

Effect of distribution system materials and water quality on heterotrophic plate counts and biofilm proliferation

Michael Le Puil¹, Young C. Chang¹, Andrew A. Randall.^{1*}, and James S. Taylor¹

¹Civil and Environmental Engineering Department, University of Central Florida, P.O. Box 162450, Orlando, FL 32826-2450

ABSTRACT

Biofilms on pipe walls in water distribution systems are of interest since they can lead to chlorine demand, coliform growth, pipe corrosion and water taste and odor problems. The study described in this paper is part of an AWWARF and Tampa Bay Water tailored collaboration project to determine the effect of blending different source waters on distribution systems water quality. This project is based on 18 independent pilot distribution systems (PDS), each being fed by a different water blend (7 finished waters blended in different proportions). The source waters being compared include groundwater, surface water and brackish water. These are treated in a variety of pilot distribution systems including reverse osmosis (RO) (desalination), both membrane and chemical softening and ozonation – biological activated carbon (BAC for a total of 7 different finished waters. The observations from this study have consistently demonstrated that unlined ductile iron was more heavily colonized by biomass than galvanized steel, lined ductile iron and PVC (in that order) and that fixed biomass accumulation was more influenced by the nature of the supporting material than by the water quality (including secondary residual levels). However bulk liquid water cultivable bacterial counts (i.e. heterotrophic plate counts or HPCs) did not increase with greater biofilm accumulation but results to date suggest high HPCs correspond with low disinfectant residual more than high biofilm inventory. Temperature affected biofilms also, and AOC was important when residual was between 0.6 and 2.0 mg Cl₂/l. An additional aspect of the work is that the potential of exoproteolytic activity (PEPA) technique was used along with a traditional so-called destructive technique in which the biofilm was scrapped off from the coupons surface, resuspended, and cultivated on R2A agar. Both techniques gave similar trends and relative comparisons among PDSs but culturable biofilm values were several orders of magnitude lower than PEPA values.

Key words: pipe material, biofilm biomass quantification, drinking water, PEPA.

*Corresponding author:

Tel: +1-407-823-6429; Fax: +1-407-823-3315; e-mail: randall@mail.ucf.edu

INTRODUCTION

One of the major concerns of drinking water producers is to provide bacteriologically safe water to the public. Controlling bacterial growth in North America largely depends upon maintaining disinfectant residual throughout the distribution systems. The disinfectant role is to provide residual in distribution systems to control microbiological degradation of water quality and protect against possible contamination. The biofilm can harbor coliform organisms, as well as viruses and opportunistic pathogens like *Cryptosporidium* oocysts (Quignon, *et al.*, 1997). A high bacterial population in potable water can be associated with an increased possibility of waterborne disease, taste and odor problems, corrosion and the need to maintain a higher disinfectant residual (Rittman and Huck, 1989). Moreover coliform bacteria have been associated with high abundance of heterotrophic bacteria and biofilm, increasing the potential for health risk (Goshko *et al.*, 1983). It is also thought that suspended cells in the liquid phase originate from the biofilm through detachment processes during the distribution of drinking water and that control of suspended cell concentrations is assisted by minimizing biofilm cells. However biofilm associated microorganisms have been shown to be less susceptible to disinfectants than their planktonic counterparts (Costerton *et al.*, 1987; Brown and Gilbert, 1993). The increasing use of monochloramines in North America, in order to prevent production of harmful disinfection by-products is an interesting alternative to free chlorine to control bacterial growth since monochloramines are thought to penetrate deeper into a biofilm matrix (LeChevallier *et al.*, 1990).

In the past, controlling bacterial regrowth in distribution systems has focused on limiting nutrient levels (AOC and BDOC) and use of secondary residual (free and combined chlorine). The effect of distribution system materials on heterotrophic plate counts and biofilm proliferation has been more extensively studied during the past decade. Recent evidence suggests that biofilm growth is closely associated with corrosion of pipe materials, through increase of disinfectant demand by corrosion products. Consequently the corrosion potential of pipe materials influences bacterial regrowth in distribution systems and ultimately downstream water quality (LeChevallier *et al.*, 1993). Recent study (Niquette *et al.*, 2000) also shows that densities of fixed bacterial biomass were dependent upon pipe material and that gray iron supported more biofilm cells than

plastic-based materials in different oligotrophic drinking water environments. It was concluded that bacteria fixed on gray iron appeared to be more protected from the chlorine residual in the water than those fixed on non-corroding materials, implying that pipe reactivity is an important promoting factor of bacterial regrowth in the distribution system. Therefore pipe material seems to have a strong influence on bacterial regrowth.

This paper investigates the inter-relationships of HPC and biofilm proliferation.

