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Water tank cleaning in Nunavik: a pilot study

Final report

July, 2009

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Table of contents

Tables

Figures

PROJECT TITLE

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ABSTRACT

This project was initiated to assess the importance, in order to maintain water quality, of cleaning in-home drinking water storage reservoirs commonly found in Nunavik. Seven reservoirs of a volume of 10L were operated at École Polytechnique de Montréal to mimic the daily bleed and feed of reservoirs in Nunavik. During a one year period, the microbial quality of the effluent of these reservoirs was characterized every 3 weeks and the biofilm was sampled every 9 weeks. Three reservoirs were intentionally contaminated with *E. coli, Giardia duodenalis* and microspheres (surrogate for *Cryptosporidium* spp.) after 6 months of operation. Two of these were then cleaned the following week to identify the best protocol to eliminate a microbial contamination (high pressure water versus cleaning with a cloth). Similarly, three other reservoirs were cleaned after being in operation for a year. This time the later weren't previously contaminated and cleaning was done to assess if biofilm detachment did cause any decrease in water quality. The obtained results demonstrate that cleaning does remove contaminants retained by the biofilm. A mass balance performed after cleaning showed that the use of high pressure water allowed the detachment of microspheres from the biofilm to a greater extent (high pressure water 47% versus cloth 13%). As for the removal of *Giardia duodenalis* only cleaning with a cloth did cause a detachment of cysts from the biofilm. Due to the low number of positive results for the removal of *Giardia* cysts by cleaning, it is impossible to conclude on the best approach to remove this contaminant. In conclusion, cleaning the interior walls and floor of in-home drinking water reservoir using both cleaning protocols is appropriate to maintain good water quality. The choice of methodology should be site specific since the implementation of both cleaning protocols present some advantages and drawbacks. We recommend that the reservoirs be cleaned on an annual basis. This could be done after spring turnover in order to minimise the accumulation of sediments on the floor of reservoirs and by consequence optimize water aesthetics after turbidity events. The procedure could be realized by a dedicated team using high pressure water, which would avoid physically entering inside the reservoirs.

INTRODUCTION

The presence of permafrost and the absence of drinking water distribution systems have led to a striking difference between methods for distributing drinking water in Nunavik and those prevailing elsewhere in Quebec. In most Inuit communities, drinking water is obtained from unfiltered chlorinated surface water. The treated water is distributed daily by a tanker truck and stored in reservoirs inside the homes.

From the data of the Inuit Health Survey, 27% of respondents mentioned that they clean their reservoir on a monthly basis (which involves disinfecting the walls of the tank with bleach), 31% every two to six months and 42% once a year or less. Generally, cleaning the tank is the responsibility of the house occupant. Currently, the efficacy of the procedures used by the tank owners is unknown.

A recent study showed a higher proportion of water tank contaminated by total coliforms in relation with the frequency of cleaning (Martin et al., 2005). It is well known that total coliforms can grow inside drinking water biofilms. Their health significance is however probably low if there origin is from the biofilm rather than the source waters. In counterpart, the data of the Inuit Health Survey seemed to demonstrate that the cleaning of the tank could protect against diarrhoea but to be possibly related to the exposure to parasites (*T. gondii, E. granulosus*) (Messier et al., 2006). Given the transversal epidemiological design of the Inuit Health Survey, these last data should be interpreted with caution and should be confirmed by more complete studies. Nevertheless, these data support the need to better document the topic of water reservoir cleaning in Nunavik.

1.0 OBJECTIVES

The general objective of this project was to assess the importance, in order to maintain water quality, of cleaning the in-home drinking water storage reservoirs commonly found in Nunavik. More specifically, the following objectives were targeted:

1. Evaluate the microbial water quality of pilot 10-L storage reservoirs over a 1-year period.
2. Confirm the need to clean in-home storage reservoirs on current scientific knowledge and current practices in the water industry.
3. If so, propose a methodology and a frequency in order to complete this procedure.
4. Evaluate the fate of a transient reservoir contamination by *Giardia duodenalis*, a protozoan parasite.

3.0 DESCRIPTION OF THE EXPERIMENTAL PROTOCOL

A. Design of pilot facilities

Due to logistic constraints related to the routine sampling and shipment of water samples for microbial analysis, storage conditions prevailing in Nunavik were reproduced in the laboratory of the NSERC-Industrial Chair at École Polytechnique de Montréal. Seven small-scale reservoirs (10 L) were operated in parallel and fed by the City of Montréal distribution system. The treated water in Montréal originates from the St. Lawrence River and is considered high quality surface water. As there is no chemically-assisted filtration in Montreal, the treatment currently in place allows for transient seasonal increase in particles, a situation probably reflecting the conditions in Nordic regions. In addition, water quality will also differ from one Nunavik community to another and it was only possible to test one source water.

The reservoirs were designed so the drainpipe would be located at the base of the wall of the reservoir. This was done to mimic reservoirs in Nunavik that can never entirely drain their storage volume. Finally, as the materials of construction of reservoirs also impact the biofilm development, the later were selected to represent what is currently used in terms of plastic, low density polyethylene (LDPE). To further study the influence of the type of material on the development of biofilm, one of these reservoirs (# 4) was made in high density polyethylene (HDPE), an alternative plastic material that could potentially be specified for the design of future storage tanks.

B. Start-up and calibration

(January 2008 to February 2008)

The pilot-scale reservoirs were installed in January 2008. Seven small scale reservoirs (10L) were calibrated in February 2008 and operated in parallel in

order to mimic the typical bleed and feed conditions of storage reservoirs in Nunavik. More precisely, the reservoirs were filled daily and then slowly emptied at a flow rate of 5 mL/min. It is important to note that the reservoirs were not completely emptied after a period of 24 hours and that they still roughly contained 30% of the storage volume when they were feed with fresh water the next day. The start-up of biofilm removal methods and microbiological methods was also done in February 2008.

C. Microbial monitoring

(March 2008 to April 2009)

C1: Water quality monitoring

Firstly, a characterization of the effluent of each reservoir started on March 11th 2008 and was done every 3 weeks over a one year period. To do so, the following microbial parameters were measured: *Aeromonas hydrophyla*, *E. coli*, Heterotrophic plate count (HPC), *Pseudomonas aeruginosa and* total coliforms. *Pseudomonas aeruginosa* and *Aeromonas hydrophyla,* are considered as opportunistic pathogens that are able to proliferate in biofilms. Opportunistic pathogens are microorganisms which may infect individuals with compromised immune system. *E. coli* is an indicator of fecal contamination. Its presence should always lead to a boil water advisory. Total coliforms and HPC bacteria are indicators of the general microbial activity in waters. They are not directly related to a health risk for the water consumers.

The methods used for the characterization of these microbial parameters are listed in Table 1. As for physico-chemical parameters, free and total chlorine residual, temperature, pH and turbidity were measured by using standard methods (American Public Health Association (APHA) and American Water Works Association (AWWA), 2005). The monitoring of the water quality of the pilot storage reservoirs ended on April 8th, 2009. Finally, a statistical analysis

(paired t-test) allowed the comparison of of the data on water quality at the effluent of the reservoirs.

Table 1: Methods used to characterize microbial parameters.

Microbial parameter	Protocol
Aeromonas hydrophyla	USEPA 1605 ADA-V (Ampicilin-Dextrin Agar with Vancomycin), incubation: 35 ^{o}C - 24h, confirmed with an oxidase test (presence of cytochrome c), fermentation of trehalose and production of indole.
E. coli	USEPA 1604 MI Agar, incubation: 35 ^{o}C - 24h, formation of blue colonies
Heterotrophic plate count	Standard methods for the examination of water and wastewater (21th Edition)*:* Method 9215 A R2A Agar, incubation: 20 ^{o}C – 7 days
Pseudomonas aeruginosa	Difco *Pseudomonas* isolation agar (protocol specified by supplier (BD) ref : 292710), incubation: 35 ^{o}C - 24h
Total Coliforms	USEPA 1604 MI Agar, incubation: 35 ^{o}C - 24h, formation of fluorescent (blue-white) colonies when exposed to fluorescent light (366nm)

C2: Biofilm monitoring

Secondly, a characterization of the biofilm present within each reservoir started on May 13th 2008 and was done approximately every 9 weeks. Biofilm sampling was done by scrapping 4 cm^2 of the interior wall of the reservoir with a sterile scalpel (30 seconds). The scalpel was immediately placed in a vial containing 10 mL sterile phosphate-buffered water and agitated. This sampling procedure was repeated twice for the same sampling area. The vial was then placed on ice and sonicated (90 seconds, 3 W, probe 3mm mm di, Ultrasonic Processor CP 70 T, Cole Palmer) to disperse the bacteria. The time of sonication was determined during preliminary assays to optimise the quantification of heterotrophic plate count bacteria, an indicator of the general bacterial population. As a result, the sonication conditions used to disperse the biofilm correspond to a specific energy of 27 kJ/L and shouldn't cause any loss of viability of bacteria (Foladori et al., 2007). After sonication, the solution was used to characterize the biofilm population by using the microbial parameters and methods listed in Table 1, i.e. the same parameters than the ones measured in water. The characterization of the biofilm ended on April 8th 2009.

