Impact of Scale Formation on Biofilm Growth in Premise Plumbing

Final Report Prepared for the Salt Institute by

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Summary: This study evaluated the effect of scaling on biofilm formation on two of the most common pipe materials used for premise plumbing. The two pipe materials used were copper and polyvinyl chloride (PVC). Biofilm formation was studied with clean coupons, coupons with hard scale and coupons with soft scale. Soft scale occurs when deposition of scale particles occurs during the scaling process. Biofilm formation was studied in biofilm growth reactors under controlled conditions, with constant flowrate, one bacterial source and a controlled nutrient source. Therefore, pipe material and scale were the only variables in the study. The effect of different pip materials was studied with clean coupons. Clean PVC coupons supported more biofilm growth than clean copper coupons. Based on surface roughness, one would expect more growth on copper coupons since it is the rougher surface. It is unlikely that PVC can release nutrients to support microbial growth, therefore, the lower biofilm growth on copper is attributed to the antimicrobial properties of copper. The study of scaled couponds revealted that scale increased biofilm growth on both pipe materials. Scale will increase surface roughness and provide shelter for microbial attachment. The increase in biofilm growth on scaled coupons was 600-620% for copper and 85-90% for PVC. The larger increase in biofilm on copper could be from the scale shielding microbes from the antimicrobial properties of copper. The increase in biofilm formation was similar for both soft scale and hard scale so there was no apparent effect from the type of scale. Since copper is the most commonly used premise pipe material in premise plumbing, the impacts of scale formation on premise plumbing could be quite pronounced.

Objectives: While a variety of studies have looked at the biofilm growth on pipe materials, no studies have addressed the impact of hard water scaling on biofilm growth in premise plumbing. The primary objective of this study was to evaluate the impact of scale formation on common premise plumbing pipe materials (copper, PVC and iron). There are many variables involved in actual premise plumbing including temperature, nutrient and bacterial sources. These variables were eliminated in this study by focusing on only pipe materials with and without hard water scale. Only one temperature, nutrient source and one species of bacteria were used.

Background: Biofilm formation and the tendency of different piping materials to enhance the multiplication of microorganisms is a well-studied topic and one that has received the attention of laboratories and related organizations around the world. Biofilms are active communities of microbes attached to surfaces and surrounded by a self-produced matrix of extracellular polymeric substances. The occurrence of biofilms can cause hygiene problems in water distribution systems. The development of biofilm depends on a variety of factors such as hydrodynamic patterns and surface materials which affect the microbial biofilm cell density through detachment phenomena [1]. A major problem arises when pathogenic or opportunistic pathogenic microbes inhabit these biofilms. Premise plumbing is an ideal ecological niche for opportunistic pathogens and also represents a front line of exposure to humans. Colonization of premise plumbing by opportunistic pathogens is well-documented, especially in hospital buildings and hotels [2;3-5;7-8].

The presence of scales, corrosion products, and associated contaminants in pipes of drinking water distribution systems (DWDS) and their release into finished water has gained a heightened scientific and societal interest in recent years [9]. Scales formed in pipes mostly consists of tubercles formed via precipitation and re-precipitation mechanisms of pipe-originating nucleating elements, such as, aluminum (Al), copper (Cu), iron (Fe), and lead (Pb). The main sources of dissolved and particulate forms of contaminants in finished water being available to nucleate scale formation or enrich existing pipe scales in DWDS are the following: a) incomplete (μm sized) particle removal by conventional drinking-water treatments, and b) dissolved divalent cations, Ca2+, Fe2+ and Mn2+ ions entering DWDS (intermittent water supply, negative pressure - intrusion, pipe leakage, etc.), which are subject to co-precipitation, oxidation and other processes that lead to nucleation and gradual crystallization of metal oxyhydroxide particles, i.e., the pipe scale precursors. Such natural particles may either remain suspended in finished water or adhere to pipe scales.

Different Pipe Material & Biofilm Formation

Most biofilm formation in DWDS occurs on the pipes, because they constitute the greatest surface area available for contamination, and no single material has been developed for use in plumbing systems which is resistant to biofilm formation [10; 11], even in the presence of high disinfectant concentrations [12]. Pipe materials affect biofilm attachment and growth due to variations in surface roughness, chemical activity, and influence on water chemistry. Metal-based materials can form corrosion products on pipe surfaces and release metals into water as a result of chemical or biological reactions.[13-15] Potential links between pipe materials and opportunistic pathogens in the drinking water environment have been explored in field surveys and laboratory experiments.

Certain pipe materials can modify and decay disinfectant residuals, leading to increased microbial regrowth in DWDS [16; 17]. Some pipe materials can also affect microbial regrowth by releasing chemical compounds such as copper, iron, and phosphorus ions, and organic compounds. Lehtola et al. (2004) reported that the formation of biofilm was slower in copper pipes than in polyethylene (PE) pipes, and that copper ions led to lower microbial numbers in water. While plastic pipes such as PE, which have recently been used as cost-effective replacements of traditional metal plumbing, may release biodegradable organic compounds and phosphorus, which can arouse microbial regrowth and biofilm formation. Roughness of pipe surface and corrosion products also affects bacterial attachment on the surface. Chang et al. (2003) found that biofilm regrowth on pipes made of rough surface materials such as cast iron, concrete-lined cast iron, and galvanized steel was greater than that on smooth-surface polyvinyl chloride (PVC) pipe [18]. Finally, pipe materials may also affect the structure of microbial community in biofilms and in the outlet water of the pipes. A recent study revealed by phospholipid fatty acid analysis (PLFA) that more gram negative bacteria are present in biofilm on copper pipe than PE pipes [17].

Antibacterial Effect of Copper Pipes

Copper is the most popular modern plumbing material [19; 20] because of its low cost and the ease with which it can be machined into a wide variety of pipes and fittings. It is also deemed highly suitable by the plumbing industry due to its perceived antimicrobial properties.

However, studies have shown that whilst the antimicrobial properties of copper are employed to good effect in, for example, copper–silver ionization biofilm control systems [21], they are over time negated at the pipe surface by the formation of biofilms largely through the chelation of metal ions within the extracellular polymeric substance (EPS) matrix [22], and the oxidation and subsequent passivation of the surface. Certain biofilm bound microorganisms may also exhibit some copper resistance [23]. The formation of biofilms on copper pipes has also been shown to induce characteristic pitting corrosion known as 'cuprosolvency' which may cause problems such as pipe failure, as well as adversely affecting public health [24-27]. Stainless steel is an alloy metal of which there are many types and grades that is viewed as a possible alternative to copper. In addition to iron, it may contain molybdenum, nickel and chromium, which facilitates the effective resistance to corrosion over long periods, due to the formation of a thin layer of chromium or potentially antimicrobial molybdenum-based oxides at the material surface [28]. Numerous studies have been carried out on its biofilm-supporting capabilities [29-33]

An inhibitory effect of copper pipe toward Legionella growth and adherence was observed in a biofilm reactor [10] and a model warm water system during the first two years of operation [34]. A negative association between copper levels >50 μg/L and Legionella colonization was also observed in real-world hot water systems [35-36]. However, the opposite effect was observed in one Legionella survey of German residences, where plumbing with copper pipes was more frequently colonized with Legionella than those made of synthetic materials or galvanized steel, and a positive correlation was observed between copper concentration and Legionella growth in hot water [37] Though the reason for the discrepancy is unknown, it is reasonable to speculate that levels of bioavailable copper concentration near the pipe surface and bulk water would differ markedly in these studies, and that copper can also increase rates of disinfectant loss,[38-39] which would also be influential on Legionella growth.

