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Laboratory Evaluation of Biofilm and Mineral Deposition on Concrete Surfaces

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Abstract:

The use of concrete structures in the potable water industry is widespread. Although a variety of finished concrete surfaces are available, the overwhelming majority employed are uncoated or unsealed. One significant challenge for water storage facilities is biological fouling which can lead to water quality compliance problems such as; loss of disinfectant residual, increased disinfection by-product formation, increased nitrification risk, and occurrence of coliforms with maturing biofilms. It was hypothesized that the high porosity and unevenness of raw concrete surfaces could be advantageous for bacterial attachment and subsequent biofilm growth.

The objective of this research was to determine if biological activity, as measured by adenosine triphosphate (ATP) concentration on concrete surfaces, could be limited or reduced by the use of commercially available protective coatings. In addition to the biological evaluation, the research evaluated mineral precipitation and any limiting effects initiated by surface coatings. Iron was selected as the indicator for mineral deposits as it is readily available in most water sources and presents an effective visual indicator of system fouling.

Our results showed that in as little as 24 hours, ATP levels and biological activity/biofilm achieve much higher growth on raw concrete surfaces compared to coated concrete surfaces. Iron concentrations in all samples corresponded to these biological activity results. This trend continued over a 30 day period, at which point the experiment was discontinued. These results indicate the use of protective coatings on potable water concrete structures can be beneficial in limiting biological fouling and mineral accumulation, thereby assisting with water quality maintenance.

Introduction:

Concrete plays a major role in providing infrastructure for storing, treating and distribution of potable water supplies. Concrete is used extensively in new construction and to repair existing infrastructure. In general, it is marketed that concrete structures require no special treatments or coatings for contact with potable water or wastewater. Subsequently, concrete pipes and tanks are typically advertised as “virtually maintenance-free”.

Occurrences of excessive bacterial populations in distribution systems can be problematic for utilities due to the hygienic safety concerns, reduced aesthic quality, and increased maintenance requirements they create. Specifically, water quality compliance problems such as; loss of disinfectant residual, increased disinfection by-product formation, increased nitrification risk and occurrence of coliforms with maturing biofilms are a few of the issues caused by biofouling. The presence of bacteria in distribution systems can be attributed to these primary mechanisms. The first is breakthrough of bacteria passing through the treatment process. The second is introduction of bacteria from growth occurring in the distribution system due to breaches in the system, contamination events, or resident biofilms. Lastly, the reduction of disinfection residuals within throughout the system.

Currently, control methods for biofouling are largely focused on the use disinfectant products, such as chlorine and chloramines. Activities designed to increase the fluid shear stress on biofilms and detach or make attachment to surfaces difficult are also used. Common examples of these methods include active tank mixing and periodic flushing of distribution lines. Additionally, physical removal and chemical cleaning are also employed to control biological fouling, but are typically engaged after fouling has become problematic due to the high costs of these procedures and the requirement to take systems offline. However, given the recent advances and new understanding of how bacteria attach to surfaces and the environments that favor biofilm growth, the need to test use of protective coatings as a preventative barrier for bacterial attachment to concrete surfaces was identified.

Background on Industry Standards Applicable to Potable Water Concrete Structures:

Concrete commonly used in large storage tanks, reservoirs and pipelines is usually a combination of cement, admixtures, curing compounds, sand, and gravel. It can also contain fly ash and other additives to strengthen the concrete and increase its durability. However, any of these additives can have contaminants that can cause compliance problems for the utility and present potential health risks to the consumer. As such, part of applicable ANSI/NSF Standard 61 for concrete usage in potable water storage tanks requires a site mix evaluation.

The use of concrete and coatings for potable water systems falls under both ANSI/NSF and AWWA standards which guide different aspects. American National Institute of Standards /National Sanitation Foundation 1996 (ANSI/NSF Standard 61) is a nationally accepted standard that protects stored water from contamination via products which come into contact with water. Products covered by ANSI/NSF Standard 61 include a variety of equipment used in the treatment and distribution of water as well as sealing materials and coatings. NSF Standard 61 was reviewed and certified by ANSI which permitted the use of the standard by other independent testing agencies such as Underwriters Laboratories.