D. Voluntary contamination

(October, 2008)

Three of the seven reservoir (# 2, 3 and 5) were deliberately contaminated with *E. coli, Giardia duodenalis* (a protozoan parasite) and microspheres after a period of 6 months of operation (October 28th, 2008). *E. coli* were used to study the fate and transport of fecal indicators. Likewise, cysts of *Giardia duodenalis,* a protozoan parasite, were used to study the effect a transient contamination. Finally, fluorescent microspheres (Fluoresbrite® YG carboxylate microspheres, Polysciences, Inc.) were used as a surrogate for protozoan parasites and their diameter (4,5 µm) was chosen to mimic *Cryptosporidium* spp., another common protozoan parasite of interest. Microspheres are easier to detect, are inert (i.e. do not loose viability) and, therefore, provide a useful tracer of contamination.

Table 2 summarizes the mean concentrations of contaminants added to each reservoir.

Table 2: Concentration of contaminants per millilitre added to reservoir 2,3 and 5.

Microbial parameter	**Mean concentration (# / mL)**
E. coli (bacteria)	906 250 +/- 44 200
Giardia duodenalis (parasite)	9.7 +/- 5.8
Microspheres	10.3 +/- 1.2

The microbial quality of the effluent of all contaminated reservoirs was assessed at time 0 (before contamination), 4, 24 and 48 hours after contamination to better understand the fate and transport of these contaminants (*E. coli, Giardia duodenalis*, microspheres). *E. coli* was measured as described in Table 1. To enable the identification of *Giardia duodenalis* by epifluorescence microscopy, cysts were filtered on a 0.22 µm membrane and then stained with FITC-labelled monoclonal antibodies. Afterwards, microspheres and cysts concentrations where determined by using a laser scanning instrument (Chemscan RDI, Chemunex, Inc.), which detected and counted the microspheres and cysts directly on the filter.

E. Evaluation of the efficacy of two cleaning methods

(November 2008 and March 2009)

Two cleaning techniques were tested to identify the best method to remove microbial contaminants and surrogates. These protocols where inspired by existing cleaning standards for water storage facilities of drinking water distribution system. The American Water Works Association (AWWA) uses scrubbing, sweeping or high pressure water as a standard procedure to clean walls and floors of storage facilities (American Water Works Association (AWWA) and American National Standards Institute (ANSI), 1993). The French guideline recommends high pressure drinking water to clean all interior surfaces of storage

facilities. This guideline also mentions that the use of cleaning products should be avoided as much as possible (Association Française de Normalisation (AFNOR), 1998).

Therefore, the first chosen protocol for this study consists in using high pressure water until 10% of the storage volume of the reservoir is filled while the second protocol consists in cleaning the reservoir by scrubbing manually the interior with a cloth using a circular motion (3 times). In the first case, the volume of 10% was selected to represent a realistic duration of a high pressure cleaning.

Both cleaning protocols were followed by chlorination (25 mg/L in 5% of the storage volume). All reservoirs were filled at maximum capacity one hour after the addition of chlorine and put back in operation. It was initially planed to use the AWWA standard disinfection after cleaning. This protocol consists in filling 5 % of the storage volume with a concentration of 50 mg/L of free chlorine for more than 6 hours and then filling the reservoir to maximum capacity and keeping it full (C_{res} = 2 mg/L) for 24 hours (American Water Works Association (AWWA) and American National Standards Institute (ANSI), 1993). Due to comments made by community members with regards to the smell and the amount of time that the reservoir was put out of use, this protocol was modified as mentioned above. Reducing the initial concentration from 50 mg/L to 25 mg/L will yield a final concentration after dilution of 1.25 mg/L. In addition, reducing the duration of stagnation from 6h to 1h was more realistic with respect to its impact on the community.

On November 3rd, 2008, **6 days after the voluntary contamination (144 hours)**, two of the three contaminated reservoirs were cleaned in order to compare cleaning protocols (high pressure versus cloth). The microbial quality of the effluent of all contaminated reservoirs was assessed by collecting composite samples every 24 hours over a period of 72 hours. Then the following microbial parameters and surrogates were measured: *Aeromonas hydrophyla*, *E. coli*,

Giardia, Heterotrophic plate count (HPC), microspheres, *Pseudomonas aeruginosa and* total coliforms. This was done to verify if cleaning did improve the water quality through time and if so, what protocol proved to be more efficient. Afterwards, a statistical analysis (repeated ANOVA) allowed to evaluate if the observed differences were statistically significant. Finally, a mass balance of the injected contaminants was also done in order to evaluate what fraction of the contaminants were removed by cleaning or simply discharged by flushing due to the daily admission of fresh water in the reservoirs. It's important to note that the reservoirs were designed so the drain pipe would be located at the base of the wall reservoirs to mimic in-home reservoirs that cannot fully empty themselves. By consequence, a residual volume of water was always present within the reservoir and it is for this reason that the release of contaminants re-suspended by cleaning occurred during more than a day. The use a mass balance to evaluate the fate and transport of contaminants did address this particularity associated to the design of reservoirs. This calculation was done by substracting from the total injected concentrations ($C_{injected}$) the total amount of discharged contaminants (*Giardia* and microspheres). The total amount of discharged contaminants ($C_{washout}$) was obtained by integrating the effluent concentrations of a reservoir at a given time after an intervention (contamination or cleaning) with the flow at which it empties itself (flow x concentration x duration = number). The concentration of contaminants still in suspension within the reservoir ($C_{residual}$) was calculated by multiplying the final effluent concentration by the residual volume still within the storage tank. Lastly, it was hypothesized that the contaminants were not accounted for were retained by the biofilm, such that:

$$C_{biofilm} = C_{injected} - C_{washout} - C_{residual}$$

On March 16th 2009, a duplicate experiment was done to compare both cleaning protocols. However, at that time, the cleaned reservoirs were not voluntarily contaminated before cleaning. In addition, the reservoirs had been in operation for a year. The microbial quality of the effluent of these reservoirs was assessed at time 0, 4, 24, 48 and 72 hours after cleaning.

Table 3 : Summary of the experimental protocol.

Reservoir	Material	Contamination*	Cleaning	
			Method	Date
1	LDPE	n /a	Control	03-17-2009
2	LDPE	**Yes**	Control	11-03-2008
3	LDPE	**Yes**	High Pressure + Cl_2	11-03-2008
4	**HDPE**	n /a	n/a	n/a
5	LDPE	**Yes**	Cloth + Cl_2	11-03-2008
6	LDPE	n /a	High Pressure + Cl_2	03-17-2009
7	LDPE	n /a	Cloth + Cl_2	03-17-2009

* October 28th, 2008 (*E. coli*, *Giardia duodenalis* and microspheres).

4.0 COMMUNITY CONTACT & COMMUNICATION

This project has been presented to the Kativik Environmental Advisory Committee, during a meeting held in Montréal on October 9th, 2008. During this meeting, the possible cleaning protocols were discussed with members of the committee. Some modifications were made to the original methodology. The chosen protocols to clean reservoirs were: the use of a cloth compared to the use of high pressure water (both methods followed by chlorination). The chlorination was also reviewed due to comments made with regards to odour and the amount of time that the reservoirs were put out of use. The original protocol was to add 50 mg/L to 5% of the storage volume and filling the reservoirs the following day (American Water Works Association (AWWA) and American National Standards Institute (ANSI), 1993). This protocol was then modified by lowering the chlorine dosage to 25 mg/L added to 5 % of the storage volume and filling the reservoir to full capacity one hour later.

5.0 RESULTS & DISCUSSION

A. Characterization of the water quality at the effluent of the reservoirs

The effluent of all reservoirs had similar physico-chemical properties throughout the year of operation. As demonstrated in Table 4, the mean pH and turbidity of all reservoir effluents were similar to values measured in the City of Montréal's distribution system (reservoir influent). By contrast, the mean chlorine residual was higher in the distribution system then in reservoir effluents. In fact, all chlorine residual measured at the effluent of all reservoirs were equivalent or inferior to 0.13 ppm. In addition, the chlorine residual in the distribution system (influent of reservoirs) was higher from November 2008 until April 2009 due to the lower water temperature within the distribution system (Figure 1).

Table 4 : Summary of physico-chemical properties of the effluent of all reservoirs from March 11th, 2008 to April 8th, 2009.

Reservoir	pH			Temperature (°C)			Turbidity (NTU)			Chlorine residual (ppm)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
DS	7.93	7.51	8.31				0.41	0.10	1.80	0.19	0.00	0.69
1	7.97	7.67	8.14	21.1	19.9	23.5	0.31	0.13	1.62	0.01	0.00	0.05
2	7.98	7.72	8.16	21.1	20.0	23.5	0.30	0.12	1.60	0.01	0.00	0.02
3	7.97	7.74	8.17	21.1	19.8	23.5	0.30	0.13	1.65	0.01	0.00	0.03
4	7.98	7.70	8.21	21.2	20.0	23.5	0.31	0.13	1.67	0.00	0.00	0.02
5	7.96	7.75	8.11	21.1	19.7	23.5	0.31	0.14	1.67	0.01	0.00	0.02
6	7.95	7.54	8.15	21.1	19.9	23.5	0.30	0.11	1.63	0.01	0.00	0.02
7	7.96	7.73	8.14	21.1	19.8	23.5	0.29	0.12	1.61	0.01	0.00	0.13

