Chapter 4

GROWTH KINETICS MONITORING OF BACTERIAL SAMPLES USING SMARTPHONE IN THE LABORATORY ENVIRONMENT.

For different microbiological and pathological studies, it is often required to monitor the growth of bacteria in a liquid nutrient medium in the laboratory environment. Conventionally, UV-VIS spectrophotometer is used to estimate the growth of bacterial cell population by measuring the absorbance at 600 nm over a period of time. Colony-forming unit (CFU) is another approach routinely performed to estimate the live bacterial cells by culturing them on semi-solid agar plates. This chapter discusses an alternative yet highly reliable smartphone-based sensing platform which has been used to monitor the growth kinetics of different bacterial species in laboratory environment. The performance of the proposed sensor has been compared with the data obtained from OD600 and CFU analysis. A good correlation of bacterial growth rates enumerated based on the proposed phone-based sensor, bench-top spectrophotometer and CFU analysis have been observed under laboratory experimental conditions. It is envisioned that the proposed sensing tool can also be utilised for measuring bio-contamination in water samples.

4.1 Introduction

Bio-contamination in drinking water is a serious threat to human beings, including other living organisms. It cause several water-borne diseases like cholera, typhoid, diarrhoea, dysentery etc. [1, 2]. Different biological pollutants such as bacteria, viruses and parasites can be present in water resources. The primary source of bio-hazards that pollutes the natural water sources is the animal and human faecal waste. According to the WHO guidelines, drinking water should not contain any bio-contaminant or its presence should be negligibly small [3]. The presence of *Escherichia coli* (*E. coli*) bacteria in the water is an indicator of faecal contamination. The study of this single-celled microorganism bears great relevance in water quality assessment. The microbiological investigations of bacteria include cell counting, study of growth kinetics, morphological characteristics and their microbial interactions [4–6]. While determining the presence of microorganisms, it may not be possible with freshly collected water samples because the bacterial concentration may be very low. Hence, the field-collected samples need to incubate in the appropriate laboratory conditions to reach the bacterial population up to a minimum detectable level. Investigating the growth kinetics of bacteria is a fundamental method to understand bacterial physiology and their metabolic functions. The growth of bacteria is directly related to the number of cells present in the growth media. Two different methods, in general, are adopted to count bacterial populations in the cultured medium namely (i) total cell count (TCC) which includes all living and dead cells and (b) viable cell count (VCC) which counts only the living bacteria in the medium. TCC can be obtained from direct microscopic counting using counting chambers or stained smears on a glass slide and optical density measurement. However, surface colony count is used to estimate the VCC whereas the colony counters are used to count the colonies formed by individual bacterium on a semi-solid agar plate [7–9].

For optical density (OD) measurement, a collimated light beam from a 600 nm peak emission source is allowed to pass through the cultured medium. The intensity of the transmitted signal depends on the concentration of bacteria present in the medium. In the CFU analysis, a certain amount of bacterial sample is cultured on a petri dish filled with a semi-solid growth medium. Depending on the number of live cells present in the medium, proportionate numbers of colonies would be formed. Ideally, each colony formed on the media surface represents one live cell in the broth which further multiplies into a larger colony via binary fission within a certain period. A colony counter is often used to count the number of bacterial

colonies grown on the surface of the solid medium. Both the commercial-grade spectrophotometer and the colony counter are relatively expensive and bulky. Generally, these tools are laboratory confined [10, 11]. In this case, compact smartphone-based bio-sensing platforms have shown a good degree of reliability in analysing various micro-organisms [12–15].

Herein, a nephelometric approach for monitoring the growth kinetics of different bacteria in a standard Luria broth (LB) medium has been presented using a smartphone. A bacteria sample on illumination with a suitable optical source scatters light signal in all directions. Light scattering by optically soft micro-organisms is a well-known phenomenon which can be understood from the Rayleigh-Gans scattering approximation [16, 17]. If the signal is recorded at a right angle to the direction of the incident light, then the scattered intensity depends on the concentration of the bacteria present in the solution. This fundamental principle of light scattering by the bacteria has been employed to develop a compact smartphone-based nephelometer. Using 3D printing technology, an optical setup for the nephelometer has been designed which can be easily coupled to the ALS of the smartphone. Using the designed platform, growth evolutions of *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*) in the cultured media have been monitored successfully. These experimental results were then compared with the data obtained from OD600 and CFU analysis of the sample. It has been observed that there is a good degree of correlation between the experimental data yielded by the designed smartphone sensor and the standard laboratory tools has been found. The performance of the designed sensor has also been evaluated by monitoring the growth of other bacterial samples in the cultured media. The designed sensor further yields reasonably low uncertainty indicating good reliability of the proposed scheme. Owing to the involvement of regular optical components and a low-cost 3D-printed plastic setup, the designed system is relatively cost-effective and performs on par with that of its commercial-grade counterparts.

4.2 Development of the nephelometric setup for monitoring growth kinetics of bacteria

As a part of the smartphone-based nephelometer, a plastic optical setup has been fabricated using 3D printing technology (*Raise 3D N2*, USA). All the required optical components have been stacked inside the plastic setup including a sample holder.

