THERMAL INACTIVATION OF VIABLE *GEOBACILLUS STEAROTHERMOPHILUS* AND *BACILLUS ATROPHAEUS* SPORES IN A BENCH-SCALE LANDFILL GAS FLARE

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ABSTRACT

JENIA TUFTS: Thermal inactivation of viable *Geobacillus stearothermophilus* and *Bacillus atrophaeus* spores in a bench-scale landfill gas flare
(Under the direction of Dr. David Leith, Dr. Jacky Rosati, and Dr. Jill Stewart)

A bench-scale, landfill flare system was designed and built to test the potential for heat-resistant spores to survive the flaring process and enter the environment. The residence times and temperatures of the flare were characterized and compared to full-scale systems. Experiments were conducted using *Geobacillus stearothermophilus* and *Bacillus atrophaeus* spores as surrogates for *Bacillus anthracis.* Spore solutions were aerosolized, dried, and sent through the bench-scale system. Sampling was conducted downstream of the flare using a bioaerosol collection device. The samples were cultured, incubated for seven days, and assessed for viability.

Results showed that the bench-scale, landfill flare system was comparable to a full-scale combustor flare with a single-orifice diffusion burner. All spores of *G. stearothermophilus* and *B. atrophaeus* were inactivated in the bench-scale system, suggesting that spores that pass through the flare in a full-scale system will become inactivated as well.

ACKNOWLEDGMENTS

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DISCLAIMER

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
B. anthracis	*Bacillus anthracis*
B. atrophaeus	*Bacillus atrophaeus*
CFR	Code of Federal Regulations
cP	Centipoise
CFU(s)	Colony Forming Unit(s)
CH_4	Methane
CO_2	Carbon dioxide
DAS	Data acquisition system
DI H_2O	Deionized water
G. stearothermophilus	*Geobacillus stearothermophilus*
HEPA	High Efficiency Particulate Absorbing
ID	Inner Diameter
Lpm	Liters per minute
MFC	Mass flow controller
mL	Milliliter
MSW	Municipal Solid Waste
N_2	Nitrogen
OD	Outer diameter
RCRA	Resource Conservation and Recovery Act
SD	Standard deviation

TSA	Trypticase Soy Agar
TC	Thermocouple
TSB	Trypticase Soy Broth
µL	Microliter
µm	Micrometer

Chapter 1: INTRODUCTION

In 2001, letters containing *Bacillus anthracis* were sent via the US Postal Service to multiple locations in the United States including the Hart Senate Office Building in Washington DC, Rockefeller Center in New York City, and the AMI building in Boca Raton, FL. When the letters were opened, *B. anthracis* was released, dispersed, deposited and re-aerosolized throughout these buildings, exposing the building occupants and contaminating building surfaces and contents (1). These exposures caused 22 confirmed cases of anthrax resulting in five deaths. The simplicity of the dispersion method exposed the vulnerability of the American public to future bioterrorist attacks, heightened governmental awareness and concerns, and emphasized the need for efficient decontamination and disposal methods of the infective materials.

The decontamination and cleanup of the 2001 attack sites produced extensive quantities of potentially contaminated wastes, including material removed from the buildings such as office furniture, computers, printers, carpets, draperies, wallboard, and ceiling panels as well as all personal protective equipment used in the decontamination process (2,3). Some debris was incinerated and some was shipped to Resource Conservation and Recovery Act (RCRA) Subtitle D solid waste landfills for final disposition (3).

Because *B. anthracis* is heat resistant and in spore form can survive for long periods under harsh conditions (4), potential exists for viable spores to escape detection and decontamination, to become lost at transfer points, or to survive multiple decontamination

processes (5). Limitations in sampling and analytical methods for viable *B. anthracis* spores further compound the disposal issue (2, 3). For example, wipe samples may not be representative of the entire building and hot spots may be missed. Also, sampling is problematic in some areas, such as the deep recesses of a computer, inside the keyboard, etc. Culturing samples is time- consuming and there may not be time to wait for analytical results before a building must be cleared of debris. Presently, incineration is the preferred method to dispose of biologically contaminated materials; however, other disposal options would likely be required in a large scale incident because the high volume of debris might overwhelm incineration facilities. One disposal option is the use of municipal solid waste (MSW) landfills.

Currently there are over three thousand MSW landfills active in the US that accept household waste, non-hazardous sludge, industrial solid waste, and construction and demolition debris (6). These landfills are made up of individual cells that are lined with clay and plastic to prevent waste from leaching into soil and groundwater (6). As each cell is filled, it is capped with clay and plastic to prevent water infiltration (6). Bacteria break down the organic wastes within each cell to produce landfill gas. Although the composition of landfill gas is unique to each location depending on the climate, moisture, and waste profile, landfill gas generally consists of about 50% methane (CH_4), 50% carbon dioxide (CO_2) and <1% non methane organic compounds (6) as well as hydrogen sulfide and other sulfur compounds. These gases, including methane, are collected through a series of pipes and are routed by fans to a landfill flare where the methane is burned to prevent its escape into the atmosphere (6).

A recent study showed that microorganisms can be transported in landfill gas (7). Other researchers have found that *Bacillus atrophaeus* strongly adheres to building material within a landfill (8), reducing the likelihood of release into the landfill gas. Overall, the fate of biological spores in the landfill is not known due to the wide variety of waste generated from the cleanup of a contaminated building, their corresponding surface and chemistry properties, and the climatic conditions.

This research attempts to investigate the fate of viable *B. anthracis* spores in the gases produced during the anaerobic breakdown of organic waste. Specifically, this research addresses the question of whether heat-resistant spores in landfill gas can be inactivated in the landfill flare. The objectives of this study were: (1) to characterize a bench-scale landfill flare system by comparing the velocities, residence times, and system temperatures with those of real-world systems, and (2) to determine the viability of heat-resistant, biological spores that pass through the flare.

Chapter 2: EXPERIMENTAL METHODS

Bacteria

The toxicity of *B.anthracis* complicates its use in bench-scale, laboratory tests. Therefore, *Geobacillus stearothermophilus* (ATCC 7953, Apex Laboratories DKT-250-8, Apex, NC) and *Bacillus atrophaeus* (ATCC 9372, Apex Laboratories RBC-343-E8, Apex, NC) spores were used as surrogates for *B. anthracis* in this study. Because these surrogates are similarly resistant to dry heat as *B. anthracis* (9) and have been used as biological indicators in sterilization processes, they are excellent indicators to demonstrate the fate of *B. anthracis* in a landfill flare. Previous work (9) determined the dry heat F-value, the time (in minutes) that causes the complete destruction of microorganisms (10), at 200 °C for *G. stearothermophilus* and *B. atrophaeus* to be 1.3 minutes and 1.1 minutes, respectively; these times are similar to the F-value of 1.2 minutes for *B. anthracis* at the same temperature. Similar to *B. anthracis,* both surrogates are gram-positive, endospore forming, rod-shaped bacteria (11). Because the circumstances of spore formation affect spore size for an endospore-forming bacterium (4), spore sizes vary. Thus, *B. atrophaeus* spores range from 2-3 µm long and are 0.7-0.8 µm wide (11), whereas *G. stearothermophilus* spores range from 2-3.5 µm long and are 0.6-1 µm wide (11). Although specific dimensions were not released to the public, most *B. anthracis* spores found in the Hart Senate Office Building ranged from 0.95 to 3.5 µm (1).

Bench-Scale Flare System

Experiments were conducted using a bench-scale landfill flare system located in a laboratory fume hood. A schematic diagram of this system is shown in Figure 1. This bench-scale system was similar to an enclosed diffusion flare, although it had a single rather than multiple burner orifices. The shroud was not insulated to allow visual monitoring of the flare. The flare measured approximately 9 cm in height and 2.8 cm in diameter at its widest point and consisted of an air-assisted diffusion burner with an ID of 0.66 cm, with air being mixed with the combustion gases after the combustion gases exited the burner orifice.

Spores were aerosolized with 0.46 Lpm of HEPA-filtered nitrogen using a low-flow nebulizer (Meinhard, model TR-50-A2, Golden, CO). Spore solution was delivered to the nebulizer using a syringe pump (New Era, model NE-1000, Farmingdale, NY) operated at 7 µL/min with a 1 mL sterile plastic syringes (BD, Franklin Lakes, NJ) with an ID of 4.78 mm. The spore solution passed from the nebulizer through a diffusion dryer before mixing with 0.42 Lpm of HEPA-filtered methane regulated by a mass flow controller (MFC). This mixture of N_2 and CH_4 was used to simulate landfill gas, which is typically comprised of about 50% CH_4 and about 50% CO_2 with other trace constituents (11). Nitrogen was used in place of CO_2 for these tests due to its lower cost and lesser environmental impact. In addition to the combustion gases, approximately 24 Lpm of dried, HEPA-filtered air flowed in an annulus around the flare, resulting in a total flow of about 25 Lpm. At the top of the flare tube, 11.9 Lpm was drawn into a BioSampler (SKC, Eighty Four, PA) while the excess gas passed through a HEPA filter before exiting through the exhaust stack. Nebulizer flows were verified with a gas flow meter (DryCal, BIOS International, Butler, NJ). The CH_4 flow was controlled with a calibrated mass flow controller (Sierra Instruments, Monterey, CA).

Combustion air flow was controlled with a calibrated rotameter (King Instrument Company, Garden Grove, CA).

Control experiments were conducted in the same manner as the spore experiments, except CH_4 was replaced with N_2 and the flare was not lit.

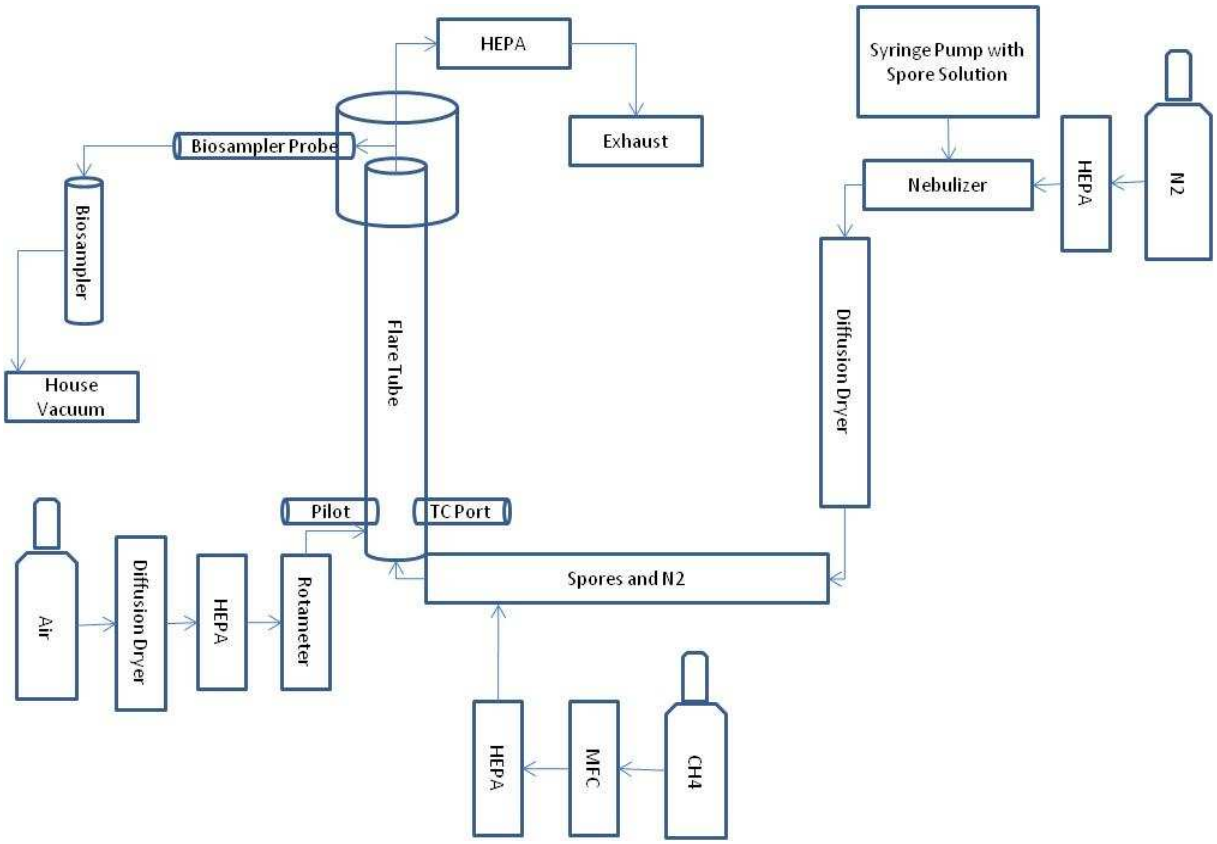

Figure 1. Bench-Scale Landfill Flare System Diagram

Flare/Stack Temperature Traverse

Figure 2 provides an enlarged schematic of the stack and flare. The flare was contained in a Pyrex glass tube 5.18 cm in inner diameter (ID). The widest point of the flare was approximately 2.5 cm from the shoulder of the flare tube, as shown in Figure 2. Two ports, situated 90° from each other, measured 2.54 cm in length with an ID of 0.9 cm and were centered 2.5 cm from the bottom of the tube, across from the widest point of the flare. The right port housed a long-neck pilot lighter that remained in place throughout testing. The left port was fitted with a Teflon plug that accommodated a type S thermocouple (TC) ceramic probe to measure temperature. The fit between the TC sheath and the Teflon plug was not tight to allow movement of the TC probe during the temperature traverse. During spore inactivation experiments, this port was capped with an air-tight Teflon fitting.

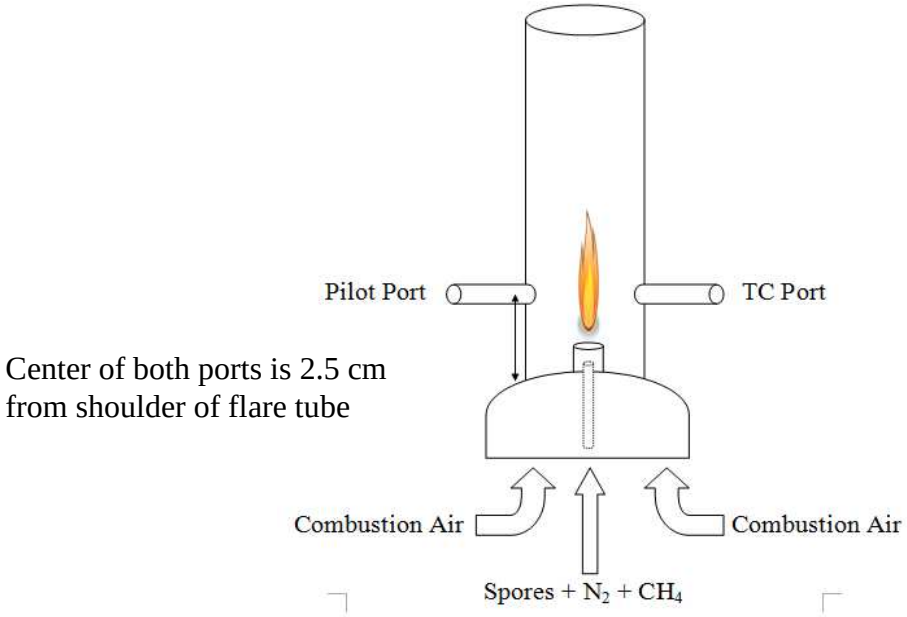

Figure 2. Detail of Flare Stack and Port Locations (not to scale)

Temperature profiles of the flare and stack were obtained using four cross-sectional traverses across the flare with a calibrated type S thermocouple. Each traverse was performed at the widest point of the flare, as determined by visual inspection. To account for potential

moisture effects from the spore solution, deionized water was nebulized through the system at 7 µL/min during the temperature profile measurements. During each traverse, temperature data were logged at ten second intervals using a data acquisition system (DAS) (IOtech cube, Omega Engineering, Stamford, CT) at fourteen sampling points, as depicted in Figure 3. Each point was sampled for 1 minute and the results averaged. When moving from a high temperature to a low temperature region, readings were allowed to stabilize before the measurements were recorded. Readings were considered stable after not falling for four consecutive readings.

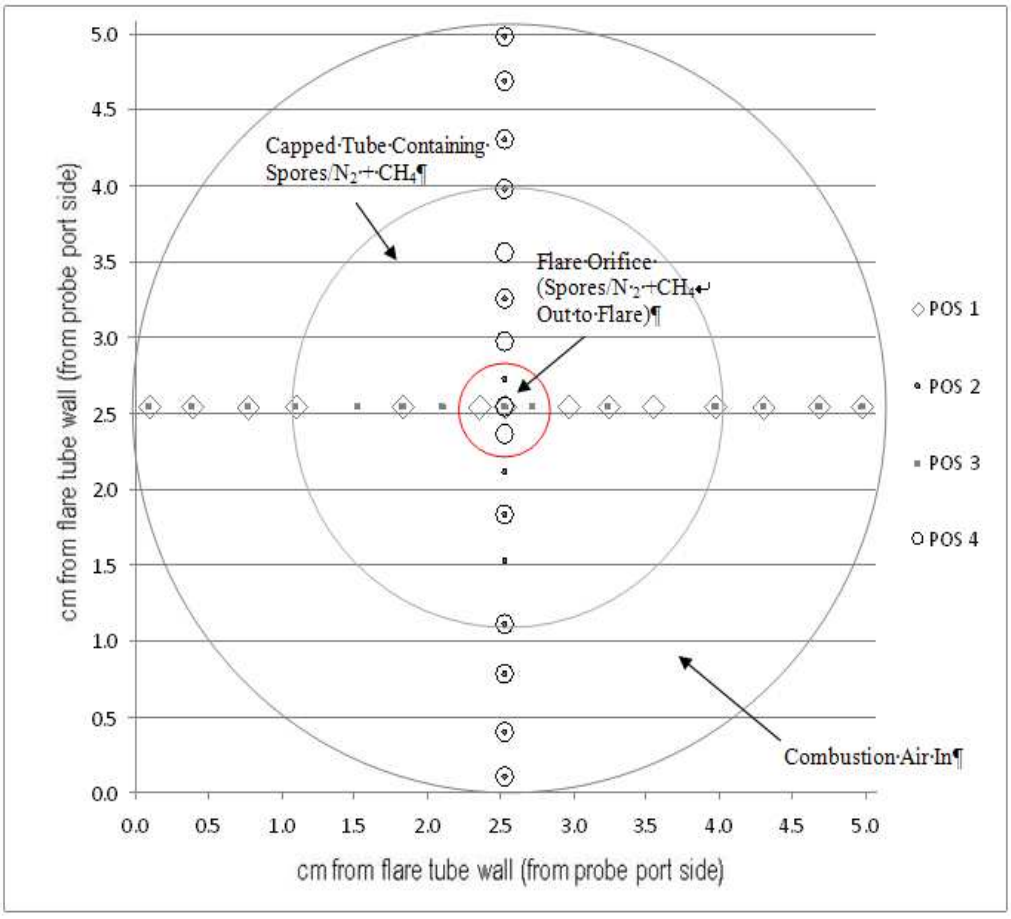

Figure 3. Top-down View of Temperature Traverse in Flare Tube.

The outermost circle in Figure 3 is the glass flare tube, the second circle is the spore/N_2/CH_4 tube and the innermost circle is the opening for the gas/spore mixture to exit and produce a flare. Combustion air enters between the outer and second circle, acting as sheath flow around the flare.

Stack Exhaust and BioSampler Temperatures

Stack exhaust temperatures and internal BioSampler temperatures were also monitored and logged at ten second intervals for the duration of test runs using a calibrated type T thermocouple. Stack exhaust temperature was measured at the top of the stack in the center at the BioSampler probe inlet when the BioSampler was not in operation. During a typical test in which the BioSampler contained 20 ml of white mineral oil (ViaTrap, SKC, Eighty Four, PA), the temperature at the BioSampler inlet was measured at 10 second intervals. Internal BioSampler temperatures were also measured at four locations in the sampler, as shown in Figure 4.

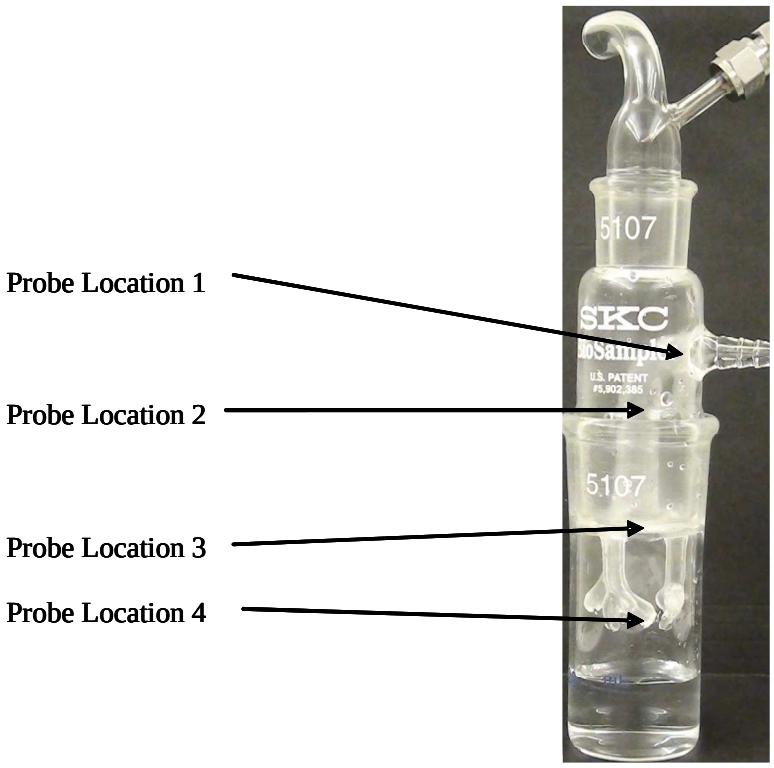

Figure 4. Biosampler Temperature Monitoring Locations

Residence Times and Turbulence Estimates

Stack residence time was estimated by dividing the stack height by the stack velocity. Similarly, spore residence time in the flare was estimated by dividing the flare height by the flare exit velocity. The flow in the flare tube included 0.46 Lpm N_2, 0.42 Lpm CH_4 and 24 Lpm of combustion air. Flows were corrected to a temperature of 1000 °C, the maximum temperature of the flare. The stack turbulence was estimated by calculating the Reynolds number for the stack at 1000 °C, using weighted density and viscosity values that accounted for the percentage of each constituent in the gas stream (*i.e.*, 96% air, 2% CH_4 and 2% N_2). The weighted density was 0.275 kg/m^3 and the weighted viscosity was 4.88 x 10^{-5} kg/m s and included the contribution of N_2 in the air.

Spore Inactivation Experiments

Before each test, the BioSamplers and sample probes were autoclaved in a pre-vacuum sterilizer (Amsco Century SV 120 Scientific, STERIS Corporation, Mentor, OH) in individual sterilization pouches using a 1-hour gravity cycle at 121 °C. After each use, the BioSamplers were autoclaved in a destruction cycle at 121°C, then washed and triple rinsed with de-ionized (DI) water. The BioSamplers were then autoclaved again on the gravity cycle in individual sterilization bags in preparation for additional testing. All white mineral oil and DI water used for testing and solution prep were sterilized for one hour using the liquid autoclave cycle at 121 °C.

Fourteen experiments were conducted to evaluate the inactivation of spores in the bench-scale system. Seven of the tests were conducted using a bacterial spore suspension of *G. stearothermophilus* with a mean population of 3.3×10^8 CFU/mL as determined by the manufacturer, and seven tests were conducted using a bacterial spore suspension of *B. atrophaeus* with a mean population of 2.1×10^8 CFU/mL, as determined by the manufacturer. A test solution concentration for each spore type, as listed in Table 1, was prepared by diluting the appropriate bacterial spore suspension with sterile DI water. These test solution concentrations were optimized to minimize the number of spores per drop of solution, and to maximize the total number of spores sent through the system.

The drop size produced by the nebulizer was taken as 10 µm based on the manufacturer's unpublished data; this assumption and the concentration of spores in the test solution allowed calculating the percentage of drops that carried spores. The estimated number of spores collected by the BioSampler per test is based on the total number nebulized per test and the fraction of the exhaust stream sampled (volume sampled by the BioSampler

divided by total exhaust flow). This calculation provides the maximum possible number of spores that could be sampled, as it does not account for spore losses in the system not related to the flare.

Table 1. Spore Concentrations

Spore Type	Test Solution Conc'n, Spores/cm^3	% of Drops Containing Spores	Nebulizer Flow, µL/min	min	Estimated Spores per Test	
					From Nebulizer	Collected by BioSampler*
GS	1.52×10^8	10	7	36	3.81×10^7	1.89×10^7
BA	1.26×10^8	7	7	36	3.15×10^7	1.56×10^7

* This number represents the maximum number of spores that could be collected by the BioSampler, based on the number per test from the nebulizer and the fraction of the gas stream entering the BioSampler.

Each test began with lighting the flare, followed by a five-minute wait to allow the flame to stabilize. Then the spore solution was nebulized upstream of the flare while a sterile BioSampler collected aerosol from the exhaust stack, (see Figure 1).

Calibration of the syringe pump that delivered spores to the nebulizer was verified by filling a syringe with water and setting the pump to dispense into a graduated cylinder for a fixed time. For each run, 250 µL of spore solution was dispensed at 7 µl/min, resulting in run durations of about 36 minutes. Syringes were filled beyond the 1 mL mark to ensure complete delivery of the solution, to prevent the syringe pump from bottoming out, and to provide samples for use as controls.

For each test, a sterile BioSampler containing 20 mL of sterile white mineral oil sampled from the stack exhaust at 11.9 Lpm for 36 minutes. Minimal mineral oil losses occurred so that the volume of mineral oil in the BioSamplers remained at about 20 mL for all

tests. During the four control runs that were performed with the flare off, a toggle switch attached to an MFC changed the CH_4 flow to N_2.