Comparison of Copper Performance to the other Pipe Materials in Biofilm Formation

Based on the detailed analysis of the Knowledge Institute for Drinking Water (KIWA) research, [40] concluded that chlorinated PVC (CPVC) consistently outperforms most other non-metallic piping materials with regard to its ability to resist the formation of biofilms. He further confirmed that there is no statistical difference between the antimicrobial performance of CPVC and copper. Both have proven to offer the best protection against biofilm formation (Figure 1).

Sturman [40] has documented that a majority of the organic carbon responsible for biofilm formation already exists in the water being tested before it flows through any piping material. In most cases, the amount of organic carbon in the water is significantly greater than what could leach from the interior of the pipe. The deviations in measurements between copper and CPVC have actually decreased, such that no significant differences are evident in recent studies.

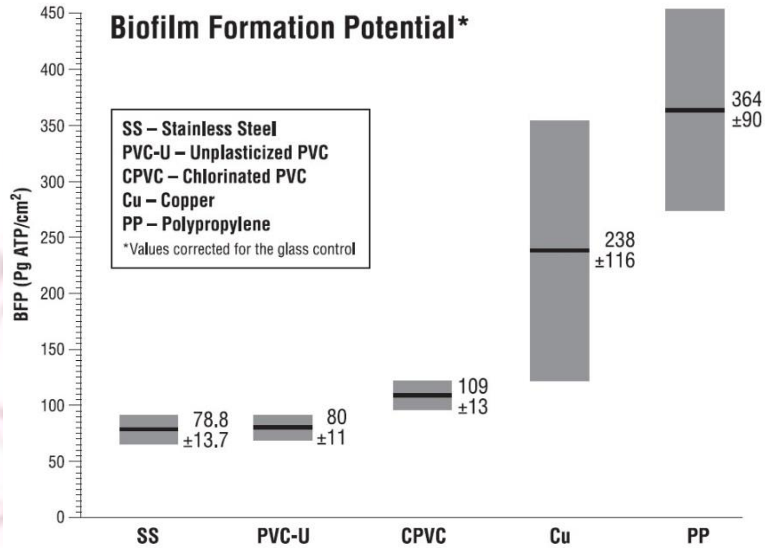


Figure 1. Biofilm formation Potential in different pipe material [40]

Yu et al [41] analyzed the effects of pipe materials on biofilm formation potential (BFP) and microbial communities in biofilms. Pipe coupons were made of six different materials (CU, copper; CP, CPVC; PB, polybutylene; PE, polyethylene; SS, stainless steel; ST, steel coated with zinc) were incubated in drinking water, mixed water (inoculated with 10% (v/v) of river water) and drinking water inoculated with Escherichia coli JM109 (*E. coli*), respectively. The highest BFPs were observed from steel pipes, SS and ST, while CU showed the lowest BFP values. Adenosine triphosphate (ATP) values of pipes incubated in drinking water increased in the order CU , CP , PE , SS , PB , ST, and ATP values in mixed water increased by CU , CP , PB , PE , ST , SS after 90 days incubation. Number of *E. coli* of pipes incubated in drinking water inoculated with the culture of *E. coli* strain increased by CU , CP , PE , PB , SS , ST. Molecular analysis of microbial communities clearly showed that the pipe materials significantly affect not only BFP but also microbial richness and diversity [41].

Waines et al [42] studied biofilm formation on four plumbing materials, viz. copper, stainless steel 316 (SS316), ethylene propylene diene monomer (EPDM) and cross-linked polyethylene (PEX), was investigated using scanning electron microscope (SEM)/confocal microscopy, ATP-/culture-based analysis, and molecular analysis. Material ‘inserts’ were incorporated into a model water distribution system. All materials supported biofilm growth to various degrees. After 84 days, copper and SS316 showed no significant overall differences in terms of the level of biofilm formation observed, whilst PEX supported a significantly higher level of biofilm. EPDM exhibited contamination by a complex, multispecies biofilm, at a level significantly higher than was observed on the other materials, regardless of the analytical method used. PCR-DGGE analysis showed clear differences in the composition of the biofilm community on all materials after 84 days [42].

Morvay et al [43] investigated the attachment of bacteria and the dynamics of microbial biofilm formation on different materials which could be used for drinking water pipeline systems. The experiment was performed using a chlorinated drinking water system and three types of materials: copper, stainless steel and polyvinyl chloride; as test surfaces. Water samples were analyzed after 21 hours of stagnation and 3 hours of continuous flow, over a period of 30 days. Fluorescent microscopy was used for counting surface attached bacteria and confocal laser scanning microscopy was used to measure biofilm thickness. The total number of viable bacteria was higher in stagnant than in flow conditions. Bacterial counts reached $8.76x10^4$ CFU/mL after 21 hours of stagnation. Stainless steel had the highest number of attached bacteria, followed by PVC. The number of bacteria attached to the surfaces ranged between $3.2x10^6$-$1.2x10^7$ bacteria/cm^2 for stainless steel and $6.2x10^6$-$9.2x10^6$ bacteria/cm^2 for PVC. On the copper surface biofilm formation was very weak, consisting of only individually attached bacteria. The biofilms formed faster on stainless steel than on PVC or copper. Minimal biofilm was found to form on the copper surface during the first 30 days of exposure to chlorinated drinking water [43].

Materials and Methods:

Biofilm Growth Reactors

The biofilm growth potential of virgin and scaled coupons was tested in biofilm growth reactors. In the initial experiments all three coupon types (Copper, PVC and Iron) were incubated in the same reactor. Co-incubation of these coupons resulted accumulation of iron oxide on the copper and PVC coupons, limiting the accurate measurement of biomass on each material type and creating scale under that was not the focus of this study. In subsequent experiments, copper and PVC coupons were incubated in independent reactors for an un-biased estimation of biofilm growth on each of these materials.

The biofilm growth reactors were made of plastic housings (approximate dimensions 5”x3”x3”) with removable covers. Each reactor contained outlet and inlet ports installed in the cover (Figure 2). The inlet and outlet ports were installed on the opposite corners of reactor lids, to facilitate unidirectional flow of water during the study period. Prior to each experiment, each reactor was disinfected by soaking in 70% propanol overnight followed by drying and rinsing (three times) with autoclaved nanopure grade water.

Each reactor was filled with 350 mL of autoclaved tap water spiked with 100cfu/mL of E coli (ATCC 25922). The biofilm reactors were sealed using packing tap to avoid water loss due to evaporation and to control cross contamination of reactors. A peristaltic pump was used to constantly recirculate (3.5mL/min) water in each reactor throughout the study period resulting in an average hydraulic residence time of 3 hours. The xxperimental setup was housed in the Environmental Microbiology Laboratory at Arizona State University which is maintained at a temperature of 27^{o}C.

Pipe material coupons were purchased from Metal Samples Company (Munford, AL) along with a coupon rack to suspend the coupons in a water solution. The coupons were weighed by Metal Samples Company and the weights were verified in the Arizona State University (ASU) environmental engineering laboratory. Each coupon is 1/8" thick x 3/4" wide x 2" long, with a 3/8" diameter hole centered for attachment to the coupon rack. Each coupon is also stenciled so that the coupons can be easily identified. Coupons purchased were of the most common premise pipe materials including PVC, copper and iron.

