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Automation and Evaluation of a Newly Developed Carbon Dioxide Evolution Monitoring System

by

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PLAGIARISM DECLARATION

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ABSTRACT

Safe drinking water is a human right and necessary for the optimal functioning of society. Current methods of testing whether water is safe to drink require a large amount of technical expertise, resources and time. This inhibits water testing in rural areas where these resources are not available. Alternative methods such as single use kits are available, but the associated cost often deters poor communities from utilizing these kits. The automated CEMS with recycle (automated Carbon Dioxide Evolution Monitoring System variant that recycles the analysed air) alarm system has the potential of being used as a cheap early warning system of microbial contamination in water that requires no technical expertise to operate, no sample preparation or consumables and has the potential to take less time than traditional methods of testing for water contamination. The CEMS with recycle uses a non-dispersive, infrared sensor that detects and displays the amount of CO₂ produced by microorganisms in the closed system. The sensor is connected to a vessel housing the water to be tested. CO₂ is pumped through the closed system using a system of valves and pumps and is measured to indirectly quantify the number of microorganisms in the water in the vessel.

The aim of the project was to detect microbial activities at low concentrations (concentrations under $10^6 \frac{CFU}{ml}$ (colony forming units per millilitre)). This was achieved by automating the CEMS with recycle, finding its detection limits and applying it in laboratory and natural settings. The utility of the automated CEMS with recycle as an alarm system was evaluated in natural and engineering settings.

The automated CEMS with recycle achieved its aim of detecting microbial activity at low concentrations (concentrations under $10^6 \frac{CFU}{ml}$). Automation was achieved using an ESP32 microcontroller to control valves and pumps to load, recycle and flush the liquid and air on demand via Wi-Fi. The detection limits were determined by running experiments in a laboratory with cultured *E.coli* and cultured river water from the Eersterivier river to determine the detection times for a range of concentrations. The system was able to detect a minimum cultured *E.coli* concentration of $10^3 \frac{CFU}{ml}$ in 220 minutes and minimum river water concentration of $10^5 \frac{CFU}{ml}$ in 820 minutes. The faster method of detection, the Kolmogorov-Smirnov test, determined these times to be 175 and 805 minutes, respectively. The system was shown to be operable in a natural setting by being able to detect microorganisms directly from a river, without culturing, 37 minutes longer than the laboratory culture's detection time at the same concentration. The automated CEMS with recycle cannot be used as an alarm system for drinking water contamination as the detection limits are too high in its current setup according to the South African Water Standards which requires it to detect concentrations as low as $10^2 \frac{CFU}{ml}$. The system, however, can confirm detections at higher concentrations significantly faster than traditional methods.

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NOMENCLATURE

Acronyms	
ATP	Adenosine Triphosphate
CEMS	CO2 Evolution Measurement System
CFU	Colony Forming Unit
E.coli	Escherichia coli
PPM	Parts Per Million
R2A	Reasoner's R2A Agar
RO	Reverse Osmosis
RMSE	Root Mean Square Error
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth

1 INTRODUCTION

1.1 Background

Access to safe drinking water is a human right and is goal number six of the UN Sustainable Development Goals (Masaaki K, 2015; World Health Organization, 2019). Despite this, 1.1 billion people globally drink unsafe water (Ashbolt, 2004) which is estimated to result in about 485 000 deaths each year due to enteric disease (World Health Organization, 2019). 58% of these deaths can be prevented through safe drinking-water, sanitation and hygiene practises (World Health Organization, 2019). Most of these deaths occur in developing countries, especially rural areas (Ashbolt, 2004) where water-born infections remain the most common cause of infectious disease (Manja, Maurya and Rao, 1982). Contamination from other sources such as chemicals like arsenic, fluoride, mercury and lead also pose health hazards.

Rural areas often have one centralized water supply within walking distance (World Health Organization, 2019). Any contamination of this water source will result in the entire community being affected. A good measure to ensure there is no contamination of drinking water is to test the water regularly for contamination. Traditional laboratory-based methods of water quality testing are infeasible in this setting due to the equipment and expertise required to conduct these tests. Easy-to-use testing kits are available, but cost at least US\$ 7.09 (R100.88) per sample which, for rural communities in developing countries, is inhibitive (Matwewe, Hyland and Thomas, 2018). Other automated systems for water quality testing exist, such as the Colifast ALARM (Colifast, 2019), but these require chemical reagents as well as technical knowledge to operate.

The introduction of low-cost, open-source hardware, such as the ESP32, (a microcontroller that can be programmed to control electronic devices) allow for easy and cheap prototyping. These devices have enabled a wide variety of devices that measure drinking water quality to be developed (Wijnen, Anzalone and Pearce, 2014).

This report introduces an easy-to-use water contamination alarm system that requires no chemical reagents or laboratory trained personnel to operate and produces no waste. This device aims to act as detection system to prevent the consumption of contaminated drinking water by detecting microbial activity at low levels. This will be achieved by measuring the CO₂ produced by microorganisms. The CO₂ will be measured by a non-dispersive infrared analyser which is part of the CO₂ Evolution Measurement System (CEMS). This system will be automated using hardware and software and operated with recycled analysed gas to improve its detection limits.

1.2 Objectives

The aim of this project is to develop and evaluate an automated system to detect microbial activities at low concentrations (concentrations under $10^6 \frac{CFU}{ml}$). To achieve this objective, the following milestones were set:

1. Develop hardware and software to automate the CEMS with recycle.
2. Apply the automated CEMS with recycle in a laboratory setting.
3. Evaluate the automated CEMS with recycle as an alarm system in natural and engineering settings by determining its detection limits over a fixed time.

1.3 Approach

The stated aim will be achieved by conducting a series of experiments to obtain a calibration curve relating the amount of concentration of microorganisms (CFU/ml) to the amount of time the system needs to be run to detect that concentration of microorganism. Carbon dioxide (CO_2) is emitted by microorganisms in a closed system and is detected by a non-dispersive infrared sensor. The minimum CO_2 levels required for a detection (significant CO_2 level) at a specific time will be determined by running baseline tests using microorganism free reverse osmosis (RO) water. The experimental runs will include inoculating the RO water in the CEMS vessel with a known concentration of a culture (determined by plating in a growth medium and counting), running the CEMS, recording the time taken for a detection to take place then confirming the concentration of microorganisms in the liquid through plating and counting. A detection is confirmed when a statistically significant level of CO_2 above the baseline is detected by the analyser. The organisms that will be used run the tests (the control variables) are a river water sample from the Eersterivier river in Stellenbosch South Africa and *Escherichia coli* (*E. coli*).

1.4 Key Questions

To achieve the above stated objectives several key questions need to be answered:

1. Does the automated rig load, flush and cycle the air without the need for human interference beyond switching on the rig?
2. What are the detection limits of the automated CEMS with recycle?
3. Is the automated CEMS with recycle a suitable method for determining the presence of microorganisms in drinking water in natural and engineering settings?

2 LITERATURE STUDY

To develop new systems of water quality testing, current systems and their merits need to be understood. Terms used in the field need also to be understood such as what constitutes safe drinking water and the guidelines that are required to be met to achieve safe drinking water status. Safe drinking water is defined as water that does not pose any health risk over a lifetime of consumption (World Health Organization, 2017). To ensure the safety of drinking water, guidelines are published that detail the drinking water quality necessary to ensure that drinking water is safe. Drinking water quality includes all physical, chemical, biological and aesthetic properties of water which determine its fitness for use (Department Of Water Affairs And Forestry, 1996).

2.1 Traditional Indicators of Drinking Water Contamination

Drinking water is regularly tested to ensure that it meets these guidelines. Testing for all pathogens is infeasible due to economic and time factors as there are such a wide variety and large number of them. Indicator organisms are used instead to determine the sanitation levels and whether pathogens are present in a sample (Thomas et al., 2005). The ideal indicator organism for water testing should (amongst other things): be present when pathogens are present, be absent from pathogen free samples, be reliably detectable at low concentrations, be higher in number than those of the pathogens and still be in numbers correlated with the degree of pollution.

The most general indicator organism is the heterotrophic plate counts (Department Of Water Affairs And Forestry, 1996). This group includes all culturable aerobic and anaerobic bacteria that use carbon sources to grow (Verhille, 2013). The culturable bacteria accounts for 1-5% of the bacterial population (Ultee et al., 2004). This low percentage of population detection is due to some bacteria not being able to grow on the medium used. The low percentage could also be due to the bacteria entering a state of being viable but nonculturable. In this state the bacteria are living but are unable to be cultured on media and have a slower metabolic rate. Heterotrophic counts, being the most general indicator organism, cannot be used as an indicator for harmful pathogens, but can be useful as an indicator of general drinking water quality (Department Of Water Affairs And Forestry, 1996). It is useful as an indicator of inadequate water treatment. A microbial count of less than 100 CFU/mL indicates a negligible risk of microbial infection (Department Of Water Affairs And Forestry, 1996).

Total coliform bacteria are another type of indicator organism that is more specific than the heterotrophic plate count. It includes bacteria of the genera *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Serratia* and *Rahnella*. It indicates general hygienic levels of water. They should not be detectable in treated water. A count of less than five CFU/100ml is deemed acceptable (Department Of Water Affairs And Forestry, 1996). Any detection indicates that there is a possibility of pathogens being present in the water. Due to these bacteria being gram-negative the appropriate growth media (such as Endo agar or MacConkey agar) is used to selectively grow these bacteria for identification (Department Of Water Affairs And Forestry, 1996).

Faecal coliforms are a subset of total coliform bacteria. They are used to detect the presence of faecal contamination from warm-blooded animals (Department Of Water Affairs And Forestry, 1996). A positive detection result may mean that bacterial pathogens such as pathogenic *E. coli*, *Salmonella* and *Shigella* (amongst others) are present. Any detection above zero CFU/100mL is indicative of a higher than negligible risk of microbial infection (Department Of Water Affairs And Forestry, 1996).

Other indicators of drinking water quality include algae, which negatively affects the taste and odour of water. The presence of algae can lead to bacteria growth after disinfection (Department Of Water Affairs And Forestry, 1996). Enteric viruses represent viruses that infect the human gastrointestinal tract (World Health Organization, 2017). This indicator differs from enteric bacteria due to the differences in sensitivity to treatment. Drinking water also needs to contain lower than specified thresholds of chemicals such as mercury, arsenic, magnesium and fluoride.

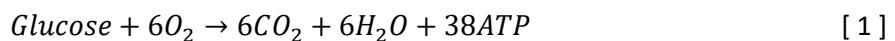
To evaluate the proposed system the traditional forms of indicators of drinking water quality can be compared to using CO₂ as an indicator. To understand when and for what organisms using CO₂ as an indicator works well the mechanism of cellular respiration needs to be understood.

2.2 Cellular Respiration

Cellular respiration is the process of breaking down a food source to gain energy (Clarke, 2013). Microorganisms can be divided into two groups based on their carbon requirements: autotrophs and heterotrophs (Clarke, 2013). Autotrophs rely on inorganic carbon to produce energy required for cellular processes. They can produce energy through photosynthesis or chemosynthesis making them a photoautotroph or chemoautotroph, respectively. Photosynthesis uses light from the sun and inorganic carbon, shown in reaction 3. Chemosynthesis uses energy from oxidation reactions of inorganic compounds such as sulphur, hydrogen, ammonia, sulphide and iron together with inorganic carbon (shown in reaction 4) (Clarke, 2013). Both processes use CO₂ as a carbon source. Heterotrophs use organic carbon to produce energy, the general respiration reactions for aerobic and anaerobic respiration is shown in reaction 1 and 2, respectively. They can be divided into photoheterotrophs and chemoheterotrophs. Photoheterotrophs use light from the sun as well as organic carbon, while chemoheterotrophs oxidise other organic compounds for energy (Prescott, 2005).

Heterotrophic cells undergo three steps to generate energy from carbon sources: glycolysis, the tricarboxylic acid cycle and oxidative phosphorylation (Clarke, 2013). The aim of the respiration process is to produce adenosine triphosphate (ATP) which is used as energy by cells. One of the by-products of this process is CO₂ for both aerobic and anaerobic respiration (shown in equation 1 and 2).

For aerobic respiration the overall chemical reaction (equation 1) is:



For anaerobic respiration the overall chemical reaction (equation 2 (Clarke, 2013)) is:

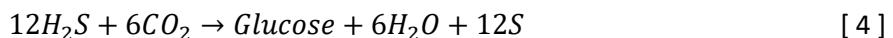


Photoautotrophs (such as algae) do not require glucose; they produce it as follows:



They then consume the glucose (Fischer *et al.*, 2010).

Chemoautotrophs usually occur in hydrothermal vents, isolated caves, volcanic activity (Madigan, Martinko and Parker, 2000). An example of a chemosynthesis reaction (reaction 4) is shown using hydrogen sulphide as the energy source.



2.2.1 Carbon Dioxide Production through Cellular Respiration

CO₂ is a common by-product of all heterotrophic respiration reactions as shown by the reactions 1 and 2 therefore the increase in CO₂ can be used as an indicator of the presence of microorganisms (Masaaki K, 2015). Photoautotrophs and chemoautotrophs use CO₂ as a reagent. These photoautotrophs will decrease the sensitivity of a system based on using CO₂ as an indicator of contamination. However, the influence of these organisms can be negated through depriving them of light as light is necessary for the respiration reaction as seen in reaction 3. Chemoautotrophs will decrease the sensitivity of a system. The presence of these organisms is due to the significant presence of the previously mentioned inorganic compounds. The presence of these organisms is already indicative of drinking water quality issues as high concentrations of the inorganic compounds necessary for chemoautotroph growth can be hazardous for consumption such as iron (Department Of Water Affairs And Forestry, 1996; Madigan, Martinko and Parker, 2000).

CO₂ production can be bypassed through the glyoxylate cycle. In this cycle the production of CO₂ is bypassed to save carbon (Ahn *et al.*, 2016). This alternative pathway occurs in conjunction with the traditional tricarboxylic acid cycle (Ahn *et al.*, 2016). This pathway is favoured when the organism is under oxidative or antibiotic stress and requires the presence of *isocitrate lyase* and *malate synthase* enzymes (which *E. coli* possesses) as well as an acetate rich carbon source (Ahn *et al.*, 2016). Lower CO₂ production can also occur when a microorganism enters a dormant or viable but non-culturable state.

The mechanism of CO₂ production from cellular respiration is understood. However, these microorganisms exist in water so the interaction of gas and liquid needs to be developed. The solubility of gas in water change depending on several factors, mainly temperature, but also agitation. It is necessary to quantify the CO₂ liberated or dissolved into the liquid for establishing the baseline.

2.3 Henry's Law

Henry's law gives the partial pressure of a dilute solute in a solvent (equation 1) (Felder, 2005). It requires that the solution behave ideally at equilibrium, the solute should be dilute and the solute should not dissociate, ionize, or react in the liquid phase (Felder, 2005). Henry's constant is a function of temperature. p_A represents the partial pressure, x_A the molar fraction of the solute. H_A is Henry's constant.

$$p_A = x_A H_A$$

[5]

2.3.1 Temperature Dependence of Gas Dissolution in Water

The solubility of gasses in liquids is temperature dependant as shown in equation 1. (Green and Perry, 2008). A, B, C and D represent the gas specific constants and x indicates the mole fraction of the solute in water. Increasing the temperature of the solvent will decrease the amount of gas dissolved in it (Dahm and Visco, 2014). This is due to the dissolving process being exothermic, increasing the amount of heat increases the energy of the gas molecules which can overcome the bond between the solvent and solute molecules allowing the gas to escape (Dahm and Visco, 2014).

$$\ln x = A + \frac{B}{T} + C \ln T + DT \quad [6]$$

Using equation 6 the estimated solubility-temperature trend of CO₂ can be quantified. Figure 1 shows the decrease of solubility as temperature increases (Green and Perry, 2008).

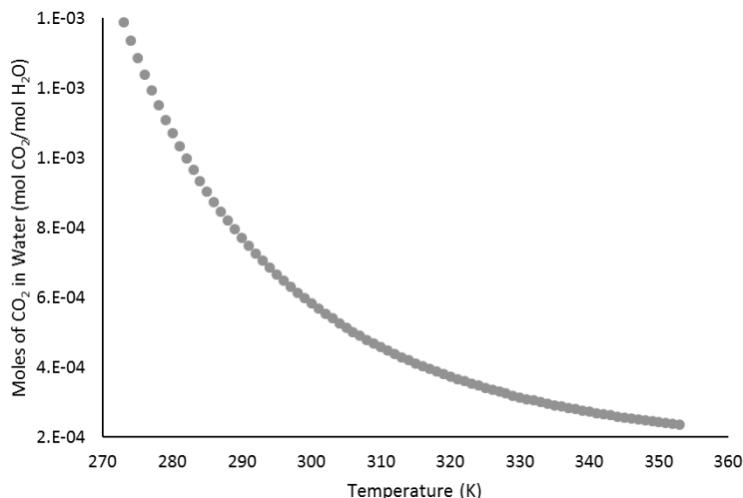


Figure 1: Solubility versus Temperature of CO₂

The background information necessary for complete understanding of the CEMS such as the indicator organisms, mechanisms of CO₂ production and CO₂'s interaction with water. The information necessary for designing the experiments will be expanded upon.

2.4 Bacterial Growth

The growth phase of bacteria will influence the future number of bacteria in the sample, thus the growth phase must be appropriately selected to conduct the experiment. If a fixed concentration of bacteria is to be investigated, it is important that the appropriate phase be selected to ensure that the concentration remains constant (Pletnev *et al.*, 2015).

2.4.1 Growth Phases

Figure 2 shows a standard growth curve. Times are not provided on the graph due to the varying times these phases can exhibit depending on, among other things, the growth medium composition, species of bacteria, temperature, pH and degree of aeration (Madigan, Martinko and Parker, 2000). The curve is

characterized by four main phases: the lag phase, the exponential growth phase, the stationary phase (also called the steady state phase) and the death phase (Zwietering *et al.*, 1990).

The lag phase is a result of the bacteria adapting to the culture conditions. The lag phase duration is specifically affected by the inoculum size, the physiological history of the cells, and the precise physiochemical environment of both the original and the new growth medium (growth medium composition, temperature, pH and degree of aeration) (Rolle *et al.*, 2012). The lag phase will increase if the growth media is changed and the principle carbon source differs between the two (Vermeersch *et al.*, 2019). It can last from about 1 hour to a number of days (Madigan, Martinko and Parker, 2000). The exponential growth phase is characterized by doubling in the total bacteria population at time intervals known as the generation time. Its duration is determined by (the previously mentioned) nutrients available to the organism as well as the temperature, pH and principally the type of organism. The generation time can last between 20 minutes and 7 hours for *E.coli* (Gibson *et al.*, 2018). The stationary phase is characterized by no change in cell concentration as the growth and death rates are equal. The entry into this phase and the next phase is dependent on the specific essential nutrient, accumulation of toxic by-products, presence of stress factors such as changes in pH, temperature, osmolarity (Jaishankar and Srivastava, 2017). Eventually the death rate exceeds the growth rate resulting in the death phase (Madigan, Martinko and Parker, 2000).

