Quantification of Escherichia coli via Analysis of β-glucuronidase Enzyme Concentrations

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ABSTRACT

Concentration of Escherichia coli can be quantified based on a digestive enzyme produced by 97% of E. coli strains called β-glucuronidase (β-GUS). When in contact with a β-glucuronide (β-GLU) molecule, the enzyme cleaves the β-GLU segment off the molecule, leaving the remaining fragment untouched. The remaining fragment can serve as a marker for the presence of the enzyme and can be quantifiably calibrated to determine the concentration of the E. coli in each sample. For a colourimetric method approach, 4-nitrophenol-β-D-glucuronide (4-NβDg) can be used as a dye for the enzyme. The remainder of the molecule after enzymatic cleavage is a 4-nitrophenol, which is blue in colour. The change in colour can be quantified based on a calibration curve. For an electrochemical method approach, 4-NβDg can also be used because 4-nitrophenol gives a characteristic cyclic voltammogram on a potentiostat. The change in resistance of 4-nitrophenol can be determined and calibrated to show the concentration of the E. coli in each sample.

1. INTRODUCTION

Escherichia coli is a highly aggressive, fast-growing and pathogenic bacteria species. It is naturally found in lower intestines of most warm-blooded organisms, most notably in cows. Due to its resistance and ability to colonize in nearly every environmental condition on earth, it is widely considered to be one of the most dangerous types of bacterial pathogens. The bacteria can cause hemorrhagic colitis and hemolytic uremic syndrome and those with a poor immune system are at higher risk. Levels of *E. coli* over 1 CFU / 25 g (colony forming unit) of ingested food are considered to be dangerous. By analyzing enzymatic byproducts rather than the bacterium itself, we can determine concentrations of *E. coli* with greater accuracy and durability for field testing.

Approximately 97% of all strains of *E. coli* produce the digestion enzyme β-glucuronidase (β-GUS). This is an intracellular enzyme responsible for the cleavage of D-glucuronic acid. By attaching a detectable marker to the D-glucuronic acid molecule, we can extrapolate a concentration calibration curve based on the presence of the marker in a sample.

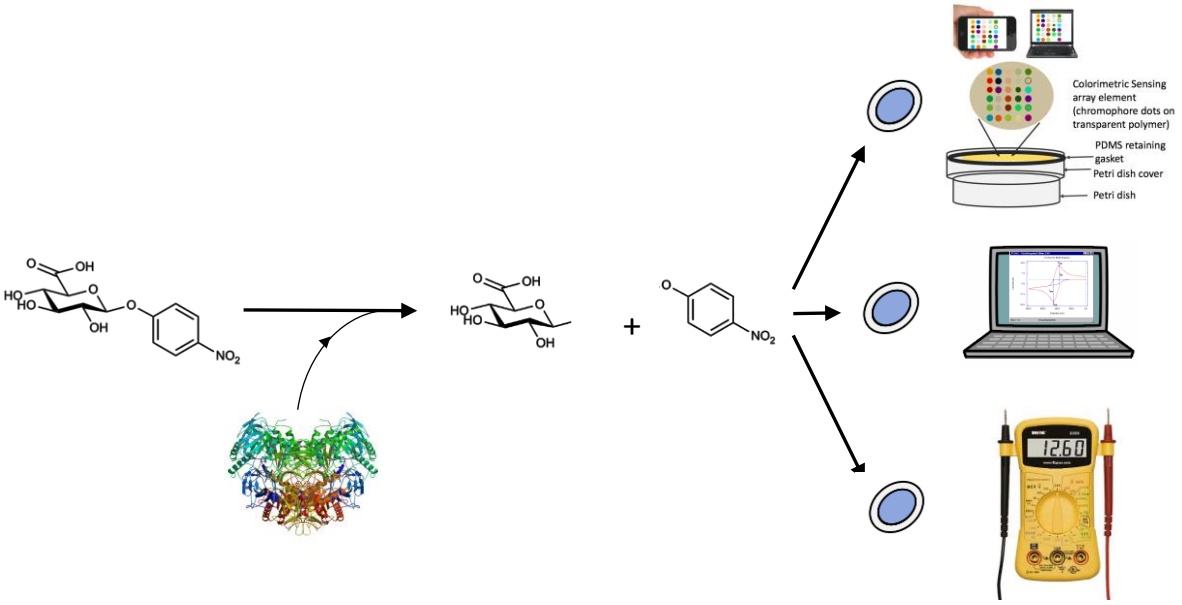

Figure 1: Graphical schematic of the breakdown of 4-NβDg to produce signals by colour change, cyclic voltammogram change and resistance change

2. MATERIALS AND METHODS

2.1.1 E. coli Culture Broth

Escherichia coli culture (strain KL25) is obtained from MacEwan University Biology department. VWR Culture Nutrient Broth is prepared at approx. 13 g/L for the cells to grow in and placed into six 250 Erlenmeyer flasks. An individual colony of *E. coli* cells is transferred using sterile inoculating loop into one of the broth-filled flasks. The flask is then placed in an incubator overnight at 37ºC and shaking at approx. 200 rpm. Once removed this flask is covered and stored at 4ºC for up to one month. The reproduction of the cells will stop at this temperature and therefore the concentration will remain the same.

