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Ultrafiltration Pilot Plant at Görväln for future WTP

Evaluation of Bacteria and Virus-particle removal

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1 Introduction

An ultrafiltration process is going to be part of Norrvatten's future drinking water treatment process. This UF process will be placed after carbon filtration, and it will be implemented as Görvälnverket's third microbiological barrier. For approximately one year, October 2022 to August 2023, a pilot study project was conducted at site at Görvälnverket.

This report is meant to serve as a complimentary report from the main report *Ultrafiltration Pilot Plant at Görväln for future WTP – determining optimal operation of ultrafiltration with in-line coagulation, purifying carbon filtrate* (Sekizovic & Warman, 2023).

Focus here is to evaluate the UF reduction of microbes in the drinking water treatment process. The UF pilot plant (manufactured by Inge GmbH, a part of the DuPont corporation) consists of two operational lines with membrane modules of the model dizzler XL 0,9 MB 80 WT. As seen in Figure 1, the membrane surface area is 80 m^2, and the pore size is 20 nm.

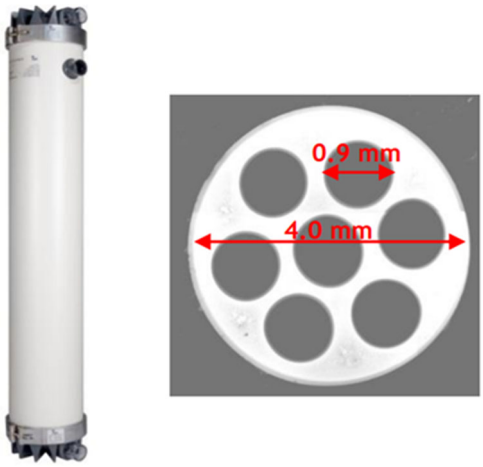

Figure 1. Membrane of the model dizzler XL 0,9 MB 80 WT. Pore size 20 nm.

In Figure 2 the pilot design is shown with water sampling points.

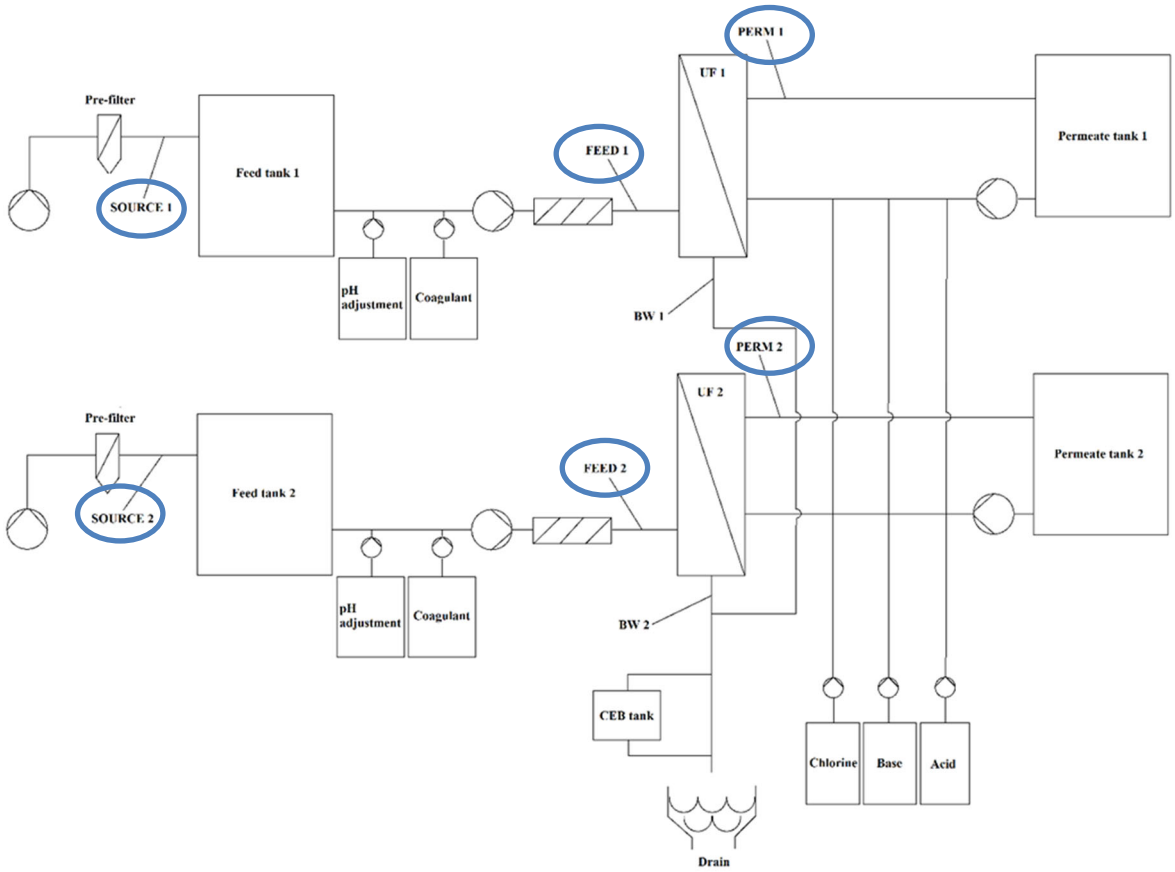

Figure 2. Pilot design with water sampling points in blue circles.

Microbes vary in size, and by filtering through membrane pore size of 20 nm, most microbes will not pass through. However, there is a risk that some microbes may pass through, especially viruses due to their smaller sizes. One example is the human enteric virus Norovirus, causing outbreaks of winter vomiting disease, with a virus-particle size of 27 nm (Robilotti *et al* 2015). And some bacteria might be able to pass through due to their motility and their adaption of their cell membrane (Männki *et al.* 2009). Also, that the filter pore size isn't static at 20 nm. For instance, temperature can have an impact on the robustness of the membranes.

In appendix A the results of flow cytometry are presented after the pilot study's plan, period 1 to period 7. Table 1 presents the testing scheme for the two lines, line 1 (L1) and line 2 (L2).

Table 1. Presents the plan of testing periods and specific adjustment of parameters.

Period and (week)	Testing parameter	Membrane line 1				Membrane line 2			
		Dosage	**Flux**	**Retention time**	**CEB**	**Dosage**	**Flux**	**Retention time**	**CEB**
1A (41 – 45)	Optimal dosage	0,5 – 2,0	70	21	48	1,0	70	21	48
1B (46 – 50)	Effect of new carbon filtrate	0,5 -2,0	70	21	48	1,0	70	21	48
2 (51 – 5)	Flux and retention time	1,5-2,0	70 – 100	21 – 30	48	1,5	70	21 – 42	48
3 (6 – 10)	Operation without coagulant	0	70	42	-	1,5	70	42	48
4 (11 – 14)	Steady state with external analysis	1,5	70	42	48	1,5	70	42	48
5 (15 – 21)	CEB adjustment	0	70	42	48+Cl	1,5	70	42	48
6 (22 – 27)	Flux during lower water quality	1,5-2,0	70 - 100	42	48	1,5	70	42	48
7 (27 – 33)	Performance recovery		70		48		70		48

2 Results and discussion – Bacteria

When looking at all experimental periods there is, as expected, a similar trend in bacterial concentrations when comparing influent and effluent water. The bacterial concentrations were measured using flow cytometric measurements (total cell count, TCC). The trend is a result of lake turn over. One question here is whether the bacterial concentration from the effluent UF water remains stable when the influent water varies so much. Looking at Figure 3, there are some bacterial spikes during autumn and spring (when the lake turn over takes place). However, the small number of cells might be an effect of instrumental measurement fluctuations. Still, in the appendix there are regression charts illustrating weak positive correlation of bacterial counts between influent and effluent water from the UF-pilot.

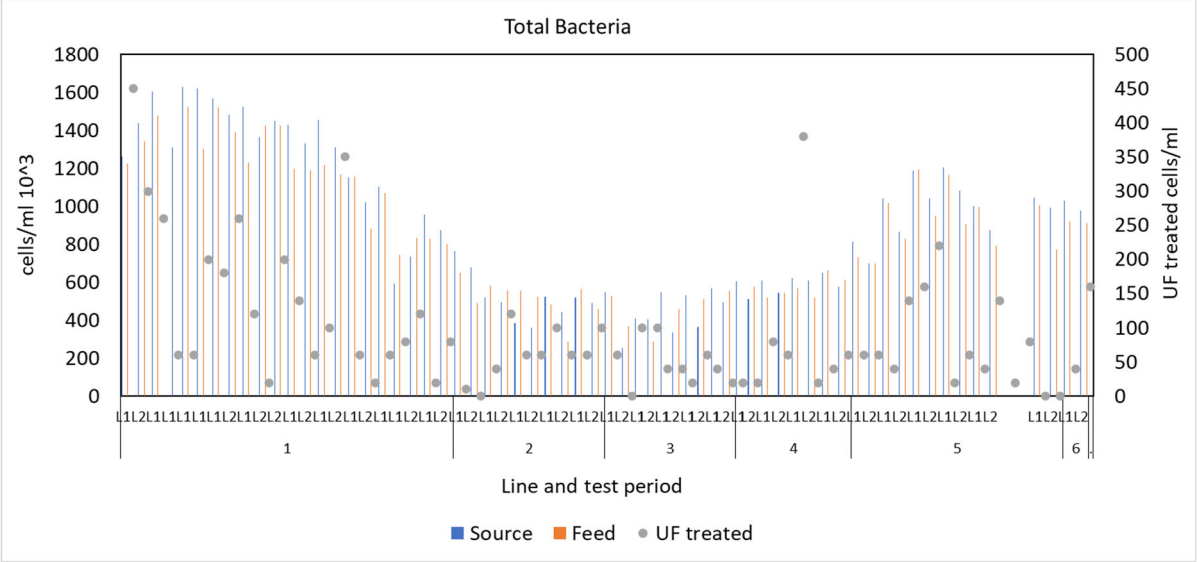

Figure 3. Manual flow cytometry measurements of line 1 (L1) and line 2 (L2) for the whole test period. The graph shows total cell count (TCC) of bacteria using a fluorofure (SYBRGreen 1) as dye.

