

Electronic Supplementary Information: Fishing, trapping and killing of *Escherichia coli* (*E.coli*) in potable water

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1 Experimental setup

Figure 1 shows the experimental setup used to test the water treatment device. A two-prong extension clamp obtained from VWR Canada, was attached to a clamp stand to hold the cardboard scaffold containing the three paper strips in position. The height of the clamp was adjusted to keep only the lower edge of the bottom glucose strip (BGS) dipped inside sample water. Similar clamp arrangements were used in tracking the liquid/air interface in control experiments using single strips of NS and GS and in determining the efficiency of different GS. Glass beakers containing 60 mL of sample water were used in all the experiments.

2 Imaging system

The images of the bacterial dispersions in the chemotaxis assay were captured with an Omax digital camera mounted to an optical microscope using a ToupView software (ToupTek Photonics). A 0.5X reduction lens was connected to the camera using an appropriate adapter for reducing the image size to match the sensor of the digital camera.

To capture images of the fluorescence distribution on paper strips, a dark enclosure was created to prevent interference from ambient light. The light source and DSLR camera were affixed to the top of the enclosure while the paper strips were placed on the bottom wooden support (see Fig. 2). In order to capture the fluorescent emissions from tagged *E. coli* cells, a 575 nm bandpass filter was attached to the camera. The LED light source was attached with a collimator lens to narrow the light rays to our region of interest.

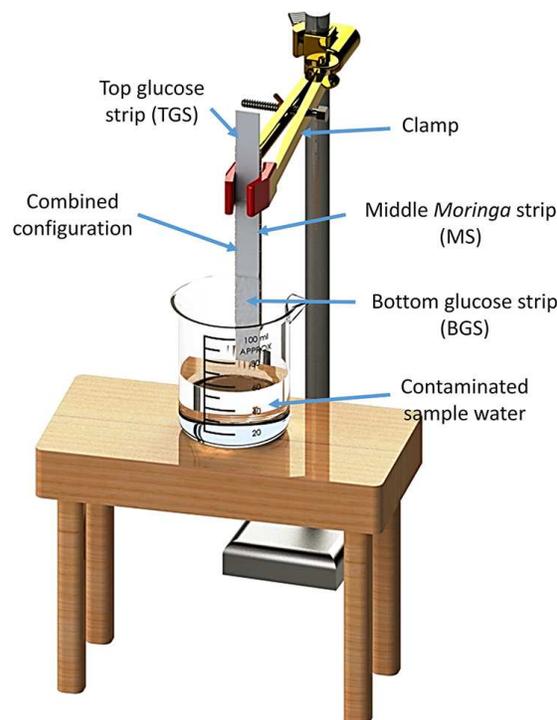


Fig. 1 The experimental setup used to demonstrate the ‘fishing, trapping and killing’ of *E. coli*.

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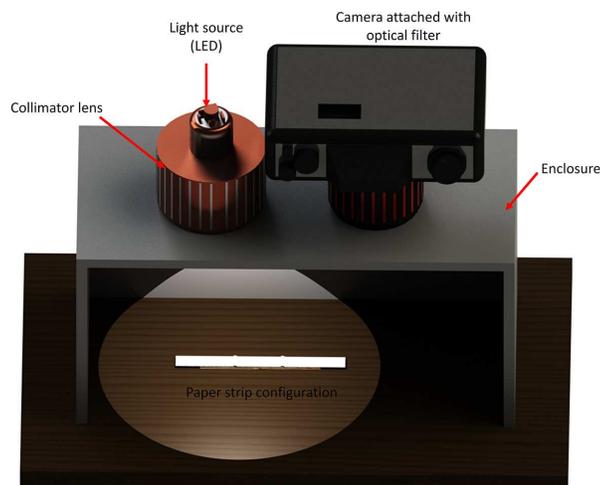


Fig. 2 The fluorescence and imaging arrangement used to capture the liquid/air interface and bacteria cells within the paper strips. A 575 nm bandpass filter is attached to the camera to capture the fluorescent emissions from the stained bacteria on paper.