2.1.2 E. coli Serial Dilution

The *E. coli* culture in the flask grows very quickly and, therefore, several serial dilutions must be performed. Each dilution is performed in a 2-mL centrifuge tube to allow

for low volumes. 100-μL of the stock broth solution is transferred into the centrifuge tube with 900-μL of 0.1 M phosphate buffer solution (PBS). This dilution has 10^{-1} times the concentration of the stock solution. A second dilution is made with 900-μL of 0.1 M PBS and 100-μL of the previous dilution. This dilution now has 10^{-2} times the concentration of the stock solution. Eight concurrent dilutions are made so that the final dilution has 10^{-8} times the stock solution. 100-μL of the last four dilutions (10^{-5}, 10^{-6},10^{-7},10^{-8}) are transferred and spread onto clean agar plates and allowed to incubate at 37ºC overnight. The result is individual colonies on the agar which, due to the incubation, started as single cells on the plate. Each colony can be counted and the number of colonies present is representative of the concentration of cells in the given volume.

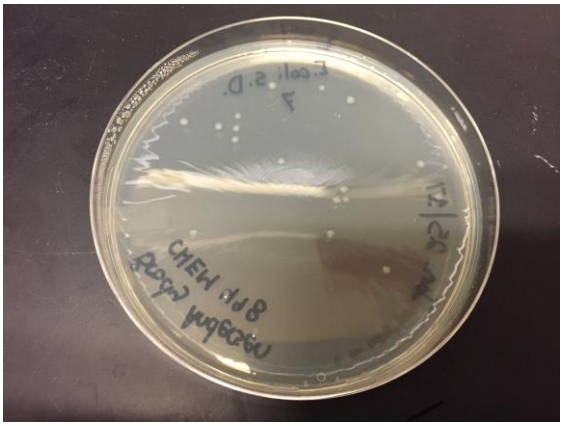

Figure 2: Serial dilution #7 on agar plate after incubation

Dilution	# of Colonies	[E.coli] / 100μL	[E.coli] / mL
5	Indeterminate	-	-
6	160	1.6×10^8	1.6×10^6
7	19	1.9×10^8	1.9×10^6
8	1	1×10^8	1×10^6

Table 1: Calculation of concentration of E. coli stock solution

2.2 Colourimetric Method

Using nanocellulose and enzyme-activated dyes, we have the potential to quantify Escherichia coli in a sample. A film is created by allowing a mixture of polyethylene oxide (PEO) and crystalline nanocellulose (CNC) in water to dry on an overhead transparency. Equal droplets (approximately 50μL) of 4-nitrophenyl β-D-glucuronide (4-NβDg) dye and of E. coli broth sample is placed on the CNC/PEO film. The cleavage of the 4-NβDg molecule occurs as below and the solution colour changes from colourless to blue. The intensity of the colour is quantifiable by image digitization, giving a figure for the deepness of the hue. This intensity produced is proportional to amount of enzyme β-

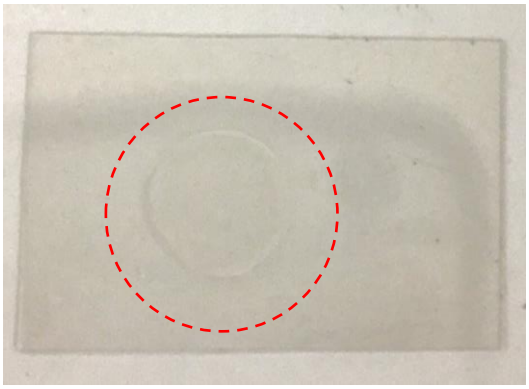

Figure 3: Hydrogel film comprised of polyethylene oxide and nanocellulose

glucuronidase (β-GUS) present and a calibration curve may be developed for the *E. coli* concentration.