Microbial reduction is (or has) often been referred to as log reduction. Table 2 shows the log reduction from incoming source water and outgoing permeate water for both L1 and L2. Log3 to log4 is the result when calculating bacterial removal from the manual flow cytometry analysis. There is an even spread for all the periods, so it doesn't seem as if the different tests during period 1 to 7 have had an impact on the log reduction.

Table 2. Manual Flow Cytometry measurements of Bacteria.

Bacterial log reduction from Source to Permeate				
Datum	**L1 %**	**L1 log**	**L2 %**	**L2 log**
2022-10-12	99,96436	3		
2022-10-20			99,97915	3
2022-10-25	99,98380	3		
2022-10-31	99,99632	4		
2022-11-01	99,98765	3		
2022-11-04	99,98853	3	99,98248	3
2022-11-08	99,99211	4	99,99853	4
2022-11-10			99,98619	3
2022-11-14	99,99020	4	99,99550	4
2022-11-15	99,99312	4	99,97334	3
2022-11-22	99,99479	4	99,99804	4
2022-11-30	99,99455	4		
2022-12-06	99,98650	3	99,98367	3
2022-12-13	99,99791	4	99,99085	4
2023-01-03	99,99869	4	100,00000	
2023-01-10	99,99232	4	99,97579	3
2023-01-17	99,98447	3	99,98339	3
2023-01-25	99,98087	3	99,98643	3
2023-01-31	99,98845	3	99,97965	3
2023-02-07	99,98902	3	100,00000	
2023-02-14	99,97548	3	99,97539	3
2023-02-21	99,99267	4	99,98804	3
2023-02-28	99,99622	4	99,98357	3
2023-03-08	99,99294	4	99,99595	4
2023-03-14	99,99670	4	99,99609	4
2023-03-21	99,98690	3	99,98896	3
2023-03-27	99,93900	3	99,99671	4
2023-04-04	99,99385	4	99,98957	3
2023-04-11	99,99262	4	99,99140	4
2023-04-18	99,99616	4	99,98381	3
2023-04-25	99,98655	3	99,97887	3
2023-05-02	99,99834	4	99,99446	4
2023-05-08	99,99600	4	99,98402	3
2023-05-23	100,00000		100,00000	
2023-06-07	99,99611	4	99,98363	3

During the test period, heterotrophic plate count has been analysed for source water and UF water, and some dates also feed water. Figure 4 shows the results of number of colony forming units after 3 days in 22°C. Even though the total number of bacterial cells is exceedingly lower than that of the present outgoing water from the plant (comparison based on flow cytometric data) there are some colonies forming from the permeate water. One theory is that the low number of cells can more easily grow on the agar plate when competition of nutrient is low. Another theory might be non-mature bacterial cells, or even spores, going through the membrane. These theories might explain why size exclusion does not result in total removal of bacterial cells. However, this uncertainty makes heterotrophic plate count not suitable as a method for quality check of UF treated water.

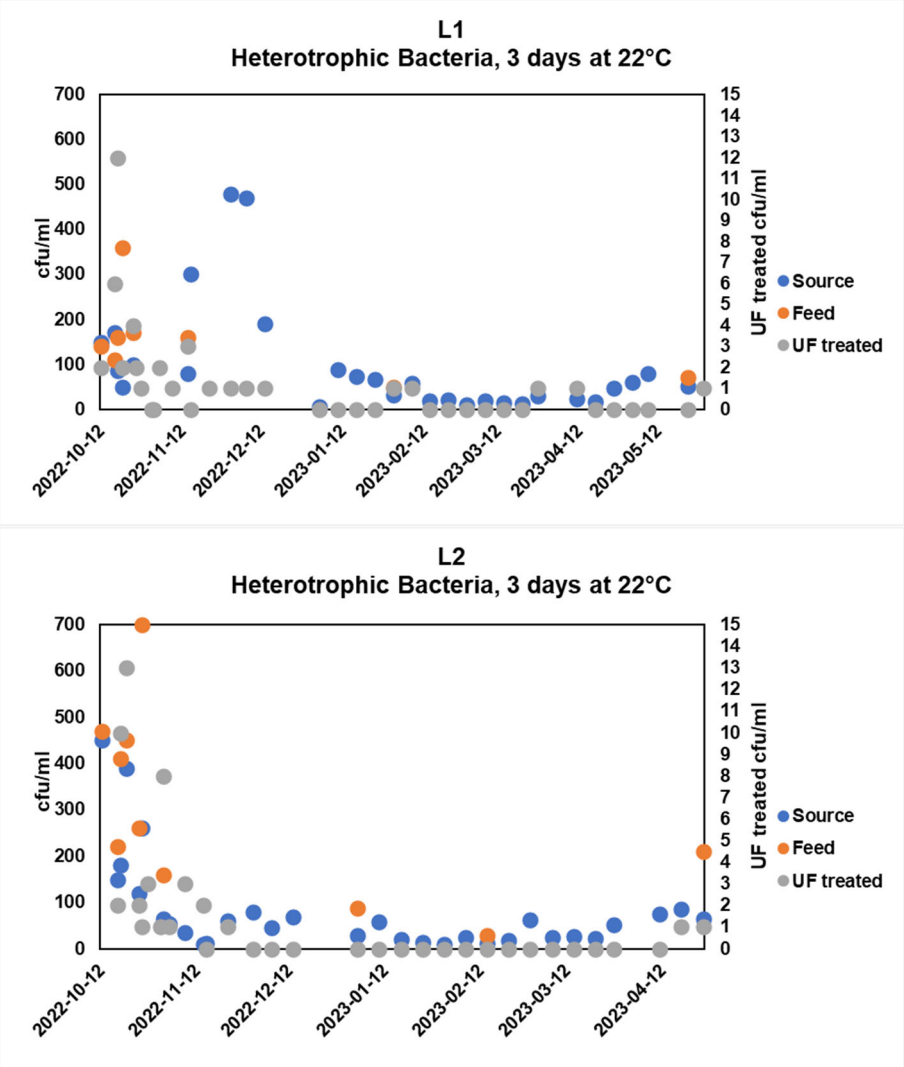

Figure 4. Heterotrophic plate count of line 1 (L1) and line 2 (L2) for the whole test period.

3 Results and discussion – Online Flow Cytometry

For period 4 and onwards, an online flow cytometer (Bactosense, bNovate) was installed to continuously measure TCC on permeate L2. Figure 5 is showing the results for both online and manual analysis.

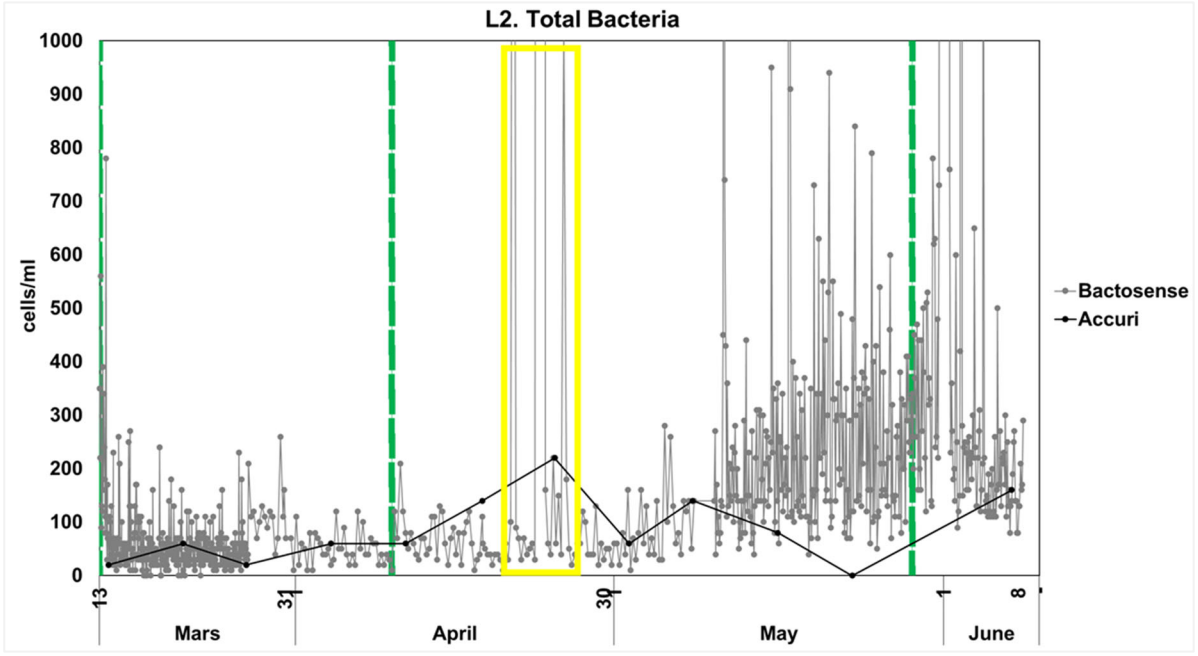

Figure 5. Flow Cytometry measurements; online (Bactosense bNovate) and manual measurements (Accuri C6+, BD Biosciences), on Permeate water from line 2. Green dotted lines indicate Period 4, Period 5 and Period 6. Different intervals of online measurement, in order from start of measurement; every hour, every sixth hour, and every other hour. Outliers in Period 5 are up to 10-fold and up to 100 times more in comparison to cell concentrations of 100 cells/ml. Yellow box are known events causing the bacteria concentration to spike. Gray box are important instrument diary notes.

Permeate measurements give similar result in total counts when looking at both online and manual data. Overlooking the outliers in the beginning of period 4 (due to some ordinary starting adjustments) other outliers have had an explanation. In period 5 the total expend of permeate and adjustment of CEB have resulted in outliers where the bacterial concentration has increased 10 folds and up 100 times when compared to bacterial concentrations of 100 cells/ml.

When the cartridge of the online cytometer was shifted, a higher bacterial concentration was measured. The differences between measurements also increased, leading to a more

fluctuating data set. There is a difference between the online and manual results. In an attempt to once again stabilize the measurements, the instrument underwent maintenance service on June 13th. Tubings, filters and pumps were changed. Despite the service, the measured cell counts were in the same range as the initial measurements of the new cartridge (these values are not shown in Figure 6). Although, an important aspect is the limit of detection that the instrument can carry out. Detection limit is 100 cells/ml and according to the manufacturer the measuring range is starting from 1000 cells/ml. So, values between 100-1000 cells/ml may not be accurate.

