# End-of-pipe treatment and regrowth of sulfonamide resistant *E. coli* in combined sewer overflow effluent

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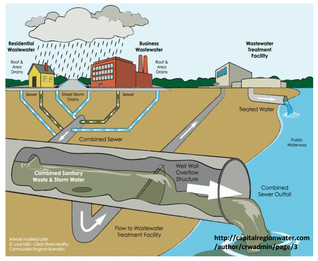
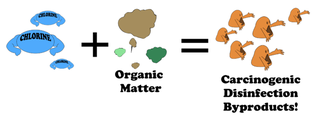
**Combined sewer overflows (CSOs) release microbial pollutants such as pathogens, fecal bacteria and antibiotic resistance genes (ARG) into waterways**.

A combined sewer system collects wastewater and wet weather runoff. A CSO occurs during times of heavy influent volume, like storms, and releases untreated storm runoff and wastewater into the adjacent surface water, degrading the water quality.




Increasing rates of antibiotic resistant infections highlight the need to understand environmental sources of community acquired infections. CSOs have been identified as a potential source for antibiotic resistance genes (ARG)[1]. Upon release into surface water, ARGs can proliferate by host cell growth or horizontal gene transfer in the environment.

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This study assesses the effectiveness of a two-step treatment using hydrodynamic separation and peracetic acid (PAA) for rapid treatment of indicator organisms, total coliform, and *E. coli* and compares it to a rapid PAA disinfection only treatment. The presence of a sulfonamide resistance gene carried by *E. coli* was also monitored during treatment to address the current gap in knowledge about whether PAA selects for ARG-carrying cells. An advantage of PAA over traditional chlorine disinfection is that there is no evidence of disinfection byproducts when reacting with the organic matter that is present in CSO effluent. PAA decomposes into acetic acid, hydrogen peroxide, oxygen, and water over time, which are essentially harmless products[2].

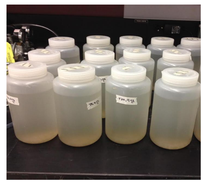
## Objectives:

1. Assess the effectiveness of a two-step process using hydrodynamic separation and rapid PAA disinfection and compare it to the effectiveness of rapid PAA disinfection alone at treating cultivable fecal bacteria, including those carrying ARG.

2. Measure regrowth potential of the above mentioned indicator organisms in surface water

## Treatment train

**1.**

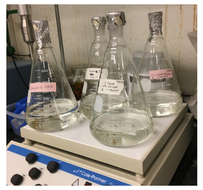


CSO effluent simulated using 40% wastewater in deionized sterile water

**2.**


Suspended cells were separated from those attached to particles simulating hydrodynamic separation of settleable particles[3]

**3.**

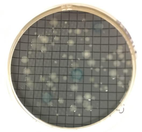


Disinfection with 5mg/L PAA for five minutes; seawater was spiked with post-disinfection subsample (1:10, v:v) and incubated for 7 days

**4.**

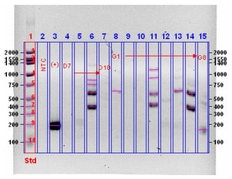
Fecal coliform and *E.coli* were analyzed using membrane filtration and MI agar according to EPA Method 1604[4]

**5.**



EPA Method 1604 used to measure colony forming units (CFU) of total coliform and *E.coli*

**6.**




PCR for *sul1* ARG on 10 colonies from each plated condition[6]

## Residual PAA after 5 minutes of treatment

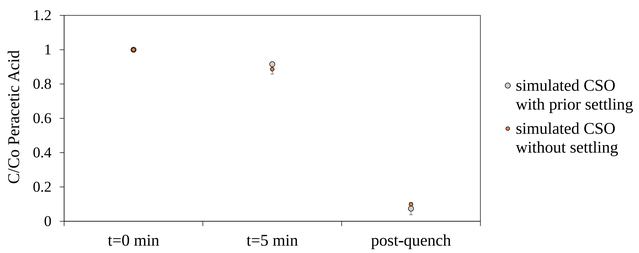



**Figure 1** PAA concentration remaining (C) compared to initial PAA concentration ($C_0$) initially (t=0min), after during 5 minutes of disinfection with 5 mg/L nominal PAA concentration (t=5min), and after quenching the disinfection reaction (post-quench).[7]