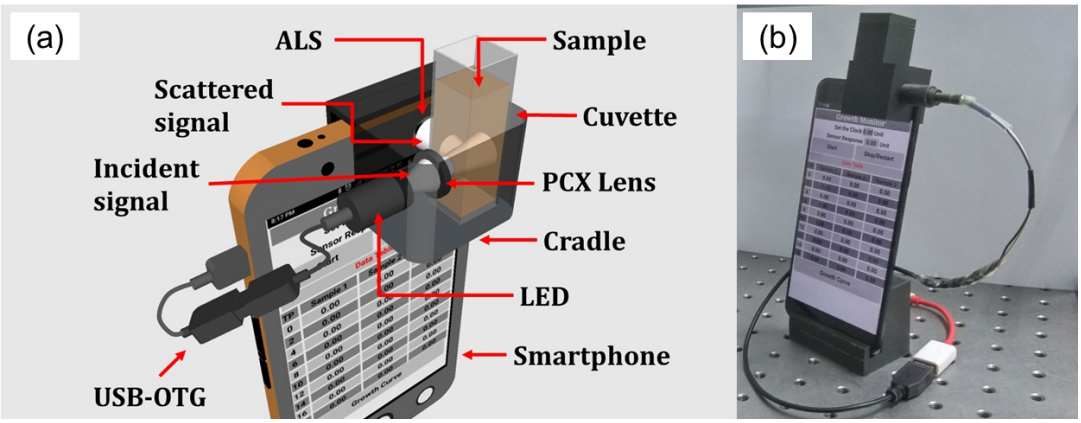


Figure 4.1: (a) Schematic of the designed smartphone nephelometer and (b) photo-image of the 3D printed setup attached to the phone.

The setup has been designed to attach with the ALS of the smartphone which acts as a photo-detector for the present sensing system. Figure 4.1 (a) shows the optical layout design of the nephelometric setup and Figure 4.1 (b) is the photo image of the developed tool. It is optically concealed so that no interference will occur due to the ambient light. An external white LED (*RS Components*, part no: VLHW4100) has been used as an optical source to illuminate the test sample. The LED is powered from the inbuilt battery of the smartphone using a USB-OTG cable. Light emitted by the LED has been collimated by using a 12 mm focal length (6 mm diameter) plano-convex lens (*Edmund Optics*, Product ID 32-471). Upon illuminating the bacteria solution with the collimated light signal, the sample solution scatters light signals in all direction. The scattered light has been collected by the ALS of the phone at an angle normal to the incoming light signal. The sample is placed in a 10×10 mm quartz cuvette (*Erma*, Part No: RE-019). In the present work a *Samsung Galaxy C9-pro*, an economically mid-range smartphone has been used to develop the proposed sensing tool. The overall dimension of the phone is 80.7×162.9×6.9 mm. This phone is equipped with a 6 inch super AMOLED display. It has Qualcomm Snapdragon 653 processor, 1.96 GHz octa-core central processing unit (CPU) and a high quality graphic processing unit (GPU). The inbuilt Random Access Memory (RAM) of the phone is 6 GB and its Read Only Memory (ROM) configuration is 64 GB [18]. The dimension of the designed sensing setup is measured to be 32 mm in length, 30 mm in width and 40 mm in height. The total weight of the designed platform including the smartphone is measured to be 250 g.