When talking to the instrument reseller, other drinking water producers have tried out reagent for measuring intact cell count (ICC) instead of TCC, giving much more stable results. So, in future testing changing to this reagent could be beneficial.

4 Results and discussion – Viruses part one

This chapter is based on the results of the master thesis *Evaluation of The Viral Reduction Potential using Ultrafiltration Membranes in the Drinking Water Treatment Process at Norrvatten* (Emma Eriksson, 2023). The tables and figures are copied from the thesis.

The project focused on certain viruses and phages, seen in table 3 to determine the UF function of virual reduction.

Table 3. Viruses investigated in the project thesis Evaluation of The Viral Reduction Potential using Ultrafiltration Membranes in the Drinking Water Treatment Process at Norrvatten (Emma Eriksson, 2023).

Virus Name	**Size**	**Shape/Morphology**	**DNA/RNA**
Norovirus [34]	27-38 nm	Icosahedral	RNA
COVID-19 [35]	70-90 nm	Icosahedral	RNA
PMMoV [36]	18 nm in diameter, 300-310 in length	Rod-shaped	RNA
Pseudomonas Phage [37]	6 nm in diameter and 3,700 in length	Filamentous	DNA
MS2 Phage [38]	23-28 nm	Icosahedral	RNA

The projects first experimental evaluation was done on four types of water samples: incoming water (raw water) to the plant, in- and outgoing water from the UF pilot plant and backwash water from the UF membrane. Approximately 9000 l of in- and outgoing water from the UF pilot plant and 400 l of raw water was sampled through electropositive filters (NanoCeram, Argonide Corporation) and then processed by ultracentrifugation. Backwash water was concentrated 80-40 ml vacuum filtration. Analysis methods used were PCR and qPCR and cultivation looking for plaque forming units (detection of bacteriophage).

No amplification in the PCR experiments came out from the raw water, probably due to too low concentration in the sample. But the backwash water, seen in table 4 gave amplification of the plant Pepper Mild Mottle Virus (PMMoV) (18 nm in diameter and 300-310 nm in length), meaning this virus comes in with incoming raw water and passes through the first microbiological barrier at Görvälnverket. Also, *Pseudophages* (phages that infect Pseudomonas sp. bacteria) seem to pass through the same way since there was plaque detection on the bacterial cultivations from the backwash water.

Table 4. Backwash water from UF pilot plant indicate Pepper Mild Mottle Virus (PMMoV) being present. First experiment had contaminated positive controls whereas the second didn't.

Experiment	Amount of Samples	Postive Samples	Ct-values
First	4	3	35,8, 37,53 38,09
Second	4	2	38,43, 45,46

Using Qubit analysis for determine the DNA concentration, seen in Figure 6, it seems like the most reduction is happening at the first microbiological barrier at Görväln plant.

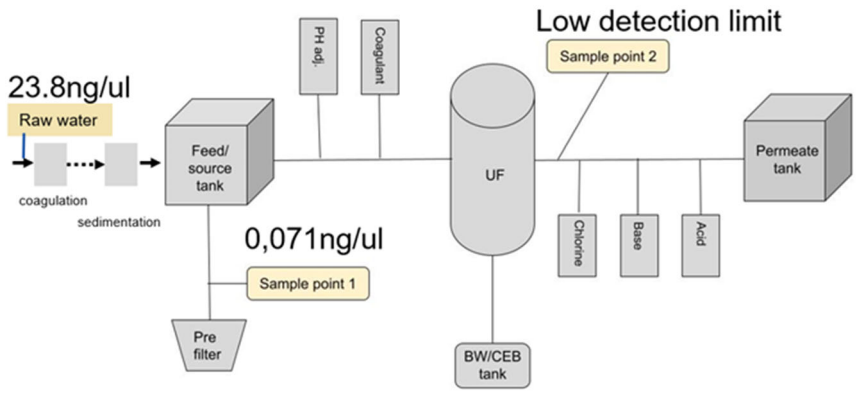

Figure 6. DNA concentration using Qubit analysis of PCR targeting Pepper Mild Mottle Virus (PMMoV) and Pseudomonas phage on water samples from incoming raw water to Görväln plant and in- and outgoing water to the ultra-filtration pilot plant.

Another evaluation from the project was done using a bench scale model (Pentair X-flow RX300 0,83 UFC) andMS2 phages (approximately 27 nm) as spiking indicator. Table 5 shows the results of cycle threshold (Ct) values from qPCR. This indicates after which cycle the target nucleic acid (MS2 phage) was detected. If the target nucleic acid is detected, it means the sample contained MS2. Therefore, Table 5 shows that some MS2 passed through the 20 nm membrane. This experiment should however be repeated due to some question marks regarding the filter's expiration best before date. However, this result is important when it comes to maintenance of ultrafilters and their functionality.

Table 5. qPCR from MS2 spiking of b*ench-scale ultrafiltration membrane (20 nm). Three positive controls containing 2,9 x 10^10 PFU MS2 phages/ml, 2,9 x 10^8 PFU MS2 phages/ml, and 2,9 x 10^6 PFU MS2 phages/ml,was used giving Ct values of 25.067, 30.339 and 30.679.*

Sample Name	Ct Replicate 1	Ct Replicate 2	Mean Ct	Standard Deviation Ct
Backwash	31,42	33,70	32,56	1,61
In	28,99	33,59	31,29	3,25
Out	38,50	35,93	37,21	1,81

Conclusions from the project is that the UF reduces PMMoV and therefore one could draw the conclusion that other virus particles of the same size and larger also is reduced. Although, to determine the exact virus reduction potential, further testing is required and to find other viral candidates of different sizes and shapes would be most needed. The bench scale experiment showed indications of viruses larger than the pore size could be present in the UF water.

Human viral pathogens that are known to be able to be transmitted through water are larger than 20 nm (Norovirus, hepatitis, and adenoviruses for instance). But there are other viruses to be concerned about: one example is parvoviruses which are much smaller and could potentially slip through and have an indirect effect on humans (bovine parvovirus).

Future perspective would be to try out more spiking experiments, both in pilot plant but also in bench scale pilot.

And still, to this date, the limitations of techniques to measure viruses which mirrors the reality are of great concern.

5 Results and discussion – Viruses part two

This chapter is based on the experimental report *Sammanställning UF barriärverkansförsök* written in collaboration with the Swedish Food Agency (Dahlén H, Eriksson R and Grubisic L. 2023).

Background and introduction

To evaluate the ultra-filters ability to reduce viruses a spike experiment was performed in collaboration with the Swedish Food Agency (SLV) and its National Emergency Laboratory (nationellt beredskapslaboratorium, NBV). MS2 coliphages was used as a model virus for small viruses like pathogenic norovirus.

The permeate was expected to have a very low concentration of MS2 and therefore a high concentration of MS2 stock were used. Sampling were done with SLV's dialysis filter method and then analysed with two different analysis methods: digital droplet PCR (ddPCR) and classic plaque assay.

Norovirus is a common human pathogenic virus that is extremely stable in aquatic environments and spreads via poor separation in sewage treatment plants to other waters. MS2 has many similarities to norovirus and is an icosahedral, positive-sense single-stranded RNA virus that infects the bacterium *Escherichia coli*. The size that is of great importance for a filter solution is approx. 23-28nm for MS2 and agrees well with the norovirus, approx. 27nm (SLV, 2023). MS2 can be grown to high concentrations and are thus suitable for high levels needed to measure high reduction.

The spiking procedure

The source tank has a capacity of 1.5 m^3 but is adjusted to 600 l (to simplify the experiment with not getting too much wastewater). A pump is used as a stirrer so that spiking material is mixed as homogeneously as possible in the tank. Samples for analysis are taken from the source tank to measure the MS2 concentration in spiked water before filtration. The spiked water is filtered through the ultrafilter and the permeate is collected in a clean tank. From this tank, samples are taken directly for analysis at SLV, but also 75 l is filtered with a dialysis filter for concentration of MS2 phages before analysis. The backwash water of the ultrafilter is also sampled. A total of four filtrations are made through the pilot plant, where one trial is without MS2 (negative control) and three with MS2. Figure 7 illustrates the pilot setup and sampling spots.

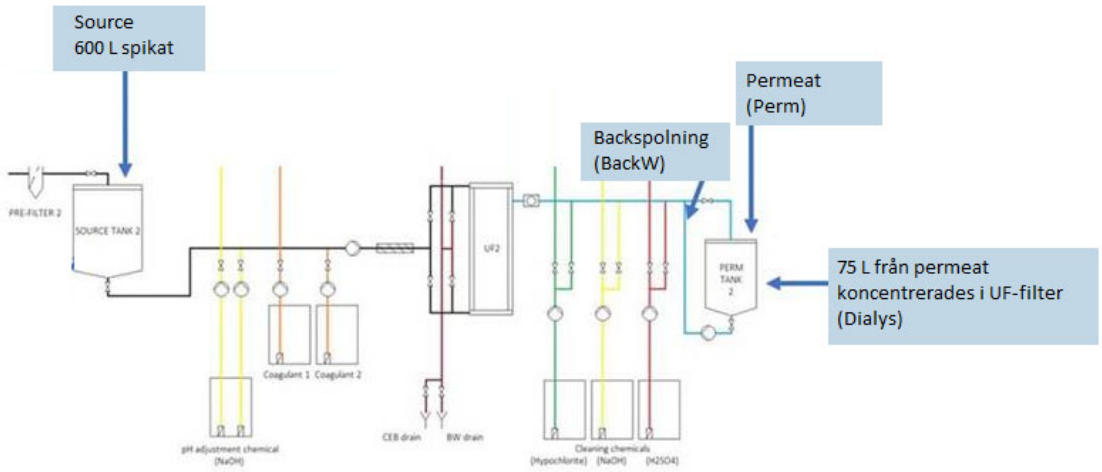

Figure 7. Overview of ultrafilter pilot plant at Görävlnverket in 2023. Arrow indicates sampling spots for spiking experiments with MS2 phages.

600 l of water in the source tank is spiked with 4.5 mL of MS2 phages with an expected initial concentration of $2.9*10^{10}$ phages/ml. This is expected to give a concentration of $2.9*10^{2}$ phages/L. The pump mixes the added phages for 10 minutes before ultrafiltration is started.

Table 6 presents the pilot's volumes and flows for the spiking experiment.

Table 6. Spiking with MS2 phages, setup of the ultrafiltration pilot model during the experiment.

