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Anil Kumar & Sudip K. Pattanayek

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Imaging of bacteria using chromonic liquid crystals

Anil Kumar and Sudip K. Pattanayek

Department of Chemical Engineering, IIT Delhi, Hauz Khas, New Delhi, India

ABSTRACT

We explored adsorption isotherms of *Escherichia coli* (*E.coli*) and Salmonella over the poly-L-lysine (PLL) coated glass surface. The detection of the adsorbed bacteria was explored using 14 wt% di-sodium-chromo-glycate (DSCG) solution in water and 5CB. The textures of the optical cells made up of LCs and the adsorbed bacteria over PLL-coated glass substrate were obtained by using a polarized microscope in the transmission mode under crossed polars. We found that DSCG solution exhibits a dark spot on the adsorbed E.coli and Salmonella. In contrast to DSCG solution, 5CB did not show any dark spot.

KEYWORDS

DSCG; *E. coli*; Lyotropic liquid crystal; Polarized microscopy; Salmonella; Thermotropic liquid crystal

Introduction

Pathogens such as *Escherichia coli (E. coli)*, Salmonella, etc., cause numerous diseases [1,2], which can even lead to death of people in many developing countries. A simple, efficient, and cost-effective method of detection of these bacteria is a thrust area of research in diagnostics. The possibility of the use of liquid crystals (LCs) has received attention of the scientific community in bio-sensing applications, especially in the detection of bacteria. The recent reports [3–10] indicate that the presence of proteins, phospholipids, DNA, cells, and viruses can orient the director of LCs such a way that one can get the optical read-outs visible to the naked eye. 4-cyano-4-pentylbiphenyl (5CB), a thermotropic liquid crystal, is widely used to distinguish gram-positive and gram-negative bacteria [11]. The lipid present in the outer membranes of the bacteria cell disrupts the orientation of the nematic phase of 5CB. This leads to the ordering transition of 5CB in the presence of *E. coli* (a gram-negative bacteria). The disadvantage of the use of 5CB is its high toxicity to bacteria [12]. The possibility of using a lesser toxic lyotropic chromonic liquid crystals (LCLCs) [12] in the detection of biological molecules has attracted various scientists. The anisotropic optical property of LCLCs phases is utilized for enhancing optical images for biosensors. Helfinstine et al. [13] have explored the use of di-sodium chromo glycate (DSCG), an LCLCs in the detection of anthrax causing bacteria *Bacillus atrophaeus* (BA). They have reported the distorted alignment of DSCG in the presence of the mixture of BA and anti BA. Xu et al. [14] have spotted a droplet of *E. coli* bacteria solution on rubbed polyethylene-imine (PEI) and subsequently the spot was covered with a droplet of 5CB. They found that a minimum number of adsorbed *E. coli* over PEI is required to distort the alignment of 5CB. To achieve the above number of adsorbed *E. coli*, 0.2 OD concentration of bacteria is required.

CONTACT Sudip K. Pattanayek sudip@chemical.iitd.ac.in Department of Chemical Engineering, IIT Delhi, Hauz Khas, New Delhi 110016, India.

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To achieve the number of bacteria above a threshold value, one can use a specifically modified surface to bind the bacteria. The orientation of DSCG near adsorbed bacteria has not been explored. To the best of our knowledge, we have not found any report on adsorption isotherm of *E. coli* and *Salmonella typhi* on poly-L lysine (PLL). Here, we have explored the adsorption isotherm of two bacteria *E. coli* and *Salmonella* on poly-L lysine (PLL) and subsequently studied the orientation of the nematic phase of DSCG over the adsorbed bacteria on PLL.

Materials and methods

Glass slides were obtained from Fisher Scientific. Poly L-lysine (0.1%), 4-cyano-4-pentylbiphenyl (5CB), DSCG were purchased from Sigma Aldrich, India. The molecular weight of PLL is about 200 kDa. *E. coli* (type DH 5 alpha) and *Salmonella typhi* were kindly provided by Professor V. Perumal of School of Biological Science, Indian Institute of Technology Delhi. All solvents, used in these experiments, were HPLC grade. A stock solution of phosphate-buffered saline (PBS) was prepared and diluted to a final concentration of 10 mM (pH 7.4).

Cleaning of glass slide. Cleaning of the glass slides was done through a severe cleaning procedure to ensure the removal of all contaminants present on the surfaces. The microscope glass slides were ultrasonically cleaned with detergent solution for 30 min and then rinsed with distilled water for several times to remove oil. It is kept in a sonication bath with freshly prepared piranha solution (mixture of H_2SO_4 and 30% H_2O_2; v/v = 7/3) for 1 hr at 80 °C. The slides were then rinsed thoroughly using de-ionized water, ethanol, and methanol in sequential steps. The cleaned slides were dried under a stream of nitrogen.