3 Analysis of fluorescence distribution on paper strips

One of the critical challenges in performing fluorescence experiments with paper strips containing both the bacteria and the *Moringa* extract is that the MOCP present in the extract also fluoresce along with the tagged bacteria within an overlapping wavelength. The inherent fluorescence of most proteins arises due to the presence of the amino acids tryptophan and tyrosine¹. When excited by a light source at 295 nm, the emission spectra of purified MOCP is found to be dominated by tryptophan emissions² with a maxima at the wavelength of 343 ± 2 nm. Moreover, existing literature suggests that the emission spectrum of MOCP and Syto 9 (used for staining *Escherichia coli* cells) overlap around the 440 nm - 450 nm region^{2,3}. Therefore, if the paper strips are excited with a light of wavelength 490 nm (outside the emission spectrum of MOCP), we would only observe fluorescent emissions from Syto 9 and propidium iodide, devoid of any contribution from tryptophan and tyrosine, which would then confirm the presence and distribution of bacteria at the fluorescing regions. Based on the studies of Stocks³ and Kwaambwa and Maikokera², we also hypothesized that a 302 nm excitation wavelength would reveal a fluorescent distribution on the paper strips with contributions from both MOCP and the stained bacteria. This was confirmed in our observations displayed in Fig. 3. The paper strips illuminated by a 302 nm ultraviolet

light source (UV Lamp, Cole Parmer, Canada) showed increased fluorescence when compared with those imaged using a 490 nm light source, the former indicating the additional fluorescent emissions from tryptophan residues within the protein. The protein, being water soluble, is transported from the MS to the TGS along with the bacterial aggregates leading to larger fluorescing zones within TGS (see images after 15 minutes dipping time in Fig. 3). The control strip (GS) did not exhibit any additional fluorescing zones under 302 nm excitation thereby confirming the absence of any protein. The fluorescence in the control strips is solely due to the fluorescent stained bacteria.

We note a couple of key points in analyzing the fluorescent distributions caused by the protein and fluorescent tagged bacterial cells. First and foremost, quantification of the live and dead cells from these images based on the fluorescence intensity distribution were not carried out because of the porous nature and the considerable thickness of the blotting paper strips. Also Syto 9 has been known to show strong bleaching effect⁴ which considerably decreases the fluorescing intensity with time giving unrealistic results. Secondly, in our study we have used crude *Moringa oleifera* seed extract on paper. The *Moringa* seeds contain nearly 27% proteins by mass⁵ of which only 1.2% is constituted by MOCP⁶. This introduces a lot of water soluble proteins unaccounted for within the seed extract, which do not possess any flocculating or antimicrobial properties but contribute towards the fluorescence owing to the ubiquitous presence of tryptophan and tyrosine. Although using a 302 nm excitation wavelength minimizes the fluorescence emissions of tyrosine, the overlapping tryptophan emissions from the other water soluble proteins, make it almost impossible to distinguish the exact locations of MOCP in the used paper strips.