OBJECT	VALUE	UNIT
Length	25	m
Pipe diameter	0.06	m
Crossection	0.0028	m^2
Pipe volume	0.0707	m^3
Tank volume	1.5	m^3
Pump tank volume (from 75 to 15%)	0.6	m^3
Released tank volume	0.9	m^3
Flow	0.0016	m^3/s
Piper inse time	45.4	s
Filtration time	578.6	s

For backflush sampling, the stagnant water in the pipes is considered and sampling takes place after the pipes have been flushed out. Consistently for all sampling is that it is the first flush water that is captured. The backflushing is relatively fast, and a complete flushing of the filter takes about 1.5 minutes.

Samples

At each filtration trial, water samples of 2 x 250 ml were taken at the Source, Permeate and Backwash (BackW) points. Also, 75 l of permeate water was taken for further concentration with a dialysis filter (Fresenius Cordiax 120dx). The dialysis filter has a cut-off of 33KDa, which corresponds to a pore size of about 8 nm. In short; 1400 ml/min filtration speed without the pressure in the filter exceeding 0.65 bar. The dialysis filters were eluted with 250 ml of PBS (pH7.2), 0.01% NaPP, 0.001% Antifoam A, 0.01% Tween80 by backflush press. The volumes obtained from the elution were A 400 ml, B 370 ml, C 495 ml and NEG 340 ml.

PEG precipitation

150 ml sample from; source tank, permeate, backwash and dialysis concentrated permeate were mixed with 2 g beef extract and 50 ml 5xPEG/NaCl. The samples are shaken until everything is dissolved and incubated in a cold room on a rotary table overnight. Incubated samples are centrifuged for 30 min, 10,000 x g before decanting the supernatant. Another centrifugation 5 min, 10,000 x g where all remaining liquid is removed with a pipette. The pellet is then dissolved in 2 ml of PBS pH7.2. Then 1 ml was used for plaque analysis and 1 ml for nucleic acid extraction.

NucliSens extraction

1 ml sample from all sampling points and concentration steps, see Appendix 1 for complete list, nucleic acid extracted with Biomeriux miniMAG and NucliSens reagents according to standard protocol. For concentration determination of MS2 from stock, 100 µl is extracted. All samples are eluted in 100 µl. A negative nucleic acid extraction control with 1 ml of PBS was extracted at the same time as the samples.

Plaque analysis

The amount of MS2 phage was analysed in all PEG-precipitated samples (Source, Permeat, Dialysis, BackW) according to ISO 10705-1. 1 ml of each sample was analysed and run in duplicate along with positive (MS2 100 PFU/ml) and negative controls (PBS). At high phage concentration, the samples were diluted 10x. The spiking material, MS2 stock from Norrvatten, was also quantified by plaque analysis.

Digital Droplet PCR

Nucleic acid extracted samples were analysed by Bio-RAD Q200 droplet digital PCR (ddPCR) to measure the amount of RNA copies from MS2 phage. For the analysis, the Bio-RAD One-Step RT-ddPCR Advanced Kit for Probes was used, which together with primers and probes for MS2 phage constituted the master mix. In a first step, 16.5 µl master mix and 5.5 µl sample were added to a plate. From this plate, 20 µl per well was then transferred to a droplet generator together with 70 µl of droplet generator oil. Droplets were generated in the Bio-RAD QX200™ Droplet Generator and 40 µl were then transferred to a PCR plate. PCR was run after which the droplets were read with the Bio-RAD Q200 droplet digital PCR. Prior to the experiment, an evaluation of the sensitivity of MS2 phage was made with the ddPCR analysis where concentrations from 200 copies per reaction down to 0.323 copies per reaction were analysed. The LOD95 for MS2 was then determined to be 3.5 copies/PCR reaction.

Appendix 1 contains a compilation of samples and results for all samples that were analysed with ddPCR. The samples from the Source tank were analysed undiluted and with dilution

1:10 and 1:100. Samples from Backwashing and dialysis were analysed undiluted and diluted 1:10. The stock solution was diluted and analysed up to 10-6.

Results/Discussion

The concentration of MS2 phages in the various sampling points measured with ddPCR and plaque analysis from the spike experiments is compiled in Appendix 1.

The concentration of MS2 stock solution was quantified by ddPCR and plaque assay and measured to be 3.07 x 10^{10} copies/ml by ddPCR and 7.3 x 10^{6} PFU/ml infectious phages by plaque assay. Such a large difference between detected RNA by ddPCR and infected plaques from the plaque assay gives indications that there are a lot of damaged or non-infectious MS2 phages in the stock solution.

MS2 phage could be detected in the samples from the Source tank (spiked water) both with ddPCR and plaque analysis, figure 8. The MS2 concentration in the source tank is on average 2.6x10^{7} copies/l (ddPCR) and on average 1.9x10^{4} PFU/l (plaque analysis).

Despite concentration with a dialysis filter, MS2 could not be detected in the permeate from the ultrafilter either with ddPCR or plaque analysis. The concentration of MS2 phage in the backwashes was 4.9x10^{6} – 1.8x10^{7} copies/l and 1.3x10^{5} – 4.7x10^{5} PFU/L.

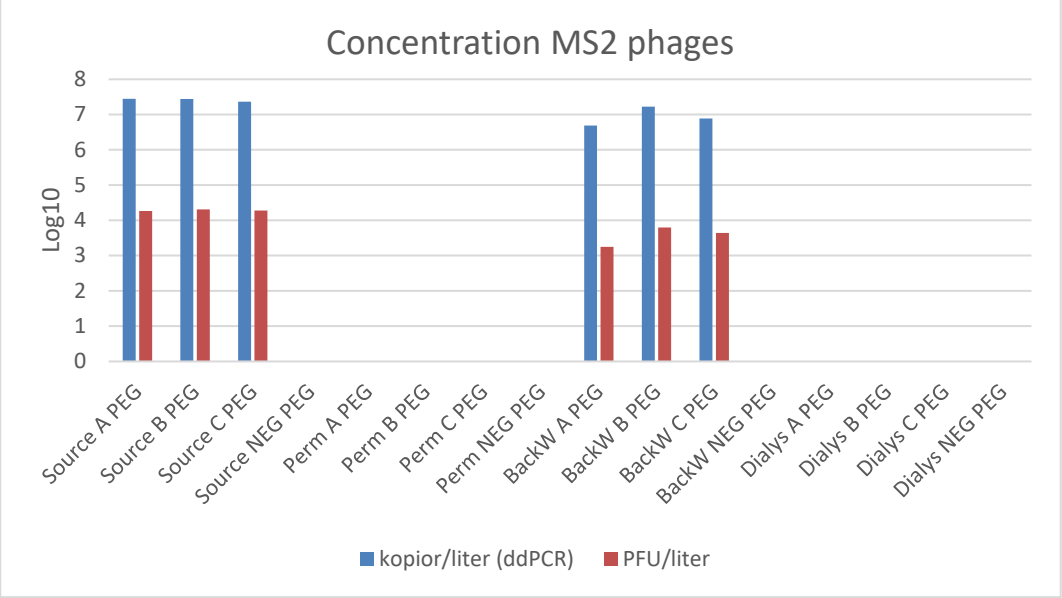

Figure 8. Log$_{10}$ of MS2 phages/l with ddPCR (blue) and plaque assay (red) from different samplings spots in spiking experiment in ultrafiltration pilot.

Since MS2 phages could not be detected in the permeate, it is difficult to comment on the level of reduction through the barrier filter. As the actual limit of detection (LOD) has not been fully evaluated, a theoretical LOD for ddPCR has been calculated. The calculation was based on the sensitivity of the ddPCR (LOD95) and approximate recoveries from setup experiments with dialysis filters and MS2. With the ddPCR's LOD95 of 3.5 copies/reaction and an approximate total recovery of 10% over both dialysis and PEG precipitation, it gives an LOD calculated per liter source sample for the different sampling steps according to

table 7. This LOD is of course very theoretical with high uncertainty but still based on real results during testing.

Table 7. Theoreticla LOD in ddPCR analysis.

Sample	Theoretical LOD (RNA copies/l original sample)
Direct sample	7,00E+05
PEG-precipitated	9,33E+03
Dialysis filter	7,50E+02
Dialys + PEG	6,16E+01

Nevertheless, assumption of log reduction can be based on that sensitivity for the ddPCR analysis from the PEG precipitated permeate is about $1x10^4$ copies/l after a 10% recovery with a spike level of $2.6x10^7$ copies/l in the source gives a barrier efficiency of up to 3.4 Log10 or more (Table X). If the same assumptions are made for the dialysis concentrate including PEG-precipitated eluate and a 10% recovery, it gives a theoretical LOD of 60 copies/l indicating a reduction/barrier effect of nearly 5.7 Log10 or more, table 8.

Table 8. Log_{10} means of copies/l in source tank compared to theoretical LOD to estimate virus reduction.

ddPCR				
Source	**Permeat (PEG)**		**Permeat (Dialys + PEG)**	
Copies/l ddPCR (Log_{10})	Theoretical LOD (Log_{10})	Reduction (Log_{10})	Theoretical LOD (Log_{10})	Reduction (Log_{10})
7,4	4	3,4	1,8	5,7

Even though the phages turned out to be weak in stock solution and a proper log reduction couldn't be made, a theoretical one could. Also, the levels from the backwash water could be seen as a receipt of the ultrafilters capacity of capturing the pahges particles. Of course, more testing should be done for accuracy and obviously on other membrane brands if they are considered as a future process step for drinking water production, however this experiment indicates a good reduction capacity of this ultrafilter.