Following each test, triplicate 1 mL samples of mineral oil were aseptically transferred from each BioSampler into three sterile 15 mL clear polystyrene culture tubes containing 10 mL of sterile Trypticase Soy Broth (TSB) (Lot 843070, Remel, Lenexa, KS). This nutritive broth was used to culture the samples because it could promote growth of spores that were injured but still viable (12). Following transfer, each sample was vortexed for 30 seconds. Samples for *B. atrophaeus* were incubated at 35 °C for 7 days, whereas samples for *G. stearothermophilus* were incubated at 58 °C for 7 days.

For each organism, one control test sample and one flare sample were also plated by serial dilution so that if spores survived the flare, a count of those surviving could be made. Plate samples were prepared by aseptically transferring 10 µL of mineral oil from the BioSampler into a microcentrifuge containing 990 µL of sterile DI water and vortexing for 30 seconds. Four serial dilutions were made resulting in 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8} dilutions for each test sample. All serial dilutions were prepared and plated in duplicate with an undiluted sample. Each serial dilution (100 µL) was transferred to sterile tryptic soy agar (TSA) plates and spread using sterile glass beads. Plated samples for *B. atrophaeus* were incubated at 35 °C for 24 hours and samples for *G. stearothermophilus* were incubated at 58 °C for 24 hours.

For both broth and plate samples, positive and negative controls were prepared. Positive controls consisted of aliquots taken from each spore solution syringe used during testing, whereas negative controls consisted of sterile TSB and sterile mineral oil. Bead blanks and DI water blanks were also prepared for the plate samples. Both positive and negative control samples were prepared to ensure that aseptic techniques were utilized, that

spore solutions were viable before going through the flare, and for use in comparing sample results. In all, eleven negative controls and twenty three positive controls were prepared.

After one and seven days, all culture tubes and plates were checked for growth. Culture tubes were held to a light and checked for turbidity (positive for growth) or lucidity (negative for growth). Plates were examined for colony growth. The turbidity of positive samples was not quantified, but was compared to negative controls to assess growth.

BioSampler Spike Tests

To ensure that spores did not become inactivated in the BioSamplers from the heat of the sample stream, another series of tests was performed where 100 µL of undiluted spore solution was spiked into sterile BioSamplers containing 20 mL of sterile mineral oil. The spiked BioSamplers were installed on the sampling port and the system started and run as usual, except that DI water was nebulized instead of a spore solution. Negative and positive controls were also prepared. Negative controls consisted of 20 mL mineral oil in a BioSampler that was not installed on the system. Positive controls consisted of BioSamplers spiked with 100 µL undiluted spore solutions that were not exposed to the heat exhaust from the flare.

Chapter 3: RESULTS

System Temperature Characterization

Temperature Traverse

Results of the temperature traverse are shown in Figure 5. Note the effect of the sampling port on temperature, which caused an area of cooling on the side of the stack with the thermocouple. This effect may have been caused by a small influx of air around the thermocouple probe; however, this influx had no visible effect on the flame. The average stack exhaust temperature measured at the inlet of the sample probe location was 215 °C (SD 16.8) over the total run time, and the flare temperature was approximately 1000 °C around the edge of the flare. Note that this profile was measured at the widest point of the flare, before complete mixing of the combustion air with the flare itself.

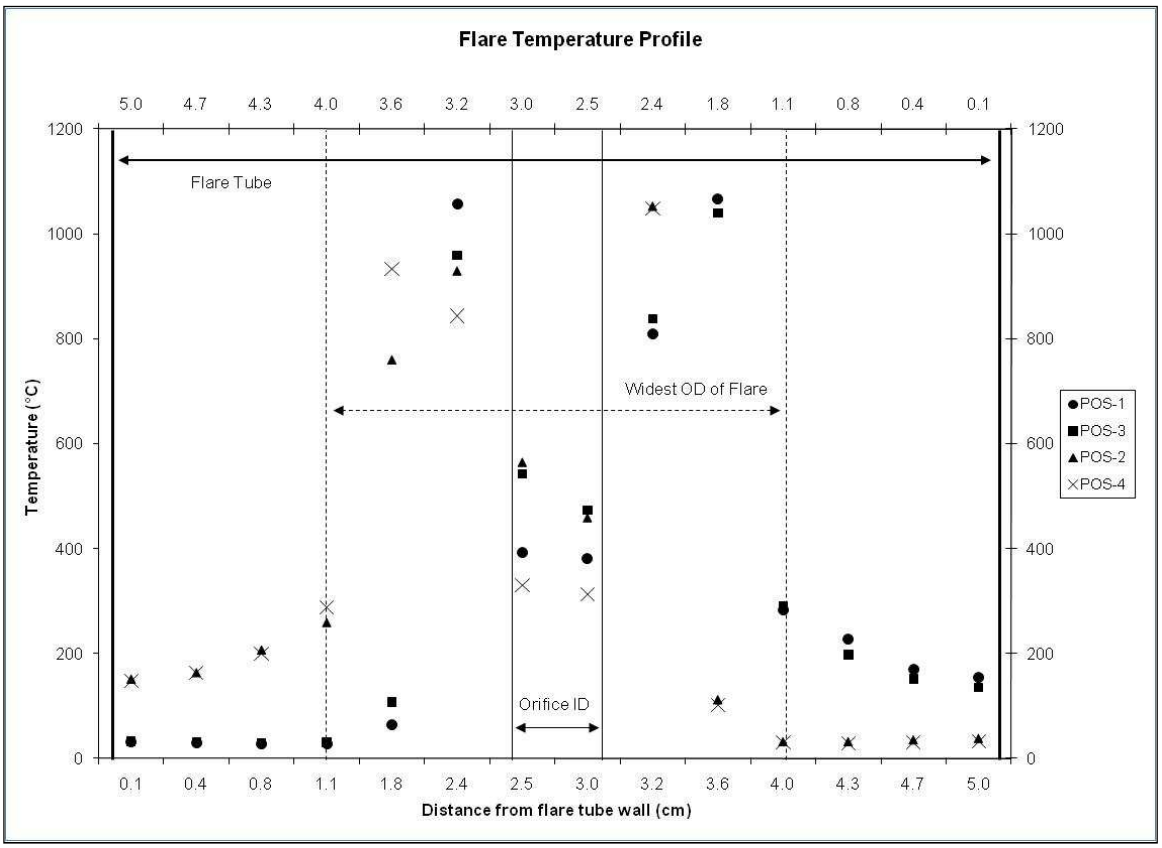

Figure 5. Temperature Profile at Widest Point of the Flare

BioSampler Temperatures

The average inlet temperature to the BioSampler was 92 °C (SD 6.8) over the total run time. Internal BioSampler temperatures were dynamic in the sampler during each 36 minute test, as shown in Figure 6; therefore, averages were not taken. Temperatures fluctuated within the BioSamplers during each test run even though the probe did not move. Limited data are available for the first test due to failure of the DAS.

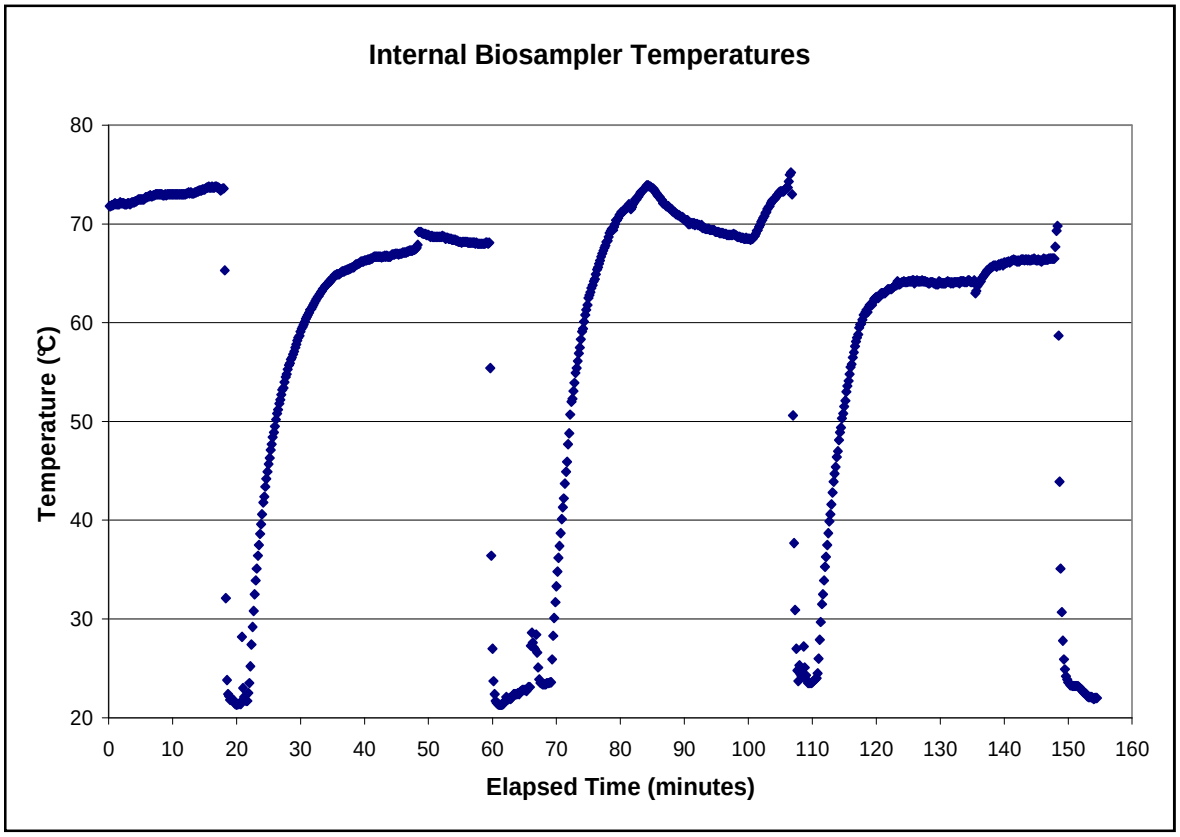

Figure 6. Internal Biosampler Temperatures (probe locations 1 – 4 are from left to right)

Residence Times and Turbulence Estimates

The flare and stack residence times were estimated to be 0.2 seconds and 0.6 seconds, respectively. These residence times are estimates since the temperature across the flare was not uniform and the degree of mixing between the flare and annular flow was difficult to determine. The Reynolds number for the stack was calculated to be 248, indicating that flow within the stack was probably in the laminar region.

Spore Inactivation Experiments

Control Samples

As shown in Figures 7 and 8, positive and negative controls were clearly differentiated in the broth samples by the cloudiness of the broth after incubation. This cloudiness is a result of precipitate formed by bacteria during growth that causes the broth to become turbid. In Figure 7, the positive controls with cloudy broth are the three samples on the left, while the negative controls are the two clear samples on the right. Photographs of all samples prepared are included in Appendix B. Figure 8 more clearly shows the differences between turbid, positive samples and clear, negative samples.

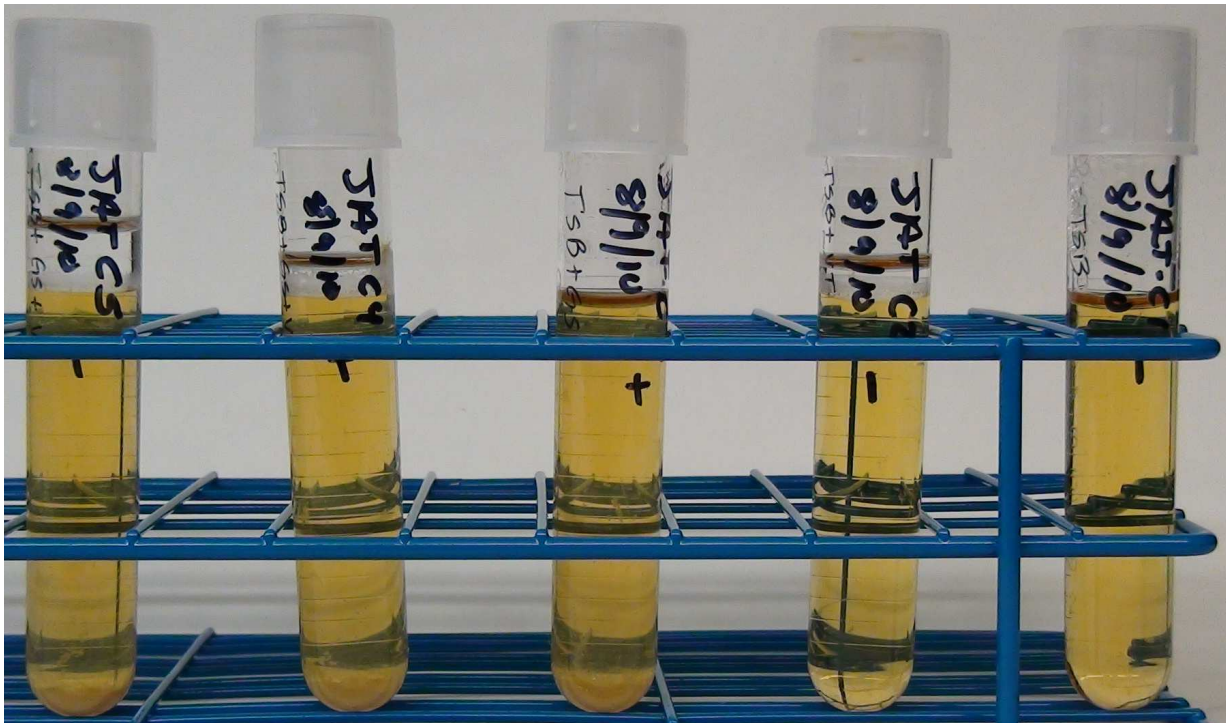

Figure 7. Positive (three at left) and Negative (two at right) Controls

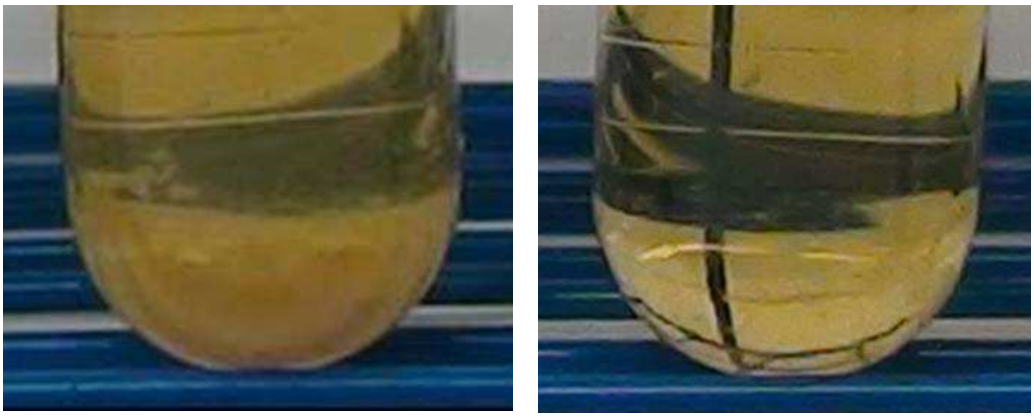

Figure 8. Positive (left) and Negative (right) Controls

All positive control samples prepared for plating and broth culture were positive for growth, confirming that the culture procedures were adequate to observe spore growth when expected. All negative control samples were negative for growth, confirming that aseptic techniques were used and that no spore contamination occurred during sampling or analysis.

The results of the BioSampler spike tests showed that spores spiked into the BioSamplers prior to a test run survived in the mineral oil, demonstrating that the viable spores sent through the system during inactivation tests were not inactivated by the heat of the oil in the BioSamplers.

All control runs with an unlit flare were positive. This finding shows that the spore solutions contained viable spores and that the bench-scale system with an unlit flare posed no impediment to the flow of spores.

Inactivation Tests

Table 1 shows that approximately 3.8×10^7 spores of *G. stearothermophilus* and 3.2×10^7 spores of *B. atrophaeus* were dispersed during each test run, of which a maximum of about 1.9×10^7 *G. stearothermophilus* spores and 1.6×10^7 *B. atrophaeus* spores entered the BioSamplers. After transfer of the 1 mL aliquot from the BioSampler, each culture tube

contained a maximum of about 9.5×10^5 *G. stearothermophilus* spores or 7.8×10^5 *B. atrophaeus* spores.

For all *G. stearothermophilus* tests with the flare on, no positive results were observed by plating or by the broth methods. These results indicate complete thermal inactivation of *G. stearothermophilus* when passing through the flare. Similarly, for all *B. atrophaeus* tests with the flare on, no positive results were observed by either culture method, suggesting complete thermal inactivation of *B. atrophaeus* when passing through the flare.

Chapter 4: DISCUSSION AND CONCLUSIONS

The objectives of this study were to characterize the bench-scale landfill flare system by comparing system parameters with those of real-world systems, and to determine the viability of heat-resistant, surrogate biological spores that pass through the flare. These objectives were met.

The bench-scale system used for these experiments incorporated an uninsulated, enclosed flare with a single orifice diffusion burner and was compared to real world systems by evaluating the net heating value of the combustion gas, exit velocity, temperature and residence times. The net heating value of the combustion gas was 33.9 MJ/scm and the exit velocity was about 0.43 m/s, both of which are within the limits of federal guidelines for landfill operations (40CFR Part 60.18). The operating temperature of the flare was about 1000 °C, which is within the 870 °C to 1037 °C operating range for an enclosed flare (13). The residence time for spores in the bench-scale flare was calculated as 0.2 s, which is much lower than the 0.6 to 1 second range of large scale enclosed flares (13) in an operating landfill and represents a worst-case scenario.

The viability of heat-resistant, surrogate biological spores were determined by sending high concentrations of both *G. stearothermophilus* and *B. atrophaeus* spores through a bench-scale landfill flare system and demonstrating the inactivation of these spores after they passed through the flare. Although *Bacillus* spore properties, including size, outer shell thickness, and heat resistance, are complicated by sporolation and preparation conditions including temperature and pH (14, 15), for enclosed landfill flares with similar flame temperatures and

longer residence times, these results strongly suggest that viable spores in landfill gas will not escape into the environment.

These data may be of interest to emergency response authorities, state and local permitting agencies, and waste management industries in the event that biological weapons residues from building decontamination residue are transported to a landfill for disposal. The methodology developed here, and the subsequent results may also be relevant to other landfill migration and release concerns, such as the release of prions from land-filled animal carcasses infected with chronic wasting disease, or the release of other toxic particulate-based contaminants

Chapter 5: FUTURE WORK

This study made use of a bench-scale landfill flare system that was designed to perform similarly to a real world system. Some of the limitations of this system include the gas mixture, which included only N_2 and CH_4. It is possible the use of N_2 instead of CO_2 had an effect on the results. It is also possible that the other gases typically present in landfill gas, such as NMOC's, would have an effect on the results. Future work on large scale landfill flare system should include the use of actual landfill gas to test whether or not differences in the gas makeup has an effect on spore viability.

Future work with this bench-scale test system may include experiments that vary organisms, residence times, and flare temperatures to determine the effects of these parameters on the results.

Appendix A: EXCEL SPREADSHEETS

Filename	Description
2010-08-20 fLARE pROFILE.xls	Flare temperature traverse data
CH4 flare flow calculations.xls	CH4 flow calculations
d and z values.xls	d and z value estimations
flame characteristics.xls	Pictures of flame used to estimate height and width
mixing chamber fill time calcs.xls	Calculations justifying removal of spore mixing chamber
Net Heating Value.xls	Net heating value calculations from 40 CFR Part 60.18
Sample Log.xls	Log of all samples collected and analyzed
spore solution concentrations.xls	spore solution concentration calculations used in Table 1 and Chapter 3.
Spores per culture calcs.xlsx	Calculations on spore concentrations in system, BioSampler and culture tubes
stack drawing for URG.xlsx	Stack drawing for URG modifications
System losses.xls	Original system loss calculations justifying system modifications to reduce dilution
Test Data Sheet V3.xls	Data Sheet used in initial system characterization tests
velocity and Reynolds calcs.xlsx	Velocity and Reynolds number calculations

The enclosed CD contains the Excel spreadsheets listed in Appendix A.

Appendix B: PHOTOGRAPHS

Appendix B Figures and Descriptions	
Figure B 1 Broth Samples – 14, 13, 10, 12, 9, 11	Figure B 32 Broth Samples – 62, 52, 60, 61
Figure B 2 Broth Samples – Tray of Samples	Figure B 33 Broth Samples – 33, 52, 34
Figure B 3 Broth Samples – 23, 24, Others	Figure B 34 Broth Samples – 45, 52, 41
Figure B 4 Broth Samples – 14, 15, 16, 20, 21, 22	Figure B 35 Broth Samples – 36, 35, 52, 23
Figure B 5 Broth Samples – Positive and Negative Samples	Figure B 36 Broth Samples – 38, 39, 35, 40, 50, 49, 48
Figure B 6 Broth Samples – 17, 18, 23, 19, 24, 20, 21, 22	Figure B 37 Broth Samples – 46, 36, 40, 44, 37
Figure B 7 Broth Samples – 8, 9, 12, 10, 13, 14, 15, 16	Figure B 38 Broth Samples – 47, 42, 40, 43
Figure B 8 Broth Samples – 4, 7, 5, 6, 3, 2, 1	Figure B 39 Broth Samples – 30, 31, 32, 27, 28, 29
Figure B 9 Broth Samples – Positive on Left, Negative on Right	Figure B 40 Broth Samples – 63, 64, 65
Figure B 10 Broth Samples – Positive and Negative Controls	Figure B 41 Broth Samples – 71, 70, 69
Figure B 11 Broth Samples – 41, 39, 45, 40, 36, 50, 37, 38	Figure B 42 Broth Samples – 72, 73, 74
Figure B 12 Broth Samples – 36, 37, 38, 39, 40	Figure B 43 Broth Samples – 72, 73, 65, 74
Figure B 13 Broth Samples – 46, 47, 48, 49, 50	Figure B 44 Broth Samples – 68, 67, 66
Figure B 14 Broth Samples– 46, 47, 48, 49, 50	Figure B 45 Broth Samples
Figure B 15 Broth Samples – 41, 45	Figure B 46 Broth Samples – 74, 73, 72, 65
Figure B 16 Broth Samples – 41, 45	Figure B 47 Broth Samples– Positive and Negative Controls
Figure B 17 Broth Samples – 42, 43, 44, 33, 34	Figure B 48 Broth Samples – Positive and Negative Controls
Figure B 18 Broth Samples - 42, 43, 44, 33, 34	Figure B 49 Broth Samples – 18, 23, 19, 24, 20, 21
Figure B 19 Broth Samples	Figure B 50 Broth Samples – 19, 17, 22
Figure B 20 Broth Samples – 27, 28, 29	Figure B 51 Broth Samples – 3, 2, 1, 4, 7
Figure B 21 Broth Samples – 30, 31, 32	Figure B 52 Broth Samples – 5, 6, 8, 16, 15
Figure B 22 Broth Samples – 26, 25, 35	Figure B 53 Plate Sample – B. Atrophaeus Stock
Figure B 23 Broth Samples – 33, 35, 36	Figure B 54 Plate Sample– 8/10/2010 Run 7
Figure B 24 Broth Samples – 26, 25, 35	Figure B 55 Plate Sample– 8/10/2010 Run 7
Figure B 25 Broth Samples – 36, 33, 35	Figure B 56 Plate Sample– 8/10/2010 Run 3
Figure B 26 Broth Samples – 30, 31, 32	Figure B 57 Plate Sample– 8/10/2010 Run 3
Figure B 27 Broth Samples – 27, 28, 29	Figure B 58 Plate Sample– 8/12/2010 Run 7
Figure B 28 Broth Samples – 59, 58, 57	Figure B 59 Plate Sample– 8/12/2010 Run 7
Figure B 29 Broth Samples – 56, 55, 34, 51	Figure B 60 Plate Sample – 8/12/2010 Run 7
Figure B 30 Broth Samples – 53, 52, 51	Figure B 61 Plate Sample – 8/12/2010 Run 3
Figure B 31 Broth Samples – 62, 52, 60, 61	Figure B 62 Plate Sample – 8/12/2010 Run 3