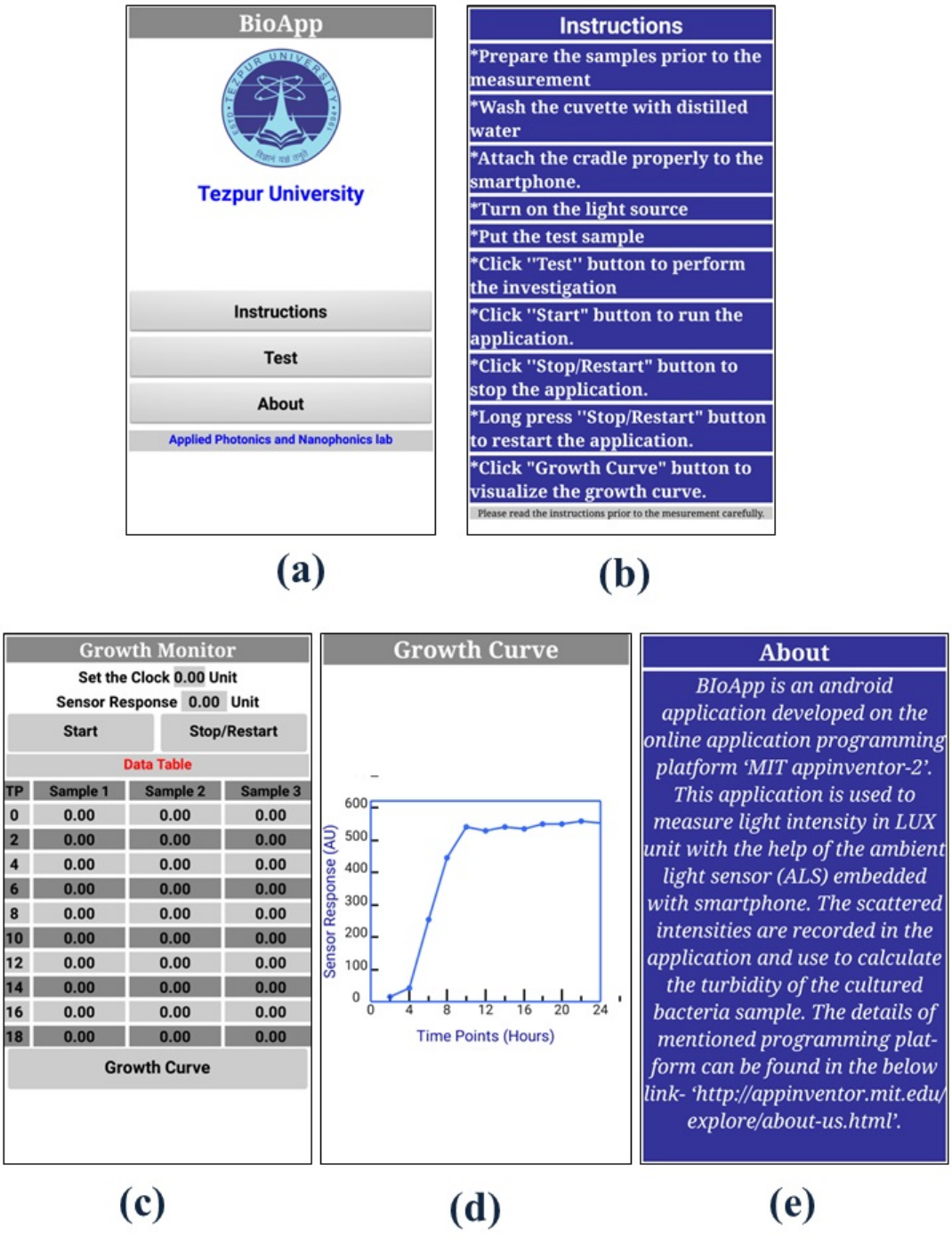


Figure 4.2: Screenshots of the Android application that has been developed for monitoring the growth kinetics of bacteria samples.

4.3 Development of the Android application

To convert the sensing signal into a readable format, a custom-designed application has been developed for Android compatible smartphones. The application has been designed in '*MIT app inventor-2*' – an open-access cloud based service readily available for all variant smartphones. The application converts the scattered light signal intensity received by the ALS into the LUX unit. It plots the characteristic growth curve of the considered bacterium for further investigation. Figure 4.2 shows the screenshots of the application's user interfaces (UI). On initiating the application, three tabs would appear for the users, '*Instruction*', '*Test*' and '*About*' (Figure 4.2 (a)). The '*Instructions*' tab contains the necessary stepwise guide to operate the tool shown in Figure 4.2 (b). On clicking the '*Test*' tab, another screen (*Growth Monitor*) pops up where the experimental data of turbidity level from a cultured medium can be recorded. To study the growth kinetics of bacteria in the liquid nutrient medium, three buttons are available for the user in the app's '*Growth Monitor*' window shown in Figure 4.2 (c). The '*Start*' button on the current screen enables the ALS of the smartphone to record the scattered signal intensity from the sample medium. It also enables the clock on the '*Growth Monitor*' screen to record the turbidity level for a specific period of time. The design sensor records 10 consecutive measurements for each sample and thereupon calculates its mean turbidity value. Prior to the investigation, the designed tool has been calibrated using standard formazin samples. Upon recording the sensor responses at different time points of a specific bacteria sample during the process of its evolution in the cultured media, the growth curve can be obtained within the phone itself. Figure 4.2 (d) shows the growth curve for *E. coli* recorded by the designed sensor. On clicking the '*Growth Curve*' tab (Figure 4.2 (c)), the characteristic curve for the considered micro-organism has been plotted. The '*Stop/Restart*' tab in Figure 4.2 (c) is used either to stop or to restart the investigation by deleting all previously stored data. The '*About*' tab provides a short description about the designed application and '*MIT app inventor-2*' platform.

4.4 Materials and methods

The research grade quality chemicals required for the present study have been purchased from different suppliers and used as received without further processing. Hydrazine sulphate and hexamethylenetetramine were procured from *Merck*, Ger-

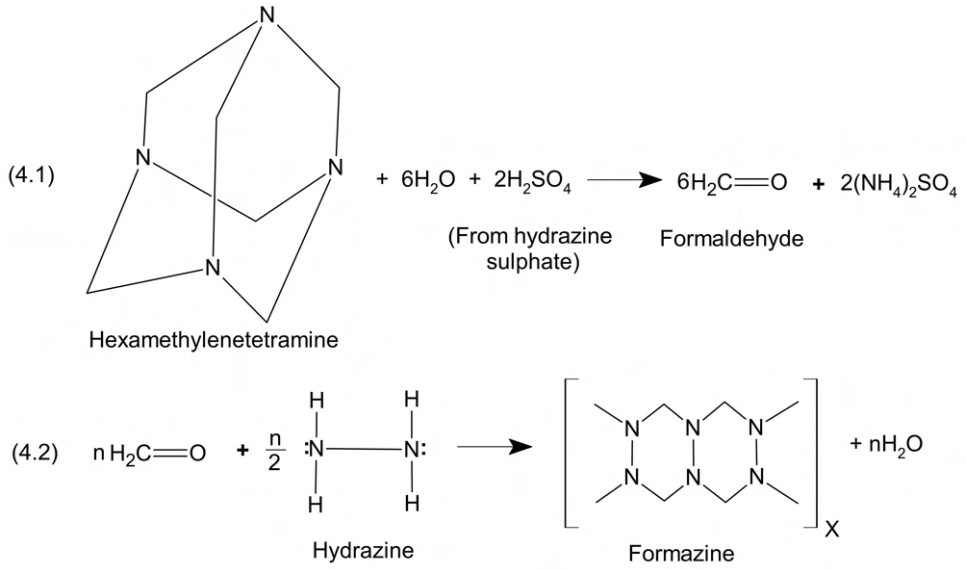
many. Luria broth and sodium chloride (NaCl) were procured from *Himedia*, India. Bacteria used in this study were acquired from Microbial Type Culture Collection (MTCC), *CSIR-Institute of Microbial Technology*, Chandigarh, India. Bacterial strains were revived by growing them in Luria-Bertani (LB) medium at 37°C in a shaking incubator for overnight and inoculum (1%) was used to study the bacterial growth kinetics for 24 hrs.

