EXPLORING FLOW CYTOMETRY FOR MONITORING OF MICROBIAL WATER QUALITY DURING MAINTENANCE OF DRINKING WATER PIPES

UNDERSÖKNING AV FLÖDESCYTOMETRI SOM METOD FÖR ÖVERVAKNING AV MIKROBIELL VATTENKVALITET VID UNDERHÅLL AV DRICKSVATTENLEDNINGAR

Måns Zamore
VA SYD, Teknik & Utveckling, Box 191, SE-201 21 Malmö, mans.zamore@vasyd.se

Abstract

Faster methods of analysing microbiological quality of drinking water after pipe breaks or new pipe installations could have beneficial implications. In this article, a summary is presented of the findings in a recent master thesis about how flow cytometry and cultivation-based techniques can be used to monitor water quality in connection to maintenance works, and how flow cytometry can aid in the decision-making around putting drinking water pipes in operation. Previously, online flow cytometry has not been used to assess microbiological water quality after pipe breaks, nor has the influence of the biofilm formation process on bacteria in the bulk water been well understood. Here, it is described how online flow cytometry combined with advanced computational methods can be used to yield nuanced microbiological information. The use of flow cytometry during maintenance work will be further evaluated in the ongoing SVU project *Safe and smart pipe installations*.

Sammanfattning

Snabbare metoder för att analysera mikrobiell dricksvattenkvalitet efter akut eller planerat underhåll av ledningsnätet skulle kunna leda till stora samhällsvinster. I den här artikeln presenteras en sammanfattning av ett nyligen slutfört examensarbete om hur flödescytometri och odlingsbaserade tekniker kan användas i samband med underhållsarbeten och hur flödescytometri kan underlätta i beslutsfattandet kring att sätta dricksvattenledningar i drift. Tidigare har inte använt online-flödescytometri använts för att bedöma mikrobiologisk vattenkvalitet efter underhåll, och inte heller har påverkan från biofilmbildningsprocessen på bakterier i vattnet varit väl förstådd. Här beskrivs hur online-flödescytometri kombinerat med avancerade beräkningsmetoder kan användas för att ge nyanserad mikrobiologisk information. Användningen av flödescytometri vid underhållsarbeten kommer att utvärderas vidare i det pågående SVU-projektet *Säkra och snabba ledningsnätsarbeten.*

Keywords: drinking water, flow cytometry, microbiology, maintenance

1 Introduction

During maintenance of drinking water pipes, either acute or planned maintenance, several microbiological challenges are presented. For instance, pathogenic microorganisms can enter the pipe during maintenance. One way to prevent this from happening is to flush the pipes with large volumes of drinking water (Van Nevel et al. 2017). This water ends up in the wastewater system. The waste of water — as well as other factors relevant to drinking water distribution network interventions, such as reduced water supply, disturbed traffic conditions, and increased workload for personnel — leads to substantial costs both for water utilities and other parts of society.

With the large costs associated with maintenance of water pipes, there is a potential benefit if maintenance works can be completed faster. A major bottleneck is the time required for microbial analysis. Current microbiological methods require incubation for 1–7 days depending on the method and which microorganisms are of interest (SLVFS 2001). Therefore, faster methods of analysing drinking water have the potential of reducing the time of maintenance with several days.

Another relevant microbiological aspect that could reduce the time spent on maintenance is a more in-depth understanding of the natural microbiological processes that occur in new or repaired pipes. It is well-established that drinking water pipes are lined with a layer of biofilm, usually formed by non-pathogenic microorganisms (Flemming 2002). New drinking water pipes will, however, not have a biofilm initially. This may affect the number of bacteria that are found in the water samples. Søborg et al. (2020) found indications that the number of bacteria measured by cultivation in new pipes follows a specific pattern, which could be a result of the formation of biofilm. If such a pattern is generally occurring in new pipes, valuable conclusions about when to take samples in new pipes, timeframe of the work and what to expect could be drawn.