Before the start of an experiment, three identical coupons of each type were selected and the same process was repeated for coupons with scale. Each coupon was individually weighed prior to incubating in a biofilm reactor. Periodically, coupons were carefully retrieved from the reactors to measure biomass accumulations. During the retrieval and biomass measurement process, coupons were carefully handled making sure that the accumulated biomass is not disturbed. For biomass measurements, coupons were incubated at 27°C for 12 hours to remove excess moisture. After 12 hour of drying, the coupons were weighed and the gain in biomass during each observation period was calculated by subtracting the initial weight from the current weight of each coupon.

Bacteria used in this study were *E. coli* (ATCC 25922) obtained from the American Culture Collection. A volume of 1.0mL from pure frozen stock was thawed and suspended in 9.0mL trypticase soy broth (TSB). The bacterial suspension was incubated in a shaker-incubator (150 rpm and 37°C) for 4 hours to achieve a log phase bacterial culture. The log phase culture was centrifuged for 10 minutes at 1000xg force and 22°C and supernatant was discarded. The bacterial pellet was washed twice with phosphate buffer (0.5xPBS) and resuspended in 2.0mL of the same buffer. A 10-fold dilution series was made from this suspension to yield a working dilution. An appropriate volume of diluted stock was added to biofilm reactor to achieve a final concentration of 100cfu/mL of water in reactor.

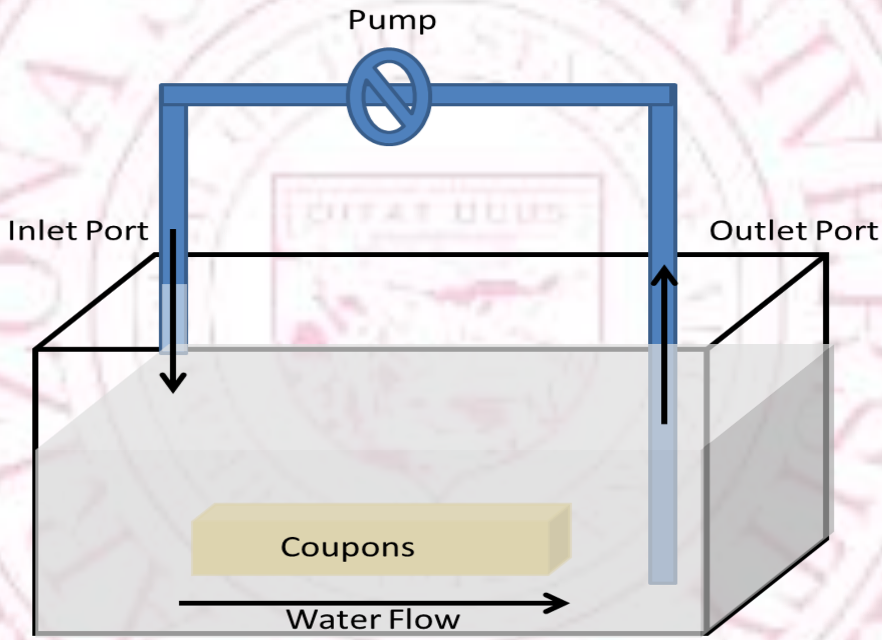


Figure 2. Schematic of biofilm reactor

Scale Formation

Biofilms were developed in the recirculating reactors described in the material and methods section. Initial testing was done in triplicate with 3 coupons of each pipe material placed at different locations in the reactor. A 3/8' diameter tygon tube was placed through the holes of each coupon to maintain the coupons in a vertical position. The sequence of coupons was copper, PVC and then iron (Figure 3.).

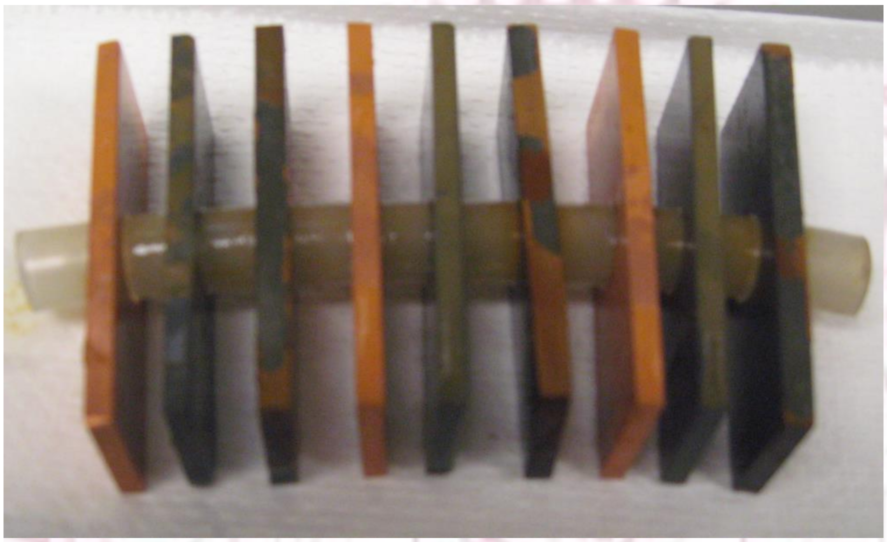


Figure 3. Sequence of Clean Coupons Placed in Recirculating Reactor

The water used in the recirculating reactor was City of Tempe tap water. The water contains 2.3 mg/l of total organic carbon and has a Biodegradable Organic Carbon concentration of 0.4 mg/l. Previous testing with the tap water has demonstrated measurable biofilm growth in time periods less than one month indicating that sufficient nutrients and organic carbon are present to support biological growth. The use of tap water also simulates the water quality that exists in premise plumbing in Tempe, AZ. The coupons were assayed for biofilm formation after 1 month and 3 months. During the initial testing, corrosion of the iron coupons was observed and the corrosion appeared to have affect biofilm formation on all the coupons as the iron oxide deposits formed a scale on all coupons in the reactor. Therefore, the test was repeated with only PVC and copper coupons in separate reactors. This was also necessary since the corrosion of the iron coupons during the scale formation procedure prevented the use of the iron coupons.

Scale was formed on coupons by placing coupons in a recirculating hot water bath maintained at a temperature of $80^{o}C$. Initial attempts to use the coupon rack supplied by Metal Samples Company were not successful. The water bath could not be covered with the coupon rack and evaporation forced the water bath to automatically shut down when water levels approached the heating coils. A test tube rack was modified to fit in the hot water bath and coupons were placed on the test tube rack. Corrosion of iron coupons was more rapid than scale formation and deposition of iron oxide occurred before scale began to form on all the coupons. Therefore, the use of the iron coupons for scale formation was not done. Scale formation was enhanced by the addition of 2 g of $CaCl_2$ along with make-up water to the water batch which holds 14 liters of water total. After three months, scale formation was observed to be uniform across all the coupons in the hot water bath.

After scale was formed, the coupons were dried and weighed to determine the mass of scale that was attached to each coupon. The coupons were then placed in the recirculating biofilm reactor and they were assayed for biofilm formation after a time period at a time scale of approximately one month. .

"Soft" scale is created by a combination of scale formation and deposition of $CaCO_3$ crystals. Therefore, "soft" scale was formed in the hot water bath by the addition of $CaCO_3$ crystals on a daily basis instead of the addition of $CaCl_2$. The coupons are currently showing scale formation that resembles "soft" scale on one side of the coupon and they are ready to be evaluated for biofilm formation. The difference between "hard" scale and "soft" scale is apparent in Figures 4 and 5. Figure 4 are calcite crystals in "hard" scale. The crystals are aligned as scale formed on the surface and the crystals are tightly attached to one another. Figure 5 are calcite crystals in "soft" scale. The crystals are not aligned as deposition on the surface occurred during scale formation. The crystals are not all tightly attached to one another and the scale can easily be wiped away.

