Nofima
Norwegian Institute of Food, Fisheries and Aquaculture Research

Desinfeksjonsmidler mot patogener og infeksjonsdynamikk i RAS

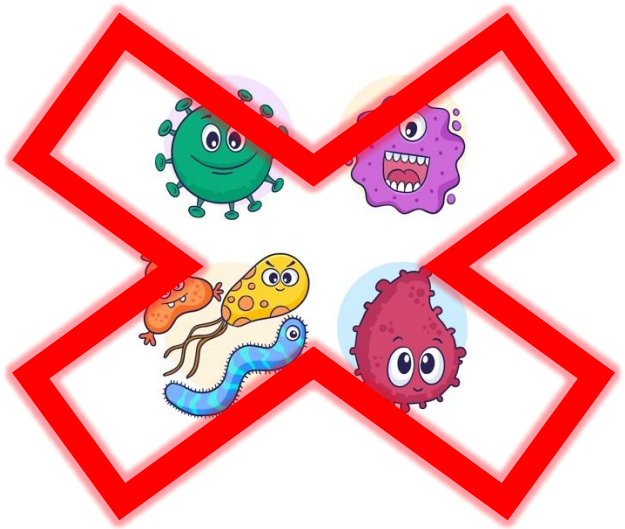
FHF-dialogmøte: Biosikkerhet i RAS, 28.okt 2026



Carlo C. Lazado

Seniorforsker Fiskehelse

Disinfection in RAS



RAS disinfection aims to control pathogens while preserving **water quality**, **biofilter performance**, **fish health,** and **personnel safety**. Unlike hospitals, complete sterilisation is neither feasible nor desirable.

"**More disinfection**" does not always mean "**better biosecurity**"



Disinfection knowledge



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Short communication

Survey findings of disinfection strategies at selected Norwegian and North American land-based RAS facilities: A comparative insight

Carlo C. Lazado[a,*,1], Christopher Good[b,*,1]

[a] Nofima, The Norwegian Institute of Food, Fisheries and Aquaculture Research, 1433, Ås, Norway
[b] The Conservation Fund Freshwater Institute, Shepherdstown, WV 25443, USA

Regional preferences differ: PAA (Perfectoxid) dominates in Norway; chlorine is most common in North America.

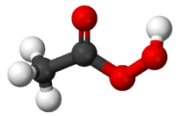
Limited validation: >80% of facilities use standardised protocols, but most lack experimental validation.

Selection driven by efficacy and safety: Both regions prioritise pathogen control and user safety; North America places greater emphasis on residual toxicity.

Inventory of disinfectants in Norway

List of Norwegian Food Safety Authority-approved disinfectants for aquaculture use in Norway

(as of October 2026)



Peracetic acid

ADDI Aqua
Aqua Des
Hydi-Des
InciMaxx Net
InciMaxx Net Foam
Kick-Start
Oxonia Active
Perfectoxid
PIPE FISH 5
VigorOx A&F 5%
VigorOx A&F 15%

Cl

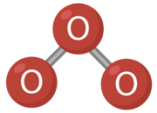
Chlorine

Ecas4-Anolyte
HL. Skjong ACA Aqua
LifeClean
LifeClean Disinfectant Concentrate
Oxitrate
Salar Des