With the development of the ANSI/NSF Standard 61, the approval and reporting for tank coatings process was standardized. State primacy agencies that previously had independent coating approval programs discontinued these programs and adopted the ANSI/NSF Standard 61.

NSF certifies individual concrete ingredients to the requirements of ANSI/NSF Standard 61: Drinking Water System Components — Health Effects. The NSF Concrete Site Mix Design Evaluation Program provides a one-time evaluation that certifies concrete consisting of non-certified cement or other ingredients against this same standard. As part of the certification, NSF requires information on each ingredient in the site mix and details of its end use structure. Testing is performed on concrete cylinders manufactured from the mix, including analysis for the potential release of regulated metals, radionuclides, volatile organics and other contaminants that may leach directly into drinking water. Per NSF, results are typically available in 30 days or less.

The American Water Works Association (AWWA) D110 Standard describes current recommended practice for the design, construction, inspection, and maintenance of wire- and strand-wound, circular, pre-stressed concrete water-containing structures with four types of core walls. Type I tanks utilize a cast-in-place concrete wall, horizontal strand pre-stressing, and vertical post-tensioning. Type II tanks are constructed with shotcrete with an embedded steel diaphragm. Type III tanks are similar yet utilize precast concrete walls with an embedded steel diaphragm. Type IV tanks are castin-place concrete with a steel diaphragm. AWWA D110-04 standards are for concrete tanks that utilize wrapped pre-stressed concrete, and AWWA D115-06 applies to tendon pre-stressed tanks. In addition to the AWWA Standards, the American Concrete Institute (ACI) 301 Standard and 350 Code requirements describe current recommended practices for the design and construction of structural concrete elements. These include cast-in-place concrete water-containing structures, such as above and below grade concrete potable water storage tanks and clearwells.

Materials and Methods:

1. TEST APPARATUS

A polyethylene plastic water storage tank was fabricated and operated using a municipal potable water source under ambient climate conditions1. The primary observation tank, a 200 gallon poly tank, measures 45.5” tall by 36” in diameter, was left open at the top for easy access and equipped with gallon indicators and a 1.25” PVC Ball valve for adjusting water levels. Approximately 200 gallons of water were used for this study which was mixed daily to insure a homogeneous solution. Mixing was accomplished by draining the primary tank into a secondary polyethylene collection tank which was then recirculated into the primary tank with a 1/3 horsepower submersible pump.

IMAGE 1

Drain and fill cycles were intended to mimic actual fluctuations in water levels encountered in operational potable water storage tanks. Concrete coupons, measuring approximately 4” x 4” x 4”, were suspended from a non-reactive metal grid which could be raised and lowered manually with limited disruption for easy access to the coupons during sampling.

IMAGE 2

In addition to concrete coupons, an uncoated carbon steel coupon was also suspended within the tank to act as a potential iron source. The observance of iron was of importance as it is widely relied upon as a visual indicator of fouling in the field. It’s extensive monitoring by municipalities due to its multiple sources, use as an indication of corrosion, and negative affect on the aesthetic nature of water made iron an even more attractive candidate for monitoring mineral deposition for this study. The carbon steel coupon measured 6” x 4” and was 0.25” thick. The ratio of iron sacrificed from the coupon to water volume was hypothesized to be greater than what is typically found in finished potable waters, but was utilized to promote mineral accumulation and entrainment within biofilm deposits on the test coupons over the relatively short study period. All coupons were sterilized with a 50 ppm chlorine solution then submerged in the tank and monitored for the build-up of biofilm and mineral precipitates on a weekly basis, over a 30-day period.

Coupons with four different types of concrete surfaces (representative of the most common finishes used for concrete structures in the potable water industry) were used in this study: Cast, Wooden Float Finish, Light Broom Finish, and Heavy Broom Finish. Each surface type was tested with and without coatings present.

IMAGE 3: COATING APPLICATION

2) COATINGS

The coatings utilized as part of this study included Tnemec's Series 22 Epoxoline and Series N140 Pota-Pox Plus. Series 22 Epoxoline is a 100% solids, solvent-free, immersion-grade modified polyamine epoxy liner. The coating is normally applied between 20 and 40 mils Dry Film Thickness (DFT) in one or two coats using airless spray or plural-component spray equipment. Series 22 has passed stringent testing for extractables in potable water applications and is VOC compliant in all air districts. The Series 22 Epoxoline is ANSI/NSF Standard 61 certified for use in potable water storage tanks, reservoirs, pipes, and valves.