Turbidity can be used to determine the phase of a culture. The stationary phase can be identified by a constant turbidity measurement. However, the turbidity remains constant throughout the death phase as dead bacteria still contribute towards the turbidity (Madigan, Martinko and Parker, 2000). This means that what is perceived as the stationary phase by the turbidity measurements may, instead be the death phase. The growth phase of a bacteria influences the concentration of microorganism likely to be present in the future. Conducting an experiment during the bacteria's stationary phase will ensure that the intended concentration of bacteria will be present in the sample.

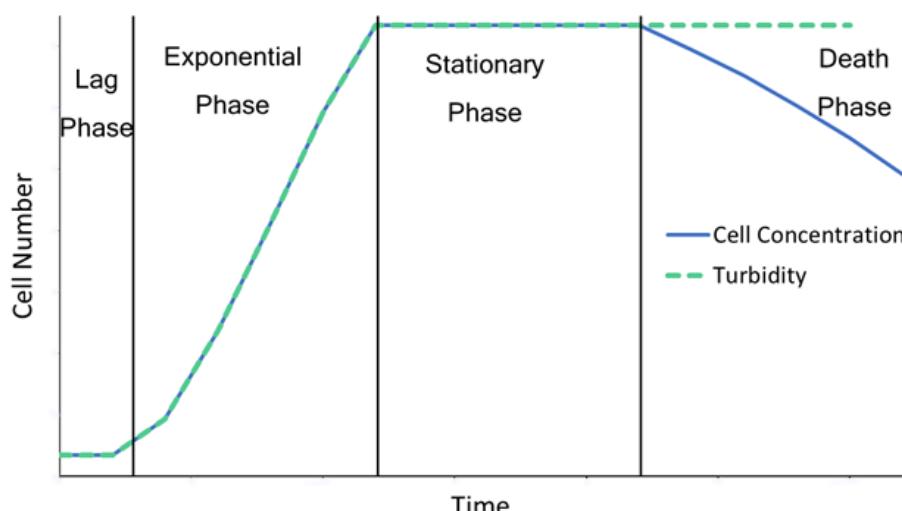


Figure 2: General Standard Growth Curve of Bacteria with Corresponding Turbidity Measurement

2.4.2 Growth Rate

Different microorganisms grow at different rates (Madigan, Martinko and Parker, 2000). The growth rate is a function of temperature, pH and growth medium composition (Madigan, Martinko and Parker, 2000). Under optimal conditions bacteria such as *E.coli* can have generation time between 17 and 20 minutes (Gibson et al., 2018; Todar, 2017). This generation time of *Vibrio cholera*, which has a generation time of 40 minutes, is double that of *E.coli* while *Pseudomonas aeruginosa* has a generation time of 30 minutes (Gibson et al., 2018). *Proteus, Arizona, Salmonella* and *Shigella spp* strains display generation times two to three times that of *E.coli* (Hendricks, 1972). According to (England, 2013) *E.coli* replicates at a rate that is close to the upper limit thermodynamically and that faster growth rates are possible, but unlikely without the creation of synthetic bacteria. *E.coli* is often used as an experimental organisms as it is fast growing, cheap and has large amounts of literature associated with it (Idalia and Bernardo, 2017).

A mixed culture will contain multiple difference species of microorganisms, thus have a range of respiration rates. Conducting tests on cultured river water is more representative of operating the CEMS in a natural setting. A mixed culture will have, on average, a longer generation time than that for *E.coli*.

The background information regarding the CEMS is completely understood such as indicators of microorganism presence, mechanisms of CO₂ production and its interaction with water as well as the information related to the experimental design. The technical aspect of the CEMS can now be explained in further detail.

2.5 Carbon Dioxide Evolution Monitoring System

The Carbon Dioxide Evolution Monitoring System (CEMS) in (Kroukamp and Wolfaardt, 2009) demonstrates the capability of measuring microorganism activity by quantifying the amount of CO₂ microorganisms produce.

2.5.1 Components and Operation Mechanism

The CEMS (shown in Figure 3) includes the experimental vessel connected to the LI-820 CO₂ gas analyser (referred to as the analyser). It consists of an inner silicone tube and an outer sealed Tygon tube. Silicone has a high relative permeability to CO₂ and O₂ compared to Tygon (Cole-Parmer Instrument Company LTD, 2018). The high concentration of CO₂ within the annulus of the silicone tube permeates the silicone to the outer annulus housed by the Tygon tube (Kroukamp and Wolfaardt, 2009). The gas in the outer annulus is transported by a sweeper gas and read by the analyser. Data from the analyser is delivered to a laptop (not shown). Software is provided with the analyser, which displays the amount of CO₂ in parts per million (ppm) along with the pressure and temperature of the analyser. All CEMS based systems comprise of at least these components: the headspace, analyser and the software. The headspace referred to is the air above the liquid in the vessel in the CEMS and the automated CEMS with recycle. The headspace is analogous to the outer annulus in the CEMS as it houses the gasses produced by the microorganisms. The CEMS with recycle is shown in Figure 4. It has the addition of an input into the vessel from the analyser output. This result is that there is no carbon dioxide loss to the atmosphere thus causing a build-up of CO₂ in the vessel.

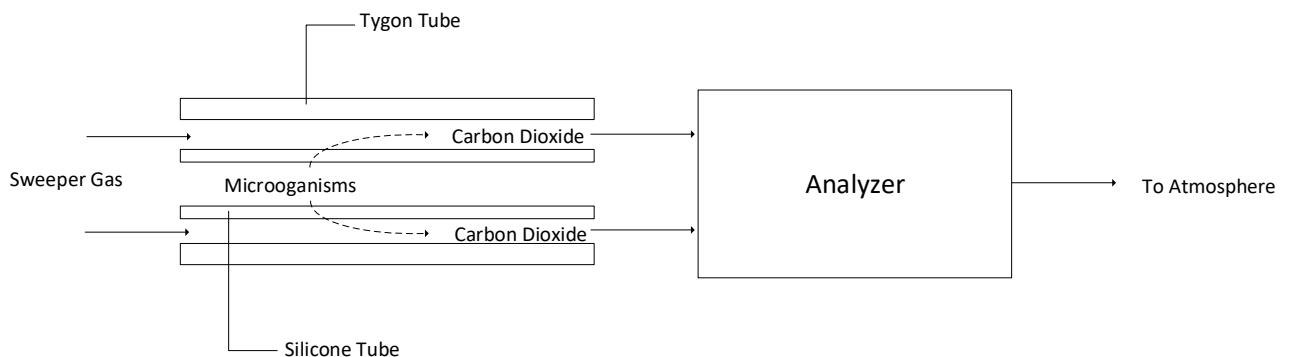


Figure 3: CEMS Experimental Setup

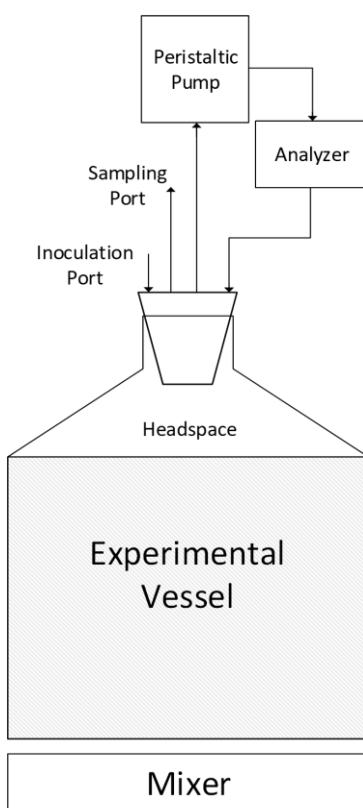


Figure 4: LI-820 CO₂ Gas Analyser CEMS with Recycle

The CO₂ in the system is transported and read by the analyser. The analyser contains a nondispersive infrared sensor (shown in Figure 5). The analyser emits a single beam with dual wavelengths from the emitter (Biosciences, 2009). CO₂ absorbs photons of certain wavelengths. The detector computes the amount of CO₂ (in Parts per Million (PPM)) in the annulus by comparing it with a reference emitted at a different band to the CO₂ detection beam. The automated CEMS with recycle (Figure 6) is explained in detail in section 3.1 Equipment Design.

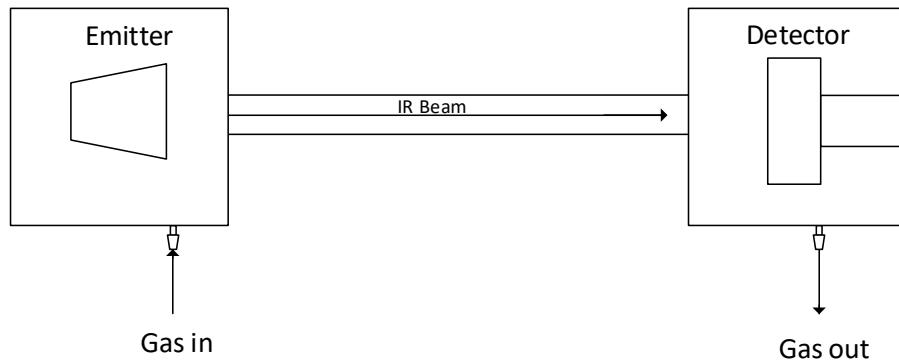


Figure 5: Analyser Schematic

2.5.2 Microorganism Detection through Carbon Dioxide Monitoring

Kroukamp and Wolfaardt, 2009, successfully utilised CO₂ measurement to measure the effect of environmental changes to the CO₂ production of *Pseudomonas bacteria* using the CEMS setup. The CO₂ was produced by the microorganisms and pumped out the headspace to the analyser. This demonstrates that CO₂ measurement is a reliable method of detecting microorganisms present in a sample.

This method of microorganism detection does not extend to photoautotrophs (such as algae) due to them not producing a net increase of CO₂. The presence of autotrophic organisms in a sample can lead to a lower CFU count than is present in the sample. During the photosynthesis phase CO₂ is consumed by these species, therefore steps (such as inhibiting light from entering the reactor) should be taken to prevent this phase from taking place.

The growth rate is shown to be strongly proportional to the respiration rate (Yu *et al.*, 2015). Higher growth rate species produce CO₂ at a higher rate than slower growing species. The detection times of a CO₂ monitoring system in a real-world setting will greatly depend on the species present in the water. It may be possible to predict the predominant species solely based on the detection time.

CO₂ monitoring is more versatile than traditional laboratory-based methods as it has the potential for detection and quantification and changes in microbe activity due to environmental changes (Kroukamp and Wolfaardt, 2009). The CEMS does not require any additional reagents or technical expertise to operate. CO₂ measurements have been shown to have the ability to detect the presence of microorganisms in a sample (Kroukamp and Wolfaardt, 2009). The research already carried out does not consider the use of the CEMS as an early warning alarm system for contaminated water, automating the entire system or adding the recycle element to the CEMS to increase detection limits.

2.5.3 Comparison with Manual Methods

Table 1 shows techniques of evaluating the quality of drinking water (Centre for Affordable Water and Sanitation Technology, 2009). Some of these techniques will be described and compared to the CEMS in this or the following section. The techniques not described are omitted as they have similar advantages and disadvantages to those mentioned.

Table 1 Examples of Laboratory-Based and Rapid Detection Techniques of Water Quality Quantification

Multiple Tube Fermentation Technique	Heterotrophic Plate Count
Membrane Filter Test	Microscopic Analysis
H ₂ S Strip Method	Presence-Absence Test (not recommended by the WHO)
UV-Vis Spectroscopy	Biosensors
Nucleic-acid based methods	Vibrational Spectroscopy
Immunoassays	Adenosine Tri-phosphate assay
MALDI/TOF mass spectrometry	Total Organic Carbon Measurement
Multi-Angle light scattering	

Laboratory-based method for detecting the presence of indicator organisms include: the multiple tube fermentation technique (United States Environmental Protection Agency, 2018a), the heterotrophic plate count and membrane filter test (Gleeson and Gray, 2002; Microscope Master, 2018).

These methods require trained operators and chemical reagents in addition to producing waste. Gas formation and incubation for the multiple tube fermentation technique can take up to 2 days to confirm a positive result (United States Environmental Protection Agency, 2018a). It also suffers from low precision (Askar and Treptow, 2011).

The heterotrophic plate count requires the use of agar and requires an incubation time of at least 48 hours (World Health Organization, 2003). This method considers only the organisms which can grow on the nutrient medium at the incubation temperature (World Health Organization, 2003). These organisms detected may not necessarily be harmful.

The membrane filter test needs at least 18 hours of incubation, a low turbidity sample and requires multiple steps including culturing, filtration and plating to confirm a positive result (Askar *et al.*, 2011; United States Environmental Protection Agency, 2018b). The membrane filter test is not a reliable method for turbid water which limits its usefulness in real-world settings (Askar and Treptow, 2011).

An alternative is presented in (Plutzer, 2012) through the use of microscopic analysis. This technique requires the sample to be filtered through a membrane and centrifuged. It has the capability of detecting organisms in a dormant state unlike the CEMS. This technique, as with other microscopy-based method requires more expensive equipment than the CEMS as well as trained operators. Operators need to have skill in identify organisms as well as using and maintaining laboratory equipment and processing the microscopic images. The report concludes that this technique is best used as a supplement to the above-mentioned laboratory methods.

The CEMS (with and without recycle) has the advantage of being able to be made completely automatic and continuous through the use of a microcontroller (Kim and Myung, 2015). This eliminates the need

for trained personnel, waste and chemicals. It has the potential to take less time than traditional methods and produce less waste than traditional methods.

The CEMS, CEMS with recycle and automated CEMS with recycle do not have the ability to identify the species of microbe present in the sample, which laboratory-based methods can do. It cannot differentiate between harmful and benign microorganisms. This disadvantage may lead to non-harmful bacteria triggering the CEMS alarm system. The presence of autotrophic organisms will lead to a decrease in sensitivity of the sample due to them using CO₂ during respiration. However, the influence of these organisms can be negated by depriving them of light. The CEMS cannot detect contaminants that do not produce CO₂, such as viruses or the presence of harmful chemicals such as arsenic and mercury.

2.5.4 Comparison with Automated Alternatives

Table 1 shows examples of detection techniques, including rapid detection techniques, commonly used to evaluate drinking water quality. A selection of these will be discussed as they tend to display similar disadvantages when compared to the CEMS with recycle.

A promising alternative to the CEMS is using a field-portable and cost-effective lensfree microscopy (Mudanyali *et al.*, 2010). It is low cost and the detection process of identifying microorganisms is automatic. The detection limit was shown to be 380 CFU/ml without pre-treatment, however this apparatus has not been tested for a natural sample, only for pure cultures. Natural samples will require tweaks to the algorithm and possibly sample preparation steps (Mudanyali *et al.*, 2010). The addition of sample preparation steps introduces the need of trained operators, which is not required for the CEMS with recycle.

Another automated alternative to the automated CEMS with recycle is available in the form of Colifast ALARM™. It can analyse for total coliforms and *E.coli* specifically. This alarm system utilizes the fluorescence given off by the enzyme (β -D-galactosidase) present in bacteria (Tryland *et al.*, 2016). This enzyme acts as a catalyst for the hydrolysis of glycosidic bonds, bonds linking sugars to other groups. Colifast ALARM™ uses the substrate 4-methylumbelliferyl- β -D-galactoside (Tryland *et al.*, 2016). This substrate has glycosidic bonds with which the bacterial enzyme reacts. The reaction between the enzyme and the substrate results in 4-methylumbelliferone (MU) which is fluorescent (Colifast, 2019). If any fluorescence is detected an alert is sent to the user. This method requires a substrate that needs to be replaced after 21 tests (Colifast, 2019).

The automated CEMS with recycle requires no reagents other than the sample to be analysed. It also has the possibility of being used to quantify the number of microbes present via a carbon balance (Kroukamp and Wolfaardt, 2009) while the Colifast ALARM does not. The Colifast ALARM™ produces waste while the CEMS with recycle does not. The CEMS will not detect any organism that does not respire such as vegetative cells, autotrophs, viruses and chemical contaminants. The Colifast ALARM™ instead focuses on detecting the possibility of pathogenic presence, while the CEMS relies on detecting the general microbial quality of the water.

The Speedy Breedy device detects the presence of microbial activity through the use of pressure sensors which monitor the positive or negative changes in pressure due to microbial activity (BACTEST, 2018). This system uses a nutrient medium that enables the growth of these microbes over time. The system enables real-time visualisation of the pressure reading to assist in interpreting the data similar to the CEMS with recycle (BACTEST, 2018). It also allows for temperature adjustment. The downside is that certain processes carried out by microbes will result in an increase in overall pressure while some other microorganism's activity result in a decrease in overall pressure. While this system can measure negative pressure, they could act against each other and result in a decreased sensitivity level of the system.

Another indirect measurement alternative is multi-angle light scattering. It differentiates organisms using the way the organisms refract and absorb light. The JMAR BioSentry™ utilizes this technology. It allows for detection and classification of organisms within minutes (Jmar, 2018) and detects vegetative cells as well as spores, which is not possible with the CEMS. This method, being related to turbidity measurements, requires that the samples be free of particulate matter. This again requires, technical expertise, reagents and it produces waste unlike the CEMS with recycle.

A more sophisticated method of carbon measurement is using the total organic carbon measurement method. These devices use an ultraviolet light or platinum catalysed combustion to oxidize organic molecules to produce CO₂ (Masaaki K, 2015). A selectively permeable membrane that only allows CO₂ through. It is then oxidised and collected in a conductivity cell. This method is extremely sensitive, however the contaminated material must be organic and have the ability to be oxidised (Masaaki K, 2015).

The automated CEMS with recycle is a more general automated detection system than those mentioned here, besides the total organic carbon device. It is much simpler to operate and requires less labour to operate and produces less waste. However, it does not detect autotrophic bacteria or any harmful substance that does not respire such as viruses or chemicals.

The CEMS function, mechanism and advantages over current systems was covered. The automation aspect of the CEMS and in industry will be covered in more detail.

2.6 Internet of Things

Industrial plants have become more integrated with the internet and technology and thus become increasingly "smart" and automated (McClements, 2018). Technological enhancements have allowed for easier operation, monitoring and fault detection by automating these processes and making them easier to view and control (Wortmann and Flüchter, 2015). Microcontroller boards like the ESP32 allow for easy and cheap prototyping of systems. These boards operate using a modular design, which keeps cost at a minimum.

Examples of the increased integration of industrial equipment and the internet is the Colifast ALARM has the ability to notify the operator through LAN or SMS (Colifast, 2019). Another example of a system, the MAS 711 Pump Monitoring System (Xylem, 2018), allows the operator to view faults, control operation and monitor pump performance from a smart device.