Preparation of poly-L lysine-coated glass slides

This is done according to the method outlined in references [15,16]. Cleaned glass slides were dipped into a solution of 0.1% aqueous poly-L-lysine (PLL) in a humid chamber. After an hour, the poly-lysine-coated glass slides were rinsed thoroughly with de-ionized water and subsequently dried under a stream of nitrogen gas. The base substrate, glass surface caries negatively charge, and can bind with positively charged PLL chain, which can adsorb the negatively charged bacteria. Various studies have shown that the structure and activities of bacteria are not affected by its adsorption. There exists a controversy on the required conditions to keep the adsorbed bacteria healthy on the PLL substrate in the literature. Reports suggest [15,16] that a thick layer of the PLL on a glass substrate and small molecules of the PLL are not desirable for the survival of the adsorbed bacteria. So, we have chosen high molecular weight PLL for our experiments.

Preparation of bacteria solutions

The received solutions of bacteria (*E. coli* and *Salmonella*) were centrifuged and re-dispersed in PBS buffer to obtain the stock solution of optical density (OD) 1. The OD of the solutions was measured using UV/Vis spectrometer with the identified peak at 600 nm. The concentration of bacteria was adjusted to the required OD by addition of buffer solution.

Bacteria decorated surfaces and its analysis

The methodology to obtain the bacteria decorated surfaces for doing experiments with atomic force microscopy (AFM), optical microscope in phase contrast mode, and polarized microscope with liquid crystals is as follows.

Atomic force microscopy

About 200 μL bacteria solutions of various OD were spotted on PLL-coated glass slides. The slides were incubated in a humid chamber maintained at room temperature to avoid evaporation of water. After about 30 min of incubation, the substrates were washed with buffer solution and subsequently with de-ionized water. The substrates were dried under a stream of nitrogen gas.

Tapping mode AFM measurement was performed using a Multi-mode SPM with controller III A (Veeco Instruments, USA) with a high-aspect ratio tapping tip. A scanning rate of around 0.7 Hz was used.

Optical microscopy in phase contrast mode

The samples were made by spotting a droplet (about 200 μL) of a bacteria solution of a required OD over PLL-coated glass surface. The assembly was kept in a humid chamber for about 1 hr, allowing the bacteria to adsorb on the PLL. The assembly was cleaned with buffer solution and observed under microscope.

The surface densities of *E. coli* or *Salmonella* were obtained by analyzing the photographs obtained from the optical microscope (Olympus IX-71) in the phase contrast mode. The total number of bacteria in each image was counted. The surface density of bound *E. coli* and *Salmonella* were calculated by an image processing software.

Polarized microscopy with bacteria solution

Two strips of spacer of thickness 10 μm were placed over a PLL-coated glass slide. A droplet of about 1 μL bacteria solution of required OD was put at the middle of the slide. The assembly was kept in a humid chamber for 30 min. It was washed with freshly prepared buffer solution and dried under nitrogen gas. The bacteria decorated glass slide was covered with poly-L lysine-coated glass slide. The cell contains two glass slides: one glass slide decorated with bacteria and another with only PLL-coated glass slide. The cell made in this way was secured with two binder clips. A few microlitre 14% DSCG solution was then drawn into the cavity formed between the two glass surfaces through capillary force. The textures of DSCG were observed using a polarized microscope (Olympus IX-71) in the transmission mode. The images were captured by a digital camera (DP-71) mounted on the microscope.

Results

Figure 1 shows optical images of *E. coli* and *Salmonella* adsorbed from different OD solution on the PLL surface. The images were analyzed using image-J software. The number of spots its area in the images, which corresponds to adsorbed bacteria, are determined by the software. The average size of *E. coli* and *Salmonella* on the surfaces are about 16 μm and 14 μm, respectively. The minimum area of spots that can be observed by this method is 6.25 μm^2. Interestingly, the reported [17] size of *E. coli* and Salmonella are 0.5 $\times$ 2 μm and 1.5 $\times$ 2 μm, respectively. The area occupied by the bacteria as indicated by optical images is much more compared to the reported area of a single bacterium. This large difference in size (spots and

