

FINAL REPORT

**“LABORATORY SIMULATION OF TRANSPORT AND DILUTION OF
HARMFUL MICROORGANISMS IN THE GREAT LAKES: IMPACT ON
HUMAN HEALTH”**

**PRINCIPAL INVESTIGATORS – A. COTEL AND J. D. SEMRAU
GRADUATE STUDENT RESEARCH ASSISTANT:
B. BATTANI**

**DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING
UNIVERSITY OF MICHIGAN**

SPONSOR:

**GREAT LAKES OFFICE
DEPARTMENT OF ENVIRONMENTAL QUALITY
STATE OF MICHIGAN**

MARCH 31, 2002

1. Introduction

Periodically, in particular during storm events, significant loading to the Great Lakes of harmful microbial organisms from fecal matter can occur from combined sewer overflows (CSO). These potential pathogens can cause a wide variety of diseases and ailments, including gastroenteritis, typhoid fever, cholera and diarrhea. If found in high enough concentrations, the presence of indicator microorganisms can lead to beach closures and an increased risk to human health. In the Great Lakes CSO discharges have been blamed for the beach closures that have occurred annually (up to 80 a year) since 1981 including in Mackinac, Macomb, Sanilac and Wayne counties (EPA, Great Lakes National Program Office). Such closures are seasonal and often occur in the summer months, which corresponds to the strongest use of the beaches around the Great Lakes and therefore provide the highest risk to the human population.

As the detection of all disease-causing agents can be time-consuming and difficult to perform reliably for some pathogens, microbial quality of water supplies, including the Great Lakes is determined by assaying for the presence of fecal coliforms that are relatively easy to detect and whose numbers correspond well with pollution. Furthermore, enterohemorrhagic *Escherichia coli* has become the leading cause of hemorrhagic colitis in the United States and Canada (Maurer, et al., 1999) and such infections have been attributed to exposure to these cells from contaminated water (Geldreich, et al., 1992). Therefore, it is very important to understand the abundance of coliforms in the Great Lakes, particularly from CSOs, and how their viability is affected by physical processes. Specifically, different mixing and dilution regimes may cause substantial and rapid changes in the cell medium (i.e., the mixing of CSO discharges with the Great Lakes) that may induce stress responses in coliforms that ultimately reduce their viability.

The objective of this project was to perform well-defined laboratory experiments to simulate CSO discharges in the Great Lakes, to correlate flow parameters that are important in mixing with the dilution and viability of coliforms as well as identify strategies that may reduce microbial viability and thus infectivity. With these studies, the relative risk and abundance of pathogens and harmful microbial microorganisms from CSO discharges in the Great Lakes can be better determined. Specifically, the fate and transport of coliforms was considered for free-flowing bacteria, in two phases of their growth cycle, exponential and stationary.

2. Literature review

CSOs have substantial impacts on ambient water quality in the United States. Over 900 communities with about 40 million people are served by CSOs, with most found in the Northeast and Great Lakes region, primarily in Ohio, New York, Illinois, Indiana, Pennsylvania, and Michigan (EPA, Office of Wastewater Management). From CSOs, the risk to human health is increased from the discharge of significant

numbers of pathogens in a short period of time and recreational activities curtailed from the closure of beaches throughout the state of Michigan. As mentioned above, coliform counts are commonly used to monitor water quality as their presence correlates well with pollution and is indicative of the presence of pathogens. The viability of such cells, however, may be affected by mixing processes. Specifically, rapid changes in environmental parameters such as pH, temperature, and nutrient sources can induce stress responses in cells that limit their growth and increase mortality (Gottschal, 1990; Chesbro, et al., 1990; Matin, 1990). It may be possible to accelerate cell death by better and more rapid mixing.

The Reynolds number is believed to have a significant effect on turbulent mixing. It is defined to be the ratio of inertial to viscous forces, the higher the value of the Reynolds number, the more turbulent a flow becomes. For water flows, there is a critical Reynolds number above which mixing is increased by orders of magnitude (Broadwell and Breidenthal, 1982), this is called the mixing transition. By improving the mixing, dilution is increased until the Reynolds number reaches a critical value of a few thousands. Above this critical Reynolds number, mixing is not significantly improved by increasing the value of the Reynolds number. Even though the effect of Reynolds number on mixing and dilution is well known, the viability of microorganisms in different turbulent conditions is not.

In these experiments, the combination of growth and non-growth techniques will allow for the better characterization of non-culturable microorganisms that may be undetected using traditional CFU analysis but may recover if environmental conditions change and thus pose a health risk. Recent studies indicate, however, that enteric bacteria such as *E. coli* do not enter a viable but non-culturable state (Bogosian, et al., 1998), rather any inability to culture *E. coli* is simply due to the fact the cells are dead. These studies proposed here will thus not only provide information how different mixing regimes affect cell viability, but can also help determine if such loss of viability is due to cell death or the inability to culture these cells in the laboratory once they enter a different metabolic state.