The CEMS system alerts the operator through e-mail. It has the potential to become even more integrated with technology by enabling remote operation of the device. This can be achieved using a microcontroller with Wi-Fi capabilities. The automation of the CEMS system is a step towards a more automated and connected workplace.

3 METHODOLOGY

3.1 Equipment Design

vessel. The experimental vessel was placed on a constantly running magnetic stirrer to ensure sufficient aeration of the liquid. The aeration provides oxygen necessary for respiration. The rubber plug ensured an airtight seal on the opening of the experimental vessel. The plug contained six ports, two for the outlet and recycle inlet of gas, one for the recycle to the headspace and one to inoculate the liquid. The sampling and inoculation ports were blocked using Tygon plugs. Tygon tubing was used to transport the gas. A two-way solenoid valve controlled whether the experimental vessel headspace was open to the atmosphere or directed the air to back to the headspace. The peristaltic pump pumped air from the headspace through the CEMS constantly. The air was diverted to atmosphere or back into the vessel depending on the position of 'Air Valve 2' in Figure 6. The pump state (on or off) and valve positions were controlled by the ESP32. The ESP32 was placed in an enclosed control box in Figure 6. The operation of the system was divided into cycles: the drainage of the experimental vessel, the loading of the experimental vessel, the flushing of the air in the headspace to atmosphere and the recycle stage. The recycle stage occurs during the experimental run where air in the headspace is recycled through the peristaltic pump and CEMS then back into the vessel.

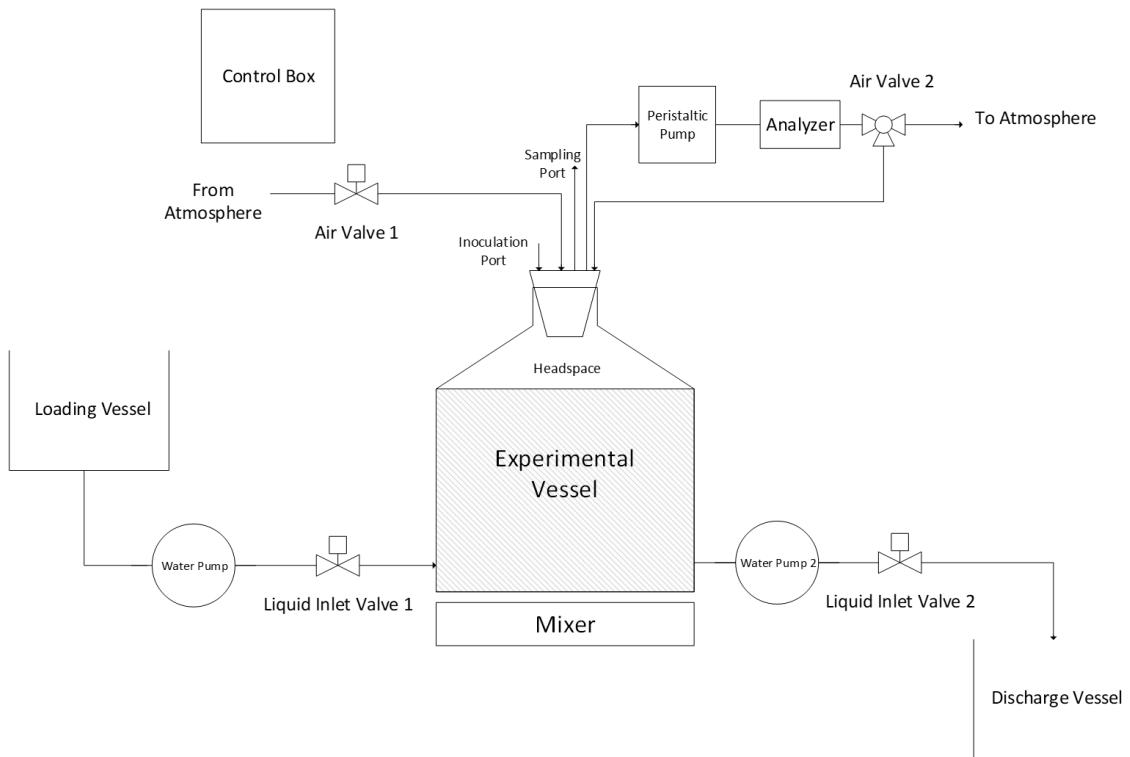


Figure 6: Automated CEMS with Recycle Experimental Setup

3.2 Experimental Design

The main objective, to determine the detection limits of the CEMS with recycle, was achieved by running the system with a range of cell concentrations (the independent variable) and recording the time taken

for a detection to be made (if at all) (the dependent variable) for a certain culture (the controlled variable). This allowed for the detection limits of the CEMS with recycle to be determined.

The experiments used a pure culture of *E.coli* to construct the calibration curve. The same was done for a mixed culture from a river. The final run was completed using water directly from a river without culturing.

Tests with non-inoculated RO water (known as baseline tests) were conducted with three repeats. Two venues' (control variable) baseline responses were tested to determine the better experimental venue: the laboratory and the 30°C oven room. The time taken to reach the stationary phase needed to be determined for both the *E.coli* and river water culture using spectroscopy. The runs for *E.coli* and river water culture were ran using concentrations of: $10^6 \frac{CFU}{ml}$, $10^5 \frac{CFU}{ml}$, $10^3 \frac{CFU}{ml}$ and $10^2 \frac{CFU}{ml}$. Two repeats were carried out per run. The system was run until the alarm is triggered (in other words, when the CO₂ level read by the CEMS was significantly higher baseline) or for one thousand minutes, whichever came first. The time taken to trigger the alarm was recorded.

The system was then run by inoculating the liquid with a cultured river water sample. The same set of concentrations of culture was used as for *E. coli*. If a concentration resulted in no detection, lower concentrations were not tested.

3.3 Experimental Method

3.3.1 Baseline Runs

The baseline tests were conducted to determine the noise of the system when RO water was free of inoculum. It is needed to quantify the minimum amount of CO₂ required to constitute a detection at a particular time. Two experimental venues (the control variables) were tested to determine if the venue influences the experiment and, if so, which was the better venue between the two. The time taken for the CO₂ response to reach a steady state was also obtained from these runs. The laboratory had a varying temperature due to the varying number of people. The amount of people and activity in the room could affect the temperature and thus the CO₂ response. The 30°C oven room that is free of people most of the day and is maintained at a constant temperature.

The vessel was filled with five litres of RO water at atmospheric temperature. The vessel was placed in the room and the experimental rig was run. The CO₂ response was recorded. From the data, the time taken for the ppm reading to reach steady state as well as the variance was obtained. This allowed for the more suitable venue (the one with lower variance and steady state time) to be determined. Readings from the CEMS were recorded every one second. The experiments were conducted for four times in each venue.

3.3.2 *Escherichia Coli* Runs

Tryptic Soy Broth (TSB) was made with a concentration of $3 \frac{g}{L}$ TSB powder (10% of the manufacturer's recommendation) into RO water. One hundred millilitres of this substance was used for the cultivation

of the microorganisms. This mixture was then sterilized in the autoclave and stored at room temperature. Tryptic Soy Agar (TSA) was used as a plating medium for *E.coli*. It was made by adding 15 g/L to the TSB mixture (which has a concentration of $3 \frac{g}{L}$). One litre of TSA was made and autoclaved. Approximately 13ml of Tryptic Soy Agar was poured in sterile Petri dish while utilizing aseptic technique and stored at 10°C.

The time taken for steady state to be reached was obtained by measuring the turbidity (at 620 nm wavelength) and performing a plate count of the sample at set time intervals. The times the turbidity is measured at are 24 hours after inoculation, 26 hours after inoculation and 28 hours after inoculation. The spectrophotometer was set to 620nm and autoclaved TSB was used as a reference. The error of the spectrophotometer was calculated by running the reference sample multiple times. Plate counts were carried out to determine the concentration of colony forming units per millilitre in the sample at these time intervals. This served to both determine the concentration at the time interval and to confirm the turbidity measurement conclusions.

The TSB was inoculated using an existing *E.coli* culture. The sample was grown in TSB until it reached steady state in a 30°C oven room while being agitated to ensure sufficient aeration (Remel, 2011). After the culture has reached steady state seven ten-factor serial dilutions are carried out. 900 µl of sterile 10% physiological saline solution was placed in each Eppendorf tube. 100 µl of the culture was placed in the same Eppendorf tube. The tube was then agitated for 20 seconds using a vortex mixer. 100 µl of the diluted mixture was placed into the next Eppendorf tube (containing 900 µl of saline solution) and agitated. This process was carried out seven times.

Two TSA Petrie dishes were divided into four quadrants, one for each serial dilution and one for the undiluted sample. They were plated using the drop plate method. 10 drops (of $10\mu l$ each) were placed in their respective quadrant. The plates were then left out at room temperature until no liquid was visible. This allowed the CFU/ml count to be verified.

The vessel was filled with RO water and allowed to stand in the 30°C room for the time determined from the baseline run. The liquid in the vessel was then inoculated with the culture by means of injecting the culture with a syringe into the sample port. The air in the headspace was continually recycled through the CEMS and back into the headspace by means of a peristaltic pump. The system was run for one thousand minutes or until a detection was made. After the completion of the run, a sample from the vessel was collected using a sterile syringe. The sample serial diluted and plated as before using the drop plate method. This secondary plating was completed to confirm the concentration of *E.coli* in the vessel. If the concentration was out from the initial reading by a factor of ten the run was discarded. The spread plate method was used instead of the drop plate method if the concentration intended to be less than $10^3 \frac{CFU}{ml}$ in the reactor. This will give a more reliable count at low concentrations. The spread plate method required $900\mu l$ of culture to be plated then counted in an agar filled Petrie dishes. The culture was spread out over the surface of the agar.

3.3.3 River Water Runs

TSB was prepared in the same way as for the *E.coli* runs. R2A agar was prepared using the manufacturer's specifications: a concentration of $18.1\frac{g}{L}$. The R2A agar did not influence plate counts, as the TSB will limit the microorganism growth before the plating. The river water was cultured in TSB for 24 hours at 26 degrees Celsius while being aerated. The time taken for steady state to be reached after the 24-hour aeration was determined in the same way as for the *E.coli* run. The turbidity was measured at 24, 26, 28, 30 and 31 hours after inoculation at a wavelength of 620nm. Plate counts were conducted for each turbidity measurement to determine the concentration.

One millilitre of volume of freeze culture was made of the cultured river water by placing the cultured river water in a mixture with $0.63ml$ of 40% stock glycerol solution and 0.375 ml of the culture.

The TSB was inoculated with $400\mu\text{l}$ of the river water from a freeze culture. It was then placed in the 30°C room and agitated until the stationary phase was reached. Seven ten-factor dilutions in saline and subsequent plating in R2A agar was carried out using the same method as the *E.coli* run. The CEMS was run as above followed by the post-run plating which was carried out in the same way as for *E.coli*.

The final test was run without growing the river water in a culture. The river water is collected in the vessel and transported to the oven room where it is allowed to equilibrate with the oven room temperature. The CEMS was run after the sample is plated in a Petrie dish. Once the run was over the sample was plated again.

3.4 Data Processing

3.4.1 Error

Standard deviation is a measure of dispersion of data (Devore, 2016). The standard deviation is used as a conservative estimation of the upper and lower bound of the CO_2 readings. It was carried out for all runs with repeats and plotted as continuous error bars (using the 'shadedErrorBar' function in Matlab written by Rob Campbell (Campbell, 2009)) together with the average of the run. The error bars were shown by the decreased saturation area.

The average standard deviation was obtained by finding the average of the variance then calculating the standard deviation.

3.4.2 Detection Limit Determination

The detection limits for each culture is determined by the lowest concentration that the system can detect. The Kolmogorov-Smirnov is a non-parametric test that was carried out to determine whether the cumulative distribution function of the run is smaller than that for the baseline (the alternative hypothesis) is true (MathWorks, 2018). A positive result (1) indicates a rejection of the null hypothesis in favour of the alternate hypothesis with a 99% confidence level. This means that the run has greater values than the baseline with a 99% confidence. This is tested using the "kstest2(x1,x2,'Tails','smaller')" function in Matlab (MathWorks, 2018). Graphs are plotted of the p-value and significance value as well as a

Boolean term testing for whether the null hypothesis can be rejected in favour of the alternate hypothesis was used. If the Boolean value was one, the null hypothesis was rejected in favour of the alternate hypothesis.

The second method of determining whether a detection has been made was visually using the ppm of CO₂ versus time plot with error bars of the baseline and inoculated run. Once the inoculated run exceeded the baseline (including error bars), a detection was concluded. This is due to a significant amount of CO₂ being released from the microorganisms that cannot be attributed to the baseline. The plot and the baseline were zeroed by subtracting the ppm reading at time equals zero from the rest of the data from that run. This enabled the baseline and experimental run to be easily compared as they have the same starting point. The determination of the automated CEMS with recycle as an alarm system will be based on comparing its detection limits to the standards present in (Department Of Water Affairs And Forestry, 1996).

4 RESULTS AND DISCUSSION

4.1 Cost Per Run

The cost of running the automated CEMS with recycle was found to be R1.74 per cycle. The one-time use water testing kits which can cost upwards of R100.88 per use which still producing waste. The sample calculations are available in Appendix C – Sample calculations.

4.2 Baseline

4.2.1 Laboratory and Oven Room Comparison

Figure 7 shows the result of the baseline tests in each venue. The plot shows the CO₂ for the experiments conducted in the laboratory response with shaded error bars over 430 minutes without any microorganisms inoculated into the water. It can be seen that the longer the time, the larger the error is. In the laboratory the standard deviation peaked at 285.92 ppm of CO₂ with an average value of 118.46 ppm of CO₂. In the oven room, shown in turquoise, the peak standard deviation was shown to be 28.91 ppm of CO₂ with an average of 14.62 ppm of CO₂. See Appendix C – Sample calculations for the sample Matlab Code and Appendix D – Raw Data for the individual runs. The Arduino code for the automation aspect is shown in Appendix B – Arduino Code.

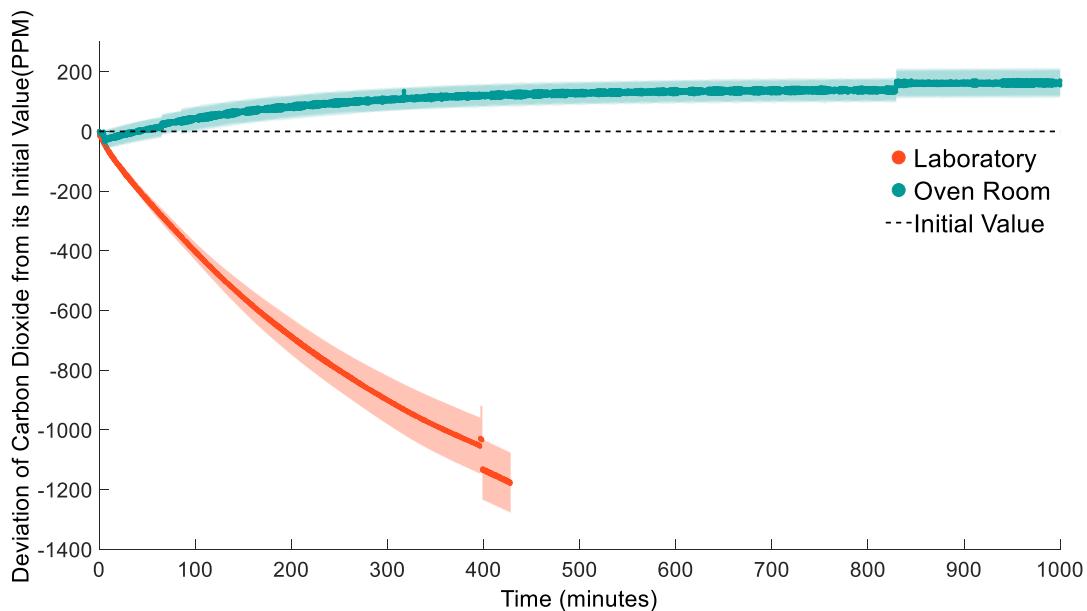


Figure 7: Laboratory and Oven Room Baseline response of the Carbon Dioxide's Deviation from its Initial Value (in Parts per Million) Emitted over Time (in Minutes)

The error (shaded portion) remained somewhat constant in the oven room. The peak standard deviation of the oven room is 194.97 ppm CO₂ less than that for the laboratory room and the average standard deviation is 107.7 ppm CO₂ less. The low variance coincided with consistent results, higher precision and better repeatability. The oven room baseline response levelled out sooner than the laboratory baseline

response. The experiments were conducted at this steady state point, thus the oven room allowed for shorter wait times between experimental runs.

It was noted that after 175 minutes (roughly 3 hours) the CO₂ ppm reading starts to level off in the oven room. This levelling off indicates that less and less CO₂ is released from the liquid. This is due to the temperature of the water equilibrating with the temperature of the oven room. Equilibrium is not reached within this time in the laboratory. From 175 to 1400 minutes the ppm reading only increases by 200 ppm of CO₂. This can be clearly seen in Figure 8. This figure shows that the CO₂ response follows a pattern of an exponential increase up until about 200 minutes, which then starts to decrease in gradient until it reaches a steady state value.

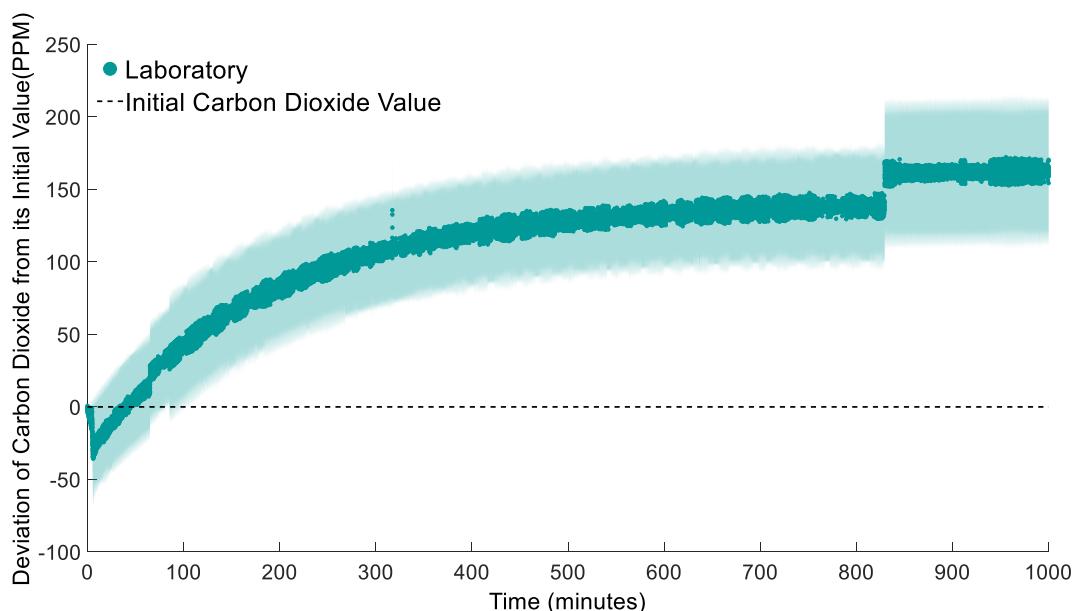


Figure 8: Deviation of CO₂ from its Initial Value in ppm of CO₂ of Uninoculated RO Water over Time in Minutes

The lower standard deviation and lower time taken to reach a steady CO₂ value can be attributed to the more rigorous temperature control in the oven room than in the laboratory. Changes in the amount of people in the laboratory, as well as intermittent running equipment such as nearby Bunsen burners will affect the laboratory temperature and hence the CO₂ response due to the link between gas solubility and temperature through Henry's law (equation 6). People respiring may cause pockets of CO₂ to enter the system during start-up or inoculation. This CO₂ will cause spikes that will negatively influence the steady state of the system.