* DS -Distribution system

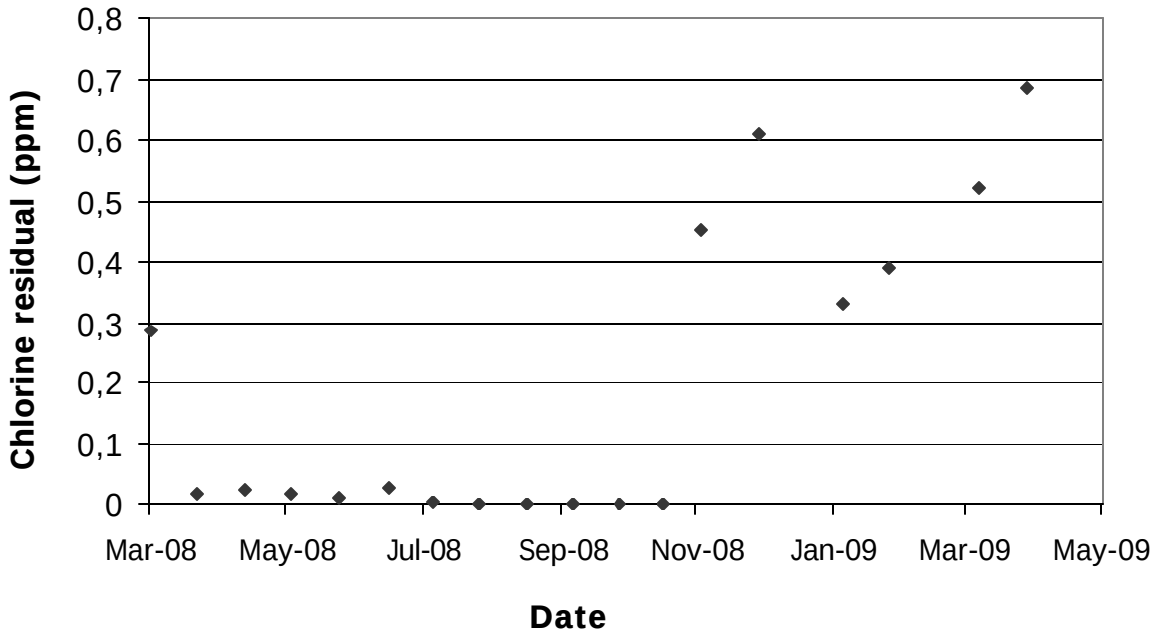


Figure 1 : Free chlorine residual of the City of Montréal's distribution system (influent of all reservoirs) from March 11th 2008 to April 8th 2009.

As for the monitoring of microbiological parameters, no opportunistic pathogenic bacteria (*Aeromonas hydrophyla, Pseudomonas aeruginosa)* or indicator bacteria (*E. coli*, total coliforms) have been detected in samples taken at the influent (City of Montreal's distribution system) or the effluent of the seven reservoirs operated at École Polytechnique de Montréal during the one year-period. By contrast, all samples from the distribution system and from the reservoirs were positive for heterotrophic plate counts (HPC) bacteria, an indicator of the abundance of bacteria. These results were expected since drinking water is never sterile, even if disinfected. As illustrated in Figure 2, the concentration of HPC of samples taken from the distribution system was significantly lower than samples taken from reservoirs throughout most of the study. The concentration at the influent varied from 1 CFU/mL up to 10 000 CFU/mL in October. Regrowth of heterotrophic bacteria in reservoirs is attributable to an increase of water temperature and to biofilm proliferation in the entire pilot setup (reservoir and tubing). Concentrations at the effluent of the reservoirs were roughly 100 to 1000

times higher than at the influent. A statistical test (paired t-test) conducted on the heterotrophic plate counts data confirmed that no significant differences were observed between all seven reservoirs before the voluntary contamination (October 28th, 2008) ($p>0.05$), therefore confirming that the reactors were equivalent before the intervention study (cleaning). Finally, during this study, the type of material of construction of reservoirs (HDPE versus LDPE) had no significant influence on the microbial quality of water ($p> 0.05$).

Table 5: Percentage of positive samples per microbial parameter taken at the influent and effluent of each reservoirs from March 11th, 2008 to April 8th, 2009.

Microbial parameter	# samples	% positive
*Aeromonas hydrophila**	18	0
*E. coli**	18	0
Heterotrophic plate counts‡	18	100
Pseudomonas aeruginosa§	18	0
Total Coliforms*	18	0

* Detection limit: < 1 CFU / 100 mL (filtered volume of 100 mL per analysis)
§ Detection limit: < 0.4 CFU / 100 mL (filtered volume of 250 mL per analysis)
‡ Filtered volume: = 10 mL per analysis

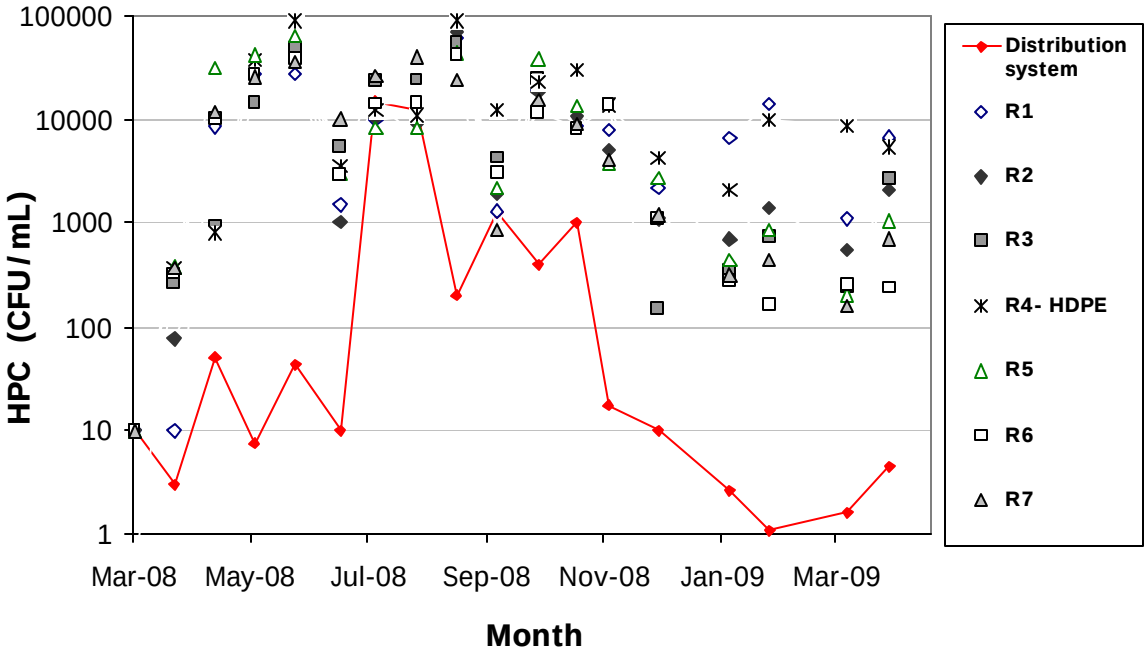


Figure 2 : Monitoring of heterotrophic plate counts (HPC) bacteria in the influent (distribution system of the City of Montréal) and the effluent of each reservoir. The doted vertical lines indicate cleaning interventions.

B. Biofilm characterization

All biofilm samples were negative for opportunistic pathogens (*Aeromonas hydrophila, Pseudomonas aeruginosa)* and microbial indicators (*E. coli,* total coliforms). However, HPC did colonise the interior walls of reservoirs, an observation coherent with the higher HPC concentrations detected in the effluent waters. Figure 3 illustrates the proliferation of the heterotrophic bacteria population (HPC) in the biofilm of reservoirs through time. Biofilm density varied largely during the project. Initial biofilm densities were high at 10^4-10^5 CFU/cm^2 and decreased as low as 1-10 CFU/cm^2 at the end of the project. The decrease of HPC was observed in October 2008 and then densities remained stable from February 2009 to March 2009. These results can be explained by a higher influent chlorine residual due to a lower water temperature in the distribution system (illustrated in Figure 1; November 2008 to April 2009). With respect to biofilm density, no significant differences in trends were measured between the

different reservoirs prior to the voluntary contamination (October 28th, 2008) ($p>0.05$). In addition, no significant differences were observed between all seven reservoirs after the first cleaning experiment (November 3rd, 2008) and the end of the sampling period (April 8th, 2009) ($p> 0.05$). Finally, no significant differences were observed with regards to the type of material (HDPE VS LDPE) and so throughout the entire project ($p> 0.05$).

Table 6: Percentage of positive samples of biofilm taken from the interior wall of reservoirs from May 13th, 2008 to April 8th, 2009.