This article describes part of the work presented in the master thesis entitled *Exploring Flow Cytometry for Monitoring of Microbial Water Quality During Maintenance of Drinking Water Pipes* (Zamore 2021). The thesis is part of a project that investigates smart and safe maintenance operations in the drinking water distribution system. Involved water utilities are VA SYD and Sydvatten, and their joint research and development company Sweden Water Research. The data used in Zamore (2021) was therefore provided by VA SYD and Sydvatten. The project has received a grant from Svenskt Vatten Utveckling (SVU), and will continue until April 2022.

2 Methods

2.1 Heterotrophic plate count (HPC)

Current methods of assessing microbiological drinking water quality after maintenance or installations of new pipes are based on cultivating bacteria on agar plates. One such method is the heterotrophic plate count (HPC), which is performed by mixing a small volume of water sample with dissolved yeast extract agar in petri dishes and then let to solidify. Thereafter, the plate is incubated for three days, after which the number of colonies are counted.

2.2 Flow cytometry

Flow cytometry is a technique which has existed for decades but has begun being used for drinking water analysis since 2008 (Hammes et al. 2008). The technique involves passing sample into a narrow chamber where particles are aligned in a single file. Laser beams are then used to strike each particle, and detectors measure how the light is scattered. For drinking water analysis, the particles of interest are often bacteria. Fluorescent dyes are available that can be used to label intact or dead cells, by binding to their DNA.

Flow cytometry has numerous advantages over the cultivation-based methods that are currently used for assessing water quality. Compared to HPC, the method is fast, sensitive, reproducible, and possible to automate (Van Nevel et al. 2017). It is possible to reach a result after only 15 minutes rather than 3-7 days that are required for cultivation-based methods. The entire bacterial community in the water can be counted rather than the approximate one percent that is measured by HPC (Van Nevel et al. 2017). However, flow cytometry

Figure 1. *The pipe break that occurred between Teckomatorp and Västra Strö. Photo by Sydvatten AB.*

has its limitations, including difficulties in clearly distinguishing between live and dead cells, that the data analysis is prone to subjective interpretations and that aggregates or clumps of bacteria are difficult to handle properly (Van Nevel et al. 2017). The method is yet to have gained regulatory status for drinking water quality assessment in any country except for Switzerland (Safford and Bischel 2019).

2.3 Collection of data

Data is presented from two different occasions: a major leak denoted the Teckomatorp pipe break and one new pipe installation in Torna Hällestad that was sampled in March-April 2021. Sampling was performed by operating personnel at the water utilities. The data presented consists of flow cytometry analyses made with online flow cytometers (for the Teckomatorp leak) and laboratory cytometers (for the Vomb leak and Torna Hällestad pipe installation), as well as HPC.

2.3.1 Teckomatorp pipe break

A major leak occurred between Teckomatorp and Västra Strö in September 2020 (fig. 1). The pipe has the diameter 1000 mm and is made of fiberglass. After the leak occurred, the pipe was closed at Västra Strö and an additional leak occurred in Teckomatorp. Repairs were thereafter made, and the pipes were then flushed with drinking water for approximately two weeks. During this time, online flow cytometers (Sigrist BactoSense) were set up at Västra Strö and Teckomatorp. The sampling was started simultaneously as the flushing of the pipe was begun, which was after the reparations were completed. Samples were taken at 4-hour intervals at Västra Strö and hourly at Teckomatorp. The distance between Västra Strö and Teckomatorp is around 10 km.

