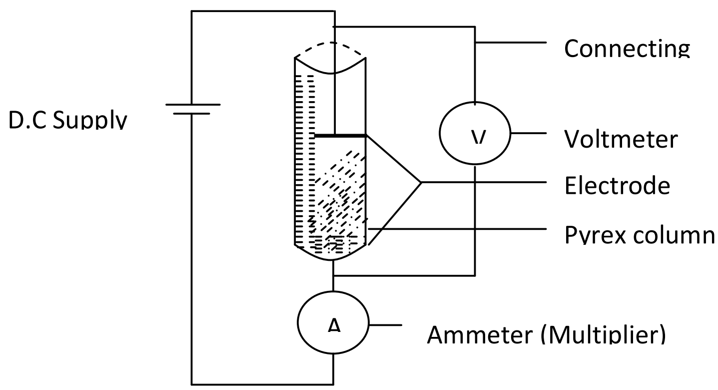


Figure 1: Experimental Setup for the Determination of Tortousity.

Bacteria collection and Preparation

The bacteria used were isolated from septic tank in the Department of Microbiology and Tedder Hall, University of Ibadan. Bacteria isolates were prepared by plating serial dilution of nutrient free water [9] on Eosin Methlene Blue Agar. The isolates with characteristic dark centre greenish metallic sheen was subcultured repeatedly by streaking on the new agar plates until pure culture were obtained. The isolates were characterized morphologically, Gram type and motility.

The pure culture of the bacteria was inoculated with nutrient broth that have been prepared by sterilization at 121^oC for 15 minutes and cooled. This was then incubated with identified bacterial for 48 hrs at 37^oC. The inoculated broth was centrifuged by dispensing them into sterilized vial and spinned at 4000 rpm to harvest the bacteria cells. The cells were washed two to three times, after centrifugation and suspended in normal saline to avoid osmotic burst. This was then kept aseptically in the refrigerator at 4^oC [7]. Exactly 1ml of the suspension was serial diluted and plated to determine the colony forming unit per milliliter (CFU/ml).

Column preparation and operation

Glass columns (1m long, 2.79cm diameter) pyrex glass were washed and disinfected with 70% ethanol and sterilized in hot air oven at 120^oC for 2 hrs. The column cylinder which was covered with muslin cloth at outlet was blocked so as to prevent the water passage. The saturated sand was then poured into the water column up to height 'h' referred to the depth equal 10cm. The column was repeatedly tapped during packaging to prevent any entrapment of air bubble with the pore space [10]. The cover was removed and water was allowed to pool down, until the dripping water from the column has a frequency of one drop per 10 seconds. Then, 2ml of bacterial suspension of known concentration was dropped onto the sand bed in the column with aids of 5ml sterile syringe and this was followed by intermittent supply of 250ml of distilled water. The effluents of the column was collected and analyzed for the bacteria load. This was done five times to have five rainfall simulations as it may occur under natural condition. Five effluents were collected differently in sterile beakers and covered immediately and subjected to microbial analysis using pour plate technique for the bacteria count. The effluents concentrations of bacteria were normalized to the respective influent concentration [11]. This process was repeated for four different depths (20, 30, 40 and 50cm).

