APPROACHES TO MONITOR AND CHARACTERIZE THE BIOLOGICAL STABILITY OF DRINKING WATER DISTRIBUTION NETWORKS

Volume III: Strategy for the Use of Flow Cytometry for Drinking Water Monitoring in South Africa

Report to the
Water Research Commission

by

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This report forms part of a set of three reports. The other reports are:

Approaches to Monitor and Characterize the Biological Stability of Drinking Water Distribution Networks. ***Volume I****: Establishing Correlations between Flow Cytometry Cell Concentrations, Heterotrophic Plate Counts and Water Quality Data* (WRC Report No. 2884/1/23), and

Approaches To Monitor And Characterize The Biological Stability Of Drinking Water Distribution Networks. ***Volume II****: Characterising the Composition of Microbial Communities and Correlations with Baseline FCM and HPC Cell Concentrations* (WRC Report No. 2884/2/23).

EXECUTIVE SUMMARY

BACKGROUND

Water utilities use various treatment strategies to ensure that the production of water will not pose a significant health risk to consumers. Water leaving the treatment plant is typically of high quality but conditions within the distribution system can lead to the deterioration of the microbial water quality. Maintaining the biological stability of drinking water is therefore one of the major challenges facing water utilities and local authorities in their endeavours to supply safe drinking water to communities. Where the original focus was on predicting potential changes by controlling parameters such as assimilable organic carbon (AOC) and disinfection residuals, the focus has shifted on the direct assessment of changes in the microbial community within the distribution network using methods such as 16S community profiling, flow cytometry (FCM) and ATP measurements.

Most water utilities in South Africa use heterotrophic plate counts (HPC) to monitor the general microbial quality of treated drinking water and to assess the biostability within the distribution network. The superiority of FCM over HPC has been demonstrated in numerous studies. These studies have shown that FCM is fast (results within 15 min), accurate and reproducible and can even be automated. It has been shown to be the most promising method for the direct assessment of changes in microbial communities in drinking water networks.

Within drinking water distribution networks, community reservoirs have been identified as one of the areas where bacterial regrowth can take place. Stagnation within these reservoirs could occur due to long residence times, sub-optimal flow dynamics and intermittent water demand. Stagnation is often associated with disinfectant decay resulting in an increase in bacterial numbers. These conditions often occur during the warmers summer months or during periods of water restrictions when a rapid deterioration of the microbial quality could put entire communities at risk.

RATIONALE

The major difficulty when implementing a direct assessment approach, such as FCM, within the water distribution environment is that there are no clear guidelines as to what constitutes a significant or relevant change in the microbial community. FCM counts have been shown to vary between different systems (chlorinated and chloraminated) and deviations or abnormal changes could only be detected once a proper baseline for both ICC and TCC values had been established for each system.

During the study we therefore endeavoured to addressed the following questions:

- What are the baseline flow cytometry TCC and ICC values (cell concentrations) for water samples collected from distribution and reticulation networks and how do they correspond with the HPC data (YEA and R2A) for the same samples?
- What is the composition of the microbial community (based on 16S profiling) of these samples and how does it correspond to the bacteria isolated using the HPC approach?
- Community reservoirs which form an important and integral part of the distribution network can be described as storage reservoirs which keep the balance between supply and demand in a distribution system. As these reservoirs store water, the water ages and the quality of the water can become questionable. Understanding the flow regimes and microbial ecology of community reservoirs will assist in the development of guidelines for the operation of these systems. For this reason, we also focused on the following questions:
- What impact does retention time (e.g. flow restrictions) in community reservoirs have on disinfectant residuals, FCM values as well as the community composition?

- What are the contributions of autotrophic bacteria to biological instability in the distribution system (as measured in reservoirs) and what functional role do they play in this ecosystem?
- What are the main functions associated with reservoir communities as determined using a metagenomic approach?

OBJECTIVES AND AIMS

The objective of the project was to provide the necessary foundation for the development of a strategy for the drinking water industry to incorporate FCM when monitoring and managing the biostability of drinking water during distribution as this is a more sensitive and rapid method compared to the HPC currently used. The project also focused on the impact of community reservoirs on the microbial quality of drinking water supplied to consumers.