$^{\bullet}O_2^{2-}$

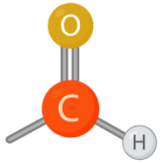
Hydrogen peroxide

Freebac 35



Ozone

AquaZone
NORMEX Desinfecta
Redoxzon



Glutaraldehyde

Aqua Omnicide
Force Seven Aqua
Grotanol 3025
Virocid

K

Pentapotassium

Virkon Aquatic
Virex Aqua

I

Iodine

Buffodine

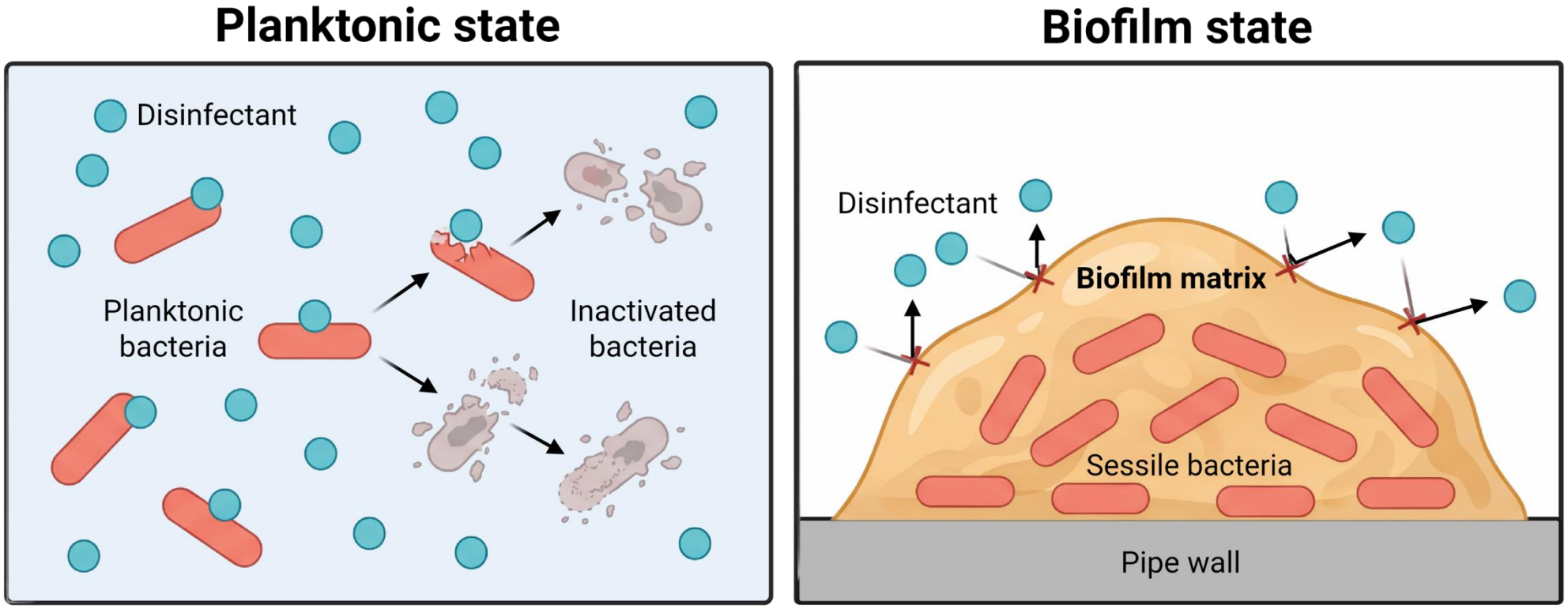
H

Hypochlorous acid

360 Desinfection med
Neuthox

List here

Bacteria in suspension and biofilm



- Free-floating bacteria are readily **inactivated/killed** by disinfectants.
- Biofilm matrices protect bacteria from disinfectant exposure.

Susceptibility of some fish pathogens to disinfectants


Listen

Research Article

Assessment of disinfectant performance against fish-pathogenic bacteria in planktonic and biofilm states

Nga Phuong Dang , Tabassum Ali , Vasco C. Mota , Carlo C. Lazado & Peter Szabor

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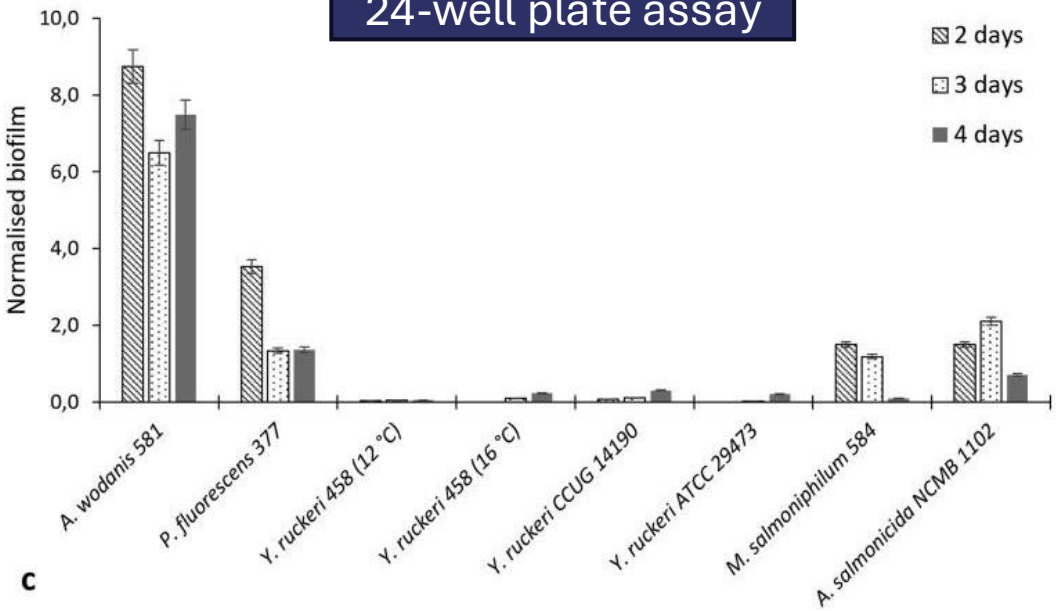
Full Article | Figures & data | References | Citations | Metrics | Licensing | Reprints & Permissions