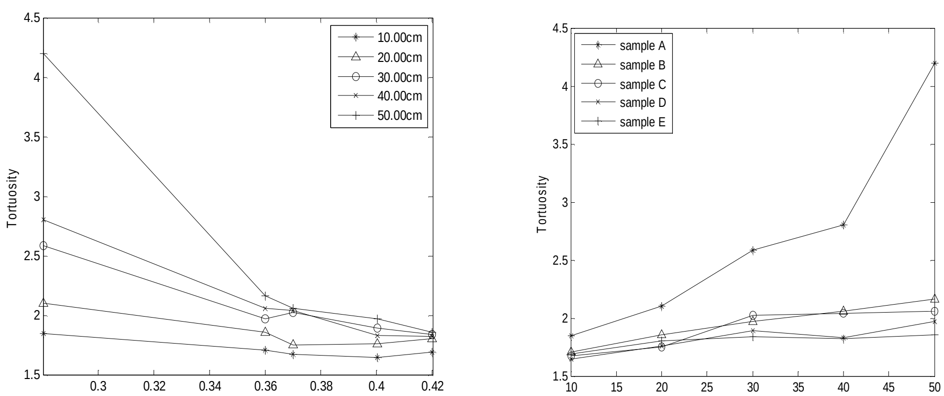
3.0 Results and Discussion

We conducted column experiment to investigate the relationship between tortuosity with porosity and depth; and later compared the results with the recovery of bacteria using the same design and column calibration. Past studies on the effect of tortuosity on the bacteria motility like that of Duffy et al.[2], did not base their observation on the experimental data of tortuosity but mere model prediction. They observed a complications in their results due to presence of porous media texture and nature of the chemotactic mechanism, their application of chemical attractants would not produce a clear relationship between pore morphology and the bacteria motility and thus cannot be use to characterize the use of porous media for groundwater supplies source protection with such parameter. In our work, we performed the experiment using an independent experiment to determine this parameter under no assay as this could be the best way by which sand media can be characterized with flow parameter. Table 1 presents the values of tortuosity at different depths for five porosities (i.e sample A(0.28), sample B(0.36), sample C(0.37), sample D(0.40) and sample E(0.42)).

Table 1: Experimentally determined values of tortuosity at different depths for five samples of varying porosities

	Tortuosity				
Porosity(ϕ)	10.00 cm	20.00 cm	30.00 cm	40.00 cm	50.00 cm
0.28	1.850	2.100	2.579	2.800	4.200
0.36	1.708	1.851	1.969	2.057	2.160
0.37	1.670	1.753	2.024	2.041	2.056
0.40	1.644	1.756	1.892	1.829	1.970
0.42	1.689	1.800	1.838	1.816	1.853

In Figure 2(a) we observed that tortuosity is approximately inversely proportional to porosity; the higher the porosity, the lower the tortousity. Porosity is function of size, shape and surface characteristic of the medium and the degree of compaction. The more rough the surface of particle the more tortuous the structure which can increase the likelihood of straining, adsorption and even die-off of bacteria that can be resulted from the effect of clogging. When the pore diameter is much larger than the run length of the bacteria, the motility is unaffected by the presence of the porous media except for the excluded volume, which is accounted for by the porosity[1]. This reflects the importance of porosity vis –a via tortuosity in quantification of bacteria migration in sand media.Also, it is critically revealed in Figure 2(b) that as depth increases, the tortuosity of all the samples increases. An increment in the tortuosity of the media would increase the travelling time of bacteria thus increases attenuation and retention which reduces the rate at which bacterial seep into groundwater supplies source.



(a) (b)

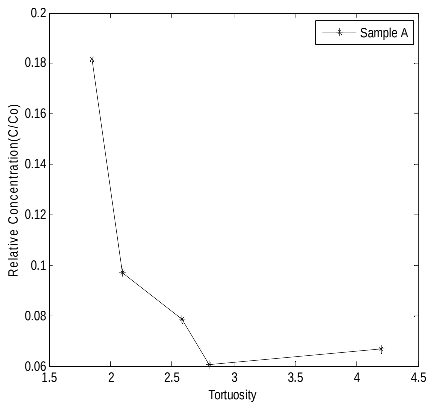
Figure 2(a) and (b) show the dependence of tortuosity on the porosity at different depths and on depth for various samples respectively. Tortuosity was found to be inversely proportional to porosity while it was approximately directly proportional to depth.

Inference on the relationship between tortuosity and migration of bacteria from column experiment were presented in Table 2. The Table showed the relative concentration C/Co of bacteria for five samples of different porosity and at different depth.

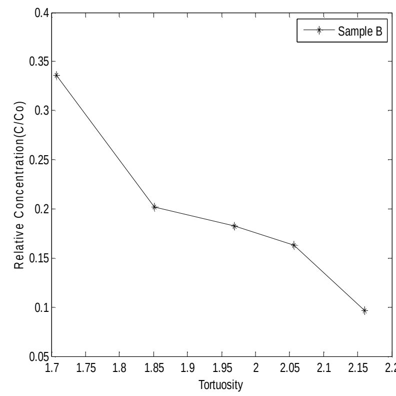
Table 2: Average values of relative concentration (C/C_o) for five drains at different porosity for the five depths considered (Escherichia coli).