MATERIAL AND METHODS

Pilot Distribution Systems

This study was part of an AWWARF and Tampa Bay Water tailored collaboration project to determine the effect of blending different treated source waters on the water quality of distribution systems. The project analysis was carried out at the University of Central Florida, Civil and Environmental Engineering Department. There were 18 independent pilot distribution systems (PDSs), each being fed by a different water blend (7 finished waters blended in different proportions). The source waters being compared include groundwater, surface water and brackish water. These were treated in a variety of pilot distribution systems including RO (desalination), both membrane and chemical softening and ozonation – BAC for a total of 7 different finished waters. The pilot distribution system (PDS) lines were constructed from actual member governments distribution systems. The first fourteen lines (PDS 1-14) were made of a combination of PVC, lined ductile iron, unlined cast iron and galvanized steel pipe sections attached in series in that order (upstream to downstream) and are referred to as “hybrid” lines. The four remaining lines (PDS 15-18) were made of a single material (unlined ductile iron for PDS 15, lined ductile iron for PDS 16, PVC for PDS 17 and galvanized steel for PDS 18) and are referred to as “single-material” lines. Each PDS had a hydraulic retention time of about 5 days. Chloramines were used for both primary disinfection and as secondary residual in the PDSs.

Incubation conditions

Coupons cut from existing pipes excavated from the ground (PVC, lined and unlined ductile iron, and galvanized steel, all from utilities in the Tampa Bay area) were incubated in these lines for a 3- month period (corresponding to a single operational phase of the project). Coupons from each material under study were incubated in the “hybrid” lines, while in the “single-material” lines coupons from the same material as the line were incubated. Coupons, which were taken from existing pipes were sometimes extensively corroded and were referred to as “aged” coupons. Coupons were always in duplicate. Duplicate coupons for both assays (HPC and PEPA) were provided. Each was 3.0 cm in diameter and affixed to a peg made from a PVC welding rod. The coupons were then placed inside of a cradle consisting of a 3-inch diameter PVC pipe cut lengthwise. The cradles had holes for the coupon pegs and were placed inside of a 4-inch diameter PVC pipe connected to the end of each PDS for incubation of the coupons. Before being placed inside of the cradles, the outer side of the coupons was taped with 1 inch wide Teflon tape in order to only expose the inner surface of the coupons to the water. The operation of the pipe cradle was similar to that of the PDSs. Specifically it normally operated at a flow of 4.7 gpd and was flushed at 1 fps once a week.

Sampling conditions

The coupon holder was carefully removed from the cradles, with the coupons still affixed and then transported inside of a closed PVC container. The humidity in the container was elevated by placing a wet sponge inside, in order to prevent desiccation of the biofilms. Upon arrival in the laboratory the coupons were analyzed during the next 24 hours.

HPC enumeration of the biofilm

The coupons were rinsed very carefully with Phosphate Buffer Solution (PBS) twice. The biofilms were detached from coupons manually using a sterile weighing

spatula (sterilized by 70% Ethanol) into 4-ml of sterile PBS, and then homogenized using a tissue blender (Tissue Tearor™, Biospec products, Inc., Racine, WI, USA) at 3000 rpm for 2 min. The sample was then serially diluted and two plates per dilution were spread on R2A agar plate (ref. 1826-17-1, Difco Laboratories, Detroit, MI, USA) and incubated for 7 days at 25°C. Typical dilution used were 10^{-3} and 10^{-4} . Finally, after incubation and enumeration, the results were expressed as cfu/cm² by taking into account the dilutions used and the surface area of the scraped coupons. Results obtained with this technique are further referred to as BFHPCs for Biofilm HPCs. This spread plate technique on R2A agar is outlined in Standard Method 9215C (APHA, 1995).

Potential of exoproteolytic activity assay

The protocol used was similar to the one described by Laurent and Servais (1995), except that it was slightly modified to use much less expensive batch reactors for the biofilm coupons. This facilitated high sample volumes at a reasonable cost. The batch reactors were cylindrical PVC cups made from 1 ¼ inch schedule 40 PVC pipe and a flat 1 ¼ inch PVC cap for the bottom. The height of the cup was about 3 to 4 inch. Upon arrival in the laboratory, the coupons were detached from the coupon holder with caution. New Teflon tape was affixed on the used Teflon tape to prevent any interactions between the bacterial biomass deposited on the outer part of the coupon and the reagent. To avoid dessication, coupons were analyzed as soon as possible. Both duplicate coupons were then placed in autoclaved PVC containers covered by aluminium foil. When the experiment was ready to be carried out, 8 ml of solution of non-fluorescent L-Leucyl-β-Naphtylamide (LL-βN, Sigma, St Louis, MO, USA) at saturating concentration of 1mM was poured into the container to submerge the coupon. The concentration was that used by Somville and Billen, 1983. At this point the enzymatic hydrolysis reaction that releases the fluorochrome β-Naphtylamine from the LL-βN molecule occurred due to the action of bacterial exoenzymes of the coupon biofilm. From this point and at 10-min. intervals, 2ml of subsample was removed and the associated fluorescence was measured at 410 nm under 340 nm excitation. Then the 2ml was returned to the PVC container.