The oven room has been determined to be the better proxy for the infield use of the CEMS with recycle. This is because the atmospheric temperature, thus CO₂ in the headspace, will not change suddenly.

4.3 Escherichia Coli

4.3.1 Stationary Phase Start and Concentration

The spectrophotometer had an average error of 0.006. Table 2 shows that the stationary phase of the standard growth curve of *E.coli* starts at 24 hours. This is due to the turbidity measurements that peak at 26 hours. This peak in turbidity signifies the end of the exponential phase and the start of the stationary phase (see Figure 2). The turbidity started to decrease slightly at 28 hours, this decrease was less than the average error of the spectrophotometer, thus insignificant. It was concluded that the stationary phase occurred at 26 hours and the concentration of *E.coli* at that time was $8 \times 10^8 \frac{CFU}{ml}$.

Table 2 Stationary Phase Determination of *E.coli* using turbidity and Plate Counts 24, 26 and 28 hours after Inoculation

Time (hours) After Inoculation	Turbidity (with TSB as a reference)	Plate Count
24	1.710	$7 \times 10^8 \frac{CFU}{ml}$
26	1.723	$8 \times 10^8 \frac{CFU}{ml}$
28	1.722	$7.8 \times 10^8 \frac{CFU}{ml}$

4.3.2 Experimental Runs

The response for the highest concentration of $10^6 \frac{CFU}{ml}$ is shown in Figure 9. A detection occurred at about 20 minutes although more tests are required to determine the variation of the response. This experiment determined the minimum time required for detection as all the flowing experiments are at lower concentrations. In the time the experiment was conducted, the *E.coli* showed no signs of decreasing CO₂ production. This can be explained by the large concentration of *E.coli* as well as the short experimental run time.

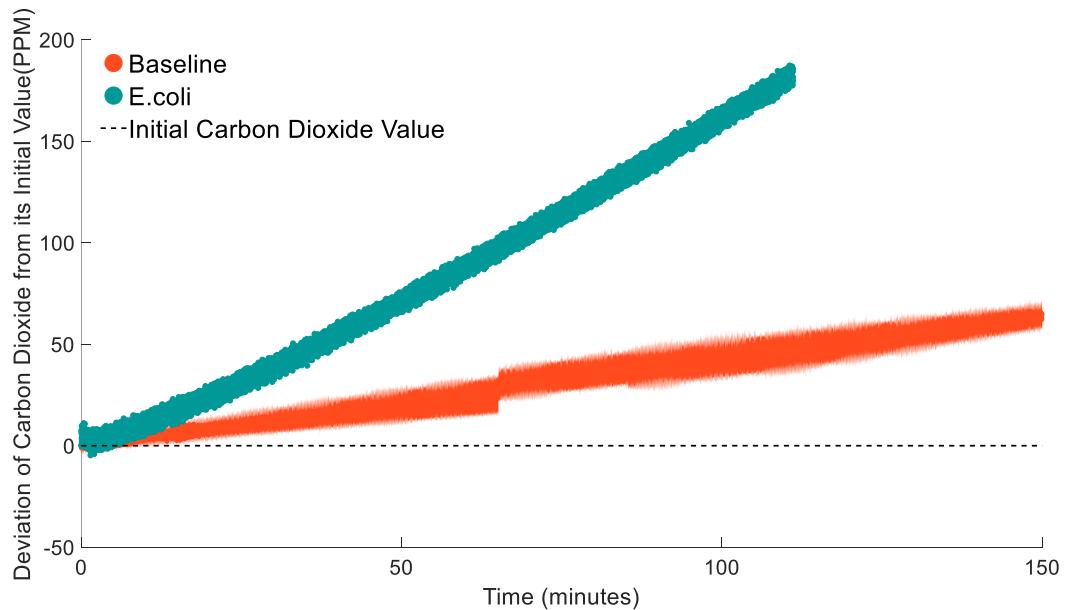


Figure 9: Deviation of Carbon Dioxide from its Initial Value (in parts per million) versus Time in Minutes of *E.coli* of Concentration $10^6 \frac{CFU}{ml}$ with the Baseline

The detection is confirmed by the Kolmogorov-Smirnov test (Figure 10). The test showed that the detection occurs at 1 minute at the chosen significance level (99%). This time is significantly less than the detection determined by the visual method in Figure 9. It can be hypothesised that the Kolmogorov-Smirnov test can confirm a detection in a shorter time than the visual method.

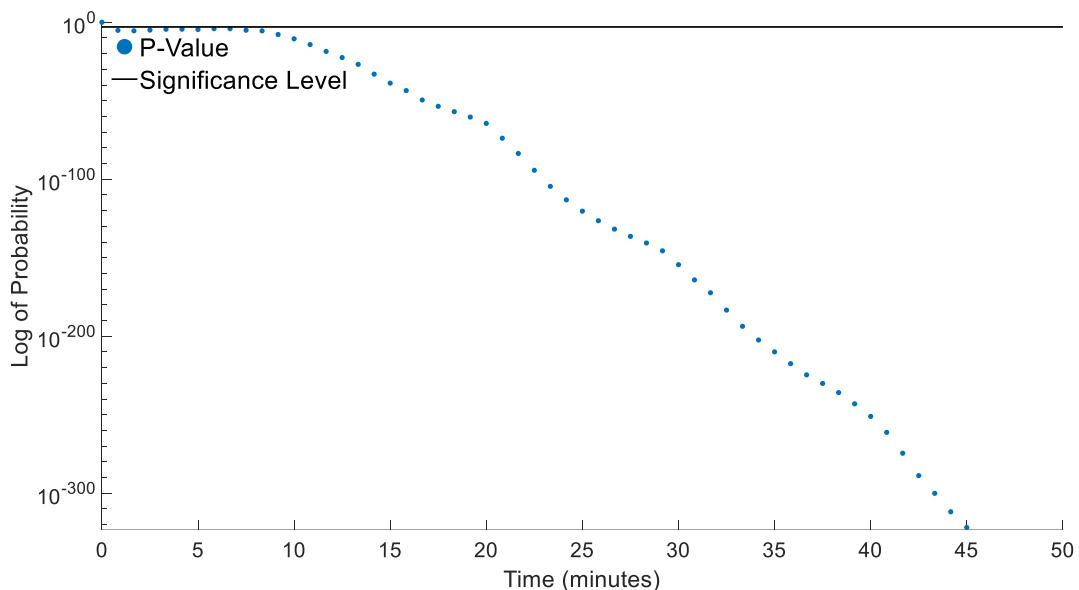


Figure 10: Kolmogorov-Smirnov Plot of Probability and Time in Minutes of P-value and the Significance Level and Detection for an *E.coli* of Concentration $10^6 \frac{CFU}{ml}$

The following run was conducted at a concentration of $10^5 \frac{CFU}{ml}$. Three repeats were carried out to quantify the error. Figure 11 shows the CO₂ response with the baseline with vertical error bars. The error bars of the two plots stop overlapping at about 40 minutes. Visually, it can be concluded that 40 minutes were required to detect $10^5 \frac{CFU}{ml}$ of *E.coli*. The detection time is double that for a concentration of $10^6 \frac{CFU}{ml}$. This is due to the lower concentration of *E.coli*, thus lower CO₂ production. The response does not increase without bound as the above run, instead it starts to level out. It shows a tendency of displaying a similar gradient to the baseline. The expected levelling in CO₂ production rate can be seen to start at about 100 minutes (Lumen Learning, 2019). This levelling can possibly be attributed to the shape of the baseline, depletion of nutrients or death of the microorganisms.

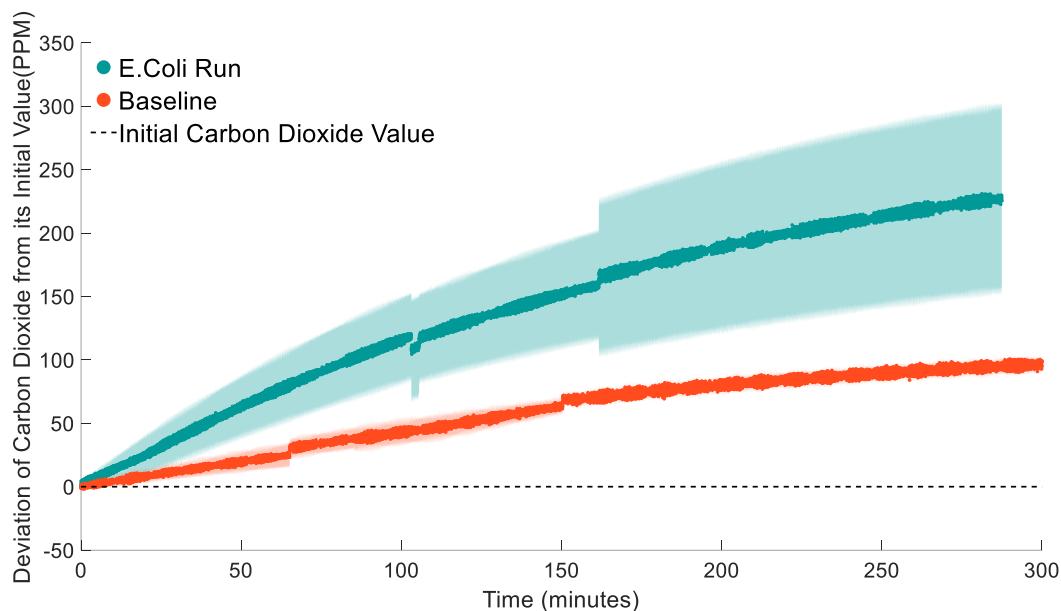


Figure 11: Deviation of Carbon Dioxide from it's Initial Value (in parts per million) versus Time in Minutes of *E.coli* of Concentration $10^5 \frac{CFU}{ml}$ with the Baseline

The detection was confirmed by the Kolmogorov-Smirnov test shown in Figure 12. This test predicted a detection at 32 minutes at the chosen significance level (99%).

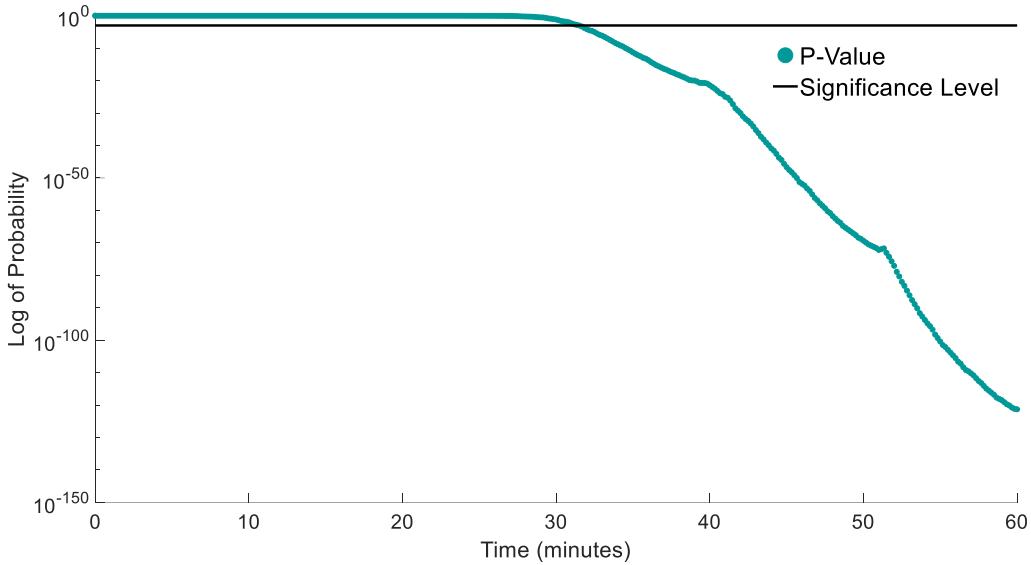


Figure 12: Kolmogorov-Smirnov Plot of the Logarithm of Probability and Time in Minutes of P-value and the Significance Level for an *E.coli* of Concentration $10^5 \frac{CFU}{ml}$

The following run was conducted using a concentration of $10^3 \frac{CFU}{ml}$. Three repeats were carried out. A significant detection was made at 220 minutes in Figure 13. The detection time increased at a lower concentration as expected. The CO₂ response continued to level at a faster rate.

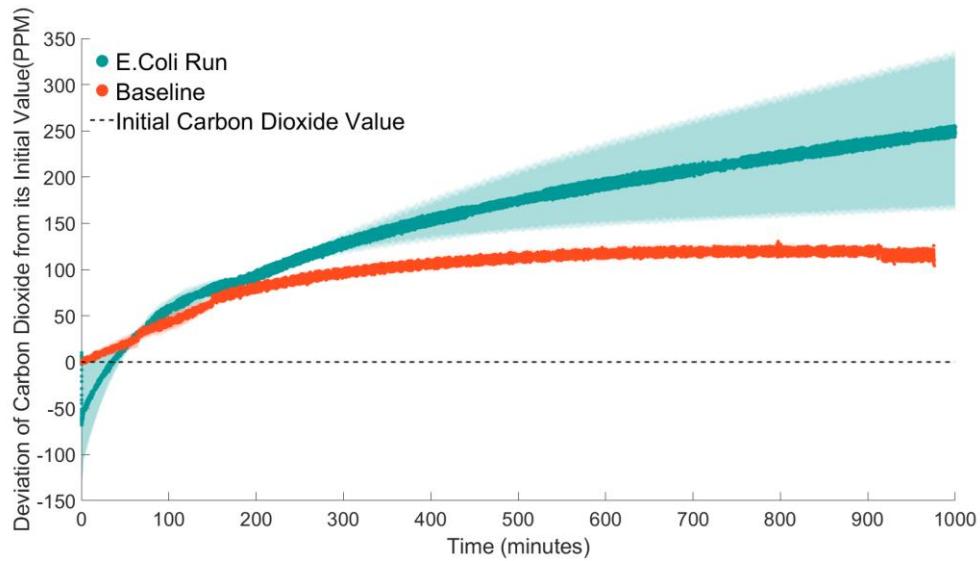


Figure 13: Deviation of Carbon Dioxide from its Initial Value (in parts per million) versus Time in Minutes of *E.coli* of Concentration $10^3 \frac{CFU}{ml}$ with Baseline

The detection was confirmed by the Kolmogorov-Smirnov test (see Figure 14). The detection time was found to be at 175 minutes.

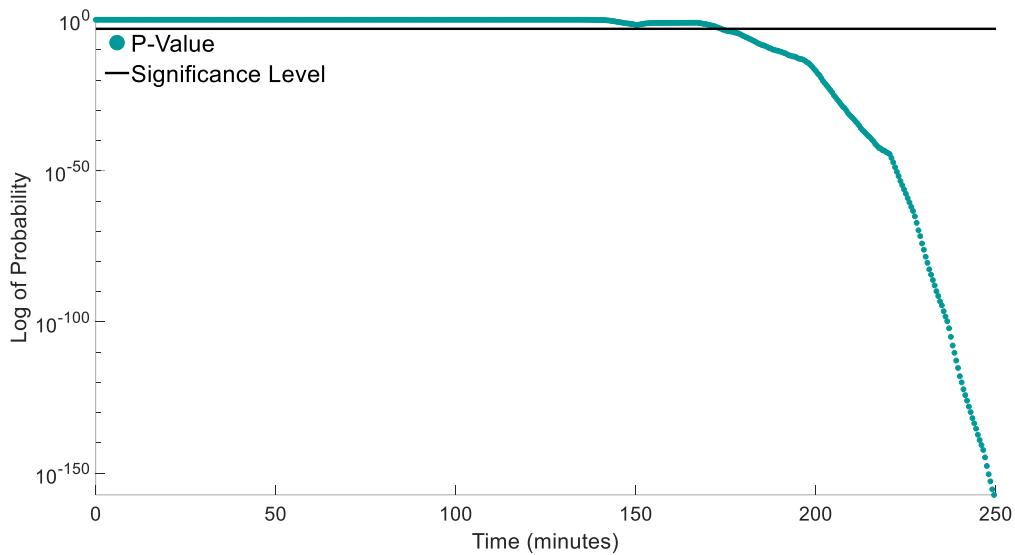


Figure 14: Kolmogorov-Smirnov Plot of the Logarithm of Probability and Time in Minutes of P-value and the Significance Level for an *E.coli* of Concentration $10^3 \frac{CFU}{ml}$

Due to the overlap of the error bars of the two scatter plots Figure 15, it can be concluded that the concentration of *E.coli* is not sufficiently high to result in a detection. At points the run data lies below the baseline error bar range. This should not occur as the run should have a baseline response at least equal to the lower error bar range of the baseline. This can be attributed to insufficient repeat runs for the baseline or *E.coli*. It could also mean that the baseline response changed during the course of the experiment. It may be that the temperature was lower when the baseline testes were carried out compared to when this experiment was completed. This would have resulted in less CO₂ in the headspace for this run. Figure 15 implies that increasing the runtime will not increase the detection limits as a result of the large error bars.