The enclosed CD contains the pictures listed in Appendix B

Photographs of Broth Culture Samples

Broth Sample 54 is missing; otherwise all broth samples are represented

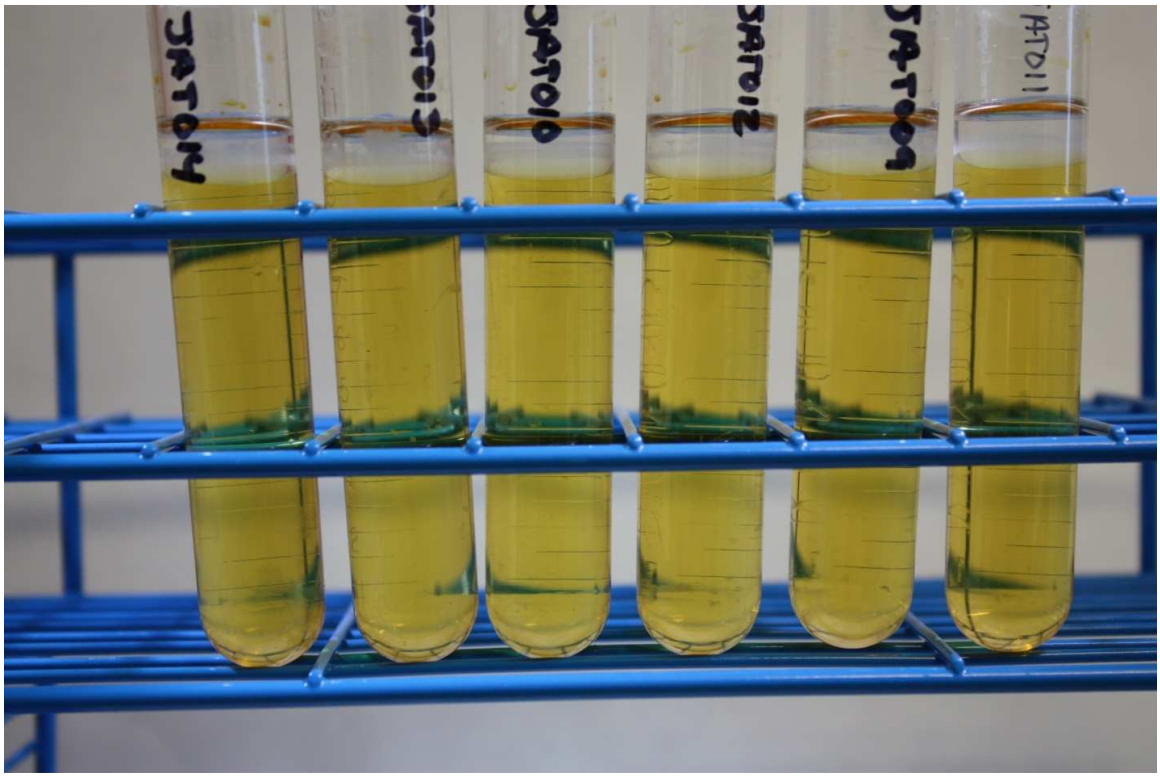

Figure B 1 Broth Samples – 14, 13, 10, 12, 9, 11

Figure B 2 Broth Samples – Tray of Samples

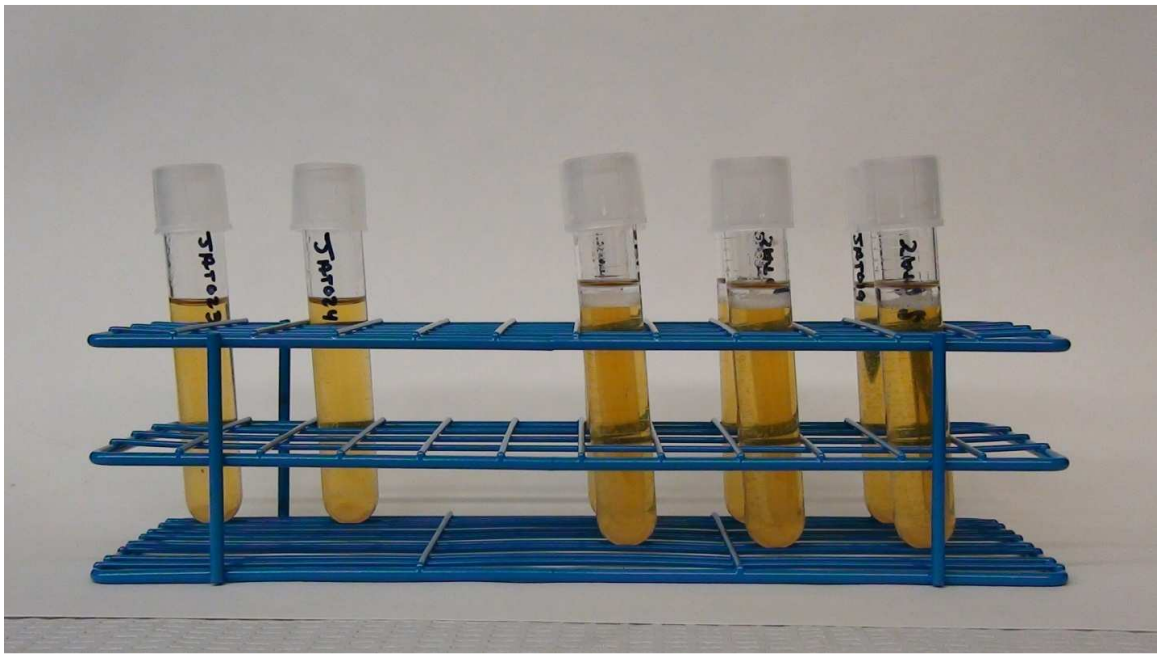

Figure B 3 Broth Samples – 23, 24, Others

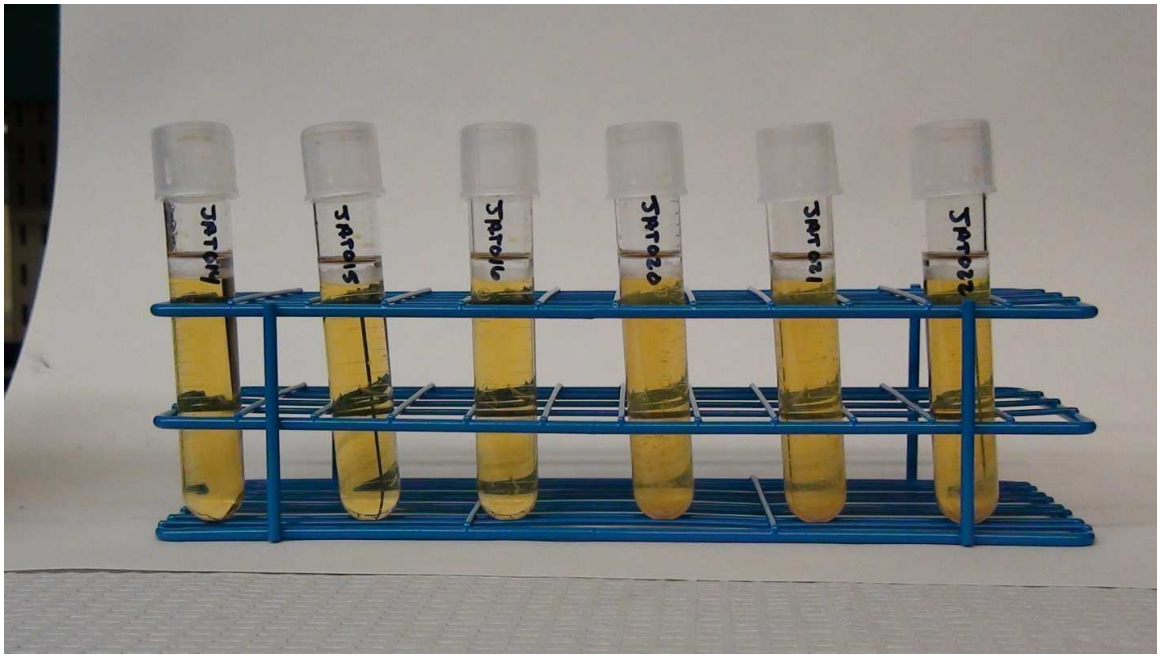

Figure B 4 Broth Samples – 14, 15, 16, 20, 21, 22

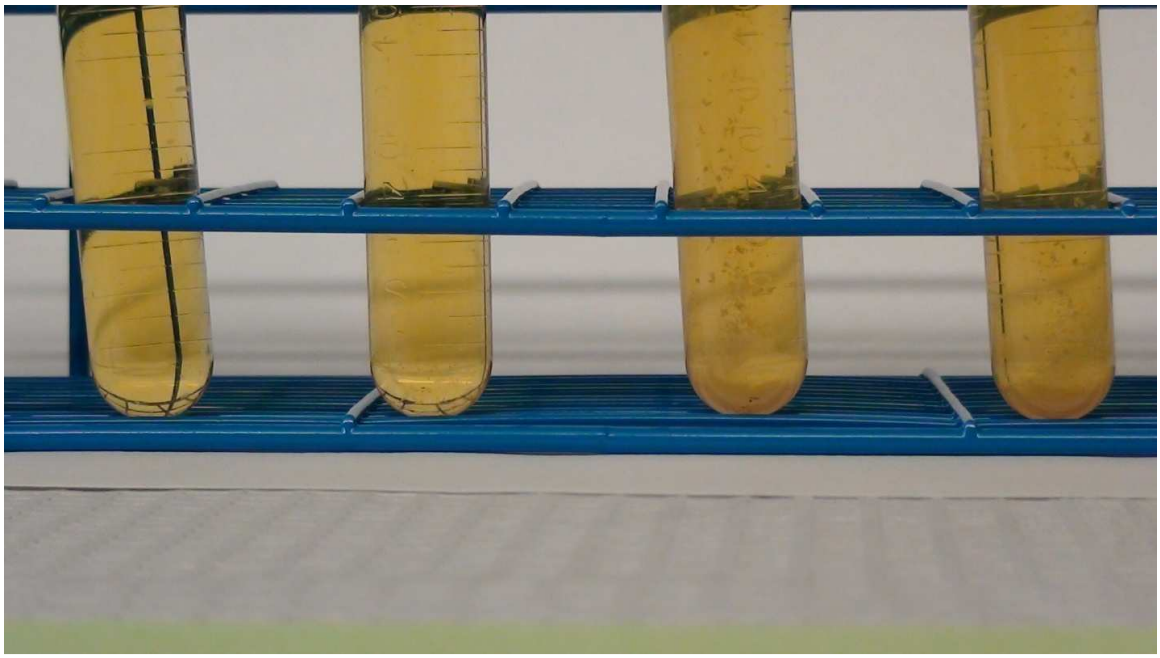

Figure B 5 Broth Samples – Positive (right two) and Negative Samples (left two)