Series N140 Pota-Pox Plus is a 67% solids, solvent-based, polyamidoamine epoxy coating used for nearly two decades in potable water immersion service. Typically applied in two to three coats (maximum 17 mil DFT) by air or airless spray application, but can also be roller-applied. Low VOC and fast-cure versions of the coating are also available. The Series N140 Pota-Pox Plus is ANSI/NSF Standard 61 certified for potable water contact when used on storage tanks, reservoirs, pipes, and valves.

3) MICROBIAL COMMUNITY AND WATER QUALITY INDICATORS

A purely cultured colony of Pseudomonas aeruginosa was selected for its widespread occurrence in soil, water, and most man-made environments throughout the world. It is known for its ability to generate significant exopolymeric slime and is extensively studied in the scientific world and easily cultured in laboratory settings, further enhancing its appeal for use in this study. A viable bacterial community of was introduced into the tank with its growth and propagation monitored via ATP analysis.

ATP testing is a culture independent method for assessing bacteria, either within a water sample or on a surface, by quantification of a universal cellular component called Adenosine Triphosphate. ATP analysis is a simple chemical test which can quickly and accurately quantify a bacterial community by exposing microorganisms to an enzyme that breaks down the cell wall. As the cell wall is lysed, the cell's ATP molecules are released and then treated with the luciferase enzyme. The enzyme catalyzes a reaction which converts the ATP into light energy.

Light output is then measured by instruments call luminometers and compared with a standard to calculate total ATP. An analysis of this type is necessary as it is estimated that less than 1% of bacteria are culturable utilizing standard heterotrophic plate evaluation methods. This type of tests is needed to ensure the entire bacterial community is accounted for.

A large bacterial population, larger than what is typically found in finished potable waters, was utilized to promote surface attachment and growth on the test coupons over the relatively short study period. Regular drain and fill cycles were conducted to simulate typical turnover rates and air exposure experienced within potable storage tanks.

In addition to bacterial quantification, an assessment of mineral accumulation potential was also conducted. Iron was selected as an indicator of mineral accumulations due to its wide presence in various water systems, common association with biofilm formations, and significance in regard to staining and fouling.

IMAGE 4: SURFACE TESTING

4) SAMPLE COLLECTION AND ANALYTICAL METHODS

Sample collection on coupon surfaces was accomplished using swabbing techniques. Starplex's Starswab II swab devices were used for all testing. The Starswab II bacteriology culture collection and transport devices were specifically chosen for their consistency and reliability in in vitro diagnostic uses and compatibility with of a wide range of bacteria. A standardized template (13.69 cm^2 or 21/8 $inch^2$) was used in order to ensure a consistent sample area was maintained during all sampling events.

All sampling was conducted on vertical coupon surfaces. Once collected, swabs were processed using a proprietary method developed in house designed specifically for extracting and testing the common waterborne contaminants found in potable water systems. Each swab was thoroughly rinsed with ATP free water and placed in a vortex centrifuge for 30 seconds to extract biological and mineral contaminants. This process was repeated in triplicate for each sample to ensure good extraction from the swab device. The extracted contents were then suspended in asterilized, non-nutritional, buffered solution compatible with rapid screening methods. All testing was accomplished within 4 hours of the sample collection.

Iron, as an indication of the potential for mineral deposition, was introduced to the research tank via a carbon steel coupon suspended within the tank. Iron was chosen as it presents a common fouling mechanism for potable water systems.

Iron analysis was conducted following Standard Methods' Phenanthioline Method #3500Fe (EPA and AWWA approved method). Iron, reported as total iron, was tested after the prepared swab solution was shaken to re-suspend iron that has fallen out of suspension. The test was run to approximate all the iron that was in the sample, including ferrous, ferric and precipitate iron compounds of each.