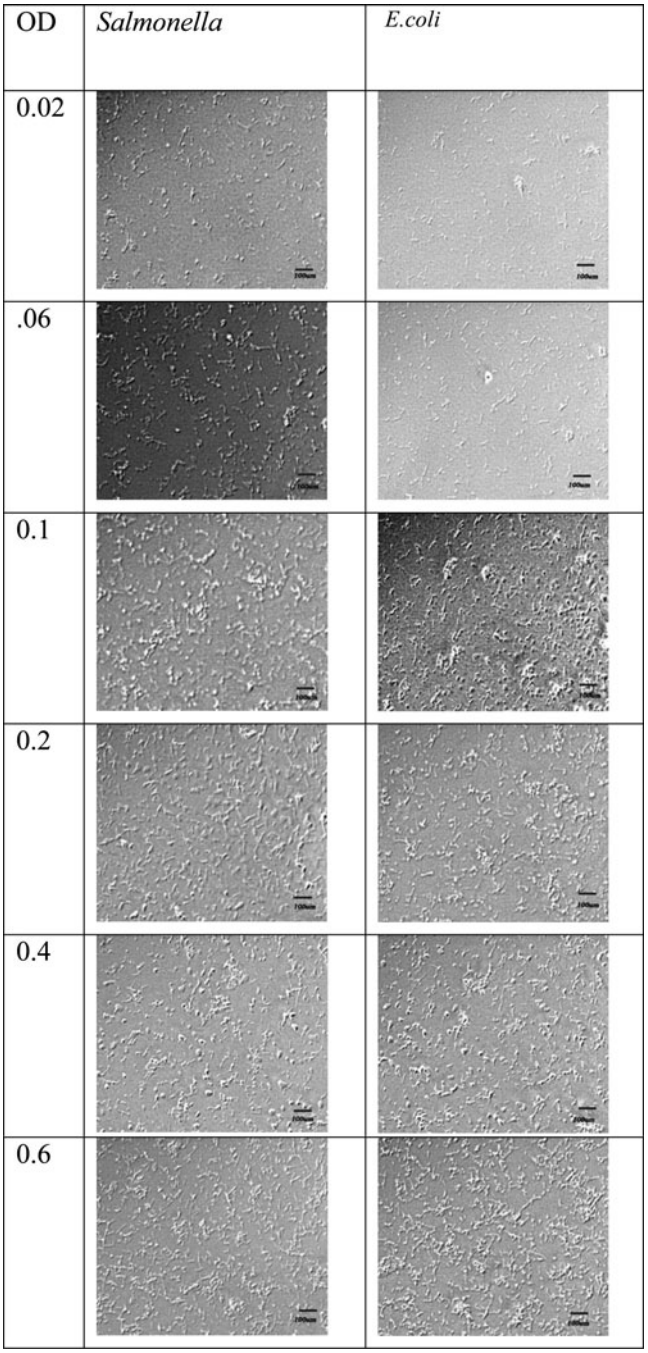

Figure 1. Adsorbed bacteria at PLL on glass slides. Salmonella (second column of panel) and E. coli (third column of panel) in phase contrast mode at different OD (first column of panel).

the size of single bacteria) indicates that the bacteria were present on the PLL surface in the form a clump.

In the above experiments, we could not account the individual bacteria at the adsorbing surface. So, we took AFM images (see Fig. 2) of the adsorbed bacteria to focus on the small size range of the bacteria at PLL surface. It is found that the small size of E. coli bacteria varies in

