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IMPACT OF DIFFERENT FREQUENCIES IN THE ENTRAPMENT OF BACTERIAL PATHOGENS FROM DRINKING WATER USING DIELECTROPHORETIC PHENOMENA

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The article has investigated the removal of water borne pathogens using dielectrophoresis (DEP) filter which is energized by varying the frequency of the applied potential from 10 kHz to 2 MHz with different voltage levels of 5; 10; 15 and 20 V. Separate experiments are conducted in artificially contaminated water samples with Escherichia coli, Staphylococcus aureus and Vibrio cholera up to 2 h. The impact of signal frequency and voltages on DEP based water treatment system has been analyzed statistically. Results have demonstrated that an ac signal of 20 V with frequency range of 500 kHz to 2 MH_Z is suitable to remove the tested bacterial population and the rate of removal of E. coli is the highest with a dielectrophoretic filtration efficiency of 77,1%.

Keywords: water borne pathogens, drinking water, frequency, dielectrophoretic phenomena.

INTRODUCTION

Water-borne pathogens have been the primary causative factor for high mortality. The World Health Organization report revealed that more than 2,5 million peoples die in a year throughout the world due to water-borne maladies.Almost 80% of diseases and over one third of mortality in developing countries are caused by the consumption of contaminated water which invoked an increased level of public and professional concern

about water safety in the light of reported outbreaks of water borne diseases and recognition of new causative agents of diseases [1]. Although inadequate drinking water and sanitation are the major causes of morbidity and mortality but appropriate treatment of water using a suitable method paves the way for obtaining pure and safe drinking water. Among many water borne pathogens, *Escherichia coli*, *Staphylococcus aureus* and *Vibrio cholera* were found to be more harmful for human health [1]. The existing water treatment filter systems which utilize pores to trap the contaminant particles have the drawback of clogging and choking of particles which require frequent maintenance and leads to increased cost of operation [2]. The systems demand a very long time to obtain the expected microbiological results [3] and not suitable for providing a fast, real time diagnosis in the event of emergency [4]. The traditional microorganism separation methods, such as electrophoresis, have their own inherent limitation that the microbes are separated based on their characteristic charge-to mass ratio and is not selective and prone to variations in various chemical environments [5]. The movement of the particles in the electrode determined by the dielectric properties (conductivity and permittivity) [6]. Dielectrophoresis (DEP) provides an alternation to conventional methods because of its ability to concentrate and separate microorganisms in a selective, rapid and reversible manner [3, 5]. The motion of a particle due to the unbalanced force present in a non-uniform electric field pulls the particle electrostatically along slope of electric field [4] which produces an unbalanced electrostatic force on the charge in a particle referred as DEP. This mechanism has non-linear dependence on electric field and is able to perform microorganism concentration as well as separation in water monitoring systems. Dielectrophoretic phenomena for removing food borne pathogens using a DEP chip with a fixed ac voltage and frequency of 20 V and 1 MHz. The signal frequencies are varied and the performance of DEP based traps for bacterial detection was analyzed in [7]. However the impact of voltage and frequency dependant properties of DEP phenomena for drinking water treatment has not been exploited yet.

In our present investigation, we have made an effort for removal of selected water borne pathogens from artificially contaminated water using Dielectrophoretic system. Moreover, quantitative analysis has been made regarding the changing properties of DEP phenomena with respect to changing frequency and voltages in trapping the pathogens from contaminated water.

Experimental

Three different bacteria *Esherechia coli, Staphylococcus aureus* and *Vibrio cholera* were isolated from drinking water to prepare artificially contaminated water samples separately [8] and known volumes of colums were added in sterile deionised water.

To check the efficiency of DEP 100; 50 and 10 μl, of 16 – 18 h aged inoculum was added in 99 ml of deionised water and prepared the dilution of 10^2; 10^3; $10^{4,}$; 10^5. From this 0,1 ml of inoculum was transferred into nutrient agar and triptic Soy agar ("Himedia", India) plates and incubated at 37°C for 24 h to obtain viable cell count with triplicates. The CFU in every dilution was quantified using colony counter. To get the load of bacterial cell, spectrophotometer observation was performed at 530 nm [2].

The dielectrophoretic chip design [2,5] consists of two conducting electrodes with thickness of 0,54 mm and a length of 15 mm copper sheets are placed in parallel with a gap of 2 mm. A transparent glass chamber with thickness of 5 mm, length of 15 mm and width of 10 mm covers them and tiny glass beads of borosilicate with 2 mm diameter are placed between the copper electrodes such that the resulting gap is filled with glass beads in single file and to find the strong electric field areas on the surfaces to trap cells. The glass chamber is provided with an entrance and exit ports for application and collection of contaminated sample. The copper electrodes are electrically energized with an AC sinusoidal waveform from function generator with adjustable frequency control and an instrumental amplifier [9] provides variable output voltage levels.