6. References

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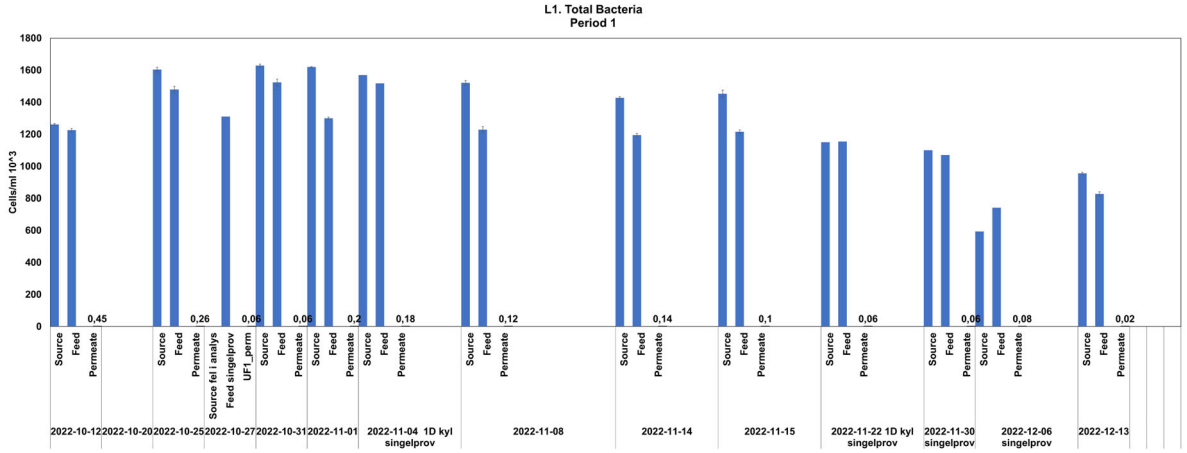
7. Appendix 1

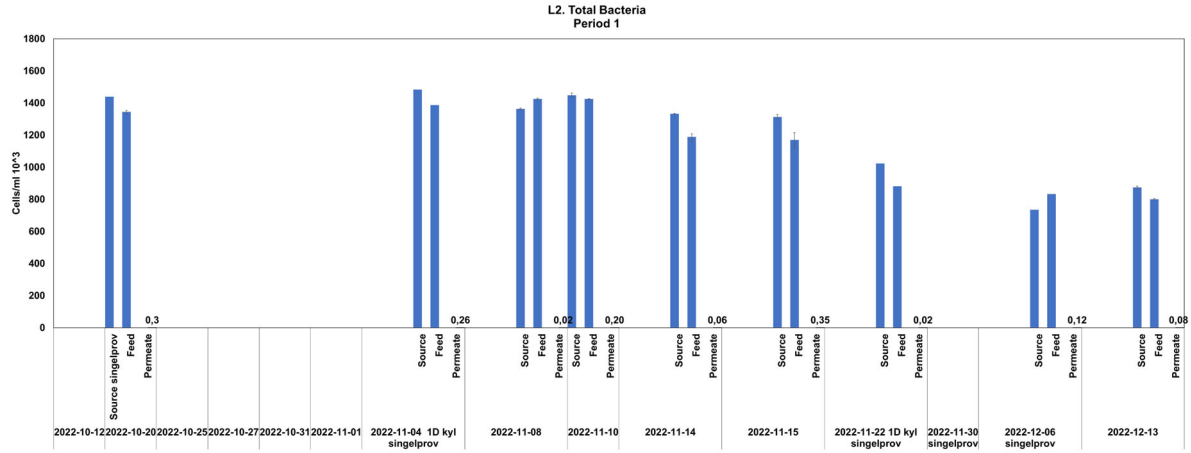
Samples analysed with ddPCR an dplack assay.

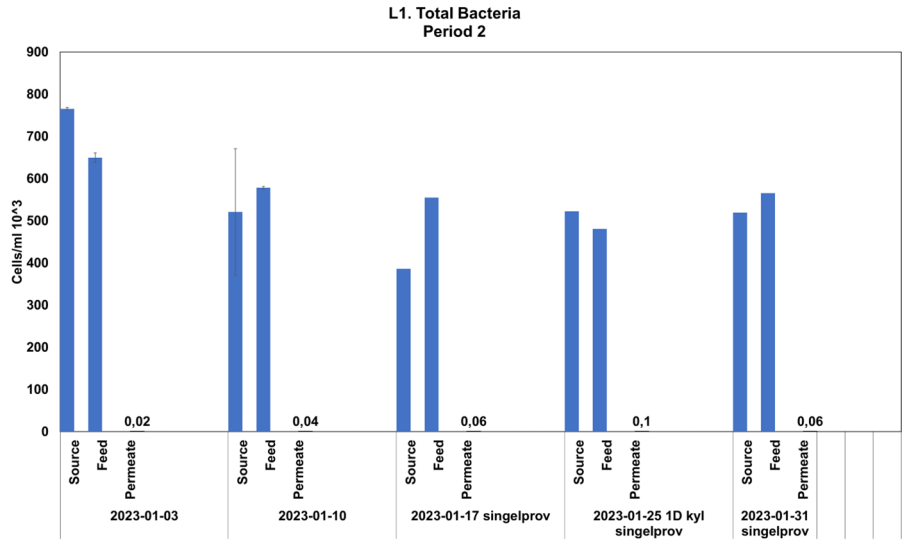
	ddPCR		Plackanalys	
Sample	**RNA copies/PCR reaction**	**RNA copies/L original samples**	**Plaque/ml**	**Plaque/L origianl samples**
Source Neg	0	0	-	-
Source A	2,99E+04	5,99E+08	-	-
Source B	3,36E+04	6,71E+08	-	-
Source C	2,92E+04	5,84E+08	-	-
Source PEG Neg	0	0	0	0
Source PEG A	1,04E+05	2,77E+07	1,37E+03	1,83E+04
Source PEG B	1,03E+05	2,75E+07	1,53E+03	2,04E+04
Source PEG C	8,60E+04	2,29E+07	1,42E+03	1,89E+04
Permeat Neg	0	0	-	-
Permeat A	0	0	-	-
Permeat B	0	0	-	-
Permeat C	0	0	-	-
Permeat PEG Neg	0	0	0	0
Permeat PEG A	0	0	0	0
Permeat PEG B	0	0	0	0
Permeat PEG C	0	0	0	0
Dialys Neg	0	0	-	-
Dialys A	0	0	-	-
Dialys B	0	0	-	-
Dialys C	0	0	-	-
Dialys PEG Neg	0	0	0	0
Dialys PEG A	0	0	0	0
Dialys PEG B	0	0	0	0
Dialys PEG C	0	0	0	0
BackW Neg	0	0	-	-
BackW A	2,67E+03	5,33E+07	-	-
BackW B	1,06E+04	2,13E+08	-	-
BackW C	5,06E+03	1,01E+08	-	-
BackW PEG Neg	0	0	0	0
BackW PEG A	1,83E+04	4,87E+06	1,33E+02	1,77E+03
BackW PEG B	6,24E+04	1,66E+07	4,66E+02	6,21E+03
BackW PEG C	2,88E+04	7,67E+06	3,28E+02	4,37E+03
Neg NA-kontroll	0	0	0	0

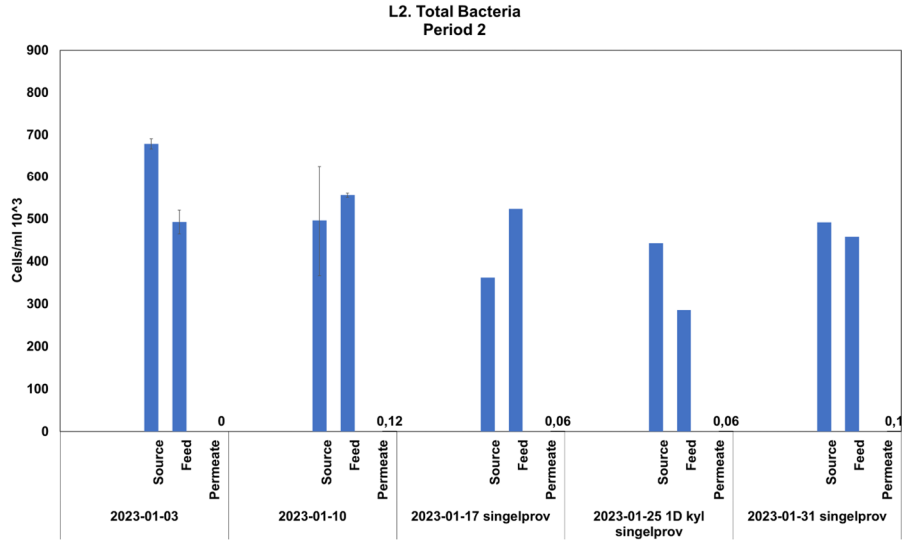
- : Sample not analysed

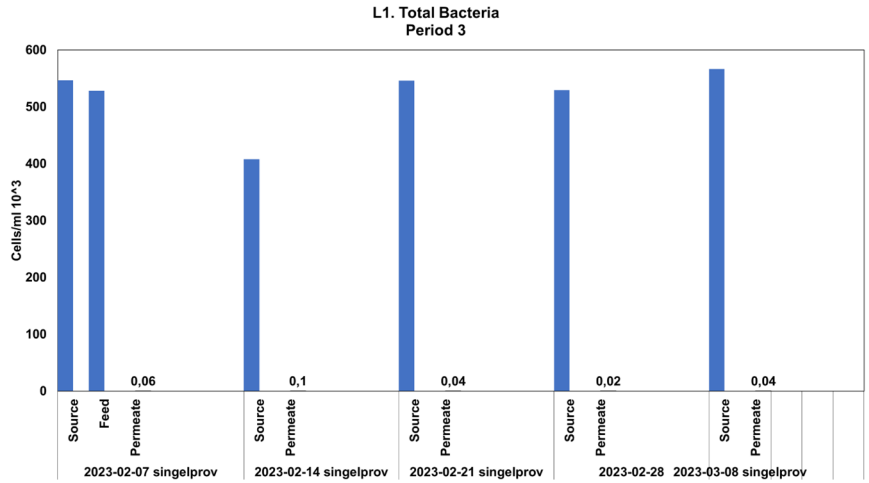
8. Appendix 2

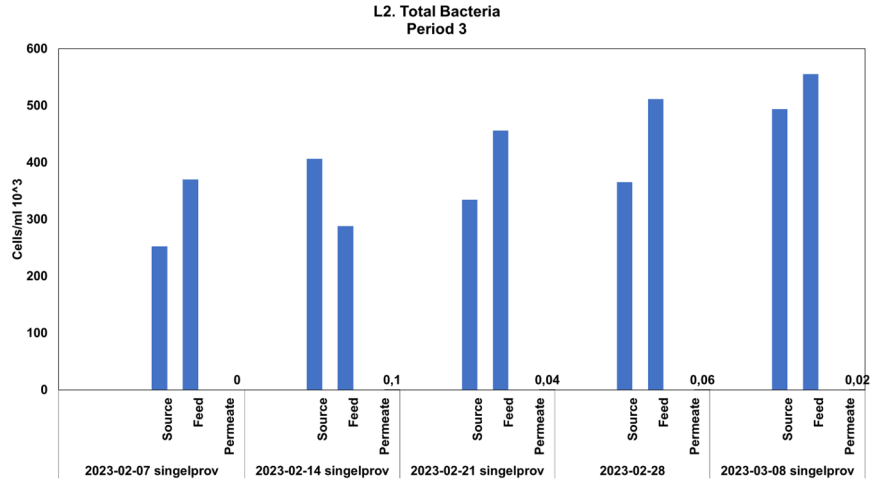

L1. Total Bacteria
Period 1
Cells/ml 10^3
1800
1600
1400
1200
1000
800
600
400
200
0
0,45
0,26
0,06
0,06
0,2
0,18
0,12
0,14
0,1
0,06
0,06
0,08
0,02
Source
Feed
Permeate
Source fel i analys
Feed singelprov
UF1_perm
2022-10-12
2022-10-20
2022-10-25
2022-10-27
2022-10-31
2022-11-01
2022-11-04 1D kyl singelprov
2022-11-08
2022-11-14
2022-11-15
2022-11-22 1D kyl singelprov
2022-11-30 singelprov
2022-12-06 singelprov
2022-12-13

