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How to Properly Sample Water Systems • Part 1

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Poorly executed sampling is the cause of most excursions from microbial control trigger values and specifications. Confusion exists over the various reasons and purposes of sampling which affect the sampling procedure that can be used. Also, the ignorance over how bacteria grow in water systems as biofilms as well as how these biofilms are affected by the shear forces of water flow velocities across their surfaces has led to misperceptions of how to use water from water systems to minimize contamination. These same misperceptions carry over into how to properly sample that water for process control and quality control purposes. In Part 1 of this article, you will learn how the flow velocity in a pipe affects the maximal thickness a biofilm can achieve and how that phenomenon can be used to our advantage in sampling. Part 2 of this article will focus on the actual procedures that should be used for sampling that will avoid most of the variability associated with microbial testing of water samples.

What is the Purpose of Your Sampling?

The purpose of your sampling determines how and where (and of course, why) that sample is collected, so it is very important to understand what you will be doing with the data that will be generated from the tests on those samples. There are usually only 4 reasons for sampling a water system:

1. To routinely monitor the water system operation to assure it is continuing to produce good quality water,
2. To routinely determine the quality of the water that is being used to assure it is still suitable for those uses,
3. To investigate operational or quality problems detected during routine monitoring, and
4. For information only (FIO).

In the case of 1, this purpose is for process control. The results of the tests on those samples can trigger remedial action if the values are out of trend for your water system based on trend-based Alert and Action Levels. Corrective and preventive actions can be executed to bring the system back under control, and avoid poor results from happening again.

In the case of 2, this purpose is quality control. The results of the tests on those samples will tell the user if the water meets the established quality standards for the water and is suitable for use, just like any other raw material used in your process.

In the case of 3, this investigative purpose hopefully occurs only episodically, typically when either the process control or quality control sampling indicate that there is a problem that needs to be corrected. This special investigative sampling is intended to help verify the problems and ferret out the root cause(s) of these problems to assist in effective remedial action.

In the case of 4, this informational purpose may be for establishing long term trends or for benchmarking a particular quality at a specific point in time for future reference. A classic example is during water system validation; one might want to specifically document the quality of the source water for specific non-routinely tested chemical or microbial attributes when the validation exercise is occurring. This testing does not have to be assessed against any performance criteria or specifications and may only have historical significance at some future point in time, such as when other investigative testing is being done to determine the source of a purification performance problem that may occur several years later.

What is the Distribution of the Water Contaminants Being Tested?

The chemical contaminants, usually expressed as conductivity and TOC levels if it is a finished compendial grade of water, are reflective of dissolved ionic and organic impurities. These tend to be homogeneously distributed throughout the system's water unless there are localized contamination sources such as leaking sub-loop or point-of-use heat exchangers or a back-flow of process fluids from a point-of-use connection to process equipment. Therefore, because of the typical homogeneity of these types of contaminants, the sampling process is usually quite simple with minimal precautions. The only precautions needed are those associated with any trace level testing: use scrupulously clean containers and avoid the outside environment affecting the test result. Because of these simple concerns, sampling to detect chemical contaminants is not the focus of this article.

The microbial-based contaminants, typically expressed as a microbial count or endotoxin level in the water, may or may not be homogeneously distributed within the system. The microbial counts detected in a water sample are planktonic or free-floating bacteria originating from biofilms, surface microbial colonizations within the system. If there are one or more biofilms growing on the piping in various locations within the recirculating distribution system or within the final purification unit operation, then these planktonic bacteria tend to be relatively evenly distributed in the circulating water, with some random variability due to episodic sloughing of biofilm flocs (clumps of slime-embedded cells) sheared from the surface of biofilms or intentionally released naked "pioneer" cells.

However, if there are also biofilms thriving within point-of-use valves or sampling ports in the distribution system or within heat exchangers, flow totalizers, or hoses or other piping connected to these sampling or use-point valves, then additional localized biofilm sloughing and pioneer cells can be added to the water stream by these "contaminated" ports as the water is leaving the system during use or sampling.