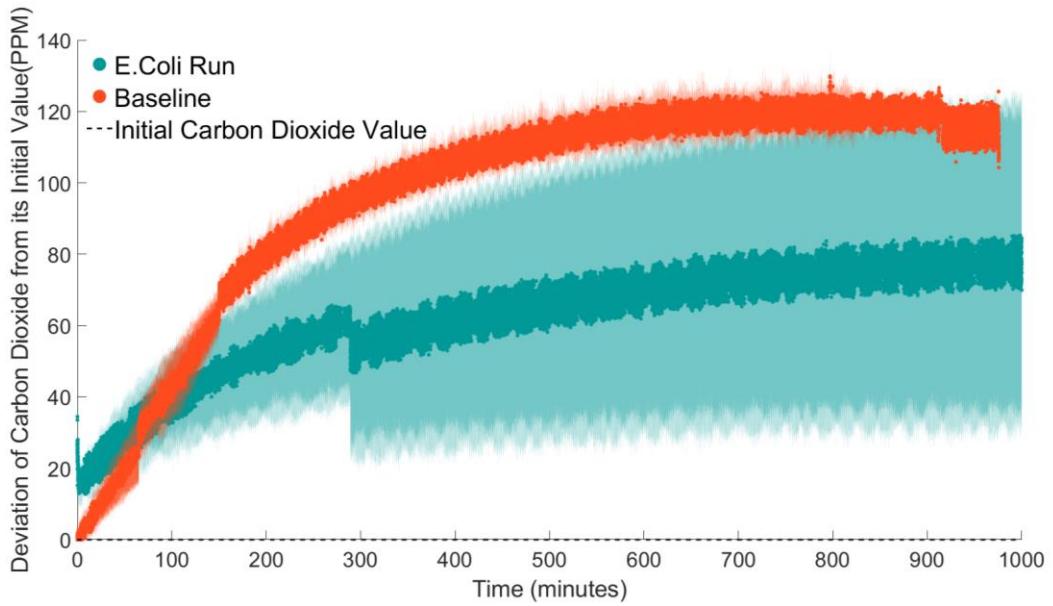


Figure 15: Deviation of Carbon Dioxide from it's Initial Value (in parts per million) versus Time in Minutes of *E.coli* of Concentration $10^2 \frac{CFU}{ml}$ with Baseline

The Kolmogorov-Smirnov test confirms the result (note Figure 16). The p-value remains well above 0.001, thus the detection value remains at zero indicating no detection.

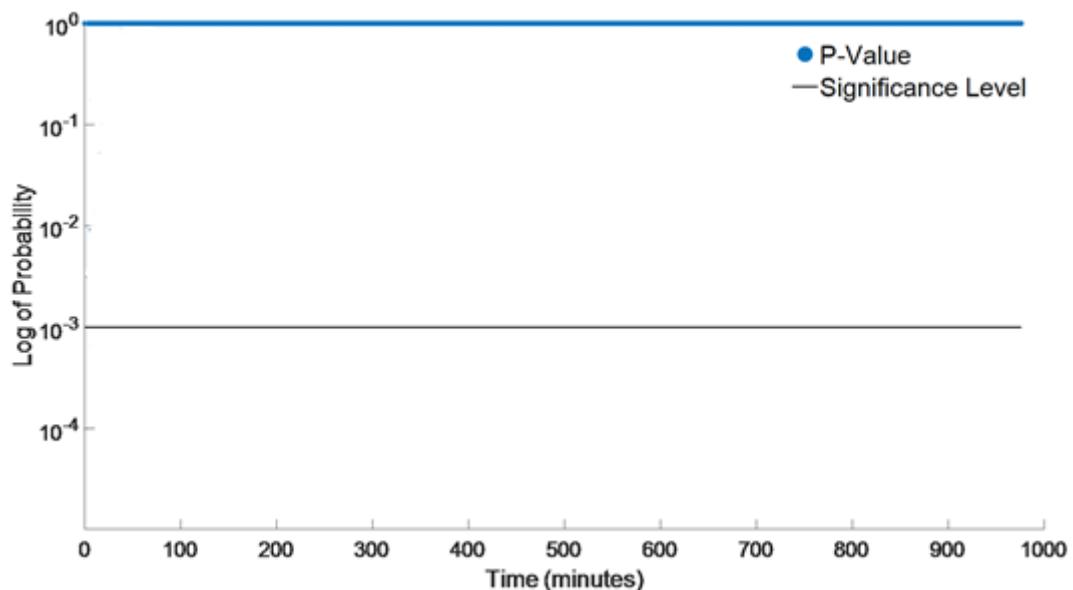


Figure 16: Kolmogorov-Smirnov Plot of Probability and Time in Minutes of P-value and the Significance Level for an *E.coli* of Concentration $10^2 \frac{CFU}{ml}$

4.4 River Water

4.4.1 Stationary Phase Time and Concentration

Table 3 shows the determination of the stationary phase time and plate counts using TSB as a reference. The time taken for the culture to reach the stationary phase was seen to be above 28 hours as seen in Table 3. Table 4 used a new reference, thus results in difference turbidity readings. This run gave a more accurate identification of the stationary phase. It was noted that steady state is reached at 29 hours. The concentration lies at about $4.3 \times 10^8 \frac{CFU}{ml}$ (the average between 29 and 30 hours after inoculation).

Table 3 Steady State Determination of a River Sample using turbidity at 24, 26, 28, 30 and 31 hours after Inoculation.

Time (hours) After Inoculation	Turbidity (with TSB as a reference)
24	0.877
26	1.771
28	1.854
30	1.766
31	1.764

Table 4 Steady State Determination of a River Sample using turbidity and plate counting at 27, 29 and 31 hours after Inoculation with a New Reference.

Time (hours) After Inoculation	Turbidity (with TSB as a reference)	Plate Count
27	2.340	$3.8 \times 10^8 \frac{CFU}{ml}$
29	2.360	$4.0 \times 10^8 \frac{CFU}{ml}$
31	2.357	$4.5 \times 10^8 \frac{CFU}{ml}$

4.4.2 Experimental Runs

The river water culture the concentration of the first Eersterivier water run was selected as $10^6 \frac{CFU}{ml}$. This allowed the lower bound of the detection time to be obtained for the following experiments. The detection time was 260 minutes (roughly 4 hours). The missing data around 150 minutes is likely due to a power cut that occurred during the run. The CO₂ response of this concentration shows a slight decrease in gradient around the 560-minute mark.

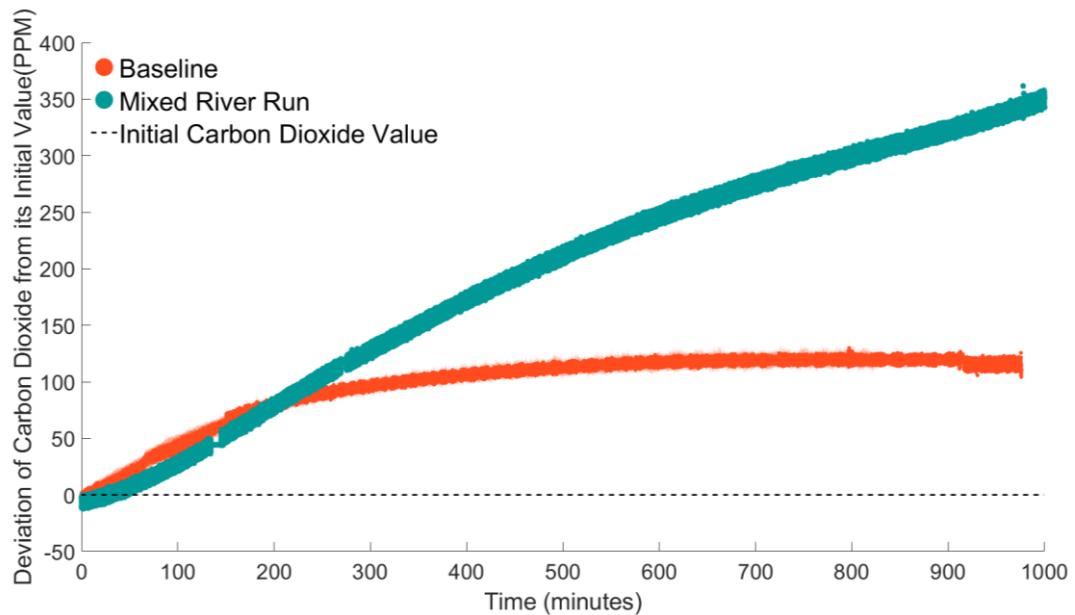


Figure 17: Deviation of Carbon Dioxide from it's Initial Value (in parts per million) versus Time in Minutes of Mixed River Water of Concentration $10^6 \frac{CFU}{ml}$ with Baseline

The Kolmogorov-Smirnov test confirmed the detection as seen in Figure 18. The detection occurred at 235 minutes, continuing the trend of having a shorter time than the visual method.

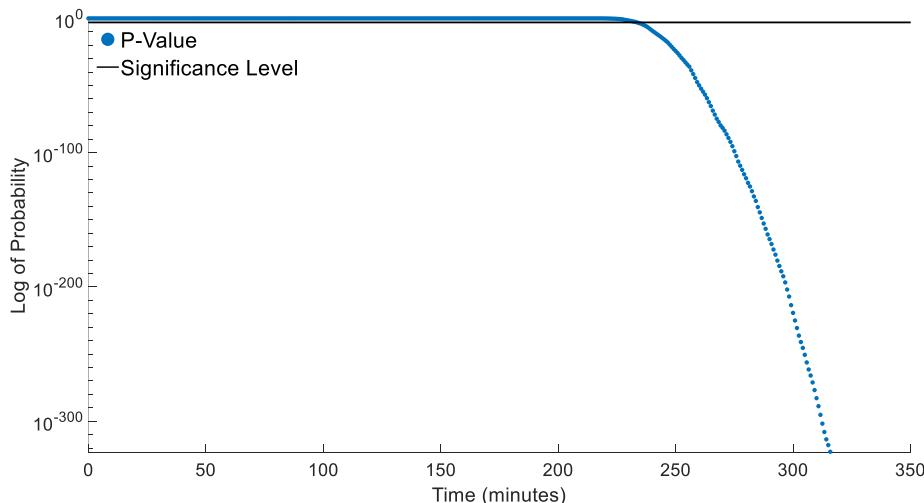


Figure 18: Kolmogorov-Smirnov Plot of Probability and Time in Minutes of P-value and the Significance for a Mixed River Water of Concentration $10^6 \frac{CFU}{ml}$

A concentration of $10^5 \frac{CFU}{ml}$ was tested with one repeat. The detection time was at 820 minutes. The plot is shown in Figure 19. The gradient of the response is similar to that of the baseline up until about 200 minutes. The gradient of the response then maintains a gradient slightly higher than that of the baseline. Finally, the gradient of the response increases to be significantly higher than the baseline where a detection is made.

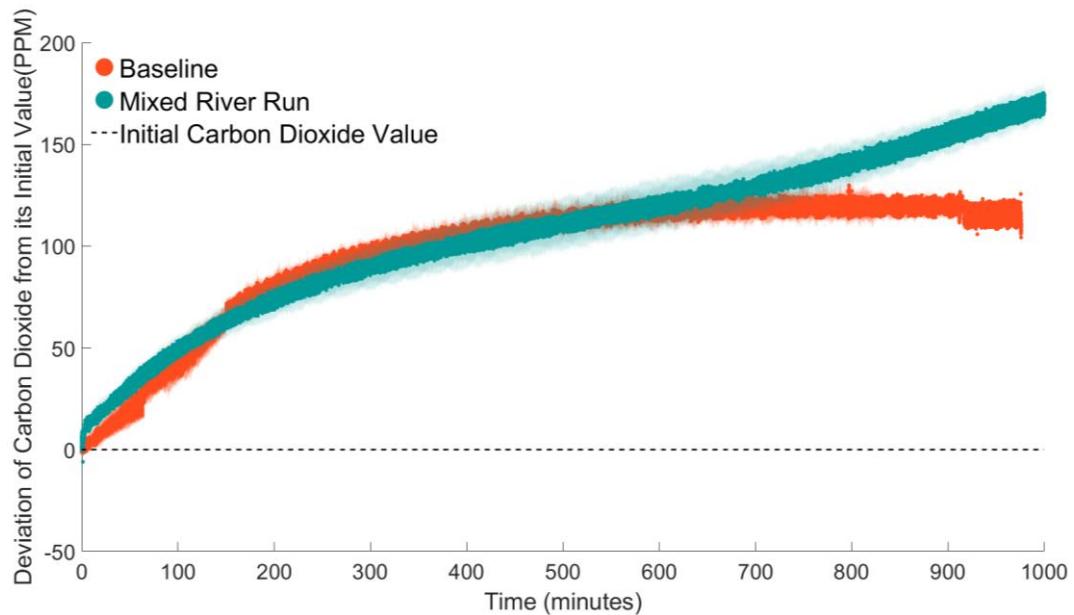


Figure 19: Deviation of Carbon Dioxide from it's Initial Value (in parts per million) versus Time in Minutes of Mixed River Water of Concentration $10^5 \frac{CFU}{ml}$ with Baseline

The Kolmogorov-Smirnov test confirmed the detection (Figure 20). There were sections where the run is larger than the baseline response (0 to 240 minutes and 805 minutes onwards). The later detection time was selected, as it is more consistent with the detection times previously determined. The initial detection can be attributed to not enough repeats being carried out, thus having too narrow error bars. It could be due to the baseline being different to what was calculated.

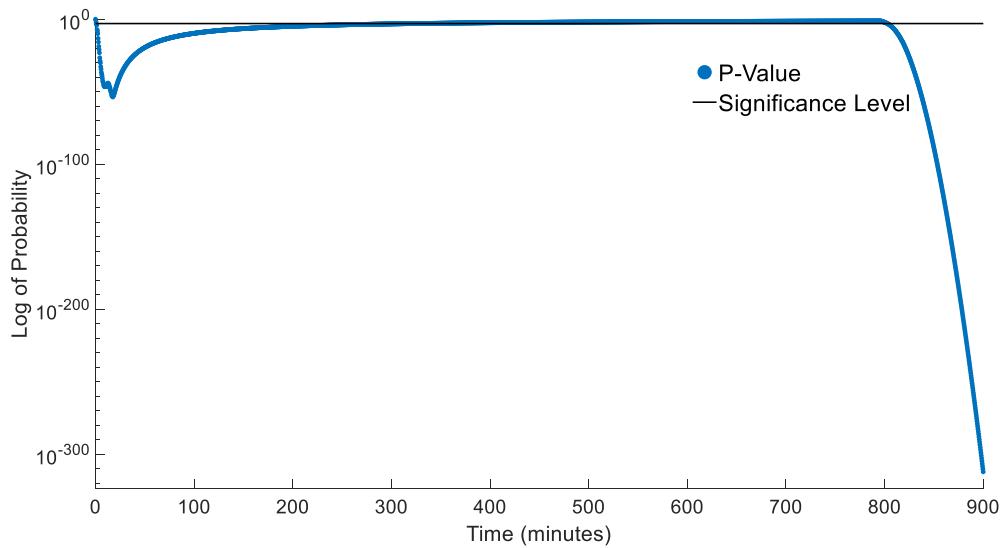


Figure 20: Kolmogorov-Smirnov Plot of Probability and Time in Minutes of P-value and the Significance Level for Mixed River Water of Concentration $10^5 \frac{CFU}{ml}$

Since the detection time for the concentration of $10^5 \frac{CFU}{ml}$ was so high, it was unlikely that a $10^4 \frac{CFU}{ml}$ concentration would result in a detection. This is confirmed by the single run shown in Figure 21. The response was below the baseline value, which could mean that the baseline response for this test was lower than that of the average calculated. Alternatively, it could be a result of a lack of repeat runs. It is predicted that once repeats are carried out the error bars will overlap. The response gradient from 600 minutes onwards is not positive. This implies that increasing the runtime will not result in a detection.

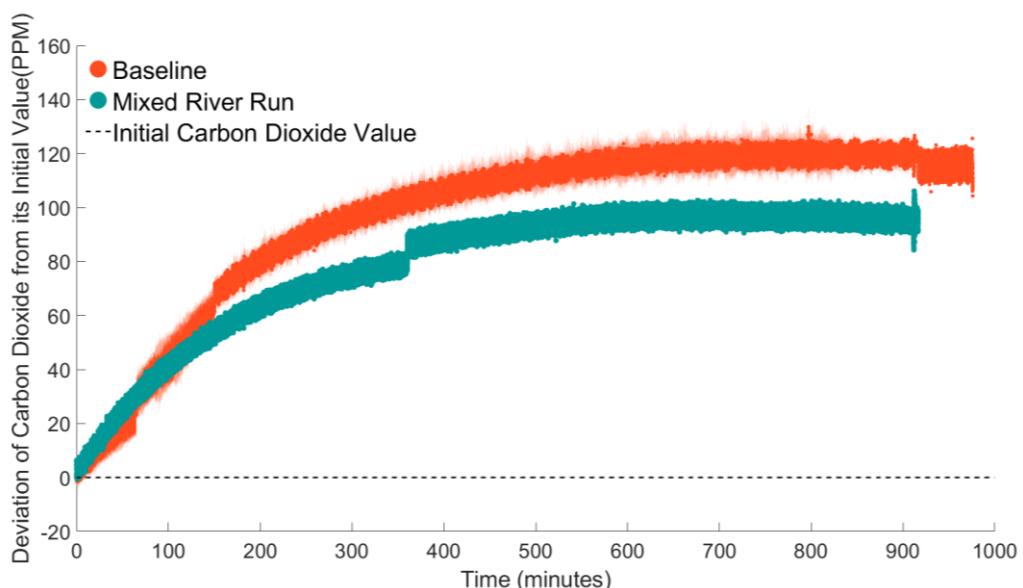


Figure 21: PPM_{CO_2} vs. Time in Minutes of Mixed River Water Run of Concentration $10^4 \frac{CFU}{ml}$ with Baseline

The Kolmogorov-Smirnov test plot (Figure 22) confirmed that no detection took place. The p-value constantly remains well above the significance level.

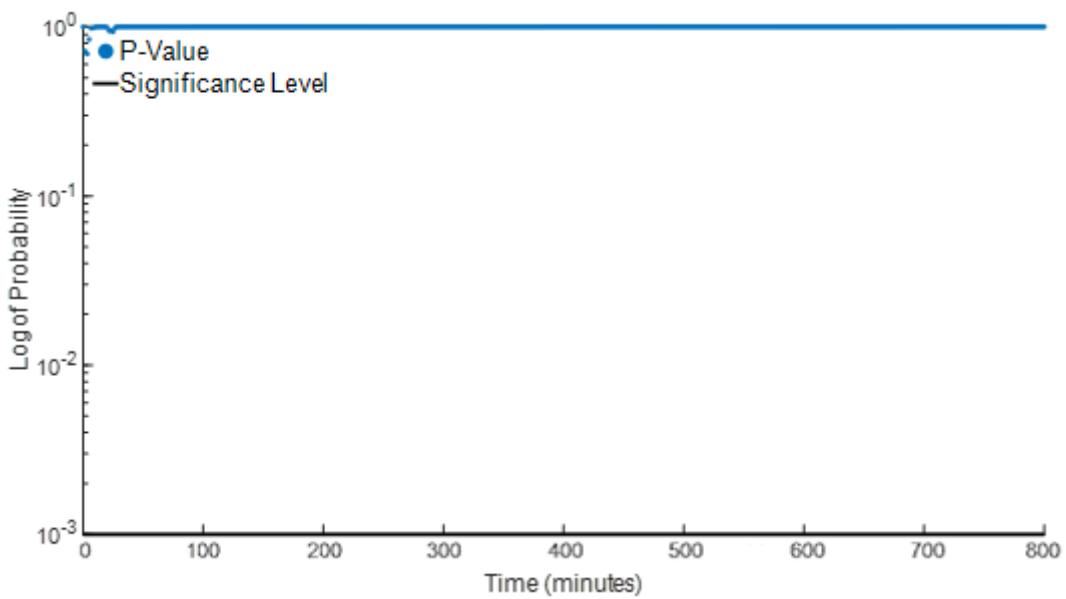


Figure 22: Kolmogorov-Smirnov Plot of Probability and Time in Minutes of P-value with the Significance Level for Mixed River Water of Concentration $10^4 \frac{CFU}{ml}$

The next run conducted was completed using a concentration of $10^3 \frac{CFU}{ml}$. The response was shown in Figure 23. The response was below the baseline, indicating no detection. However, the mixed river water run response did not lie within the error bars of the baseline response at all points. This was likely caused by the lack of repeat runs. Initially the gradient of the experimental run was slightly lower than that of the baseline. It climbed to just above the gradient of the baseline before tapering off again. It seems possible from this figure that increasing the runtime may result in a detection.