3. Experimental procedure

In these experiments, the fate and transport of coliforms from CSO discharges into the Great Lakes will be determined through of a laboratory model of a typical discharge from a combined sewer overflow.

For the case of free floating bacteria, the Reynolds number will be varied from a value value of 3,000 to 56,000 by changing flow velocity and/or pipe diameter. These experiments will allow us to determine how eddy size affects microbial dilution and viability. Cell dilution and viability will be assayed using traditional colony-forming units assays.

1. Experimental setup

Flow was delivered from a small plexiglass head tank of varying height. The elevation of the base of the head tank varied from 38.75 cm to 145.42 cm, in 15.24 cm increments, above the base of the experimental reactor, a 225 L plexiglass tank (dimensions 1 m x 0.5 m x 0.5 m). Both the head tank (approximately 20 L) and the reactor (approximately 225 L) were filled with water and treated with 0.01% v/v of a 30% H₂O₂ solution the night before an experiment to disinfect the water. Samples of the treated water were taken immediately prior to an experiment and spread on nutrient agar plates to ensure no microbial activity would be observed as background.

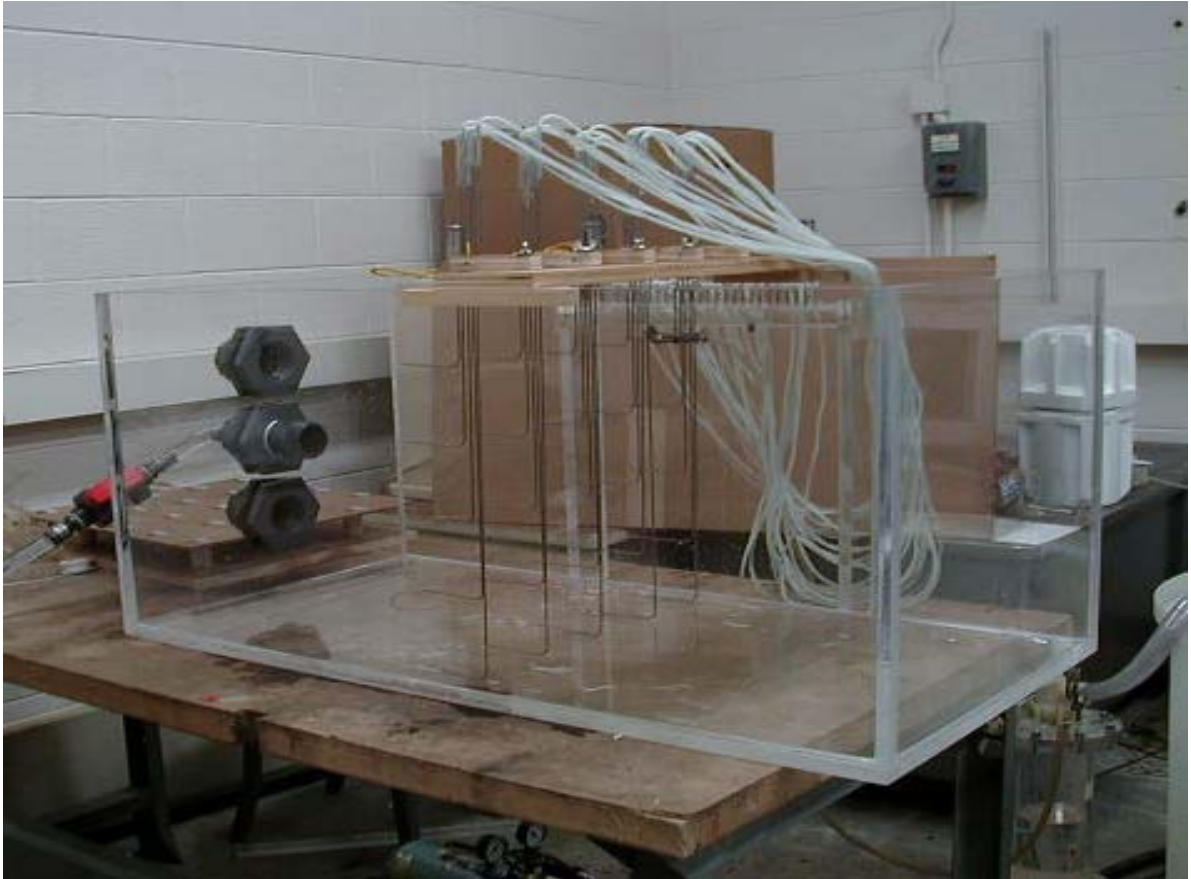


Figure 1: Experimental set-up

For experiments, a 1 L batch of exponential-phase *Escherichia coli* DH5 α or a 100 mL batch of stationary phase DH5 α was added to the head tank along with 1 L of sterile TSB media and allowed 30 minutes for acclimatization. Reference samples of the exponential-phase *Escherichia coli* DH5 α (prior to addition to head tank) and the head tank/*E. coli* mixture after 30 minutes were taken immediately prior to release into the reactor.