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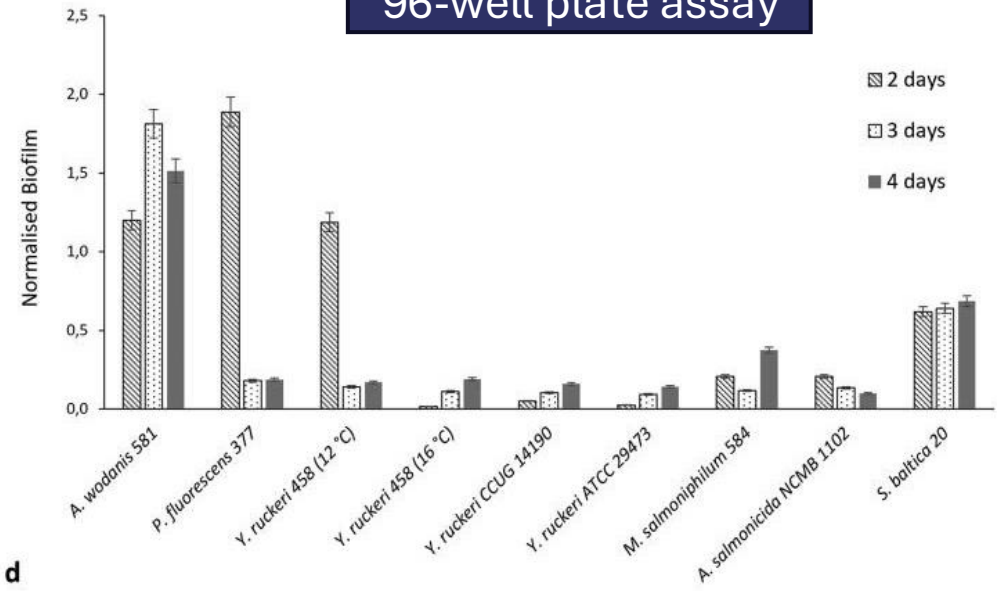
Biofilm formation *in vitro*



24-well plate assay



96-well plate assay



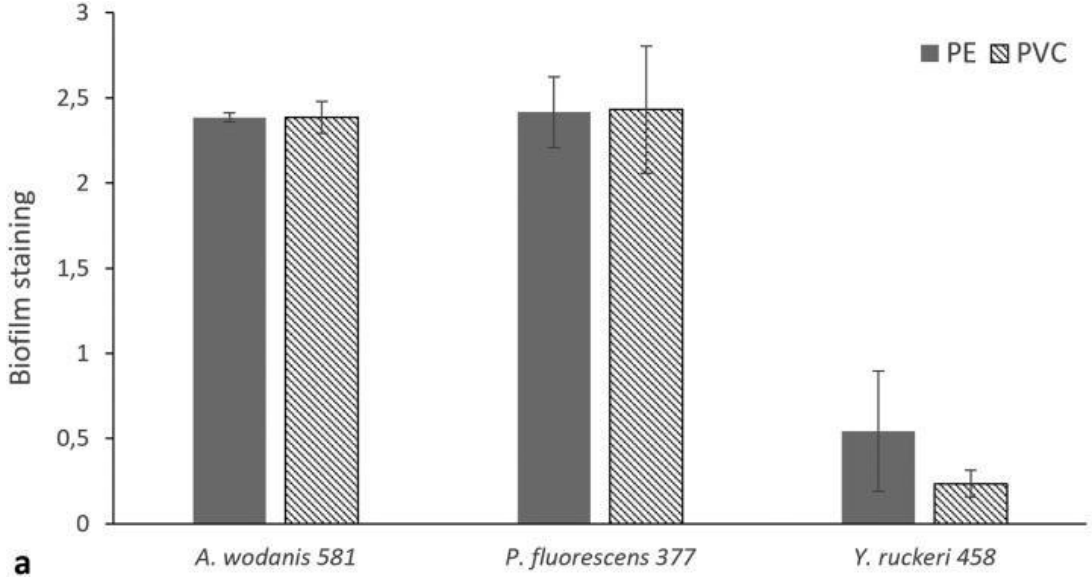
Bacterial strains were provided by Pharmaq Analytiq AS

Biofilm formation on plastic materials

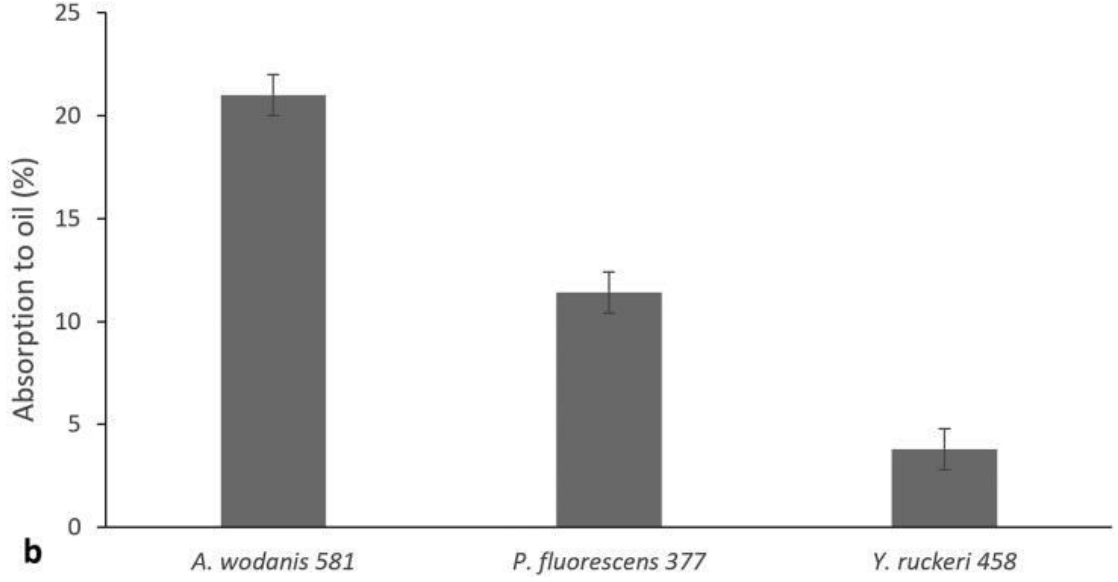
Material properties such as material type, hydrophobicity, and surface roughness are shown to influence the formation of biofilm on the material surface.