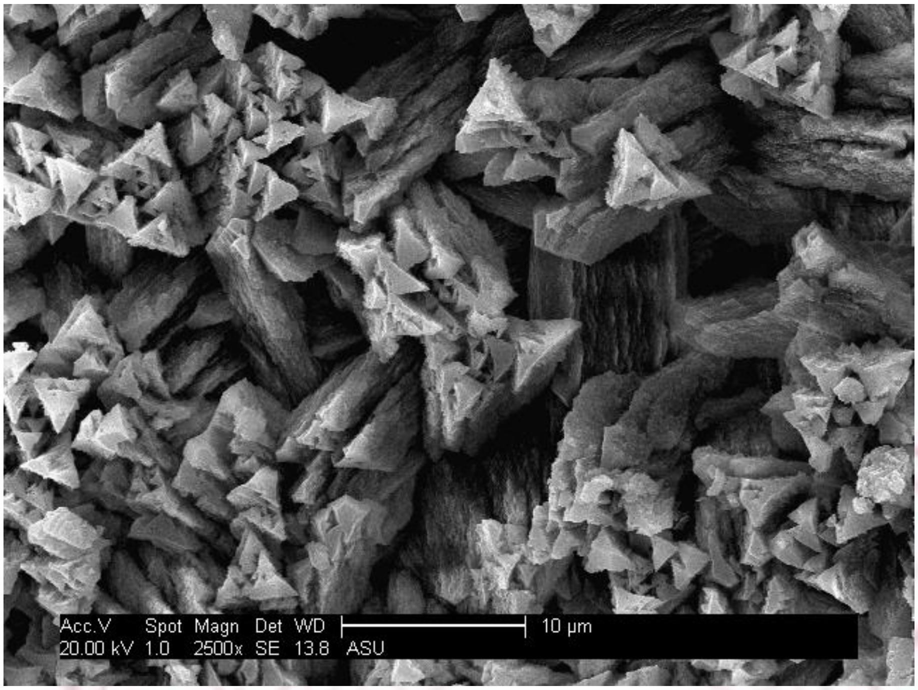


Figure 4. Electron Micrograph of “hard” scale. The calcite crystals are aligned as scale formed directly on the surface.

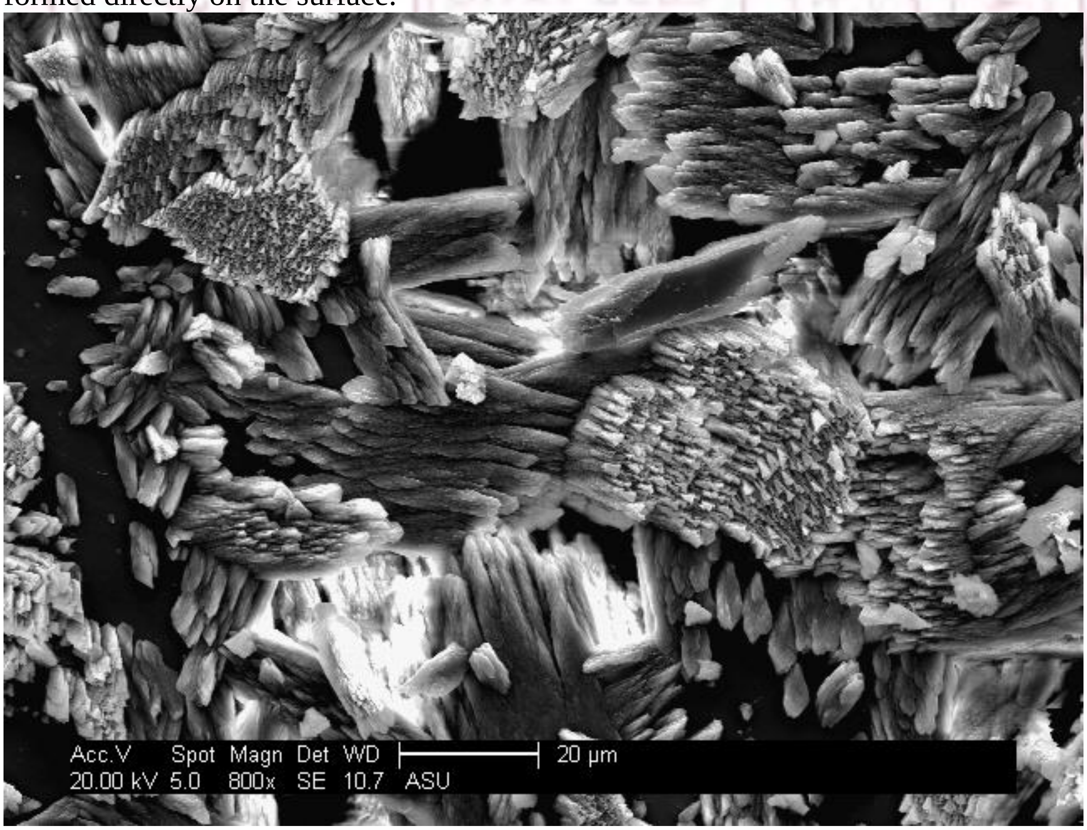


Figure 5. Electron Micrograph of “soft” scale. The calcite crystals are not aligned as deposition occurred on the surface during scale formation.

Results: The results for the mass of biofilm formed on the coupons initially placed in the recirculating reactor are presented in Figures 6, 7 and 8. The data is presented in terms of the time for growth (1 month and 3 months) and the placement in the reactor. The coupons placed furthest from the inlet had the greatest amount of growth. This is most likely a consequence of turbulence near the reactor inlet, although the recirculation rate was designed for laminar flow conditions. The least amount of growth was observed on PVC and biofilm formation between one month and three months was not significant on the PVC coupons (Figure 4). The PVC coupons are considered to be the smoothest of the pipe materials with a negligible sand roughness coefficient. The copper coupons had approximately twice the biofilm growth as the copper coupons and the majority of growth also occurred during the first month (Figure 5). The iron coupons had an order of magnitude greater biofilm formation and the majority of growth occurred between 1 month and 3 months (Figure 6). Corrosion of the iron coupons increased surface roughness and appears to have created an excellent environment for biofilm formation. Figure 10, 11 and 12 are pictures of the coupons after different time periods in the reactor. Corrosion is clearly apparent for the iron coupons and biofilm formation can be observed on all the coupons.