A standard curve correlating fluorescence intensity to β N concentration was required for each set of samples. After conversion using the standard curve, β N concentration was plotted against time and the slope, i.e. the β N concentration increase (nmol of β N produced per min. and per cm^2) was obtained by simple linear regression on the linear portion of the response curve. Then it was converted into biomass expressed in $\mu\text{g C}/\text{cm}^2$ by multiplying it by 6.57 (reciprocal of the slope of the correlation straight line established by Laurent and Servais, 1995). Finally this was converted into cells/ cm^2 by considering an average carbon content of 20×10^{-15} g C/cell (Laurent and Servais, 1995).

Water quality analyses

Most water quality analyses for the bulk liquid were carried out with respect to Standard Methods (APHA, 1995). Residual concentration of free and total chlorine were measured on site with a portable spectrophotometer (Hach 46700; precision ± 0.03 mg Cl_2/l) and recommended reagents (Hach No. 21055-69, N,N-diethyl-*p*-phenylenediamine (DPD); Hach No. 21056-69, DPD and potassium iodide).

The sampling bottles and 40-mL vials for AOC analysis were muffled at 525°C for 5 h after cleaning. AOC was measured using the rapid method of LeChevallier et al. (1993), except that plate counts were used to enumerate bacteria rather than ATP fluorescence, in conjunction with Standard Methods 9217 (1995) and the method of van der Kooij (1982).

RESULTS AND DISCUSSION

The results presented summarize four operational phases of the TBW-AWWARF project, i.e. twelve months of study.

The observations from this study have consistently demonstrated that unlined ductile iron was more heavily colonized by biomass than galvanized steel, lined ductile iron and PVC (in that order). Figure 1 presents a representative example of this observation, for the second phase of the study, for the hybrid lines. In the hybrid data the secondary residual was equal for all four coupons in a single hybrid PDS. Thus in this case it can be seen that cast iron had a higher biomass inventory (as much as one order of

magnitude) than PVC, even when residual was equal. Holden *et al.* (1995) documented similar results, comparing biofilm inventories on cast iron and medium-density polyethylene (MDPE). It can be noted that the hybrid lines were fed with different blends. This did not seem to have an effect on the observed trend. Therefore fixed biomass accumulation was more influenced by the nature of the supporting material than by the water quality. Figure 2 presents the combined results of the four phases of the study for the pure lines (fed by same blend but consisting of different single materials). The trend in Figure 2 is the same (e.g. PVC lowest, CI greatest) if average values are compared, even though combined residual levels were different from one line to the next. The fixed biomass inventory was fairly insensitive to residual levels, which were much lower for unlined metals. In contrast bulk liquid bacterial counts (i.e. heterotrophic plate counts or HPCs) varied inversely with disinfectant residual (Figure 3). The highest HPCs were observed in the unlined cast iron and galvanized steel lines, which had very low residuals. Analysis of both hybrid and pure PDS data throughout the length of the study showed that combined residual had a significant effect on bulk liquid HPCs, particularly when levels were below 0.6mg Cl₂/l (Figure 4). HPCs above 100,000 cfu/ml were not seen if residuals were above 0.6 mg/l (chloramines were being used), but very low counts (<100 cfu/ml) were sometimes seen in systems with negligible residual. Lower counts were consistently seen with residuals in excess of 0.6 mg/l, however. Below residual levels of 0.6 mg Cl₂/l HPCs were high (10⁵ or greater being common) and this seemed to be true regardless of AOC concentrations or stability. However when residuals were between 0.6 and 2.0 mg Cl₂/l HPC counts correlated directly with AOC stability (and more loosely with AOC concentration). Waters showing changes in AOC levels (between influent and effluent) greater than 37 µg C/l tended to have elevated HPC counts (>10⁴ cfu/ml; Figure 5). The data was ambiguous above 2.0 mg Cl₂/l of residual with respect to AOC since there was not a broad range of influent AOC values in that data set, and none above 109 µg C/l. AOC and HPCs seemed stable with residual above 2.0 mg Cl₂/l, but whether that would have been true for a higher AOC finished water could not be evaluated. With biofilm inventory there was no significant difference in the hybrid lines even though HPCs varied from 100 to 100,000 cfu/ml. There did appear to be a correlation in the pure lines, but because of the confounding effect of material and

residual concentration this correlation could not be exclusively attributed to either residual or material. Low residuals increased the probability of high HPC counts significantly, while the effect of material on HPC counts was inconclusive. And probably more a result of residual consumption associated with the material.