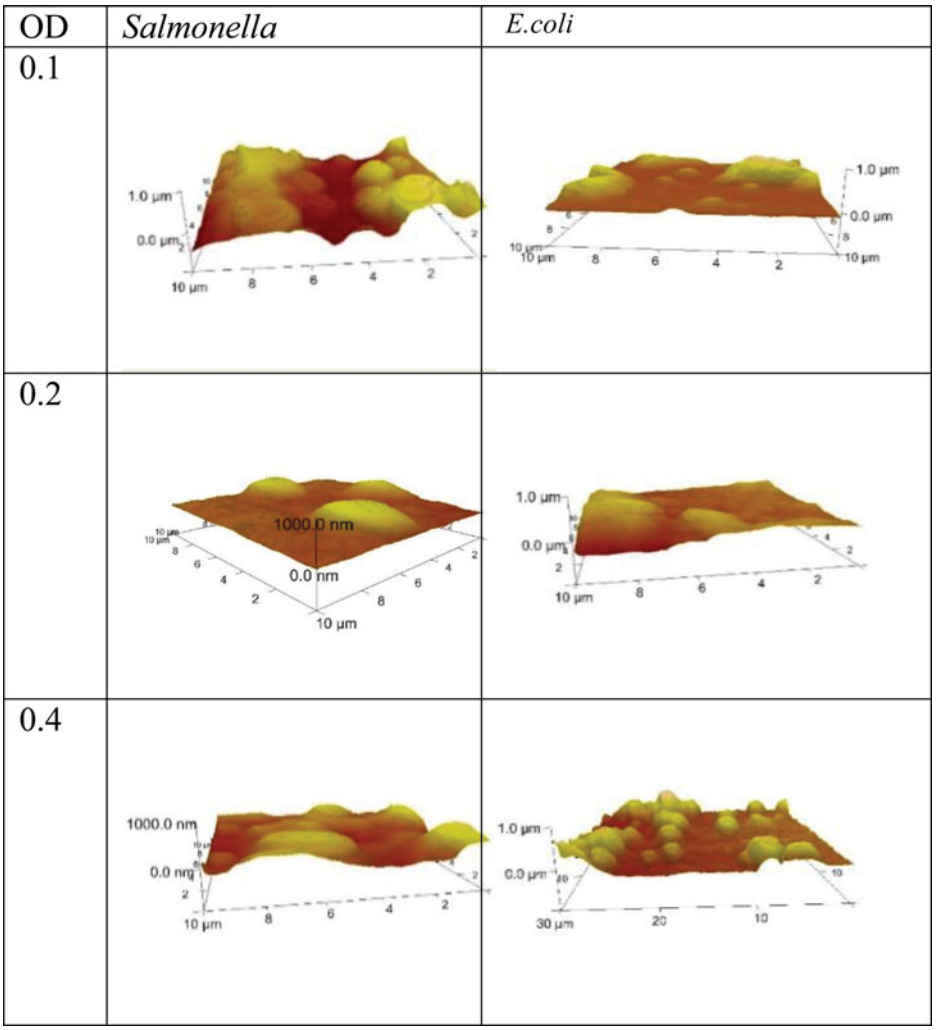

Figure 2. AFM images of adsorbed bacteria at PLL over glass slides. Salmonella (second column of panel) and *E. coli* (third column of panel) at different OD (first column of panel).

multiple of 2 μm and that of Salmonella varies in multiple of 1.5 μm. In addition, we observed that the thickness of *E. coli* and Salmonella are roughly 0.7 μm. These data match well with the reported [17] data.

Figure 3 shows the optical behavior of DSCG or 5CB inside a cell containing two glass plates or two PLL-coated surfaces under crossed polars. Glass surfaces are hydrophilic, which leads to the formation of Schlieren texture for the both LCs as shown in Figs. 3(a) and (c). Ideally, one would expect that 5CB would align homeotropically on the glass substrate due to interactions of silanol and CN group. As this interaction is very weak, Schlieren structure is observed. A similar observation is reported in reference [18]. On the other hand, the presence of PLL on the glass surface leads to the formation of fairly uniform texture for both the LCs (see Figs. 3(b) and (d)).

Figure 4 shows the texture of DSCG and 5CB on adsorbed *E. coli* and Salmonella from different OD solutions on PLL-coated glass surface. The first and second columns show the optical texture observed for DSCG on *Salmonella* and *E. coli*, respectively. The dark black spot appears for both Salmonella and E. coli, if they are adsorbed from a solution of concentration

