Dealing with Microbial Levels in Cold WFI Production Systems

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Membrane-based production of Water for Injection (WFI) is now approved by the European Authorities with the caveat of 2 membrane barriers and heightened control of the system microbiological levels.
Electrolytic Scale Reduction (ESR) and Hydro Optic Disinfection (HOD) Reverse Osmosis (RO) pretreatment is demonstrated in the following case studies, which is media-free and without chemicals. The ESR-HOD combination controls bacteria by constantly and actively disinfecting the water with chlorine, when there is high chloride in the feed water, and high doses of UV radiation from the HOD for Constant Bacterial Reduction (CBR).
As is demonstrated in case studies 1–3, a regulated municipal feed will enable a system based on ESR-HOD-RO-RO-Continuous Deionization (CDI) or ESR-HOD-RO-CDI-Ultrafiltration (UF) to manufacture WFI reliably. Problems ensue when the city water quality fluctuates with high levels of micro and pathogens. In this case there was a low generation of chlorine in the ESR due to low levels of chloride in the municipal feed. The problem was finally solved by a chemical rinse combined with manual cleaning of filter and RO housings and, in addition, by 2 weekly hot water sanitizations (up from the usual 1 weekly sanitization) and free chlorine dosage on the city water feed.
Case 4 also demonstrated that unchecked growth of the bioburden in RO feed is liable to lodge in the RO concentrate compartment and to grow through to the permeate.
A Pseudomonas biofilm was easily removed from the system once detected, as the system is media-free and fully Stainless Steel (SS) fabricated. The only contamination that builds up is on the filters that need to be replaced proactively once every 2 weeks and not per pressure drop.
Some of the systems in the case studies were inspected onsite by US Food and Drug Administration (FDA) authorities and others were inspected by European Medicines Agency (EMA) off-site. *All inspections were passed very well with no comments/requests/warnings.*

Introduction

In Europe, up to 2017, Water for Injection (WFI) systems had to be based on expensive thermal distillation.

WFI production with thermal processes was always used as this was the unequivocal European Pharmacopeia (EP) requirement. Even though the United States Pharmacopeia (USP) has permitted membrane based WFI production for decades, most of the pharma production was based on thermal processes as few companies produce only for the US market [1].

After the latest update of the EP – that came into effect 2017 – that includes membrane production of WFI, there is no further regulatory impediment to widespread production of WFI without thermal distillation in US and Europe. In the words of the European Pharmacopoeia commission on water for injection:

> *"Any non-distillation technology for producing WFI should be equivalent in quality to that produced by distillation, where equivalence in quality does not simply mean compliance with a specification but also takes into account the robustness of the production method"* (underlined words: emphasis by the author) [3].

The robustness of water production with Reverse Osmosis (RO) membranes is high, as the cut off size of an RO membrane is far below the size of a bacterium. Although in practice, bacteria can pass from the concentrate to the permeate side.