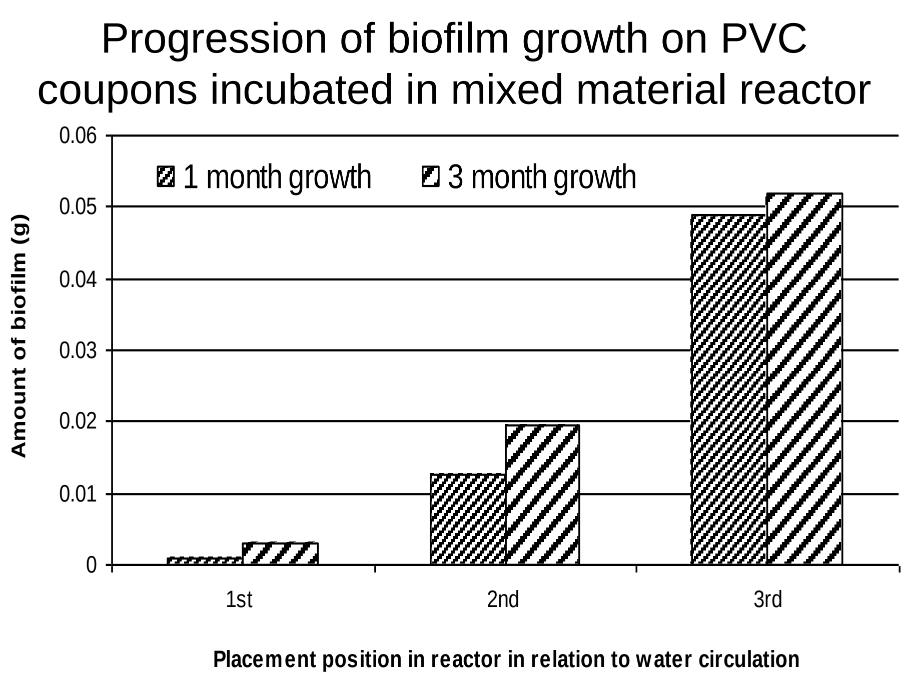


Figure 6. Biofilm Growth on PVC coupons.

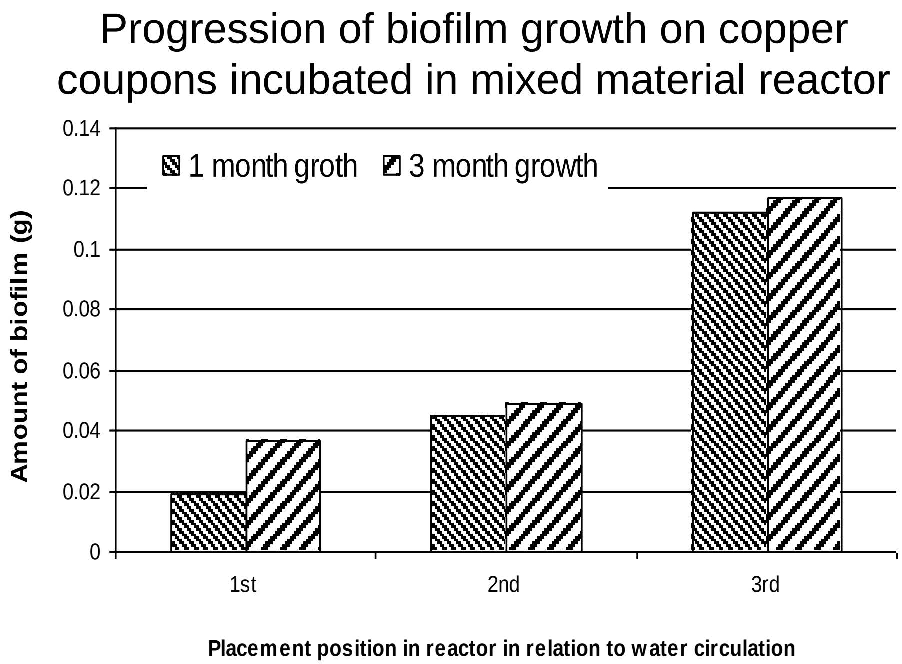


Figure 7. Biofilm growth on copper coupons.

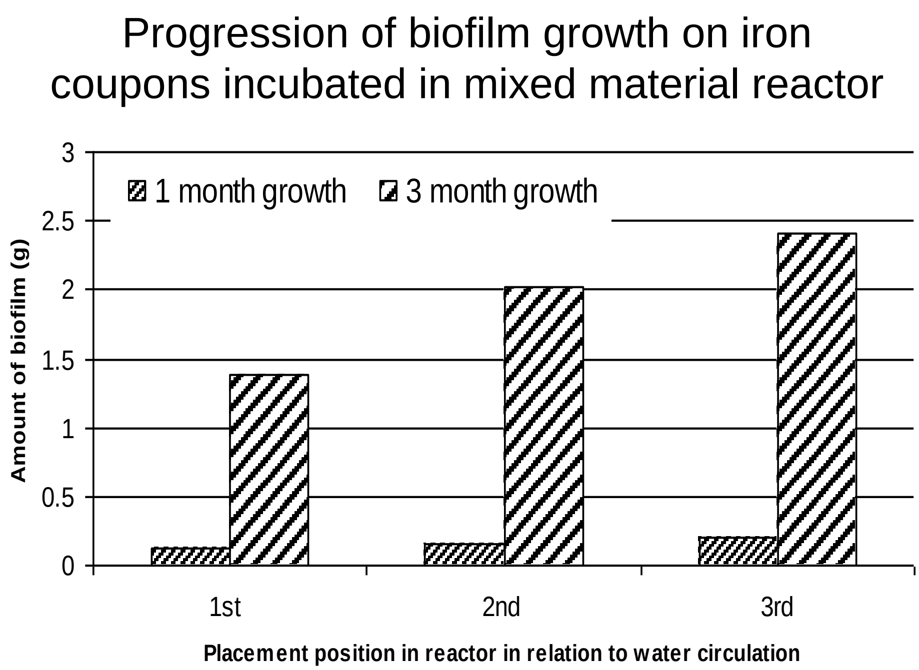
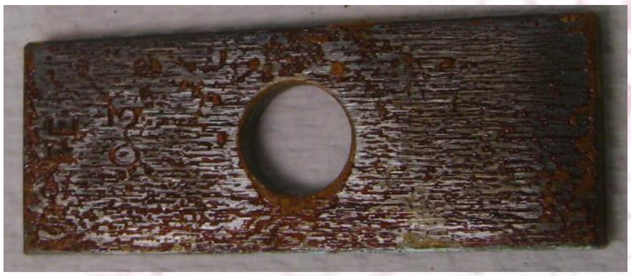
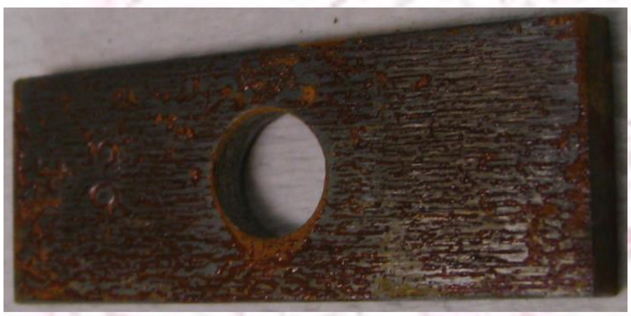


Figure 8. Biofilm growth on iron coupons.

3 MONTHS

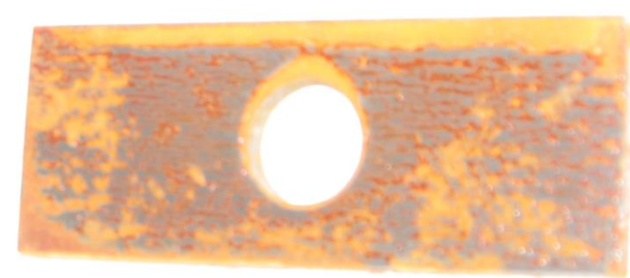


2 MONTHS



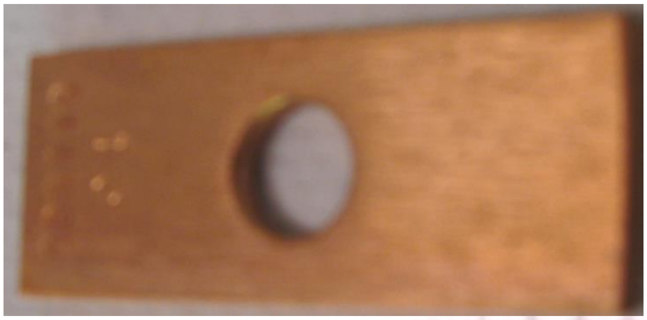
1 MONTH

Figure 10. Iron coupons after 1 month, 2 months and 3 months. The corrosion is clearly evident and biofilm formation was greates.