PE and PVC, the most used materials in RAS, for biofilm testing.

Biofilm formation (4 days)

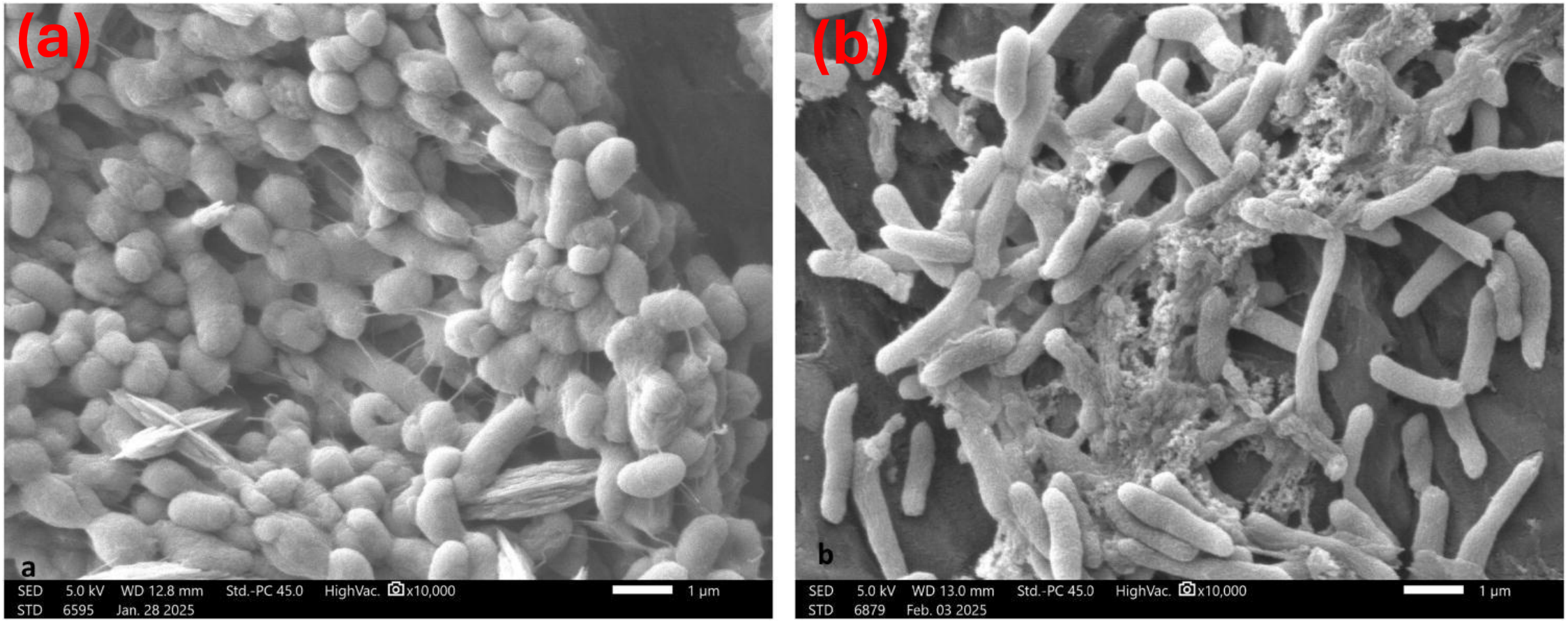


Hydrophobicity of bacterial cell surface



Bacterial cells with adhesion to n-hexadecane greater than 20% are considered hydrophobic

Biofilms of *A. wodanis* 581 and *P. fluorescens* 377 on PVC carrier



SEM images of A. wodanis 581 biofilm **(a)** *and P. fluorescens 377 biofilm* **(b)** *that formed on PVC carrier after 6 days of incubation at 12 °C.*

Tests of different disinfectants

Disinfectants	Manufacturer	Dose for bacteria (%)	Contact time (min)	Chemical composition
Aqua Des	Aquatiq Chemistry AS Lillehammer, Norway	0.5-1	30	Hydrogen peroxide, peracetic acid, acetic acid
Freebac® 35	Aquarmar International B. V. Flevoland, Nederland	0.0017 (water); 1-5 (surface)	2-530	Hydrogen peroxide
Addi Aqua	Lilleborg Profesjonell Oslo, Norway	0.5-1	30	Hydrogen peroxide, peracetic acid, acetic acid
Perfectoxid	Aquatiq Chemistry AS Lillehammer, Norway	0.5-1	30	Hydrogen peroxide, peracetic acid, acetic acid
Virkon™ AQUATIC	Antec International Ltd Suffolk, United Kingdom	1	30	Pentapotassium bis(peroxymonosulphate) bis(sulphate), sodium dodecylbenzenesulfonate, malic acid, sulphamidic acid
Virocid	CID LINE Ieper, Belgium	0.25-1.5	30	Glutaraldehyde, quaternary ammonium compounds, didecyldimethylammoniumchlorid, isopropanol