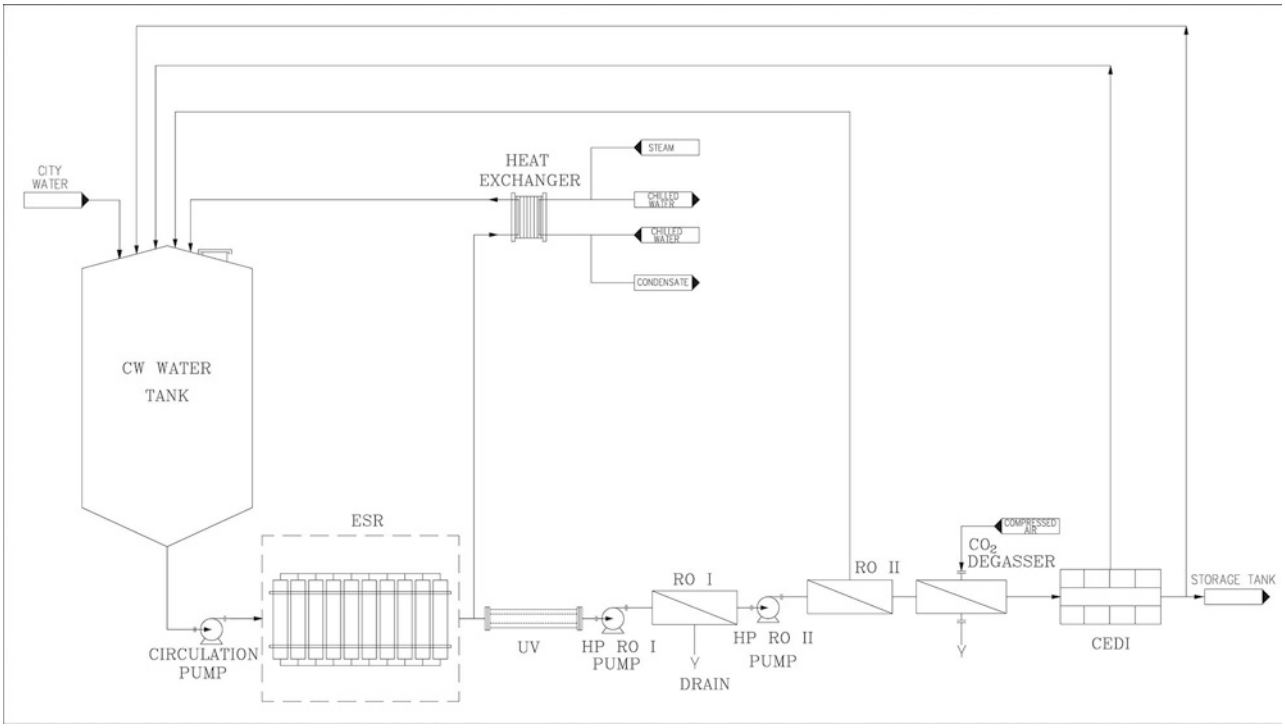

Figure 1: Case Study 1, WFI Production with ESR-HOD-RO-RO-Degasser-CDI (source: all figures were made by the author, Biopuremax Ltd).

This is why the permeate is not usually sterile.

Downstream contamination after the RO is rationalized by an imperfection in the membrane or less than perfect sealing gaskets between feed and product [4]. Even during proper operation, biofilm can form on the product side of a RO membrane and will result in biological growth in the permeate. For Purified Water (PW) this low number of Colony Forming Units (CFU) will not usually cause Out of Specification (OOS) results. As WFI has a much lower limit on the CFU levels, this biofilm usually will push the results OOS.

To minimize permeate contamination, it is crucial to keep the microbial levels of the RO feed water to a minimum as high levels of incoming bacteria will result in high levels of biofilm downstream. This is further complicated by a variation in feed water bacterial levels differing per yearly season.

The engineering challenge is not only to achieve the minimum levels of bacteria, but also to minimize maintenance and down time.

Commonly, softening ion exchange is utilized for PW and WFI pretreatment. If chlorine removal is needed an Active Carbon Filter (ACF) is installed or a dosing station with Sodium Bi Sulfite (SBS).

Softeners replace easy to scale "hard" ions with more soluble "soft" ions to prevent scale deposition on RO membranes. If chlorine is present, the ACF/SBS remove/neutralize the free chlorine to protect RO membranes and, even more sensitive, downstream.

A different method of pretreating RO feed water can deal with contamination in a better manner. There is no media for softening and no active carbon for chlorine removal. Not only does the new method abolish the contamination build up in softeners and carbon, but the pretreatment will also actively reduce bacterial levels incoming from the city water and throughout the system.

Background

■ WFI Specifications

The specifications of PW and WFI both overlap and differ. Typically, the chemical parameters of both PW and WFI are easily achieved. In contrast, the microbial targets of the WFI are more challenging.

The EP WFI microbial specification calls for less than 10 CFU/100 ml which the EP has stated that is not reliably achieved with *single* pass RO even if the RO feed water bacteria level is controlled.