3 MONTHS

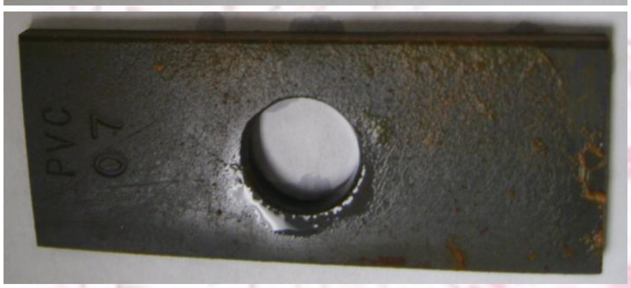
2 MONTHS



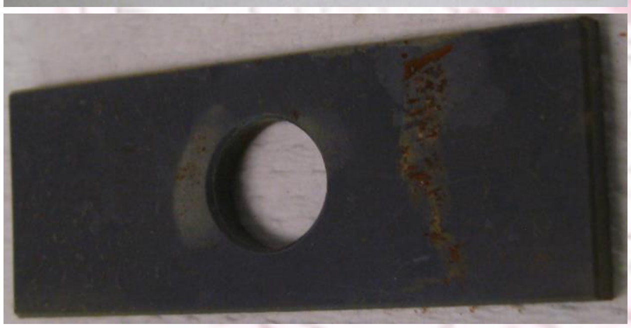
1 MONTH

Figure 11. Copper coupons after 1 month, 2 months and 3 months.

3 MONTHS



2 MONTHS



3 MONTHS

Figure 12. PVC coupons after 1 month, 2 months and 3 months.

The results for the second set of biofilm formation tests without the iron coupons are presented in Figures 13 and 14. The amount of biofilm formed was significantly less and as the previous measurements included both biofilm and iron oxide. Therefore, these measurements are of biofilm only and they are accurate. The copper coupons had less than 0.003 grams of biofilm after 3 months while the PVC coupons had less than 0.2 grams of biofilm after 3 months. Both pipe materials are very smooth as Copper has a sand roughness coefficient of 0.0015 mm while PVC is considered smooth. Copper is known to inhibit biological growth and copper nanoparticles are used to inhibit biofilm formation in other materials. One would normally expect the biofilm growth to be greater on the rougher surface consistent with Figure 1.

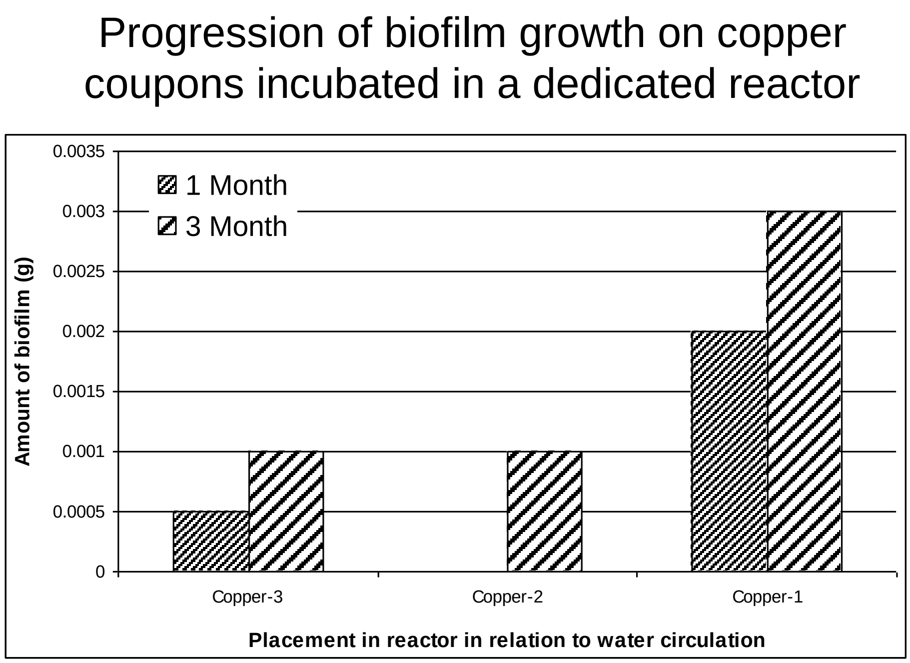


Figure 13. Biofilm formation on copper coupons during testing without iron.

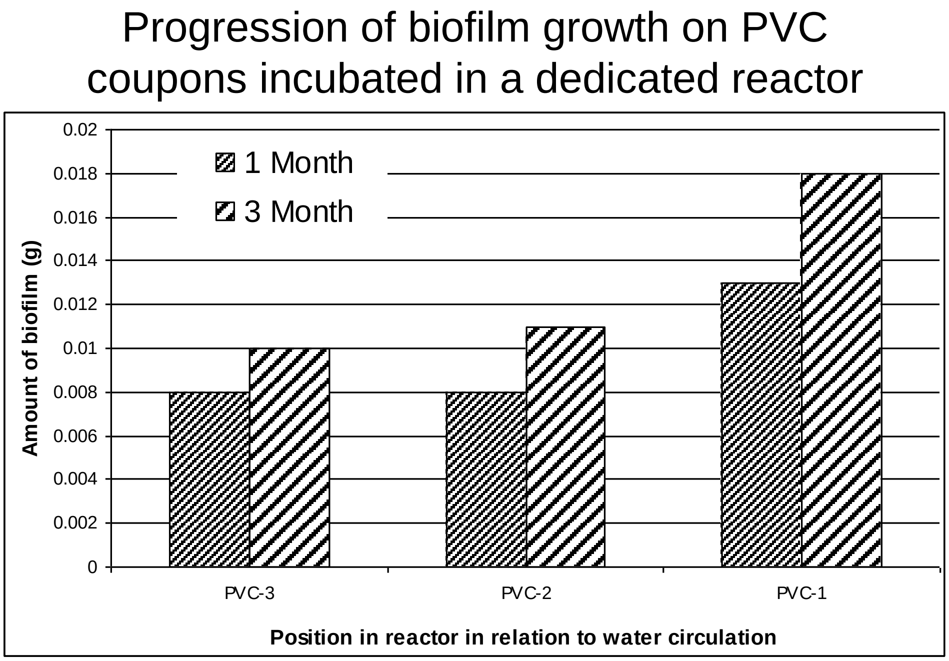


Figure 14. Biofilm formation on PVC coupons during testing without iron.

Scale was formed in a uniform fashion on both PVC and copper coupons (Figure 15). The scale appears to be primarily calcite based on optical microscopic observation (Figure 16.). Based on previous experience with the water used to form the scale, calcite would be expected to be the primary crystalline form of $CaCO_3$.