Effect of disinfectants in suspension test

*recommended dose

Not considered effective

Disinfectant recommended dose (%)	Dose (% v/v)	Log reduction achieved						
		A. wodanis	*P. fluorescens*	*Y. ruckeri*	*Y. ruckeri*	*Y. ruckeri*	*A. salmonicida*	*M. salmoniphilum*
		581	377	458	ATCC 29473	CCUG 14190	NCMB 1102	584
Aqua Des (0.5-1)	0.25	8.0 ± 0	4 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	2.6 ± 0
	0.5*	8.0 ± 0	8.9 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0
	1*	8.0 ± 0	8.9 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0
Freebac® 35 (-0.0017)	0.005*	3.00 ± 0	4 ± 0	4 ± 0	nd	nd	nd	2.6 ± 0
	1	8.0 ± 0	4 ± 0	4 ± 0	nd	nd	nd	3.3 ± 0
	2	8.0 ± 0	4 ± 0	4 ± 0	nd	nd	nd	3.3 ± 0
Addi Aqua (0.5-1)	0.25	8.0 ± 0	4 ± 0	4.1 ± 0	7.0 ± 1	9.0 ± 0	9.0 ± 0	2.6 ± 0
	0.5*	8.0 ± 0	5.9 ± 0.4	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0
	1*	8.0 ± 0	8.9 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0
Perfectoxid (0.5-1)	0.25	8.0 ± 0	5.1 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	2.6 ± 0
	0.5*	8.0 ± 0	7.2 ± 2.5	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0
	1*	8.0 ± 0	8.9 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0
Virkon (1)	0.5	6.9 ± 1.5	3.0 ± 0	4.1 ± 0	4.1 ± 0	5.3 ± 0	5.1 ± 0	3.3 ± 0
	1*	8.0 ± 0	7 ± 2.5	5.1 ± 0	5.1 ± 0	6.3 ± 0	6.1 ± 0	3.3 ± 0
	2	8.0 ± 0	8	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0
Virocid (0.25–0.5)	0.25*	8.0 ± 0	3.0 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	2.6 ± 0
	0.5*	8.0 ± 0	3.0 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0
	1	8.0 ± 0	3.0 ± 0	9.0 ± 0	8.0 ± 0	9.0 ± 0	9.0 ± 0	3.3 ± 0

Log reduction after treatment with disinfectants. The number of bacterial cells in the test suspensions were 10^8–10^9 CFU/mL. Log reduction of lower than 5 (99.999%), which were highlighted in bold, is considered not effective according to standard method EN1656.

Effect of disinfectants on biofilm

Within the recommended dose

*recommended dose

Disinfectant	Dose (% v/v)	Biofilm viability reduction (%)		
		Y. ruckeri 458	*A. wodanis 581*	*P. fluorescens 377*
Aqua Des (1%)	0.025	81.6 ± 9	100 ± 0	–
	0.25	98.7 ± 0.55	100 ± 0	98.4 ± 1.2
	1*	99 ± 0.36	100 ± 0	98.5 ± 0.39
	2	99.2 ± 0.96	100 ± 0	98.6 ± 0.22
	5	100 ± 0.32	100 ± 0	–
Freebac® 35 (1-5%)	0.025	1.93 ± 3.8	100 ± 0	–
	0.25	4.54 ± 0.46	100 ± 0	56.2 ± 1.48
	1*	99.5 ± 3.2	100 ± 0	97.1 ± 1.2
	2*	100 ± 0.24	100 ± 0	98.9 ± 0.5
	5*	100 ± 0.79	100 ± 0	–
Addi Aqua (1%)	0.025	94 ± 4.4	100 ± 0	–
	0.25	97.2 ± 0.2	100 ± 0	98.9 ± 0.27
	1*	98 ± 0.39	100 ± 0	99 ± 0.52
	2	98.4 ± 0.5	100 ± 0	99.6 ± 0.27
	5	99.9 ± 0.5	100 ± 0	–
Perfectoxid (1%)	0.025	5.2 ± 0.5	100 ± 0	–
	0.25	100 ± 2.1	100 ± 0	96.7 ± 1.39
	1*	100 ± 0.4	100 ± 0	98 ± 0.04
	2	100 ± 0.6	100 ± 0	98.1 ± 0.2
	5	100 ± 0.23	100 ± 0	–
Virocid (0.25–0.5%)	0.025	87.6 ± 0.57	96.3 ± 0.12	–
	0.25*	97.4 ± 0.35	100 ± 0	97.6 ± 1.0
	1	100 ± 0.2	100 ± 0	100 ± 0.57
	2	100 ± 0.1	100 ± 0	100 ± 0.13
	5	100 ± 0.23	100 ± 0	–

The standard methods that are used for disinfectant approval for surface cleaning are EN 14349 (for non-porous surface) and EN 16437 (for porous surface). To pass the tests, a log reduction of ≥ 4 (able to kill 99.99% of bacteria) is required.