This is the reason that the EP has specified the *production equipment* needed to generate WFI and not just the final product specification. The EP has specified a minimum of 2 membrane barriers for WFI production, either double pass RO or a single pass RO with an additional Ultra Filtration (UF) step.

■ Meeting Specifications

The bacterial levels through the pretreatment stages and the production RO stages should decrease at every

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Figure 2: Case Study 1.

stage to achieve the low levels of bacteria need to meet the WFI specification reliably. The operational levels of the system should be constant even when incoming bacterial levels fluctuate per season.

Control of the system is key, as even if the WFI quality is being achieved by the final production process, if bacterial levels increase after different stages, instead of decreasing, there will be probable future specification excursions [5].

When the feed water has high average CFU levels, intensive routine maintenance is needed to control the contamination in the pretreatment. Possible microbial control plans can include regeneration with biocides or full sanitization with hot water [2]. The risk of contamination is heightened with elevated feed water temperatures.

Media free pretreatment

■ ESR, HOD, HWS RO-EDI

Electrolytic Scale Reduction (ESR)

The ESR is a Stainless Steel (SS) pipe/reactor that dissociates some of the water molecules into OH^- and H^+ ions.

An electrical current is passed through the water and through electrolysis some of the molecules break up into H^+ and OH^- ions. The high concentration of OH^- ions on one side of the reactor will cause hardness precipitation [6].

In this manner the RO is protected from hardness scaling.

As a side effect of the electrolytic precipitation, residual levels of free chlorine will be generated from incoming chlorine in the feed water without having to add hypochlorite. The free chloride will disinfect and will actively reduce bacteria from the city water tank and up to the HOD (see below) that will remove it.

Hydro Optic Dechlorination (HOD)

Free chlorine can be removed by use of high-intensity UV radiation [7, 8].

The HOD is a high-powered unit that can generate the needed UV dosage levels needed to break up the free chlorine molecules in the HOD feed water.

As can be expected, due to the extremely high UV dosage levels, the HOD also continuously disinfects and reduces the bacteria levels of the water flowing through it.

Both the ESR and HOD do not have any moving parts and do not need chemicals or regeneration.

When a system continuously reduces bacteria with no need for sanitization or cleaning, this is called Continues Bacterial Reduction (CBR).

After down time or maintenance, the ESR-HOD combination can be hot water sanitized together as both are made of hot water-resistant materials: SS and quartz.

Hot Water Sanitization (HWS) RO–Electro Deionization (EDI)

RO and EDI have become the standard for pharma water production processes. HWS RO and HWS EDI have been implemented for critical microbial control [9]. The standard implementation of HWS RO and EDI is common but usually these systems are sanitized in isolation to the pretreatment. It is not common to heat sanitize softeners and carbon filters at the same time as the RO as this can cause particulates, fines, and endotoxin to slough off the media and to clog the delicate membranes. The usual practice is not to sanitize the pretreatment or to sanitize offline without combining the RO and EDI in the process.

The HWS is that it is much more effective than chemical disinfectants without using hazardous substances [4].

Case Studies

The following case studies are all real installations, and the microbial results were gathered by inhouse sampling and analysis.

The results presented in tables are subsets of the total results. Only the essential partial results are presented for brevity and clarity to demonstrate the performance of the system.

Table 1a

Total Count Micro PQ Levels for Case Study 1.

System description	Points location/ description	Spec.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Pre-Treatment System	Before city water tank	Action limit ≤50 000 [cfu/100 ml]	700	0	700	0	500	1 700	400	0	0	80
	After city water tank circulation pump		400	0	100	0	0	0	100	0	0	40
	After filter 8µ	Alert limit ≥40 000 [cfu/100 ml]	300	0	0	0	12	0	0	0	0	0
	After filter 3µ		0	0	0	0	0	0	0	0	0	0
RO System	After HOD	Action limit ≤100 [cfu/100 ml]	0	0	13	0	19	7	0	*48*	14	19
	RO (First Pass)		0	0	0	0	0	0	0	0	0	0
	RO (Second Pass)	Alert limit ≥50 [cfu/ 100 ml]	0	0	0	0	0	1	0	0	0	0
CDI	After Degasser before CDI	Action limit ≤10 [cfu/100 ml]	0	0	0	0	0	12	1	0	0	0
	After CDI (Product)	Alert limit ≥5 [cfu/100 ml]	0	0	0	1	0	0	0	0	0	0

All microbial values in the table are on a scale of cfu/100 ml and not cfu/ml.
All the zero values in the table were permuted from the original <1 for clarity.