The project aims were to:

1. Adapt the current FCM methodologies for the analysis of disinfected samples with low bacteria levels.
2. Create a baseline FCM (TCC and ICC values), HPC (YEA and R2A) and 16S community profile databases for water samples from chloraminated distribution and reticulation networks.
3. Investigate the main biological functions associated with communities that deviate from the baseline FCM values.
4. Develop a strategy for the drinking water industry and regulatory authorities for the incorporation of FCM as a technique when managing the biological stability of drinking water during distribution.
5. Investigate the contribution of autotrophic bacteria to biological instability in distribution system (as measured in reservoirs) and establish the functional role of these bacteria in the ecosystem.
6. Investigate the impact of increased retention time in community reservoirs on disinfectant residuals, FCM values and community composition in order to assist the development of procedural guidelines for the management of these reservoirs.

MAIN FINDINGS

Development of a strategy for the drinking water industry and regulatory authorities for the incorporation of FCM in managing the biological stability of drinking water

Flow cytometry has great potential as a rapid, reproducible and relevant process parameter for a diverse range of applications in drinking water systems. As with any new technology, there are various issues that need to considered before implementing this analysis at water utilities. The most important would be the interpretation of the result as there are no set alert limits. Each system and sampling point need to be evaluated based on its own available baseline data before an appropriate response can be initiated. Without such technical support, implementation would not deliver the all the obtainable benefits and could result in failure. The current proposal is that implementing flow cytometry should be *voluntary* and that the data collected by a utility will be for their own in-house use to manage their treatment and distribution systems. Once some of the larger utilities have implemented flow cytometry successfully wider implementation within the industry could be considered. It is not foreseen that flow cytometry would be included as part of SANS 241 in the near future.

CONCLUSIONS

During this project we studied the use of various analytical tools to study and ultimately manage the biostability of drinking water in distribution and reticulation systems. The analytical methods included culturing (HPC), an approach which has been used by the industry over many years as well as more recent molecular tools such as 16S profiling and metagenome analyses. We also explored flow cytometry as an additional process indicator. This project clearly demonstrated that these newer technologies have developed to such a level that they can now easily be incorporated into microbial drinking water quality studies. The costs

associated with the sequencing-based techniques is also decreasing to a level where it can be considered for more routine use.

From a research perspective the vast amount of information which is collected when applying these molecular approaches provides a detailed view of the microbial community and its members. When compared with samples taken at other time points or sampling points this data could be used investigate the interactions and dynamics within the distribution system or reservoir. Combined with other water quality parameters this information provides a better understanding of the microbial ecology of such systems.

RECOMMENDATIONS

Interpretation and integration of the various sets of information and how to apply it when managing large networks remain the main challenge. Implementation of these analyses for routine purposes within the industry should only be considered after a careful cost benefit analysis. The main cost associated with these analyses is not necessarily linked to the direct costs of the analyses or the required infrastructure but often lies with the human resources component. This type of data interpretation requires a highly skilled team of scientists with a detailed understanding of the system, its associated microbiology as well as bioinformatic analyses.

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ACRONYMS & ABBREVIATIONS

AOC	Assimilable organic carbon
ATP	Adenosine triphosphate
DWDS	Drinking water distribution system
DWTP	Drinking water treatment plant
FCM	Flow cytometry
HPC	Heterotrophic plate counts
ICC	Intact cell counts
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
PI	Propidium iodide
SANS	South African National Standard
SG	SYBR Green
SLMB	Schweizer Lebensmittelbuch (Swiss Food Book)
TCC	Total cell counts
WHO	World Health Organisation

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Currently the biostability of water in drinking water distribution systems is achieved either through the removal of nutrients that could enhance regrowth, e.g. biofiltration systems or by reducing the concentration of microorganisms by means of disinfection and maintain a disinfection residue (Proctor *et al.*, 2015; Prest et al., 2016). The stability of these system was is typically monitored based on heterotrophic plate counts. Since the first report by Hoefel and colleagues in 2003 (Hoefel *et al*., 2003) on the use of flow cytometry for the enumeration of water-borne bacteria, this technique has been applied as an alternative method to monitor the biostability of both type of systems. In Switzerland which relies mainly on groundwater and biofiltration systems, flow cytometry has been used as a process control measure (ref) for 10 years. The first Swiss standard analytical method (333.1) was already published in 2012 (SLMB, 2012) and implementation started in 2013. One of the first reports of the use of flow cytometry in chlorinated systems was from the UK (Scottish Water) and Latvia (Gillespie *et al*. 2014; Nescerecka *et al*., 2014). A comprehensive study in collaboration with Scottish Water (Cheswick *et al*., 2019) also included 40 systems that used chloramine as a residual disinfectant.