The effect of temperature was also investigated. It was observed that fixed biomass was sensitive to increase of temperature (Figure 6). An increase of 5°C (from 18 to 23°C) led to increases of about one order of magnitude in biofilm inventory while increasing from 23 to 26°C multiplied the fixed cell densities by a factor of 5 to 10. The effect of temperature on HPCs was inconclusive, even though depletion of residual was observed at higher temperatures. No straightforward correlation was found connecting temperature and HPCs. However the impact of higher temperatures driving increased consumption of combined residual levels affected HPCs in a very significant way.

An additional aspect of the work to date is that in addition to the PEPA technique the biofilm was scrapped off from an identical coupon surface for the pure material lines, resuspended, and cultivated on R2A agar (BFHPC). Figure 7 (second phase, representative of the other phases) shows that the BFHPC technique yielded much lower estimates of the biofilm inventory on the coupons (2 to 4 orders of magnitude lower than the PEPA results). However the trend observed for the effect of material on biofilm accumulation, e.g. that cast iron was more heavily colonized than PVC, was the same using both techniques. This strengthens the conclusion that material was a major factor in determining the biofilm inventory.

Biofilm bacteria are thought to be more resistant to disinfectant due to a physiological state associated with low growth rates (Brown and Gilbert, 1993) or due to increased diffusional resistance created by the extracellular polymers (Costerton *et al.*, 1987). Works by De Beer *et al.* (1994) using chlorine sensitive microelectrodes demonstrated that chlorine did not fully penetrate into the biofilm matrix because chlorine reacted with the biofilm constituents faster than it could diffuse into the biofilm. This phenomenon has also been documented by more recent studies (Stewart and Raquepas, 1995; Chen and Stewart, 1996). This resistance to disinfectant levels can explain the insensitivity of biofilm inventory to combined residual observed in this study.

In terms of bulk liquid HPCs, the effect of materials was most significant in terms of material impact on residual levels. Unlined metals depleted residual levels and this resulted in elevated HPCs. Any intrinsic effect of material was of a much smaller magnitude than residual effect and was not observable if present.

CONCLUSIONS

The major findings of the experiments discussed herein are summarized below:

- Biofilm inventory was a function of the material and was relatively insensitive to secondary residual levels or to variations in water quality.
- HPC counts could not be directly correlated to biofilm inventory. HPCs were most significantly impacted by residual. As secondary residual decreased from 0.6 to 0 mg Cl₂/l the probability of high HPC counts (>100,000 cfu/ml) increased very significantly.
- AOC stability affected HPC proliferation when residuals were between 0.6 and 2.0 mg Cl₂/l.
- Unlined metals and higher temperature (summer time) resulted in significantly greater residual consumption, and this increased the probability of high HPC counts if residual consumption was nearly complete.

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Figures Legends

Figure 1. PEPA End of Phase 2 (sort by blends).

Figure 2. LogPEPA and Effluent combined residual vs material – pure lines only.

Figure 3. LogHPC and Effluent combined residual vs material – pure lines only.

Figure 4. HPC effluent vs Effluent combined residual.

Figure 5. HPC effluent vs Absolute value of Delta AOC.

Figure 6. PEPA vs Average temperature (influent/effluent)

Figure 7. PEPA and BFHPC vs material – pure lines only.