Table 1b

Micro PQ Levels for Case Study 1 (continuation of table 1a).

System description	Points location/ description	Spec.	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
Pre-Treatment System	Before city water tank	Action limit ≤50 000 [cfu/100 ml]	200	300	204	256	281	175	300	300	300	300
	After city water tank circulation pump		18	31	48	38	32	17	82	60	66	200
	After filter 8µ	Alert limit ≥40 000 [cfu/100 ml]	0	4	0	1	0	0	15	9	33	9
	After filter 3µ		0	1	0	2	0	0	9	1	6	2
RO System	After HOD	Action limit ≤100 [cfu/100 ml]	*45*	14	12	83	4	35	15	5	0	6
	RO (First Pass)		0	0	0	0	0	0	0	0	0	0
	RO (Second Pass)	Alert limit ≥50 [cfu/100 ml]	0	0	0	0	0	0	0	0	0	0
CDI	After Degasser before CDI	Action limit ≤10 [cfu/100 ml]	0	0	0	0	0	0	1	0	0	5
	After CDI (Product)	Alert limit ≥5 [cfu/100 ml]	0	0	54	0	0	0	0	1	0	0

All microbial values in the table are on a scale of cfu/100 ml and not cfu/ml.
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Figure 3: Case Study 2.

Case study 1: 1 000 l/hr Cold WFI Double Pass RO

Case study 1 is a combination of Electrolytic reduction of scale using ESR and UV chlorine neutralization using HOD as a pretreatment for double pass RO and a final stage CDI (fig. 1).

It has a WFI Product flow rate of 1 000 l/hr.

The system in case study 1 is a SS manufactured unit as in fig. 2. A Sanitization with hot water, minimum of 80 °C, is performed automatically at weekends for 60 min.

Table 1a summarizes Total Count results for this system during PQ phase 1 and phase 2.

The system results for all chemical parameters were met during the PQ. The conductivity, Total Organic Carbon (TOC) were measured online and not noted in the table. Also, the endotoxins were well below the alert limits.

As can be seen from table 1a and 1b, the levels of microbiological growth are steadily reduced as the water advanced through the system.

The only exception to this is in the results from the RO system after the HOD. The levels exceeded the alert limit or action limit in 3 instances out of the 20-day sampling. The results were tracked down to a contaminated sample valve that was corrected by draining hot water through it during a hot water sanitization. Both the alert limit and action limits are very low as the scale is cfu/100 ml and not cfu/ml.

The WFI product had 1 reading above action limit on day 13. This was similarly corrected in the same manner by draining hot water

Table 2

Total Count Micro PQ Levels for Case Study 2.

System description	Points location/ description	Spec.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Pre-Treatment System	Before city water tank	Action limit ≤500 [cfu/ml]	1 300	900	400	500	100	100	100	600	200	500
	After city water tank circulation pump	Alert limit ≥ 400 [cfu/ml]	1 100	1 000	1 000	1 400	3 100	1 900	900	200	500	400
RO System	RO (First Pass)	Action limit ≤100 [cfu/ml]	1	12	1	0	1	0	0	1	0	0
	RO (Second Pass)	Alert limit ≥50 [cfu/ml]	0	0	0	0	0	0	0	0	0	0
CDI	After CDI (Product)	Action limit ≤10 [cfu/100 ml] Alert limit ≥5 [cfu/100 ml]	0	0	1	0	0	0	0	0	0	0

All microbial values in the table are on a scale of cfu/ml, only CDI product is on a scale of cfu/100 ml.
All the zero values in the table were permuted from the original <1 for clarity.