A number of reviews have been published which covered the advantages of implementing flow cytometry in drinking water system (van Nevel *et al*., 2017; Safford and Bischel, 2019). Some of these advantages include that it provides an unbiased quantification of suspended particle and their properties including bacteria (van Nevel et al., 2017), it is rapid and more sensitive compared to culture methods (Prest *et al*., 2014; van Nevel *et al.*, 2017) and apart from the initial investment in the instrumentation, it is a cheap analysis (van Nevel *et al.*, 2017). Flow cytometry methods can easily be standardized to provide reproducible results (Prest et al., 2013).

Note 1: Supplementary literature information

For supplementary literature review information on the factors influencing microbial growth and approaches for monitoring the biological stability of drinking water in distribution networks and use of flow cytometry in drinking water quality monitoring refer to Volume I report of this project, and can be accessed as a separate electronic file.

1.2 PROJECT OBJECTIVES AND AIMS

1.2.1 Study objective and main research questions

The objective of the project was to provide the necessary foundation for the development of a strategy for the drinking water industry to incorporate FCM when monitoring and managing the biostability of drinking water during distribution as this is a more sensitive and rapid method compared to the HPC currently used. The project also focused on the impact of community reservoirs on the microbial quality of drinking water supplied to consumers. Therefore, the study was designed to address the following questions:

- How can the current FCM methodologies be adapted for disinfected drinking water samples with low bacterial numbers?
- What are the baseline flow cytometry TCC and ICC values (cell concentrations) for water samples

collected from distribution and reticulation networks and how do they correspond with the HPC data (YEA and R2A) for the same samples?

- What is the composition of the microbial community (based on 16S profiling) of these samples and how does it correspond to the bacteria isolated using the HPC approach?
- What are the main functions associated with communities that are deviating from the baseline FCM values as determined using a metagenomic approach?
- What impact does retention time (e.g. flow restrictions) in community reservoirs have on disinfectant residuals, FCM values as well as the community composition?
- What are the contributions of autotrophic bacteria to biological instability in the distribution system (as measured in reservoirs) and what functional role do they play in this ecosystem?

1.2.2 Aims of the study

The project aims were to:

1 Adapt the current FCM methodologies for the analysis of disinfected samples with low bacteria levels.
2 Create a baseline FCM (TCC and ICC values and fluorescent fingerprints), HPC (YEA and R2A) and 16S community profile databases for water samples from chloraminated distribution and reticulation networks.
3 Investigate the main biological functions associated with communities that deviate from the baseline FCM values.
4 Develop a strategy for the drinking water industry and regulatory authorities for the incorporation of FCM as a technique when managing the biological stability of drinking water during distribution.
5 Investigate the impact of increased retention time in community reservoirs on disinfectant residuals, FCM values and community composition in order to assist the develop procedural guidelines for the management of these reservoirs.

1.3 STUDY DESIGN AND METHODS

1.3.1 Scope and limitations

Although all the systems used as case studies were from the Gauteng area, it would be possible to apply the main findings of the project to other drinking water utilities in South Africa as the treatment as well as distribution conditions and management practices are fairly representative of South African systems.

1.3.2 Sampling and sample analysis

The main focus of the first part of the project was to investigate the value of flow cytometry as a process indicator when managing water distribution networks. Samples were collected from a large distribution network at six different sampling locations on a bi-weekly basis over a period of 8 months. For reticulation samples (point of use), water was collected from different residential locations in Tshwane district. Six points were sampled on a bi-weekly basis over the same period of 8 months.

Heterotrophic plate counts were performed using yeast extract and Reasoner's 2 agar (R2A) following standard protocols. Flow cytometry concentrations were determined using SYBR Green I and propidium iodide stains to obtain total and intact cell concentrations. The pH and chlorine concentration of the samples were also determined. In addition, part of each sample was concentrated with membrane filtration, followed by DNA extraction and 16S profiling. Based on colony differences at least 3 colonies were picked from the R2A

plates of each sample. These bacteria were identified based on partial 16 rRNA sequencing and phylogenetic analyses. Based on this dataset potential correlations between the different parameters were analysed.