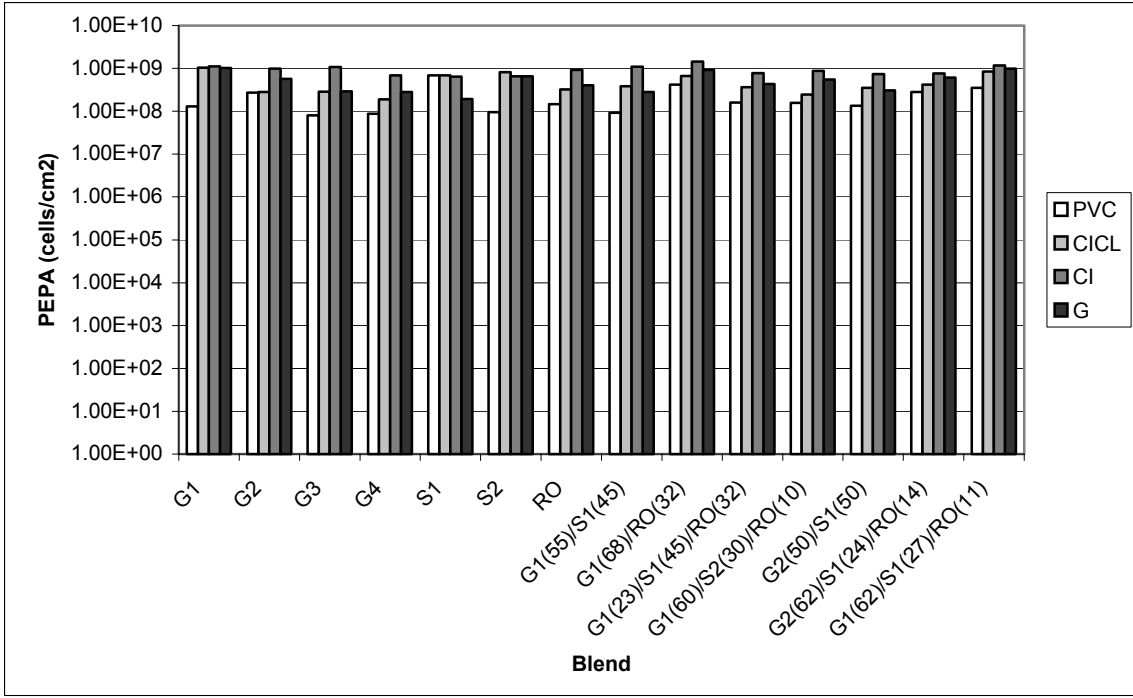


Figure 1.

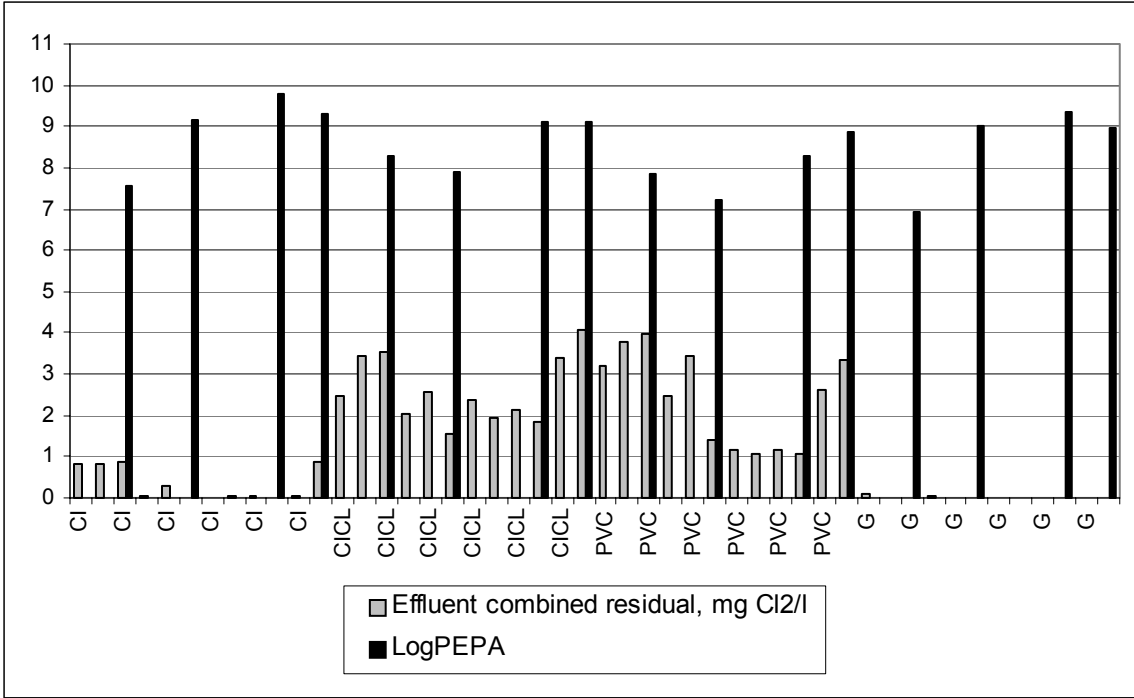


Figure 2.