Microbial parameter	# samples per reservoir	% positive*
E. coli	6	0
Total Coliforms	6	0
Pseudomonas aeruginosa	6	0
Aeromonas hydrophila	6	0

*Detection limit: 2.5 CFU /cm^2

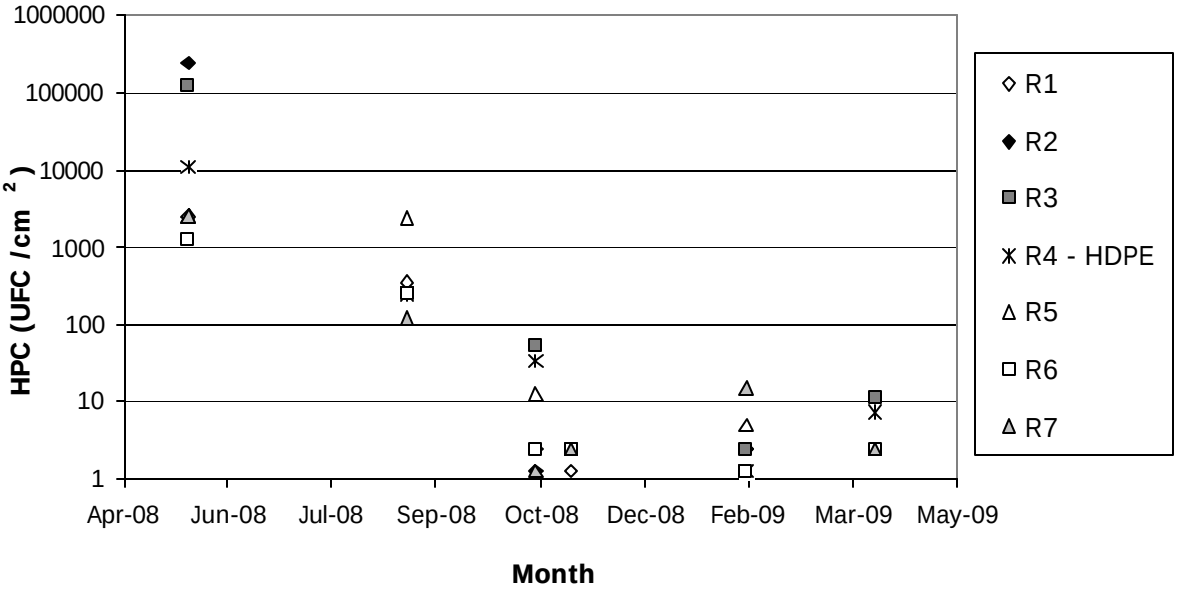


Figure 3 : Monitoring of heterotrophic plate counts (HPC) in biofilm samples.

C. Voluntary contamination of reservoir 2, 3 and 5

Most communities in Nunavik are deserved by unfiltered water supplies and experience seasonal variations of water quality (e.g. turbidity and microbial quality). In order to reflect this vulnerability, an intentional contamination was done at pilot-scale to simulate an adverse transient event of water contamination. Therefore, reservoirs 2, 3 and 5 were deliberately contaminated on October 28th, 2008. These three reservoirs were inoculated with *E. coli, Giardia duodenalis* and microspheres. These contaminants were chosen to evaluate the fate and transport of microbial indicators (*E. coli*), protozoan parasites (*Giardia duodenalis*) and surrogates for protozoan parasites (microspheres). Following the contamination, the free chlorine residual of the contaminated reservoirs was adjusted to 0.5 mg Cl_2/L.

After inoculation, the effluent concentrations of microspheres and *Giardia duodenalis* decreased through time due to flushing and, possibly, settling and adsorbing to the reservoirs (Figure 4). The contamination procedure was reproducible as all three reactors did behave similarly to a transient contamination ($p > 0.05$). However, it is important to note that these contaminants were still measured at the end of the sampling period (48 hours) since the inoculated concentrations were quite high. The microspheres effluent concentration decreased on average of 1.3 logs in 48 hours (20-fold). As for the effluent concentration of *Giardia duodenalis*, it decreased on average of 2.2 logs in 48 hours (160-fold). Finally, the *E. coli* added to the three reservoirs was rapidly inactivated by the chlorine residual and no samples were positive during the sampling period. This result reinforces the importance of free chlorine for the control of bacterial contamination.

In addition, to better understand the fate of the contamination transient, a mass balance was made to identify the fraction of micropheres and/or *Giardia* cysts that did attach to the biofilm which had formed over the six months of operation. It was

hypothesized that the difference between the injected concentration and the discharged concentrations (including the residual volume left in the reservoirs) were in fact adsorbed and/or settled in the reservoirs. As listed in Table 7, a higher percentage of cysts did attach to the biofilm compared to microspheres. This lack of attachment of microspheres is consistent with their surface properties. The later are more negatively charged than *Giardia* cysts which result in a lower attachment efficiency.

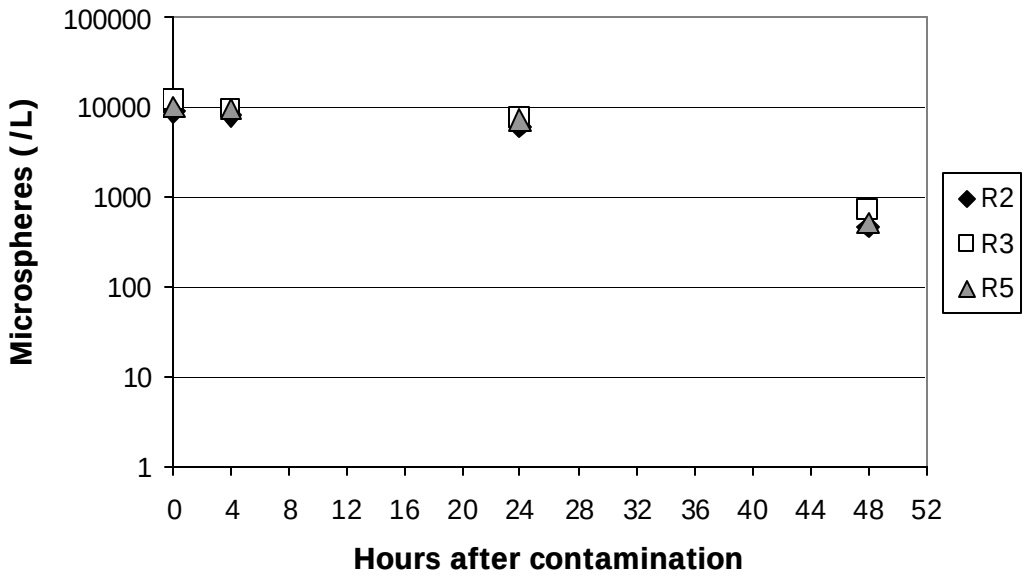
Results from Table 7 indicate that 60-84% of microspheres and 27-37% of *Giardia* cysts are washed from the reservoirs in 48 hours. Since reservoir 5 did empty itself a lower flow rate than reservoir 2 and 3 due to operational variability, less contaminants were discharged form the later through the same period of time. For both contaminants, less than 2% were still in suspension in the reservoir waters. The important information from this experiment relates to the high proportion of organisms which remains adsorbed to the inner wall or floor of the reservoir. This fraction varied from 14-38% to 63-73% for microspheres and *Giardia*, respectively. Such result reinforces the need to put in place a systematic cleaning procedure in the event of an accidental contamination of in-home storage tanks.

Table 7 : Mass balance of microspheres (MS) and *Giardia* cysts 48 hours after the contamination.

Description	Reservoir 2		Reservoir 3		Reservoir 5	
	MS	***Giardia***	**MS**	***Giardia***	**MS**	***Giardia***
Contaminants in suspension	85,4%	35,7%	85,7%	37,2%	61,7%	26,7%
i) Contaminants discharged from the reservoir	84,1%	35,4%	84%	37%	59,8%	26,6%
ii) Contaminants still in suspension within the reservoir	1,3%	0,3%	1,6%	0,2%	1,9%	0,2%
Contaminants attached to the biofilm	14,6%	64,3%	14,3%	62,8%	38,3%	73,3%

MS-Microspheres

(a)



(b)

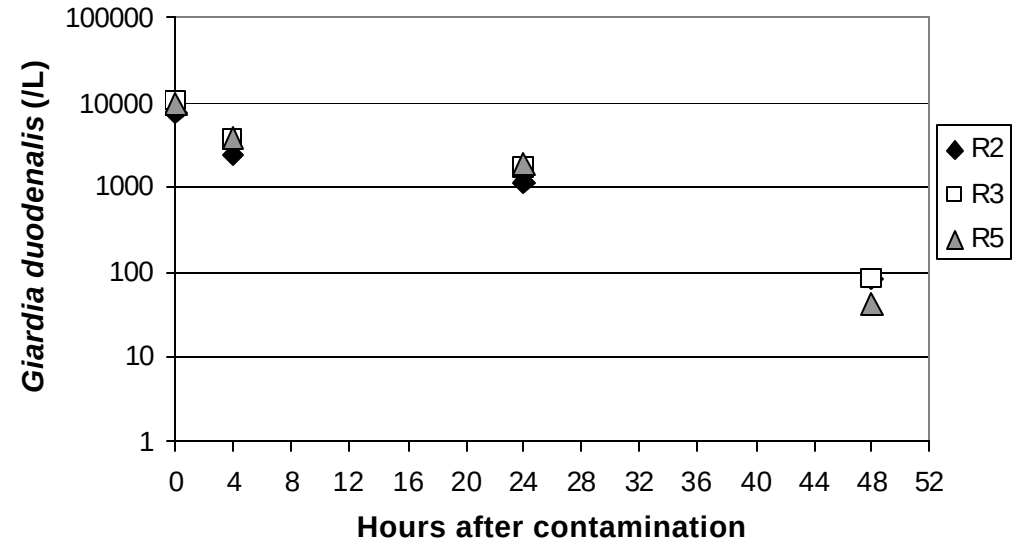


Figure 4: Monitoring of the effluent concentrations of microspheres (a) and of *Giardia duodenalis* (b) for reservoirs 2, 3 and 5 after the voluntary contamination (October 28th, 2008).