For the second part of the project permission was obtained from two of the local municipalities in Gauteng to include one of their community reservoir in this study. Reservoir A was situated in an area known to experience water quality problems. Water leaving the purification plant is typically of acceptable quality but by the time it reaches the consumer the water quality may have deteriorated significantly. This reservoir was sampled over several months to determine the interplay between design and flow patterns of the reservoir on the microbial quality within the reservoir.

Measurements such as flow, temperature and water levels within the reservoir were collected as input to create a crude model of the hydrology within the reservoir using the Computer Fluid Dynamics (CFD) system. This information was used to identify possible stagnation zones in the reservoir. This data served as input for the location of sampling points within the reservoir. To ensure that enough biological material was collected, all the samples were concentrated with membrane filtration. This was followed by DNA extraction from each sample which was used for 16S profiling and metagenome studies.

Sampling was also done at an additional community reservoir (Reservoir B) to determine the effect of residence time on the microbial water community in the reservoir. This reservoir received treated drinking water from a large water treatment works. After the filling of the reservoir, the inlet to the reservoir was closed and not refilled until the reservoir dropped to a level of 35%. This was done to allow for the longest possible residence time in the system. Sampling was conducted over a period of a week. Samples were processed and analysed as indicated above.

1.4 MAIN RESEARCH FINDINGS

1.4.1 Investigating the value of flow cytometry as a process indicator when managing water distribution networks

To address the first three aims of the project, multiple trial runs were conducted to determine the best concentrations and volumes of stains as well as the gating to be used for the FCM analysis. The controls to be included were also confirmed and a final procedure was established which was used throughout the study. Overall, the results showed no strong correlations between the ICC and HPC or chlorine levels. All the points had generally higher R2A concentrations as expected and FCM concentrations were almost 10 times higher than any of the plate counts observed. The results are consistent with what was seen in other studies with weak correlations between the two approaches. HPCs only detect a fraction of the bacteria present in the sample as not all bacteria grow on these general media.

The 16S profiling results showed that the bacterial diversity is high amongst all these samples. The bacterial communities were rather unique among samples and the abundance of specific species varied. Various parameters could be responsible for the differences in the bacterial diversity across all sampling points. When looking at each sampling location, one would expect that the bacterial community present would be fairly consistent but a temporal influence played a vital role in the variation in the observed diversity. None of the samples where the FCM count deviated from the norm had any specific group dominating the sample.

A large diversity of bacteria was recovered from the plate counts as the selected isolates belonged to 53 genera and 28 families of which the majority are well known to be present in drinking water. The family *Sphingomonadaceae* had the most representatives with most belonging to the genus *Sphingomonas.* Several

others grouped with isolates from the genera *Sphingopyxis* and *Sphingobium,* which are known to be important environmental bacteria. The *Methylobacteriaceae* was the second most common family with isolates belonging to the genera *Methylobacterium* and *Methylorubrum*. Another prominent family was the *Mycobacteriaceae*. Some of these isolates grouped closely with some of the opportunistic pathogenic *Mycobacterium* species. The last prominent family was the *Comamonadaceae.* The diversity of these families varied across the sampling locations and some families had more isolates from specific points. Together they represented 2/3 of the total number of isolates obtained during this study.

In summary, the flow cytometry results confirmed the presence of a large number of bacteria in the samples. The numbers ranged between $5x10^2$ cells/ml for the production sample to 10^5 cells/ml for some of the point of use samples. It showed that FCM could be used as a reliable process indicator to provide additional information at each sample point but that the results were site specific. FCM could also rapidly detect significant changes or clear trends linked to these communities.

1.4.2 Investigating the contribution of autotrophic bacteria to biological instability in distribution system

An analysis of Reservoir A was conducted to investigate the contribution of autotrophic bacteria to biological instability in distribution system and establish the functional role of these bacteria in the ecosystem. The assessment of the design of Reservoir A showed that depending on the fill-draw cycle, regions of stagnation could be predicted. It is believed that a late fill-draw cycle could have a larger stagnation zone directly opposite the inlet on the other side of the reservoir. The microbial population based on 16S profiling looked very similar at point and different depths throughout the reservoir indicating a homogenous bacterial community was present in the reservoir.