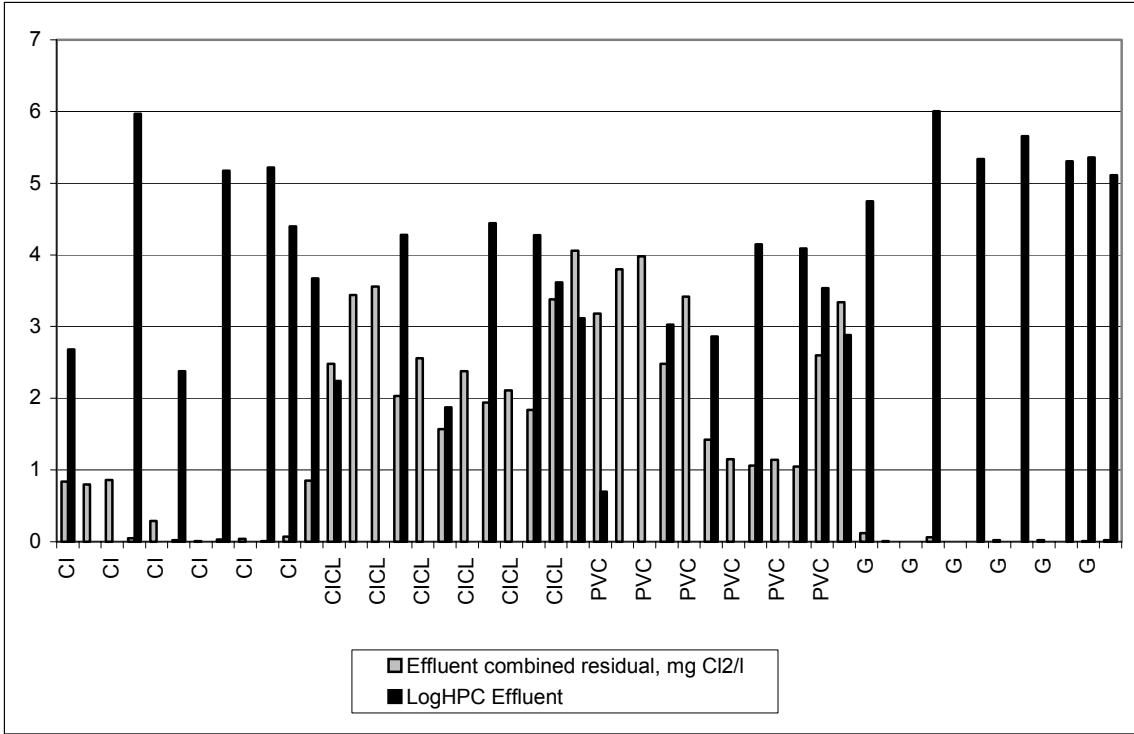


Figure 3.

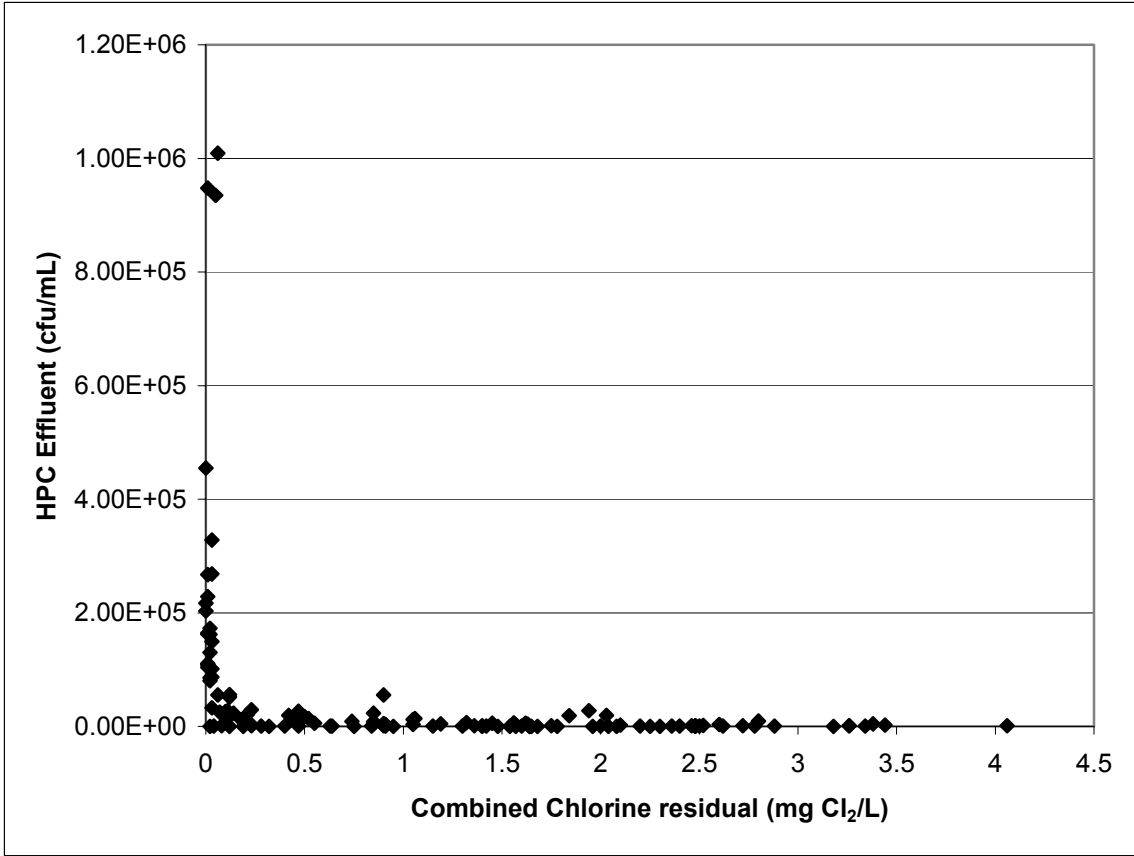


Figure 4.