Based on the height of the head tank above the reactor and the reactor nozzle size, the microbes were delivered at different rates to the reactor through a 3/4" inner diameter Fisher™ plastic tube at Reynolds numbers ($Re = VD/\nu$) ranging from 3000 to 56000. The Reynolds number is defined in terms of the flow quantities at the nozzle exit, i.e. nozzle exit velocity (V), nozzle diameter (D), and kinematic viscosity (ν) of the injected solution into the tank. Samples were collected at different locations in the reactor and taken back to the laboratory where they were spread on nutrient agar plates and incubated for 48 hr at 30°C.

Coliform preparation and storage

Escherichia coli DH5 α ($\sim 10^7$ CFU/mL) was grown in Trypticase Soy Broth (TSB) media and stored at 30°C. Every seven days, 10 mL would be aseptically transferred to a sterile 1 L solution of TSB agar (autoclaved for 30 minutes at 121°C) to keep the culture fresh.

Instantaneous Sampling of coliform survival

A vacuum pump setup was used to collect samples corresponding to the centerline of the flow and at 10 cm and 20 cm vertical and horizontal from the centerline, according to the following cross-sectional grid pattern:

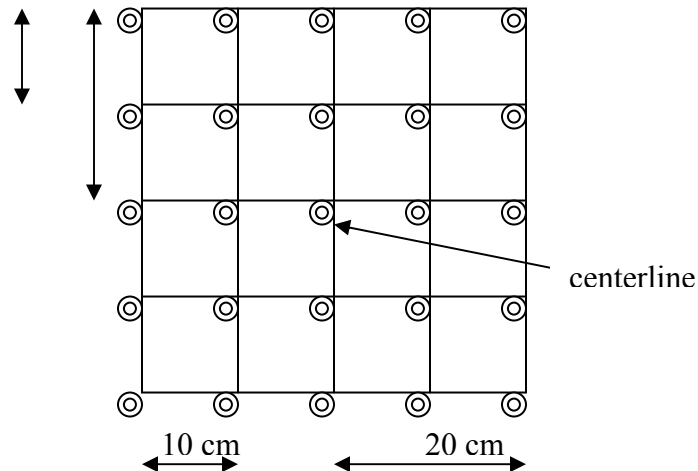


Figure 2: Initial design of the sampling matrix

Samples were drawn from a constant far-field location of 50 cm downstream from the edge of the nozzle.

The first few samples were of little use when it was discovered that the turbulent jet was not expanding laterally to several of the sampling locations. At the highest Reynolds numbers, bacteria were only observed at location C3 (the centerline) and C2 (the location just above the centerline). The sampling matrix was compacted so that distances were only 5 and 10 cm apart instead of 10 and 20, effectively shrinking the sampling area to 1/4 its original size while maintaining the same number and relative location of sampling ports.

A dummy sample was run in the dark, using fluorescein-dyed water and a slide projector to create a sheet of light, to observe the jet expansion and verify that the new sampling locations would indeed be capturing jet fluid and not unmixed ambient fluid. From this experiment came the observation that the extreme sampling points were not capturing any jet fluid and thus would not demonstrate any turbulent mixing of ambient and jet fluid. As such, 12 of the 25 locations were discontinued, and samples were only taken at the centerline, all locations directly surrounding the centerline, and at points 10 cm above, below, to the left, and to the right of the centerline.

The next few experiments provided equally puzzling results, as it was noticed (by the research assistant) that more than one bacteria type was present on the plates that should have contained only *E. coli*. Bacteria with the following characteristics were observed:

1. The round, white, opaque *E. coli*
2. A species that initially looks like *E. coli* but after 24 hours attains a characteristic sharp yellow color
3. A large orange species about twice the size of *E. coli*

Both the orange and yellow unidentified species, hereafter referred to as the “contaminant” species, were present in roughly the same count as *E. coli*. The counts were noted but competition issues may have prevented representation of the actual *E. coli* behavior. After repeating experiments while isolating different components of the setup, the source of the contamination was identified as one of the valves regulating flow between the head tank and the reactor. To address this issue, all valves were soaked in 70% ethanol overnight and all tubes that contained flow were sterilized beforehand.