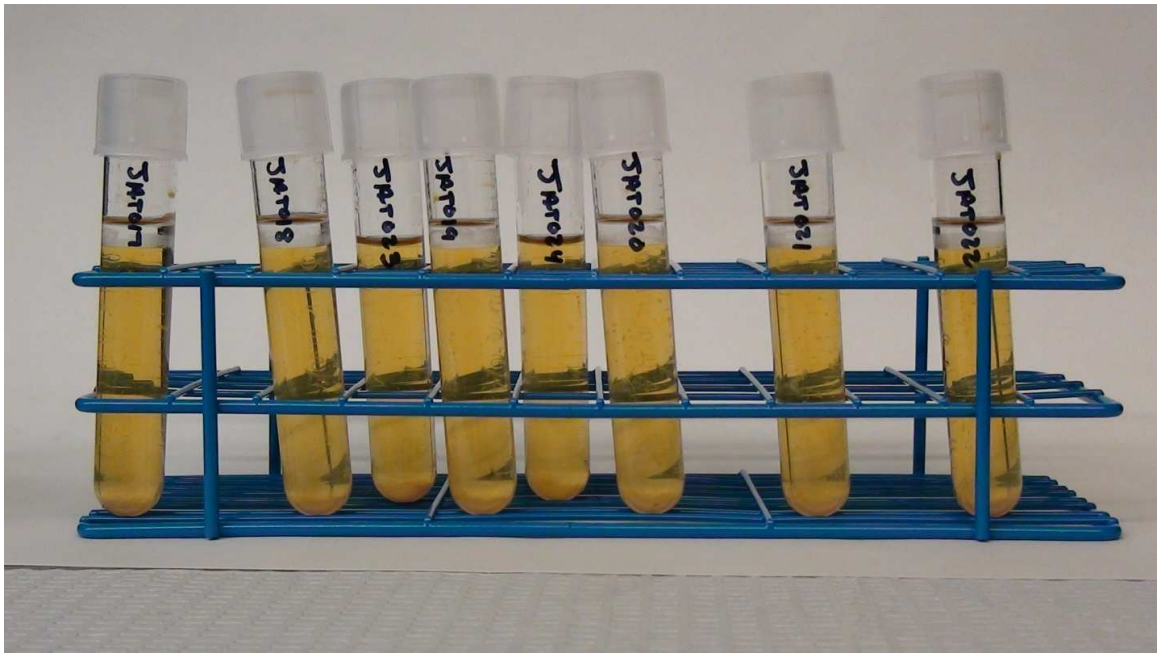

Figure B 6 Broth Samples – 17, 18, 23, 19, 24, 20, 21, 22

Figure B 7 Broth Samples – 8, 9, 12, 10, 13, 14, 15, 16

Figure B 8 Broth Samples – 4, 7, 5, 6, 3, 2, 1

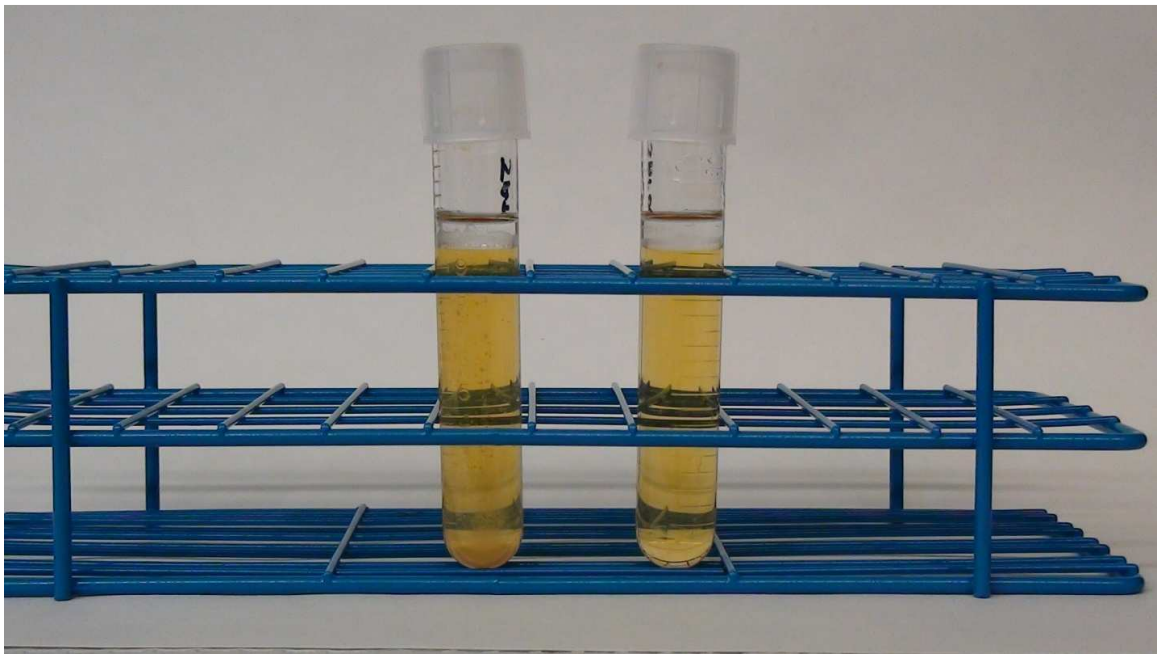

Figure B 9 Broth Samples – Positive on Left, Negative on Right

Figure B 10 Broth Samples – Positive and Negative Controls

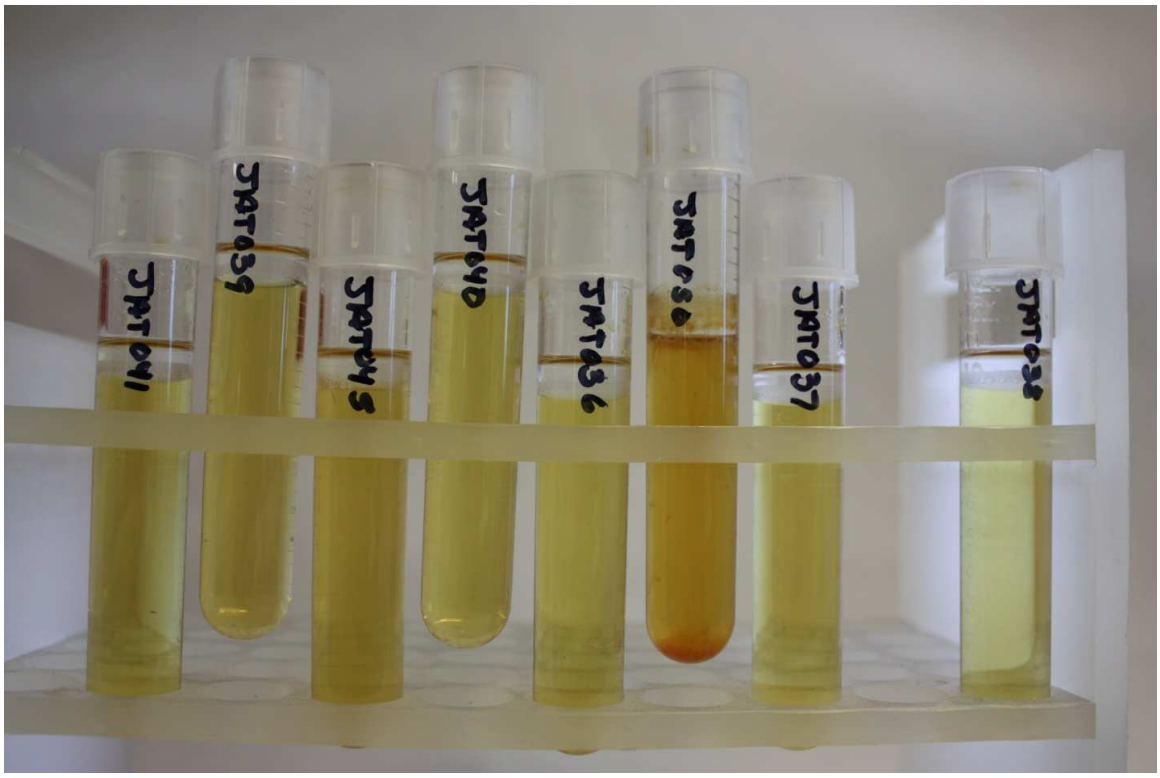

Figure B 11 Broth Samples – 41, 39, 45, 40, 36, 50, 37, 38

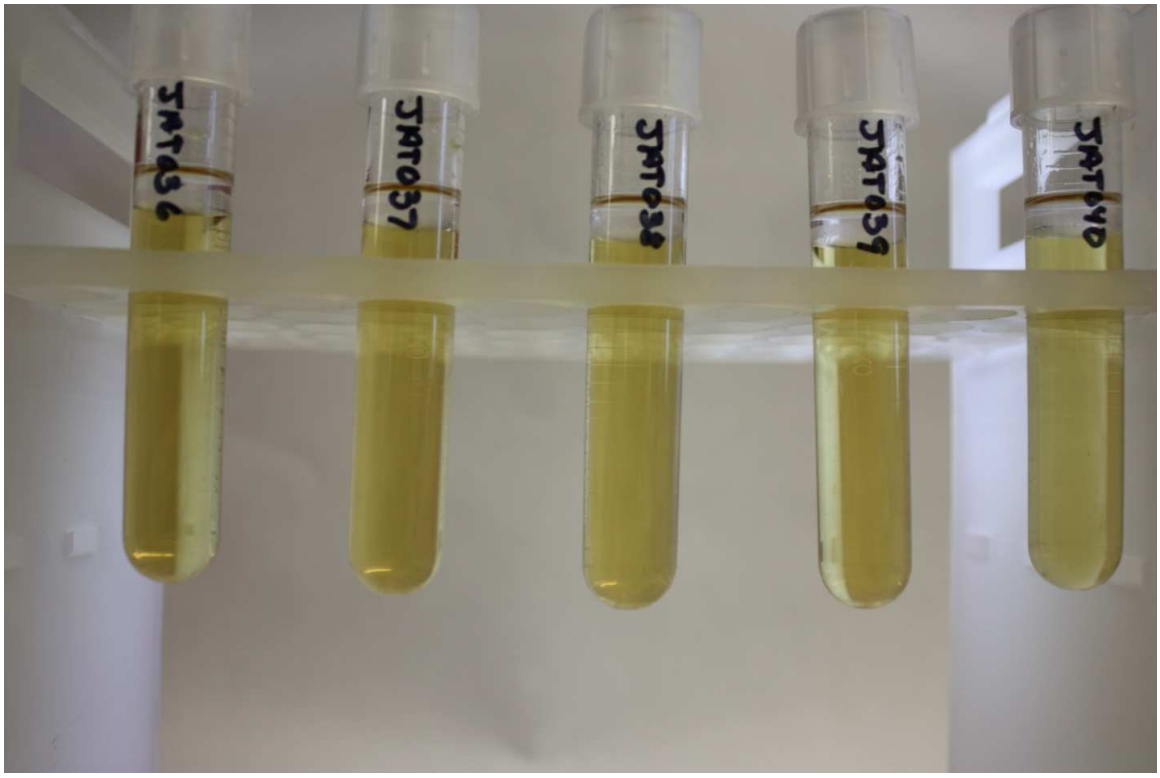

Figure B 12 Broth Samples – 36, 37, 38, 39, 40

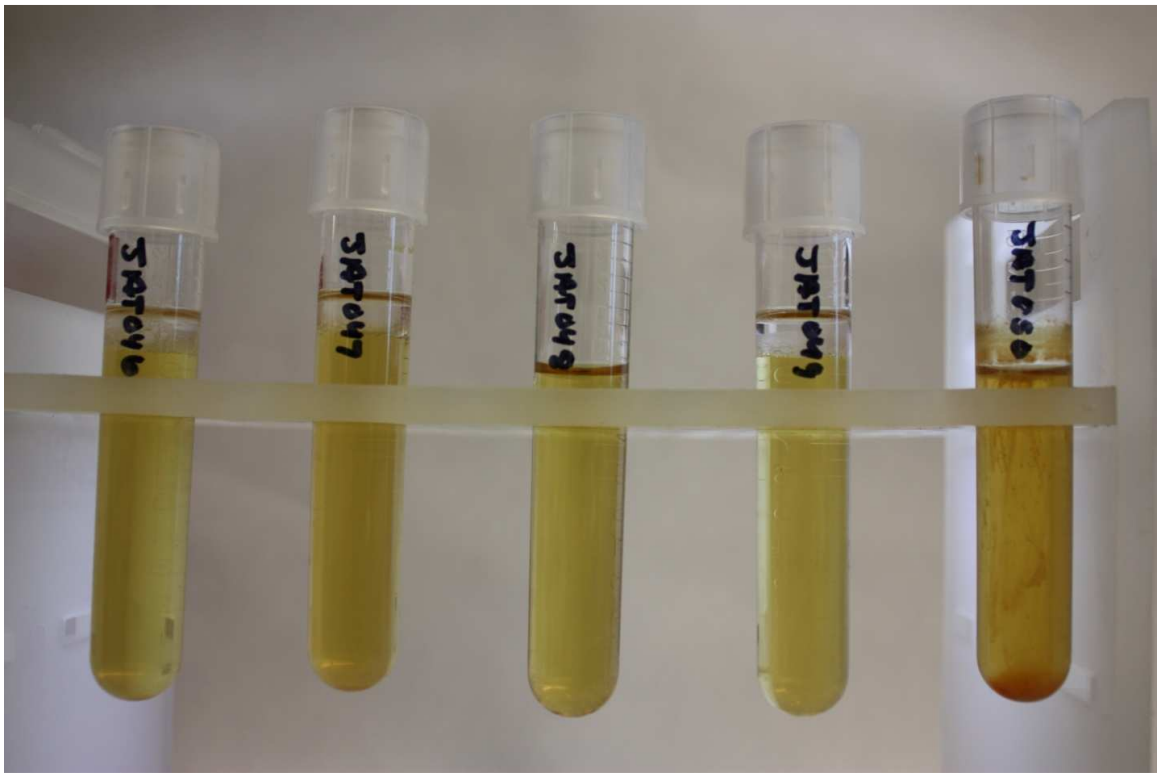

Figure B 13 Broth Samples – 46, 47, 48, 49, 50

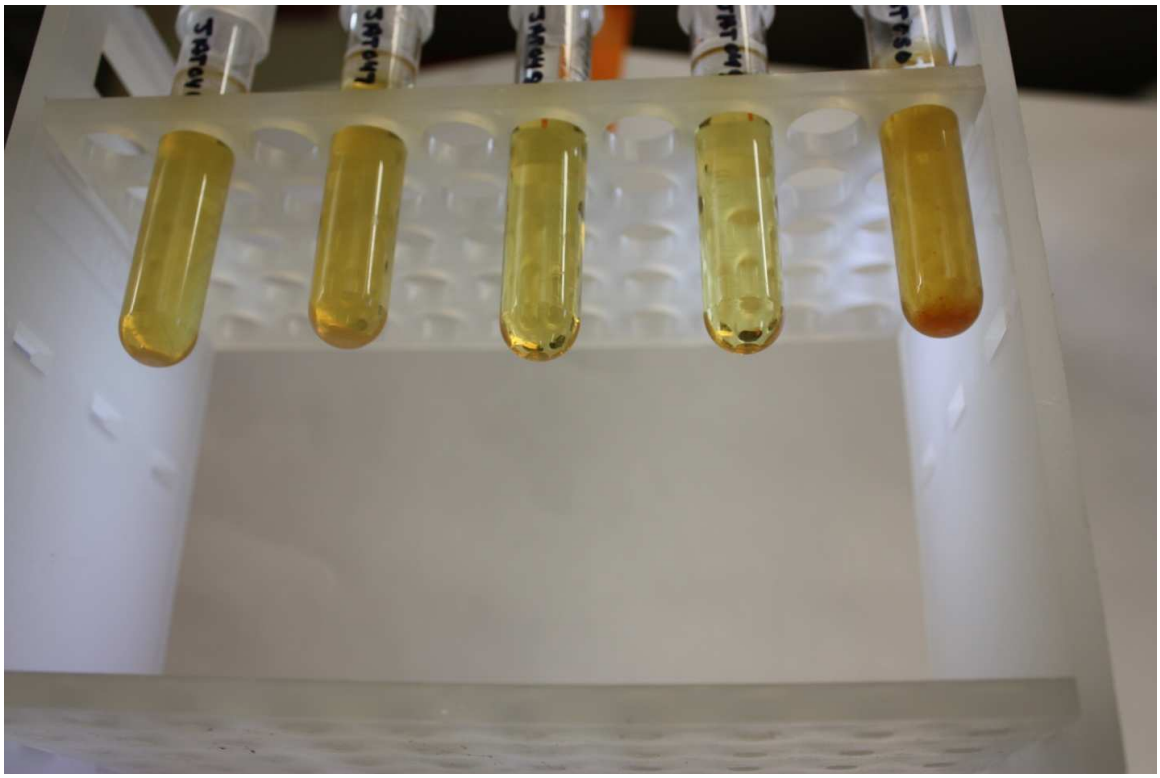

Figure B 14 Broth Samples– 46, 47, 48, 49, 50

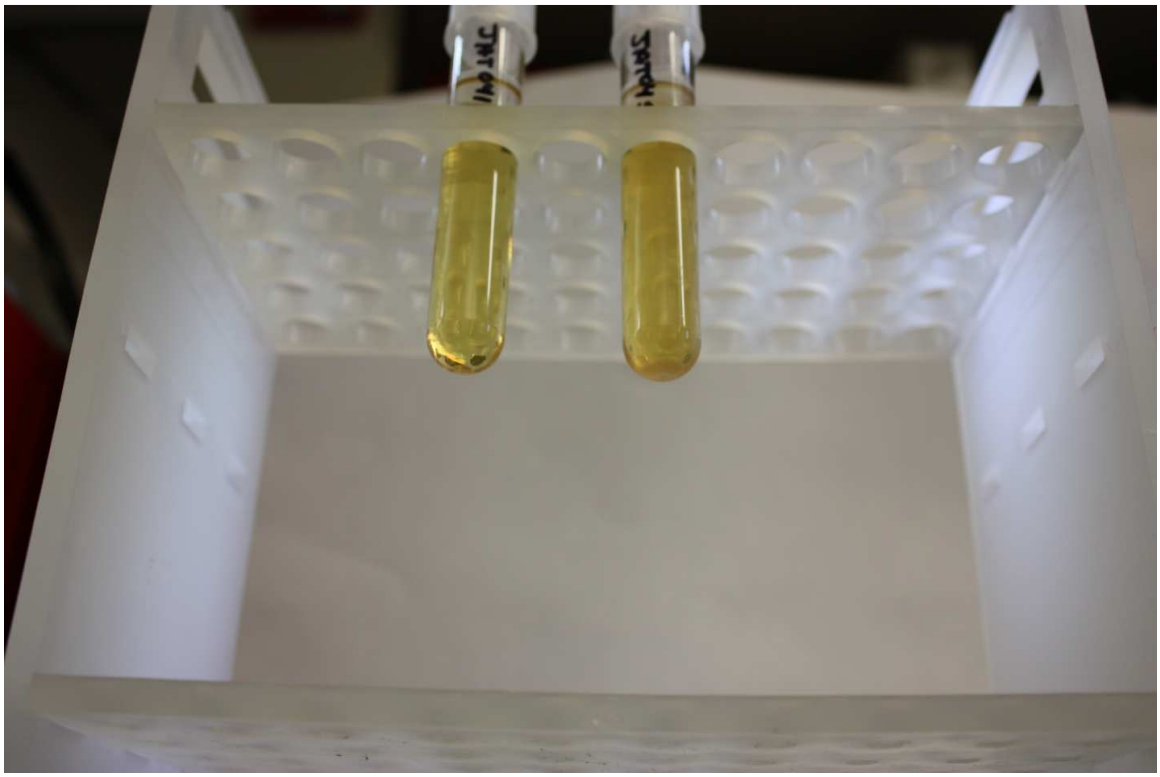

Figure B 15 Broth Samples – 41, 45

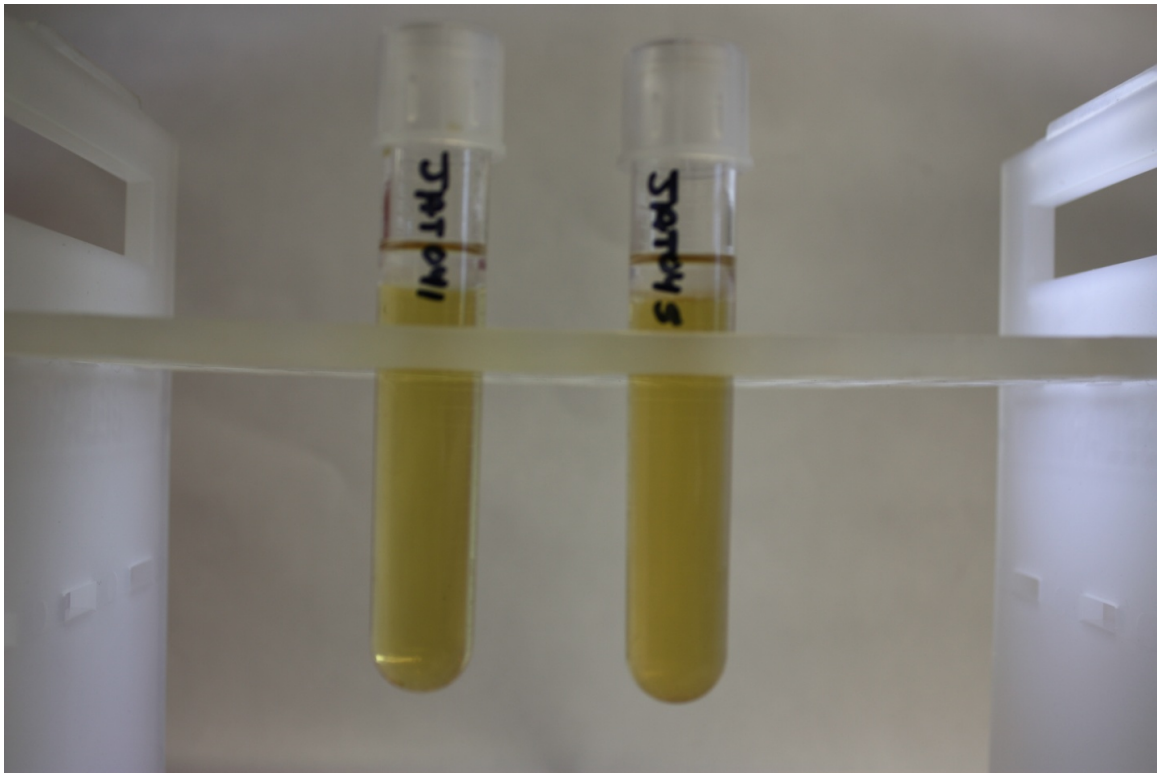

Figure B 16 Broth Samples – 41, 45

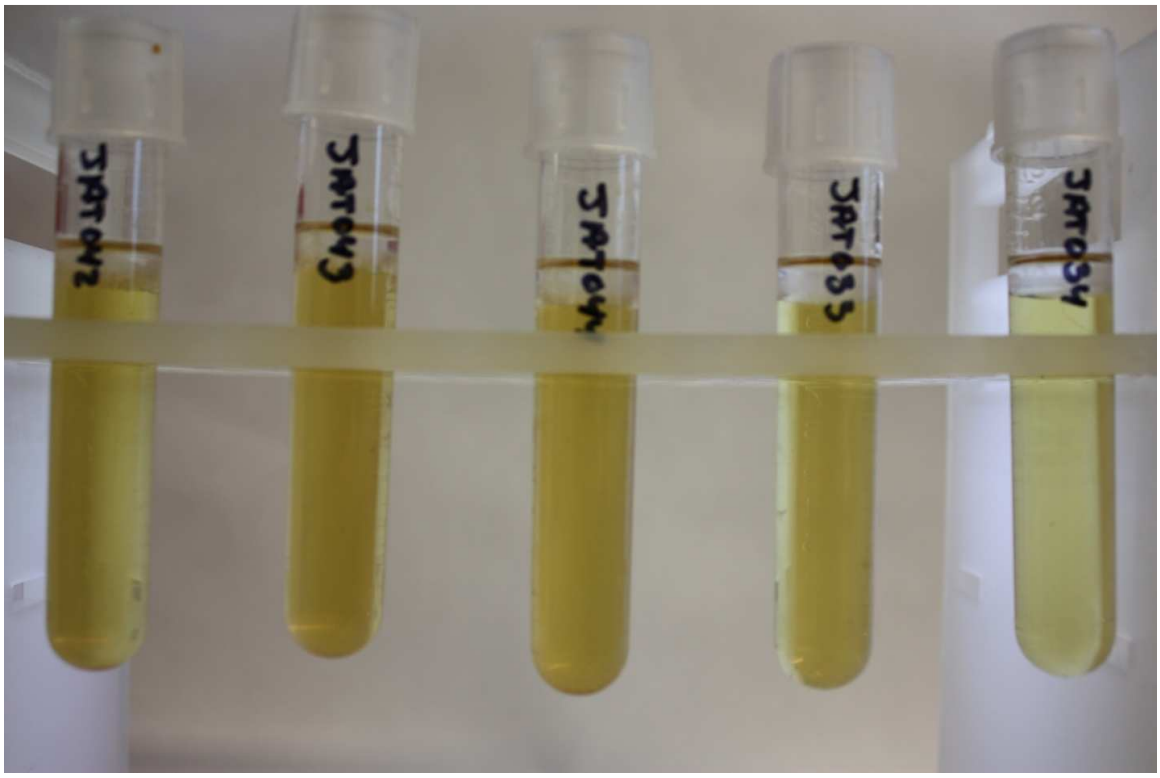

Figure B 17 Broth Samples – 42, 43, 44, 33, 34