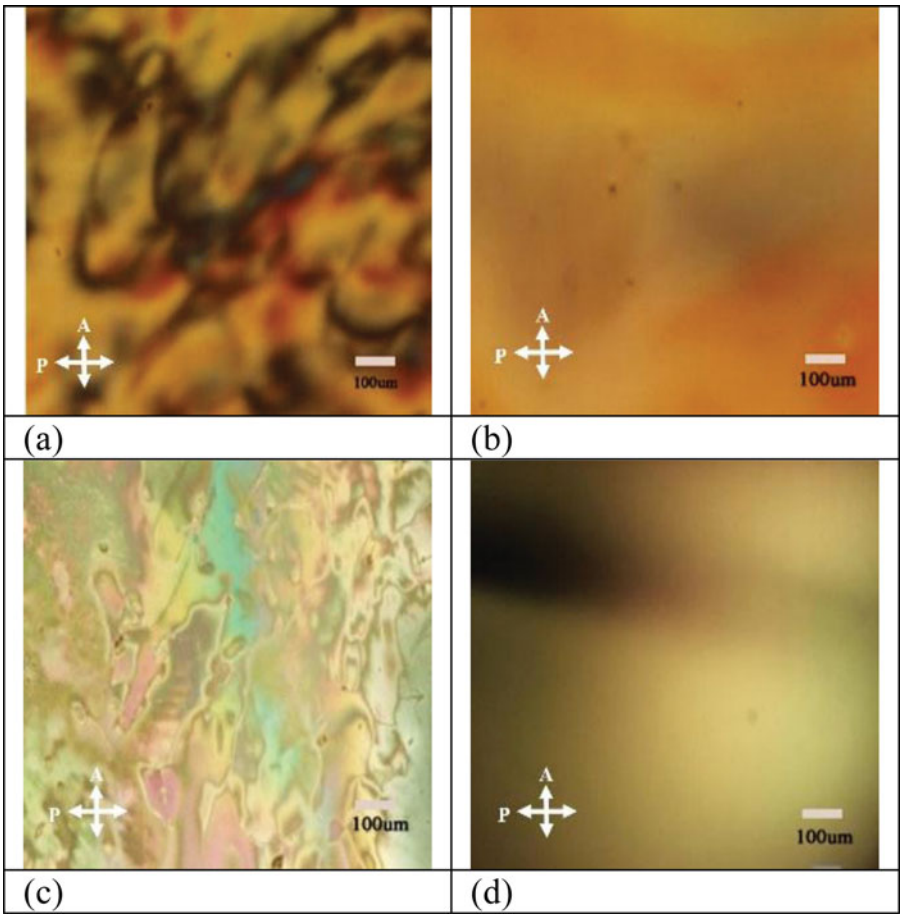

Figure 3. Texture of DSCG and 5CB in between two glass slides of cell as observed by using polarize microscope under cross polar conditions: (a) DSCG inside two cleaned unmodified glass slides. (b) DSCG inside two polylysine coated glass slides. (c) 5CB inside two cleaned unmodified glass slides. (d) 5CB inside two polylysine-coated glass slides.

above a threshold value. The threshold concentration of *Salmonella* and *E. coli* are 0.4 OD and 0.6 OD, respectively. The reason of lower threshold concentration for Salmonella is further investigated in the discussion section.

The observed optical texture of 5CB on the adsorbed *Salmonella* and *E. coli* is shown in the third and fourth columns of panel in Fig. 4. The concentration of solution of bacteria from which the adsorption took place was indicated in the figures. We found that the uniform planar arrangement of 5CB over PLL surface changes to nonuniform planar arrangement due to the presence of adsorbed bacteria. The absence of nonuniformity in the alignment of 5CB is due to the presence of randomly adsorbed bacteria on the surface.

Discussion

The dark black spots for DSCG over both *Salmonella* and *E. coli* adsorbing from solution containing bacteria concentrations above the threshold concentration (see Figs. 4(a) and (b)) is due to homeotropic arrangements of mesogen. This is confirmed by the following observation during our experiments. On rotating the sample in cross polar condition, no change of dark