Redesign of sampling technique - Average concentration measurements of E. coli:

The sampling matrix was further reduced to nine probes linked to three peristaltic pumps that sampled at the jet centerline and five centimeters in each direction. The steel probes were supported by 1/8" food-grade Masterflex® tubing and fed through Cole-Parmer® polycarbonate precision pump heads into test tubes.

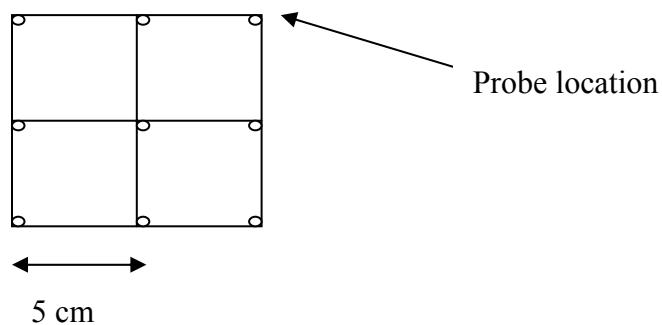


Figure 3: Final design of sampling matrix

2. Experimental techniques

Assay of coliform numbers and viability:

For the laboratory studies, coliforms are used as indicator organisms of the fate and transport of harmful microorganisms in the Great Lakes. The common microbial technique of colony forming unit assay was used to determine the distribution and viability of a group of coliforms under different hydraulic regimes. The cells were grown to the mid-exponential phase on LB medium to insure that viability was not affected by the metabolic state. *E. coli* is directly added to the model CSO discharge at a constant concentration.

Test tubes containing the fluid drawn from each sampling location, along with reference tubes containing fluid from the head tank/*E. coli* mixture and from the reactor (before release of head tank fluid) were immediately diluted and spread onto nutrient agar plates. Plates were incubated for 48 hours at 30°C and colony growth was counted and reported as colony forming units per mL. Dilution factors varied according to growth phase ($\sim 1:10^3$ for exponential, $\sim 1:10^5$ for stationary) and sampling location (centerline concentrations were approximately one to two orders of magnitude higher than outlying points). The dilution amount for each sample was based on the dilution ratio of each sampling point to the reference sample as measured by the Spec 20.

Dilution measurements were performed with a Spec 20 using fluorescein as a passive scalar. For the near field experiments, each sample analyzed for *E. coli* concentration also contained enough fluorescein to obtain dilution measurements. This procedure allowed us to obtain simultaneous measurements of pure dilution and *E. coli* concentration for the exact same conditions.

3. **Hydrodynamic model**

a) Background on turbulent spectrum and the concept of energy cascade

Accurate and fundamental predictions about turbulence were first developed by A. N. Kolmogorov. In a series of papers beginning in 1941 he described a new model for isentropic turbulence in incompressible flow. Following the earlier notions of L. F. Richardson concerning the transport of energy from the largest to the smallest eddies, Kolmogorov showed that the energy spectrum of the turbulent velocity fluctuations should be proportional to the minus five-thirds power of the wavenumber over a certain range of wavenumber. This "inertial subrange" consists of those eddies which are small enough to have forgotten their origin, yet large enough that viscosity has no direct effect on them. It follows that the largest eddies contain the bulk of the kinetic energy of the turbulence and that there must be some smallest eddy at which viscosity directly transforms the kinetic energy into thermal energy. Taking this smallest

(Kolmogorov microscale) eddy to be λ_0 , it must have an eddy Reynolds number of approximately unity,

$$\text{Re} = \frac{\lambda_0 v_{\lambda_0}}{\nu} = 1$$

According to the cascade process, energy is supposed to be transferred from one eddy to a smaller eddy via an inviscid process. Without dissipation in stationary turbulence, the rate of energy transfer from one eddy size to another must be constant. The kinetic energy per unit mass associated with an eddy is v_λ^2 . The time to transfer energy from an eddy of size λ is proportional to its rotation time λ / v_λ . Therefore, in the inertial subrange, the energy flux, or rate at which energy flows down the cascade from larger to smaller eddies is

$$v_\lambda^3 / \lambda = \varepsilon$$

where ε is the energy flux per unit mass down the cascade. Since there is no dissipation in the inertial subrange, the energy flux there is a constant.

Within the inertial subrange, the flux of energy from larger to smaller scales should be self-similar in the limit of large Reynolds number. That is to say, there is no preferred or distinguished eddy size in this range. Only when the Kolmogorov microscale is reached at the bottom end of the inertial subrange is the kinetic energy destroyed.

Eddy lifetimes and vorticity

The rotation period of a turbulent eddy in a self-similar flow must be proportional to its chronological age. There simply is no other time scale available. The rotation period is approximately the inverse of the vorticity. This must be true for both the large scale vortices, as well as smaller ones continually born during the cascade process. Consequently, every turbulent eddy that is self-similar in this way should rotate approximately once in its lifetime.