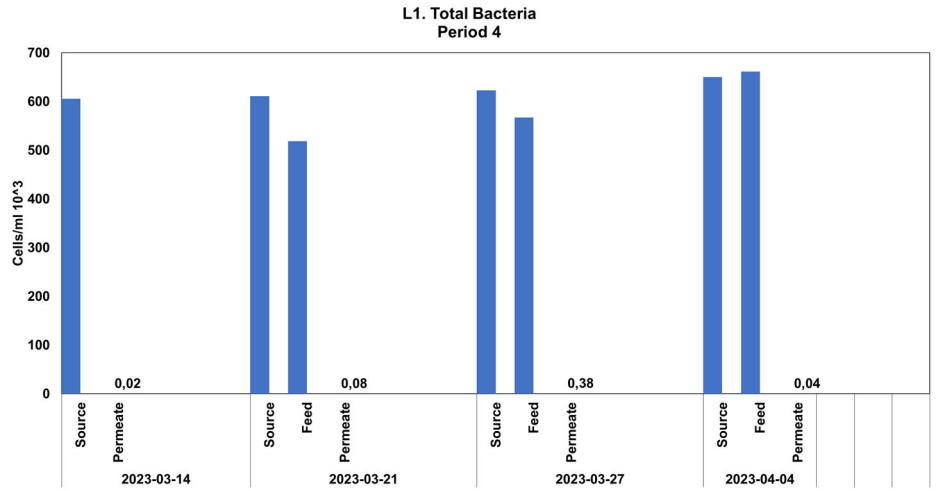

L2. Total Bacteria
Period 1
Cells/ml 10^3
1800
1600
1400
1200
1000
800
600
400
200
0
0,3
0,26
0,02
0,20
0,06
0,35
0,02
0,12
0,08
Source singelprov
Source
Feed
Permeate
2022-10-12
2022-10-20
2022-10-25
2022-10-27
2022-10-31
2022-11-01
2022-11-04 1D kyl singelprov
2022-11-08
2022-11-10
2022-11-14
2022-11-15
2022-11-22 1D kyl singelprov
2022-11-30 singelprov
2022-12-06 singelprov
2022-12-13

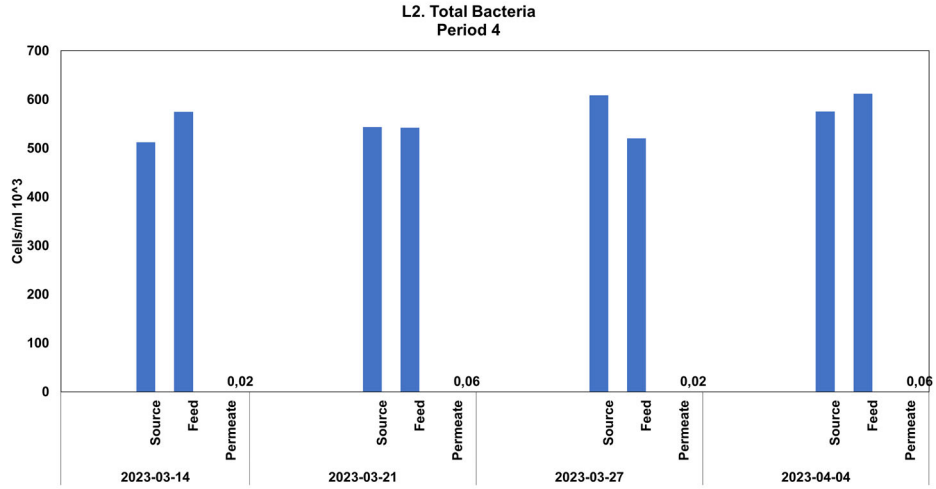

L1. Total Bacteria
Period 2
Cells/ml 10^3
900
800
700
600
500
400
300
200
100
0
0,02
0,04
0,06
0,1
0,06
Source
Feed
Permeate
2023-01-03
2023-01-10
2023-01-17 singelprov
2023-01-25 1D kyl singelprov
2023-01-31 singelprov

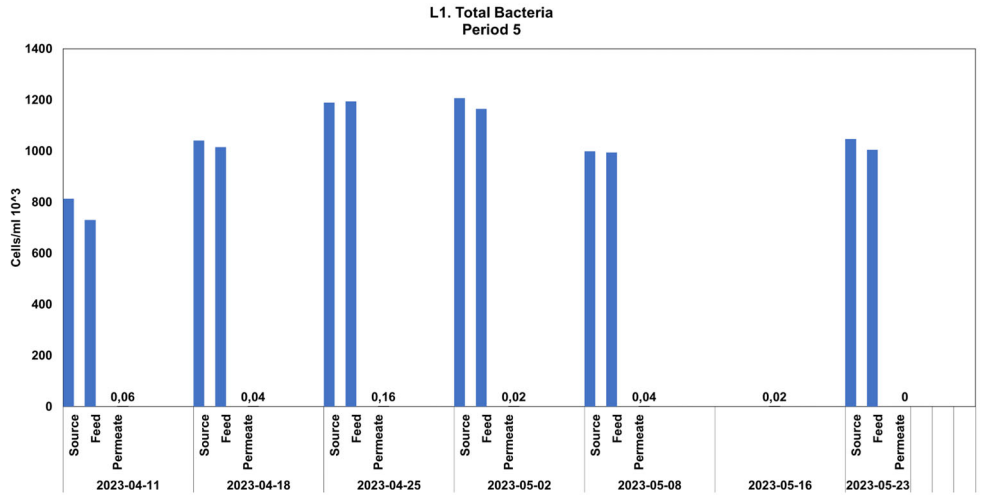

L2. Total Bacteria
Period 2
Cells/ml 10^3
900
800
700
600
500
400
300
200
100
0
0
0,12
0,06
0,06
0,1
Source
Feed
Permeate
2023-01-03
2023-01-10
2023-01-17 singelprov
2023-01-25 1D kyl singelprov
2023-01-31 singelprov

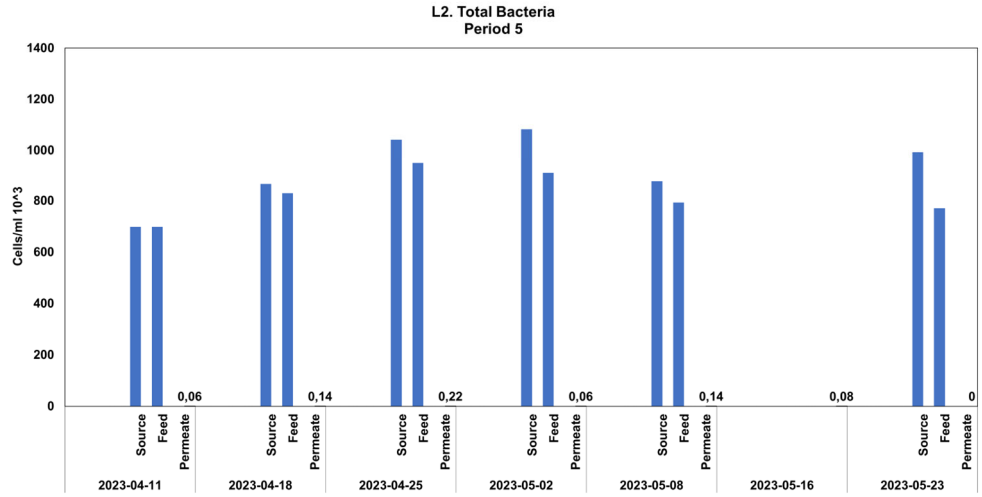

L1. Total Bacteria
Period 3
Cells/ml 10^3
600
500
400
300
200
100
0
0,06
0,1
0,04
0,02
0,04
Source
Feed
Permeate
2023-02-07 singelprov
2023-02-14 singelprov
2023-02-21 singelprov
2023-02-28
2023-03-08 singelprov


L2. Total Bacteria
Period 3
Cells/ml 10^3
600
500
400
300
200
100
0
0
0,1
0,04
0,06
0,02
Source
Feed
Permeate
2023-02-07 singelprov
2023-02-14 singelprov
2023-02-21 singelprov
2023-02-28
2023-03-08 singelprov