D. Comparison of the efficacy of cleaning methods

The microbial quality of the effluent of the three contaminated reservoirs was assessed after cleaning at time 24, 48 and 72 hours in order to evaluate if any detachment of biofilm occurred and had caused a decrease in water quality. **Reservoir 2** was used as a control: it had voluntarily been contaminated during the previous week but had not been cleaned. **Reservoir 3** was cleaned with high pressure water and **reservoir 5** was cleaned with a cloth. After cleaning, none of the samples were positive for opportunistic pathogens or microbial indicators in the effluent of any of these reservoirs (Table 8). Therefore, cleaning did not cause any detachment of indicator bacteria or opportunistic pathogens present in the biofilm. This is consistent with results of the microbial monitoring of the biofilm showing that none of these bacteria did proliferate in the biofilm of these reservoirs supplied by the City of Montréal's distribution system.

Table 8 : Percentage of positive samples per microbial analysis taken at the effluent of each reservoirs after cleaning (t = 24h, 48h and 72 h).

Reservoir	*E. coli**		Total Coliforms*		*Pseudomonas aeruginosa*§		*Aeromonas hydrophyla**	
	#	% positive	#	% positive	#	% positive	#	% positive
2 (control)	3	0	3	0	3	0	3	0
3 (pressure)	3	0	3	0	3	0	3	0
5 (cloth)	3	0	3	0	3	0	3	0

* Detection limit: < 1 CFU /100 mL (filtered volume of 100 mL per analysis)
§ Detection limit: < 0.4 CFU/100 mL (filtered volume of 250 mL per analysis)

Variations of heterotrophic plate counts (HPC) were observed after cleaning. As portrayed in Figure 5, the effluent concentration of heterotrophic bacteria of reservoir 3 and 5 (24 hours after cleaning) where significantly higher ($p < 0.05$) than in reservoir 2 (control). These results confirm that both cleaning protocols did cause a detachment of biofilm. A significant decrease ($p<0,05$) of HPC was

observed in the reservoir 5 (cloth-washed), 48 hours after cleaning, and then remained stable at a similar concentration than of reservoir 2 (control) until 72 hours. Likewise, the effluent concentration of HPC of reservoir 3 (high pressure water) did significantly decreased ($p<0.05$), 24 hours after cleaning, compared to its initial concentration. It then stabilized at a similar concentration as observed before cleaning.

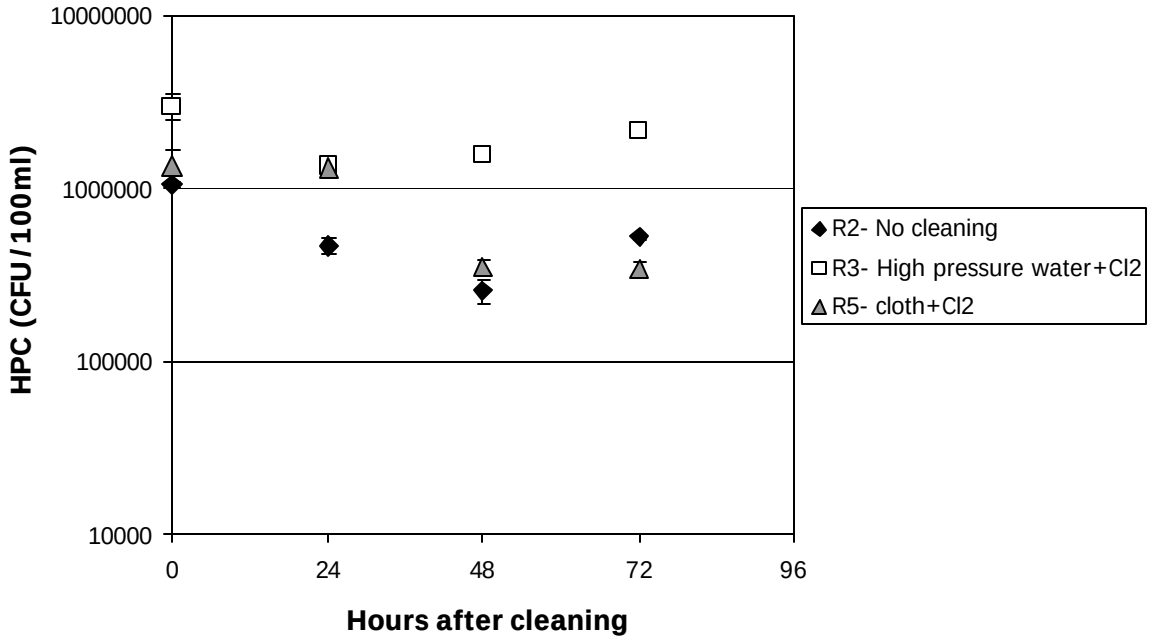


Figure 5 : Monitoring of effluent concentrations of heterotrophic plate counts (HPC) after cleaning. Error bars represent the minimum and maximum measured values of duplicate analysis.

Both cleaning protocols did cause a detachment of microspheres from the biofilm. This detachment was shown by a higher effluent concentration of microspheres in reservoir 3 and 5 than in reservoir 2 (control), 24 hours after cleaning (Figure 6). These results show that a fraction of the microspheres attached to the biofilm prior to cleaning were removed by both cleaning protocols. Since the reservoirs cannot entirely empty themselves, some of the microspheres re-suspended by cleaning were still contained in the residual volume within the reservoir when the later were first emptied (24 hours after cleaning). These microspheres were then flushed during the following day. Two days after cleaning (48 hours), the effluent

concentration of these reservoirs did start to decrease. At the end of the sampling period (72 hours), reservoir 3 (high pressure) and 5 (cloth) had statistically lower concentrations then measured 24 hours subsequent to cleaning ($p < 0.05$). As illustrated in Figure 6, the use of a cloth seems to be more efficient to detach the microspheres from the biofilm as it offered lower effluent concentrations than when cleaned with high pressure water 48 and 72 hours after cleaning. However, these differences amongst cleaning protocols were not statistically significant ($p > 0.05$). Furthermore, the effluent concentration of all three reservoirs 72 hours after cleaning were similar ($p > 0.05$). This shows that even if the water quality first decreased after cleaning (both protocols) due to the wash out microspheres, it returned to normal three days latter. Finally, it is important to note that reservoir 2 was put out of service during the same period as reservoir 3 and 5 but wasn't cleaned. The increase of the effluent concentration of microspheres in reservoir 2 through time is most likely caused by the hydraulic perturbations caused to the biofilm when it was put back in operation. This increase observed in reservoir 2 was minimal and proved to be statistically insignificant ($p > 0.05$).

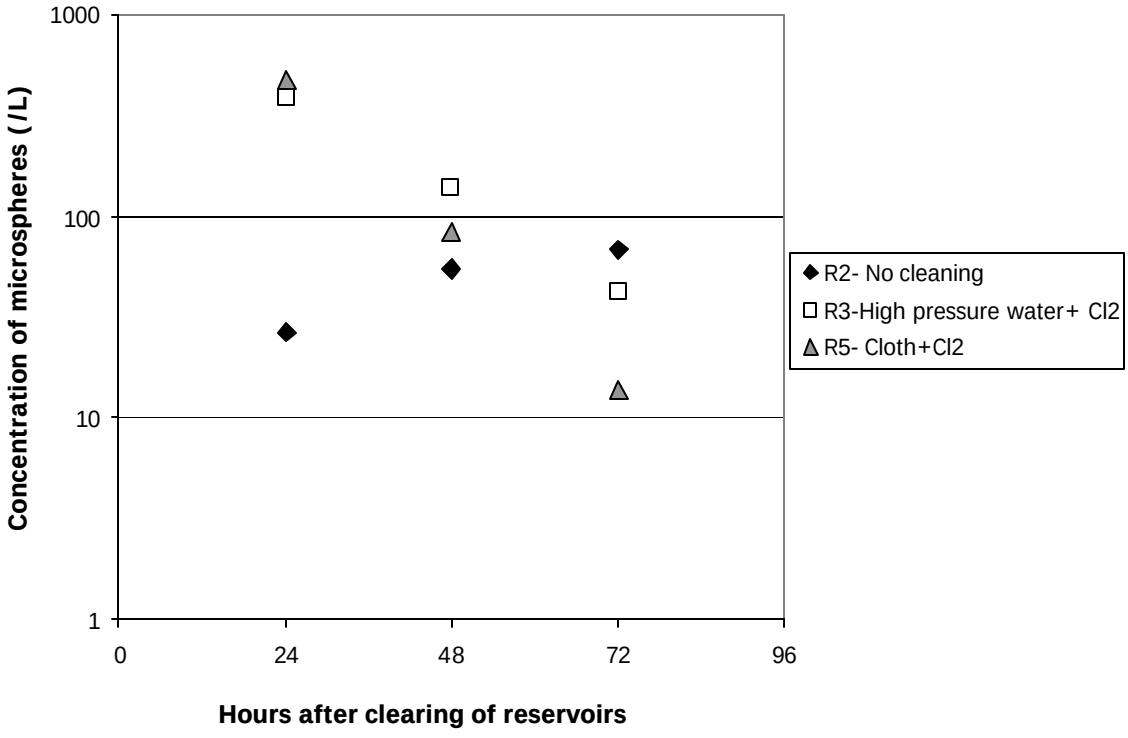


Figure 6: Monitoring of effluent concentrations of microspheres after cleaning.

As demonstrated by the mass balance after contamination (Table 7), a higher percentage of *Giardia* cysts did attach to the biofilm compared to microspheres. Only method 2 (cleaning with a cloth) did cause the detachment of *Giardia duodenalis*. However, this detachment was observed only during the first 24 hours subsequent to cleaning in reservoir 5 and all other samples were negative (Table 9). As for reservoir 3, cleaned with high pressure water, *Giardia duodenalis* was undetected in all samples. Therefore, it is difficult to conclude on the superiority of one cleaning method over another based on only one positive data.