How Water Flow Affects Biofilm Height

When a biofilm grows on a water system surface, the velocity of the water flow at that location determines the maximum thickness that can be attained by that biofilm as well as its tenacity with the surface. If the water is turbulent, it tends to shear off the fragile tops of biofilms. However, even with high velocity, highly turbulent flow, it is not turbulent near the pipe surface. The closer you get to the surface, the less turbulent and slower the velocity of the water is. As the pipe surface is approached, the flow changes from turbulent to laminar. Biofilm best grows and remains intact within the laminar flow region next to the surface. The transition point between turbulence and laminarity is called the laminar sublayer or boundary layer, and that usually demarks the maximum height that biofilm can achieve at that flow velocity if given enough time to grow to that height. Any part of the biofilm structure that extends above the laminar sublayer into the turbulent zone is at high risk of being sheared off by that turbulent flow. Of course, if biofilm development is interrupted or inhibited or even killed by intermittent hot water or other sanitizer treatments, it may never achieve an easily shearable thickness.

A study by Pittner and Bertler* calculated this boundary layer or laminar sublayer thickness as a function of water velocity in various diameters of piping as shown below:

Laminar Sublayer Thickness (microns)

Pipe Size	Velocity (ft/sec)					
E, I , RDS	0.2	1.0	2.0	5.0	8.0	12.0
0.428" IS	*	*	125	55	37	26
1/2" Sch 80	*	*	136	60	40	28
1" Sch 80	*	265	146	65	43	30
2" Sch 80	537	291	158	69	46	32
3" Sch 80	563	305	165	74	48	33
4" Sch 80	582	312	170	75	50	34

**Pittner, G.A.; Bertler, G "Point-of-use Contamination Control of High Purity Water Through Continuous Ozonation", Ultrapure Water 5(4), pp. 16-22 (May/June 1988).*

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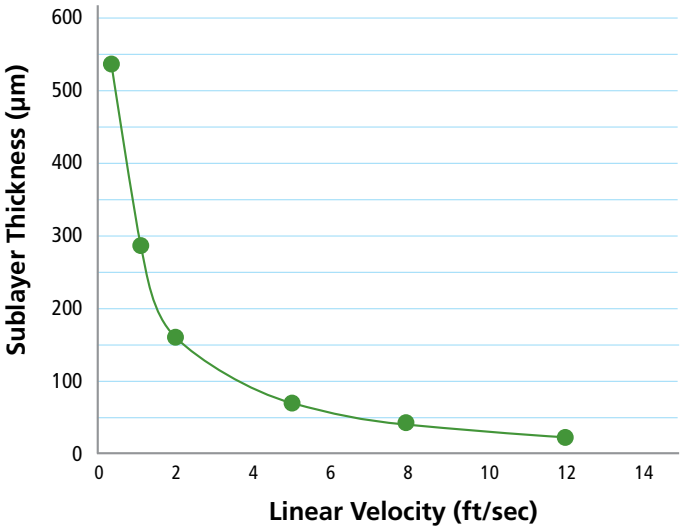
A plot of the above data showing the laminar sublayer thickness in a 2 inch diameter pipe as a function of linear water velocity is shown below:



These data say several things:

1. At velocities above 12 ft per second, an increase in linear velocity does not substantially reduce the thickness of this boundary layer. And there is only a minor change in boundary layer thickness at velocities above 8 ft per second.
2. At the reasonably commonly designed flow velocity for pharmaceutical water systems of about 4 feet per second, that boundary thickness (and therefore maximal biofilm height) could be as much as about 100 micrometers if given enough time to grow between sanitizations. An astronomical number of biofilm bacteria with a typical 1 micron dimension could be packed into a 100 micron thick biofilm.
3. At lesser flow velocities, the boundary layer substantially thickens until where there is barely any flow at all or stagnation; given enough time between growth-inhibiting treatments or sanitizations, the biofilm could become as much as 1 mm thick or more. Such a condition could exist within little-used valves, even sanitary ones. A cubic millimeter of this biofilm slime could contain many millions of bacteria waiting to be partially sloughed off into the water flow during the next opening of that valve. This is an important concept that will be germane to discussions in Part 2 of this article.

Conclusions

Maximal biofilm thickness is a function of water velocity since the biofilm will always be limited by the boundary layer. Artificially reducing the thickness of a biofilm in a given location (such as within a sample port) by substantially increasing the flow rate offers the opportunity to remove substantial amount of biofilm from a sample port before sampling and therefore reduces the amount of water contamination contributed by that valve. This is a well-known phenomenon. The problem is that this pre-flushing is often not done with sufficient velocity or in a variable manner from sampler to sampler or location to location.

In the next newsletter, Part 2 of this article will delineate how to standardize this sampling procedure, the advantages of doing so, and why the optimal procedure may not usable for QC sampling. To download Part 2, click here.

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Opportunity

Opportunity is missed by most people because it is dressed in overalls and looks like work.

- Thomas Edison

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