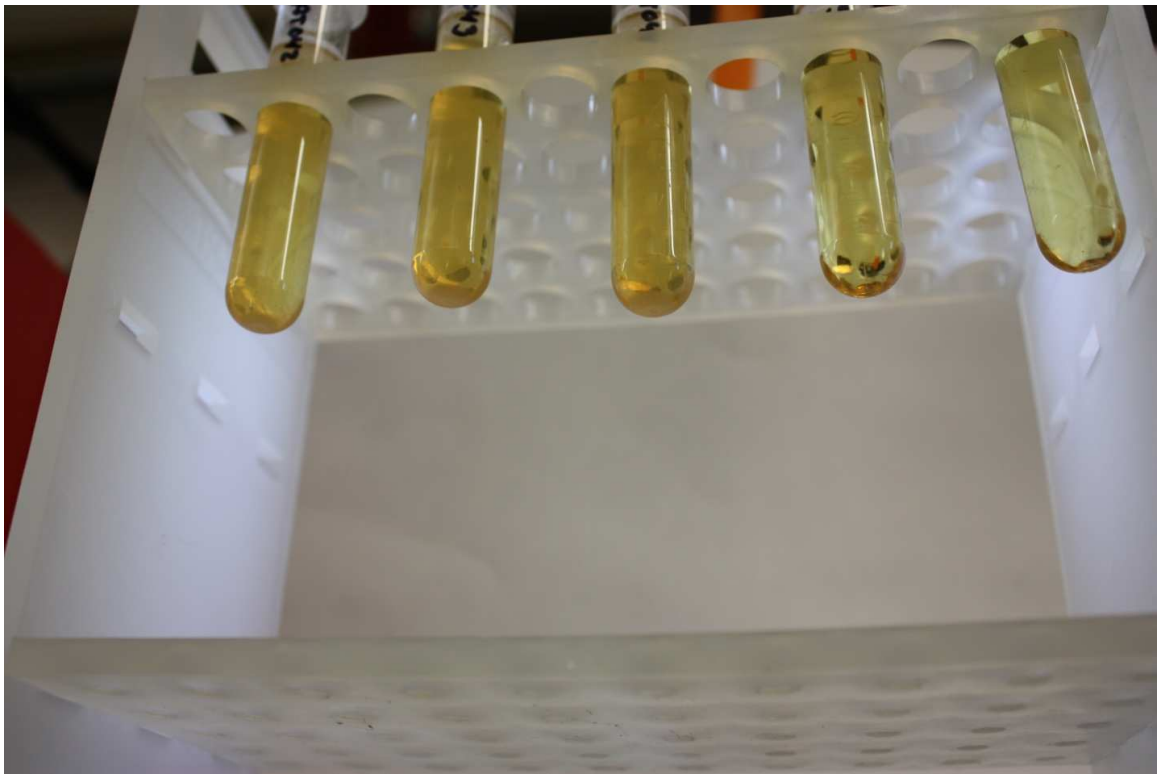

Figure B 18 Broth Samples - 42, 43, 44, 33, 34

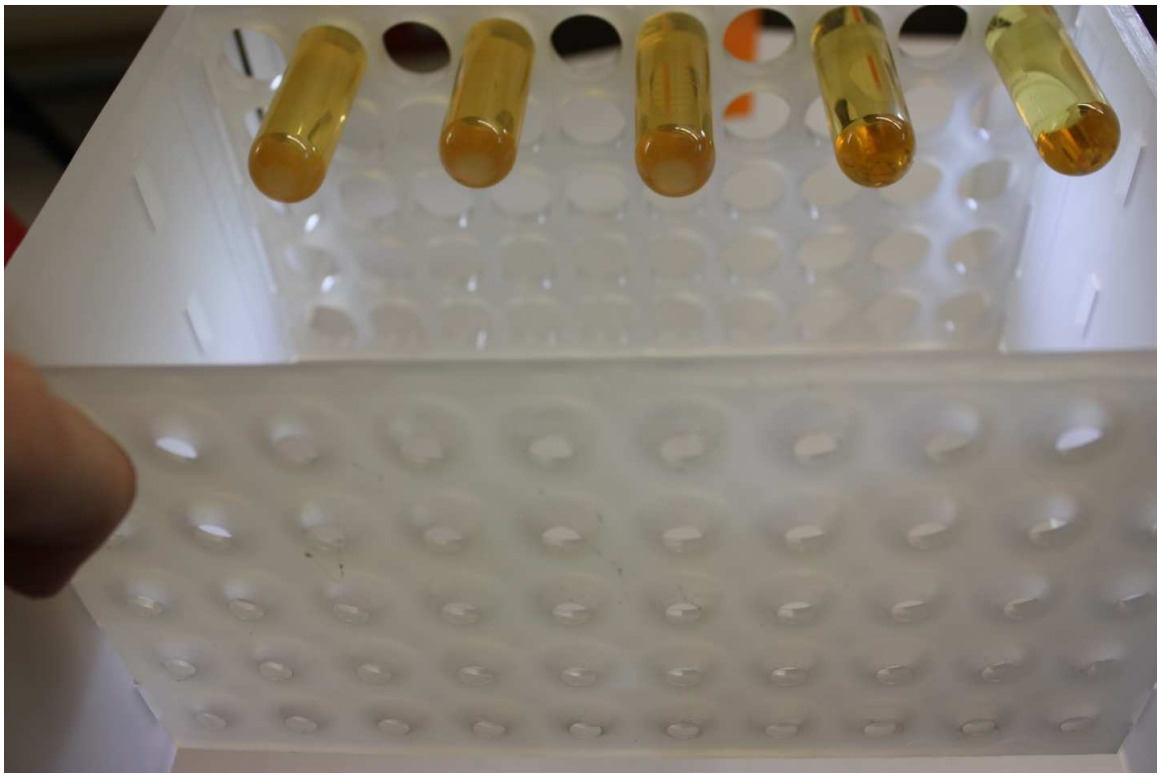

Figure B 19 Broth Samples

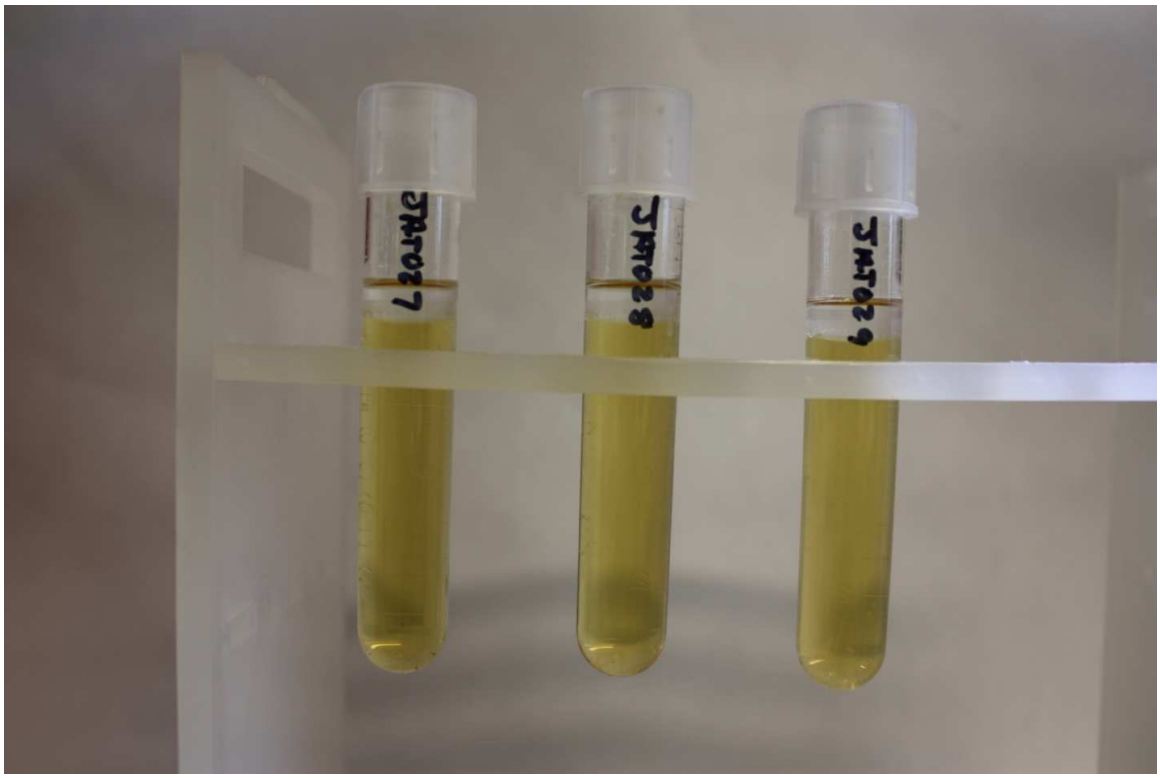

Figure B 20 Broth Samples – 27, 28, 29

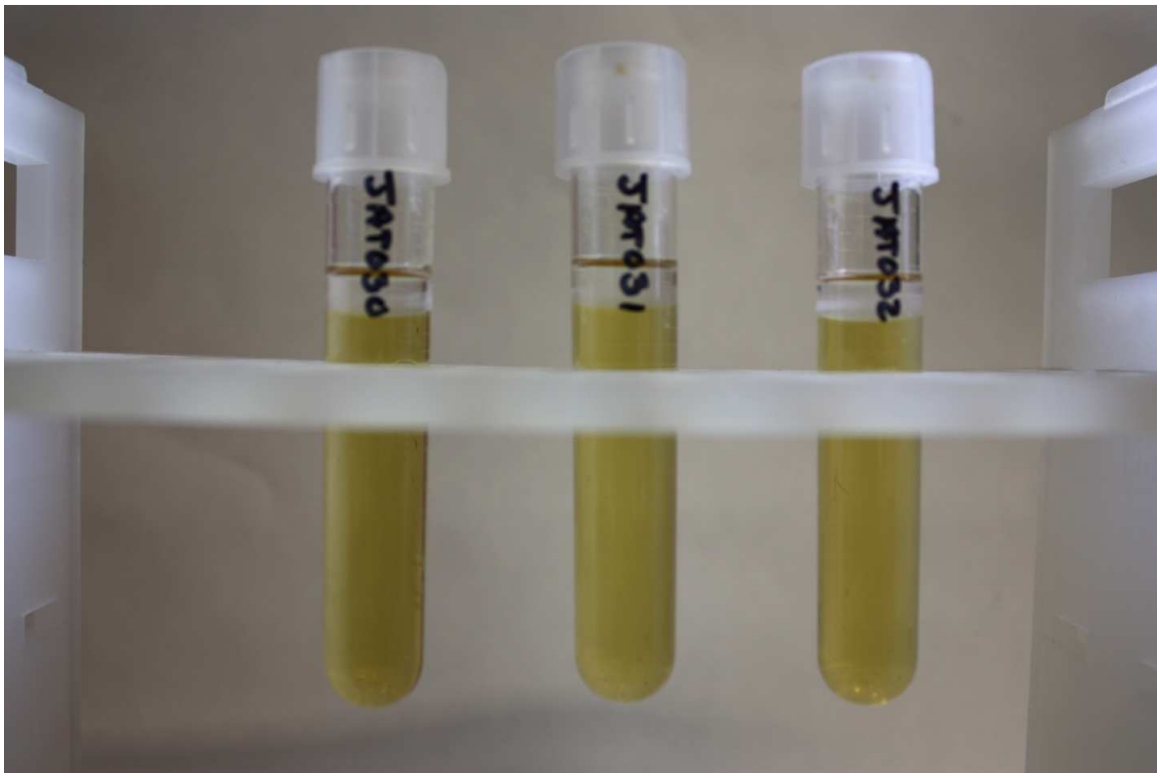

Figure B 21 Broth Samples – 30, 31, 32

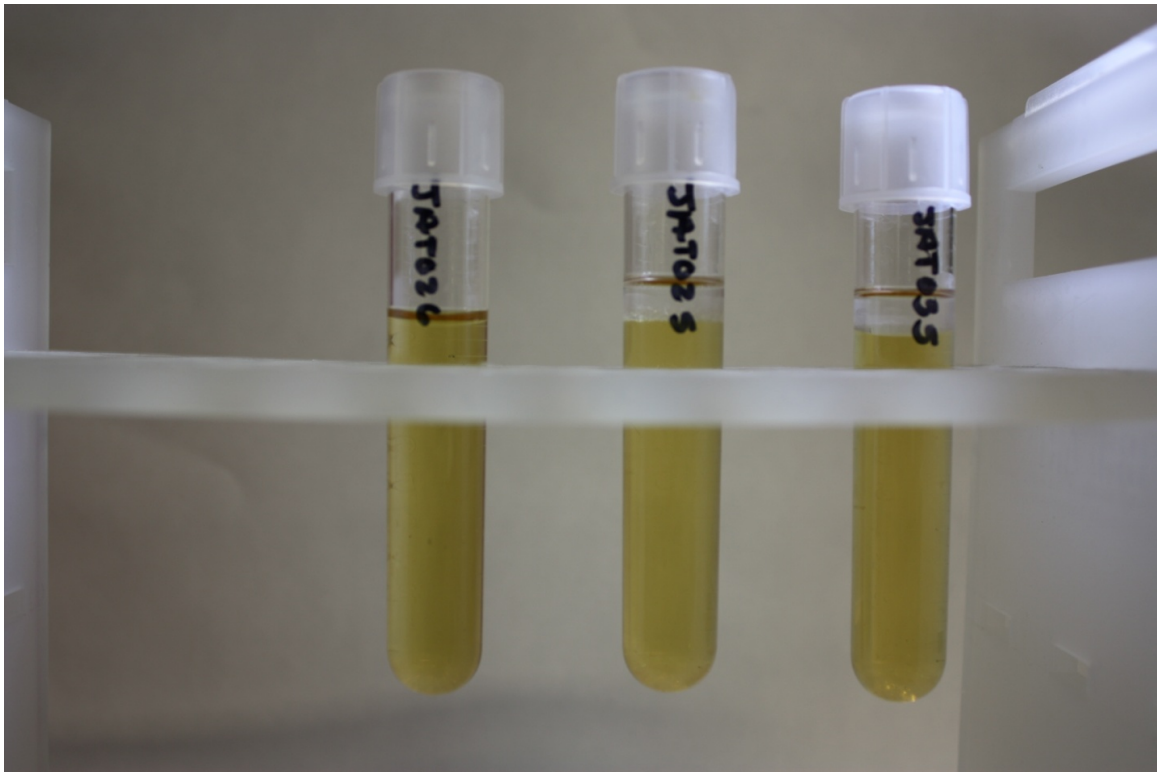

Figure B 22 Broth Samples – 26, 25, 35

Figure B 23 Broth Samples – 33, 35, 36

Figure B 24 Broth Samples – 26, 25, 35

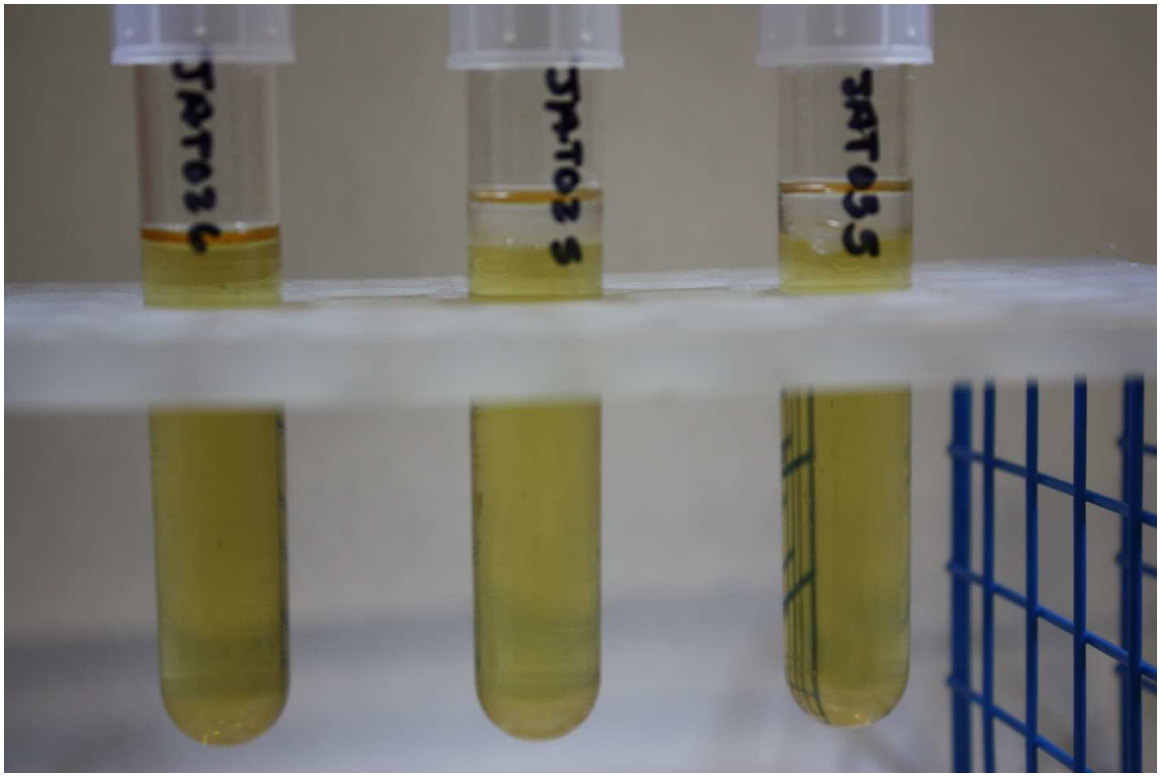

Figure B 25 Broth Samples – 36, 33, 35

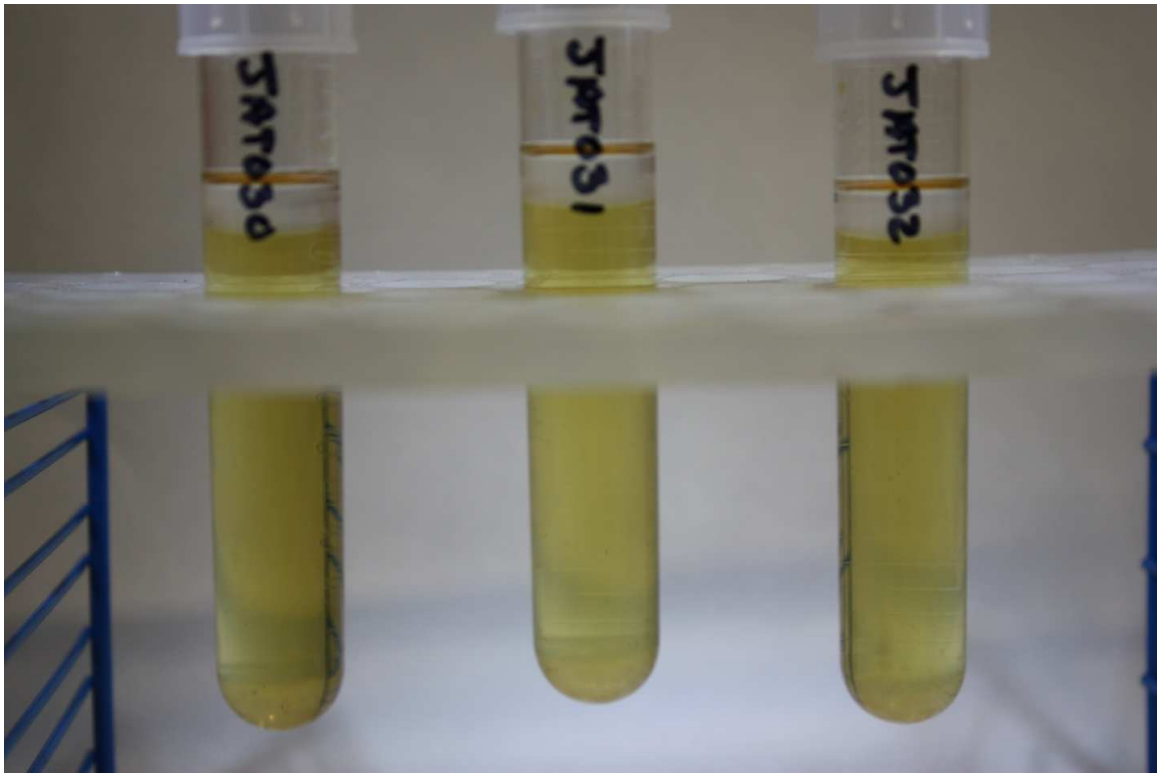

Figure B 26 Broth Samples – 30, 31, 32

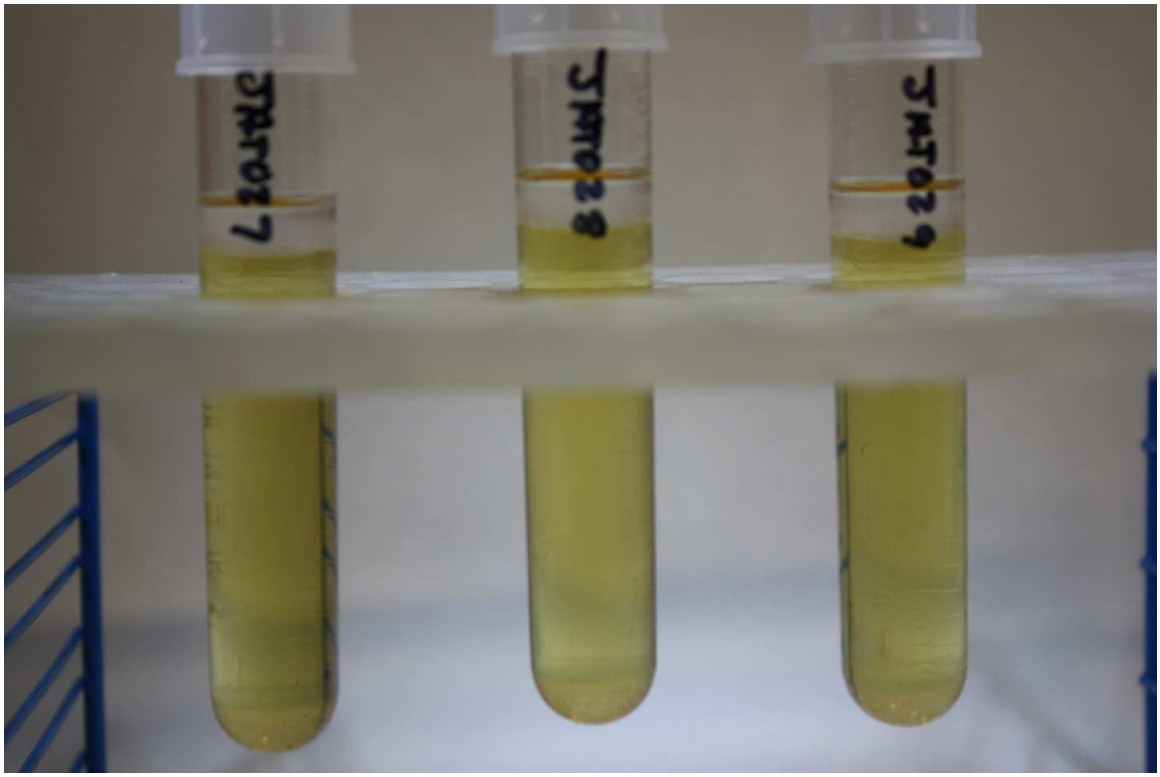

Figure B 27 Broth Samples – 27, 28, 29

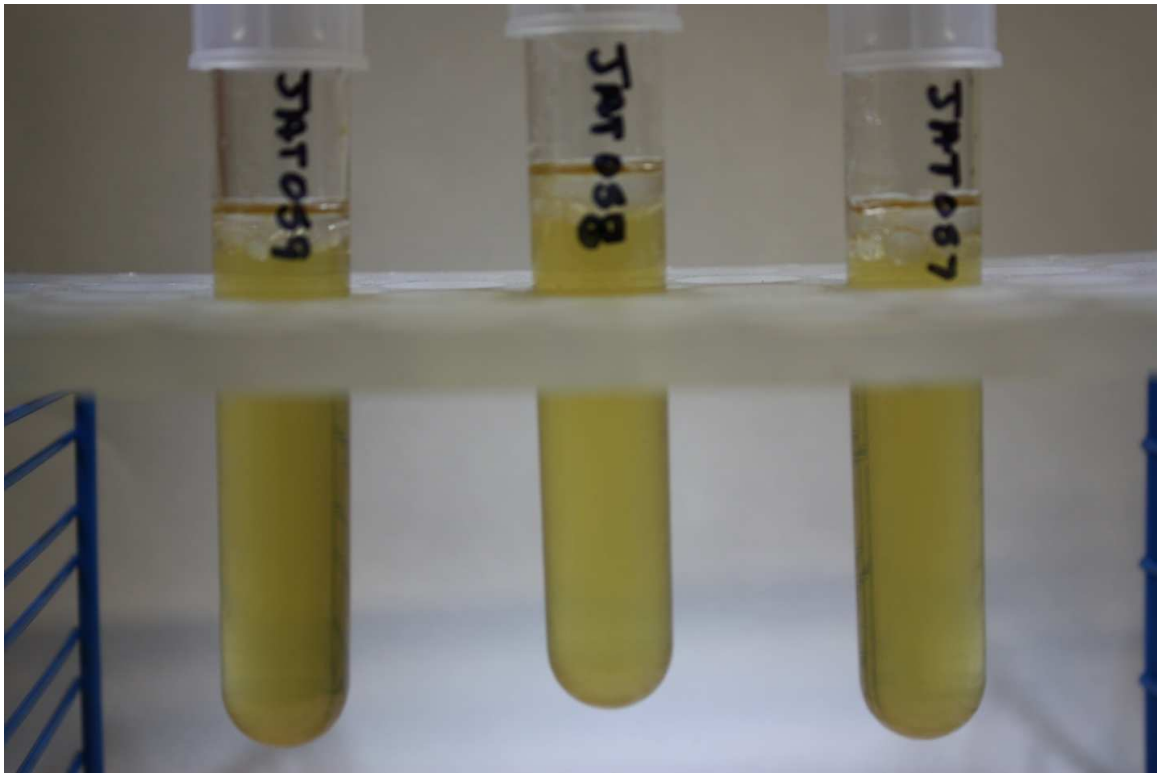

Figure B 28 Broth Samples – 59, 58, 57

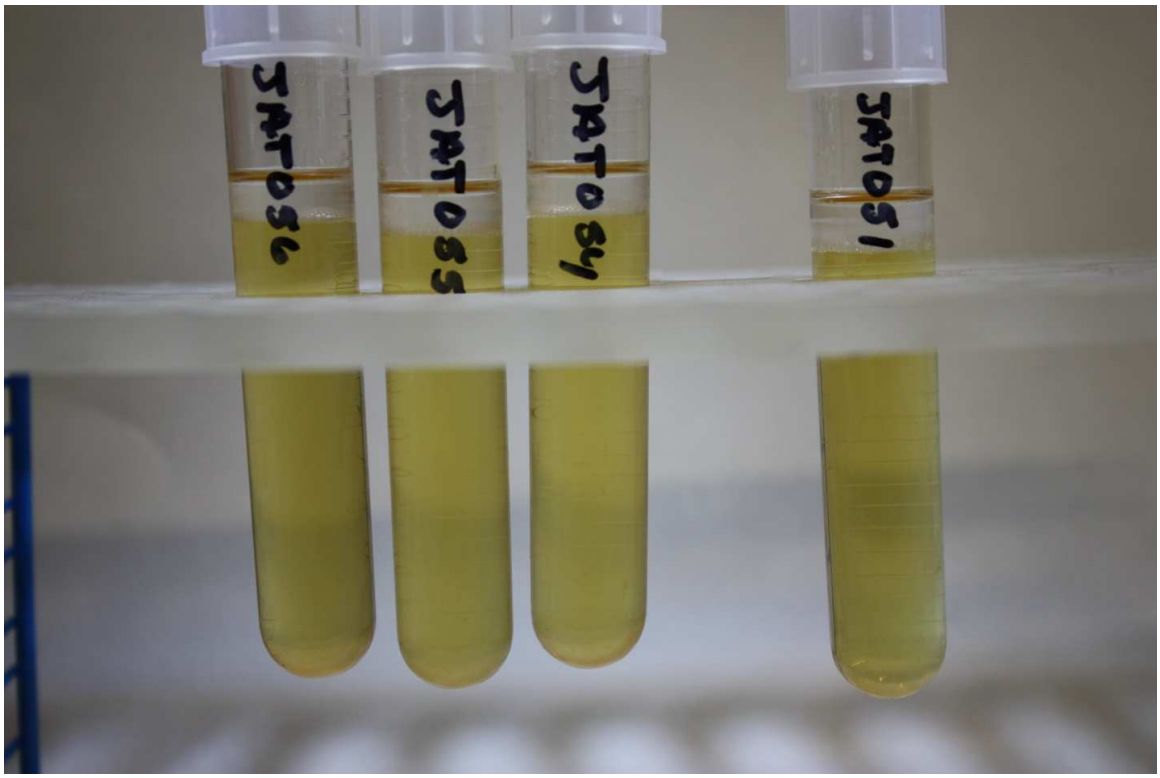

Figure B 29 Broth Samples – 56, 55, 34, 51

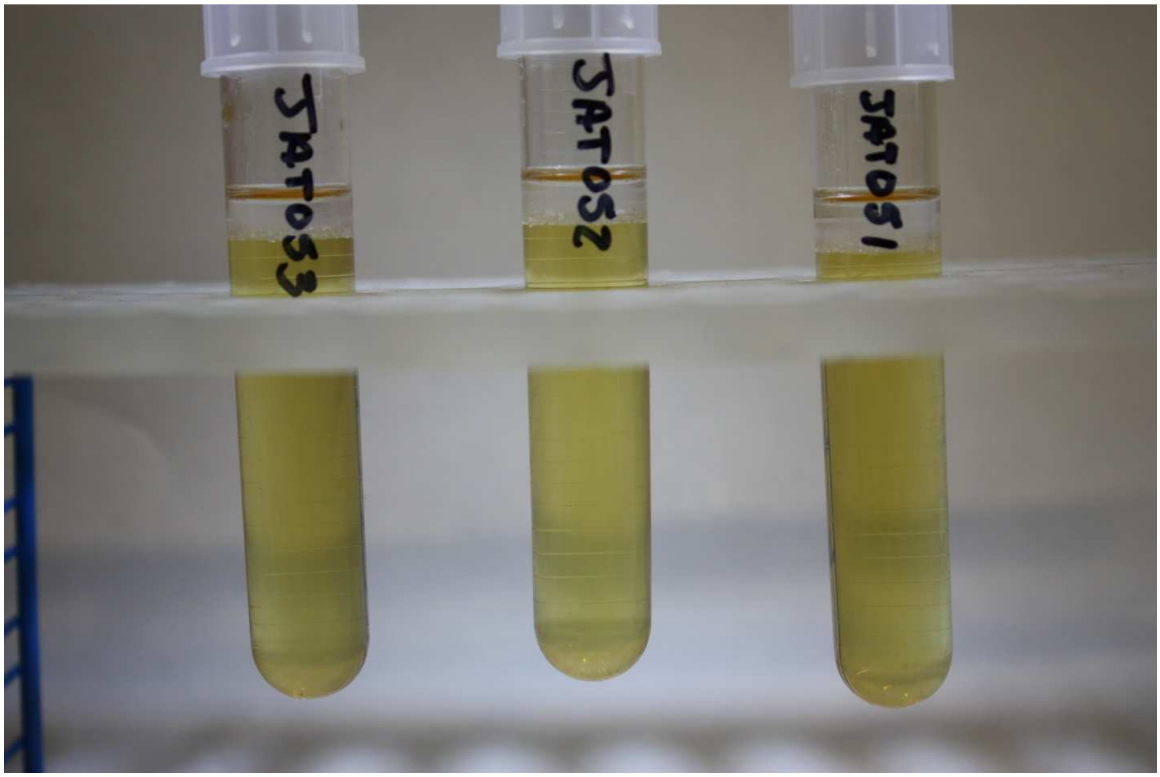