Proteobacteria and *Bacteroidetes* represented the highest abundances with members belonging to *Flavobacterium*, *Polynucleobacter*, *Burkholderiaceae*, *Porichthyaceae, Nitrospira* and *Sediminibacterium*. All these bacteria are commonly seen in drinking water systems. Genera such as *Flavobacterium* and *Nitrospira* are known to include autotrophic members.

The metagenome study of Reservoir A also showed that the *Proteobacteria* and *Bacteroidete*s were the most abundant members of the community. Deep taxonomy classification proved to be demanding of the bins compiled, indicating that most of them represented potential novel species, even genera. The only bin that could be identified to species level was Metabat240 which represented *Mycobacterium arupense*. This species is known as an opportunistic pathogen.

The functional analysis of the metagenoe data revealed that the community performed all the basic enzymatic functions but that some enzymes were not present in any samples. Regardless of the month sampling was performed and although the species composition changed, the microbial functionality remained constant, showing microbial stability and functional redundancy.

1.4.3 Impact of increased retention time in community reservoirs on disinfectant residuals, FCM values and community composition

The reservoir community seemed to be similar regardless of the time of day the samples were taken. This implied that the retention time (tested for up to 3 days) didn't influence the community composition. Dominant bacteria seen at all sample points included *Nitrosomonas*, *Phrarobacter, Sphingomonas* and *Sphaerotilus*. These bacteria are common in drinking water systems.

1.5 SUMMARY

In this project the main focus was to collect data for disinfected distribution systems. The results supported the findings of previous studies demonstrating the value of flow cytometry as a sensitive process parameter to monitor changes in the microbiology of distribution networks (Prest *et al.*, 2013; Proctor *et al.*, 2015). In line with other studies we also found that there was no correlation between HPCs and flow data (van Nevel *et al.*, 2017), that the values differed depending on the sampling site (Cheswick *et al*., 2019), that intact cell counts ICC) should be used instead of total cell counts (TCC) and that the data had limited hygienic relevance (Proctor *et al*., 2018; Cheswick *et al*., 2019; Favere *et al*., 2021). It is clear from these results that flow cytometry could over time replace HPCs for the enumeration of bacteria in drinking water (Manickum, 2020) but that this transition should be carefully managed and will only be of value as a process parameter for those in charge of managing the distribution network. The data is very site specific and cannot be evaluated using shared limits as values are depending on a number of factors which could impact on the microbial community (Safford and Bischel, 2019).

Note 2: Detailed experimental work and results
For detailed information on the experimental work that was conducted under this project, refer to Volumes I and II, which can be accessed as a separate electronic file.

CHAPTER 2: STRATEGY FOR THE USE OF FLOW CYTOMETRY FOR DRINKING WATER MONITORING IN SOUTH AFRICA

2.1 INTRODUCTION

The primary use of flow cytometry in the drinking water environment is as a process parameter for routine analysis to detect any abnormal changes in the level of bacteria in the system. This can only be done with confidence once a utility established a solid database of measurements for a particular system to support decisions. Abnormal changes could be indicative of regrowth due to high organic loads or low levels of disinfectant residual, treatment failure, deterioration of the source water quality, stagnation during storage (e.g. reservoirs or building plumbing) or infiltration into the system (van Nevel *et al*., 2017). All of these scenarios would require a rapid response to ensure the safety of the end-users.

The most basic application of flow cytometry in the South African context will be to measure the number of bacteria present in a sample after treatment. As most systems rely on disinfection as part of the treatment and the maintenance of a disinfectant residual to maintain the biostability in the distribution network, the focus should be on determining the number of potential intact (= viable) cells. The measurement of ICC is typically based on staining with both SYBR Green 1 and propidium iodide.

2.2 IMPLEMENTATION OF FLOW CYTOMETRY IN SOUTH AFRICA

Implementation of flow cytometry could be done using two approaches in South Africa.