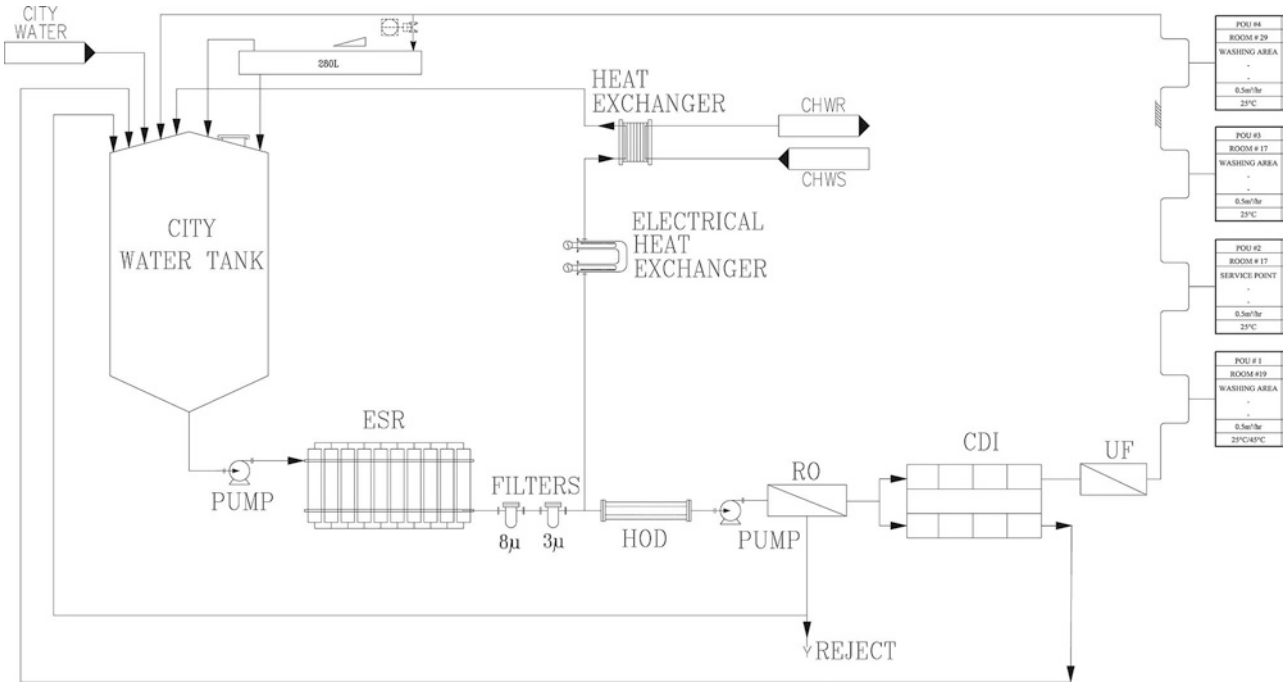

Figure 4: Case Study 3, WFI Production with ESR-HOD-RO-CDI-UF-Circulation.

through it the sample valve during a hot water sanitization.

No further Out of Specification (OOS) instances were detected anywhere in the production system even though the PQ was finished over 1 year ago.

■ Case study 2: 500 l/hr Cold WFI Double Pass RO

Case study 2 is the same system as case study 1, but with a WFI product flow rate of 500 l/hr (fig. 3).

A sanitization with hot water, minimum of 80 °C, is performed automatically at weekends for 60 min.

The system results for all chemical parameters were met during the PQ. The conductivity, TOC were measured online and are not noted in table 2. Also, the endotoxins were well below the alert limits.

As can be seen from table 2, apart from the feed water and city water tank, the levels of microbiological growth are steadily reduced as the water advanced through the system.