For determination of total ATP, the BacTiter-Glo System (Promega, Madison, WI, USA) was used. The BacTiter-Glo Buffer was mixed with the lyophilized BacTiter-Glo Substrate and equilibrated at room temperature[1]. A sample of 50 µL of the prepared swab solution and an equal volume of BacTiter-Glo-reagent were then mixed and the luminescence of the sample was immediately measured with a luminometer (Model TD-20/20, Turner BioSystems, Sunnyvale, CA, USA). Cell quantities were calculated from total ATP measurements as follows:

$$\text{Population (cells/mL)} = \frac{\text{Total ATP concentration (fg/mL)}}{\text{ATP-per-cell (fg/cell)}}$$

* fg =

1- Room temperature was maintained at 20.5° Celsius (69° Fahrenheit)

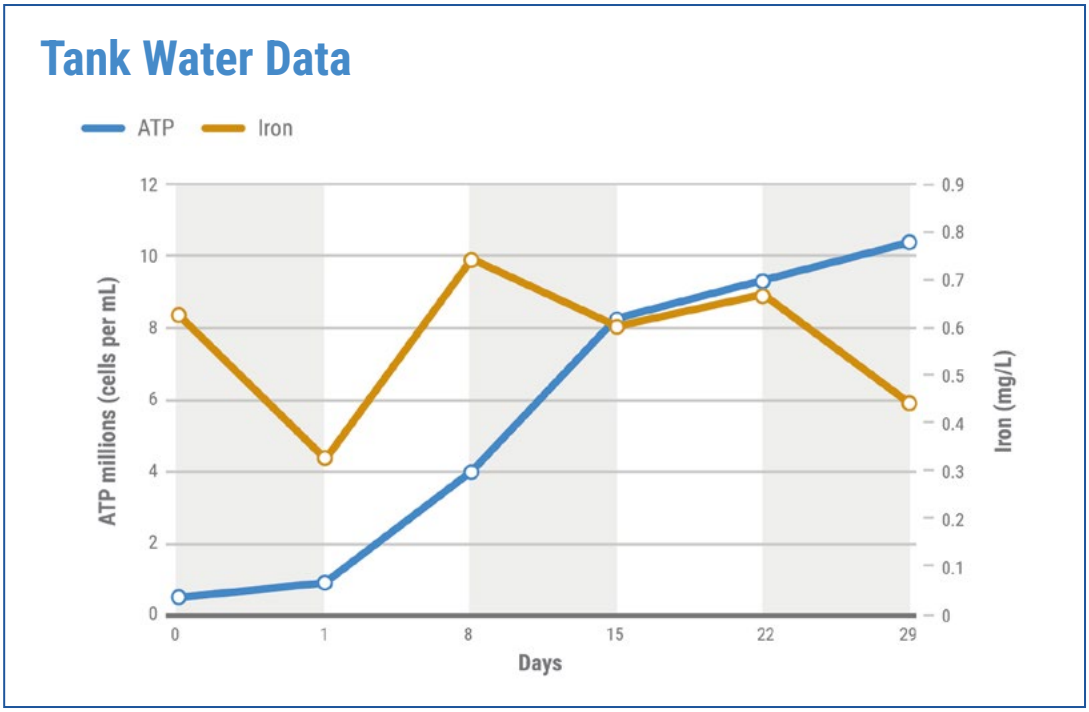
Results:

A total of 78 water and swab samples were collected and analyzed over 30 days. ATP and iron data obtained from coupon swab samples were averaged according to coating type. For example, ATP levels for all raw concrete surfaces (includes four different surface finishes mimicking current construction techniques) were averaged together for each day sampling occurred.

Prior to submersion, all coupons were sterilized using a 50 ppm chlorine solution to reduce all bacterial activity as much as possible, and swabbed to verify disinfection efforts. Following disinfection, average cell counts on all uncoated concrete surface types were ≤40,000 cells per milliliter and less than 30,000 cells per milliliter on coated surfaces. Total disinfection was not found to be necessary considering completely sterile surfaces are very rarely found outside of laboratory settings. In this case, pre-submersion cell counts were found to be representative of field conditions.

Analysis of the tank water prior to submersion found elevated levels of planktonic, or free swimming, bacteria present. As mentioned earlier, elevated bacteria counts within the water were desired to promote cell attachment and biofilm growth over the relatively short span of the study. Average cell counts for the tank water for the 30-day period were calculated at approximately 7.6 million cells per milliliter, as shown in Figure 1.