2.2.1 Utility specific implementation

Large water supply utilities should be encouraged to implement flow cytometry to assist them with the management of their supply systems, specifically critical systems serving large populations. Rapid results for these systems, characterized by high volumes and short retention times is crucial for the management of such system and HPCs are of limited use. The benefits of fast decision making in order to address problems is a major motivation for the inclusion of flow cytometry as an additional process parameter. It however, requires a system specific database as well as a dedicated team who fully understands the technique and its limitations to analyse and interpret the result and decide on an appropriate response. who suggested (van Nevel *et al*., 2017) that implementation of flow cytometry would require the parallel measurement using flow cytometry and HPC to build a solid databased for the confident interpretation of the cytometry results.

Implementing flow cytometry would be *voluntary* and the data collected will be for their own in-house use to manage the treatment and distribution systems. Once a utility has made the financial investment to obtain a flow cytometer and has implemented flow cytometry as an analytical method, the data or technique could be used for any of the additional applications mentioned in Section 8.4. The implementation of on-line measurement systems can also be considered (Hammes and Besmer, 2018).

2.2.2 Implementation as an alternative mandatory process risk indicator

Currently most utilities use HPCs as a process indicator to monitor their water supplies. It is one of the mandatory process risk indicators specified in the current version of SANS 241 (SABS, 2015). As defined in the standard "HPC is a process indicator that provides information on the efficacy of the treatment processes (including disinfection), and the integrity of the distribution network (after growth due to inadequate disinfectant residuals). Changes to the range of HPC counts from a regular sampling point may indicate a problem with treatment or changes within the distribution." Research has shown that flow cytometry could perform better than HPCs when used to provide similar information for water supply systems. It is therefore not unreasonable to suggest that it could be included as an alternative mandatory process risk indicator to HPCs.

The inclusion of any analytical method as part of a national or international drinking water standard requires that the method also be validated and standardized / accredited either locally or through one of the international bodies such as the International Organization for Standardization (ISO). A recent review by Manickum (Manickum, 2020) from Umgeni Water, provides a detailed account of the routes to be followed for the standardization and accreditation of such a method. Currently the only official method for flow cytometry is the Method 333.1 published by the Federal Office of Public Health in Switzerland (SLMB, 2012). Prest and colleagues (Prest *et al*., 2013) demonstrated that the use of strict staining protocol and fixed gating parameters could provide consistent results independent of the instrument used. In spite of various studies on the application and benefits of flow cytometry as well as its use as the basis for risk assessment as part of water safety plans, the lack of standardisation (in methodology and data interpretation) remains a major obstacle for the implementation of flow cytometry as part of any national drinking water standard (Schönher *et al*., 2021). It is therefore not foreseen that flow cytometry would be included in SANS 241 in the near future.

2.3 OTHER FLOW CYTOMETRY APPLICATIONS IN DRINKING WATER QUALITY ASSESSMENT

The focus of this study was primarily on the use of flow cytometry to monitor the biostability of drinking water during distribution based on ICCs. From the workshop and the current literature, it is clear that there are currently several additional applications for basic flow cytometry analyses (TCC and ICC) linked to drinking water. These include:

- Monitoring of water sources to be able to adjust the treatment regime (Safford and Bischel, 2019; Schönher et al., 2021)
- Evaluating the conventional treatment processes which include disinfection (Cheswick *et al*., 2020; Li *et al*., 2021; Cheswick, *et al*., 2022)
- Evaluating biofiltration as an alternative treatment (Lautenschlager *et al.*, 2014; El-Chakhtoura *et al*., 2015)
- Evaluating desalination using reverse osmosis (Sousi *et al.*, 2020)
- Fully automated online monitoring to detect potential fluctuations at short time scales (Farhat *et al*., 2020)
- Growth potential tests of the raw or treated water (Sousi *et al*., 2021)

The use of flow cytometry fingerprints. For these analyses additional data collected during flow cytometry are analysed. These applications include:

- Community profiles and diversity (De Roy *et al*., 2012; Props *et al*., 2016)
- Event detection (Favere *et al.*, 2020; Favere *et al.*, 2021)
- Physiological characterization of bacteria (Buysschaert *et al*., 2017)

One of the main challenges of interpreting flow cytometry data is that a skilled operator who understands the

system is required. Current developments focus on the automatic detection of abnormal changes in the data using approaches such as Bollinger bands to develop models adapted to each sampling point. These approaches are best suited for large datasets collected at a high frequency, e.g. online system (Amalfitano *et al*., 2017).