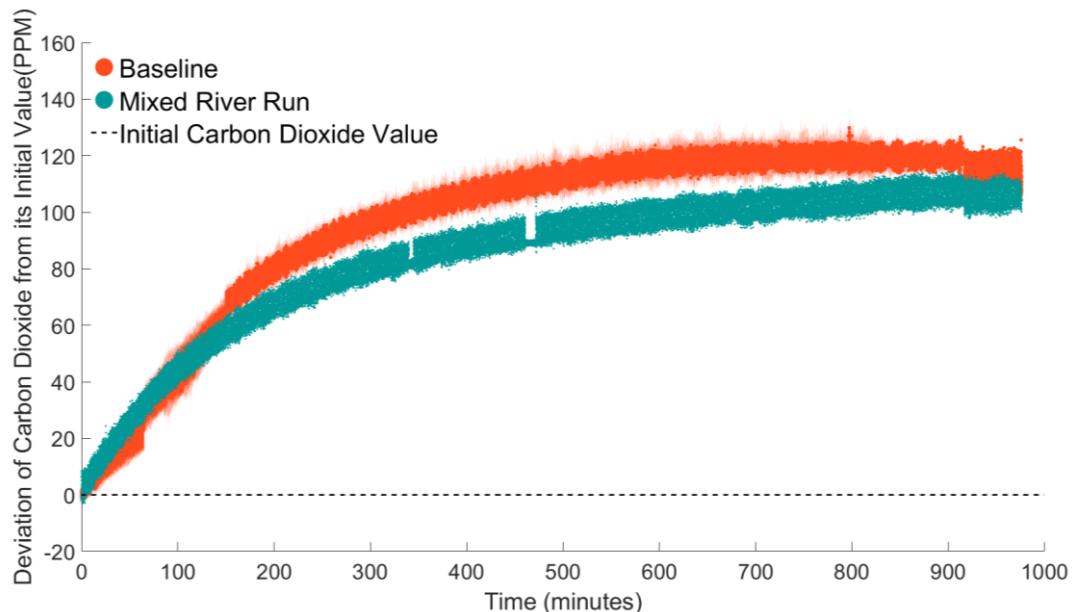


Figure 23: Deviation of Carbon Dioxide from it's Initial Value (in parts per million) versus Time in Minutes of Mixed River Water of Concentration $10^3 \frac{CFU}{ml}$ with Baseline

The Kolmogorov-Smirnov test backed up the lack of detection (Figure 24). The p-value was consistently above the significance level. Initially the p-value nears the significance level before reaching the maximum distance away.

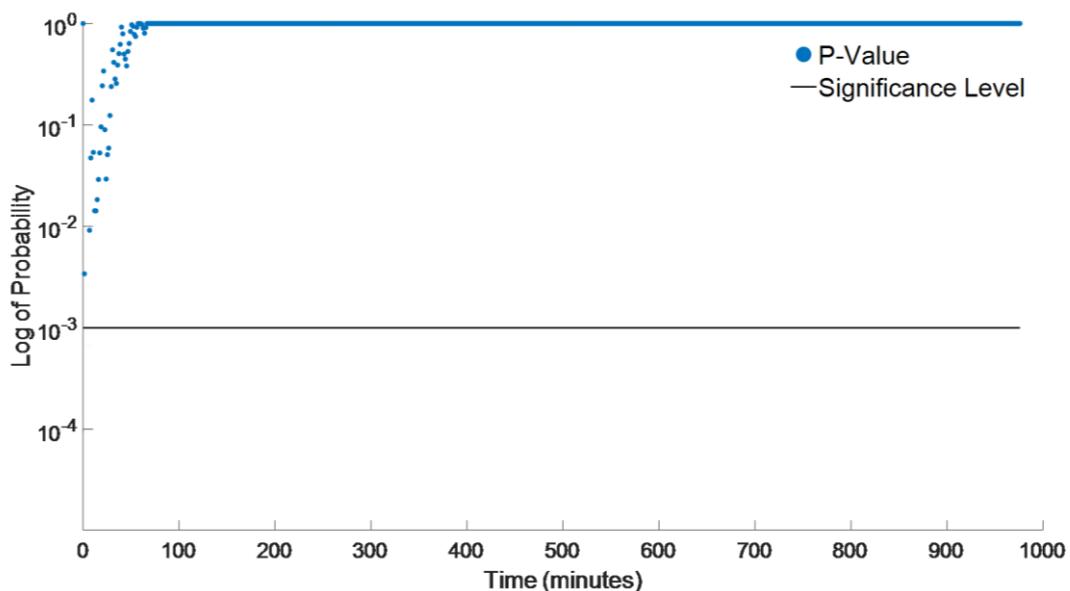


Figure 24: Kolmogorov-Smirnov Plot of Probability and Time in Minutes of P-value and the Significance Level with Corresponding Points of Detection for Mixed River Water of Concentration $10^3 \frac{CFU}{ml}$

The final test was conducted using river water without culturing it (direct river water run). The plate counts before and after were in the range of $10^6 \frac{CFU}{ml}$. The detection time is 300 minutes. The gradient of the response was slightly below that of the baseline initially, but it carried on steadily for the duration of the experiment.

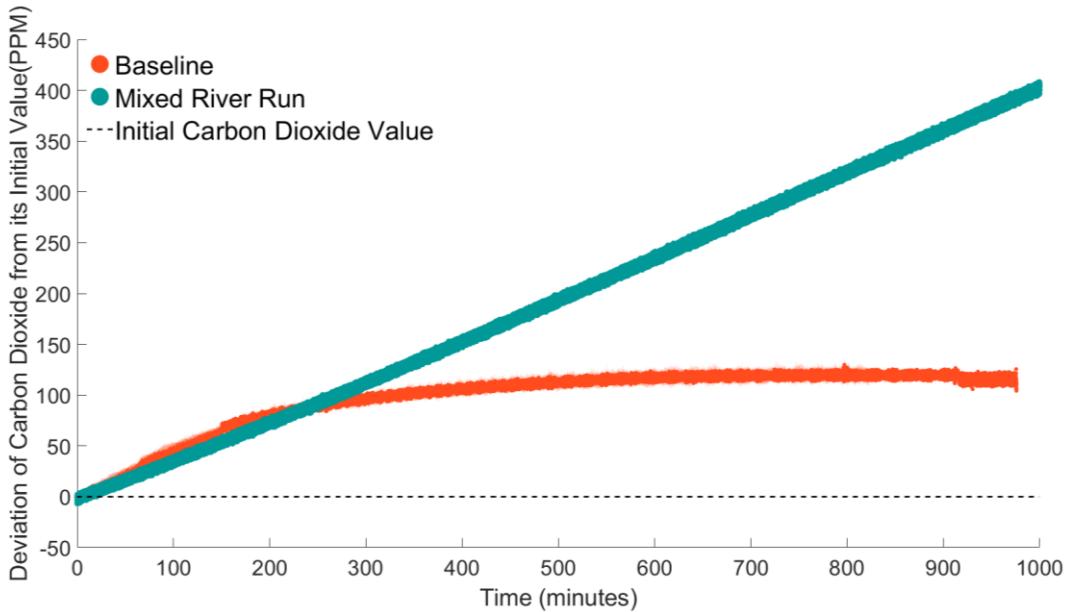


Figure 25: PPM_{CO_2} vs. Time in Minutes of Direct River Water Run without Growth in a Medium of Concentration $10^6 \frac{\text{CFU}}{\text{ml}}$ with Baseline

The Kolmogorov-Smirnov test confirmed the detection (Figure 26). The detection time for this method was 272 minutes.

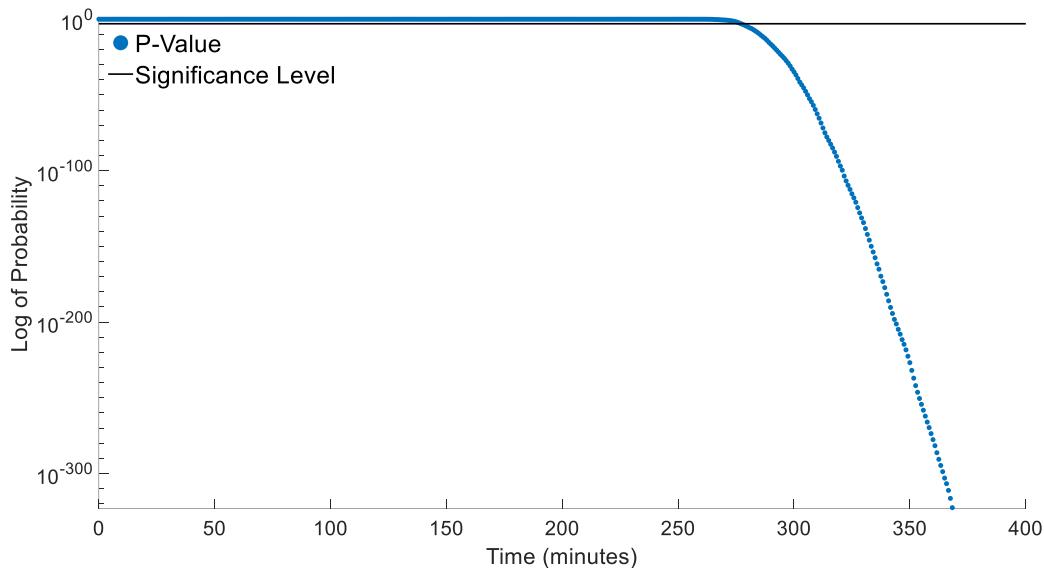


Figure 26: Kolmogorov-Smirnov Plot of Probability and Time in Minutes of P-value and the Significance Level for Mixed River Water of Concentration $10^6 \frac{\text{CFU}}{\text{ml}}$

4.5 Escherichia Coli Compared to Eersterivier Water

It is noted that the river water detection times are between 15 and 200 times longer than that for *E.coli* as seen in Table 5. This can be attributed to *E.coli*, a fast growing microorganism, not being predominant in the river water sample. Slower growing organisms exist in water. Examples of such organisms include

Pseudomonas aeruginosa and *Shigella* spp. The presence of predominantly fast or slow growing organism is reflected in their respiration rate, thus CO₂ production. The Kolmogorov-Smirnov is a faster method of determining whether a detection took place than the visual method as it detected contaminations 23 minutes sooner on average. Both the *E.coli* and mixed river water responses showed more and more similar patterns to the baseline the lower the concentration

Table 5 Comparison of Detection Times for Escherichia coli and Eersterivier Water.

Concentration ($\frac{CFU}{ml}$)	Detection Time (minutes)	
	Visually Kolmogorov-Smirnov Test	
	Escherichia coli	Eersterivier Water
1×10^6	20 1	260 235
1×10^5	40 32	820 805
1×10^4	N/A	No detection
1×10^3	220 175	No detection
1×10^2	No detection	No detection

The mixed river water culture generally showed a lower overall rate of CO₂ production than *E.coli* but had a more sustained CO₂ production rate. Figure 13 shows the limit of detection of *E.coli* (which occurs at a concentration of $10^3 \frac{CFU}{ml}$). The initial gradient of CO₂ production of *E.coli* is significantly greater than that for the baseline. This high gradient was a result of the fast growing *E.coli*, which produces high amounts of CO₂ quickly. This can be compared to Figure 23, which shows the CO₂ response for a concentration of $10^3 \frac{CFU}{ml}$ of river water. In this run, the initial gradient of the CO₂ response was not much greater than that for the baseline however; it carried on for a longer time than its *E.coli*, about 140 minutes, before decreasing in gradient. This more sustained production of CO₂ is a result of the varying generation times of the mixed river water culture. This range of generation time resulted in a range of CO₂ production rates. Further examples of this is shown for a concentration of $10^5 \frac{CFU}{ml}$. For the mixed river water culture (Figure 19) the gradient of the response was barely greater than the baseline initially. The response maintained a gradient barely greater than the baseline up until about 700 minutes where the gradient increased. This contrasts with the *E.coli* run (Figure 11) which immediately displayed a gradient much greater than that of the baseline. A final example was the direct river water run's gradient (Figure 25) which is low (relative to the positive detection plot for *E.coli*), however it maintained this gradient for a long time in contrast to the corresponding concentration of *E.coli* (Figure 9).

4.6 Direct River Water Run

The detection time of the direct river water run compared to the cultured river water at the same concentration shows that the direct river water run has a detection time that is 37 minutes longer than

its cultured counterpart using the Kolmogorov-Smirnov test. This could be due to the growth medium being more nutrient rich than the river water, thus resulting the microorganisms have a lower generation in the culture than in the river water. This effect is shown in detail (Gibson *et al.*, 2018) where generation times of some species is up to 50 times slower. The difference is not as high here, possible due to the low concentration of TSB that was used. Autotrophic microorganisms were unlikely to increase the detection times as the experiments were conducted in an enclosed, dark room.

4.7 Baseline Migration

It is noted that for the river water runs the baseline has shown a tendency to be lower than the established baseline. This can be attributed to the increase in atmospheric temperature. This increase leads to a lower amount of CO₂ being released in the headspace of the vessel.

5 CONCLUSIONS AND RECOMMENDATIONS

The automated CEMS with recycle was shown to require no sample preparation steps, require no technical expertise, produce no waste and is cheaper per run than single use water testing kits. The automated CEMS with recycle was found to be cheaper than the one-use test kits by about R99,14 while also producing no waste.

The stated aim of the project was achieved: an automated system to detect microbial activities at low concentrations (concentrations under $10^6 \frac{CFU}{ml}$) was developed and evaluated. The CEMS was automated using a system of valves, pumps and controlled by the ESP32 microcontroller. It was made operable wirelessly through Wi-Fi. The CEMS with recycle was operated successfully in the laboratory (using cultured samples of *E.coli* and a mixed river water culture) and in a natural setting (by using cultured and direct (uncultured) water from the Eersterivier river) over a time of 1000 minutes. The detection limits of the CEMS were determined for a pure and mixed culture. The *E.coli* detection limit was found to be at a concentration of $10^3 \frac{CFU}{ml}$ and it took 220 minutes to confirm the detection using the CO₂ response plot and 175 minutes using the Kolmogorov-Smirnov plot. The mixed culture detection limit was found to be $10^5 \frac{CFU}{ml}$ at a time of 820 minutes using the CO₂ response plot and 805 minutes using the Kolmogorov-Smirnov method. The direct river water run took 37 minutes longer than its cultured counterpart at the same concentration. The use of the automated CEMS with recycle as a drinking water alarm system is not feasible, according to the South African Water Quality Guidelines, as the detection limits are too high. The guidelines states that above a concentration of $10^2 \frac{CFU}{ml}$ there exists a, albeit slight, risk of microbial infection.

Although the system cannot act as a drinking water contamination alarm, it can identify contamination of higher concentration faster than traditional methods such as the multiple tube fermentation technique. These methods (for a concentration of $10^6 \frac{CFU}{ml}$ of direct river water) can take up to 48 hours to confirm a result compared to the 272 minutes from the automated CEMS with recycle.

The Kolmogorov-Smirnov test was found to detect a contamination 23 minutes earlier, on average, compared to the visual method. The Kolmogorov-Smirnov test method of detection consistently verified detections earlier than the visual method with only one false positive. Due to this the Kolmogorov-Smirnov test should be used as the method in the automation algorithm to confirm the presence of microorganisms in the sample.

In a natural setting the apparatus should be opaque to inhibit the growth of photoautotrophs (such as algae) which will lower the sensitivity of the system. The system will continuously cycle the air in the headspace over 1000 minutes then flush the liquid. Baseline tests will have to be carried out to quantify the amount of CO₂ liberated during the runs in the natural setting. This baseline should be much flatter than those presented here due to the more consistent temperature. Ideally the system would be run at a similar time each day to ensure that the temperature change (thus baseline) is constant with each run. Alternatively, a database of baselines for each start and end temperatures can be obtained which will be

used to compare the experimental run to. The baseline can be selected based on the start temperature which will allow for a detection to be confirmed in real time. If further accuracy is desired a baseline and experimental run can be ran simultaneously, however this will require an additional sensor.

5.1 Further Testing

Future experiments need to be carried out using pure cultures of slower growing microorganisms such as *Pseudomonas aeruginosa* and *Shigella spp*. This will allow for the hypothesis that the predominant species present in mixed sample can be identified from the detection time and plot. A more optimistic hypothesis to test is to determine if the predominant species and concentration can be inferred by the response curve and detection time.

Repeat tests need to be carried out on experiments with one or no repeats. The Kolmogorov-Smirnov plots for mixed river water of concentration $10^5 \frac{CFU}{ml}$ shows a premature detection. This may be rectified once more repeats have been carried out.

Whether increasing the runtime increases the detection limits needs to be examined. This is unlikely, however, as all the run response figures with no detection (except Figure 23) indicate that this will not occur due to the flat gradient of the response plot.