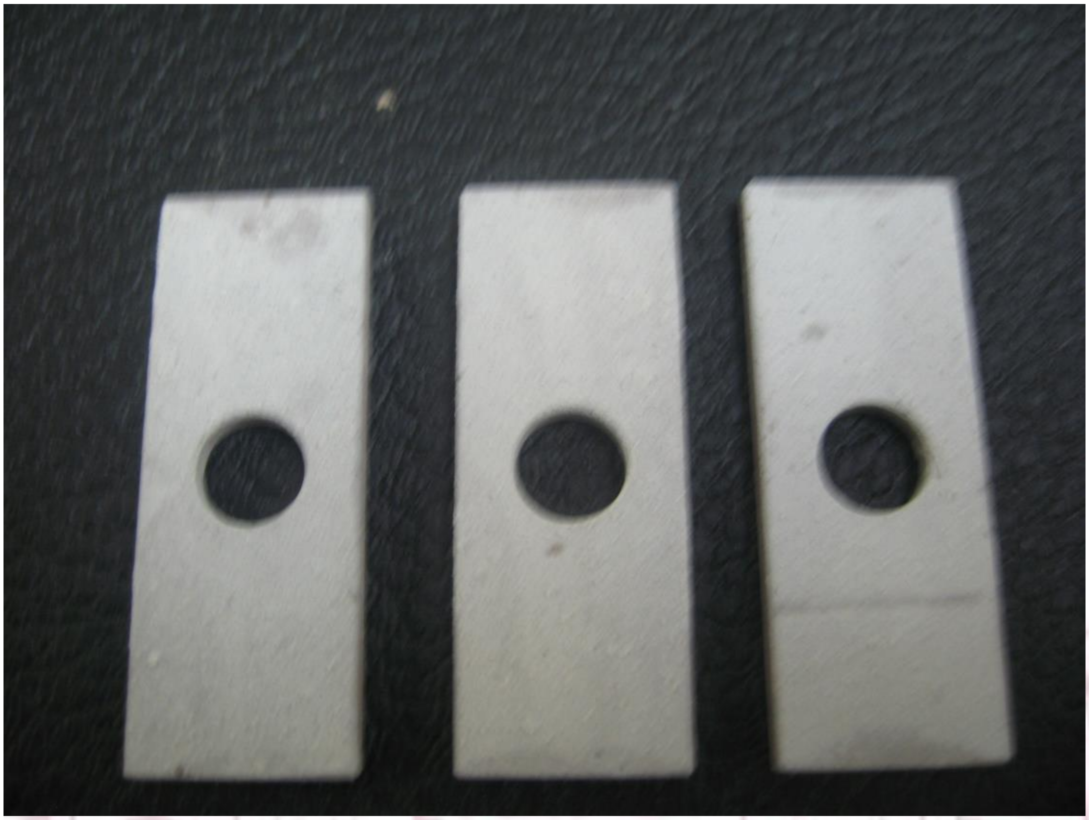


Figure 15. Copper coupons with scale.

Figure 16. Optical microsope picture of calcite crystals along the center of a coupon.

The results for biofilm formation on coupons with scale are presented in Figures 17 and 18. There is a significant increase in biofilm growth for both PVC and copper between 1 month and 2.5 months. For the PVC coupons, the scaled coupons had up to 0.018 g of biofilm which is an 85% increase in biofilm growth in comparison to the clean coupons. For the copper coupons, the scaled coupons had up 0.021 g of biofilm which is a 600% increase in biofilm formation as compared to the clean copper coupon. Both sets of coupons had a significant increase in biofilm growth when scale was attached. The large increase for copper coupons could be from the scale shielding the microbes from the antimicrobial effects of the copper.

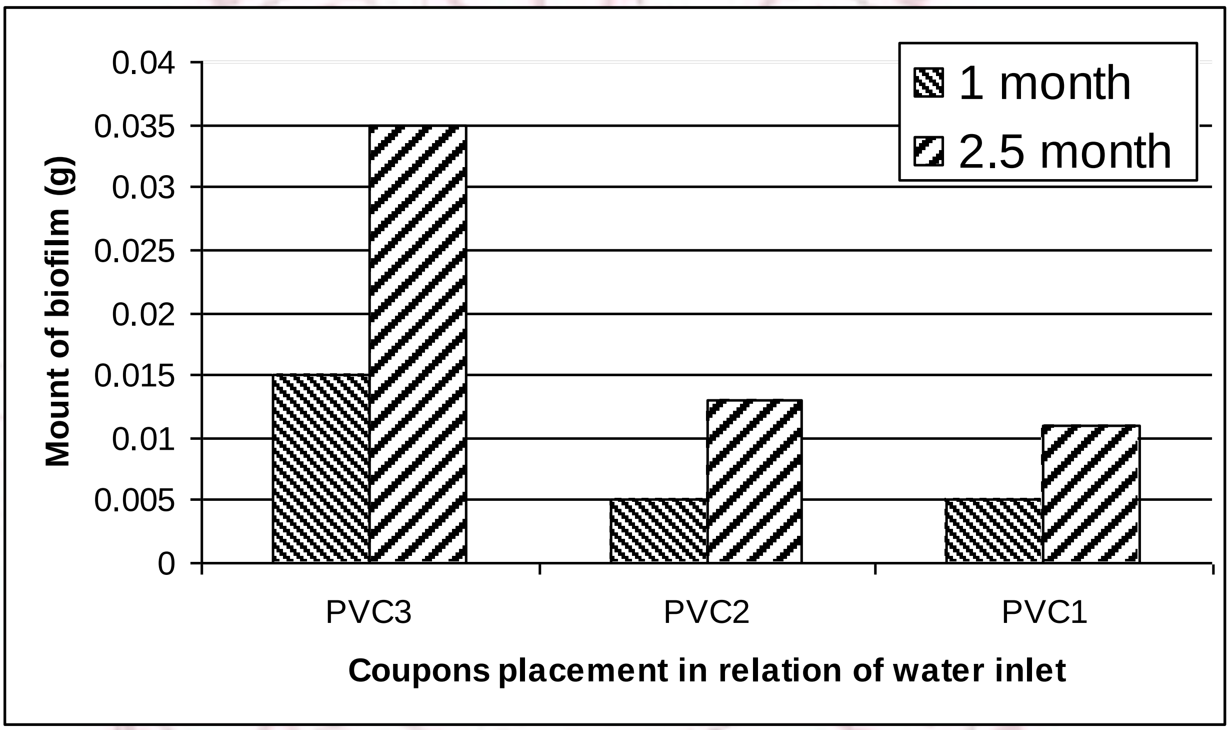


Figure 17. Biofilm growth on PVC coupons with scale.