Apart from the conventional stains which are used in drinking water related projects, such as SYBR Green (detection) and propidium iodide (viability), flow cytometry could also be used to determine enzyme activity, cell functions using GFP reporters as well as to detect specific groups of organisms or species using either fluorescently labelled antibodies or FISH probes. Although this approach is ideally suited for the detection of specific pathogens in water, the measurement threshold of flow cytometry (200-2000 cells/ml) is too high for the detection of pathogens at low concentration within samples with high levels of other bacteria. These samples will first have to be selectively concentrated using approaches such as magnetic labelled antibodies. The detection of viruses in water using flow virometry is also an active field of research (Rockey et al., 2019).

2.4 TECHNOLOGY TRANSFER

The implementation of flow cytometry as a water quality parameter to be measured on a routine basis cannot be undertaken without creating an awareness of the requirements and limitations of this method. For this reason, the project team organised a technology transfer workshop which was held on 26 July 2022 at the University of Pretoria (Appendix A). The workshop was followed by a practical training session on the next day presented by Dr Frederik Hammes from the Department of Environmental Microbiology, EAWG – Swiss Federal Institute of Aquatic Science and Technology, Zürich, Switzerland. Dr Hammes was also the main speaker at the workshop. As the workshop was presented in a hybrid format (in person and on line) speakers representing several of the key international research groups focusing on drinking water flow cytometry could participate. The hybrid format also allowed for the capturing of the presentations which are now available via a private YouTube link (https://youtu.be/VdFFVPLDaeA).

2.5 SUMMARY

Flow cytometry has great potential as a rapid, reproducible and relevant process parameter for a diverse range of applications in drinking water systems. Each system and sampling point need to be evaluated based on its own available baseline data before an appropriate response can be initiated. Without such an in-depth understanding, implementation would not deliver all the obtainable benefits and could result in failure. Once some of the larger utilities have implemented flow cytometry successfully wider implementation within the industry could be considered.

CHAPTER 3: CONCLUSIONS AND RECOMMENDATIONS

3.1 CONCLUSIONS

During this project we studied the use of various analytical tools to study and ultimately manage the biostability of drinking water in distribution and reticulation systems. The analytical methods included culturing (HPC), an approach which has been used by the industry over many years as well as more recent molecular tools such as 16S profiling and metagenome analyses. We also explored flow cytometry as an additional process indicator. This project clearly demonstrated that these newer technologies have developed to such a level that they can now easily be incorporated into microbial drinking water quality studies. The costs associated with the sequencing-based techniques is also decreasing to a level where it can be considered for more routine use.

From a research perspective the vast amount of information which is collected when applying these molecular approaches provides a detailed view of the microbial community and its members. When compared with samples taken at other time points or sampling points this data could be used to investigate the interactions and dynamics within the distribution system or reservoir. Combined with other water quality parameters this information provides a better understanding of the microbial ecology of such systems.

The assessment of the design of Reservoir A showed that depending on the fill-draw cycle regions of stagnation could be predicted. It is believed that a late fill-draw cycle could have a larger stagnation zone directly opposite the inlet on the other side of the reservoir. Using 16S profiling we could demonstrate that in spite of this potential stagnation zone, the bacterial community in the reservoir was homogenous regardless of the depth or location within the reservoir. Observed changes in the community were linked to temporal differences, often with large month to month variation. Following the same approach, we showed that a retention time up to 3 days didn't change the community within Reservoir B. While there has been a concern due to the potential for increased microbial growth over time this study has shown that the community remains rather stable.

16S profiling provided a detailed view of the bacterial communities associated with the water samples. When compared with the cultivation methods such as HPC, the limitations of this latter approach were clearly highlighted. HPCs only detect a small fraction of the microbial community and results are only available after one or more days. Similarly, to other reports we have also shown that the cultured bacteria are not necessarily the main members of the community when their identities are compared with the 16S profile for the same sample.

The metagenomic study indicated that most of the genomes that could be recovered after sequencing of the total DNA, represented potential novel species, even genera, and that very little information is available in terms of their biology. The functional analysis revealed that the community performed all the basic enzymatic functions. Regardless of the month the sampling was performed and although the species composition changed, the microbial functionality remained constant, showing microbial stability and functional redundancy in the reservoir community.