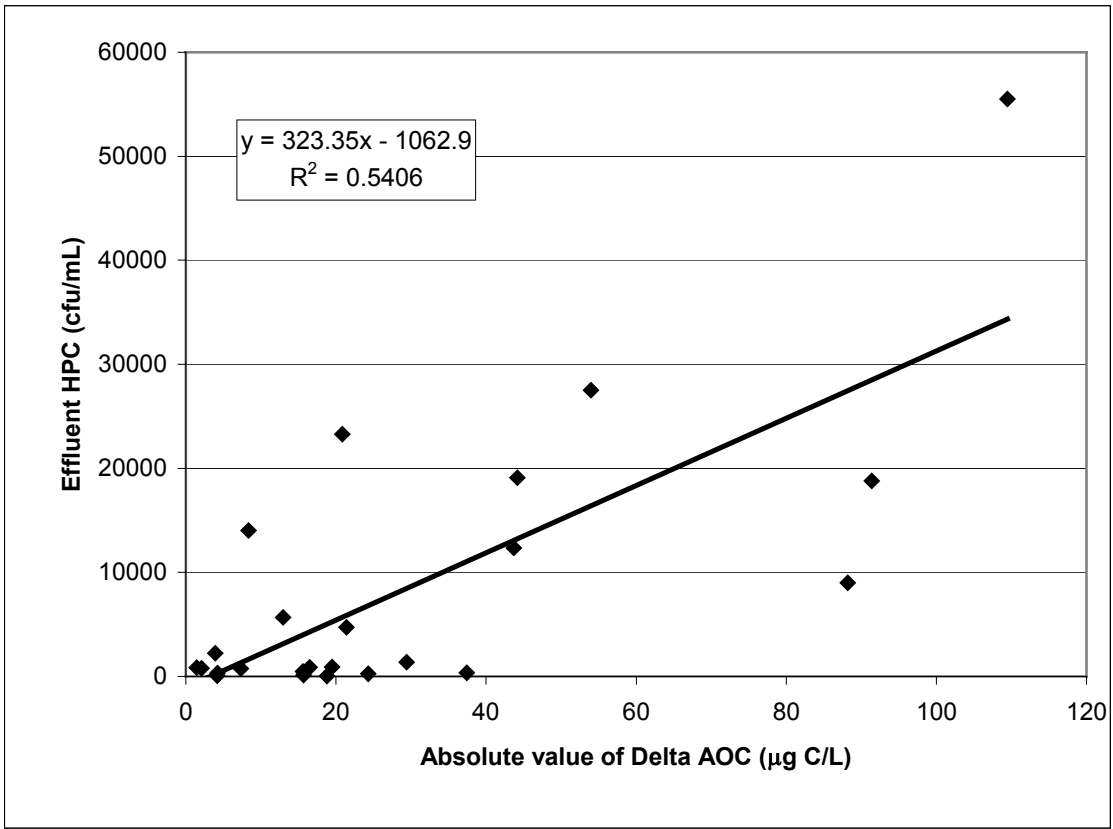


Figure 5.