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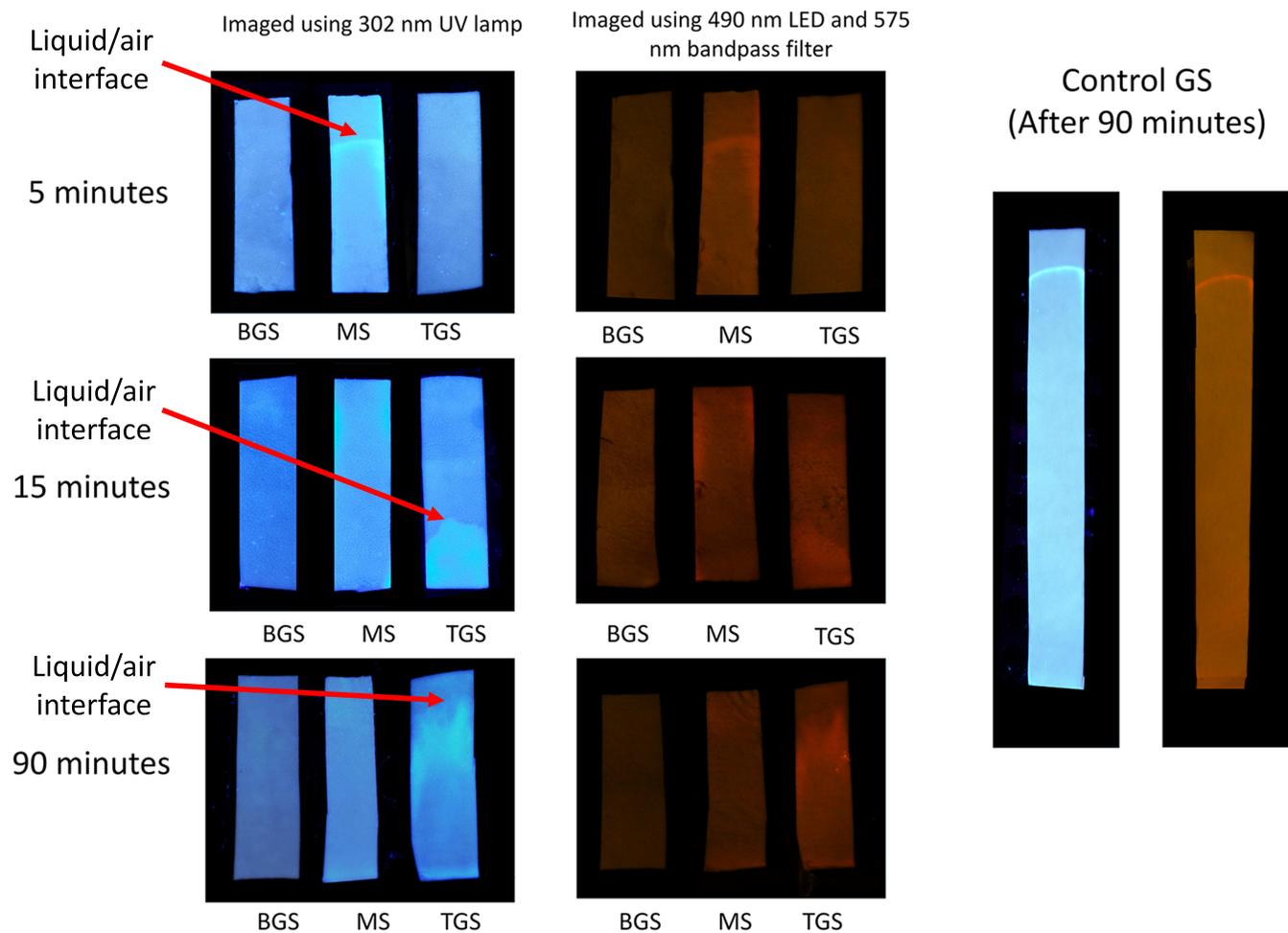


Fig. 3 Fluorescence observed on paper under different set of excitation wavelengths for different time instants during the experiment. The intact configuration was dismantled and laid out as three separate strips - bottom glucose strip (BGS), middle Moringa strip (MS), and top glucose strip (TGS) for visualization purpose. In the first 5 minutes, the liquid/air interface has moved into MS, as indicated here with appreciable fluorescence for both the light sources. After 15 minutes, the front has moved in the TGS, as indicated by the position of the liquid/air interface. At the end of experiment (90 minutes), a broader spread of fluorescence indicates the presence of bacterial clusters along with water soluble MOCP that has transported from MS to TGS. Comparison between the the left and the right panels for the dismantled strips shows greater intensity of fluorescence under UV light indicating the contribution of MOCP. The control strip (GS) at the far right panel did not show any extra fluorescing regions under UV light confirming that no additional fluorescence arises in the absence of *Moringa* seed extract.