In the designed DEP filter system, 1 ml of bacterial inoculum added in deionized water (Average no of CFU/mL) was circulated for 2h (flow rate of 1 mL/min) at room temperature (34°C). Absorbance (530 nm) and viable cell counts of the bacterial suspension were measured before and after circulation.

An AC signal applied in the voltage range of 5; 10; 15 and 20 V with different frequencies ranging from 10 kHz to 2 MHz for 2 h with artificially contaminated water. The processed sample was plated again to obtain the viable cells and spectrophotometer reading was also taken in all samples. Dielectrophoretic filtration efficiency (DFE, %) is calculated by [2]:

$$DFE = [(N_i^v - N_0^v) - (N_i - N_0)]/ N_i^i \cdot 100.$$

The total filtration efficiency (TFE,%), also known as cell elimination efficiency, is calculated as,

$$TFE = [(N_i^v - N_0^v)] / N_i^i \cdot 100 ,$$

where N_i^v and N_0^v are the cell count before and after circulation with voltage application. N_i , N_0 the counts without voltage application N_i^i is the initial cell numbers.

The acquired data was analyzed using statistical tool SPSS 17. To describe the degree of relationship between two variables; the signal frequency and the spectrophotometer reading, we find the correlation using Karl Pearson's coefficient. To find the area of convergence we have classified the frequency range into three groups and standard deviation is applied for the groups of signal frequencies 10 to 50 kHz (Low_ Group), 100 to 300 kHz (Mid_ Group) and 500 kHz to 2MHz (High_ Group).

Results and discussion

In the present study, the ability of DEP filtration (DF) system to capture *E. coli, S. aureus and V. cholera* was examined in artificially contaminated water sample. Filtration is the most important method to remove the bacteria from liquid form of any sample. The earlier works [2, 10, 11] insisted upon the need of an alternative method to the existing ones due to the drawbacks such as cost, clogging of filter, time and periodical monitoring filtration system. The scarcity of the water and prevalence of water borne diseases and pollutants thereby increases the health hazards through water borne pathogens.

The initial bacterial count was estimated at 0 hour in colony counter and spectrophotometer (at 530 nm) (data not shown). Without the application of voltage, the microbial cells were not captured in the DF system [9]. The relationship between the migrations of charged unicellular bacteria when placed in an electric field was observed [11]. When the electrode voltage was increased, more cells were collected around the glass beads and also results increased rate of entrapment [3, 4, 12]. The initial value of *E. coli* at 0 V was 0,865 and it is reduced to 0,003 with a DFE of 77,1% and TFE of 82,08%. For *S. aureus*, the initial value of 0,094 is reduced to 0,003 with a DFE of 75,53% and TFE of 91,49 % and the amount of *V. cholera* is also diminished from the initial value of 0,912 to 0,009 with a DFE of 73,8% and TFE of 77,41%. The results are presented in Table 1 and the curves shown in Fig 1 – 3 graphically depict the comparison of decreasing bacterial population by the impact of changing applied frequency and voltages. The graphical illustration suggests that 20 V is

the most suitable voltage for curbing bacterial population effectively in all the three cases and also it is observed that beyond 1 MHz there is no noticeable reduction of bacterial count.

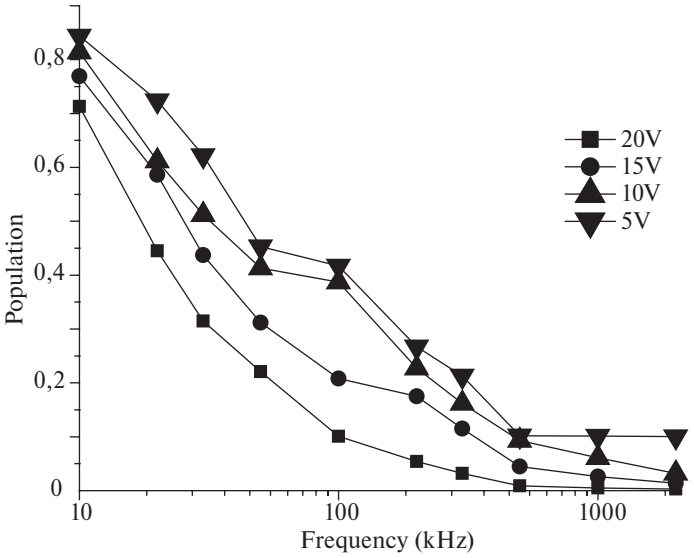

Fig. 1. Comparison of E. coli population.