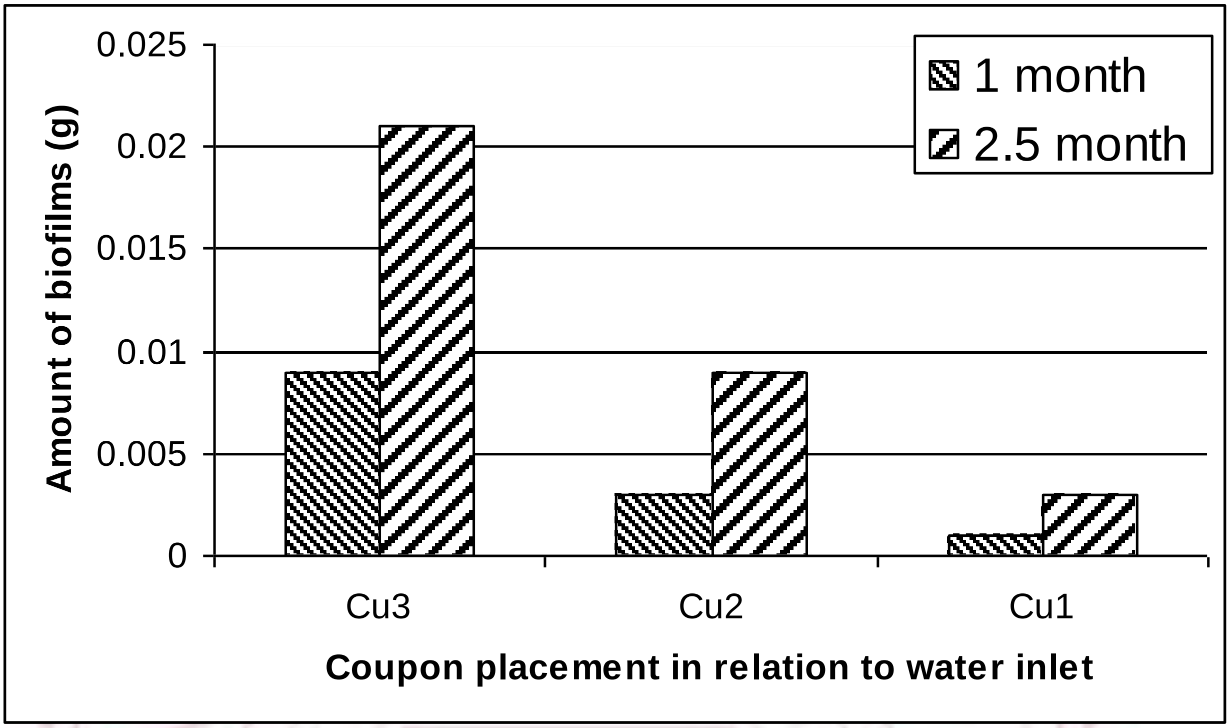


Figure 18. Biofilm growth on copper coupons with scale.

The results for biofilm formation on coupons with soft-scale are presented in Figures 19 and 20. The results are quite similar to the results oberseved with the hard-scale that are presented in Figures 17 and 18. There is a significant increase in biofilm growth for both PVC and copper between 1 month and 2.5 months. For the PVC coupons, the soft scaled coupons had up to 0.037 g of biofilm which is a 90% increase in biofilm growth in comparison to the clean coupons. For the copper coupons, the soft scaled coupons had up to 0.022 g of biofilm which is a 620% increase in biofilm formation as compared to the clean copper coupons. There was no significant difference between the soft scale and the hard scale in terms of biofilm formation. Both sets of coupons had a significant increase in biofilm growth when scale was attached regardless of the type of scale. Both soft scale and hard scale could shield the microbes from the antimicrobial effects of the copper.

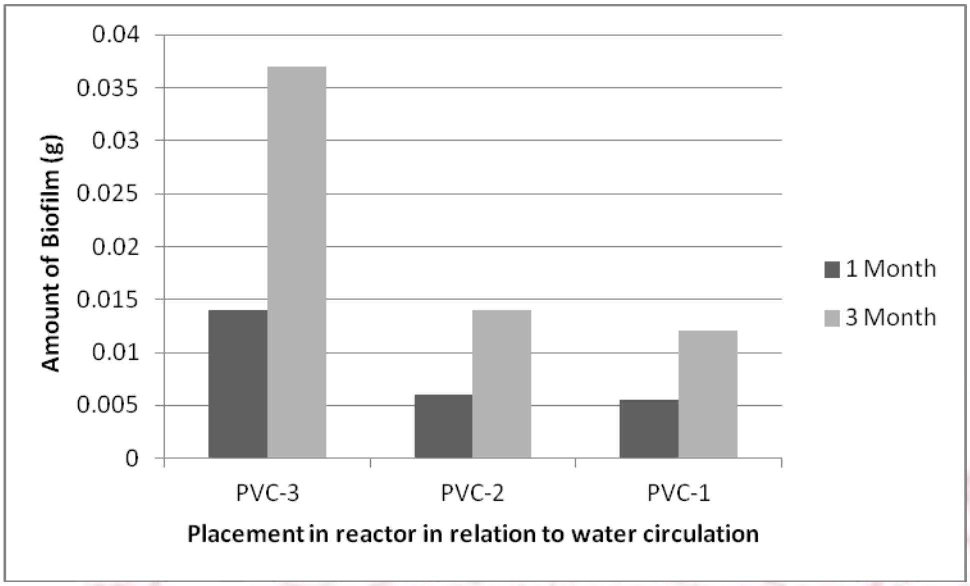


Figure 19. Biofilm growth on PVC coupons with soft scale

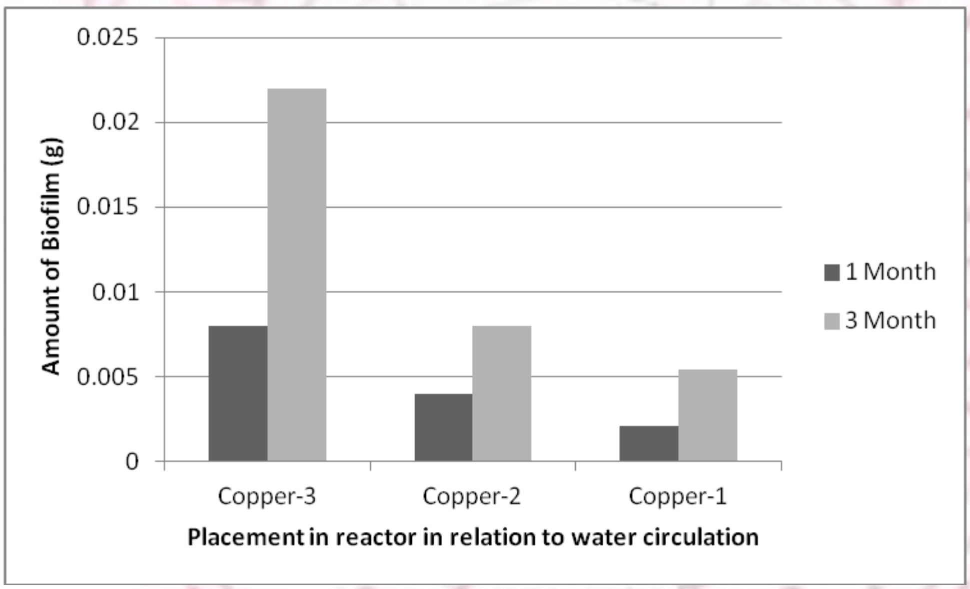


Figure 20. Biofilm growth on copper coupons with soft scale.

Conclusions

- Clean PVC coupons supported more biofilm growth than clean copper coupons. Based on surface roughness, one would expect more growth on copper coupons since it is the rougher surface. It is unlikely that PVC can release nutrients to support microbial growth, therefore, the lower biofilm growth on copper is likely from the antimicrobial properties of copper.
- Scale increased biofilm growth on both pipe materials. Scale will increase surface roughness and provide shelter for microbial attachment.

- The increase in biofilm growth on scaled coupons was 600-620% for copper and 85-90% for PVC. The larger increase in biofilm on copper could be from scale shielding the microbes from the antimicrobial properties of copper.
- The increase in biofilm formation was similar for both soft scale and hard scale.

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