Figure B 30 Broth Samples – 53, 52, 51

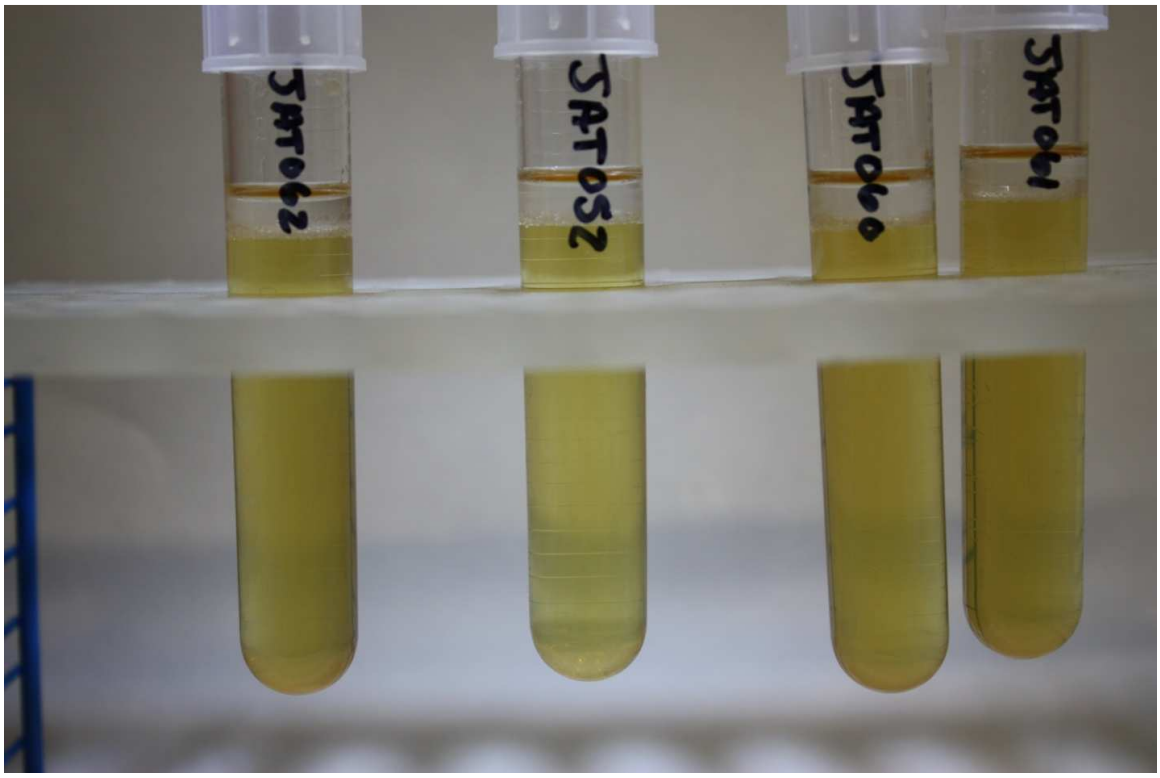

Figure B 31 Broth Samples – 62, 52, 60, 61

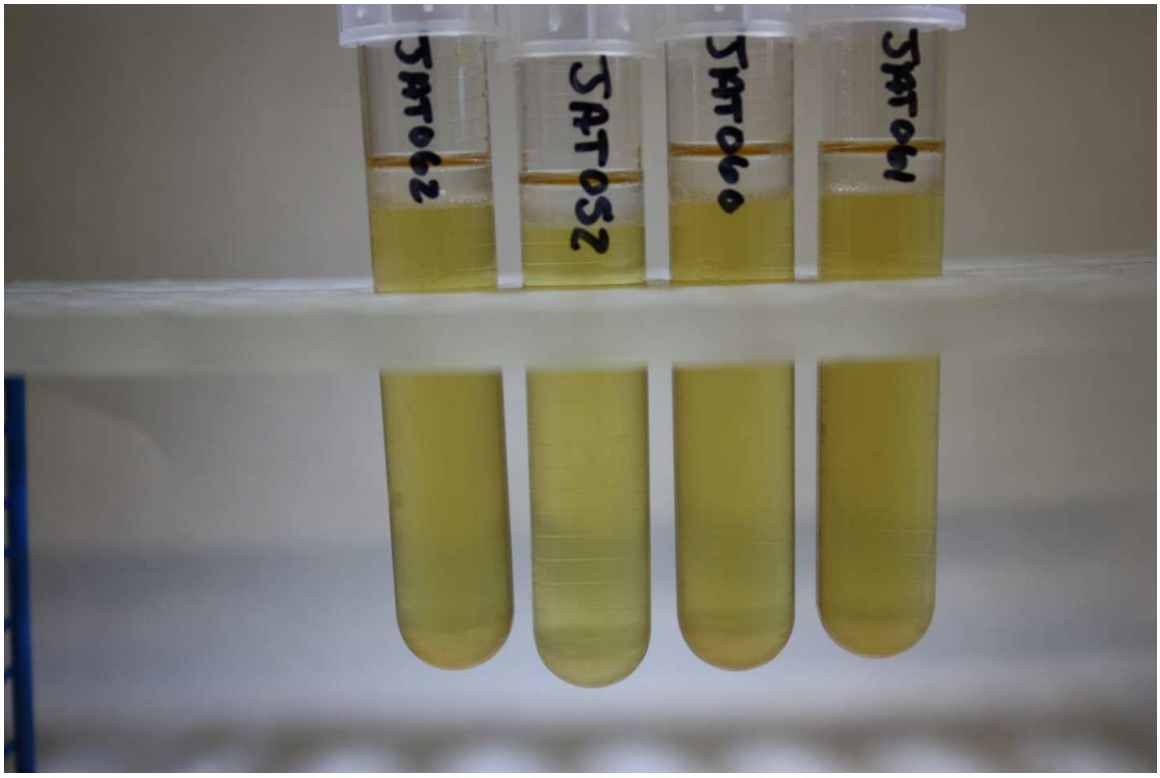

Figure B 32 Broth Samples – 62, 52, 60, 61

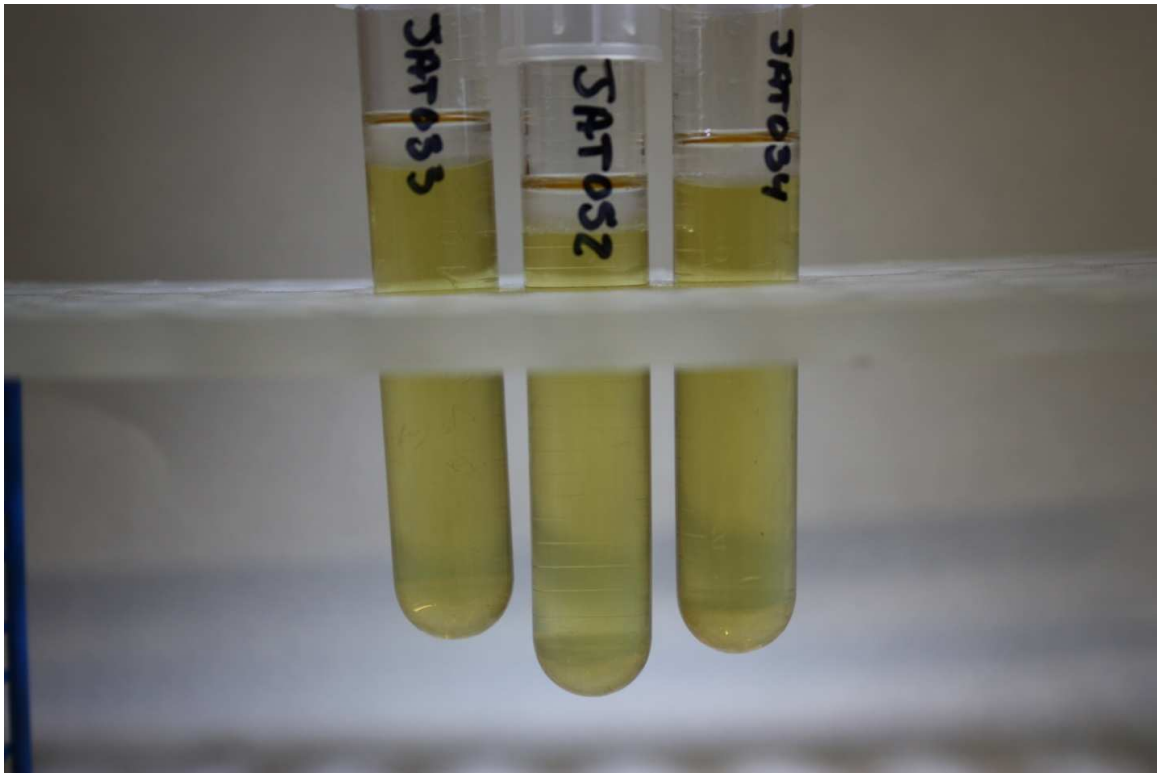

Figure B 33 Broth Samples – 33, 52, 34

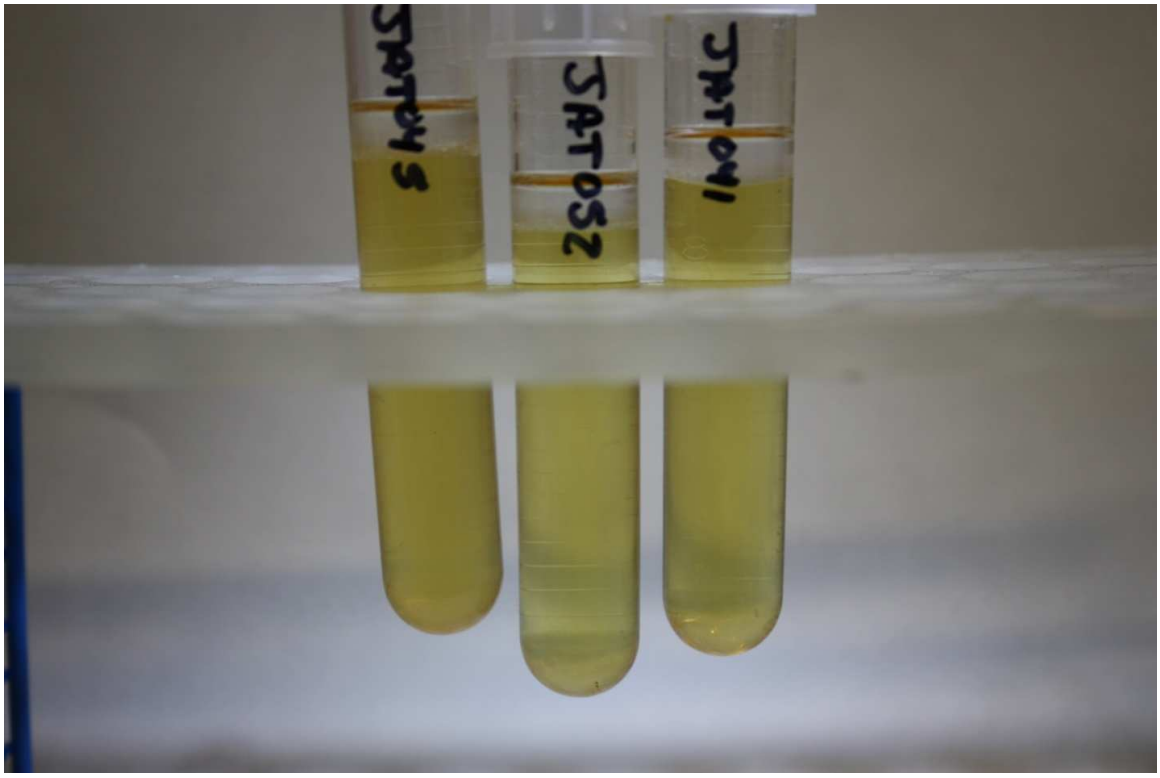

Figure B 34 Broth Samples – 45, 52, 41

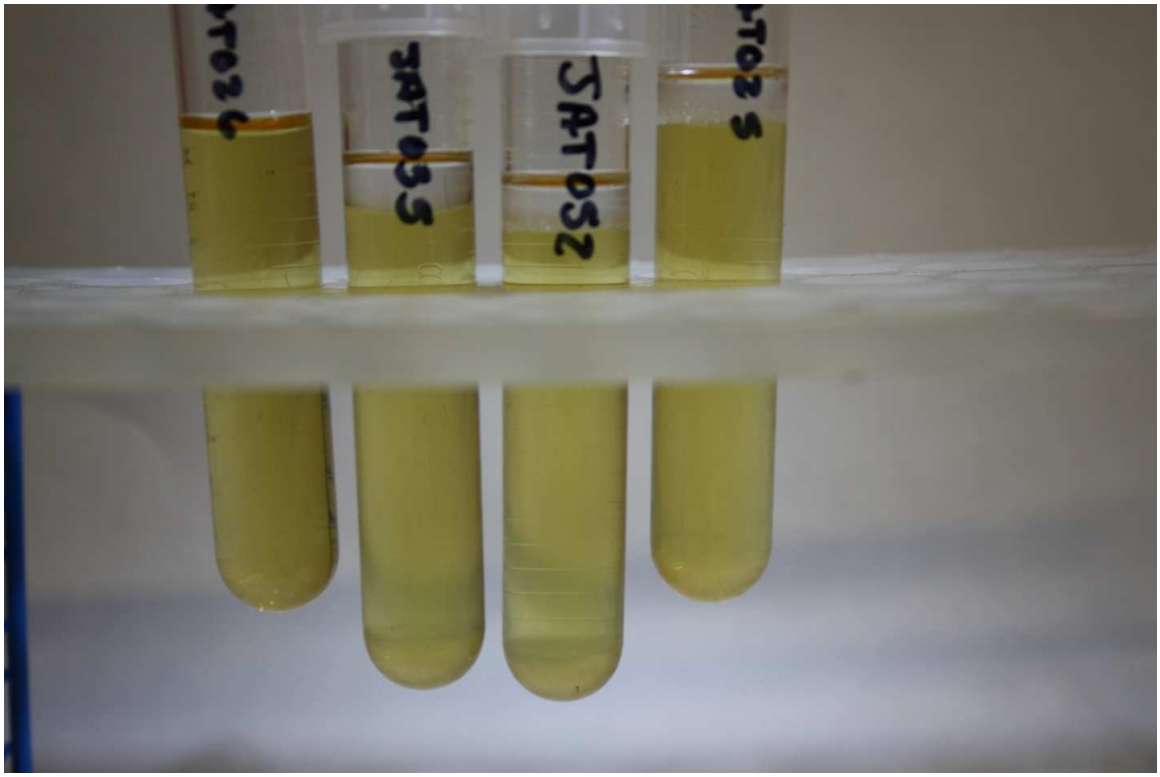

Figure B 35 Broth Samples – 36, 35, 52, 23

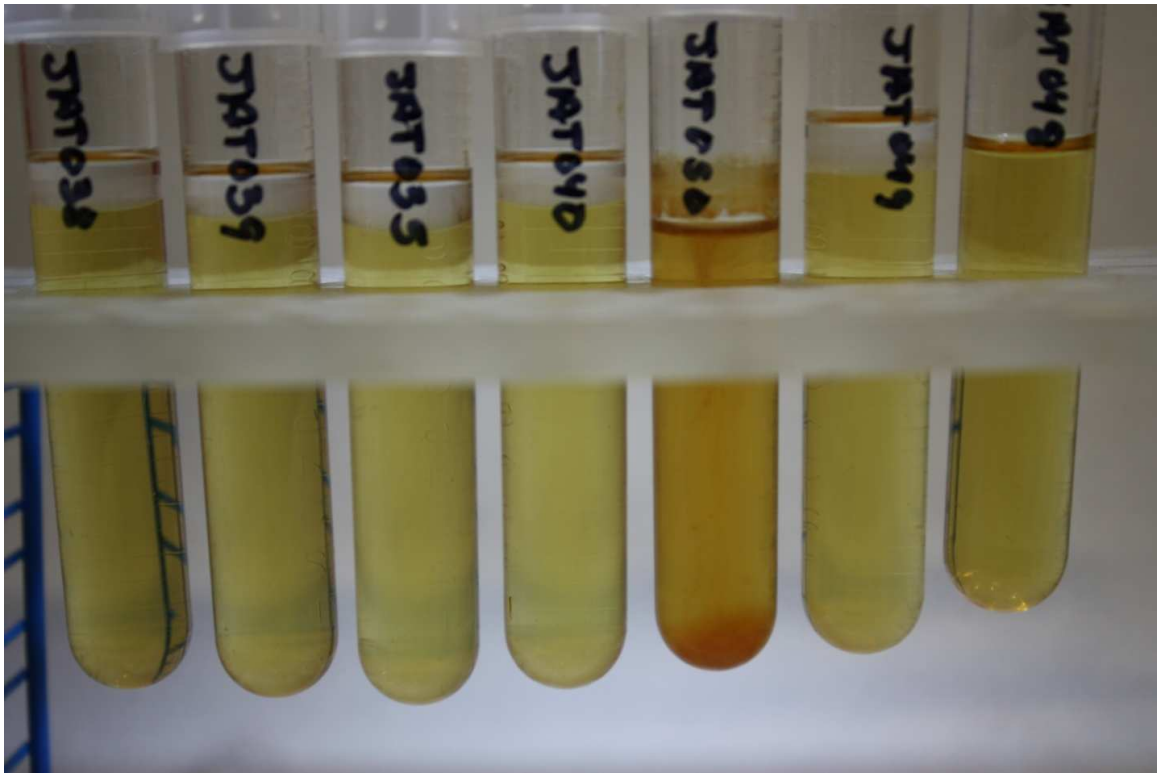

Figure B 36 Broth Samples – 38, 39, 35, 40, 50, 49, 48

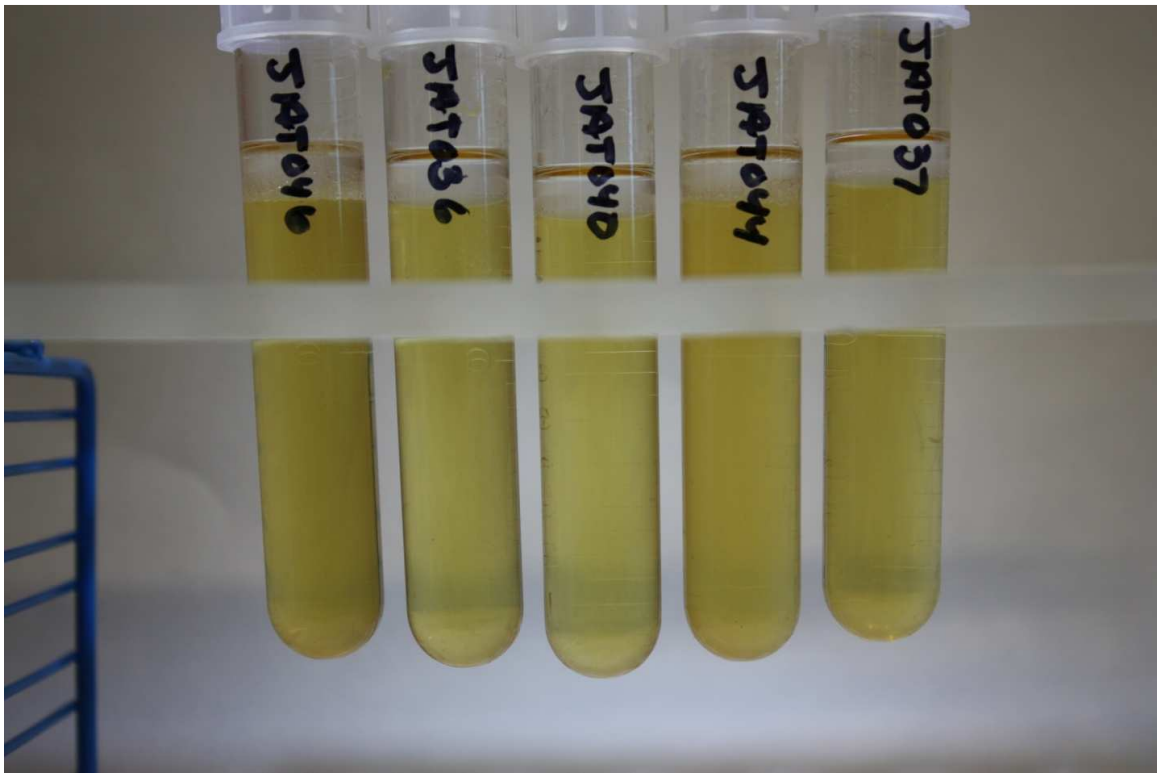

Figure B 37 Broth Samples – 46, 36, 40, 44, 37

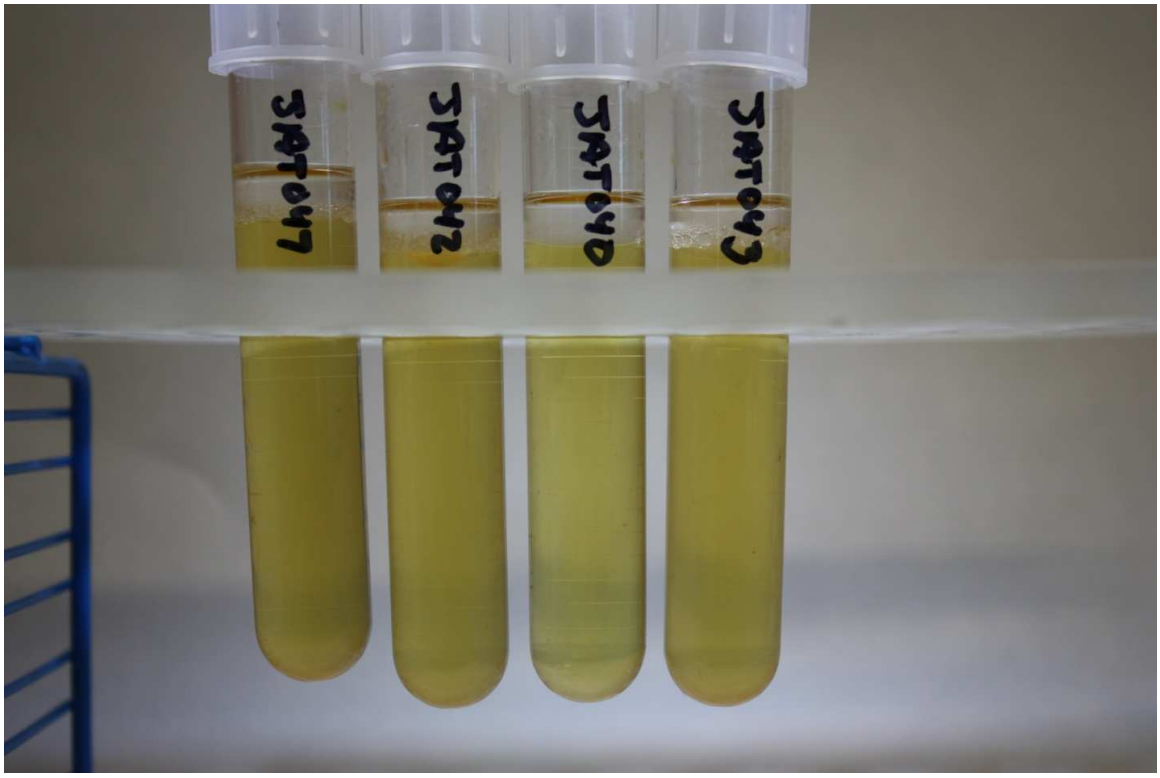

Figure B 38 Broth Samples – 47, 42, 40, 43

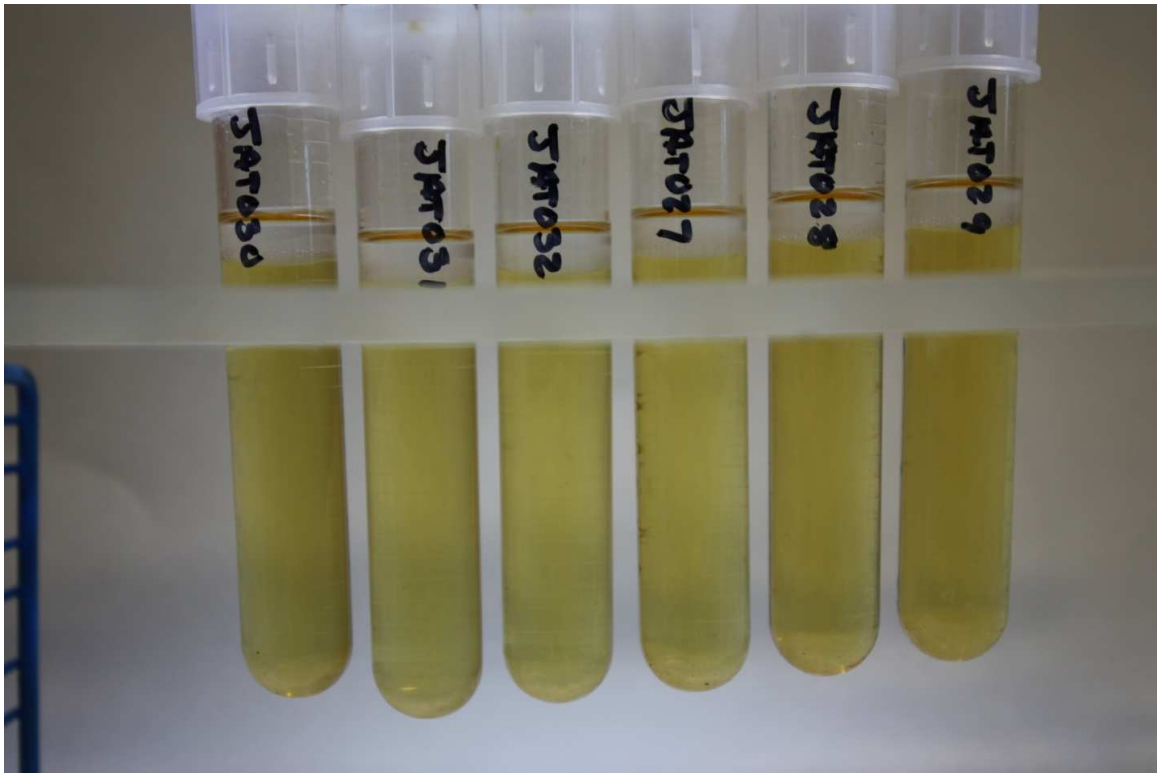

Figure B 39 Broth Samples – 30, 31, 32, 27, 28, 29

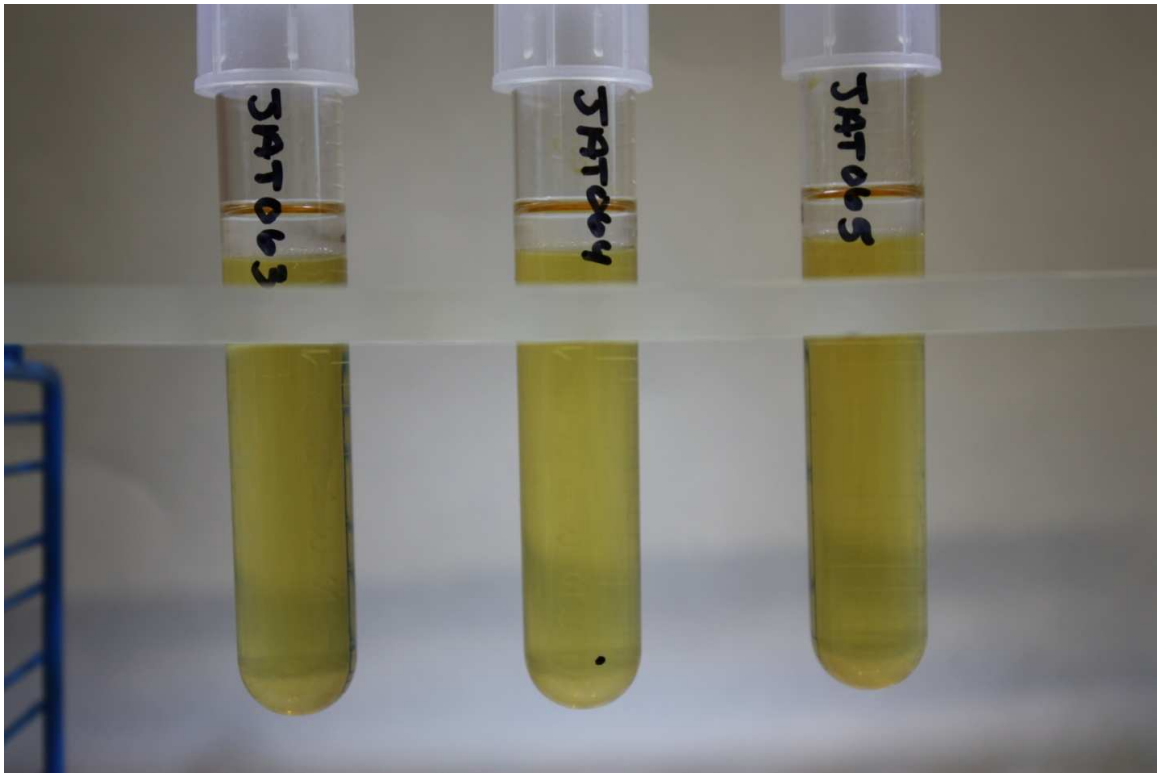

Figure B 40 Broth Samples – 63, 64, 65

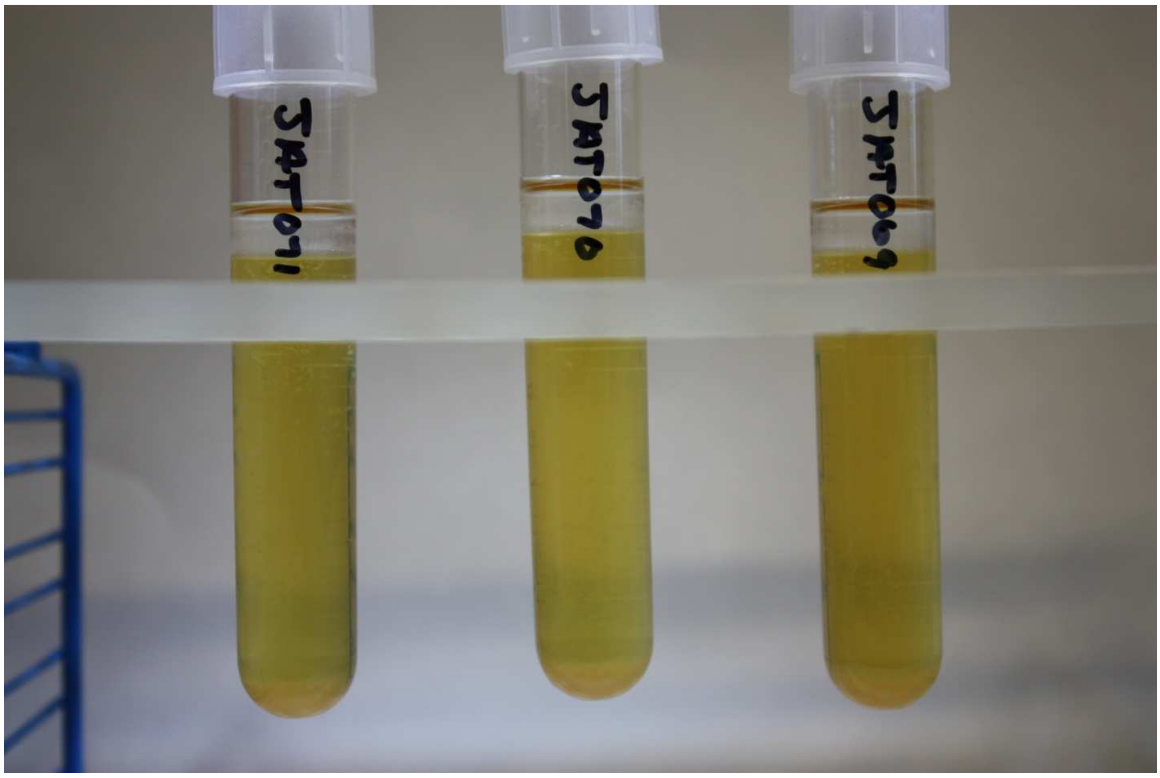

Figure B 41 Broth Samples – 71, 70, 69