2.3 Conductometric Method

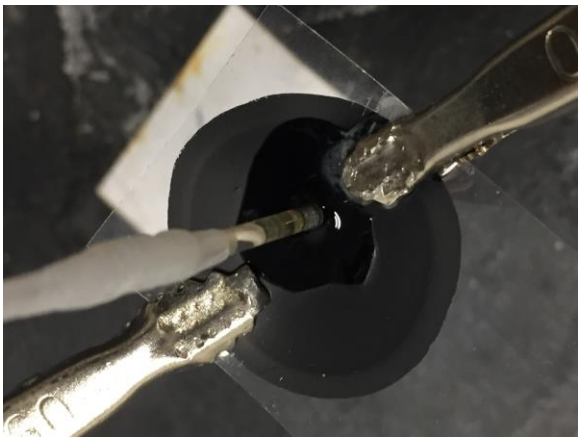

Figure 4: Hydrogel film comprised of polyethylene oxide, nanocellulose and multi-walled carbon nanotube

Using nanocellulose and a potentiostat, we can create another method to quantify *Escherichia coli* in a sample. A similar film created by allowing a mixture of polyethylene oxide (PEO), crystalline nanocellulose (CNC) and multi-walled carbon nanotube (CNT) in water to dry on an overhead transparency. The initial resistance can be seen by measuring the cyclic voltammogram of the film. Equal droplets (approximately 50μL of 4-nitrophenyl β-D-glucuronide (4-NβD_g) dye and of *E. coli* broth sample are placed on the CNC/PEO/C film. By applying a voltage across the film, we can obtain cyclic voltammogram using the potentiostat. The observed change in current is due to the detection of 4-nitrophenol in the sample. This change is proportional to amount of enzyme β-glucuronidase (β-GUS) present and a calibration curve can be developed for determining the *E. coli* concentration. In addition, the film can be coated with a solution of 0.05 M aniline and 1.00 M sulfuric acid to create a polymerized aniline (PANI) surface on the hydrogel. By doing so the change in current in the film itself is minimized in a cyclic voltammogram and the changes more related to the addition of the bacteria rather than internal structure resistance.

2.4 Resistometric Method

Using the same film as in the electrochemical method, we can attach the film to a multimeter in order to determine the resistance of the film. In theory, the resistance of the porosity of the film should change when exposed to the bacteria. Using a multi-walled carbon nanotube in the film, the bacteria tends to embed itself into the nanotube due to an electrostatic charge between the PEO and bacteria. The resistance of the film is measured first, followed by the change in resistance when 4μL of phosphate buffer is placed on the film, and finally the change in resistance when 4μL of the *E. coli* sample is placed on the film.

3. RESULTS AND DISCUSSION

Many tests have been run so far with many variation of film compositions and method of testing. Thus far, none of our data has shown that the signal provided is quantifiable and that any sort of calibration can be produced. The colourimetric method, regardless of the variations tested, yielded no colour change whatsoever in any test. This may be a result of an issue with the dye 4-nitrophenyl β-D-glucuronide used. If this dye is not valid then no colour change could occur. We could not verify the validity of the dye itself because there was no standard to check against. The electrochemical method also showed no positive results as the cyclic voltammograms contained no observable peaks for reduction-oxidation. Finally the direct resistance method appeared to show that there was a change in resistance due to the porosity of the film, but was not quantifiable once measurements at varying concentrations of *E. coli* were tested. Additional testing required in order to show that there is a correlation between the concentration of the *E. coli* and the signal given.