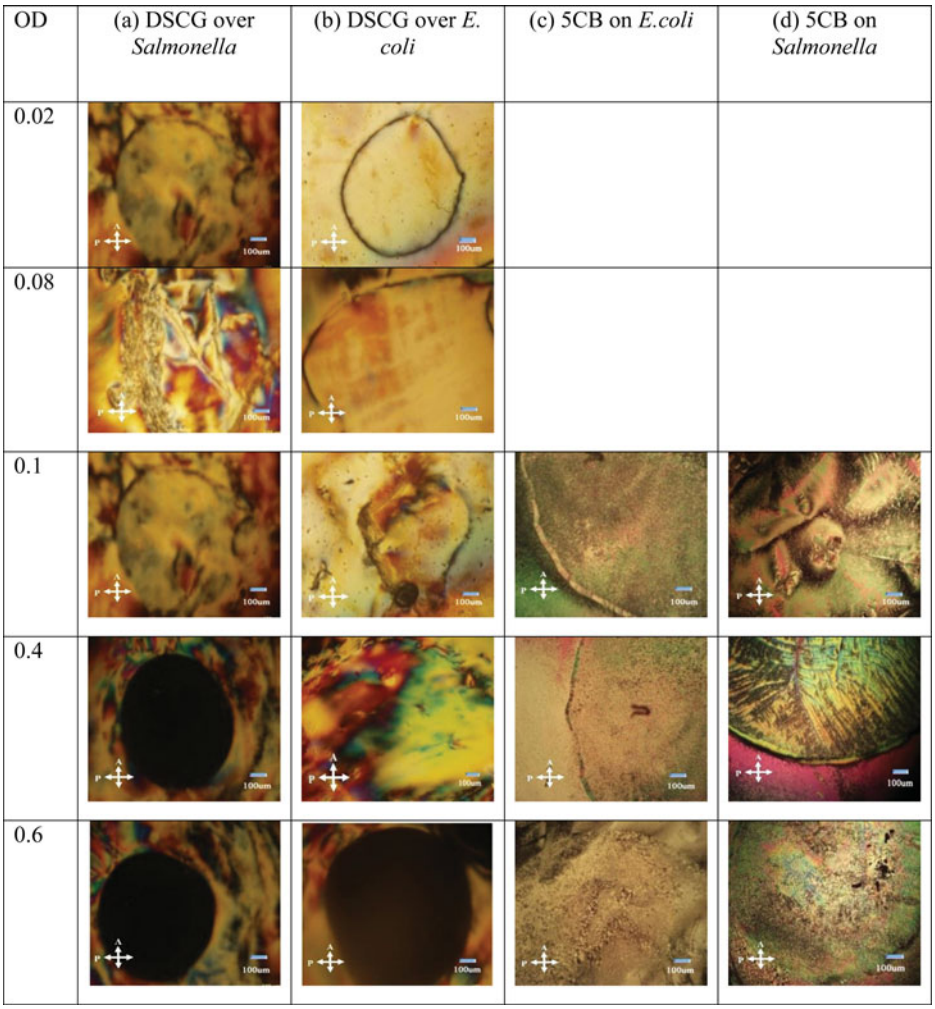

Figure 4. Texture observed by using polarized microscope in cross polar for liquid crystals inside the cells containing one glass slide with adsorbed bacteria over PLL-coated glass slides from solution of different OD (as indicated in first column of panel) and other glass slide with PLL: (a) DSCG over Salmonella. (b) DSCG over *E. coli*. (c) 5CB over Salmonella. (d) 5CB over *E. coli*.

spot was observed. This indicates that the black spot is due to homeotropic arrangements, i.e., perfect perpendicular arrangement of mesogen over bacteria at the surface. The bright regions outside the black spots in the optical textures of the above samples are due to a near planar azimuthal orientation of the LCs on the surface. The difference gives a clear distinction in the optical appearance of LCs as either dark or bright images.

The reason of lower threshold concentration of *Salmonella* than *E. coli* for the appearance of dark black spot is probably due to two factors: (a) a higher number of adsorbed *Salmonella* than *E. coli* on PLL-coated surfaces at the same concentration of bacteria and (b) higher negative charge on *Salmonella* than *E. coli*. The effect of the surface charge of bacteria is reported by Barak et al. [19]. They have found, the adhesion of Salmonella typhi is more than E. coli on various sprouts due to the higher negative surface charge of Salmonella. The number of *Salmonella* and *E. coli* at the adsorbing surface is determined from the analysis of Fig. 1. From the total area occupied by bacteria, we have calculated the fraction of the total adsorbing surface area occupied by bacteria (the maximum value be 1).

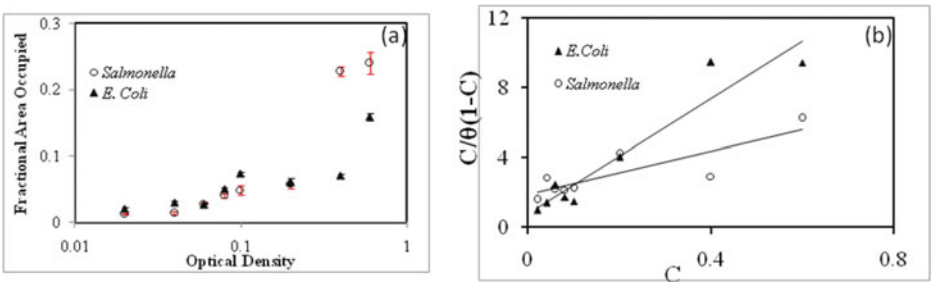

Figure 5. Adsorption isotherm of two bacteria. (a) Variation of bacteria density on surface with bulk concentration of bacteria, (b) variation dimensionless fractional area and concentration, C.

Figure 5(a) shows the variation of the fraction of total surface area covered by the bacteria adsorbed on the PLL surface with change in the concentration of bacteria in solution (OD of the solution). The fraction of the covered area by *Salmonella* and *E. coli* is similar at around 0.1 OD. At a higher OD, fraction of the area covered by *Salmonella* and its number is significantly higher. The visual observations of Fig. 1 also suggest that the number of spots on the surface increases with increase in OD. The area of a spot in Fig. 1 is proportional to the number of the adhered bacteria as a clump. We found out the area of spots, which was used to calculate the total number of adsorbed bacteria by dividing it with area of individual bacteria at the surface. At the highest concentration of bacteria, the total number of the adsorbed *Salmonella* and *E. coli* are 3.5×10^5 and 1.75×10^5, respectively. We note that the reported [14] number of adsorbed *E. coli* over PEI, a positively charged polymer to distort the alignment of 5CB is 5.19 $\times$ 10^4 mm^{-2}.