The challenge to case study 2 is far higher than for case study 1. The inlet microbial values in case 2 exceed the inlet microbial values in case 1 by 3 orders of magnitude. As the feed water data in tables 1a and 1b are presented with a resolution of cfu/100 ml, as opposed to table 2 where the feed water data is presented with a resolution of cfu/ml.

Even so, the RO 1, RO 2, and CDI show exceptionally low levels of microbial growth.

The site suffers from chronic low quality feed water and the microbial levels from the municipal supply are consistently high.

Apart from the feed water and city water tank, no further out of specification instances were detected in the production system even though the PQ was finished over 2 years ago.

■ Case study 3: 500 l/hr Cold WFI Single pass RO

Case study 3 is a combination of electrolytic reduction of scale and UV chlorine neutralization as a pretreatment for single pass RO, CDI and UF as shown in fig. 4.

There is a WFI Product flow rate of 500 l/hr.

There is no WFI storage tank, and the product water is circulated around the plant and returned to the city water break tank.

The system in case study 3 is a SS manufactured unit as in fig. 5. A sanitization with hot water, minimum of 80 °C, is performed automatically at weekends for 60 min.

The total amount of produced water, 500 LPH of WFI, is available online to any of the users, but concurrent use is limited. That is why this type of system is perfect for smaller R&D or scale-up plants. The unit can be installed in the first step of production when consumption is low. When expansion is needed, a WFI storage tank can be added without change to the production system.

The system results for all chemical parameters were met during the PQ.

The conductivity, TOC were measured online and not noted in table 3. Also, the endotoxins were well below the alert limits.

As can be seen from table 3, the micro levels are extremely low as the system continuously circulates the

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■ **Table 3**

Total Count Micro PQ Levels for Case Study 3.

System description	Points location/ description	Spec.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Pre-Treatment System	Before city water tank	Action limit ≤500 [cfu/ml]	–	1	2	–	–	–	–	49	–	–
	After city water tank circulation pump	Alert limit ≥400 [cfu/ml]	–	0	17	–	–	–	1	1	–	–
RO System	After HOD	Action limit ≤100 [cfu/ml]	0	0	0	0	0	12	4	1	1	1
	RO 1 Outlet	Alert limit ≥50 [cfu/ml]	0	0	0	0	0	0	0	0	0	0
CDI	After CDI (Product)	Action limit ≤10 [cfu/100 ml] Alert limit ≥5 [cfu/100 ml]	0	0	0	0	0	0	0	0	0	0
Distribution	POU 1	≤10 [cfu/100 ml] Alert limit ≥5 [cfu/100 ml]	0	0	0	0	0	0	0	0	0	0
	POU2		0	0	0	0	0	0	0	0	0	0
	POU 3		0	0	0	0	0	0	0	0	0	0
	POU 4		0	0	0	0	0	0	0	0	0	0

All microbial values in the table are on a scale of cfu/100 ml and not cfu/ml.
Valves that were not sampled are noted by "–".
All the zero values in the table were permuted from the original <1 for clarity.

product water through all the water processing equipment: ESR, HOD, RO, CDI, and UF. This situation is optimal for the users in the plant.

The system was not hot water sanitized at all during the PQ period, 4 weeks without sanitization, demonstrating CBR.

No OOS instances were detected in the production system even though the PQ was finished over 4 years ago.

■ Case study 4: Cold WFI 500 l/hr Double Pass RO

Case study 4 is the same system as case study 1, but with a WFI product flow rate of 500 l/hr.

A sanitization with hot water, minimum of 80 °C, is performed automatically at weekends for 60 min (fig. 6).

This site is supplied with feed water from a local well which is not well maintained. The feed water is commonly contaminated with high total count and Pseudomonas.

The system results for all chemical parameters were always met. The conductivity, TOC were measured online and are not noted in tables 4a and 4b. Also, the endotoxins were well below the alert limits.

Problems arose after PQ as in table 4a.

At this stage the system was taken offline for 2 days for cleaning. Filters and RO membranes were removed, and the filter/RO housings were manually cleaned. NaOH was circulated 2 % at 45 °C for 60 min.