Flow cytometry showed the greatest potential as a rapid, reproducible and relevant process parameter for a diverse range of applications in drinking water systems. Our results supported the findings of previous studies demonstrating the value of flow cytometry as a sensitive process parameter to monitor changes in the microbiology of distribution. In line with other studies we also found that the values differed depending on the

sampling site, that intact cell counts (ICC) should be used instead of total cell counts (TCC) and that the data had limited hygienic relevance.

3.2 RECOMMENDATIONS

Interpretation and integration of the various sets of information and how to apply it when managing large networks remain the main challenge. Implementation of these analyses for routine purposes within the industry should only be considered after a careful cost benefit analysis. The main cost associated with these analyses is not necessarily linked to the direct costs of the analyses or the required infrastructure but often lies with the human resources component. This type of data interpretation requires a highly skilled team of scientists with a detailed understanding of the system, its associated microbiology as well as bioinformatic analyses.

The current proposal is that the implementation of flow cytometry should be *voluntary* and that the data collected by a utility will be for their own in-house use to manage their treatment and distribution systems. Once some of the larger utilities have implemented flow cytometry successfully wider implementation within the industry could be considered. To encourage the uptake of this technology funding for such an implementation project should be considered. It is not foreseen that flow cytometry would be included in SANS 241 in the near future.

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CAPACITY BUILDING

WRC Report No K5/2884//3: Approaches to monitor and characterize the biological stability of drinking water distribution networks.

1. K Moodley (PhD Microbiology) – 2018
   Title: Molecular approaches to study the microbial ecology of drinking water reservoirs.

2. Luveshnie Gounden (MSc Microbiology) – 2018-2020
   Title: Monitoring bacteria in a large South African drinking water distribution and reticulation system.

3. Workshop presentation:
   Gounden L. and Venter S.N. 2022. Monitoring bacteria in a large South African drinking water distribution and reticulation system. UP / WRC Workshop: Flow cytometry: Drinking water applications, 26 July 2022, Pretoria.

4. Annelie (Dodd) Scholtz (MEng Water Resources Engineering) – 2020
   Annelie Dodd (BEng (Hon) Water Resources Engineering) – 2019

5. Mashudu Enica Munyai (BSc (Hon) Microbiology) – 2019

APPENDIX A: WORKSHOP PROGRAMME

Flow Cytometry: Drinking water applications

(Workshop presented by the University of Pretoria on behalf of the Water Research Commission)

Date: 26 July 2022
Time: 9h00-13h00
Venue: University of Pretoria
Recording (https://youtu.be/VdFFVPLDaeA)

This workshop was aimed at introducing flow cytometry as an additional technique when managing and monitoring the biological stability of drinking water during distribution. Presentation covered various aspects such as the basic principles of flow cytometry, potential benefits of using this approach based on actual examples as well as the potential of flow cytometry for on-line monitoring.

Programme:

9h00 – 9h10: Welcome
Prof Fanus Venter – University of Pretoria
Dr Nonhlanhla Kalebaila _ Water Research Commission
9h10 – 10h00: Introduction to Flow cytometry
Dr Frederik Hammes – Department of Environmental Microbiology, EAWG – Swiss Federal Institute of Aquatic Science and Technology, Zürich, Switzerland
10h00 – 10h20: South African pilot study
Luveshnie Gounden – University of Pretoria, South Africa
10h20 – 10h40: The use of flow cytometric fingerprinting in microbial water quality monitoring
Fien Waegenaar – University of Ghent, Belgium
10h40 – 11h10: Break
11h10 – 11h30: Flow cytometry in chlorinated drinking water systems
Dr Francis Hassard, Cranfield University, United Kingdom
11h30 – 11h50: Microbial water quality in a reverse osmosis drinking water distribution system
Ratna Putri – King Abdullah University of Science and Technology, Thuwal, Saudi-Arabia
11h50 – 12h10: Building plumbing-associated microbial communities after extended periods of altered water demand
Dr Solize Vosloo – Northeastern University, Boston, United States of America
12h10 – 12h30: On-line flow cytometry: Drinking water applications
Dr Marisa Silva – onCyt Microbiology, Zürich, Switzerland
12h30 – 13h00: Q and A / discussion session
Dr Frederik Hammes – Department of Environmental Microbiology, EAWG

A ***practical introduction to flow cytometry analyses for drinking water,*** an in-person training session was held on Wednesday 27 July 2022, at the University of Pretoria.