2.3.2 Torna Hällestad pipe installation

A new pipe installation in Torna Hällestad was sampled after being put in operation, from 23rd of March to 16th of April 2021. The pipe installation was performed to accommodate for a newly built residential area. The sampling was begun after the new pipe had been installed. The pipe was continuously flushed with drinking water during the sampling period. Samples were taken approximately every two days, excluding weekends and holidays. Sampling points included were a reference point and a sample point at the end of a new sec-

tion of pipe. The reference point is from a nearby pipe that, according to the projectors, should not have been affected by the new pipe installation. The pipe is of the material polyethylene (PE) and of the diameter 160 mm. The pipe has connections to another older pipe of diameter 80 mm making it possible (albeit unlikely) that water can temporarily flow in both directions, which is typical for smaller pipe systems feeding residential areas. The sampling was performed via a valve connected to a small hose in plastic. Samples were analysed with HPC and one of two separate laboratory flow cytometers (BD Accuri C6 Plus) – a few samples were analysed at Sydvatten's laboratory in Stehag but most samples were analysed at VA SYD's laboratory in Malmö.

3 Results

3.1 Teckomatorp pipe break

To construct an overview of the microbial communities in the samples of the Teckomatorp and Västra Strö sampling points using flow cytometry data, PCoA plots of the Bray-Curtis dissimilarity matrixes were made. PCoA plots were made both resampled to the minimum number of cells in the dataset (fig. 2), and non-resampled (not shown). Resampled plots are thereby independent of changes in the number of cells, unless these changes are also accompanied by microbial community changes. Both the resampled (fig. 2) and non-resampled (not shown) plot had a similar appearance, with two separate clusters for the two sampling points and the first seven samples from Teckomatorp deviating from these clusters. The plot is clear in that the measured fluorescence is different among the Teckomatorp and Västra Strö sampling points. Furthermore, it is clear that the samples from the first 7 hours at the Teckomatorp sampling point were different since these samples appear outside the cluster (fig. 2).

The Bray-Curtis dissimilarity metric was also calculated for each sample in this dataset (fig. 3). The underlying principle behind this figure is similar to the PCoA plot (fig. 2) but by plotting the

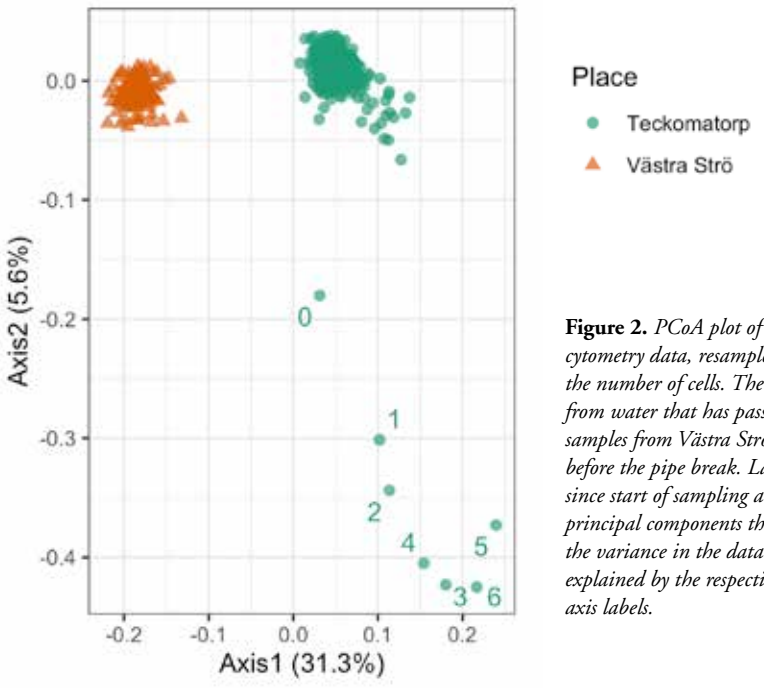

Figure 2. *PCoA plot of the Teckomatorp pipe break flow cytometry data, resampled and thereby independent of the number of cells. The Teckomatorp samples were taken from water that has passed the pipe break section, while samples from Västra Strö were used as a reference point before the pipe break. Labels indicate number of hours since start of sampling and flushing. The axes represent principal components that explain a certain part of the variance in the data. The percentage of variance explained by the respective components is stated in the axis labels.*