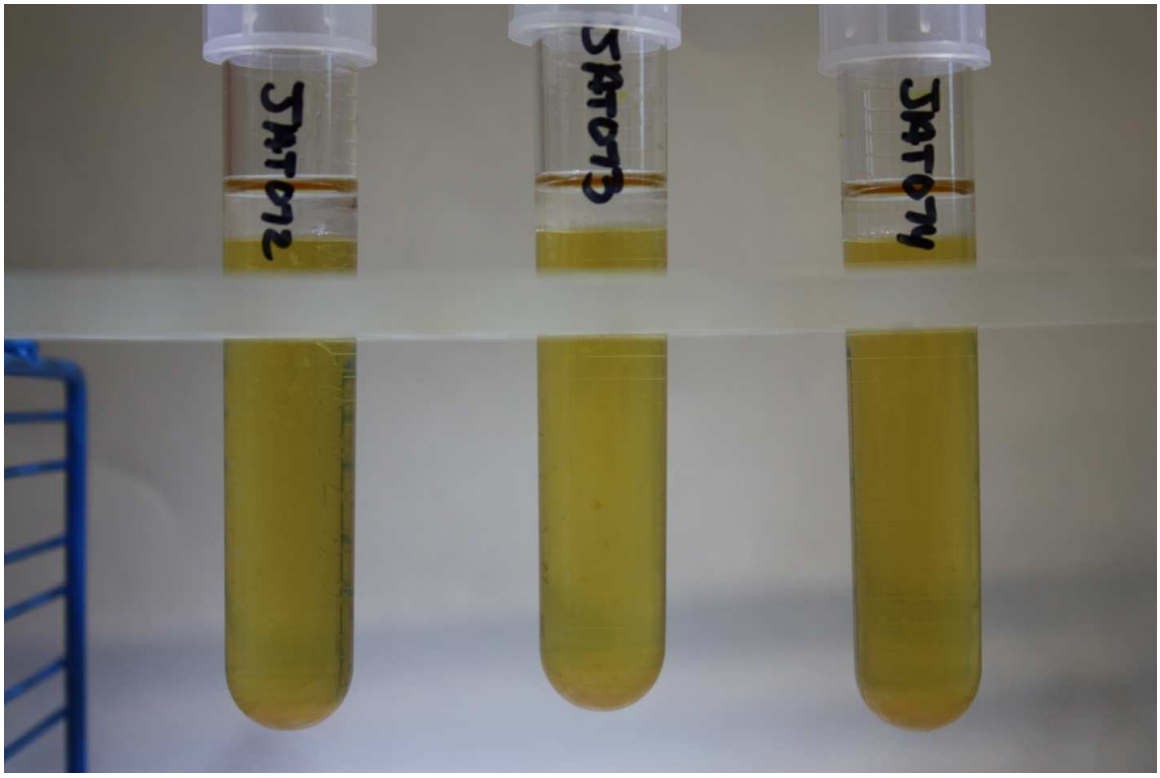

Figure B 42 Broth Samples – 72, 73, 74

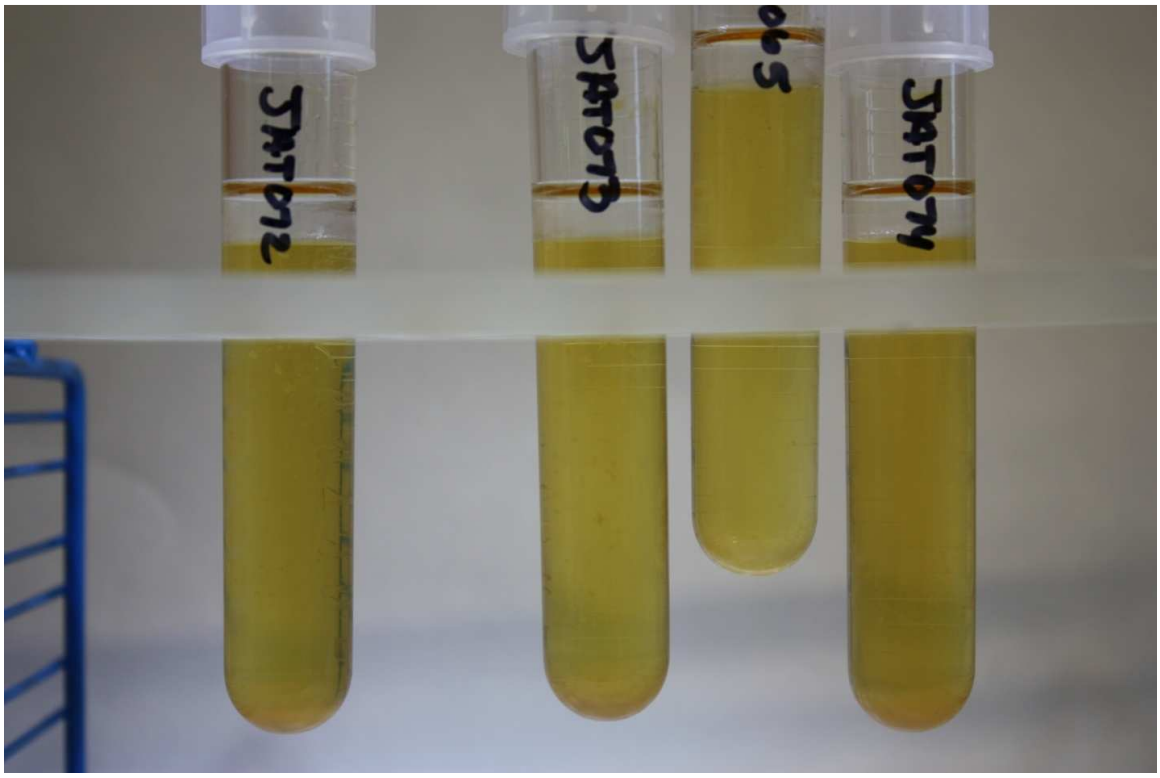

Figure B 43 Broth Samples – 72, 73, 65, 74

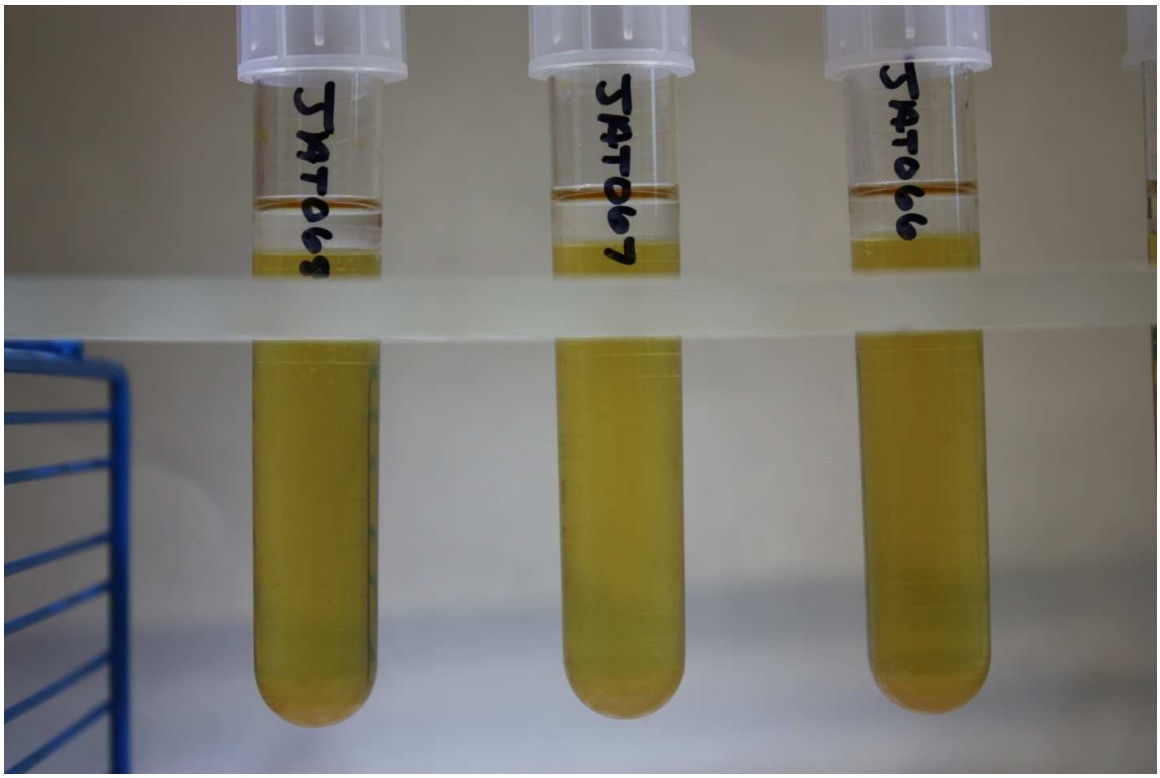

Figure B 44 Broth Samples – 68, 67, 66

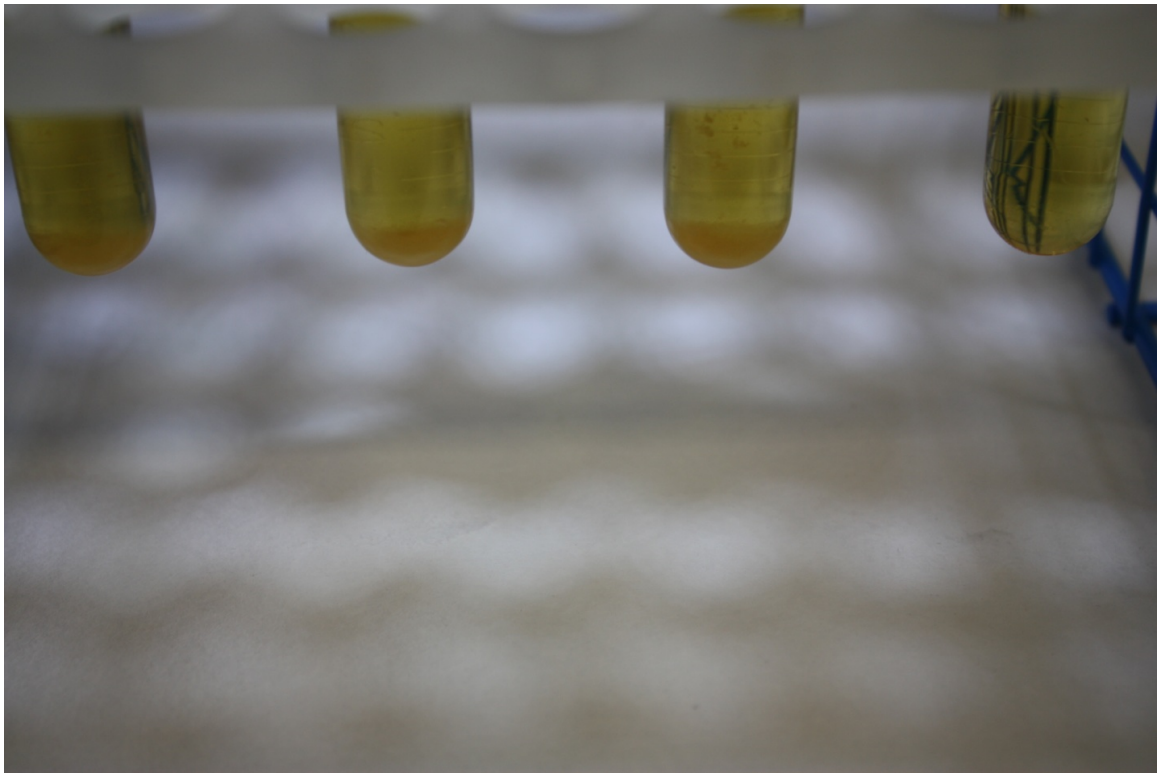

Figure B 45 Broth Samples

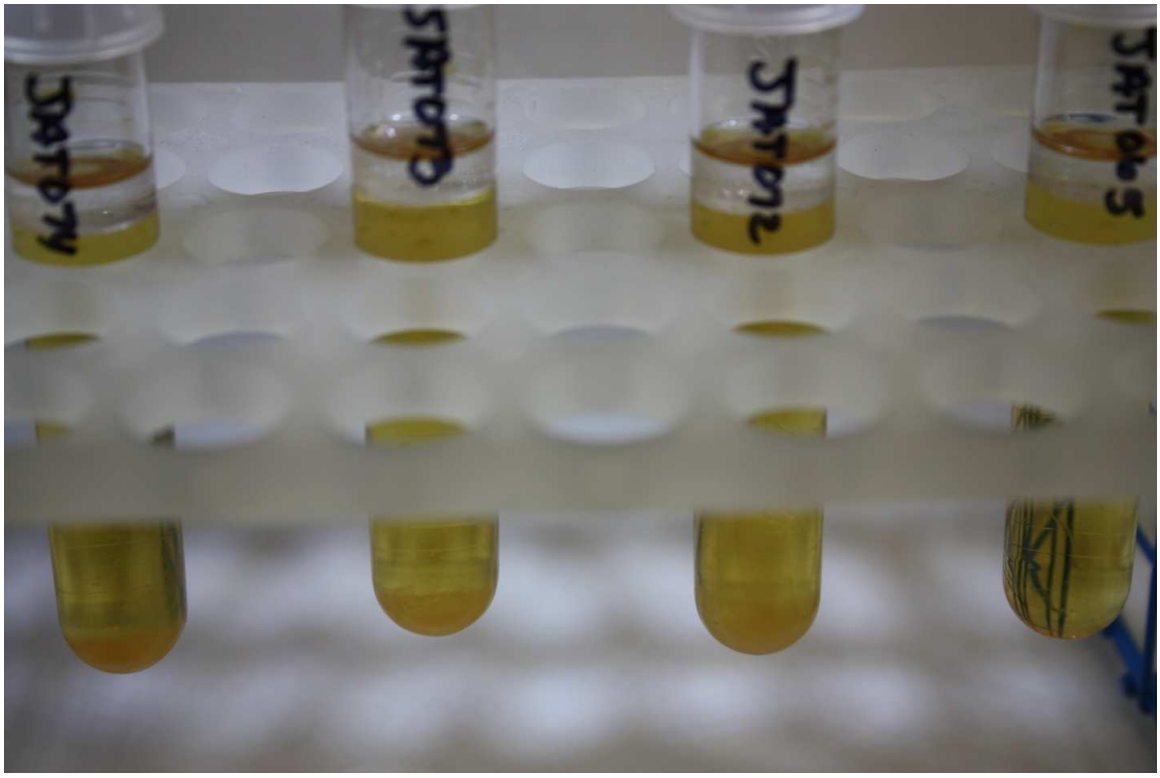

Figure B 46 Broth Samples – 74, 73, 72, 65

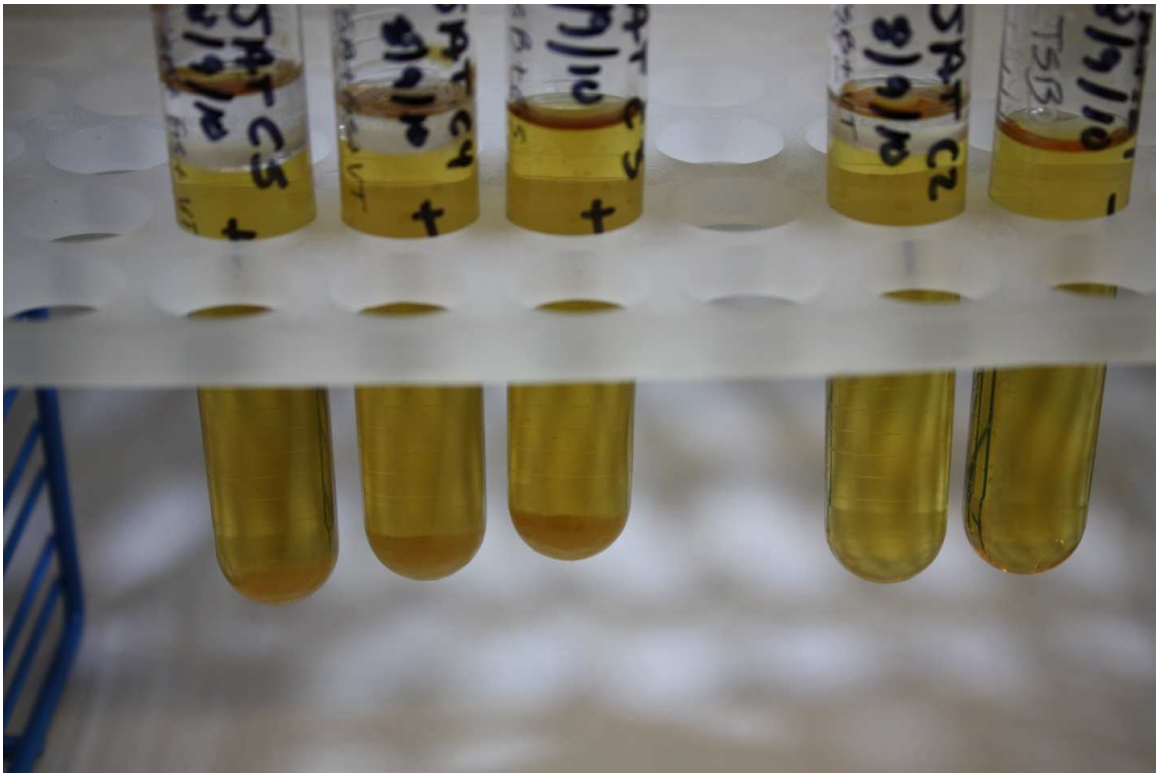

Figure B 47 Broth Samples– Positive and Negative Controls

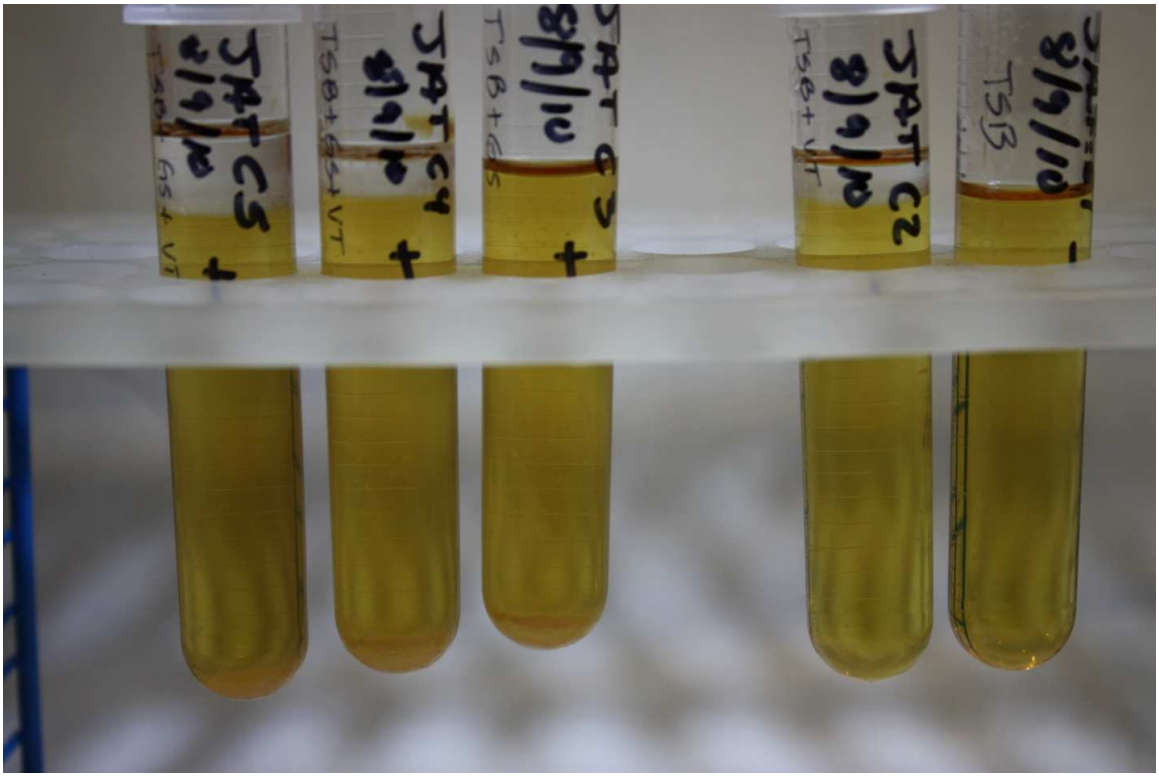

Figure B 48 Broth Samples – Positive and Negative Controls

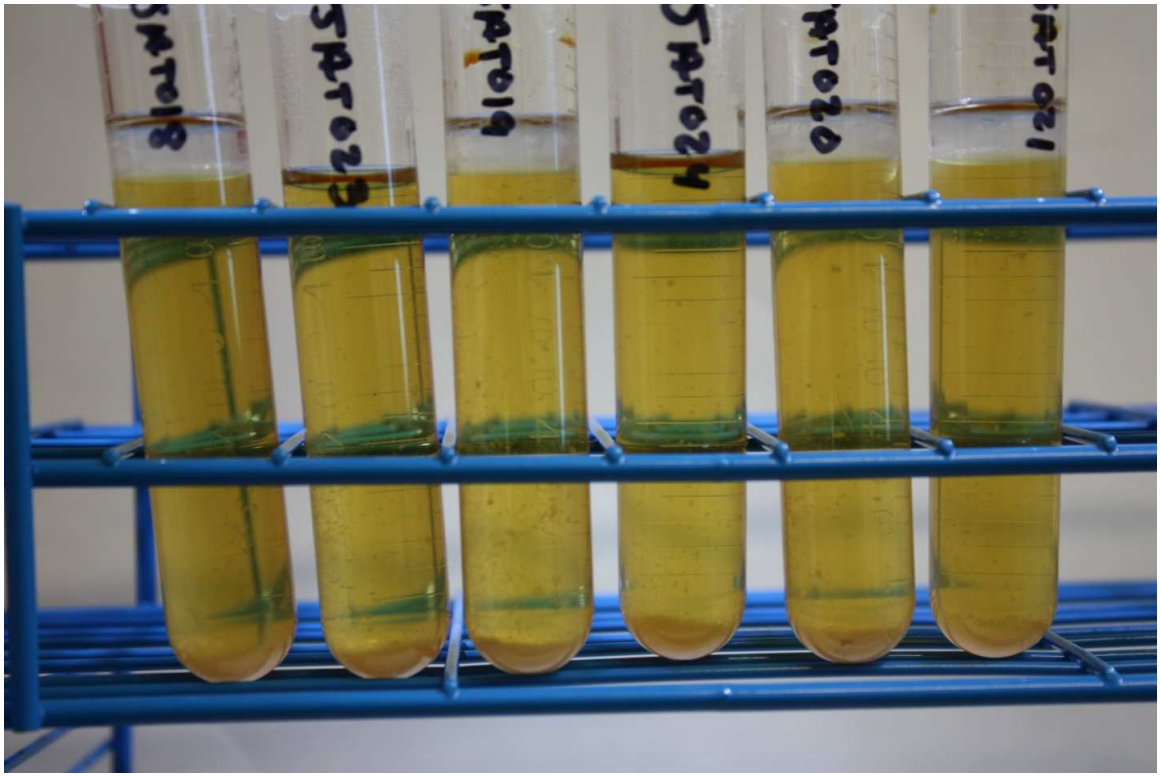

Figure B 49 Broth Samples – 18, 23, 19, 24, 20, 21

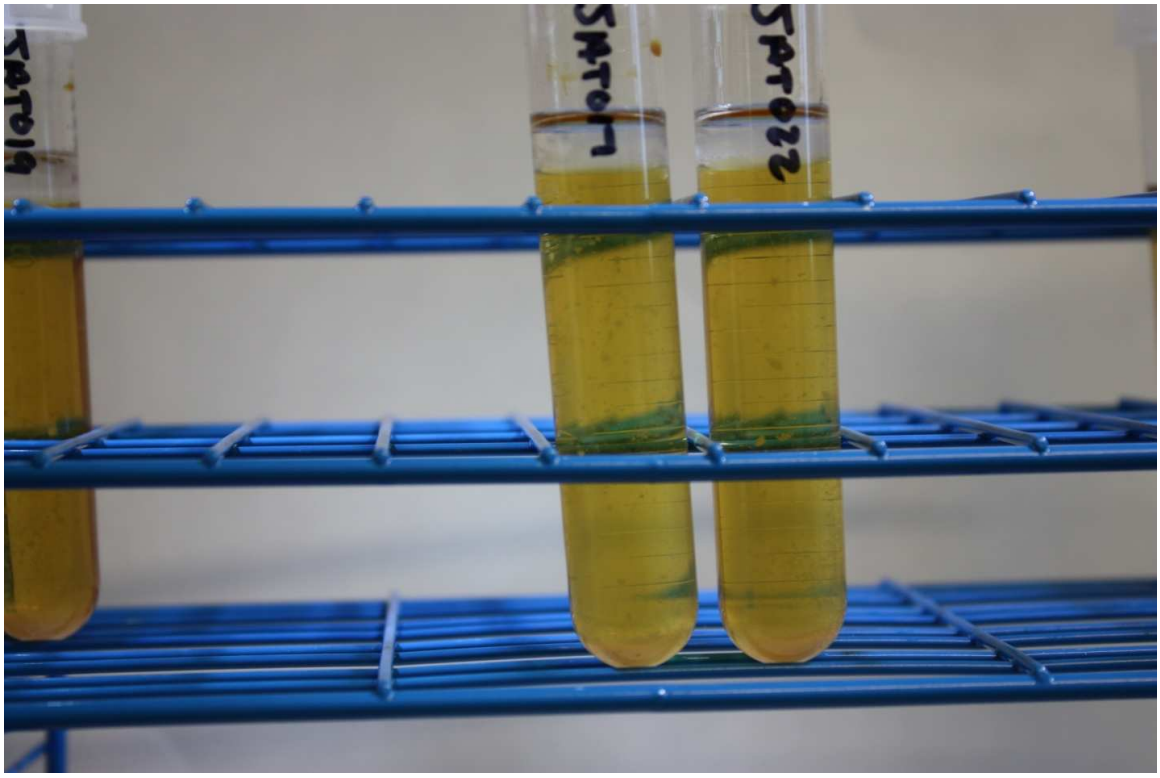

Figure B 50 Broth Samples – 19, 17, 22

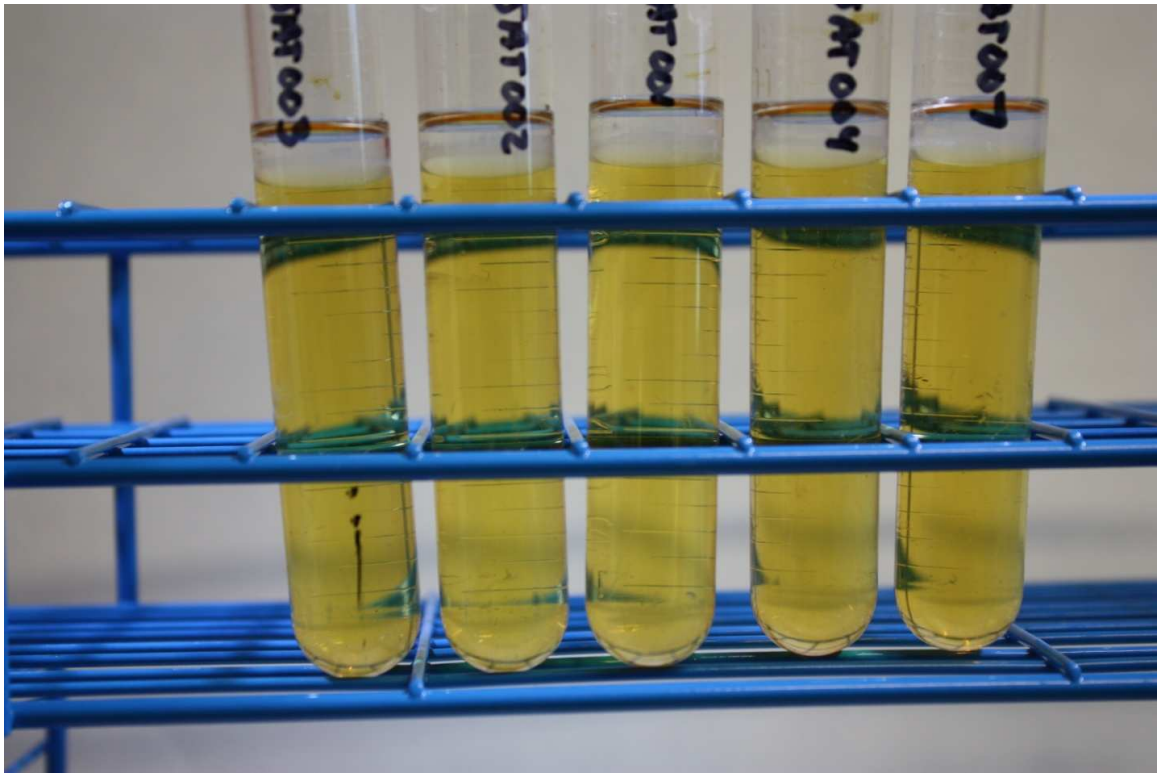

Figure B 51 Broth Samples – 3, 2, 1, 4, 7

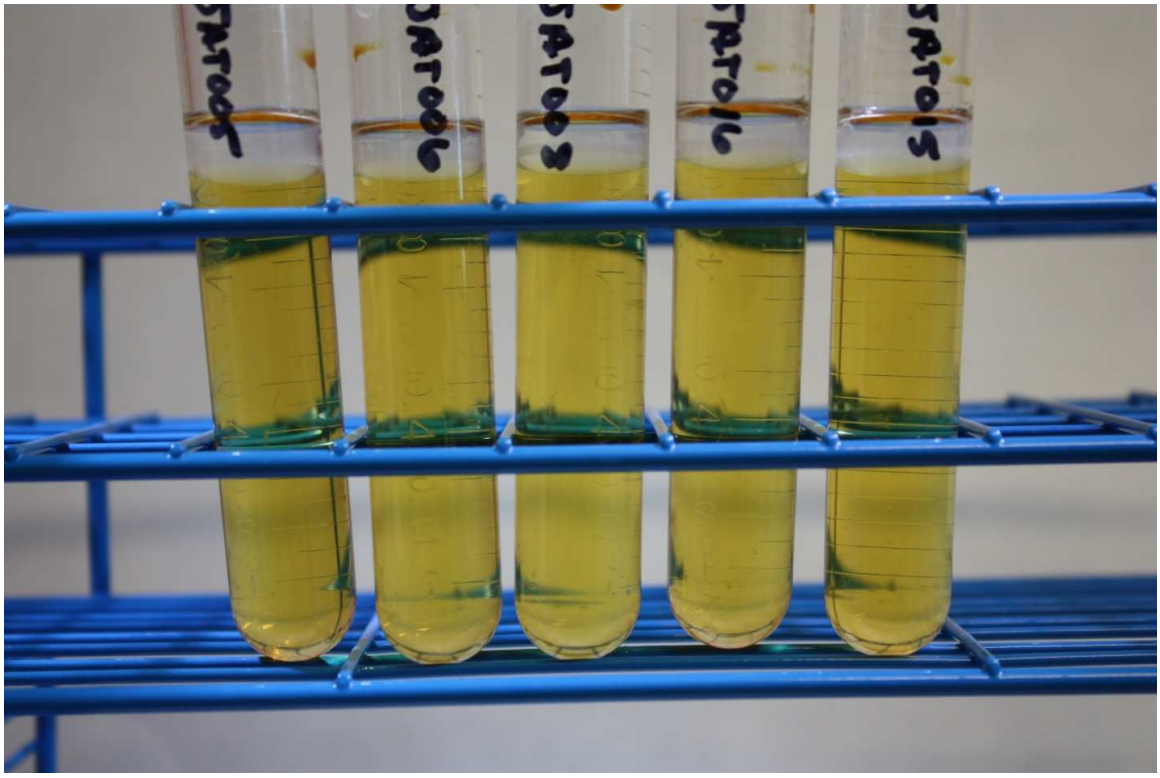

Figure B 52 Broth Samples – 5, 6, 8, 16, 15

Pictures of Plate Samples