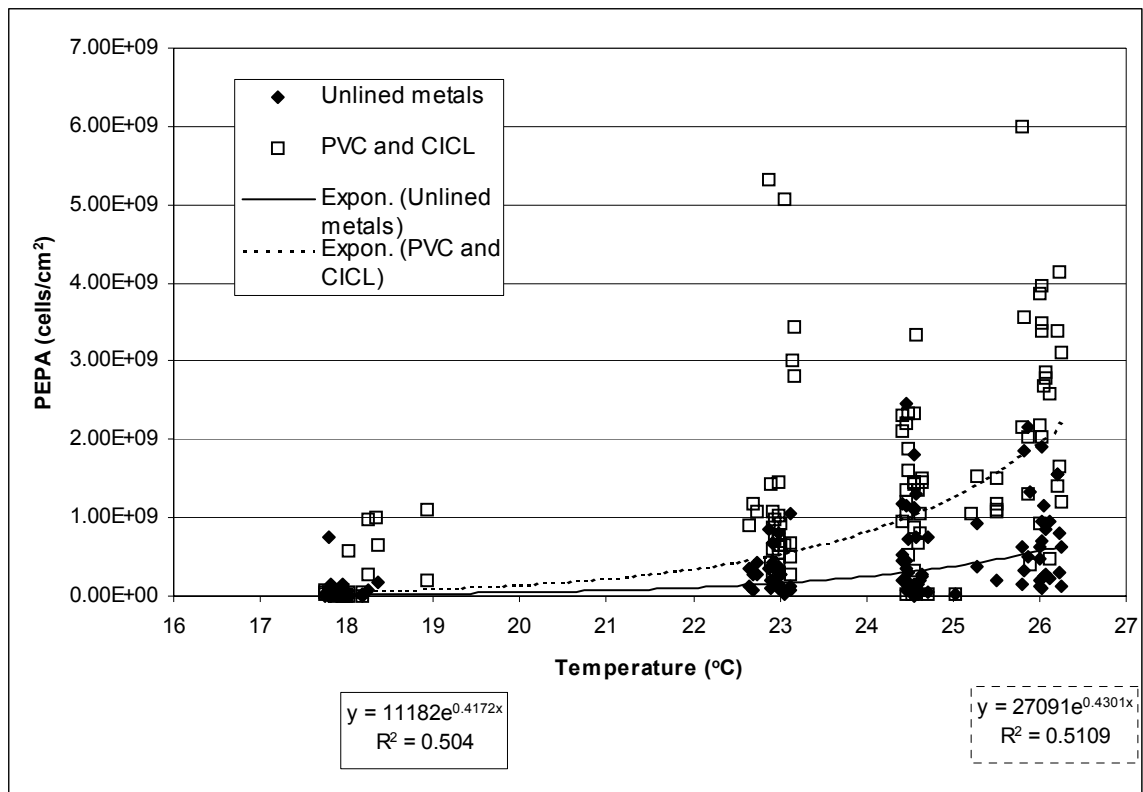


Figure 6.

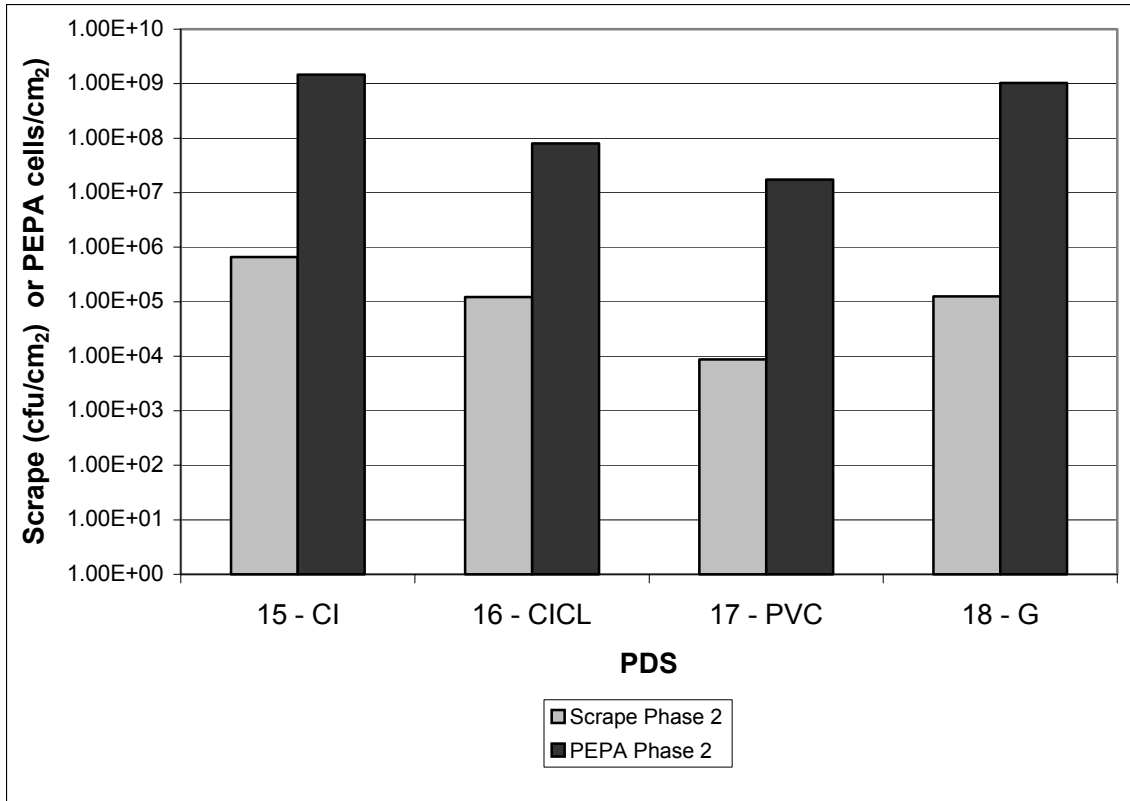


Figure 7.