Two Special Eddy Sizes: The Largest and the Smallest

There are two unique and therefore important eddy sizes, the largest and the smallest. The former has the greatest tangential speed, but ironically the longest rotation period. The rotation period τ_λ of some arbitrary eddy of size λ is

$$\tau_\lambda = \frac{\lambda}{v_\lambda}$$

This equation yields the ratio of the rotation periods of the largest to the smallest eddies to be

$$\frac{\tau_\delta}{\tau_{\lambda_0}} = \text{Re}^{1/2}$$

where δ is the large eddy size.

b) Application to *E. coli* viability

The strain rate associated with each eddy size (σ_λ) will be inversely proportional to the eddy rotation period, or proportional to its vorticity. The strain rate is the important parameter to be tested in this project as it could lead to *E. coli* cells being lysed due to excessive strain on the cells membrane.

Following from the definition of strain rate, the ratio of the strain associated with the smallest eddies (Kolmogorov eddies) to that associated with the largest eddies is:

$$\sigma_{\lambda_0} = \sigma_\delta \text{Re}^{1/2}$$

Where the strain rate is the greatest is the more likely location for *E. coli* cells to be lysed and unable to survive the discharge conditions. The largest strain rate associated with the largest eddy will occur near the nozzle, since by definition,

$$\sigma_\delta = \frac{\Delta U}{\delta}$$

For a fixed ΔU (defined as the velocity difference across an eddy), the strain rate associated with the Kolmogorov eddies will be proportional to $\delta^{-1/2}$. This implies that the highest strain rate will occur in the vicinity of the nozzle. However, to have Kolmogorov eddies present in the turbulence spectrum, the boundary layer needs to roll up, form large vortices, which themselves have to perform 2 to 3 vortex pairings in order to create Kolmogorov eddies. Therefore the optimal distance for highest strain rate will be as close as possible to the nozzle with the presence of Kolmogorov eddies.

According to this model, dilution (reported in terms of fluorescein concentration) and *E. coli* concentration would be different in the near field. The difference between the two concentrations (fluorescein and *E. coli*) would not increase in the far field.

4. Results

The first set of experiments was performed at constant temperature and specific gravity for both the discharge and receiving fluids. Two different locations were tested: five nozzle diameters and 25 nozzle diameters downstream of the discharge point. These experiments were performed for a wide range of the Reynolds number, from 3,000 to 56,000.

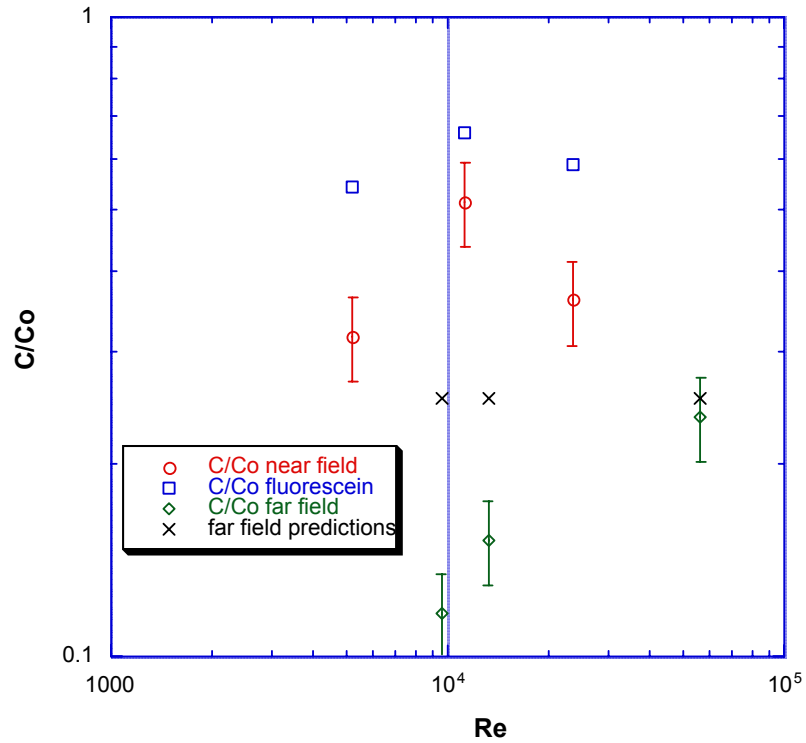


Figure 4: Normalized *E. coli* concentration versus Reynolds number

Figure 4 shows the effect of Reynolds number in terms of dilution and *E. coli* concentration. Each concentration measurement is normalized by the initial discharge fluid concentration, for both fluorescein and *E. coli*. As long as the Reynolds number is above the mixing transition, there is no dependence of Reynolds for the concentration measurements. The difference in concentration between fluorescein and *E. coli* seems to be controlled by the parameter representing the downstream distance, i.e. the number of diameters downstream of the discharge location.