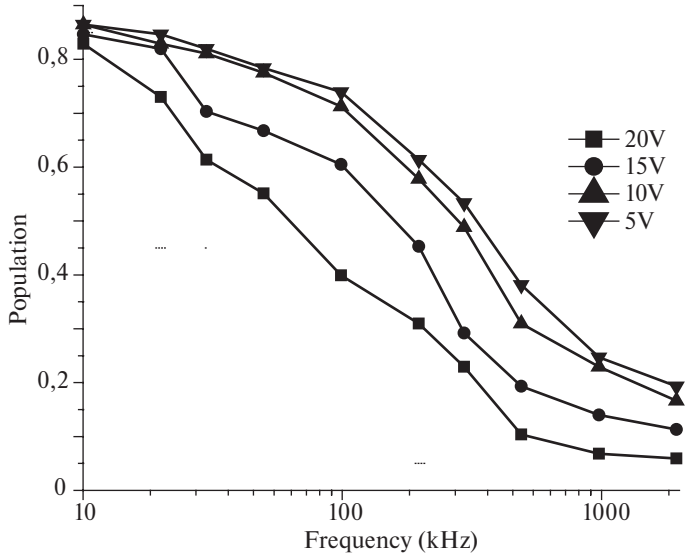

Fig. 2. Comparison of S. aurues population.

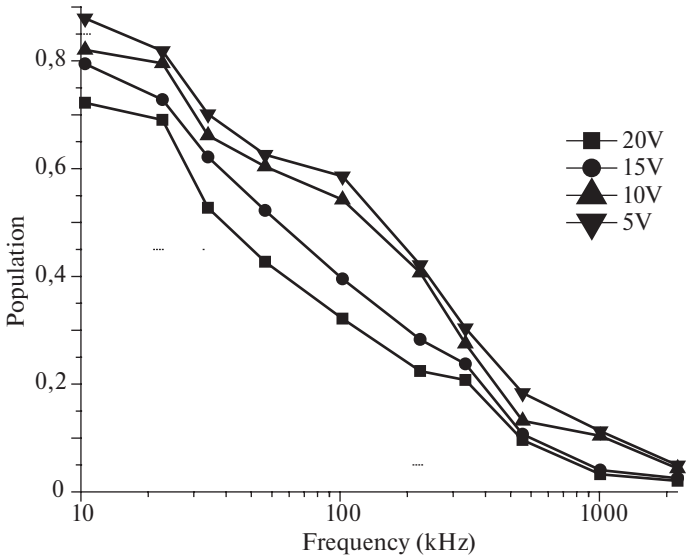

Fig. 3. Comparison of V. cholera population.

Further, we have found that the application of ac voltage has a negative correlation with the bacterial count in all the three samples. The correlation is more negative in *S. aureus* (−0,877) with 5 V and it is less negative in *E. coli* (−0,528) with 20 V applied. The result (negative correlation of −0,5 to −0,8) demonstrates the effective elimination of bacterial population (Table 2). The negative correlation between the applied voltage and bacterial count is graphically shown in Fig. 4. Moreover, the obtained values of filtration efficiency are also dissimilar for different tested bacteria and the bacterial size, morphology and the movement of the cell under electric field may probably be the reasons behind the differentiation of entrapment of tested bacterial cells. Since high Joule heating take place due to the immersion of electrode at high electric field, the behavior of biological cell is not only influenced by DEP but also by the thermal convection of flow of liquid in the system [4, 13, 14] which altogether leads to high entrapment of biological cells at high voltage. The applied signal voltage is increased from 5 V and up to 20 V in order to enhance the generated DEP force to overwhelm the drag force exerted by liquid flow in the DF, thus the particles which are suspended could be trapped [4, 15, 16] and eliminated from the flowing liquid.