4.4.1 Bacterial growth kinetics experiments

The detailed experimental procedure involved in the present study has been discussed here. The designed smartphone-based nephelometer has been used to monitor the growth kinetics of *E. coli* and *B. subtilis* in the laboratory environment. Two standard microbiological tools: optical density (OD) and colony-forming unit assay have been used to monitor and to validate the proposed tool's performance. After reviving the bacteria inoculum, the resultant bacterial suspensions were washed in phosphate buffered saline and re-suspended in medium to get initial OD600 of one. Bacterial inoculum of 1% was added into 250 ml of Erlenmeyer flask to obtain a uniform mixture of the culture and subsequently transferred 3 ml of the initial bacterial culture in a 20 ml test tube and incubated at 37°C shaking incubator at 180 rpm. Respective OD and nephelometric measurements were carried out using a bench-top spectrophotometer and the smartphone nephelometer from 0 to 24 hrs at half-hour interval. Similarly, the number of CFUs were analysed by culturing the bacteria at different time points by plating serial dilutions on LB plates and surviving colonies were enumerated at 0, 1, 2, 3, 4, 5, 6 hrs of growth.

4.4.2 Synthesis of standard turbid sample

Following the standard US-EPA method-180.1, formazin standard sample was synthesized to calibrate the smartphone-based nephelometer [19]. According to this protocol, 1 g of $(NH_2)_2$-H_2SO_4 and 10 g of $(CH_2)_6N_4$ were dissolved in 100 ml of distilled water in two separate glass beakers. After sometime, both the solutions were mixed in equal amounts and stored in a dark place for 24 hrs to form the formazin suspension. The final turbid solution obtained from this step has turbidity level of 4000 nephelometric turbidity units (NTU). The prepared stock sample can be diluted to lower turbidity levels by adding proportionate amount of distilled water. The interaction between various reagents have been been shown in the chemical reactions 4.1 and 4.2.



Formation of formazin suspension

4.5 Calibration of the developed platform with formazin sample

The designed smartphone-based nephelometer was initially calibrated with a standard turbid medium. Here, formazin sample was used as the standard turbid medium. The designed sensor records the scattered signal intensities from the turbid medium and evaluates a calibration equation. Usually, all standard turbidimetric tools follow this protocol, while calibrating the device prior to study of the turbidity level of an unknown sample. In the present work, the obtained calibration equation has been applied to monitor the growth of bacteria in the nutrient medium (LB broth). Figure 4.3 represents the characteristic responses of the device when the turbidity of the standard formazin solution is varied in between 0 to 500 NTU. The sensor responses (scattered intensity) are found to be linear with the turbidity of the medium and the coefficient of regression (R^2) is estimated to be 0.99. The calibration equation obtained from the regression analysis is given by 4.1-

$$T = (2.824 \times I) - 1.062 \tag{4.1}$$

where, T implies the turbidity of the sample and I indicates the amount of light intensity scattered by the turbid medium.