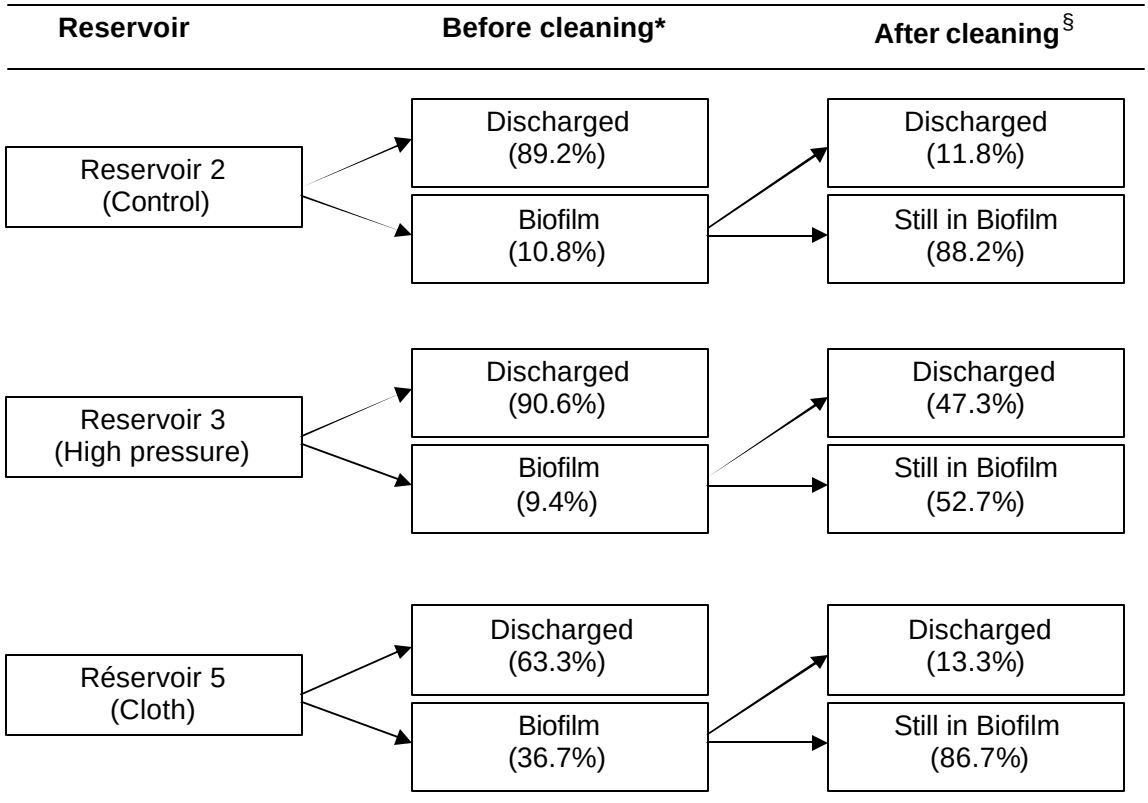
Table 9: Effluent concentrations of *Giardia duodenalis* subsequent to cleaning.

Reservoir	Time after cleaning (hrs)	*Giardia* /L
2 Control	24	< 13
	48	< 10
	72	< 10
3 Pressure-washed	24	< 13
	48	< 10
	72	< 10
5 Cloth-washed	24	247
	48	< 10
	72	< 10

In order to fully evaluate the efficiency of both cleaning protocols, a mass balance was completed with the data obtained 72 hours after cleaning. This was done to identify which protocol removed the greater amount of contaminants from the biofilm of reservoirs and to what extent. The advantage of using a mass balance is that it takes into account the exact number of contaminants added to each reservoir as well as the number attached to the biofilm. In addition, a mass balance also takes into account the contaminants released from the reservoir as they empty themselves. By consequence, a mass balance allows a more

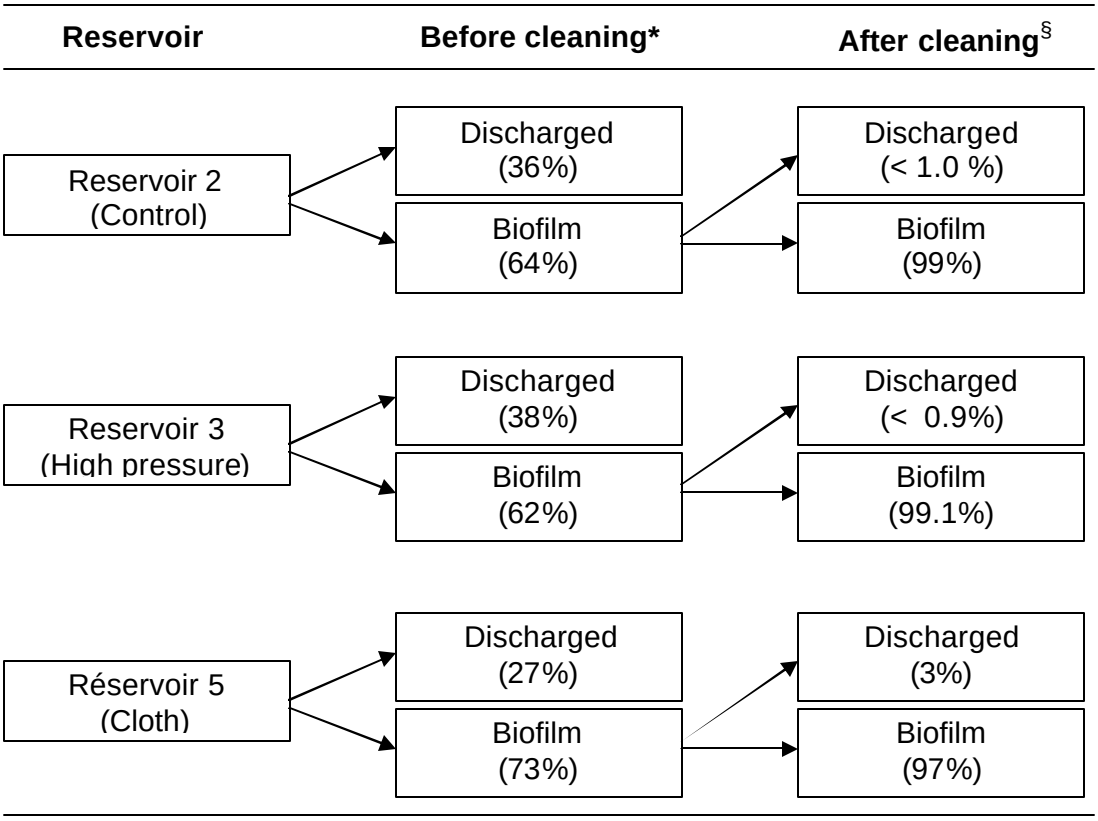
accurate comparison of cleaning protocols then effluent concentrations. To do so, the concentrations of contaminants still attached to the biofilm of each reservoir before/after cleaning were calculated as described in Table 10 and Table 11. As listed in Table 12, the use of high pressure water caused a greater percentage of detachment of microspheres retained by the biofilm prior to cleaning (47%) than the use of a cloth (13%). The use of high pressure water didn't cause any detachment of *Giardia duodenalis*. To the contrary, the use of a cloth did allow the removal of some *Giardia* cysts. This removal was minimal since it only represents 3% of the total cysts that were attached to the biofilm of reservoir 5 prior to cleaning. This detachment of *Giardia* measured in reservoir 5 might also be linked to number of cysts that adhered to the biofim after contamination. As listed in Table 11, the percentage of the initially dosed cysts attached to the biofilm prior to cleaning in reservoir 5 (73 %) did exceed the percentage of attached cysts in reservoir 3 (62.5 %). In fact, the biofilm of reservoir 5 theoretically contained 4715 more cysts than reservoir 3. This might explain why samples taken from reservoir 3 (pressure) were under the limit of detection compared to samples taken from reservoir 5 (cloth). Therefore, we do not feel that these results are sufficient to discard the use of a cloth to wash the reservoirs.

Table 10: Summary of percentage of microspheres retained by the biofilm and discharged from the reservoir prior and subsequent to cleaning.



*144 hours after contamination, § 72 hours after cleaning

Table 11: Summary of percentage of *Giardia* cysts retained by the biofilm and discharged from the reservoir prior and subsequent to cleaning.



* 144 hours after contamination, § 72 hours after cleaning

Table 12 : Fate of microspheres (MS) and *Giardia duodenalis* (G) detached from the biofilm during the first 72 hours subsequent to cleaning.

Description	**Reservoir 2** (Control)		**Reservoir 3** (High pressure)		**Reservoir 5** (Cloth)	
	MS	**G**	**MS**	**G**	**MS**	**G**
Fraction of contaminants present within the biofilm detached by cleaning*	11,8%	<1,1%	47,3%	<0,9%	13,3%	3 %
i) Discharged from the reservoir	10,3%	<0,7%	45,9%	<0,5%	13,2%	3%
ii) Still in suspension within the reservoir§	1,5%	<0,4%	1,4%	<0,4%	0,1%	<0,4%

*Calculated in function of the number of contaminants retained by the biofilm prior to cleaning (144 hours after contamination).
§72 hours after cleaning.