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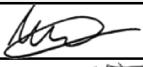
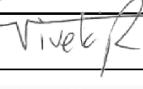
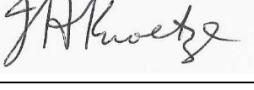
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APPENDIX A – PROJECT PROPOSAL AND MEETING MINUTES

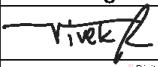
Department of Process Engineering Final Year Project (CE 478 | MP 478) – Project Approval Form

Name of Supervisor	Student Name and Number
Dr TM Louw	Raga, VV - 18643450
Project Title	
Automation and evaluation of a newly developed Carbon dioxide Evolution Monitoring System	
Project Description and Objectives	
<p>An online, real-time Carbon dioxide Evolution Monitoring System (CEMS) has been developed to quantify microbial activity in a variety of settings, including wastewater treatment plants, polluted rivers, grey water, etc. However, the sensitivity of the current CEMS implementation limits its utility to wastewater treatment plants. The detection limits can be enhanced by automating the system such that the integrated carbon dioxide production over a fixed period of time can be determined. The goal of this project is to develop an automated CEMS capable of detecting microbial activities at low concentrations.</p>	
Project Outcome and Deliverables	
<p>The following requirements have to be met for the project to be considered successful:</p> <ul style="list-style-type: none"> • Development of a process control system (hardware and software) to automate the CEMS; • Application of the automated CEMS to monitor microbial activity in a controlled lab setting; • Evaluation of the utility of the CEMS as an alarm system in a variety of natural and engineered settings. 	
Project Characteristics and Requirements	
<p>Applicability to Chemical Engineering: The student must engage with selected knowledge in the research literature of the chemical engineering discipline. Briefly describe the relevance of this project to chemical engineering.</p> <p>The project focusses on the development and implementation of a process control system for the continuous monitoring of microbial activity. The project requires knowledge of process control and is directly relevant to environmental engineering and wastewater treatment.</p>	
<p>References: Are sufficient literature sources available and accessible to support this topic? Provide at least two examples.</p> <ol style="list-style-type: none"> 1) Kroukamp, Otini, and Gideon M. Wolfaardt. "CO₂ production as an indicator of biofilm metabolism." <i>Appl. Environ. Microbiol.</i> 75.13 (2009): 4391-4397. 2) Kim, Kyukwang, and Hyun Myung. "Sensor node for remote monitoring of waterborne disease-causing bacteria." <i>Sensors</i> 15.5 (2015): 10569-10579. 	
<p>Infrastructure, Resources and Funding: Are there special infrastructure, resource or funding requirements for this project? If so, specify how these will be provided.</p> <p>The CEMS is available for use. Appropriate training on the system, as well as lab space, will be provided by active collaborators at the Department of Microbiology. No additional funding is required.</p>	
ECSA Exit Level Outcomes	
<p>2. Application of scientific and engineering knowledge: Apply knowledge of mathematics, natural sciences, engineering fundamentals and an engineering speciality to solve complex engineering problems.</p> <p>Knowledge of the following areas of mathematics and/or natural science and/or engineering fundamentals shall be applied:</p> <ul style="list-style-type: none"> • Process control; • Bioprocess engineering; • Environmental engineering; • Analytical chemistry. <p>Sufficient complexity shall be captured in:</p> <ul style="list-style-type: none"> • The evaluation of the proposed method in a variety of undefined natural and engineered settings. 	

Department of Process Engineering
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4. Investigations, experiments and data analysis: Demonstrate competence to design and conduct investigations and experiments.		
Investigate the use of various hardware/software alternatives for the automation of the monitoring device. Planning and execution of a series of experiments to determine the sensitivity of the CEMS to different microbial loads. Analysis of results to determine the utility of the CEMS to various natural and engineered settings.		
6. Professional and technical communication: Demonstrate competence to communicate effectively, both orally and in writing, with engineering audiences and the community at large.		
A well-structured, well-written, professional technical report shall be submitted for assessment. In addition, oral and poster presentations shall be delivered to examiners as part of the formal assessment.		
8. Individual, team and multidisciplinary work: Demonstrate competence to work effectively as an individual, in teams and in multidisciplinary environments.		
The student shall work under the guidance of a supervisor. The supervisor shall provide sound, professional advice and administrative support, but shall not do the work on behalf of the student. The student shall therefore demonstrate competence to interact, devise and conduct the investigation effectively as an individual, as follows: <ul style="list-style-type: none"> • Regularly meet with the supervisor (including taking minutes of meetings) and interact with laboratory and other technical support staff. • Design and implement an automated method for the real-time use of the CEMS • Plan and conduct a series of experiments. • Analyse and interpret results. • Assess the utility of the proposed method for application in a variety of settings. • Present findings in a report and during oral presentations, all though individual efforts. 		
9. Independent learning ability: Demonstrate competence to engage in independent learning through well-developed learning skills.		
The student shall engage, independently and without formal lecturing, with new theoretical and/or practical concepts. Key concepts and skills to be mastered independently are: <ul style="list-style-type: none"> • Online, real-time, automated sensing; • Microbial carbon cycling; • Microbial measures of environmental impact 		
Criteria for continuation of project (items to be delivered by June)		
Prototype automated CEMS Completed planning and gathered all resources for experiments		
Sign-off: This project has been registered and reviewed by the Department of Process Engineering, and accepted as suitable for a Final Year Project.		
	Signature	Date
Supervisor		2019/10/30
Student		2019/10/30
Coordinator		04/11/2019

Department of Process Engineering
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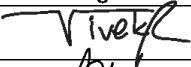
Name	Signature	Date
Vivek Raga		19/02/2019
Dr Tobie Louw	 Digitally signed by Tobi Date: 2019.02.19 14:34:40 40200	19/02/2019

Problems experienced and progress made since previous meeting
The hand in dates were discussed as well as milestones that should be achieved throughout the year.
General expectations were highlighted.
Explanations of two project topics and their suitability were discussed.

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Project allocation	Dr Louw	1 March
Biweekly meetings to track progress starting on 01/03/2019 at 11:00am	Vivek Raga	Every two weeks
Progress Report Hand-in	Vivek Raga	24 June
Oral Presentation	Vivek Raga	25-26 July
Final Report Hand-in	Vivek Raga	5 November

All parties present must sign these short minutes at the end of the meeting. Scan the signed document and send an electronic copy to your supervisor within 48 hours after the meeting. The original must be included in an appendix of your final report.

Department of Process Engineering
Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting

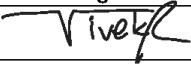
Name	Signature	Date
Vivek Raga		06/03/2019
Dr. Wendy Stone		07/03/2019

Problems experienced and progress made since previous meeting
This first meeting was used to clarify the scope and expectations from the microbiology part of the assignment.

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
A meeting between my supervisors and myself to further clarify the scope of the project.	Vivek Raga Dr. Wendy Stone	08/03/2019
Lab safety training	Vivek Raga	Around June/July <i>(provisional)</i>
Literature data is to be sent as well as a description of the lab work to be carried out.	Dr. Wendy Stone	07/03/2019

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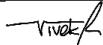
Name	Signature	Date
Vivek Raga		08/03/2019
Dr. Tobi Louw		08/03/2019
Dr. Wendy Stone		08/03/2019

Problems experienced and progress made since previous meeting
The project was clearly defined and expectations were given. The skripsi project registration form was made available.

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Attending a workshop on the topic of low cost technology to get an idea of how to use a micro-controller (Arduino or Raspberry PI) for the purposes of automating the CEMS system.	Vivek Raga	End of March
Investigate methods to detect microbial levels in sample	Vivek Raga	Before the end of June
Decide on how much is the maximum time for allowing the microbes to reside in the tank.	Vivek Raga	Before the end of June
Decide on the method of detecting CO ₂ released from microbes	Vivek Raga	Before the end of June
Develop an automated process control system to automate the CEMS	Vivek Raga	Before the end of June

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Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting

Name	Signature	Date
Vivek Raga		15/03/2019
Dr. Tobie Louw		15/03/2019

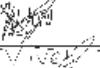
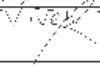
Problems experienced and progress made since previous meeting

The topic registration form was submitted.
 Literature sources were added. The contents of the report's literature review were explained.
 Different forms of measurement of microbes are to be compared to the method to be used in this project. The strengths and weaknesses of this project and its methods need to be compared to existing methods.

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Water standards need to be obtained and compared	Vivek Raga	
The components required as well as their layout need to be determined	Vivek Raga	
Research needs to be done on water sanitation methods	Vivek Raga	

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Department of Process Engineering
Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting

Name	Signature	Date
Dr Stone		12/04/2019
Vivek Raga		12/04/2019

Problems experienced and progress made since previous meeting

The design and its requirement have been clarified. The device was decided to run on a timer with additional investigation being done on operation via WiFi.

The air pump was provided for the assignment.

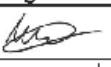
Preliminary pipe sizing was carried out.

The CEMS was investigated in terms of it's incorporation with the circuit.

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Design was altered, loading and flushing streams need to be removed	Vivek Raga	May
Final pipe sizing needs to be carried out and necessary parts ordered.	Vivek Raga	May
The peristaltic air pump needs to be investigated for use in the system	Vivek Raga	May
Noted that circulating water via pump is not necessary, since real-world application involves testing headspace only	Vivek Raga	May

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Department of Process Engineering
Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting

Name	Signature	Date
Dr Louw		12/04/2019
Vivek Raga		12/04/2019

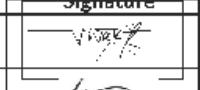
Problems experienced and progress made since previous meeting

Questions regarding the equipment to be used were answered including: what the reactor will look like, would the CEMS system need to be connected to the Arduino in any way and the types of solenoid valves necessary for the system.

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Remedy the Literature review to increase focus on CO2 monitoring and limit focus on the Arduino	Vivek Raga	June
Alter Design to keep air pump active throughout the cycle	Vivek Raga	May

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**Department of Process Engineering
Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting**

Name	Signature	Date
Vivek Raga		26/04/2019
Dr. Louw		26/04/2019

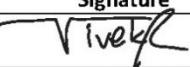
Problems experienced and progress made since previous meeting

Vessel material was decided on. Flushing method was finalized.
Ordering process was discussed.

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Request quotes from valve suppliers. Make sure they are correctly specified.	Vivek Raga	26/04/2019
Confirm valve, pump and vessel suitability with Mr. Peterson and Mr. Jooste	Vivek Raga	06/05/2019
Confirm orders for solenoid valves	Vivek Raga	09/05/2019

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Department of Process Engineering
Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting

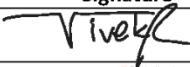
Name	Signature	Date
Vivek Raga		02/09/2019
Dr.Louw	Tobias Louw <small>Digital signature by Tobias Louw Date: 2019.11.04 14:07:40 +02:00</small>	02/09/2019

Problems experienced and progress made since previous meeting
Ecoli Runs were completed and Data interperated

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Mixed water run experimental preparation	Vivek	05/09/2019

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Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting

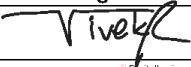
Name	Signature	Date
Vivek Raga		04/10/2019
Dr.Louw	Tobias Louw Digitally signed by Tobias Louw Date: 2019.11.04 14:08:10 +02'00'	04/10/2019

Problems experienced and progress made since previous meeting
Experimental results were obtained

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Finish the Draft	Vivek	28/10/2019
Final Experimental Run Finalization	Vivek	21/10/2019

All parties present must sign these short minutes at the end of the meeting. Scan the signed document and send an electronic copy to your supervisor within 48 hours after the meeting. The original must be included in an appendix of your final report.

Department of Process Engineering
Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting

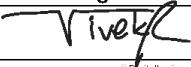
Name	Signature	Date
Vivek Raga		17/10/2019
Dr.LOUW	Tobias Louw <small>Digital signature by Tobias Louw Date: 2019.11.04 14:08:22 40200</small>	17/10/2019

Problems experienced and progress made since previous meeting
The preliminary data processing was conducted. The first set of runs were completed.

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Use the Kolmogorov–Smirnov test to determine whether the run is statistically similar to the baseline	Vivek	28-Oct
Use standard deviations to display variation in runs and baseline	Vivek	28-Oct
Complete the writeup	Vivek	28-Oct
Run mixed river water run to find the smallest detection concentration	Vivek	25-Oct

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Department of Process Engineering
Final Year Project (CE 478 | MP 478) – Short Minutes of Meeting

Name	Signature	Date
Vivek Raga		31/10/2019
Dr.LOUW	Tobias Louw <small>Digital signature by Tobias Louw Date: 2019.11.04 14:08:34 40200</small>	31/10/2019

Problems experienced and progress made since previous meeting
Draft Report was further amended KS test was completed

Decisions, as well as actions to be taken after meeting	Responsible	Deadline
Display a plot of the K-S test in addition to the error bar plot	Vivek	01/11/2019
Further Correct Report, specifically the Results and Discussion section	Vivek	01/11/2019

All parties present must sign these short minutes at the end of the meeting. Scan the signed document and send an electronic copy to your supervisor within 48 hours after the meeting. The original must be included in an appendix of your final report.

APPENDIX B – ARDUINO CODE

```

WiFi.begin(ssid, password);
Serial.println(WiFi.macAddress());
while (WiFi.status() != WL_CONNECTED) {
    delay(500);
    Serial.print("\n connecting");
    Serial.print(".");
}

// Print local IP address and start web server
Serial.println("");
Serial.println("WiFi connected.");
Serial.println("IP address: ");
Serial.println(WiFi.localIP());
server.begin();

}

void loop(){
 WiFiClient client = server.available(); // Listen for incoming clients

if (client) { // If a new client connects,
    Serial.println("New Client.");
    String currentLine = ""; // make a String to hold incoming data from the client
    while (client.connected()) { // loop while the client's connected

        if (client.available()) { // if there's bytes to read from the client,
            char c = client.read(); // read a byte, then
            Serial.write(c); // print it out the serial monitor

            header += c;
            if (c == '\n') { // if the byte is a newline character
                // if the current line is blank, you got two newline characters in a row.
                // that's the end of the client HTTP request, so send a response:
                if (currentLine.length() == 0) {
                    // HTTP headers always start with a response code (e.g. HTTP/1.1 200 OK)
                    // and a content-type so the client knows what's coming, then a blank line:
                    client.println("HTTP/1.1 200 OK");
                    client.println("Content-type:text/html");
                    client.println("Connection: close");
                    client.println();

                    // turns the GPIOs on and off
                    if (header.indexOf("GET /26/on") >= 0) {

                        Serial.println("GPIO 26 on");
                        output26State = "on";
                        digitalWrite(output26, HIGH);
                        wflush();
                        fill();
                    } else if (header.indexOf("GET /26/off") >= 0) {

                        Serial.println("GPIO 26 off");
                        output26State = "off";
                        digitalWrite(output26, LOW);
                    } else if (header.indexOf("GET /27/on") >= 0) {
                        Serial.println("GPIO 27 on");

                        output27State = "on";
                        digitalWrite(output27, HIGH);
                    } else if (header.indexOf("GET /27/off") >= 0) {
                        Serial.println("GPIO 27 off");
                        output27State = "off";
                        digitalWrite(output27, LOW);
                    }
                }
            }
        }
    }
}

// Display the HTML web page
client.println("<!DOCTYPE html><html>");
client.println("<head><meta name=\"viewport\" content=\"width=device-width, initial-scale=1\">");
client.println("<link rel=\"icon\" href=\"data:,\">");
// CSS to style the on/off buttons
// Feel free to change the background-color and font-size attributes to fit your preferences
client.println("<style>html { font-family: Calibri; display: inline-block; margin: 0px auto; text-align: center; }");
client.println("button { background-color: blue; border: none; color: black; padding: 16px 40px; }");
client.println("text-decoration: none; font-size: 20px; margin: 1px; cursor: pointer; }");
client.println(".button2 {background-color: #555555;}</style></head>");

// Web Page Heading
client.println("<body><h1>CEMS Controller</h1>");

// Display current state, and ON/OFF buttons for GPIO 26
client.println("<p>Run- State " + output26State + "</p>");
// If the output26State is off, it displays the ON button
if (output26State=="off") {
    client.println("<p><a href=\"/26/on\"><button class=\"button\">Run</button></a></p>");
```

```

    } else {
        client.println("<p><a href=\"/26/off\"><button class=\"button button2\">Running...</button></a></p>");
    }

    // Display current state, and ON/OFF buttons for GPIO 27
    // client.println("<p>GPIO 27 - State " + output27State + "</p>");
    // If the output27State is off, it displays the ON button
    // if (output27State=="off") {
    //     client.println("<p><a href=\"/27/on\"><button class=\"button\">ON</button></a></p>");
    // } else {
    //     client.println("<p><a href=\"/27/off\"><button class=\"button button2\">OFF</button></a></p>");
    // }
    // client.println("</body></html>");

    // The HTTP response ends with another blank line
    client.println();
    // Break out of the while loop
    break;
} else { // if you got a newline, then clear currentLine
    currentLine = "";
}
} else if (c != '\r') { // if you got anything else but a carriage return character,
    currentLine += c;      // add it to the end of the currentLine
}
}

// Clear the header variable
header = "";

// Close the connection
client.stop();
Serial.println("Client disconnected.");
Serial.println("");
}
}

///////////////////////////////void wflush() //int soutout, int airin, int airout, int flushtime
{
    Serial.println("starting flush");
    delay(500);          // wait for a 1/2 second to avoid any em interferance
    digitalWrite(solutout, HIGH); //Switch outlet Solenoid ON to allow water to flush
    digitalWrite(airin,LOW);   //Assuming air valve is NO
    digitalWrite(airout,HIGH); // Depends on Conception !! make sure this flushes to atmosphere!

    delay(flushtime);      //allow for all/most of the water to exit the vessel
    digitalWrite(solutout, LOW); //Switch outlet Solenoid OFF to allow start of filling procedure

    delay(500);          // wait for a 1/2 second to avoid any em interference
}
///////////////////////////////void fill() //int soutout, int airin, int airout, int flushtime
{
    digitalWrite(solin, HIGH); //Switch inlet Solenoid ON
    delay(500);           // wait for 1/2 a second

    digitalWrite(relay, HIGH); //Switch inlet pump ON

```

```

    // Close the connection
    client.stop();
    Serial.println("Client disconnected.");
    Serial.println("");
}

void wflush() //int solout, int airin, int airout, int flushtime
{
    Serial.println("starting flush");
    delay(500);           // wait for a 1/2 second to avoid any em interferance
    digitalWrite(solout, HIGH); //Switch outlet Solenoid ON to allow water to flush
    digitalWrite(airin,LOW);   //Assuming air valve is NO
    digitalWrite(airout,HIGH); // Depends on Connecction !! make sure this flushes to atmosphere!

    delay(flushtime);      //allow for all/most of the water to exit the vessel
    digitalWrite(solout, LOW); //Switch outlet Solenoid OFF to allow start of filling procedure

    delay(500);           // wait for a 1/2 second to avoid any em interference
}
void fill() // int solout, int airin, int airout, int flushime
{
    digitalWrite(solin, HIGH); //Switch inlet Solenoid ON
    delay(500);             // wait for 1/2 a second

    digitalWrite(relay, HIGH); //Switch inlet pump ON
    delay(pumptime);        //Allow Pumping for determined duration seconds

    digitalWrite(relay, LOW); //Switch inlet pump OFF
    delay(500);             // wait for a 1/2 second to avoid any em interference
    digitalWrite(solin, LOW); //Switch in Solenoid OFF to closed position
}

void flash()
{
    digitalWrite(output26, HIGH);
    delay(500);
    digitalWrite(output26, LOW);
    delay(500);

    digitalWrite(output26, HIGH);
    delay(500);
    digitalWrite(output26, LOW);
    delay(500);

    digitalWrite(output26, HIGH);
    delay(500);
    digitalWrite(output26, LOW);
    delay(500);
}

```

APPENDIX C – SAMPLE CALCULATIONS

Calculation of the Resistor in Series with the One-Way Air Valve

The voltage of the power source is 12 Volt. The voltage required by the solenoid valve is 3 Volts. The current required by the valve is 0.16A

$$\begin{aligned}V &= I * R \\R &= \frac{V}{I} \\R &= \frac{12 V - 3V}{0.16 A} \\R &= 56 \text{ Ohm}\end{aligned}$$

Therefore, a 56-Ohm resistor will be used in series with the solenoid valve.