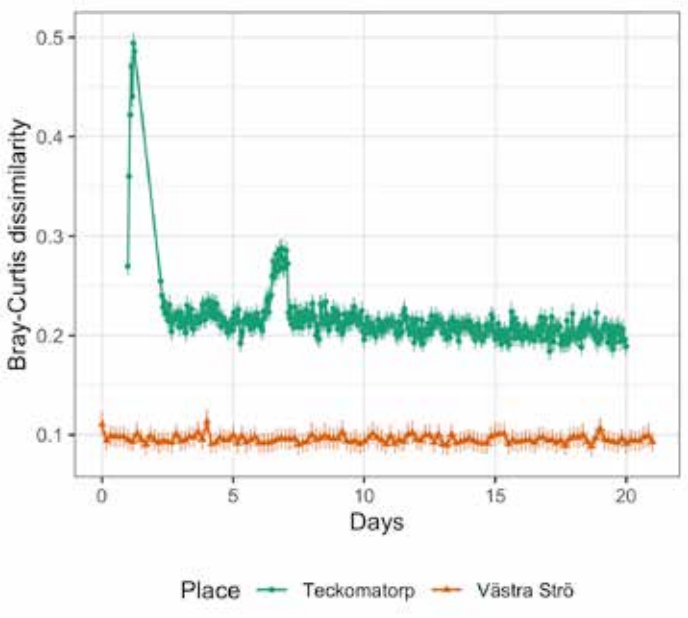

Figure 3. *The Bray-Curtis dissimilarity metric calculated from gated data from online flow cytometers set up at the Teckomatorp and Västra Strö sampling points. Day 0 was when the flushing began, after the repairs had been completed. Error bars corresponding to the standard deviation are displayed in semi-transparent colors.*

dissimilarity as a function of time, further insights can be gained. The figure (fig. 3) shows that the first seven samples differed from the other samples at the sampling point, with their dissimilarity metric differing from the subsequent samples. It is evident that elevated values appear around day 6–7 in the Teckomatorp sampling point. The plotted lines for Teckomatorp and Västra Strö do not overlap, which implies that the microbial communities at the two sampling points were not the same. This can be compared with the PCoA plot (fig. 2), which shows two clusters that do not overlap. In other words, the results from the PCoA plot and the Bray-Curtis dissimilarity metric are the same, but the presentation of the dissimilarity metric may be more appropriate when the variable of interest is time.

3.2 Torna Hällestad pipe installation

The data from the Torna Hällestad pipe installation consists of flow cytometry samples and HPC from two different sampling points: a reference sampling point and a sample point at the end of a new section of pipe. Both HPC and flow cytometry total cell counts are plotted (fig. 4). Notably, the HPC increased 8-fold after day 14 and doubled in the subsequent sample, reaching values well above the threshold accepted by regulations (100 cfu/ml). For the flow cytometry total cell counts, the values were between 375 000 and 740 000 cells/ml, and the difference between reference and sample was at most two-fold for day 20. The increases in HPC at the sample point were accompanied by increases in flow cytometry total cell counts (for instance, with HPC increasing from 22 cfu/ml to 180 cfu/ml from day 14 to 18 and total cell counts increasing from 525 000 to 575 000 the same days). Note that the total cell counts for the reference point varied considerably between 380 000 cells/ml for day 4 to 520 000 cells/ml for day 14.

Further analysis was also performed on the flow cytometric data, including calculation of the Bray-Curtis dissimilarity metric (fig. 5). All three of these methods may elucidate changes in the microbial communities. Some insights could be derived from this Bray-Curtis dissimilarity metric figure (fig. 5), for instance the microbial communities are clearly different in the last three samples between reference and sample points. These samples also differ considerably in the number of total cells (fig. 4).