Figure 1. Tank Water ATP and Iron Levels over Time



Iron analysis of the tank water showed gradually increasing levels over the course of the study, corresponding to the gradual introduction of iron being shed from active corrosion of the suspended carbon steel coupon. The purpose of the iron coupon was to provide a known and constant source of minerals for deposition and evaluation. As with the supply of bacteria, the carbon steel coupon remained within the tank to facilitate iron mobilization and promote any potential fouling on the surfaces being analyzed.

After 24 hours of submersion within the inoculated tank, all coupons were temporarily raised from the tank and swabbed on a designated vertical surface. ATP analysis showed that after the 24 hour period, average cell counts across uncoated concrete surfaces grew to nearly 300,000 cells per milliliter, while counts on both coated surfaces remained at ≤30,000 cells per milliliter. Increases of this magnitude in uncoated concrete surfaces indicated a much higher degree of bacterial attachment and biofilm formation across those surfaces as compared to surfaces utilizing protective coatings. This trend continued over the next two sampling events conducted at Day 8 and Day 15. Over the first 15 days of the study average bacterial counts reached approximately 6.6 million cells per milliliter on uncoated samples. During the same period all coated surfaces saw much lower but consistent population growth. Average counts on both coating types remained under 575,000 cells per milliliter through the first half of the study.

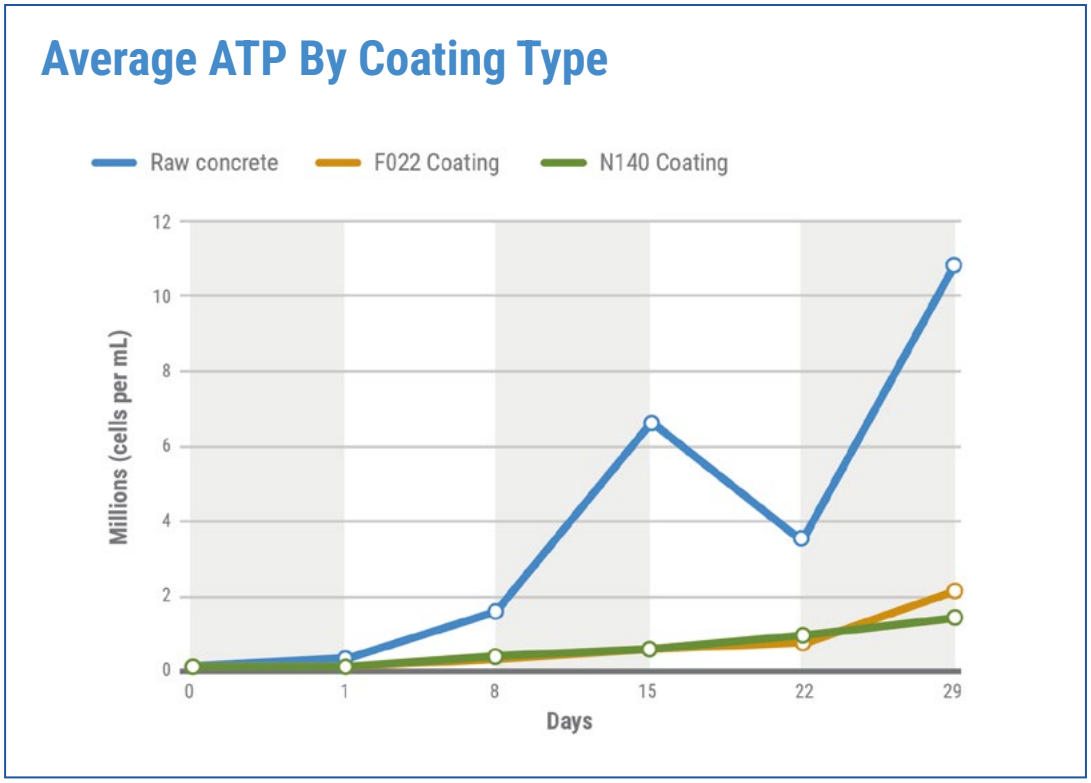


Figure 2. ATP Levels on Coated and Uncoated Coupons over Time

ATP analysis conducted on Day 22 indicated a drop off of average bacterial populations on uncoated concrete surfaces. At the same time, concrete samples with either coating maintained their low but steady growth rates. Anomalies such as this are not uncommon to bacteriological studies as biofilm is not a uniform deposit and sample collection location could be involved with such results.