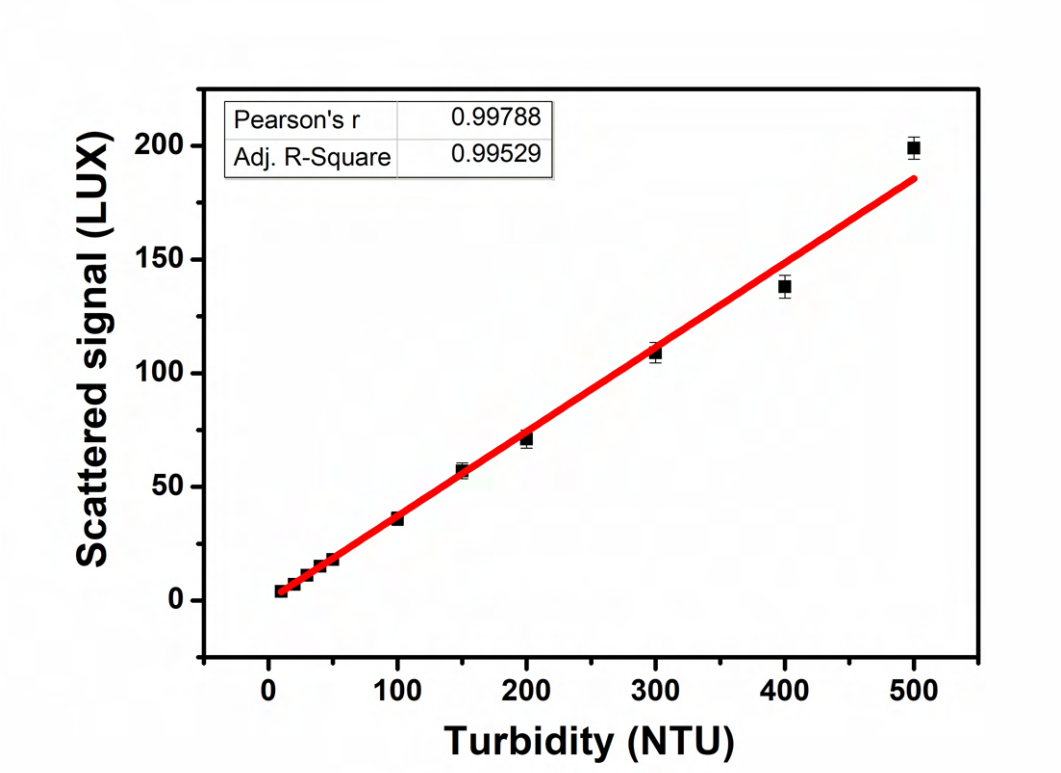


Figure 4.3: Regression analysis of the designed sensor response while measuring turbidity level from different formazin standard samples. Error bar in the figure indicates the standard deviation in sensor responses.

4.6 Monitoring of growth kinetics of *E. coli* and *B. subtilis* using the designed sensor

In the next step, the growth kinetics of a gram-negative bacterium *E. coli* (MTCC 40) and gram-positive bacterium *B. subtilis* (MTCC 121) cultured in standard LB medium have been monitored with the designed smartphone platform. Figures 4.4 (a) and (b) illustrate the characteristic growth kinetics of these two bacterium samples recorded for 24 hrs. Under the same experimental condition, optical densities (OD) of the cultured media have been monitored by a bench-top spectrophotometer. In the figure, the obtained responses from the designed smartphone device (turbidity) have been normalised and compared the results with the standard spectrophotometer data (OD600). The experimental results indicate a good correlation between both the tools' responses (Figure 4.4 (a) and (b)). The three distinct phases of growth namely the '*lag phase*', '*log phase*' and '*stationary phase*' of bacterial cells can be clearly recorded by the designed platform. Tables 4.1 and 4.2, show the char-

Table 4.1: %RSD in estimating turbidity of *E. coli* bacterial samples

Time Points	**Mean Turbidity (NTU)**	**Standard Deviation**	**% RSD**
0	6.24	0.63	10.14
2	14.67	0.82	5.57
4	87.33	5.89	6.74
6	152.67	3.74	2.45
8	184.67	12.03	6.51
10	181.33	7.26	4.00
12	185.00	8.04	4.35
14	183.00	1.41	0.77
16	188.00	6.68	3.55
18	188.00	3.74	1.99

Table 4.2: %RSD in estimating turbidity of *B. subtilis* bacterial samples

Time Points	**Mean Turbidity (NTU)**	**Standard Deviation**	**% RSD**
0	8.00	0.56	7.00
2	12.00	0.89	7.42
4	20.67	1.41	6.82
6	42.67	8.83	20.70
8	60.00	11.52	19.20
10	87.00	2.83	3.25
12	118.33	8.04	6.79
14	125.33	7.12	5.68
16	128.66	7.12	5.53
18	131.66	8.04	6.11

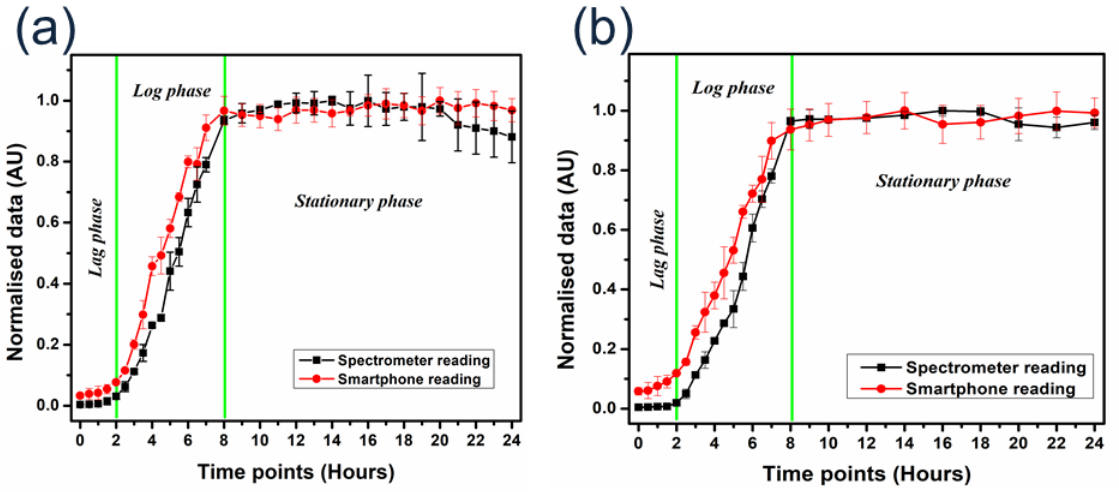


Figure 4.4: Comparison of the growth kinetics recorded by the standard spectrophotometer and by the designed smartphone sensor for (a) *E. coli* and (b) *B. subtilis*. The three phases of the growth process for both the considered bacteria can be distinctly recorded by the designed sensor.