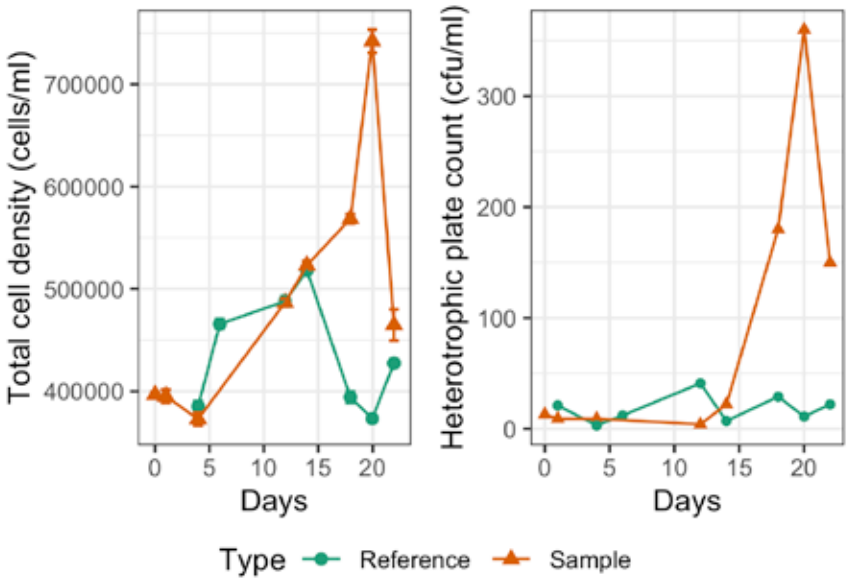

Figure 4. *Total cell density and HPC for Torna Hällestad. Total cell densities were measured in triplicates with one of two separate laboratory flow cytometers (either at VA SYD's laboratory or at Sydvatten's laboratory). Error bars correspond to the standard deviation. HPC values were determined by cultivation. The reference point is from a nearby pipe that should not have been affected by the new pipe installation.*

4 Discussion and conclusions

It was of interest to investigate whether faster microbiological analysis methods can replace those currently used after maintenance works or new pipe installations. Some insights about the use of flow cytometry have been gained. Online flow cytometry has proven to be especially useful due to the short intervals of sampling, and novel computational methods have also proven to be useful for analysing the flow cytometry data.

4.1 Flow cytometry

4.1.1 Teckomatorp pipe break

To begin with, both the PCoA plot (fig. 2) and the Bray-Curtis dissimilarity (fig. 3) metric calculation for the Teckomatorp pipe break provide valuable characterisations of changes in the microbial communities. It is clear that the first seven hours of sampling downstream of the pipe break deviate microbiologically. After another 5 days, the dissimilarity metric increased again, and was relatively high for approximately 1 day. The cause of this increase is unknown and was not reflected in the total cell counts nor in the HPC or the slow-growing bacteria. The increase of the dissimilarity metric clearly indicates that there was a shift of the microbial community during this time, which then returned to normal. Possible explanations may be changes in the flow, stagnation of water at the sampling point, contamination with soil or other foreign matter, or malfunctioning of the instrument. This increase is an example of potentially valuable information that can be gained from using online flow cytometers in maintenance settings.

4.1.2 Torna Hällestad pipe installation

The Torna Hällestad pipe installation is different from Teckomatorp primarily in that it is not an example of a major pipe break which is repaired, but rather an installation of a new pipe. This implies that, contrary to in the Teckomatorp case, no biofilm existed before the start of sampling, although some organisms could be present from the manufacturing and transport of the pipe. Another difference is that the diameter of the pipe was smaller than in the Teckomatorp case – at 160 mm com-