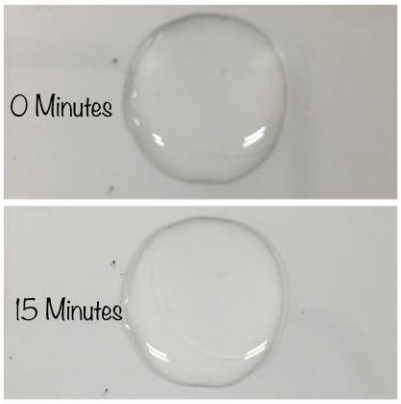

Figure 5: No colour change observed at 15 minute elapse

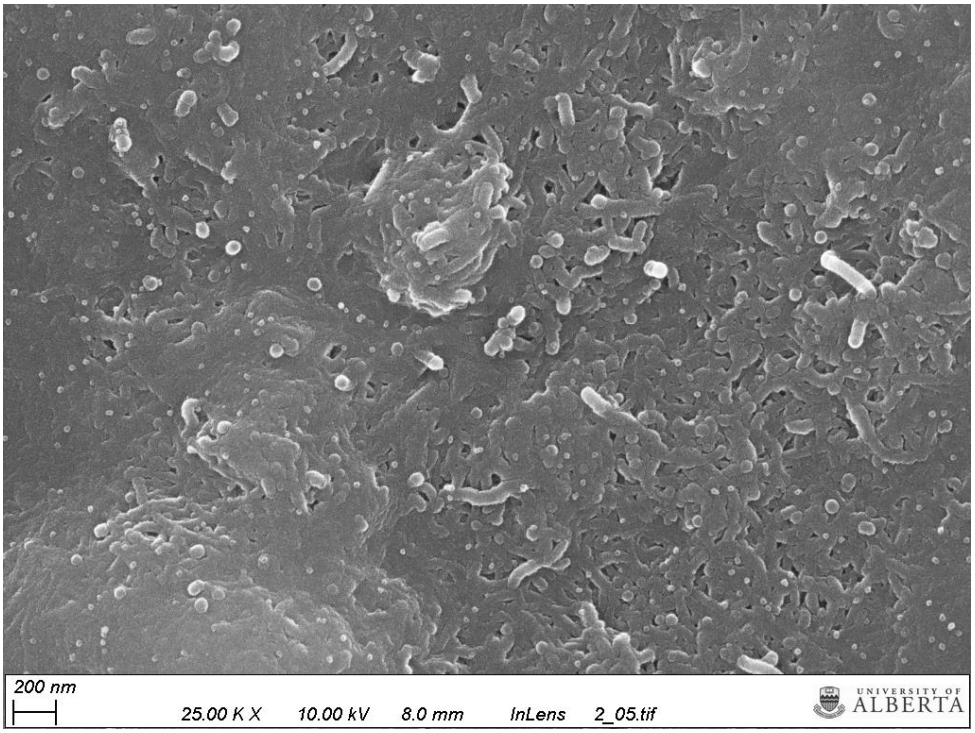

Figure 6: E. coli on the surface of the colourimetric CNC/PEO film. Due to the difference in electronegativity, the bacteria is attracted to the surface of the film rather than its surrounding solution.

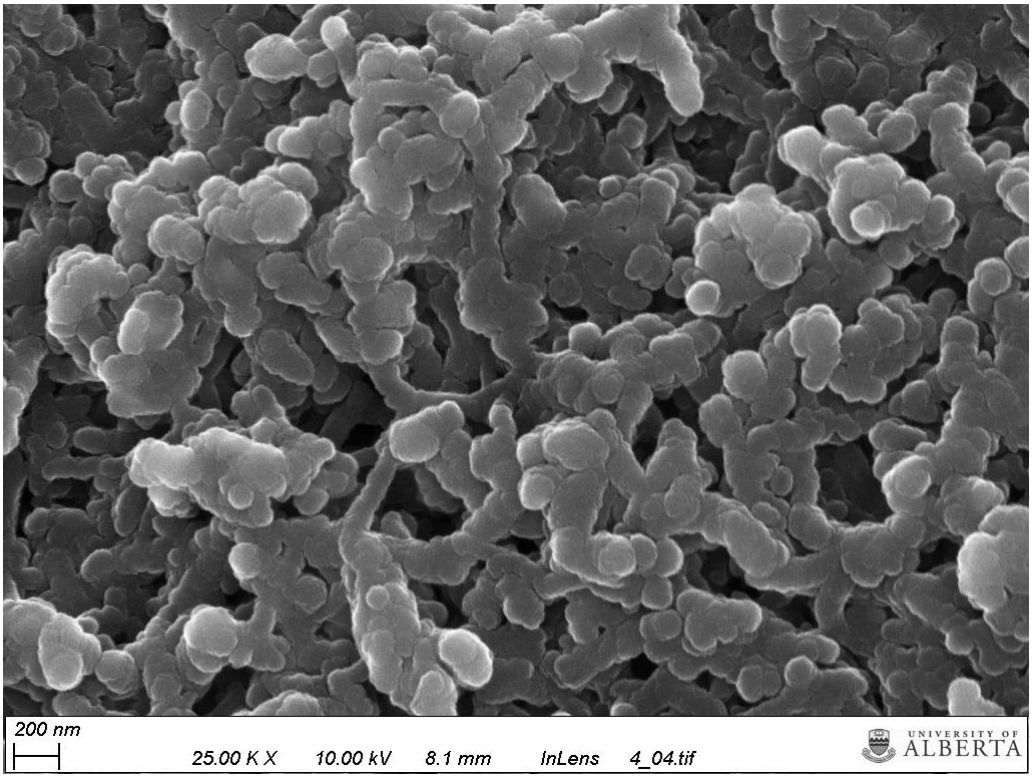

Figure 7: E. coli on the surface of the conductometric CNC/PEO/CNT film. Once again due to the difference in electronegativity, the bacteria is attracted to the surface of the film rather than its surrounding solution.

4. CONCLUSION

The previous research has shown that it is plausible for all three methods to observe changes in the colour and electrochemistry of the created films. The tests done in literature show repeatability in the analysis of these enzymes and relatively low error in doing so. However our results are inconclusive for the testing of β-GUS. This could be a result of faulty materials or bacteria enzyme production. Due to the lack of time, we are unable to specify which of these issues is most likely but the research does still show promise. Moving forward, I recommend the purchase of fresh materials in order to guarantee the viability of the compounds used and develop additional method stabilization from the obtained positive results.

Acknowledgements

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