The observed adsorption isotherms of both bacteria (Fig. 5(a)) are similar and can be classified as type-IV adsorption isotherm. The area occupied by the bacteria reaches saturation quickly at lower bulk concentration. The area continues to remain constant up to certain high concentration of bacteria. With further increase in concentration, the occupied area increases rapidly and saturates at a high concentration. This type of adsorption is observed for the multilayer adsorption cases where mixtures of attractive and repulsive interactions are present. In general, the type-IV adsorption isotherm can be fitted by a modified BET equation [20] and valid for adsorption of small molecules. The BET equation on rearrangement can be written as

$$\frac{C}{\theta(1-C)} = \{1 + (K-1)\,C\}\,\frac{1}{\theta_m K}, \tag{1}$$

where θ is dimensionless surface coverage, θ_m is the surface coverage at saturation for monolayer, C is the dimensionless concentration, K is the parameter related to the binding intensity for all layers and is the ratio of equilibrium constants of adsorption for first layer and upper layer. The dimensionless concentration is defined as the ratio of equilibrium concentration and equilibrium concentration at complete saturation. Assuming OD of solution as equilibrium concentration and the concentration for complete saturation be 1 OD, we obtain the C value. The values of C and θ (corresponding to fraction of area occupied) are utilized to calculate the left side of the above equation. Fig. 5(b) shows the validity of above equation -1, for the bacteria adsorption. The graph is utilized to estimate the parameters of the equation. The obtained K value from the slope and intercept of lines (Fig. 5(b)) of the adsorption isotherms of *Salmonella* and *E. coli* are 4.3 and 21.8, respectively. These data indicate that Salmonella binds tightly to the surface through first layer and as well as in the upper layers; *E. coli* binds more tightly at first layer than that at the upper layers. For adsorption of *Salmonella* and *E.*

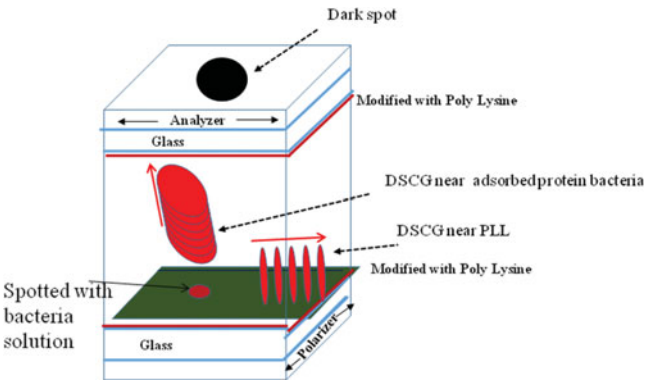

Figure 6. Schematics of arrangement of DSCG in cell over adsorbed bacteria and near polylysine (PLL). The red arrow indicates the direction of director.

coli, θ_m is calculated to be 0.12 and 0.06, respectively. The value of θ_m indicates that the area occupancy of Salmonella is twice than that of *E. coli*.

The reason of the homeotropic arrangement of DSCG on the adsorbed bacteria from an interaction perspective is discussed here. A sheet like structure of DSCG due to interaction of four DSCG molecules has been proposed by researchers [21]. The sheets stack together to give direction of the director, which is decided by the interactions among DSCG, surface, and bacteria. The alignment of DSCG near the adsorbed bacteria leads to homeotropic texture. Presumably, the negatively charged bacteria forces DSCG, which has negatively charged groups at the periphery of the molecule, to align its sheet perpendicular to the surface. The schematics of arrangement can be shown in Fig. 6. We note that the dark spot is found in the spotted region with the bacteria solution. The area outside the spotted place contains only polylysine and is expected to show planar arrangement of DSCG (see also Fig. 3). Thus, ideally one would expect the homeotropic arrangement at the location of droplet of bacteria, surrounded by the planar arrangement of LC. The upper PLL-coated plate is expected to give planar arrangement of DSCG. The planar arrangement is not going to affect the underlying homeotropic arrangement due to the presence of bacteria. We note that Fang et al. [22] have done experiments with 5CB inside an optical cell containing one of the substrates with PLL and another substrate with a biological cell covered over PLL.

On the contrary, had we used the glass surface as upper plate, one would have observed the Schlieren structure of DSCG. This might have affected the observation of texture due to the presence of bacteria. So, we have used the PLL-coated surface, instead of glass surface, as the upper plate. In addition, we have used both the plates bacteria decorated PLL substrates for some of the experiments (as shown in the appendix). Figure A1 in the appendix shows that nonuniform texture all over the cell. It is difficult to get any information due to our incapability of putting spots of bacteria symmetrically on both PLL-coated glass slides making the cell.

A report of similar system, Shiyanovskii et al. [23] have used an optical cell with two glass slides coated with the rubbed polyimide. They filled the cell with DSCG solution, antibody, and antigen-bearing particles. The planar arrangement of DSCG is reported to prevail for small, isolated particles, but distorted due to the growing immune complexes for antigen–antibody binding. Whereas we have observed that the presence of sufficient number of bacteria at the surface changes the planar director field of the mesophase to perpendicular arrangement.