Samples taken on Day 29, the final day of the study, showed bacterial populations on uncoated concrete surfaces had returned to previous growth rates seen in the first half of the study. ATP analysis showed an average of 11.1 million cells per milliliter in the prepared swab solutions from uncoated surfaces. Coupons with the coating type F022 did show a small uptick in cell populations but remained relatively low at an average of 2.1 million cells per milliliter across all four surface types. The average population size of coupons coated with N140 was only 1.4 million cells per milliliter.

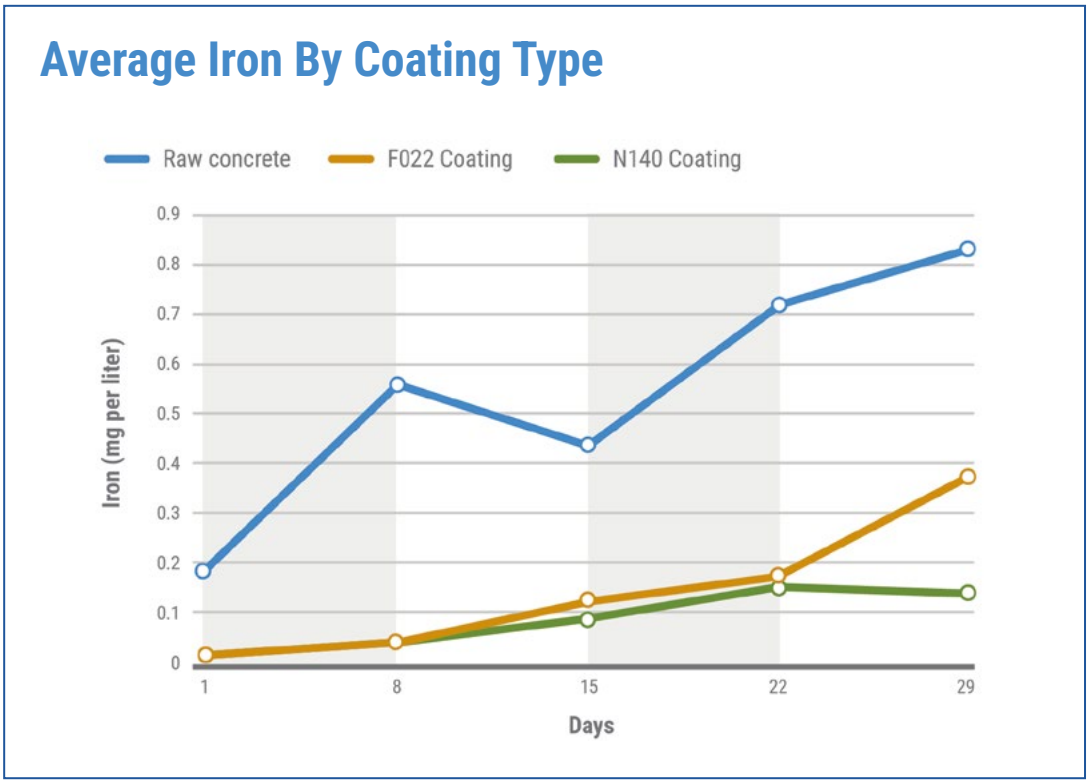


Figure 3. Iron Levels on Coated and Uncoated Coupons over Time

IMAGE 5

Overall, averaged iron levels, introduced from the raw steel coupon, in all samples corresponded to bacterial population growth obtained via ATP analysis. The involvement of iron entrainment into biofilm is a common activity and can be an indicator or mineral deposition. The presence of iron staining is a visual indicator of system fouling that can impact system disinfection and water quality. As noted in figure 3 both coating types appeared to maintain much lower iron concentrations throughout the course of the study, as compared to uncoated surfaces.

A comparison of iron levels on the coupon surfaces to those seen in the tank water analysis showed that iron levels deposited on the uncoated concrete coupons were nearly equal to the concentrations present in the water, throughout the course of the study.