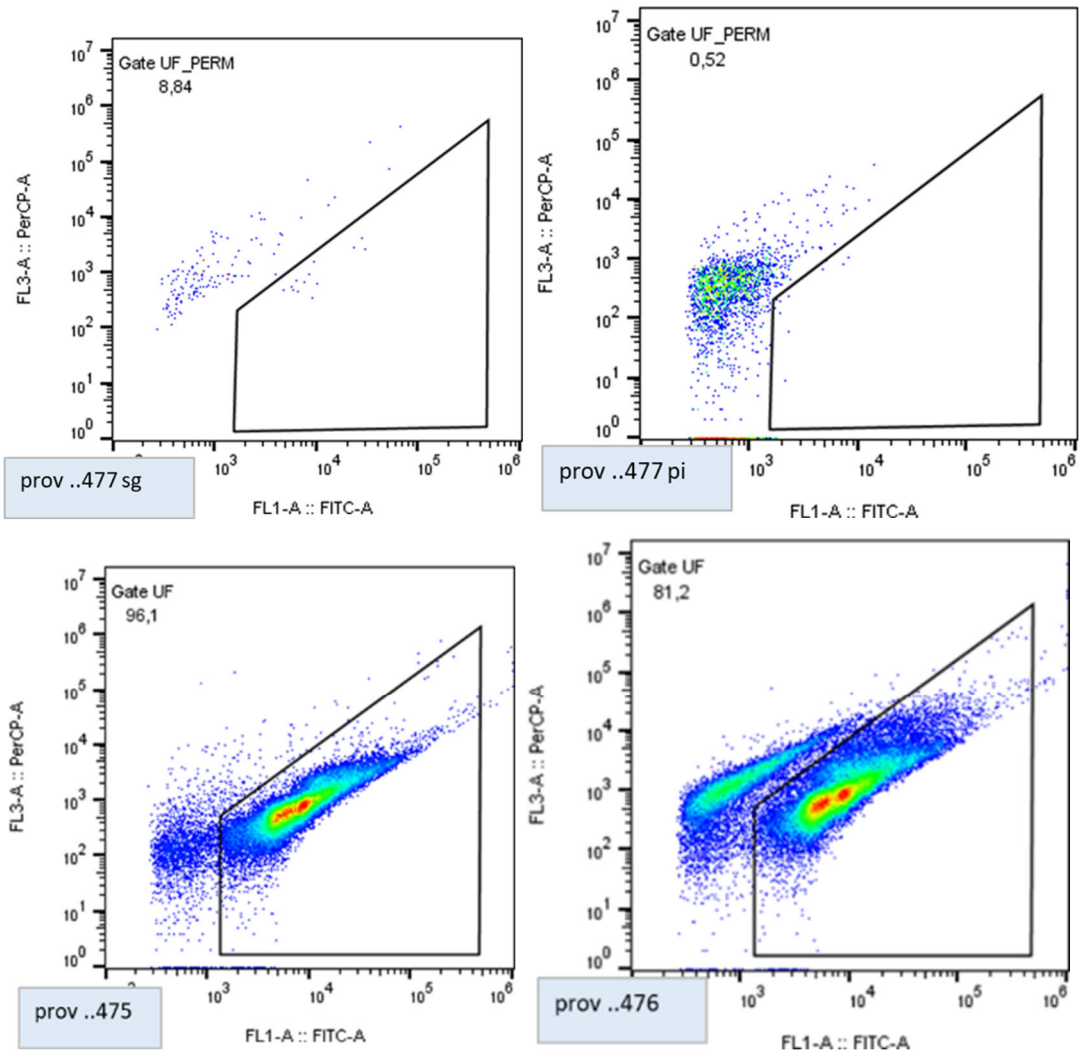

L1. Total Bacteria
Period 4
Cells/ml 10^3
700
600
500
400
300
200
100
0
0,02
0,08
0,38
0,04
Source
Permeate
Source
Feed
Permeate
Source
Feed
Permeate
Source
Feed
Permeate
2023-03-14
2023-03-21
2023-03-27
2023-04-04

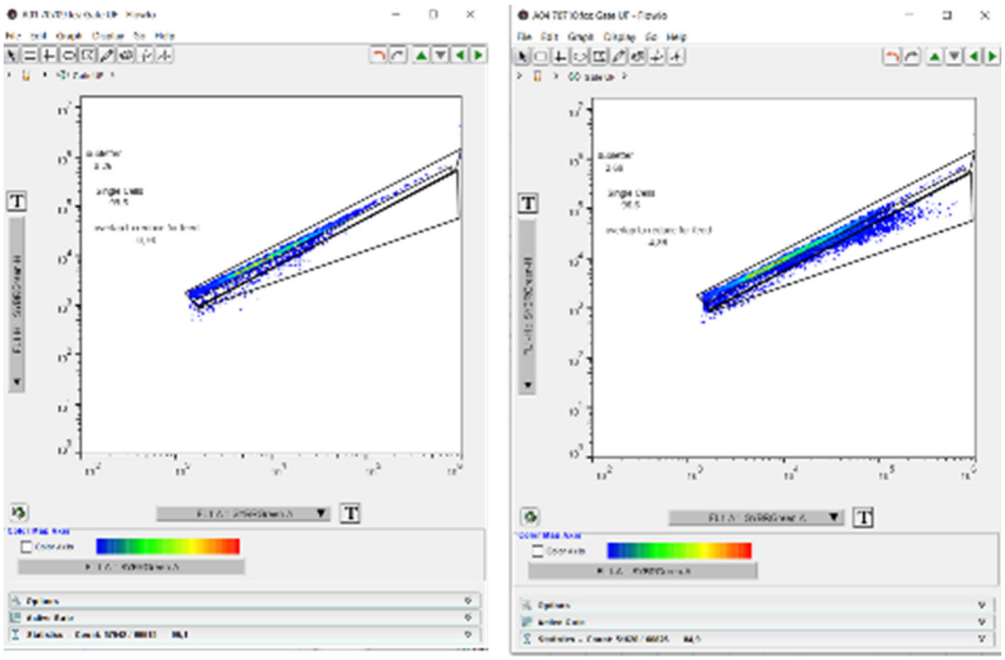

L2. Total Bacteria
Period 4
Cells/ml 10^3
700
600
500
400
300
200
100
0
0,02
0,06
0,02
0,06
Source
Feed
Permeate
Source
Feed
Permeate
Source
Feed
Permeate
Source
Feed
Permeate
2023-03-14
2023-03-21
2023-03-27
2023-04-04


L1. Total Bacteria
Period 5
Cells/ml 10^3
1400
1200
1000
800
600
400
200
0
0,06
0,04
0,16
0,02
0,04
0,02
0
Source
Feed
Permeate
2023-04-11
2023-04-18
2023-04-25
2023-05-02
2023-05-08
2023-05-16
2023-05-23

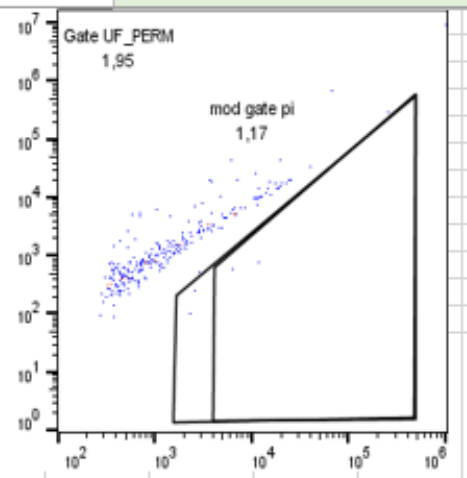

L2. Total Bacteria
Period 5
Cells/ml 10^3
1400
1200
1000
800
600
400
200
0
0,06
0,14
0,22
0,06
0,14
0,08
0
Source
Feed
Permeate
2023-04-11
2023-04-18
2023-04-25
2023-05-02
2023-05-08
2023-05-16
2023-05-23

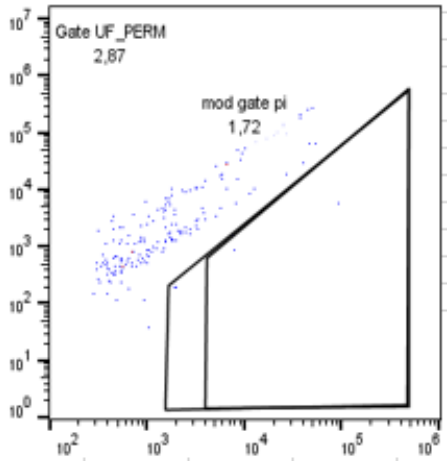
8.1 Flow cytometry gates

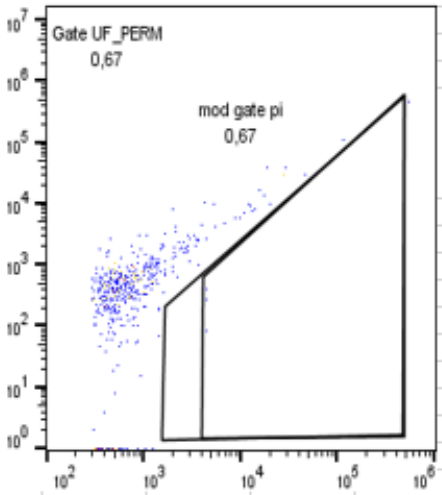


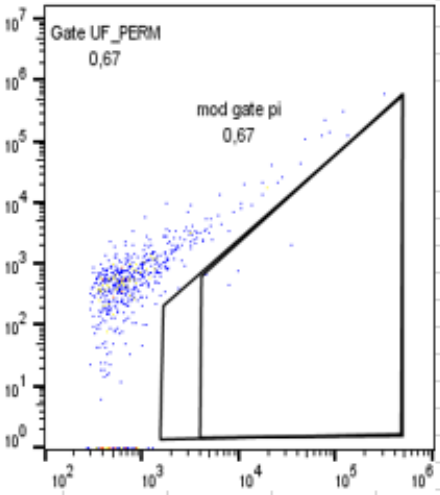


SG (dubbletter) och PI (dubbletter) för prov 70522. Som exempel på hur det ser ut att använda "mod gate pi".