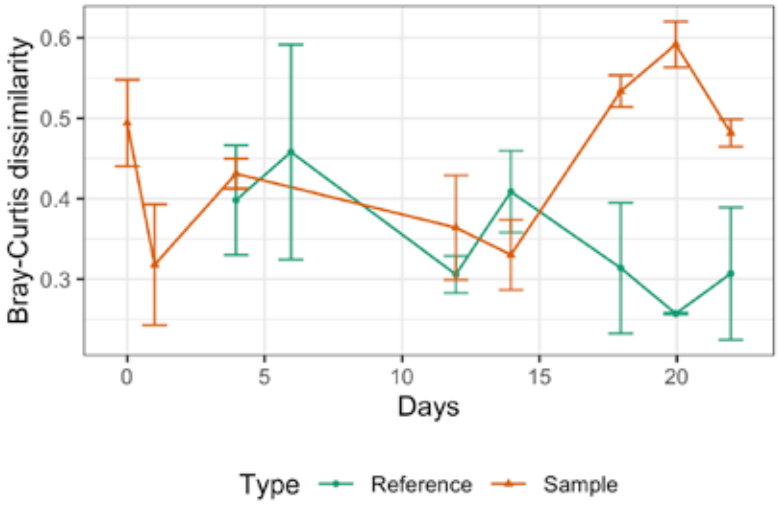

Figure 5. *The Bray-Curtis dissimilarity metric calculated from reference samples and samples from the Torna Hällestad pipe installation. Samples were analysed with one of two separate laboratory flow cytometers. Error bars corresponding to the standard deviation of triplicate samples are displayed in semi-transparent colors. The reference point is from a nearby pipe that should not have been affected by the new pipe installation.*

pared to 1000 mm. This leads to an increase in surface-to-volume ratio, i.e. the inner surfaces of the pipe have an increased contact with the bulk water compared to larger diameter pipes. Consequently, it can be expected that the biofilm formation has a larger effect in smaller pipes than larger pipes, whatever that effect may be.

As the cell count appears to vary independently of time, the observed variations in the flow cytometry data from Torna Hällestad did not seem to primarily be a function of time since start of sampling (fig. 4). Factors that may have a larger impact, and which may obscure any patterns that are of interest related to biofilm formation, may include time of sampling, flow rates, stagnation of water etc. (Gabrielli et al. 2021). Further supporting this hypothesis is the fact that the reference point exhibits large variations between 380 000 cells/ml to 520 000 cells/ml. Clearer results may have been gained if such factors could be kept constant and controlled, and if the sampling frequency could be increased to sample for instance hourly with online flow cytometers. In other words, it is preferable to choose online flow cytometers above laboratory flow cytometers due to the increased sampling interval that is possible with online cytometers.

4.1.3 Computational methods of analysing flow cytometry data

It has become clear that flow cytometers generate vast datasets with a wealth of microbiological information that may be difficult to interpret. The field is rapidly evolving with interesting methods of analysing flow cytometric data being published the last few years. Efforts are underway to apply such computational methods to drinking water monitoring, as by Sadler et al. (2020) and Favere et al. (2020). Future research areas can include evaluations of different advanced fingerprinting methods for drinking water monitoring, as well as more intricate computational analysis methods including machine learning. Such advancements may increase the usefulness of flow cytometry for drinking water analysis, providing more nuanced information than the currently used HPC.

4.1.4 Comparison between HPC and flow cytometry

It can be argued that HPC is no longer the most appropriate method for analysing bacteria in drinking water. Flow cytometry has numerous advantages over HPC, including speed, reproducibility, automation and sensitivity. Therefore, the method should be able to replace or at least complement HPC for evaluating drinking water quality after maintenance or new pipe installations. It can be noted that the results from the methods cannot be expected to correlate (Van Nevel et al. 2017), but this should not serve as an argument against flow cytometry. Rather, the lack of correlations elucidates flaws in the HPC method.

4.2 Conclusions

To conclude, flow cytometry has shown to be a sensitive and feasible method of assessing changes in microbial water quality. Recent computational methods, including calculations of the Bray-Curtis dissimilarity metric from flow cytometric fingerprints, present a more nuanced analysis than merely cell counts. New computational methods can likely improve the usefulness of flow cytometry even more. The use of flow cytometry during maintenance work has the potential of reducing the time required for flushing of water pipes, and consequently also the volumes of water needed for flushing. Furthermore, indirect costs for society may be reduced by shortening the time of maintenance. Flow cytometry and applications of the method in maintenance work will be further evaluated in the ongoing SVU project *Safe and smart pipe installations.*

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