Table 1. Efficiency of DF in removal of bacteria at 530 nm

Organism	Voltage(V)	Frequency (kHz/MHz)									
		10	20	30	50	100	200	300	500	1	2
E. coli	20	0,713	0,445	0,315	0,221	0,101	0,054	0,032	0,009	0,005	0,003
	15	0,769	0,586	0,437	0,312	0,208	0,175	0,115	0,045	0,026	0,014
	10	0,814	0,612	0,512	0,413	0,387	0,227	0,162	0,093	0,061	0,032
	5	0,844	0,724	0,622	0,453	0,417	0,267	0,213	0,102	0,1012	0,1005
S. aurues	20	0,089	0,078	0,065	0,058	0,041	0,031	0,022	0,008	0,004	0,003
	15	0,091	0,088	0,075	0,071	0,064	0,047	0,029	0,018	0,012	0,009
	10	0,093	0,089	0,087	0,083	0,076	0,061	0,051	0,031	0,022	0,015
	5	0,093	0,091	0,088	0,084	0,079	0,065	0,056	0,039	0,024	0,018
V. cholera	20	0,715	0,683	0,519	0,418	0,312	0,214	0,197	0,085	0,021	0,009
	15	0,788	0,721	0,614	0,514	0,386	0,273	0,227	0,096	0,029	0,014
	10	0,814	0,789	0,654	0,596	0,534	0,398	0,265	0,121	0,093	0,032
	5	0,873	0,812	0,694	0,618	0,578	0,412	0,294	0,173	0,102	0,038

Table 2. Correlation between spectrometer reading (530 nm) Vs signal frequencies

Bacteria	Voltage (V)	Correlation (*r*)
E. coli	5	-0,668
	10	-0,694
	15	-0,626
	20	-0,528
S. aureus	5	-0,877
	10	-0,855
	15	-0,777
	20	-0,723
V. cholerae	5	-0,798
	10	-0,786
	15	-0,745
	20	-0,719

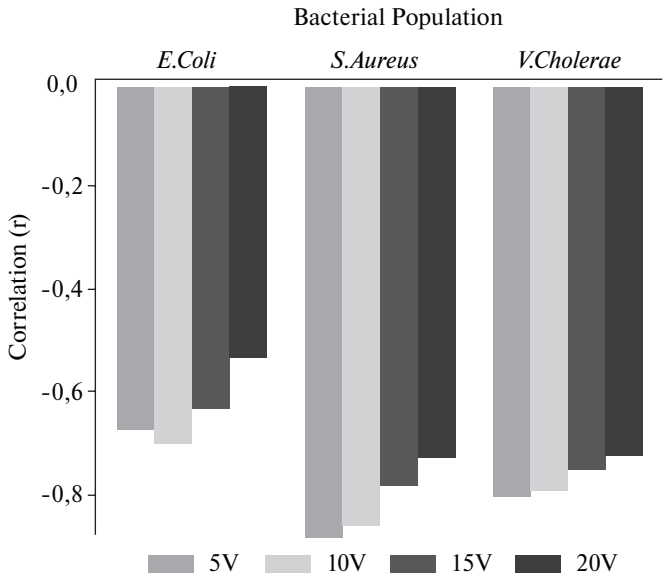

Fig. 4. Correlation among bacterial population.

The area of convergence of maximal elimination of bacterial population over the entire frequency range is estimated by assorting the frequency into three regions; namely, Low_ Group, Mid_ Group and High_ Group. The Mean and Standard deviation of spectrometer reading for the three groups of frequencies are listed in Table 3. The minimum deviation is present in the High Group of frequencies (500 kHz to 2 MHz) with a deviation of ±0,0065 and a mean of 0,0169 with 0,024 and 0,011 as maximum and minimum readings for *S. aureus* which indicates the area of convergence.

Table 3. Mean and Standard deviation of spectrometer reading of different groups of signal frequency

Bacteria	Frequency classification	Mean ± Std Dev(Max, Min)
E. coli	Low_ Group	0,5495±0,1855 (0,785; 0,349)
	Mid_ Group	0,1965±0.0751 (0,278; 0.13)
	High_ Group	0,0492±0,0125 (0,062; 0,037)
S. aureus	Low_ Group	0,0826±0,0078 (0,091; 0,074)
	Mid_ Group	0,0518±0,0127 (0,065; 0,039)
	High_ Group	0,0169±0,0065 (0,024; 0,011)
V. cholera	Low_ Group	0,6763±0,1197 (0,797; 0,536)
	Mid_ Group	0,3408±0,1043 (0,452; 0,246)
	High_ Group	0,0677±0,048 (0,119; 0.023)

CONCLUSIONS

We have investigated an innovative approach for water treatment using Dielectrophoretic phenomena. Exceptional properties of electric signals such as frequency and voltage were utilized in our study in order to ameliorate the performance of DEP system in removing water-borne pathogens and ensure safe drinking water. We have tested the performance of our DEP system using artificially contaminated water samples with *E. coli*, *S. aureus* and *V. cholera*. Statistical analysis indicated that changing the signal properties contribute to the improved performance of the DF in removing bacteria. The highest

efficiency of DF for trapping tested organisms has been observed at 20 V and around 500 kHz to 2 MHz frequency.

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