E. Comparison of cleaning protocols and detachment of biofilm in reservoirs operated for a one year period

To confirm previous results, the cleaning protocols were tested on reservoirs that had been in operation for one year, (while results in Section D were based on reservoirs in operation for 6 months). It is important to note that these reservoirs were not deliberately contaminated during previous experiments. **Reservoir 1** served as a control (i.e. not cleaned but put out of operation during cleaning), **reservoir 6** was cleaned with high pressure water and finally **reservoir 7** was cleaned with a cloth. In order to minimise the risk of contamination, the synthetic cloth (Hero ^{TM}, All Purpose Reusable Towels) used for this experiment was autoclaved to ensure its sterility.

The effluent concentrations of all bacterial parameters (*A. hydrophila, E.coli*, heterotrophic plate counts, *P. aeruginosa* and total coliforms) were evaluated at time 0, 24, 48 and 72 hours. As demonstrated in Table 13, none of these reservoirs tested positive for opportunistic pathogens (*A. hydrophila, P. aeruginosa)* and for microbial indicators (*E. coli* or total coliforms) to the exception of heterotrophic plate counts.

Table 13 : Percentage of positive samples per microbial analysis taken at the effluent of each reservoirs after cleaning (no previous contamination).

Reservoir	*E. coli**		Total Coliforms*		*Pseudomonas aeruginosa*§		*Aeromonas hydrophyla**	
	#	% positive	#	% positive	#	% positive	#	% positive
R2	4	0	8	0	4	0	4	0
R3	4	0	8	0	4	0	4	0
R5	4	0	8	0	4	0	4	0

*Detection limit: < 1 CFU / 100mL (filtered volume of 100 mL per analysis)
§ Detection limit: < 0.4 CFU / 100 mL (filtered volume of 250 mL per analysis)

Furthermore, variations of heterotrophic plate counts (HPC) were observed after cleaning. Figure 7 illustrates the variations of HPC through time in function of the

cleaning protocol. In fact, contrarily to what was observed during the first cleaning experiment, both cleaning protocols did significantly lower the effluent concentration of HPC, 24 hours after cleaning ($p < 0.05$). An increase of HPC concentration, 48 hours after cleaning, was then observed in reservoir 6 (2.2 logs or 160-fold) and reservoir 7 (3.0 logs or a 1000-fold). It is important to note that the HPC concentration of reservoir 6, 48 hours after cleaning, was not significantly different than it's initial concentration (before cleaning) ($p > 0.05$). The later remained stable until the end of the sampling period (72 hours). On the other hand, the HPC concentration in reservoir 7, 48 hours after cleaning, was significantly higher than its initial concentration ($p < 0.05$) but like reservoir 6, it remained stable until the end of the sampling period. Finally, after 72 hours, the HPC concentration of both reservoir 6 and reservoir 7 were still significantly lower than in reservoir 1 which wasn't cleaned to serve as a control ($p < 0.05$).

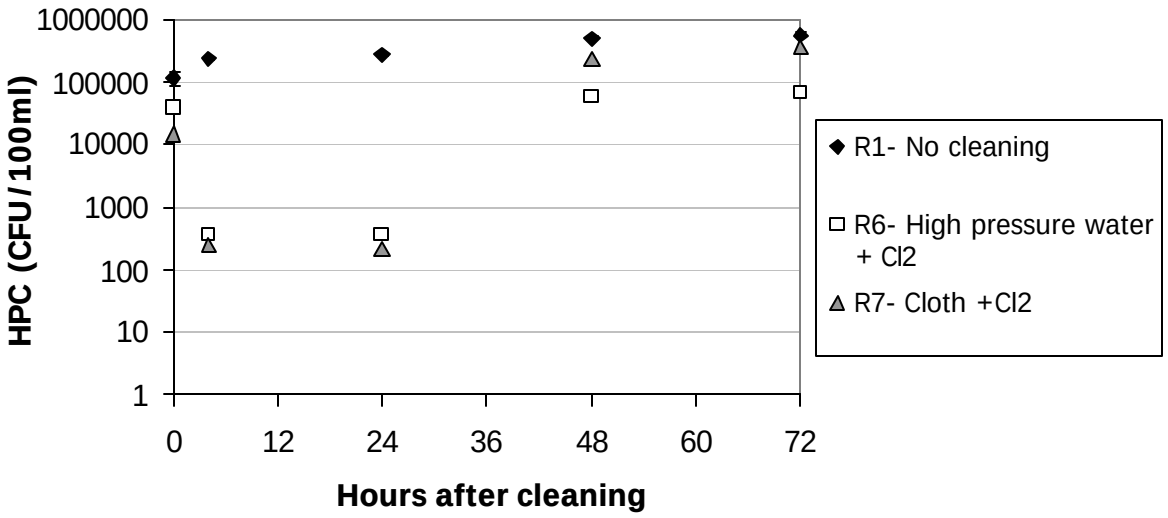


Figure 7: Monitoring of effluent concentration of heterotrophic plate counts (HPC) after cleaning.

To better assess the variations of HPC, free chlorine was measured at time 0, 2, 4, 24, 48 and 72 hours subsequent to cleaning. At time zero, the data obtained differed greatly between reservoir 6 (1.23 mg/L; high-pressure) and reservoir 7

(<0.03mg/L; cloth) to which the same amount of chlorine had been added subsequent to cleaning. To understand these discrepancies, total chlorine was analysed for all other samples taken from reservoir 7 (cleaned with cloth). Total chlorine results showed that in reservoir 7, chloramines were formed due to the presence of nitrogen (ammonia). The new synthetic cloth was then identified as a source of nitrogen (ammonia) and the cause of this bias. In fact, when further investigated with method NF T 90-015 (Association Française de Normalisation (AFNOR), 1990), the synthetic cloth contained a nitrogen (ammonia) concentration that exceeded 500 µg/L.

Table 14 : Free and total chlorine residual measured after cleaning (March 16th, 2009).

Time (hr)	Reservoir 1 (Control)		Reservoir 6 (High pressure)		Reservoir 7 (Synthetic cloth)	
	Free chlorine (ppm)	Total chlorine (ppm)	Free chlorine (ppm)	Total chlorine (ppm)	Free chlorine (ppm)	Total chlorine (ppm)
0	0.16	n/a	1.23	n/a	< 0.03	n/a
2	0.01	n/a	0.98	n/a	< 0.03	0.54
4	0.01	n/a	0.69	n/a	< 0.03	0.44
24	0.01	n/a	0.24	n/a	< 0.03	0.28
48	0.01	n/a	0.05	n/a	< 0.03	0.05
72	0.12	n/a	0.19	n/a	< 0.03	0.13

This result is of importance. The product that was used to wash the reservoirs is the standard Hero™ , All Purpose Reusable Towels which is commonly used and machine washable (please consult Figure 9, for a picture). The labelling of the product does not mention the presence of ammonia. Ammonia is frequently used as a cleaning product. It can be found, for example, in Windex™. The presence of ammonia is a significant interference to the efficacy of chlorine. Therefore, we advise that this type of product should not be used to clean residential reservoirs.

The interference of ammonia on chlorination has the following consequence. The efficacy of cleaning methods cannot be based on the measurement of HPC bacteria, which are sensitive to free chlorine. Therefore, our conclusions will be mainly targeting on our evaluation of microspheres and *Giardia* release (Section

9.0 D), and so, particularly since no other microbial parameter were positive after cleaning (Table 8 and Table 13).

6.0 KEY FINDINGS / CONCLUSIONS

During this project, three main objectives were targeted.

1. **Evaluate the microbial water quality of pilot (10-L) storage reservoirs over a 1-year period.**

   Characterizing the effluent of reservoirs over a one year period showed that all reservoirs evolve in a similar fashion through time. Opportunistic pathogens and microbial indicators remained undetected in all samples to the exception of heterotrophic plate counts bacteria (HPC). Similarly, no opportunistic pathogens proliferated in the biofilm of reservoirs supplied by the City of Montréal's distribution system, before or after cleaning. It is normal to find HPC bacteria in stagnating drinking water. In 2002, the World Health Organization sponsored a workshop to address the issue of the health significance of HPC in drinking water. Their conclusions were that there is no strong scientific evidence that HPC represents a health concern for individuals. For example, more than 97% of HPC ingested weekly by US citizens actually come from food rather than drinking water.

   The material of construction (HDPE or LDPE plastics) made no significant differences in water quality or biofilm proliferation. Therefore, the choice of HDPE (high density polyethylene) versus LDPE (low density polyethylene) should be based on other technical or economical considerations. Although the literature indicates that the type of material can influence the biofilm density, it must be pointed out that the largest differences are observed for ferrous versus non-ferrous materials rather than from one plastic type to another.

2. **Evaluate the fate of a transient reservoir contamination by *Giardia duodenalis*, a protozoan parasite.**

The intentional contamination of the reservoirs with *E. coli*, *Giardia* cysts and microspheres indicates that free chlorine would play an important role in protecting the public against chlorine-sensitive organisms such as *E. coli* bacteria. However, more resistant organisms would be mainly removed due to the natural emptying/filling of the reservoirs. In addition, in the event of a contamination, some microbial contaminant will be adsorbed in the biofilm, as was evidenced with our mass balance calculations. This fraction was not negligible, accounting for 10-70% of the total number of microbial contaminants.

3. **Confirm the need to clean in-home storage reservoirs on current scientific knowledge and current practices in the water industry. If so, propose a methodology and a frequency in order to complete this procedure.**

After 6 months of operation, three reservoirs were deliberately contaminated with microspheres, *Giardia duodenalis* (a protozoan parasite) *and E. coli* (bacteria). Six days after the contamination, two out of the three contaminated reservoirs were cleaned to compare cleaning protocols (high pressure water versus cleaning with a cloth, both followed by chlorination). Both cleaning procedures did cause a detachment of biofilm.