## End-of-pipe treatment removes cultivable indicator organisms from CSO

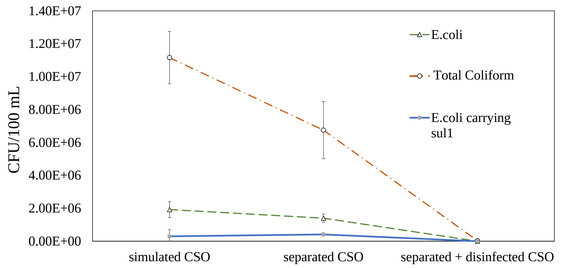



**Figure 2.** *E. coli* and total coliform concentrations in the source waste stream (simulated CSO), after removing settleable particles (separated CSO), and after disinfection (separated + disinfected CSO).[7]

## Total coliform and *E. coli* regrowth not observed in sea water

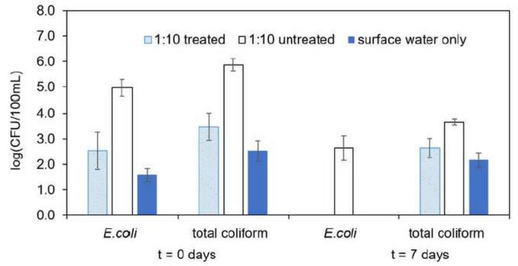



**Figure 3.** Total coliform concentrations in reactors with surface water spiked with a 1:10 dilution (v:v) of treated and untreated simulated CSO in surface water and surface water only controls.[7]

## End-of-pipe treatment selects for ARG

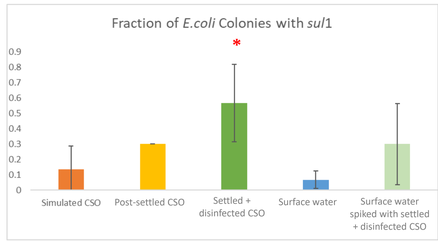



**Figure 4.** Fractions of *E.coli* colonies containing *sul*1 ARG in each sample condition following PAA treatment (n=10).[7]
*Post-disinfection < simulated CSO, p = 0.002

## Discussion

Hydrodynamic separation did not significantly reduce *E. coli* or TC concentrations (p>0.95) while PAA treatment, both post-separation and without separation, did significantly reduce *E. coli* and TC ($p < 5.7x10^{-10}$ for both). However, hydrodynamic separation did significantly reduce total suspended solids ($p=2.1x10^{-3}$), although this did not significantly impact PAA demand (p=1.0). Our lab previously demonstrated that hydrodynamic separation could remove 0.5 -0.9 log of *sul*1 ARG in actual CSO effluent[5] and that PAA was effective at disrupting the membrane of cells carrying *sul*1 ARG with applied doses as low as 5 mg-L/min but was not effective at destroying this harmful DNA.[1] Therefore, there is evidence that both treatment steps tested are helpful in reducing the hazard posed by ARG in CSO effluent. The potential for regrowth of fecal indicators in surface water after a seven day incubation period was found to be significantly higher ($p<1.9x10^{-7}$) in reactors spiked with untreated effluent compared to the other two reactors.

Further studies should be conducted to assess if PAA preferentially selects for other ARG-containing cells, as only one ARG was assessed in this study. A more comprehensive assessment could better inform treatment options.

## Acknowledgements & References

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(2) Kitis, M. (2004) *Environ. Int.* (1), 47-55.
(3) Krometis et al. (2007). *Water Research* 41(2), 506-516.
(4) United States Environmental Protection Agency (2002). *Office of Water*. EPA-821-R-02-024.
(5) Eramo et al. (2017b.) *Frontiers in Microbiology*
(6) Pei et. al. (2006). *Water Research* 40, 2426-2435.
(7) Eramo et al. (accepted) *ASCE Journal of Environmental Engineering.*