acteristic percentage relative standard deviation (%RSD) readings of the designed sensor while recording the growth evolution of *E. coli* and *B. subtilis* for 18 hrs. For each sample medium, the designed sensor records 10 consecutive measurements and estimates %RSD value in terms of the maximum variation of the sensor readings from its mean turbidity value. The maximum value of %RSD for *E. coli* and *B. subtilis* were estimated to be 10% and 20%, respectively. The relatively low %RSD suggests an acceptably well accuracy of the proposed sensor while monitoring the growth kinetics of bacteria in the laboratory conditions.

4.7 Evaluation of sensor performance with Colony Forming Unit (CFU) analysis

Another commonly used analytical method for monitoring bacterial growth is the CFU analysis, using which the number of bacterial colonies formed on a semi-solid agar medium are estimated by a colony counter. The number of colonies formed on the plate gives the count of VCC of the sample. In the present study, CFU data have been correlated with the designed sensor responses. Figure 4.5 shows the graphical representation of turbidity measurement recorded by the designed sensor against the CFU counts of the bacterial sample at different time points of growth. CFU counts of the bacterial medium implies the number of live cells present in the medium while

the turbidity measurements refer to the intensity level of the scattered signals that contain both live and dead cells. The relation between CFU counts and turbidity values recorded by the designed smartphone sensor follows a nonlinear relationship which is shown in the equation 4.2. The experimental observations reported in this study are implicative of the proposed sensor that has been demonstrated as an alternative tool for studying the growth kinetics of bacteria in standard laboratory condition along with OD and CFU investigations.

$$N_c = 1284 + (-37.14) \times T + (0.30) \times T^2 \tag{4.2}$$

where, N_c implies the number of colonies formed on the agar plate and T indicates the turbidity of the bacteria sample.

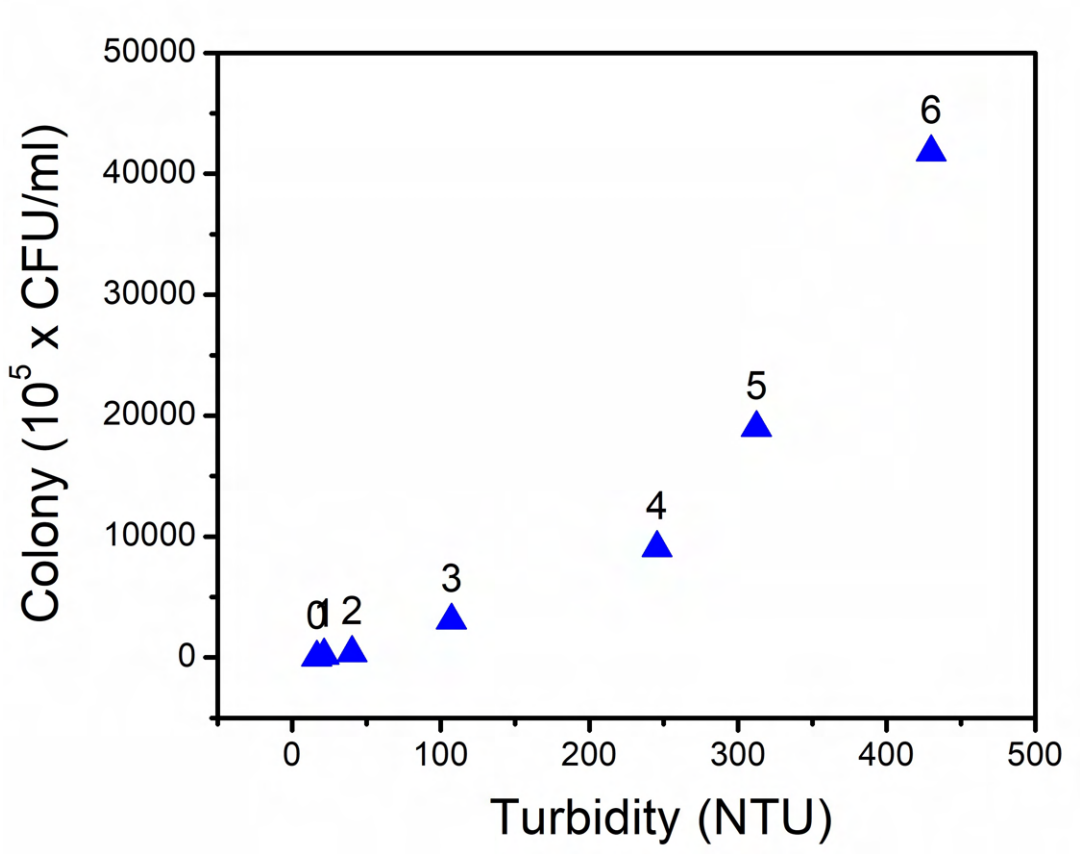


Figure 4.5: Variation of cell populations (CFU/ml) vs. turbidity of *E coli* bacteria in the cultured medium.