Within the scatter of the data, we can assume that the concentration of fluorescein is constant versus Reynolds in the near field. It was impossible to measure the concentration of fluorescein in the far field because the fluorescein concentration in that case was below detection level.

A standard equation (Fischer et al. 1979) was used to obtain predicted values of dilution:

$$C/C_0 = 5.64Q/(M^{1/2} * Z)$$

where Q is the volumetric flow rate and M is the momentum flow rate, V^2A . This equation predicts the centerline concentration as a function of downstream distance, Z , and is only valid for far field conditions, i.e. Z (downstream location) $\gg D$ (nozzle diameter).

There is a significant amount of scatter in the *E. coli* counts for the far field. Boundary layer behavior at high Reynolds is believed to be responsible for the observed scatter. Because of the geometry of the discharge itself, i.e. similar to a pipe, there is no contraction in the nozzle cross-sectional area which would keep the boundary layer attached to the walls and therefore produce a nice clean flow out of the nozzle. In our case, due to both geometry and high Reynolds effect, there is separation of the boundary and onset of instabilities at the nozzle exit, therefore producing variability in the concentration data.

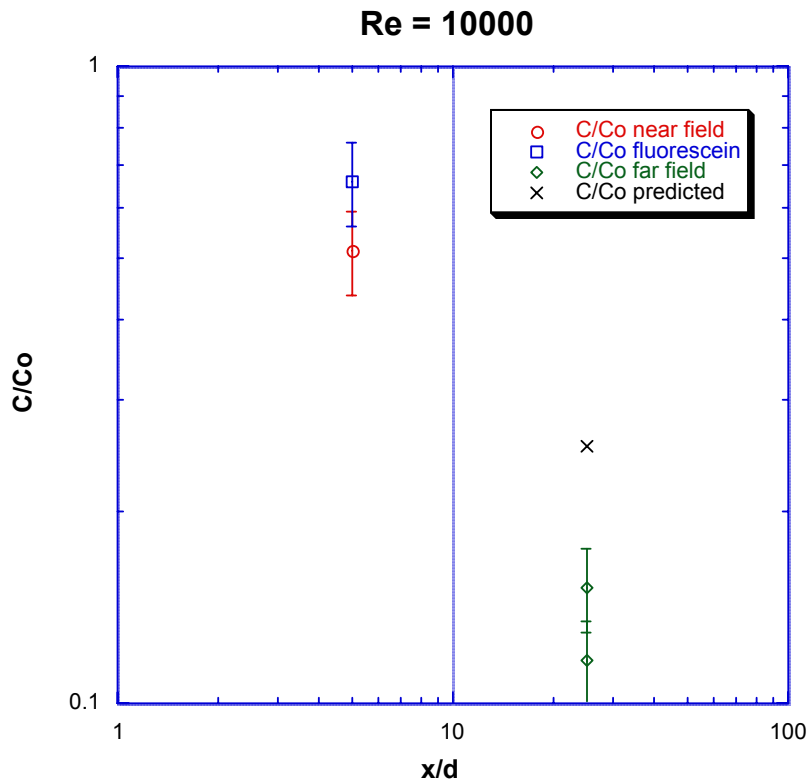
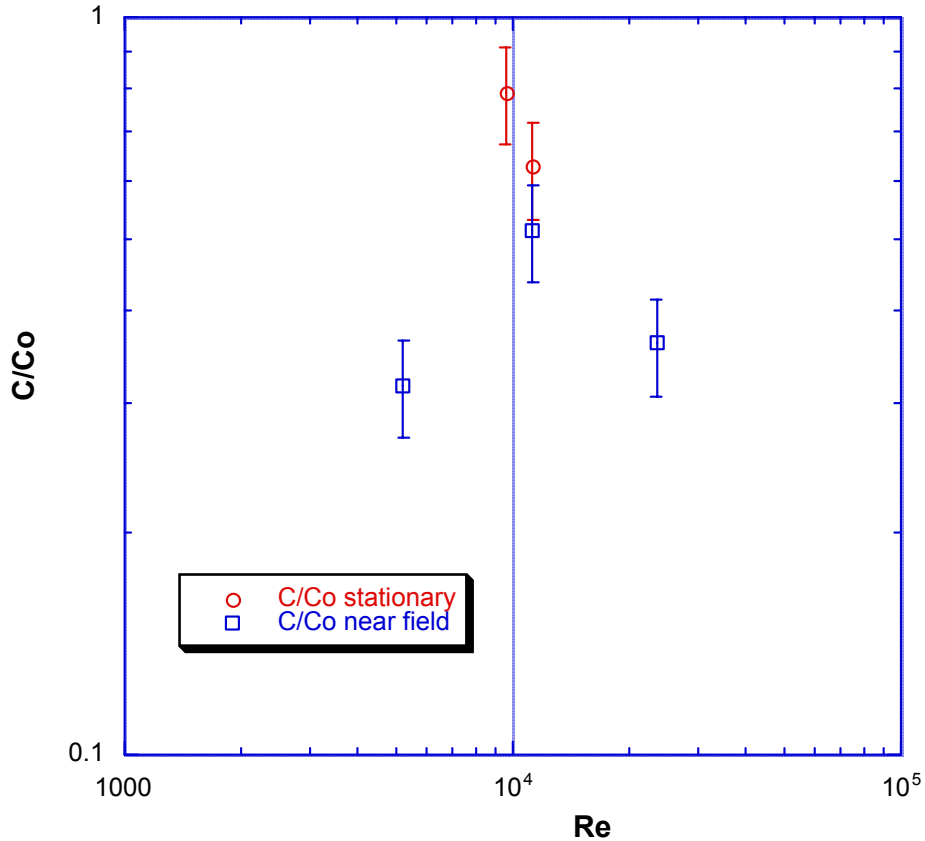