The system was then rinsed and hot sanitized.

The results were satisfactory for another 3 weeks vis-à-vis the Pseudomonas but still residual high levels were detected in total count. This was in conjunction with higher levels of total CFU in feed water (not shown in the table).

Even though the levels on the outlet of the HOD was always zero, the reject from the RO was measured to be Too Numerous To Count (TNTC) on several occasions.

This was while still sanitizing with hot water once a week but no other treatment.

After the 3 weeks had passed the situation took a turn for the worse with the measured total count of CFU as in table 4b but Pseudomonas was *always zero at all points.*

From table 4b one can see the microbial contamination moving along the system and infecting RO 2 feed and reject. Even the previously sterile RO 2 permeate is now showing signs of growth.

Throughout the infestation, the system had no OOS in permeate

Table 4a

Total Count Micro/Pseudomonas Levels for Case Study 4.

System description	Points location/ description	Spec.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Pre-Treatment System	City water	Action limit ≤500 [cfu/ml] Alert limit ≥400 [cfu/ml]	100/0	–/0	75/0	10/192	15/0	–/–	–/0	–	–	5
	After city water tank circulation pump		–/0	1 510/–	–/4	15/80	1 030/0	5/–	–/0	–	–	365
	Inlet filters		116/0	–/–	–/1	65/67	430/0	30/–	–/0	–	–	765
	After filter 8µ		–/–	1 336/–	–/2	195/29	225/0	–/–	–/0	–	–	–
	After filter 3µ		–/–	–/23	267/0	75/39	180/0	–/2	–/0	–/0	–/0	–/0
	After HOD		–/–	–/–	–/0	0/0	0/0	–/–	0/0	–	–	–
RO System	Feed RO1	Action limit ≤100 [cfu/100 ml] Alert limit ≥50 [cfu/100 ml]	–/–	100/–	–/0	40/0	100/0	–/0	–/0	–/0	–/0	–/0
	Reject RO 1		2.5/0	–/3	3.7/13	4.6/0	100/1	3.5/TNTC	730/0	–/0	160/0	2 090/0
	Permeate RO 1		0/0	–/0	–/0	0/0	0/0	0/–	–/0	–	–	0.3
	Feed RO 2		–/0	15.7/0	–/0	0.3/0	0.4/0	0.1/–	–/0	–	–	–
	Reject RO 2		0/0	–/0	0/0	0.6/0	0.1/0	–/–	–/0	–	–	–
	Permeate RO 2		0/0	–/0	0/0	0/0	0/0	–/–	–/0	–	–	0
CDI	After Degasser before CDI	Action limit ≤10 [cfu/100 ml] Alert limit ≥5 [cfu/100 ml]	–/–	0/–	0/0	0/0	0/0	0/–	–/0	–	–	–
	After CDI (Product)		0/–	0/–	0/0	0/0	–/0	0/–	–/0	–	–	–

All microbial values in the table are on a scale of not cfu/ml, only CDI product is on a scale of cfu/100 ml.
Valves that were not sampled are noted by "–".

RO 2 or in product, but this was assumed to be temporary.

At this stage, the system was stopped for 5 working days and the following actions taken:

- The system was cleaned as before.
- HWS was stepped up from once a week to twice a week.
- Filters were replaced every 2 weeks and not every 4–6 weeks.
- Free chlorine dosing was added to the city water tank, 0.2–0.4 ppm.

The third round of testing gave results in table 4c.

From table 4c it can be seen that the system is now stable and has *been since this intervention over 2 years ago.*

The most indicative sample point is the RO 1 reject that will have the worst possible CFU levels of the system. The RO 1 reject is very much the "canary in the coal mine" and is the first point to show contamination.

Conclusions

Cold WFI production is a viable process but limiting microbial growth in the pretreatment production sys-

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■ **Table 4b**

Total Count Micro Levels for Case Study 4, Three Weeks Later.