Conclusions

We have demonstrated that PLL-coated surfaces are capable of capturing negatively charged *Salmonella* and *E. coli* through electrostatic attraction at neutral pH. The number of adsorbed bacteria depends on the bulk concentration of bacteria and its type. The strains of bacteria used are unable to give the directional arrangement of 5CB but capable of changing the orientation of DSCG in the perpendicular direction, which leads to a dark spot in the optical texture. The appearance of the dark spot depends on the number of the adsorbed bacteria on the PLL-coated surface.

Adsorption isotherms of the bacteria indicate that all adsorbed layers of Salmonella are tightly bound on the surface, but only the first layer of adsorbed *E. coli* is tightly bound on the surface. In addition, the study shows that the nematic phase of DSCG can give the homeotropic arrangement over the adsorbed bacteria. The system may be utilized as the LC-based bacteria identification method.

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Appendix

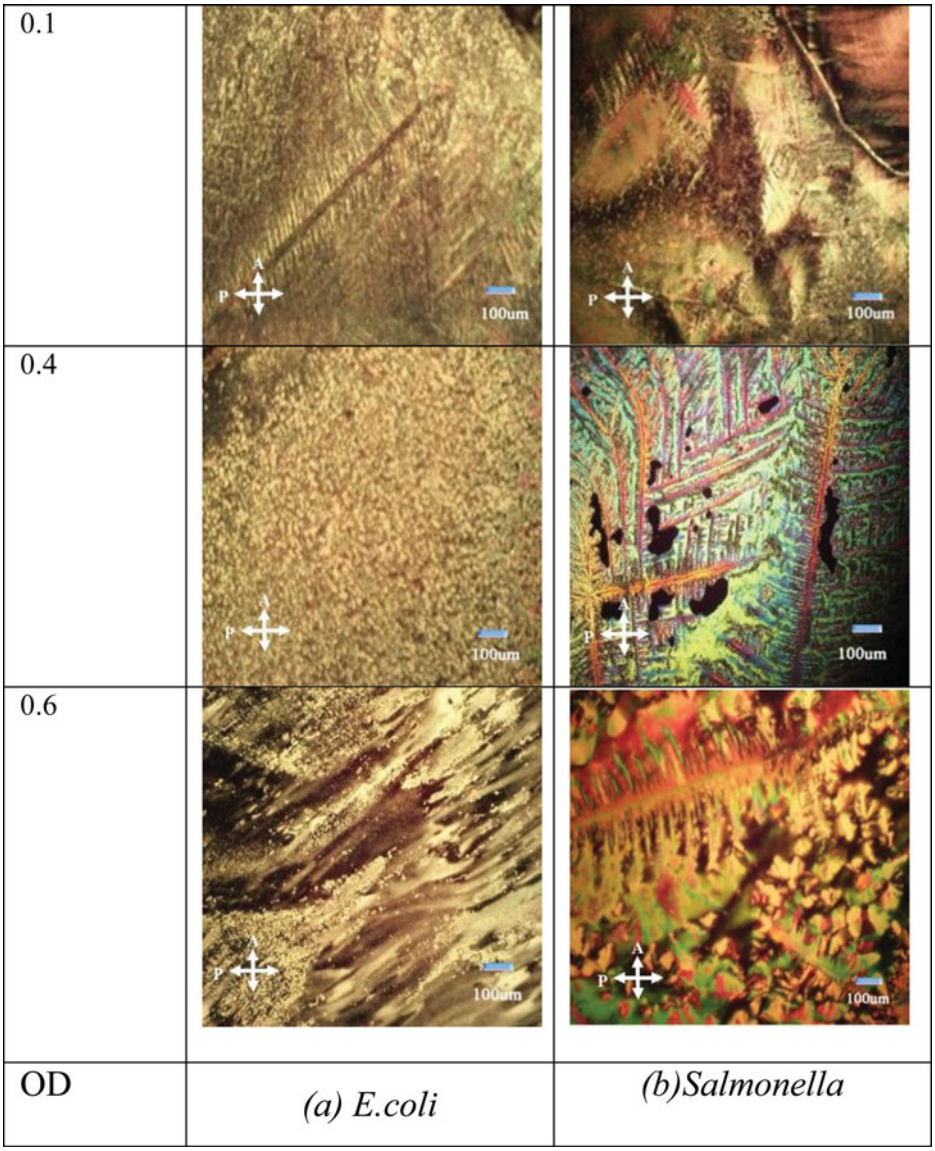

Figure A1. Texture observed by using polarized microscope in cross polar for 5CB inside the cells containing both glass slides with adsorbed bacteria over PLL-coated glass slides from solution of different OD (as indicated in first column of panel). (a) Using *E. coli*. (b) Using Salmonella.