Figure 5: Normalized concentration measurements as a function of downstream distance for a fixed Reynolds number of 10,000.

No significant decrease in the number of *E. coli* colonies is observed in the far field (figure 5). This supports the argument that the decrease in *E. coli* colonies happens in

the near field. These data corroborate the hypothesis that high strain rate is responsible for destroying *E. coli* bacteria in the near field.

For most of the experiments, the *E. coli* bacteria were in the exponential growth (4 hours of incubation at 30°C) phase. Some conditions were repeated to evaluate the effect of growth phase. In those cases, the *E. coli* was incubated for 20 hours to



represent the stationary growth phase.

Figure 6: Exponential versus stationary phase

As expected, *E. coli* in the stationary phase were more resistant to stress than in the exponential phase (figure 6). Because of their suspected added resistance, they will not be so susceptible to the consequences of high strain rate in the near field.

Lastly, the effect of temperature was tested. A couple of experiments were performed with a difference in temperature between receiving and discharge fluid of 10 °C and 20 °C respectively. These experiments were performed in the near field at relatively low Reynolds numbers.

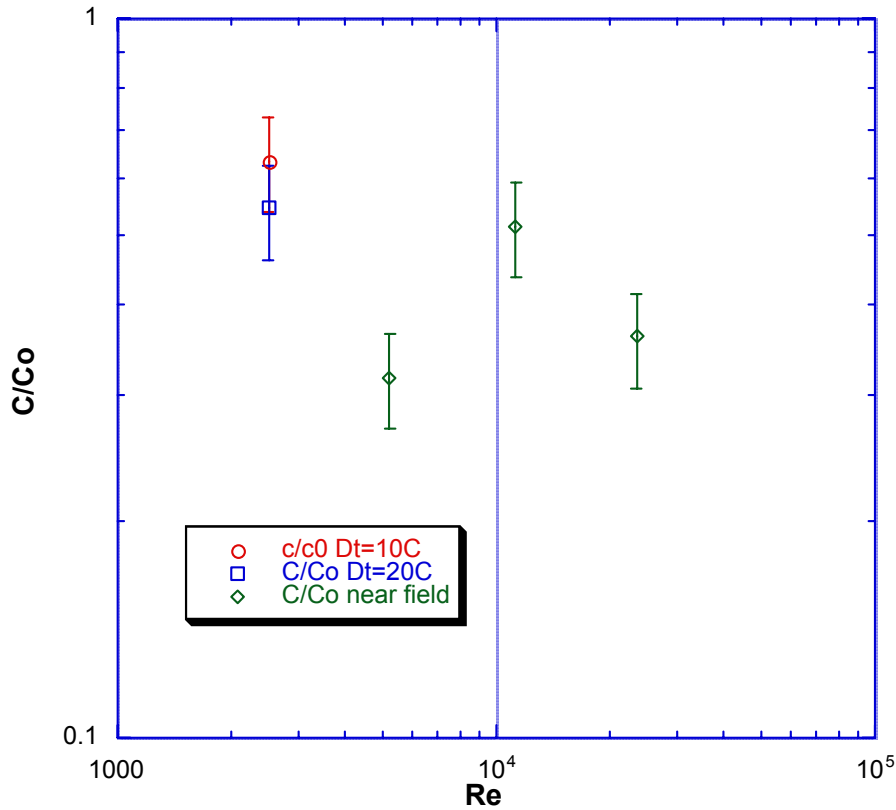


Figure 7: Effect on temperature on *E. coli* concentration

No strong effect of temperature on the viability of *E. coli* was observed (figure 7). The concentration measurements with a temperature difference were within error bars of the measurements performed with the same temperature. It is known that temperature has an effect on cell growth and viability. However, in these experiments, the cells are in contact with the colder environment only a short time before a sample is drawn. Also, the change in temperature was not large enough to produce a large density difference between the jet and receiving water, therefore not affecting the dilution rate.