Amount of CO₂ Dissolved at a Specific Temperature

$$\begin{aligned}\text{Molecular weight of Water} &= 18.02 \frac{\text{g}}{\text{mol}} \\ \text{Molecular weight of CO}_2 &= 44.01 \frac{\text{g}}{\text{mol}}\end{aligned}$$

Solubility constants for CO₂ for use in equation 6 provided in (Green and Perry, 2008):

$$A = -159.9$$

$$B = 8741.7$$

$$C = 21.7$$

$$D = 1E - 3$$

At a temperature of 273 K.

$$\begin{aligned}\ln x &= A + \frac{B}{T} + C \ln T + DT \\ \ln x &= -159.9 + \frac{8741.7}{273} + 21.7 \ln(21.7) + 1E - 3 \times 21.7 \\ x &= 1.387407E - 3 \frac{\text{moles CO}_2}{\text{moles water}}\end{aligned}$$

Cost to Run the Automated CEMS with Recycle

Total watt usage of the system

Sensor: 14W

Pumps: 22W

Laptop: 62W

Total: 98W

Time ran in a run: 1000 minutes or 16.7 hours

$$\begin{aligned}kWh &= \text{Power} * \text{hours} \\kWh &= 98W * 16.7hr \\kWh &= 1.63 kWh\end{aligned}$$

$$\text{Electricity Cost} = 106.8 \frac{\text{cents}}{\text{kWh}}$$

Cost per run:

$$\begin{aligned}\text{Run Cost} &= \text{Electricity Cost} * \text{kWh} \\ \text{Run Cost} &= 106.8 * 1.63\end{aligned}$$

$$\text{Run Cost} = R 1.74$$

Cost of Testing Kits

Dollar to rand:

$$\$1 = R14.23$$

Cost of water testing kits:

$$\$7.09$$

Without accounting for shipping cost, the cost per use is:

$$\begin{aligned} \text{Test Kit Cost} &= \frac{\$}{\text{kit}} * \frac{R}{\$} \\ \text{Test Kit Cost} &= 7.09 * 14.23 \\ \text{Test Kit Cost} &= R100.88 \end{aligned}$$

Experimental Run Sample Calculations

E.coli Concentration of 10^5 Colony Forming Units per Millilitre

```
%Run 1 plots for an Ecoli concentration of 10^5 CFU/ml

%calculate the single side standard deviation
actstand=0.5*stdR1

%Plot the response using a graphing function by Rob Campbell
%Set plot aesthetics
e = shadedErrorBar(timin(1:length(aveR1)),aveR1,actstand, 'lineprops', {'o','color',[0/255
153/255 151/255],'markerfacecolor',[0/255 153/255 151/255],'markerfacecolor',[0/255 153/255
151/255],'Markersize',3}, 'patchsaturation',0.33) ;
%disable edge error bar line
set(e.edge,'LineWidth',2,'LineStyle','none')
set(gca,'FontSize',18);
xlabel('Time (minutes)', 'FontSize',25);
ylabel('Carbon Dioxide (PPM)', 'FontSize',25);
xlim([0 300]);
hold on

%Plot the baseline using the same function with standard deviation
%alter plot visually
e1 = shadedErrorBar(timin(1:length(aveBL)),aveBL,(0.5*stdevBL), 'lineprops',
{'o','color',[255/255 75/255 30/255],'markerfacecolor',[255/255 75/255
30/255],'markerfacecolor',[255/255 75/255 30/255],'Markersize',3}, 'patchsaturation',0.33) ;
set(e1.edge,'LineWidth',2,'LineStyle','none')
xlim([0 100]);
legend('E.Coli Run', 'Baseline'); % Add a legend
set(legend,'FontSize',20);
legend boxoff
```

```
%Display of Kolmogorov-Smirnov Plot
clc;
%calculate the single side standard deviation
actstand=0.5*stdR1;
%get the length to which the test will be conducted by using the shortest
%variable
lgth=actstand;
%variable initialization
pv=1;
yn=0;
inc = 10;
newtime=0;
%obtain lower bound of experimental run
aveR1lower = (aveR1-actstand);

%Run for loop on increasing cumulative ranges of the experimental run and
%baseline
for cc = 1:inc:lgth
    %get the boolean detection value and the p-value
    %set the alpha value and ensure a single sided test is carried out to
    %determine whether the run is greater than the baseline
    [h,p]=kstest2(aveR1lower(1:cc),baselineupper(1:cc),'Tail','smaller','Alpha',0.001);
    %store p vals in matrix
    pv=[pv;p];
```

```

%store boolean vals
yn=[yn;h];
%get time values where the kstest2 was evaluated
newtime=[newtime;timin(cc)];

end
%Get a column matrix equal to 0.001 to plot vs the p-val
siglevel(1:length(newtime))=0.001;
siglevel=siglevel';

%plot the p-val; boolean detection val and the significance value on the
%same plot
hold on
scatter(newtime,pv,'MarkerFaceColor',[0/255 153/255 151/255],'MarkerEdgeColor',[0/255 153/255
151/255]) %plots the p-value
hold on
scatter(newtime,yn);
hold on
plot(newtime,siglevel,'LineWidth',2,'color',[255/255 75/255 30/255])

xlim([25 35]);

%aesthetic changes
set(gca,'FontSize',18);
xlabel('Time (minutes)', 'FontSize',25);
ylabel('Probability', 'FontSize',25);

legend('P-Value','Detection','Significance Level'); % Add a legend
set(legend,'FontSize',20);
legend boxoff

```

E.coli Concentration of 10³ Colony Forming Units per Millilitre

```

%%Run 2 plots with std dev x10^3
clc;

%calculate the single side standard deviation
%Plot the response using a graphing function by Rob Campbell
%Set plot aesthetics
e=shadedErrorBar(timin(1:length(aveR2)),aveR2,(0.5*stdRun2), 'lineprops', {'o','color',[0/255
153/255 151/255],'markerfacecolor',[0/255 153/255 151/255],'markerfacecolor',[0/255 153/255
151/255], 'MarkerSize',4}, 'patchsaturation',0.33) ;
set(e.edge,'LineWidth',2,'LineStyle','none')
set(gca,'FontSize',18);
xlabel('Time (minutes)', 'FontSize',25);
ylabel('Carbon Dioxide (PPM)', 'FontSize',25);
xlim([0 1000]);
hold on

%Plot the baseline using the same function with standard deviation
%alter plot visually
e1 = shadedErrorBar(timin(1:length(aveBL)),aveBL,(0.5*stdevBL), 'lineprops',
{'o','color',[255/255 75/255 30/255],'markerfacecolor',[255/255 75/255
30/255],'markerfacecolor',[255/255 75/255 30/255], 'MarkerSize',4}, 'patchsaturation',0.33) ;
set(e1.edge,'LineWidth',2,'LineStyle','none')

```

```

%Legend!
legend('E.Coli Run', 'Baseline'); % Add a legend
set(legend,'FontSize',20);
legend boxoff

%Display of Kolmogorov-Smirnov Plot
clc;
%calculate the single side standard deviation
actstand2=(0.5*stdRun2)
%get the length to which the test will be conducted by using the shortest
%variable
lgth=baselineupper;
%variable initialization
pv=0;
yn=0;
inc = 30;
newtime=0;

%obtain lower bound of experimental run
aveR2lower = (aveR2-actstand2);

%Run for loop on increasing cumulative ranges of the experimental run and
%baseline
for c = 1:inc:lgth
    %get the boolean detection value and the p-value
    %set the alpha value and ensure a single sided test is carried out to
    %determine whether the run is greater than the baseline
    [h,p]=kstest2(aver2lower(1:c),baselineupper(1:c),'Tail','smaller', 'Alpha',0.001);
    %store p vals in matrix
    pv=[pv;p];
    %store boolean vals
    yn=[yn;h];
    %get time values where the kstest2 was evaluated
    newtime=[newtime;timin(c)];
end

%Get a column matrix equal to 0.001 to plot vs the p-val
siglevel(1:length(newtime))=0.001;
siglevel=siglevel';

%plot the p-val; boolean detection val and the significance value on the
%same plot
hold on
scatter(newtime,pv,'MarkerFaceColor',[0/255 153/255 151/255],'MarkerEdgeColor',[0/255 153/255
151/255]) %plots the p-value
hold on
scatter(newtime,yn);
hold on
plot(newtime,siglevel,'LineWidth',2,'color',[255/255 75/255 30/255])

set(gca,'FontSize',18);
xlabel('Time (minutes)', 'FontSize',25);
ylabel('Probability', 'FontSize',25);

legend('P-Value','Detection','Significance Level'); % Add a legend

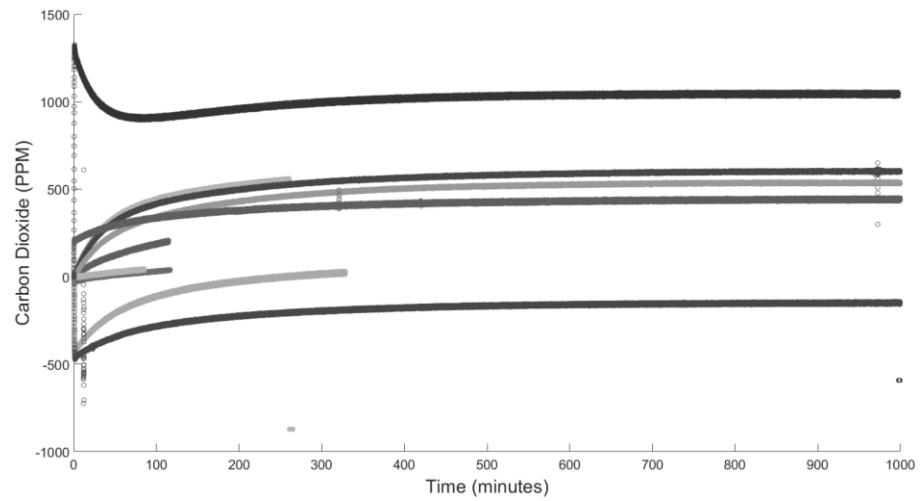
```

```
set(legend,'FontSize',20);  
legend boxoff
```

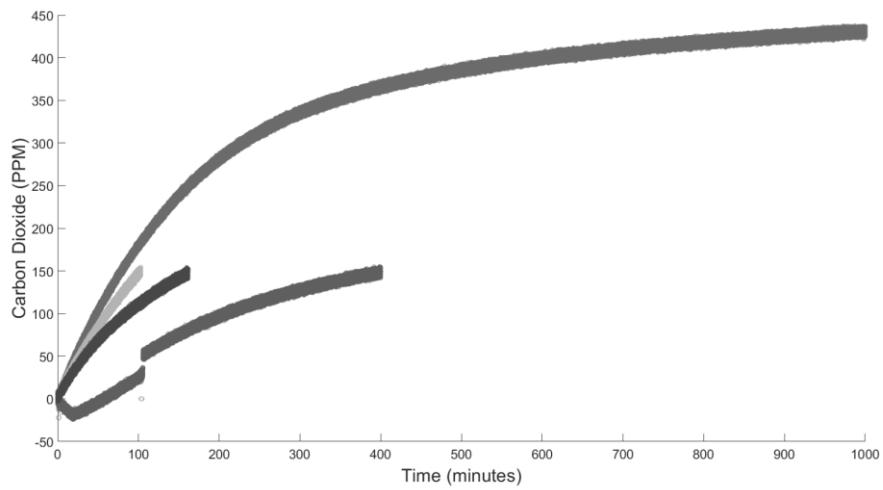
The rest of the calculations and plots followed the same procedure with the appropriate data used for each calculation

APPENDIX D – RAW DATA

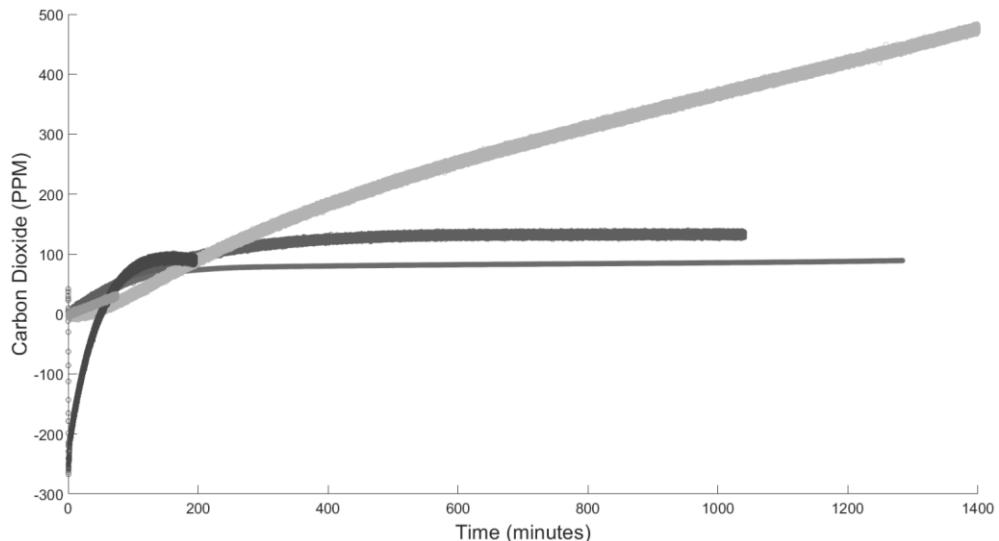
Baseline runs in the 30°C oven room:



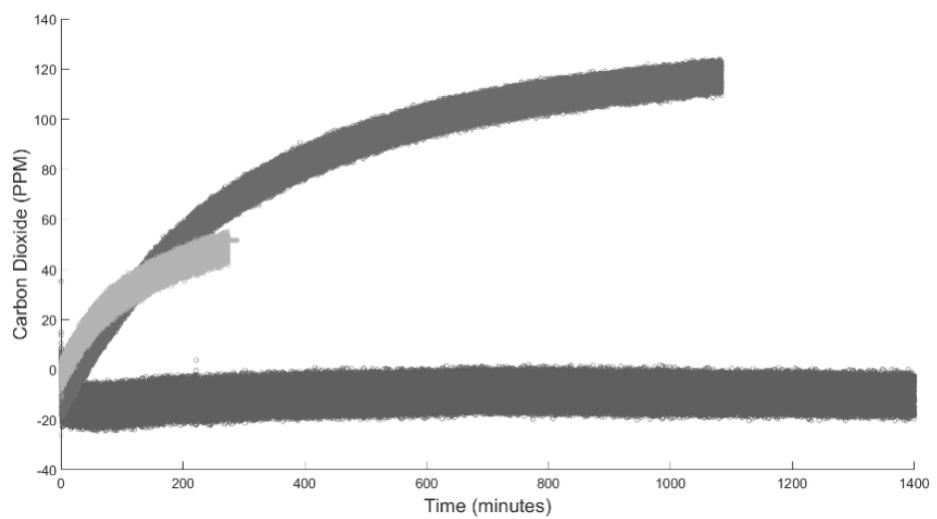
E. coli runs of concentration $10^5 \frac{CFU}{ml}$



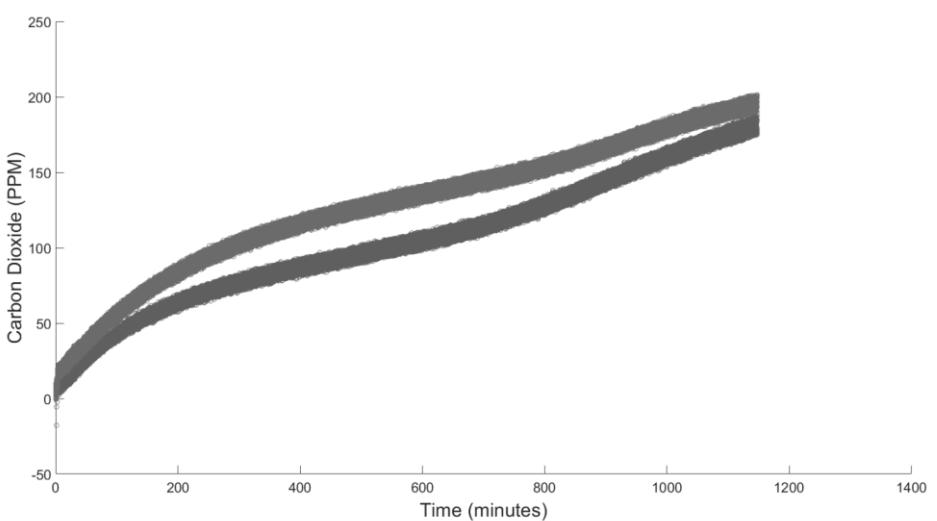
E. coli runs of concentration $10^3 \frac{CFU}{ml}$



E.coli runs of concentration $10^2 \frac{CFU}{ml}$



River water concentration of $10^5 \frac{CFU}{ml}$



APPENDIX E – HOUSEKEEPING FORM



Housekeeping:

Final year project 478 | Finalejaar projek 478

I hereby confirm that I have thoroughly cleaned the laboratory equipment and work area used by me during the course of my project and that I have returned all relevant equipment and tools to the responsible person at the Department of Process Engineering.

Hiermee bevestig ek dat ek die laboratorium-toerusting en werksarea wat ek gebruik het tydens my projek deeglik skoongemaak het en dat ek alle betrokke toerusting en gereedskap teruggegee het aan die verantwoordelike personeellid by die Departement Proses-ingenieurswese.

Student

VIVEK RAGA

Print Name

Signature

Technical Officer /
Facilities Manager

Jane de Kock

Print Name

Signature

Technical Officer
(Analytical Labs)

WENDY STONE

Print Name

Signature

Supervisor

Tobi Louw

Print Name

Signature

Additional notes:

NOTA / NOTE:

The official completion of a final year project requires that the equipment and work area be cleaned and that all equipment be returned. Therefore, the final mark will only be released when the convenor receives the signed form of the relevant student (included as last page in the final report).

Die ampelike afhandeling van 'n finalejaar projek vereis dat die toerusting en werksarea skoon sal wees en dat toerusting teruggehandig sal wees. Derhalwe sal die finale punt slegs vrygestel word indien die sameroeper die getekende vorm van die betrokke student ontvang (ingesluit as laaste bladsy in die finale verslag).





Final Year Project Inventory 2019

Please list all the equipment, materials, etc. used for the duration of your final year project.

Student Information	
Student Name:	Vivek Raga
Student Number:	18643450
Supervisor:	Dr Louw
Lab:	Microbiology

Equipment Inventory		
Asset Number (if available)	Item Description	Quantity
	Stirrer	1
	CEMS	1
	Peristaltic Pump	1
	LiCor CO ₂ gas analyzer	
	Laptop (data logging)	
	Tubing and connectors	
	Pipettes	
	Autoclave	

Glassware Inventory		
Item Description	Volume	Quantity
Vessel (5L)	6 L	1
250 mL Erlenmeyer flasks	250ml	



Chemicals Inventory

Extra/Miscellaneous Items



Student Signature

WENDY STONE 
Technical Officer Signature

29/10/19 Date