System description	Points location/ description	Spec.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Pre-Treatment System	City water	Action limit ≤500 [cfu/ml] Alert limit ≥400 [cfu/ml]	–	–	–	5	–	–	–	–	125	–
	After city water tank circulation pump		–	–	–	1 280	–	–	–	–	TNTC	30
	Inlet filters		–	–	–	835	–	–	–	–	TNTC	65
	After filter 8µ		–	–	–	1 025	–	–	–	–	TNTC	–
	After filter 3µ		–	–	–	–	–	–	–	–	1 440	–
	After HOD		–	–	–	–	–	–	–	–	2	–
RO System	Feed RO1	Action limit ≤100 [cfu/ 100 ml] Alert limit ≥50 [cfu/ 100 ml]	–	–	–	0.72	–	–	–	–	TNTC	–
	Reject RO 1		1 760	TNTC	TNTC	TNTC	TNTC	0	0	155	TNTC	TNTC
	Permeate RO 1		–	–	–	–	–	–	–	–	8.9	1
	Feed RO 2		–	–	–	8.1	–	–	–	–	7.2	TNTC
	Reject RO 2		–	–	–	1.5	–	–	–	–	6.6	–
	Permeate RO 2		–	–	–	0.3	–	–	–	–	0	–
CDI	After Degasser before CDI	Action limit ≤10 [cfu/100 ml]	–	–	–	0	–	–	–	–	0	0
	After CDI (Product)	Alert limit ≥5 [cfu/100 ml]	–	–	–	0	–	–	–	–	0	0

All microbial values in the table are on a scale of not cfu/ml, only CDI product is on a scale of cfu/100 ml.
Valves that were not sampled are noted by "–".

■ **Table 4c**

Total Count Micro Levels for Case Study 4, after Second Cleaning.

System description	Points location/ description	Spec.	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Pre-Treatment System	City water	Action limit ≤500 [cfu/ml] Alert limit ≥400 [cfu/ml]	–	0	–	0	–	5	–	40	–	–
	After city water tank circulation pump		0	–	0	–	5	–	0	–	0	–
	Inlet filters		–	–	10	–	–	–	5	–	–	–
	After filter 8µ		–	–	–	0	–	–	–	0	–	–
	After filter 3µ		0	–	–	–	0	–	–	–	0	–
	After HOD		–	0	–	–	–	0	–	–	–	–
RO System	Feed RO1	Action limit ≤100 [cfu/ 100 ml] Alert limit ≥50 [cfu/ 100 ml]	0.1	–	0.4	–	0	–	1.9	–	0	–
	Reject RO 1		0	0	0	0	0	0	0	0	0	–
	Permeate RO 1		–	–	0	–	–	0	–	–	0	–
	Feed RO 2		0	–	0	–	0	–	0	–	0.1	–
	Reject RO 2		0.1	–	0	–	0	–	0	–	0.3	–
	Permeate RO 2		–	–	0	–	0	–	0	–	–	0
CDI	After Degasser before CDI	Action limit ≤10 [cfu/100 ml]	–	0	–	0	–	0	–	0	–	–
	After CDI (Product)	Alert limit ≥5 [cfu/100 ml]	–	–	–	–	–	–	–	–	–	–

All microbial values in the are on a scale of not cfu/ml, only CDI product is on a scale of cfu/100 ml.
Valves that were not sampled are noted by "–".

Figure 5: Case Study 3.

Figure 6: Case Study 4.

tems is essential for meeting specifications.

Different combinations of cold WFI production equipment were studied: ESR-HOD-RO-RO-EDI, ESR-HOD-RO-EDI-UF, both systems produce stable WFI parameters with full control throughout the system over multiple seasons and over multiple years.

There is no justification for the extra investment costs, floor space, energy, and maintenance for the Multiple-effect (ME) distillation as the RO based system consistently produces WFI even when challenged by problematic feedwater. When adding that those systems that were inspected by the regulatory authorities were accepted as legitimate for cold production of WFI, then the conclusion is that there is no necessity for thermal WFI production.

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