4.8 Study of sensor's performance for other bacterial samples

In the final step, performance of the designed tool has been demonstrated by estimating the turbidity of other bacterial samples. Three more bacteria namely *Bacillus cereus* (*B. cereus*), *Listeria monocytogenes* (*L. monocytogenes*) and *Staphylococcus aureus* (*S. aureus*) have been considered for the present study. Upon revival of the bacterial strains, each sample has been incubated at 37°C and 180 rpm for three hours. The bacterial media were then diluted to 50% and 25% respectively by adding proportionate amount of sterilized saline water into it. Figure 4.6 shows the raw data of scattered signal intensities of all the bacterial samples at different concentrations recorded by the designed sensor. The smartphone sensor records different turbidity value for different bacterial samples in the medium that suggests the growth multiplication rate of each bacteria is unique. This study again reveals that with the proposed smartphone sensor, qualitative analysis of different bacterial samples can also be demonstrated.

4.9 Summary

In the present chapter, the working of a simple smartphone sensing system for monitoring the growth kinetics of bacteria under standard laboratory conditions has been demonstrated. Using 3D printing technology, a low cost and a robust optical setup has been designed that houses the necessary optical components and can be coupled easily to the ALS of the smartphone. The present sensing tool is based on light scattering principle, using which, it is also possible to monitor the concentration of non-biological and inorganic samples such as clay, sand particles, sulphate and chloride compounds which are commonly present in various water bodies [20–22]. The net cost involved of the prototype design including the smartphone is approximately $450 (approx. 35,000.00 INR). An Android application with a user-friendly interface has been developed to analyse the scattered signal intensity from the sample within the phone itself thus making the tool a truly standalone system. The same application can be utilised to share the acquired data to any place in the world by using the mobile communication network. In this investigation, the growth kinetics of *E. coli* and *B. subtilis* in LB media under standard laboratory conditions has been successfully monitored. Performance of the designed sensor has been compared with the laboratory-grade spectrophotometer (OD600) and a good correlation of the ex-

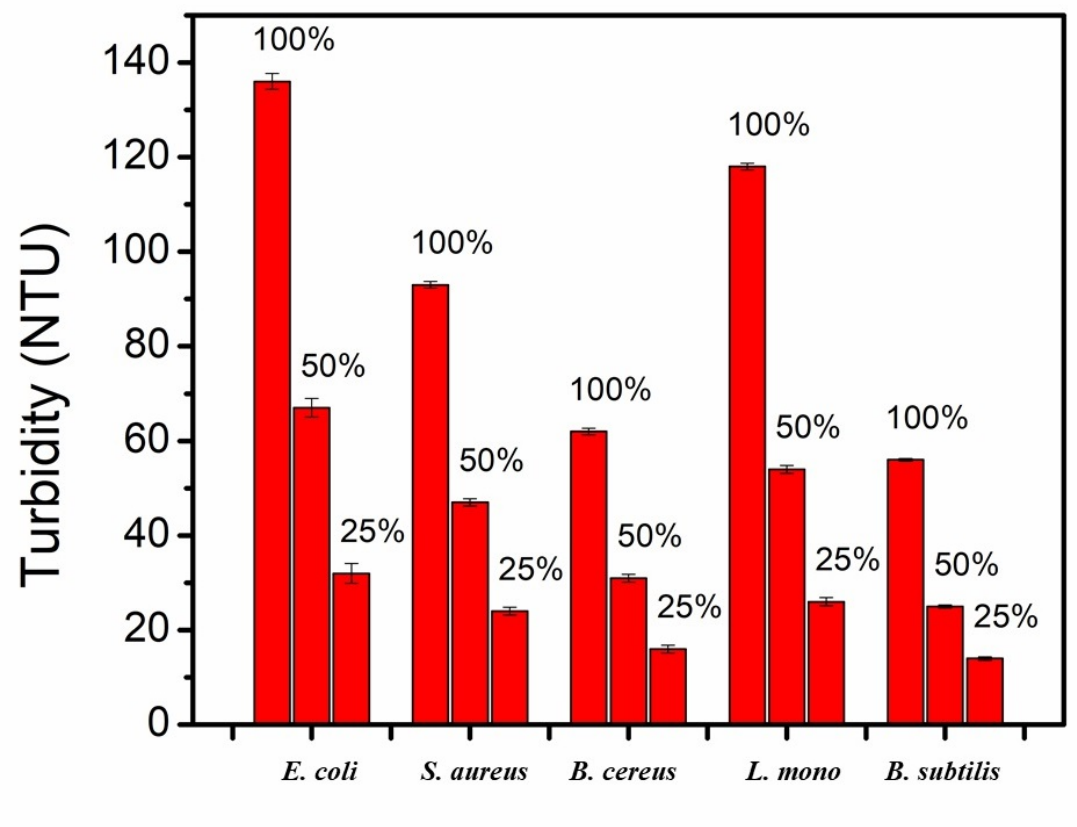


Figure 4.6: Histogram representation of turbidity measurements of five different bacterial samples in three distinct dilutions.

perimental data has been observed. Furthermore, the smartphone sensor responses have also been compared with the CFU data. Again a good degree of correlation of the results yielded by both the tools has been noticed. The %RSD in measurements of the designed sensor for *E. coli* and *B. subtilis* has been evaluated and the maximum values for these two samples were found to be 10% and 20% respectively. It has been anticipated that the proposed scheme could emerge as a cost-effective and an alternative platform which can be used for reliable monitoring of growth kinetics of any bacterial cells in standard laboratory conditions. For reliable operation of the setup in different environmental conditions, several modifications should be made which are as listed as follows. Based on the nature of the target samples to be detected in environmental conditions, the optical source of the present setup may need to be changed accordingly. Also, depending on the temperature and the relative humidity level in the environment the sensing setup needs to be re-calibrated with standard samples for in-field sensing studies.