- Data on HPC bacteria release after cleaning were strongly influenced by the free chlorine residual concentration and are therefore not deemed a reliable indicator of cleaning efficacy.
- As for the removal of *Giardia*, no detachment of cysts was observed when high pressure water was used and only a small fraction of the cysts were removed from the biofilm by cleaning the reservoir with a cloth. Therefore, it's impossible to conclude on the effectiveness of

this protocol (cloth) with only one positive result although we do not think these results are sufficient to discard the use of a cloth to clean in-home reservoirs. It is also important to note that due to the small surface of reservoirs (10L) operated at École Polytechnique de Montréal, the use of a cloth might have been more efficient than in full sized reservoirs since it was easier to clean its entire surface and the scrubbing procedure had a lower probability of missing an area than in a large tank.

- Data obtained by a mass balance subsequent to cleaning showed that a greater removal of microspheres (surrogate for *Cryptosporidium spp.,* a chlorine resistant parasite) was achieved with high pressure water. This dataset is the most convincing since microspheres are not impacted by free chlorine (as opposed to HPC) and reliable data were obtained before/after cleaning (as opposed to *Giardia*). Consequently, we feel that cleaning with high pressure water is more efficient to promote the detachment microspheres from the biofilm than cleaning with a cloth.
- Finally, due to the location of the drainpipe, the reservoirs were not able to fully empty themselves. As a result, contaminants removed by cleaning were not entirely drained when the reservoir was first emptied (24 hours after cleaning). This situation is similar to what is currently happening in Nunavik reservoirs (due to their design). The management of this remaining wash volume is key to the success of the cleaning procedure. In fact, chlorination after cleaning is particularly important to inactivate pathogenic microorganisms removed by cleaning and that are still in suspension in the residual volume within the reservoir. After cleaning and before chlorination, the removal of this residual volume by pumping could be considered as a possible avenue to shorten the time of the washout of contaminants. Furthermore, to minimise this problematic in the

future, newly acquired reservoirs should be designed so that they can be entirely drained by gravity.

In summary, in our opinion, both cleaning procedures represent a viable alternative to clean in-home storage tanks, although we noted that the pressure wash was more efficient. However, other factors should also be included in the decision as both approaches have advantages and drawbacks.

Regarding the use of the wash cloth:

Currently, washing with a cloth is the procedure which is used in the communities of Nunavik.

- The results of the second cleaning experiment did demonstrate that if the reservoirs are cleaned with a cloth, it is essential not to use any product containing ammonia since it will compromise the final disinfection with chlorine. Our cleaning experiment did also demonstrate that some synthetic fabrics do contain nitrogen (ammonia) even if it is not listed on the packaging. It is for this reason that the use of a clean cloth made in natural fibres (e.g. cotton) is more appropriate.
- Washing with a cloth also implies that someone has to physically enter the reservoir. This situation conduces to the potential risk of bringing external contamination.
- In addition, the cleaning procedure is usually done with strongly chlorinated waters. The person in charge of the cleaning can become irritated by the chlorinated smell arising from the wash water.
- On the other hand, washing with a cloth require much less water than the cleaning with a high pressure jet. This can be an advantage if it is not possible to properly drain the reservoir. In such cases, cleaning with a cloth is a better alternative. In order to solve the issue of chlorine fumes, it is recommended that the wash water used by the

cleaning person be only mildly chlorinated (4 ppm or less). The whole idea of this step of the procedure is to physically remove the sediments and the biofilm and therefore, the presence of highly chlorinated waters, is not deemed essential. The final chlorination step will disinfect the reservoir and can be performed at higher chlorine dosage since the individual is no longer inside the reservoir.

Regarding the use of the pressure washer

- Results of this study demonstrate that the use of high pressure water is appropriate to clean the interior of in-home drinking water reservoirs as it allowed the removal of more contaminants attached to the biofilm and is probably more efficient than a cloth at the scale of a full-scale reservoir.
- Some reservoirs have their drain located a few inches above the floor. In such case, cleaning with a high pressure jet will lead to the accumulation of biofilm/sediment at the bottom of the reservoir. A possible avenue to avoid this problematic associated to the wash water remaining within the water tank, could be to remove the latter by pumping before performing the final chlorination.
- Apart from the necessity to drain the wash water generated by the pressure washer (discussed previously), the pressure washer is a more invasive procedure for the residents. Realistically, it could only be applied if the communities were to train a team in charge of washing storage tanks on a routine basis. This approach represents a solution which would be efficient (in terms of cleaning) and effective (in terms of implementation in the community).

Regarding the frequency needed to wash the storage tanks

There are three situations which could call for cleaning the residential storage tanks: (i) cleaning following a proven or suspected contamination, (ii)

cleaning following aesthetic complaints by the users and (iii) routine preventive maintenance

In the two first cases (contamination or complaints), the cleaning shall be done when needed. Regarding routine preventive maintenance, the pilot study has shown that the general microbial community present inside reservoirs will rapidly re-establish following a cleaning procedure. However, there are two valid reasons to justify cleaning these reservoirs on a routine basis. For one, most Nunavik communities are deserved by unfiltered surface waters. Therefore, seasonal increase in turbidity is to be expected. Particles can settle at the bottom of these reservoirs. These sediments in conjunction with the biofilm present on the inner surface of the reservoirs are favourable to the formation of taste and odours. Based on the current knowledge on the health risk related to biofilm, the communities should be more concerned about the water quality that feds these reservoirs rather than what becomes of it during its stagnation within a reservoir.

In essence, the frequency of cleaning is site-specific as it should relate to the particles content fed to the reservoir. For example, feeding a reservoir on a yearly basis with a high turbidity water (5 NTU) should translates in the accumulation of 100 mL – 400 mL depending on the fraction of particles settling to the bottom (assumed here as 10-50%). A volume of 400 mL represents 0.2 mm of sludge at the bottom of a 2 m^2 reservoir. Therefore, the accumulation of sediments should not be that important if the turbidity of the feed water is lower than the regulatory limit of 5 NTU.

Current practices for cleaning municipal water storage tanks will vary from 1 to 5 years, depending on the municipality. Some large reservoirs in the City of Montreal have not been cleaned for several decades due to the impossibility to put them off-line. Even though the Montreal distribution system experiences seasonal peak events in turbidity, the accumulation of

sediments in one of these reservoirs was minimal during a sampling campaign performed in the late 1990s by our research group.

Although the accumulation of sediment is limited and cleaning could be done less frequently, we recommend that the reservoirs be cleaned on an annual basis. One of the rationale supporting an annual frequency is related to the ease of implementing annual maintenance as opposed to, for example, a bi-yearly frequency. In addition, the activity could be scheduled after the spring turnover when increased turbidity is often experienced. Reduced frequency could potentially be achievable in some communities. For example, a team could be trained to wash the reservoirs with high pressure water. Half of the community reservoirs could be washed by this team on one given year while the other half is washed the subsequent year, therefore achieving a frequency of 2 years without compromising the team habit of cleaning every year. Finally, additional site-specific studies would be needed to support site-specific frequencies based on treated water quality.

7.0 REFERENCES / PERTINENT LITERATURE

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8.0 IMAGES / PHOTOS / PRODUCTS

Figure 8: Experimental setup located at École Polytechnique de Montréal

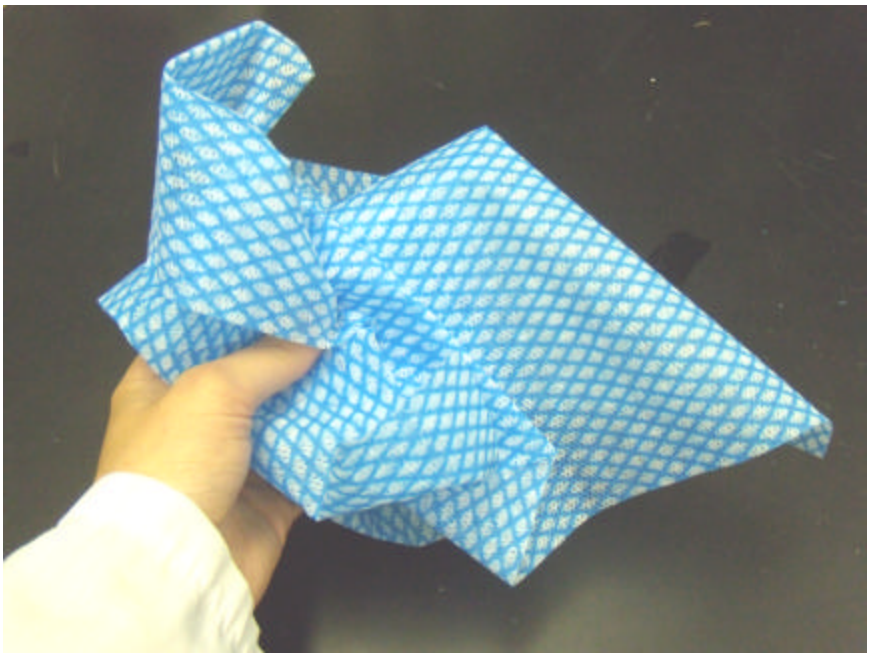

Figure 9 : Synthetic cloth (Hero ^{TM}, All-purpose reusable towels) used for cleaning experiments