Porosity (ϕ)	10.00cm	20.00cm	30.00cm	40.00cm	50.00cm
0.28	0.1818	0.0970	0.0788	0.0606	0.0667
0.36	0.3365	0.2019	0.1827	0.1635	0.0962
0.37	0.3067	0.2067	0.1667	0.1000	0.0800
0.40	0.5234	0.3738	0.3363	0.2533	0.1869
0.42	0.6916	0.6355	0.4953	0.4393	0.2804

Figure 3(a-e) presents the relative concentration of bacteria recovered for different samples compared with tortuosity. The figures depict a Brownian motion. The average reduction in the migration of bacteria cells is as a result of irreversible adsorption of bacteria to filter matrix which depends on the contact time between the bacteria and the porous medium. The more tortuous the path length taken by bacteria, the less the conductance properties of the fluid carrying the cells, hence the more the hindrance render to the flow and this will increase the adhesive between the bacteria cells and media matrix. Sample A showed the highest trend of bacteria migration attenuation and reduction with increase tortuosity followed by sample B up to sample E. For sample C, D and E, we observed a sudden decrease in C/Co as tortuosity increases



(a)



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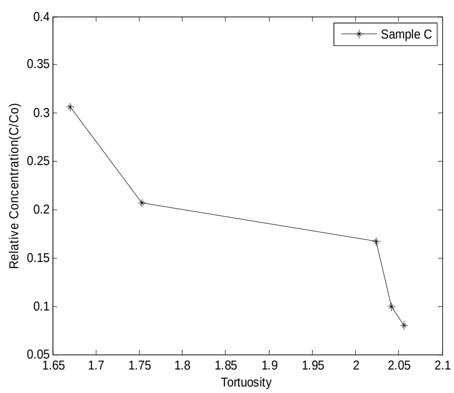

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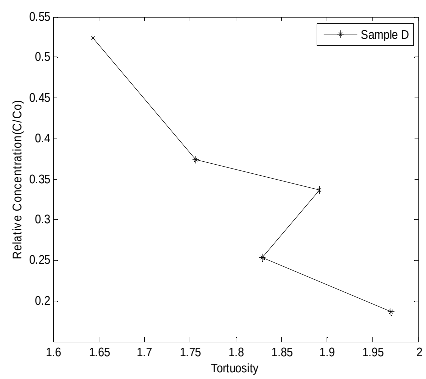
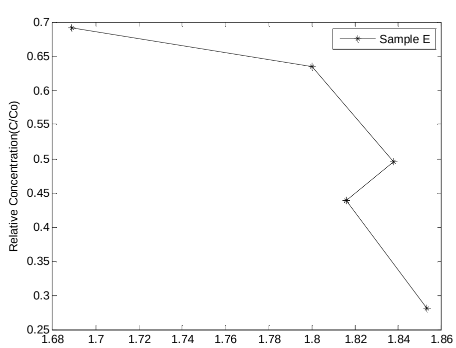


(d)



(e)

Figure 3(a-e): Plot of relative concentration of bacteria (E coli) versus tortuosity for various samples beyond 1.85. This showed that when the flow path is highly tortuos, then, the flow distance will increase; consequently, long time of travelling will subsequently increases the number of contact between the bacteria cells and the media grains, thus high sticking efficiency and increasing the adsorption to the grain surfaces. This would increase the coefficient of attenuation. High tortuous path length will increase the number of collision between bacteria cells and porous media hence increase the rate of adsorption as this is an important mechanism influencing bacteria transport in porous media [12, 13, 14]. The high difference observed in tortuosity between sample A and the rest is due to high margin between their porosity. We observed that as porosity increases, say sample C, D and E, the response of bacteria elusion is fluctuating with tortuosity showing that tortuosity is most relevant when quantifying the transportation of bacteria in fine to medium size particles.

4.0 Conclusion

We conducted column experiments to investigate the effect of tortousity on the migration of bacteria in saturated sand media. First, we examine the influence of media depth and porosity on the tortuosity and we observed that tortuosity is inversely proportional to the porosity and directly proportional to the depth. We further compared the relative bacteria concentration with toutuosity and concluded that increase in the tortuosity of the porous media reduces the migration of bacteria. The implication of these experiments with respect to attenuation and retention of bacteria in saturated porous media is elucidated (i.e the mass concentration of bacteria that could be retained was purely depends on the geometry of the media of which tortuosity quantified).

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