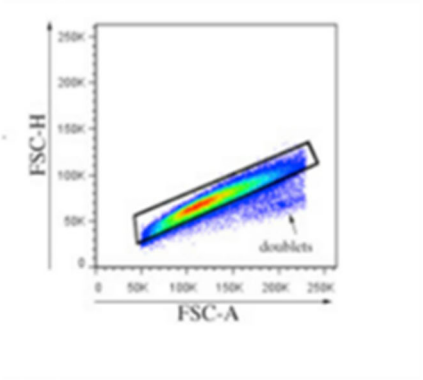




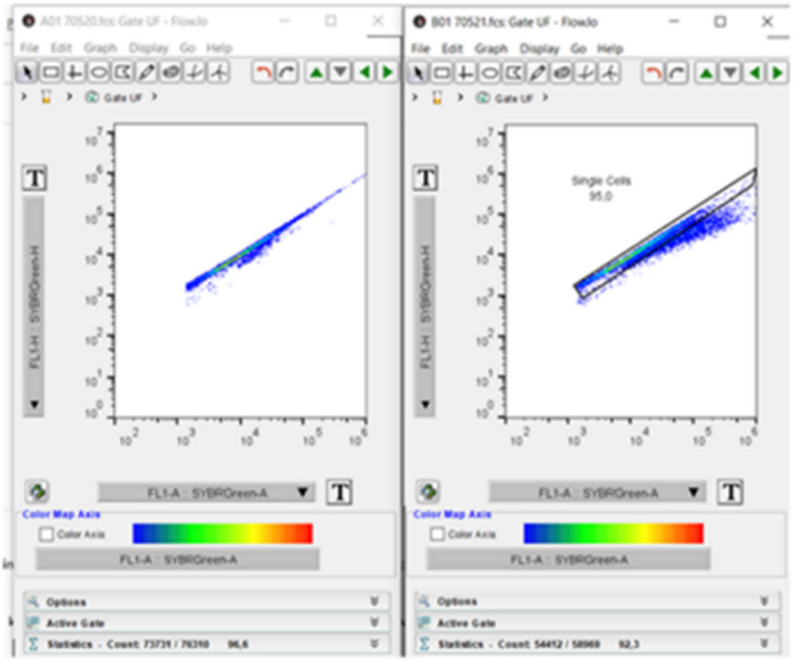




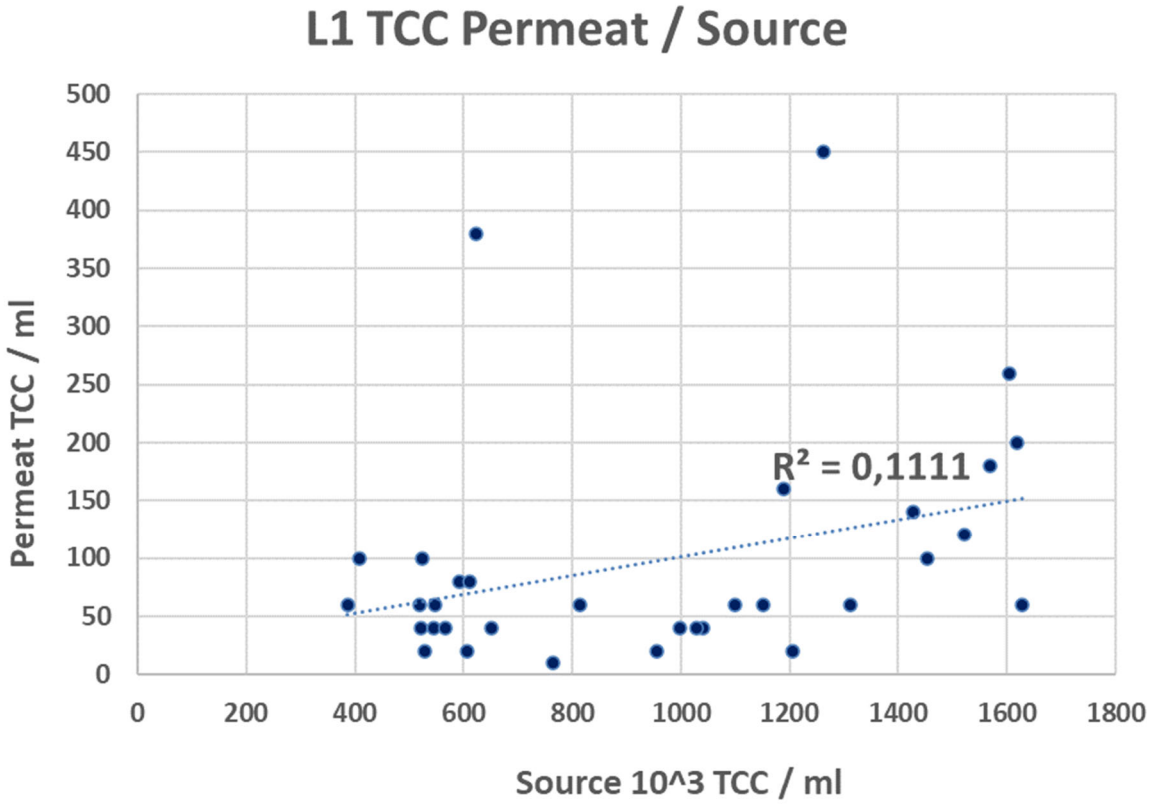
Exempel från litteraturen:

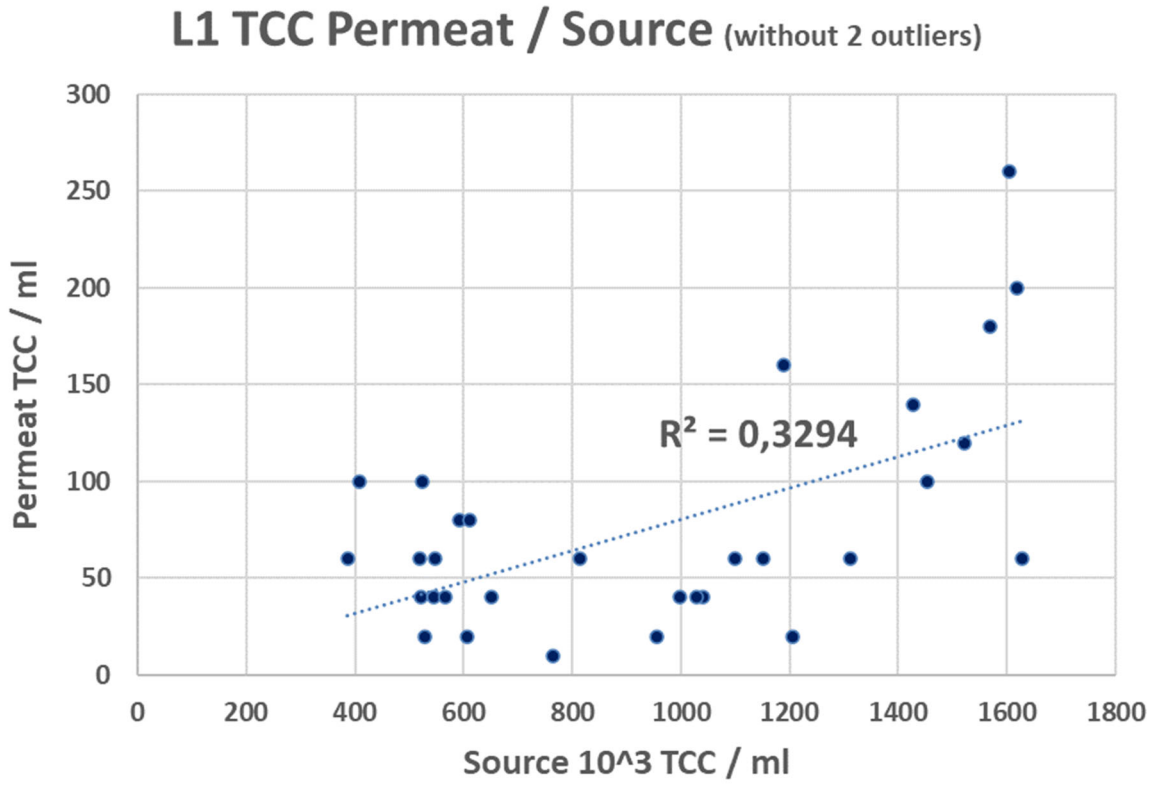


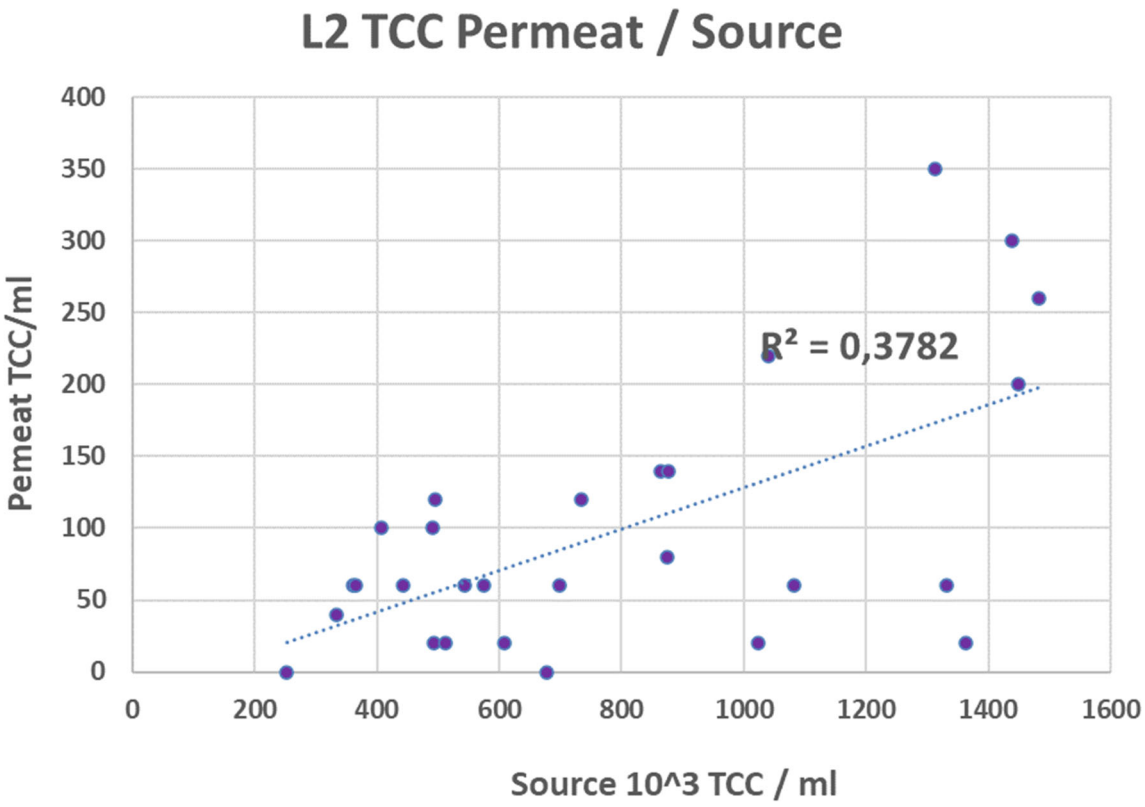
Våra körningar:
70520 är source och 70521 är feed. Ni ser hur det spretar mer i feed (=dubbletter).



8.2 Regression charts






L2 TCC Permeat / Source
Pemeat TCC/ml
Source 10^3 TCC / ml
R² = 0,3782
0
50
100
150
200
250
300
350
400
0
200
400
600
800
1000
1200
1400
1600