Summary of Results:

In summarizing the project results, we note that all coupon surfaces were disinfected and tested for bacterial presence utilizing the ATP bacterial quantification test method. The initial level of bacterial cells per milliliter on all coupons was < 40,000.

At the initial testing interval of 24 hours, or one day, the bacterial concentration on the coated concrete coupons was noted to have no change while the concentration on the uncoated coupons had increased from approximately 30,000 to over 300,000 cells per milliliter. These one day results on the uncoated coupons do show that the planktonic organisms free swimming in the tank water are capable of immediate attachment and proliferation into the commonly referred to biofilm. The test results from the next four intervals (Day 8, Day 15, Day 22, and Day 29) show a similar trend difference in the coated versus the uncoated concrete coupons. Results also indicated the bacteria initiated the formation of biofilm on the coated coupons in subsequent testing intervals. Testing conducted at the completion of the study showed an average of six times higher concentrations of bacteria on the raw concrete coupons than the coated coupons.

Correspondingly, the iron levels, recorded as total iron, increased from below the detection limit at the initial reading to an average of over 0.2 mg/l on the day one analysis of the uncoated coupons. The coated coupons showed an average of 0.02 mg/l iron on the day one analysis. This trend difference is somewhat similar throughout the 30 day testing period with the overall average results showing and average of 5 times more iron concentration on the uncoated coupons than the coated coupons. Noted in the results is the comparison that the iron concentration on the uncoated concrete coupons is the same as the iron concentration of the tank water. These results would indicate that the available iron is easily deposited out of the system water without some influence to its attachment and can result in system staining and fouling.

Conclusion:

The presence of biofilm deposits within a water system can impact supply, treatment, storage, and distribution. The issues can range from impacts on system efficiency, to disruption of treatment process, to degradation of produced water quality. Monitoring of the biological community is often largely limited to regulated coliform testing and evaluation of disinfection residuals within the distribution system.

Quantification of the bacteria present, or assessment of the accumulation and development of biofilm, is not currently a requirement and therefore not commonly included in system monitoring. With regards to the bulk accumulation of natural organic carbon that makes up the biofilm; these natural organic carbons can produce undesired disinfection-by-products when reacting with the halogen chemicals used in system disinfection. Additionally, disinfection chemicals react with the organic composition of the biofilm, which includes the multitude of biological communities, subsequently depleting residuals of the disinfection chemicals and challenging the regulated control limits.

The implications of biofilm accumulation within water storage facilities is not unknown. EPA research reported this in their 2002 report on Finished Water Storage Facilities. In this study, it was assessed that “Water quality problems associated with finished water storage facilities with direct potential human impact include disinfectant decay, microbial regrowth, chemical contamination, nitrification, pathogen contamination, disinfection by-product formation, and sediment accumulation (US DPA).” As research has shown finished water storage facilities are susceptible to biofilm growth and development, including concrete surfaces.

The objective of this research was to determine if biological activity, as measured by adenosine triphosphate (ATP) concentration and iron precipitants, on concrete surfaces could be limited or reduced by the use of commercially available protective coatings. The benefits of any inhibition to the development of biofilm are noted in the multiple negative effects that the presence of biofilm has within a potable water system. Concrete water storage facilities have long been touted as standalone entities with no proven need for coating or regular maintenance including cleaning and disinfection.

The results of this research, shown graphically in Figure 2, are inconsistent with the "standalone" beliefs regarding concrete storage facilities. As shown in the resulting test data of ATP levels on the various concrete coupons the raw coupons exhibited a considerably higher level than either of the coated coupons. The difference in bacterial concentration was established in the initial testing interval of 24 hours showing ten times more bacteria on the raw uncoated concrete coupon than on the coated coupons. This difference continued throughout the thirty day project with an overall difference of over six times more bacteria on the raw uncoated concrete coupons. Therefore, this research has shown that raw uncoated concrete surfaces are more susceptible to biofilm development and accumulation compared to coated surfaces due in part to the increased surface area and porous nature of the finished structure and that coating the surfaces of concrete structures can inhibit the formation and accumulation of biofilm and the resulting negative effects.

Footnote:

References:

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