References

[1] Anand, A., Rajchakit, U., and Sarojini, V. Detection and removal of biological contaminants in water: the role of nanotechnology. In *Nanomaterials for the Detection and Removal of Wastewater Pollutants*, pages 69–110. Elsevier, 2020.

[2] Inamori, Y. and Fujimoto, N. Microbial/biological contamination of water. *Water Quality and Standards-Volume II*, 7:194, 2010.

[3] Organization, W. H., WHO., and Staff, W. H. O. *Guidelines for drinking-water quality*, volume 1. World Health Organization, 2004.

[4] Baev, M. V., Baev, D., Radek, A. J., and Campbell, J. W. Growth of escherichia coli mg1655 on lb medium: monitoring utilization of sugars, alcohols, and organic acids with transcriptional microarrays. *Applied microbiology and biotechnology*, 71(3):310–316, 2006.

[5] Huang, L. Growth kinetics of escherichia coli o157: H7 in mechanically-tenderized beef. *International journal of food microbiology*, 140(1):40–48, 2010.

[6] Salehizadeh, H., Vossoughi, M., and Alemzadeh, I. Some investigations on bioflocculant producing bacteria. *Biochemical engineering journal*, 5(1):39–44, 2000.

[7] Mir, M., Wang, Z., Shen, Z., Bednarz, M., Bashir, R., Golding, I., Prasanth, S. G., and Popescu, G. Optical measurement of cycle-dependent cell growth. *Proceedings of the National Academy of Sciences*, 108(32):13124–13129, 2011.

[8] Hedges, A. J. Estimating the precision of serial dilutions and viable bacterial counts. *International journal of food microbiology*, 76(3):207–214, 2002.

[9] Hammes, F., Berney, M., Wang, Y., Vital, M., Köster, O., and Egli, T. Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. *Water research*, 42(1-2):269–277, 2008.

[10] Henn, R., Schwab, A., and Huck, C. W. Evaluation of benchtop versus portable near-infrared spectroscopic method combined with multivariate approaches for the fast and simultaneous quantitative analysis of main sugars in syrup formulations. *Food Control*, 68:97–104, 2016.

[11] Barter, P. J. and Deas, T. Comparison of portable nephelometric turbidimeters on natural waters and effluents. *New Zealand Journal of Marine and Freshwater Research*, 37(3):485–492, 2003.

[12] Dou, L., Luo, L., Zhang, X., Zhang, W., Liu, L., Yin, X., Liu, S., Sun, J., Zhang, D., and Wang, J. Biomimetic cell model for fluorometric and smartphone colorimetric dual-signal readout detection of bacterial toxin. *Sensors and Actuators B: Chemical*, 312:127956, 2020.

[13] Hong, X., Lu, T., Fruzyna, L., and Yu, B. A dual-modality smartphone microendoscope for quantifying the physiological and morphological properties of epithelial tissues. *Scientific reports*, 9(1):1–9, 2019.

[14] Zhang, J., Khan, I., Zhang, Q., Liu, X., Dostalek, J., Liedberg, B., and Wang, Y. Lipopolysaccharides detection on a grating-coupled surface plasmon resonance smartphone biosensor. *Biosensors and Bioelectronics*, 99:312–317, 2018.

[15] Liang, P.-S., Park, T. S., and Yoon, J.-Y. Rapid and reagentless detection of microbial contamination within meat utilizing a smartphone-based biosensor. *Scientific reports*, 4(1):1–8, 2014.

[16] Katz, A., Alimova, A., Xu, M., Rudolph, E., Shah, M. K., Savage, H. E., Rosen, R. B., McCormick, S. A., and Alfano, R. R. Bacteria size determination by elastic light scattering. *IEEE Journal of Selected Topics in Quantum Electronics*, 9(2):277–287, 2003.

[17] Koch, A. L. and Ehrenfeld, E. The size and shape of bacteria by light scattering measurements. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 165(2): 262–273, 1968.

[18] Samsung. Specification of samsung smartphone. URL `https://www.samsung.com/in/smartphones/`. Accessed on: 2022-07-15.

[19] Sadar, M. J. Stabilized formazin composition, 1 1995. US Patent 5,777,011.

[20] Hussain, I., Ahamad, K., and Nath, P. Water turbidity sensing using a smartphone. *Rsc Advances*, 6(27):22374–22382, 2016.

[21] Omar, A. F. B. and MatJafri, M. Z. B. Turbidimeter design and analysis: a review on optical fiber sensors for the measurement of water turbidity. *Sensors*, 9(10):8311–8335, 2009.

[22] Sheen, R., Kahler, H., Ross, E., Betz, W., and Betz, L. Turbidimetric determination of sulfate in water. *Industrial & Engineering Chemistry Analytical Edition*, 7(4):262–265, 1935.