5. Conclusions

Our measurements show conclusively that there is more to the problem than simple dilution. It is possible to lower *E. coli* concentration with high strain rate associated with the turbulent structure of the flow. This is however only prevalent in the near field, where the highest Kolmogorov strain rate occurs. Once *E. coli* enter the stationary growth phase, they become more resistant to the stress associated with the

turbulent field. Also, there seems to be no stress associated with large temperature difference during the discharge process. Temperature will only have an effect for long contact times.

6. Future work

Now that it is known that the near field of a discharge is a vulnerable location for *E. coli* survival, changes to pipe geometry or flow conditions should be focused on this particular region of a discharge.

One approach would be to add vortex generators at the outlet of the discharge pipe in order to improve near field mixing in the vicinity of the discharge and also increase Kolmogorov strain rate, as well as reducing the minimum downstream distance for the location of highest Kolmogorov strain rate. Devices such as vortex generators have been used in Aerospace industry for a couple of decades to control boundary layer separation and therefore reduce drag on airplane wings. Vortex generators act as disrupters of the flow in the boundary layer. They create vortices that inject momentum into the boundary layer, therefore making the boundary layer turbulent but more resistant to adverse pressure gradient and separation (Anderson, 1991).

The effect of scale should also be investigated, either by building a larger laboratory facility or using the existing wave tank at the University of Michigan to reproduce a larger scale beach environment. Field measurements would also be welcome to test the applicability of the strain rate argument to real environments. Issues regarding background concentration would have to be resolved in these cases.

7. References:

- Anderson, J.D. (1991) Fundamentals of Aerodynamics, Mc Graw-Hill.
- Bogosian, G., Morris, P.J.L, and O'Neil, J.P. (1998) A mixed culture recovery method indicates that enteric bacteria do not enter the viable but non-culturable state, Applied and Environmental Microbiology, vol. 64, pp. 1736-1742.
- Broadwell, J.E. and Breidenthal, R.E. (1982) A simple model of mixing and chemical reaction in a turbulent shear layer, Journal of Fluid Mechanics, vol. 125, pp. 397-410.
- Chesbro, W., Arbidge, M., Eifert, R. (1990) When nutrient limitation places bacteria in the domains of slow growth: metabolic, morphologic and cell cycle behavior, FEMS Microbiology letters, vol. 74, pp. 103-120.
- EPA, Great Lakes National Program Office,
<http://www.epa.gov/glnpo/beach/index.html>.
- EPA, Office of Wastewater Management, <http://www.epa.gov/owm/cso.htm>.
- Geldreich, E.E., Fox, K.R., Goodrich, J.A., Rice, E.W., Clark, R.M., and Swerdlow, D.L. (1992) Searching for a water supply connection in the Cabool,

- Missouri disease outbreak of *Escherichia coli* O157:H7, Water Research, vol. 26, pp. 1127-1137.
- Gottschal, J.C. (1990) Phenotypic response to environmental changes, FEMS Microbiology letters, vol. 74, pp. 93-102.
- Kolmogorov, A.N. (1941) Local structure of turbulence in an incompressible viscous fluid at very high Reynolds numbers, Dokl. Akad. Nauk SSSR, vol. 30, pp. 299.
- Kolmogorov, A.N. (1962) A refinement of the previous hypotheses concerning the local structure of turbulence in a viscous incompressible fluid at high Reynolds number, J. Fluid Mech., vol. 13, pp. 82-85.
- Matin, A. (1990) Molecular analysis of the starvation response in *Escherichia coli*, (1990) FEMS Microbiology letters, vol. 74, pp. 185-196.
- Maurer, J.J., Schmidt, D., Petrosko, P., Sanchez, S., Bolton, L., and Lee, M.D. (1999) Development of primers to O-antigen biosynthesis genes for specific detection of *Escherichia coli* O157 by PCR, Applied and Environmental Microbiology, vol. 65, pp. 2954-2960.
- van Loosdrecht, M.C.M., Lyklema, J., and Norde, W. (1990) Influence of interfaces on microbial activity, Microbiological Reviews vol. 54, pp. 75-87.
- Zhou, J., Bruns, M.A., and Tiedje, J.M. (1996) DNA recovery from soils of diverse composition, Applied and Environmental Microbiology, vol. 62, pp. 316-322.