Note that not all plate samples were photographed

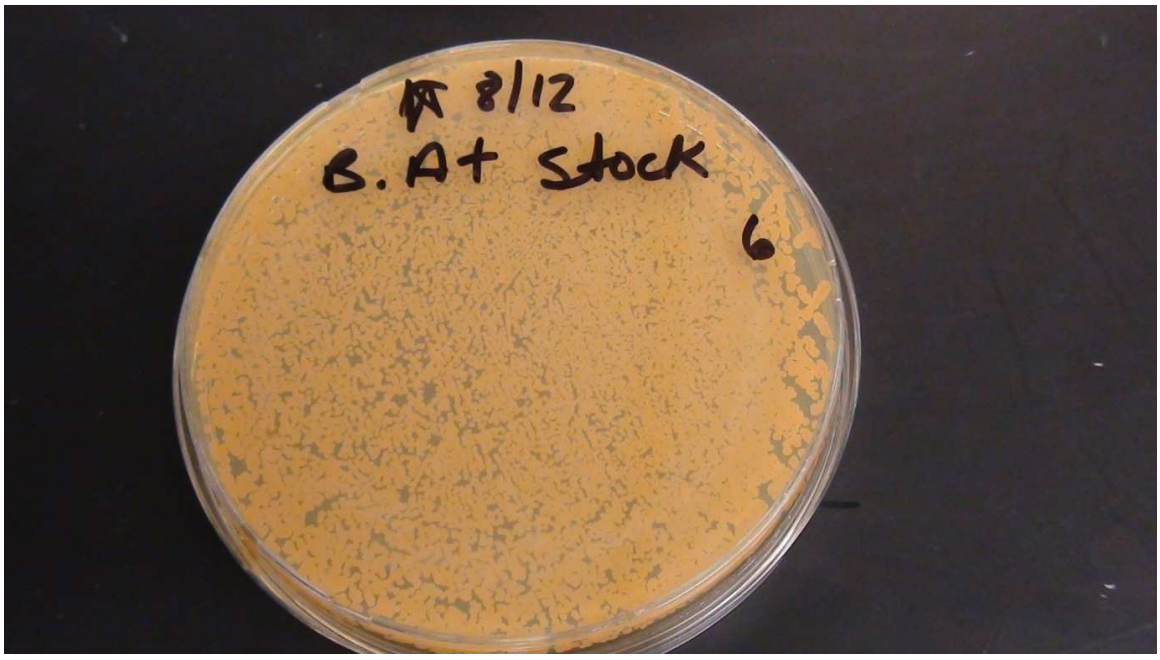

Figure B 53 Plate Sample – B. Atrophaeus Stock

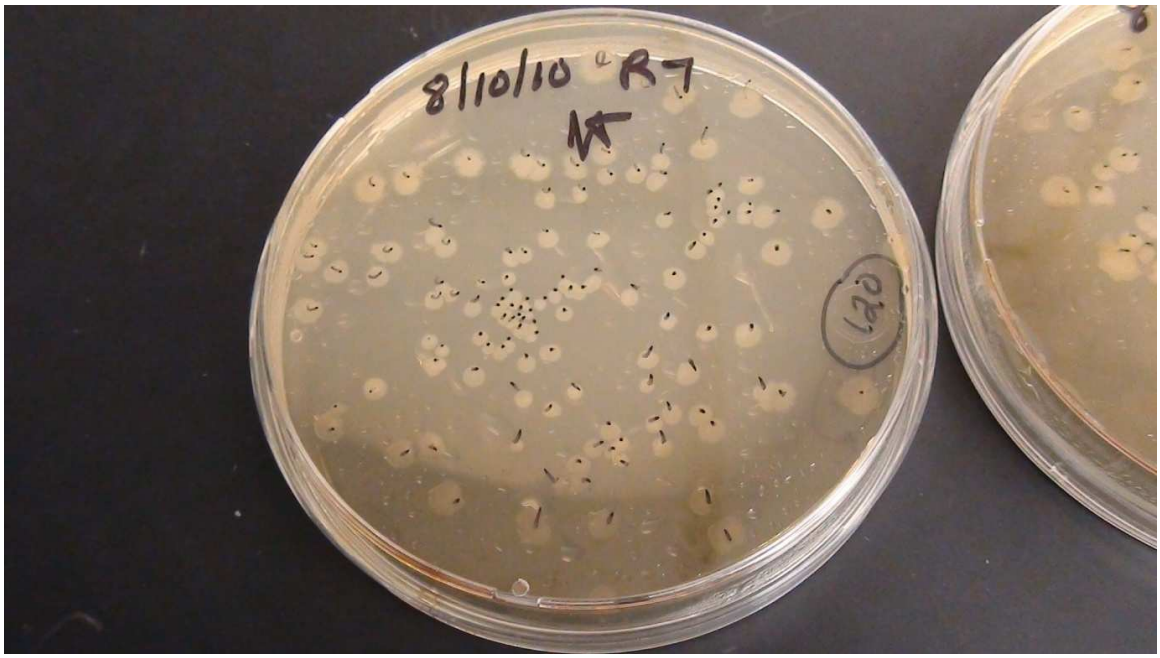

Figure B 54 Plate Sample– 8/10/2010 Run 7

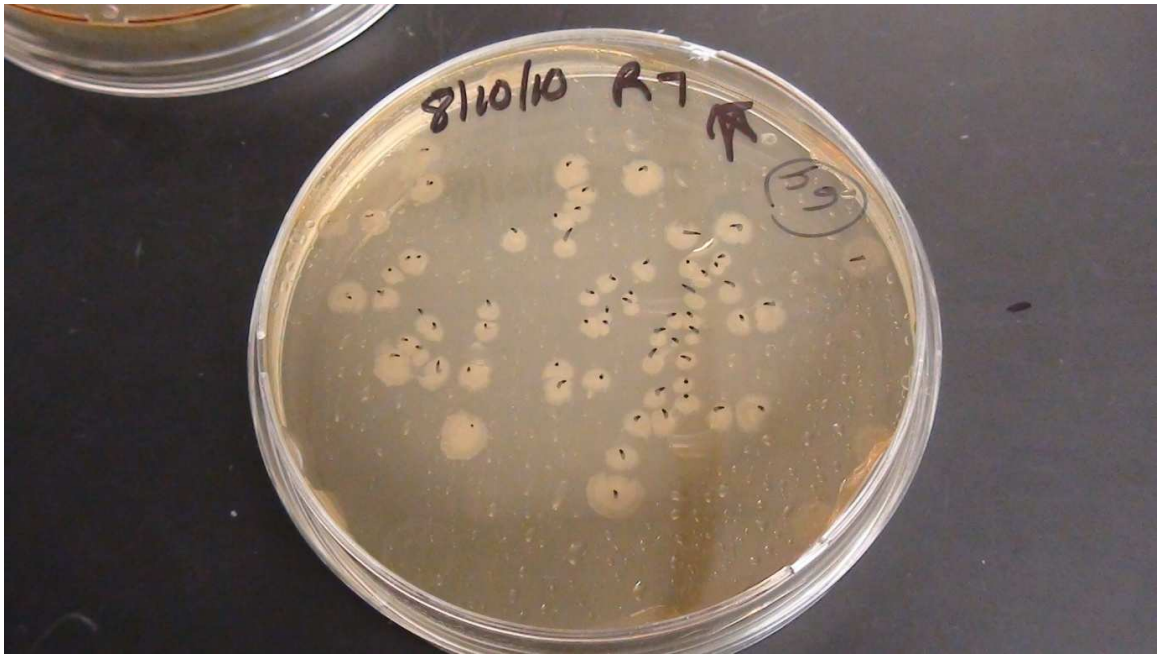

Figure B 55 Plate Sample– 8/10/2010 Run 7

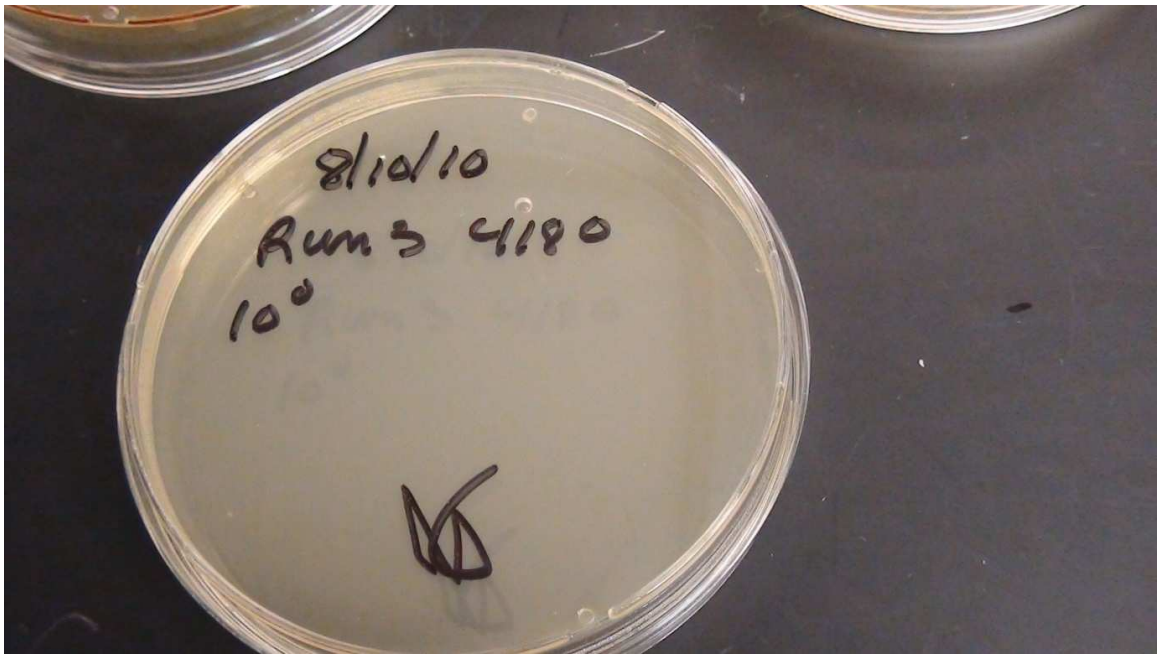

Figure B 56 Plate Sample– 8/10/2010 Run 3

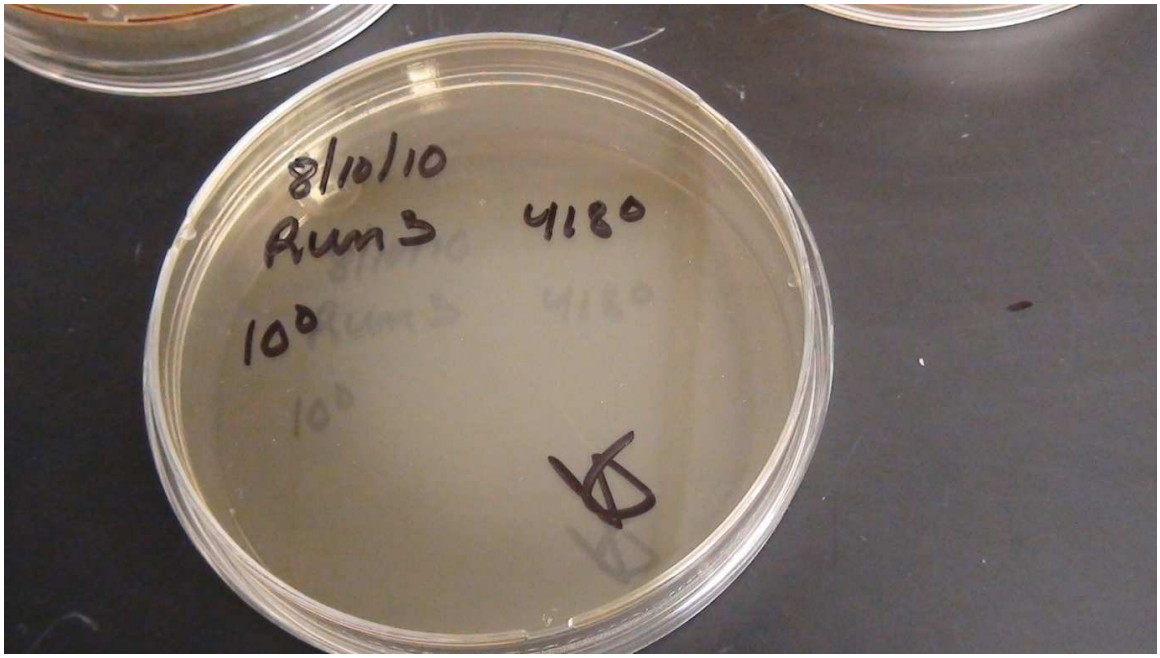

Figure B 57 Plate Sample– 8/10/2010 Run 3

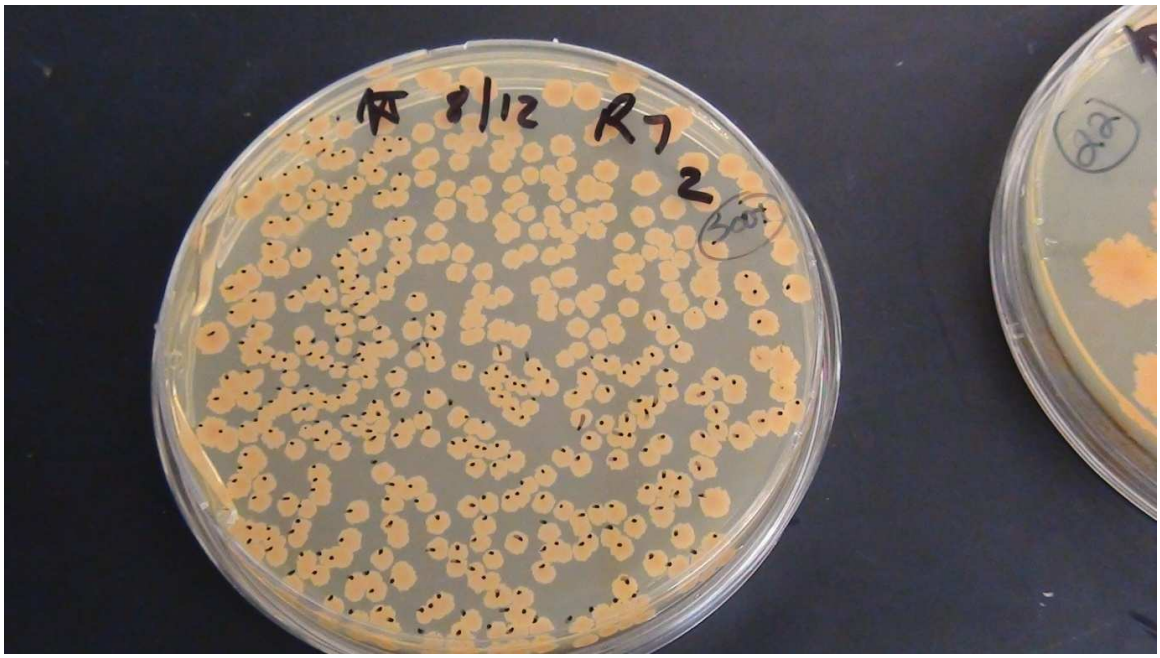

Figure B 58 Plate Sample– 8/12/2010 Run 7

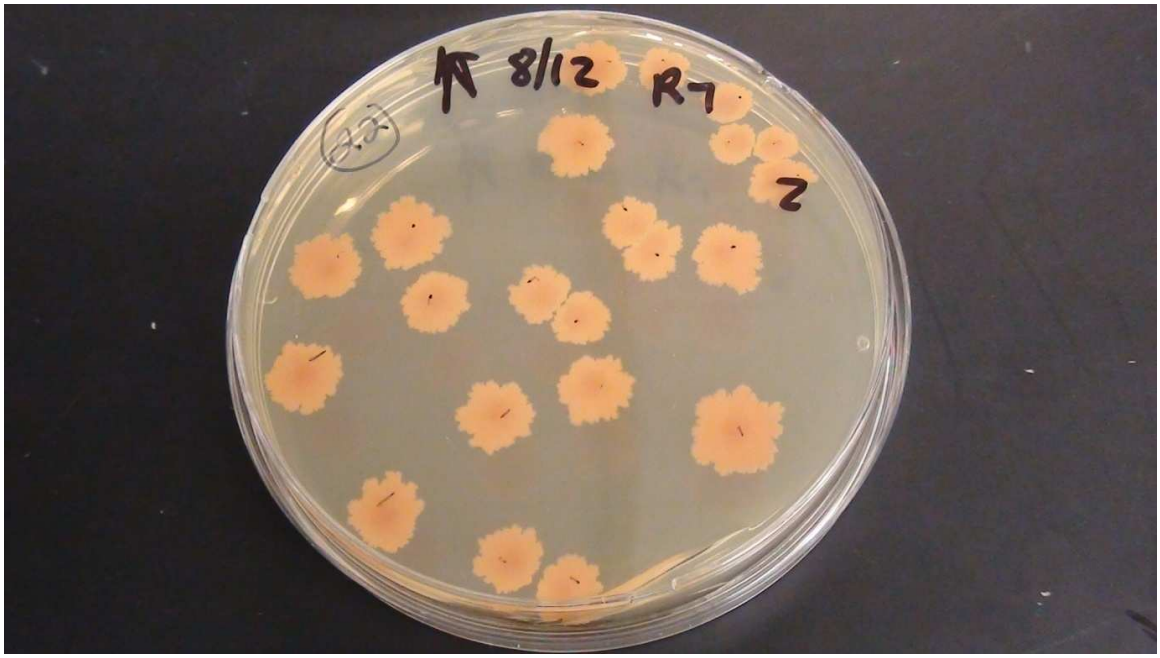

Figure B 59 Plate Sample– 8/12/2010 Run 7

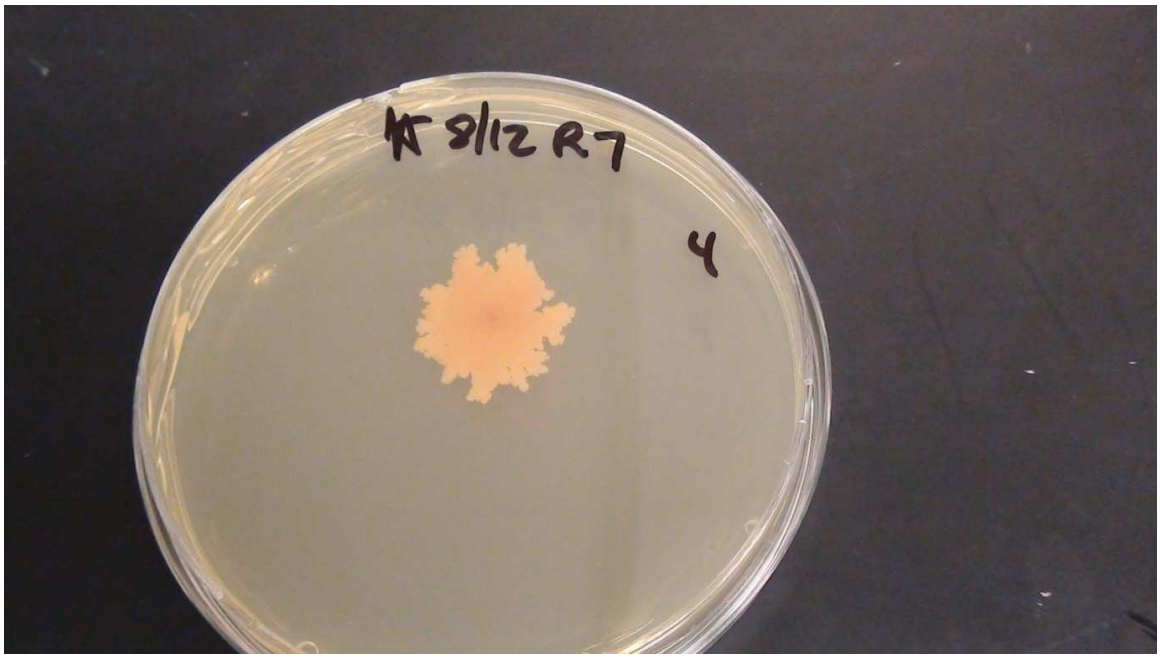

Figure B 60 Plate Sample – 8/12/2010 Run 7

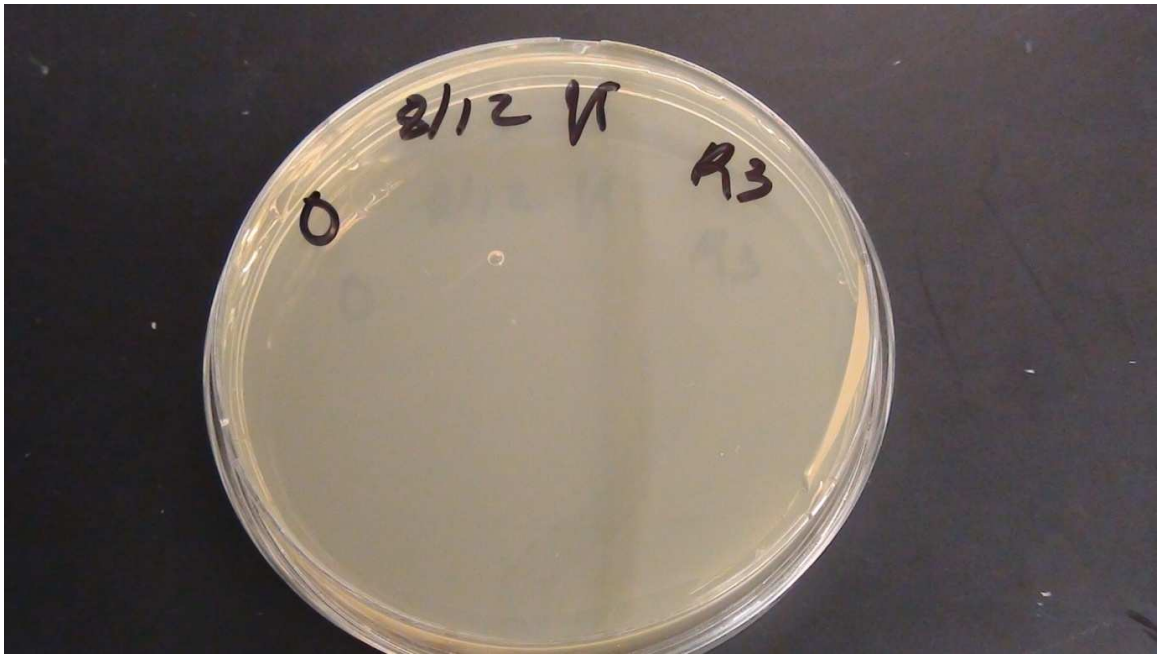

Figure B 61 Plate Sample – 8/12/2010 Run 3

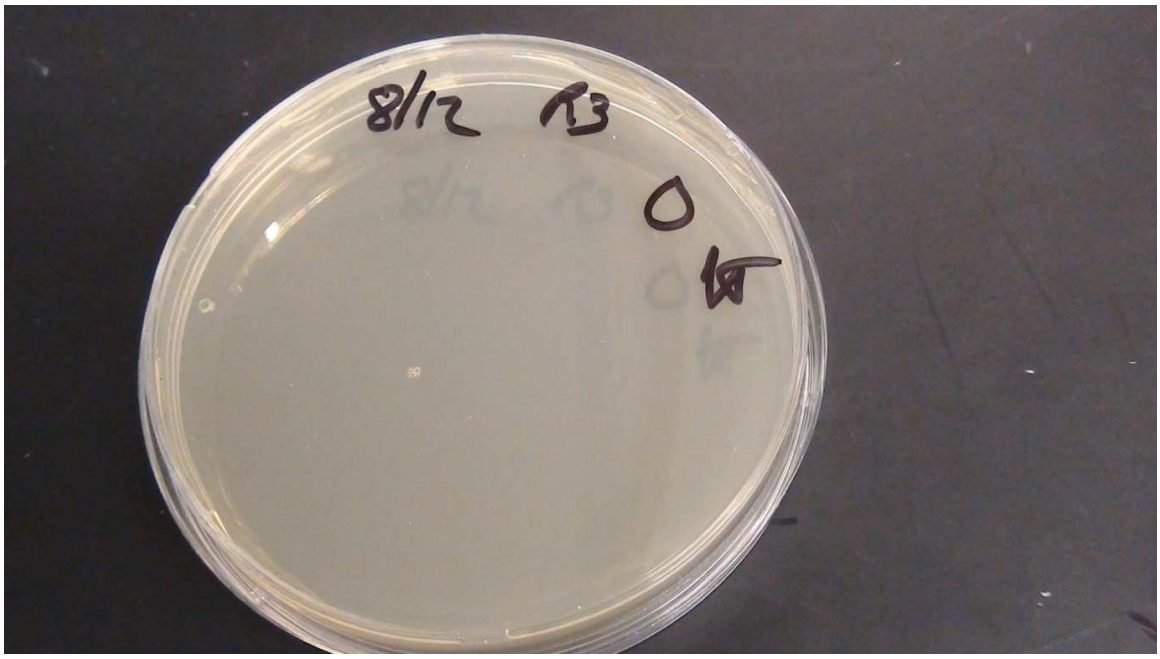

Figure B 62 Plate Sample – 8/12/2010 Run 3

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