Effect of disinfectants on biofilm

Below the recommended dose

*recommended dose

Disinfectant	Dose (% v/v)	Biofilm viability reduction (%)		
		Y. ruckeri 458	*A. wodanis 581*	*P. fluorescens 377*
Aqua Des (1%)	0.025	81.6 ± 9	100 ± 0	–
	0.25	98.7 ± 0.55	100 ± 0	98.4 ± 1.2
	1*	99 ± 0.36	100 ± 0	98.5 ± 0.39
	2	99.2 ± 0.96	100 ± 0	98.6 ± 0.22
	5	100 ± 0.32	100 ± 0	–
Freebac® 35 (1-5%)	0.025	1.93 ± 3.8	100 ± 0	–
	0.25	4.54 ± 0.46	100 ± 0	56.2 ± 1.48
	1*	99.5 ± 3.2	100 ± 0	97.1 ± 1.2
	2*	100 ± 0.24	100 ± 0	98.9 ± 0.5
	5*	100 ± 0.79	100 ± 0	–
Addi Aqua (1%)	0.025	94 ± 4.4	100 ± 0	–
	0.25	97.2 ± 0.2	100 ± 0	98.9 ± 0.27
	1*	98 ± 0.39	100 ± 0	99 ± 0.52
	2	98.4 ± 0.5	100 ± 0	99.6 ± 0.27
	5	99.9 ± 0.5	100 ± 0	–
Perfectoxid (1%)	0.025	5.2 ± 0.5	100 ± 0	–
	0.25	100 ± 2.1	100 ± 0	96.7 ± 1.39
	1*	100 ± 0.4	100 ± 0	98 ± 0.04
	2	100 ± 0.6	100 ± 0	98.1 ± 0.2
	5	100 ± 0.23	100 ± 0	–
Virocid (0.25–0.5%)	0.025	87.6 ± 0.57	96.3 ± 0.12	–
	0.25*	97.4 ± 0.35	100 ± 0	97.6 ± 1.0
	1	100 ± 0.2	100 ± 0	100 ± 0.57
	2	100 ± 0.1	100 ± 0	100 ± 0.13
	5	100 ± 0.23	100 ± 0	–

The standard methods that are used for disinfectant approval for surface cleaning are EN 14349 (for non-porous surface) and EN 16437 (for porous surface). To pass the tests, a log reduction of ≥ 4 (able to kill 99.99% of bacteria) is required.

Benchtop RAS water disinfection

Both biomedia and water were originally from a mature RAS stocked with Atlantic salmon smolt (Havbruksstasjonen i Tromsø)

Disinfectant	Used dose (%)	Log reduction	
		TSA medium	CIN medium
Addi Aqua	0.5	5.8 ± 0.57	4.5 ± 0.4
Aqua Des	0.5	5.8 ± 0.24	3.5 ± 0.15
Perfectoxid	0.5	6.6 ± 0.3	4.5 ± 0.58
Virocid	0.25	5.5 ± 0.53	4.4 ± 0.27
Virkon	1	6.1 ± 0.11	4.3 ± 0.39
Freebac®35	0.0017	0.5 ± 0.03	0.1 ± 0.06

Perfectoxid and Virkon showed the strongest performance, achieving >6-log reductions.

Virocid was highly effective at the lowest dose tested (0.25%).
Freebac®35 showed limited efficacy, with only 71% (TSA) and 16% (CIN) reduction.



1.8L of water and 1.2L of biomedia were filled in each tank.

In vivo trial – simulation of a biosecurity breach

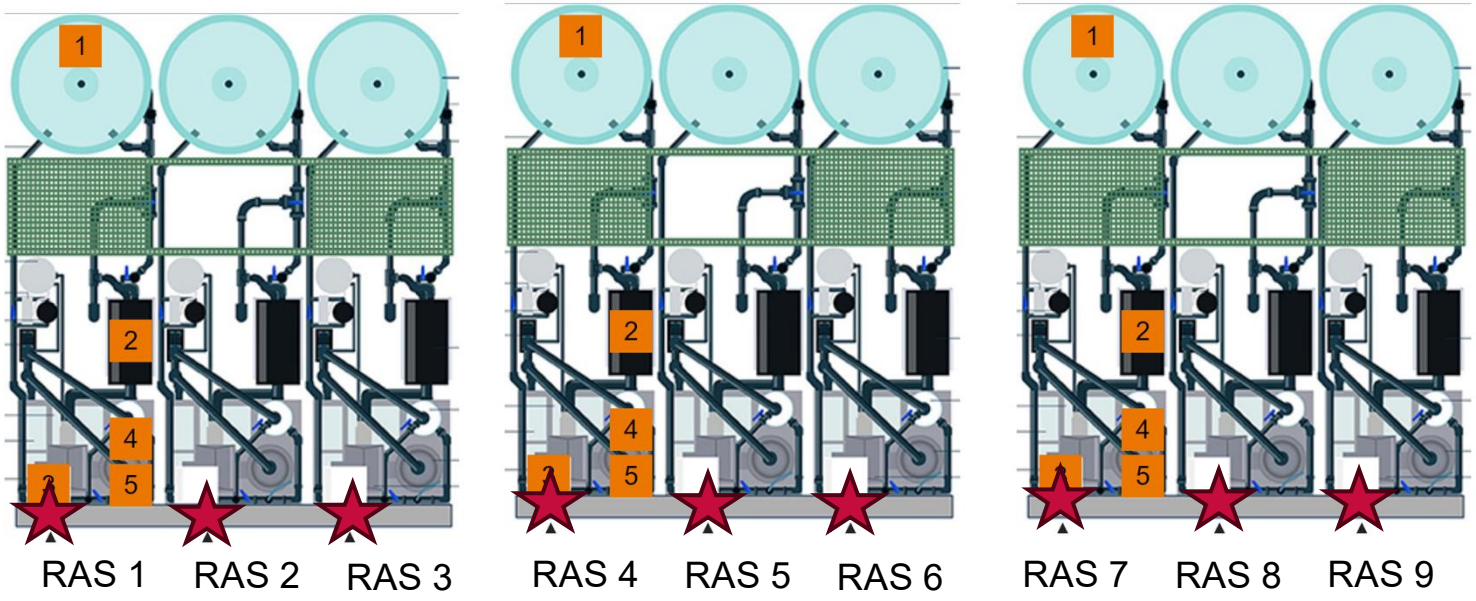
Key information:

Smolts (70-80 g)
Brackishwater 12 ppt, 450L tank
12°C
MBBR
Yersinia ruckeri (10^8), 1% (v/v) per total daily volume (ca. 20 L/day) once

Ctrl: RAS feed
water stable; no infection

Non-RAS feed
less water stable; infection

RAS feed
water stable; infection



Water samples from each tank

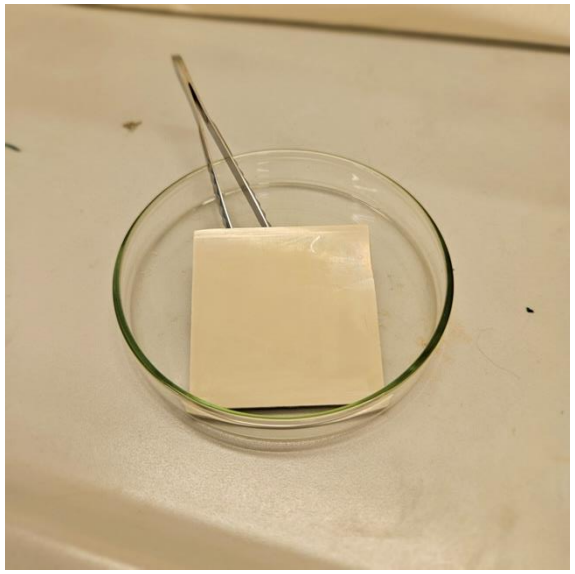
Biofilm samples:

1. Tank wall
2. Drum filter
3. Sump tank
4. Biofilter wall
5. Biomedia (3-5 pcs pr system)

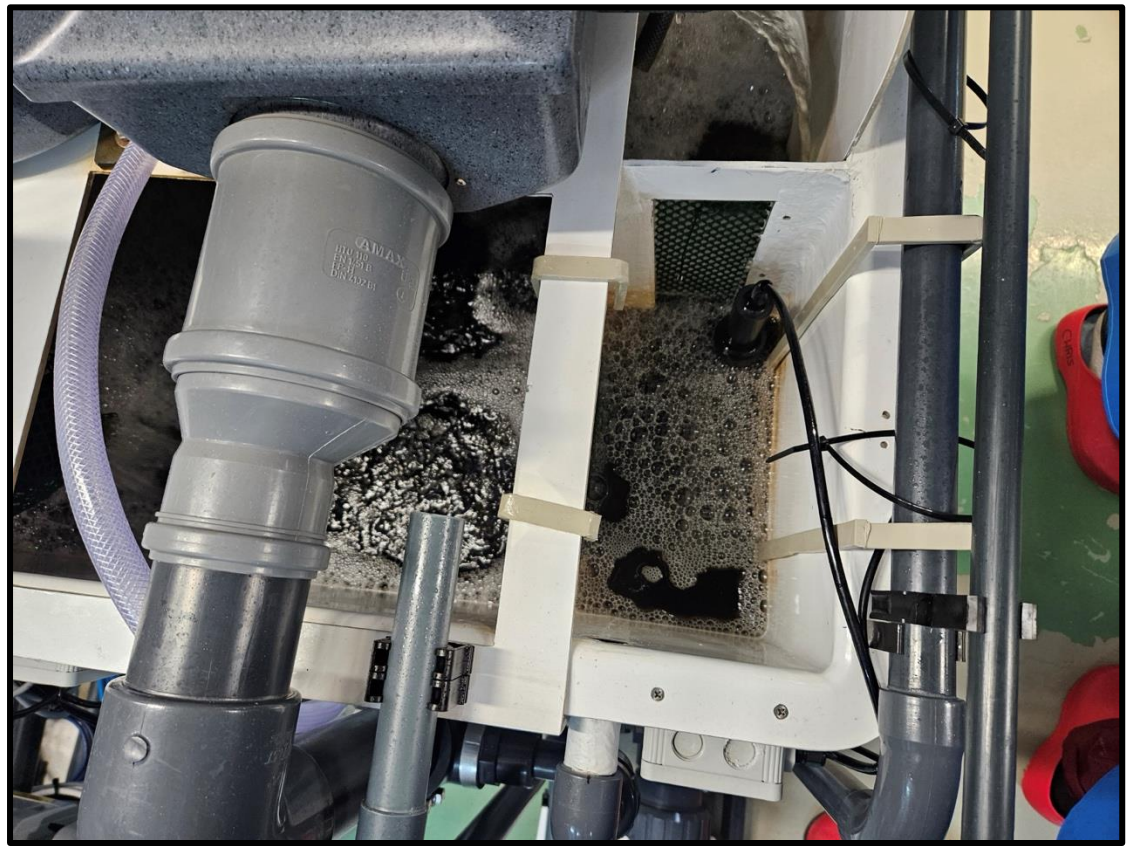


★ **Pathogen breach**

Biofilm formation in RAS

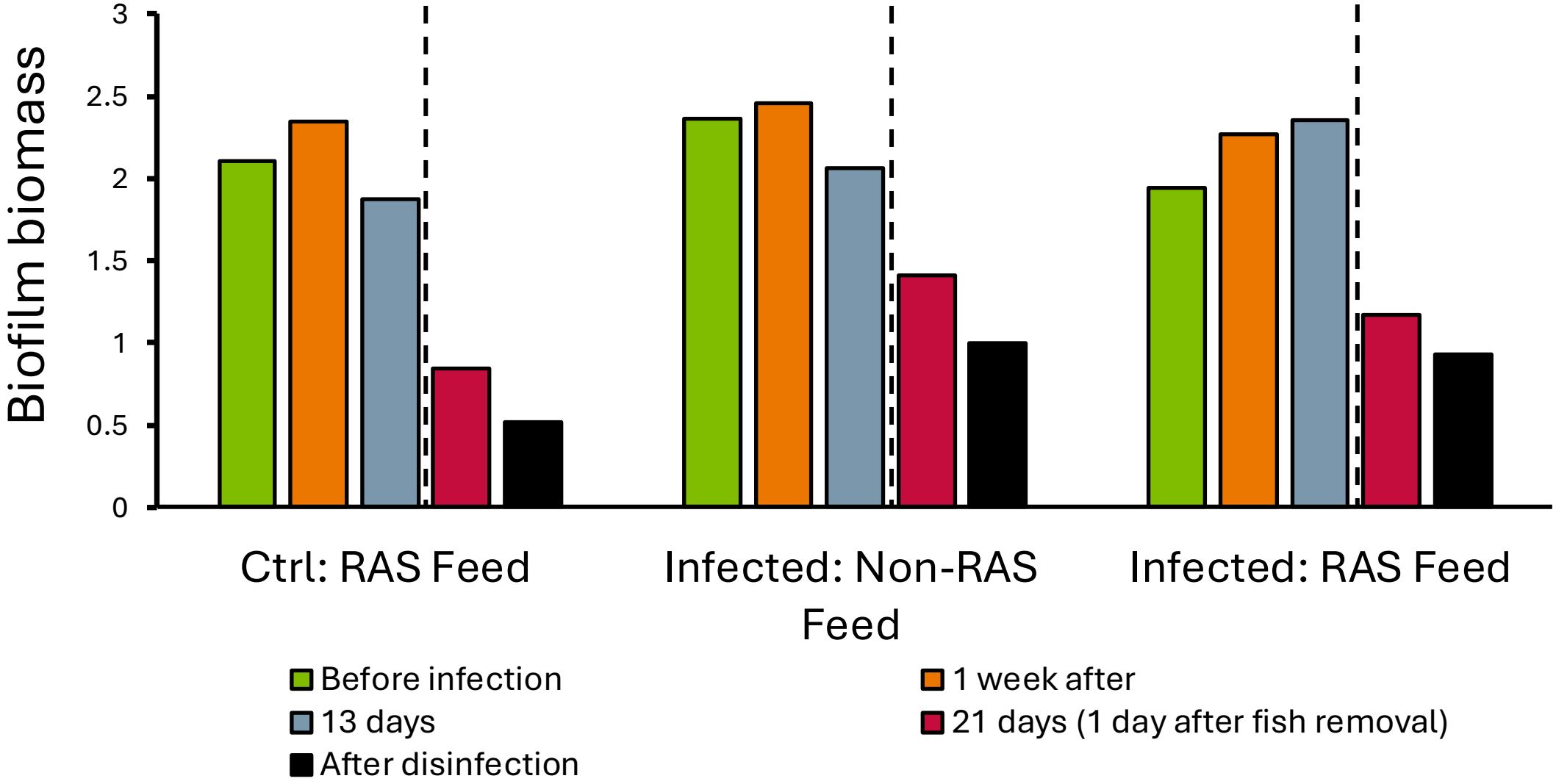






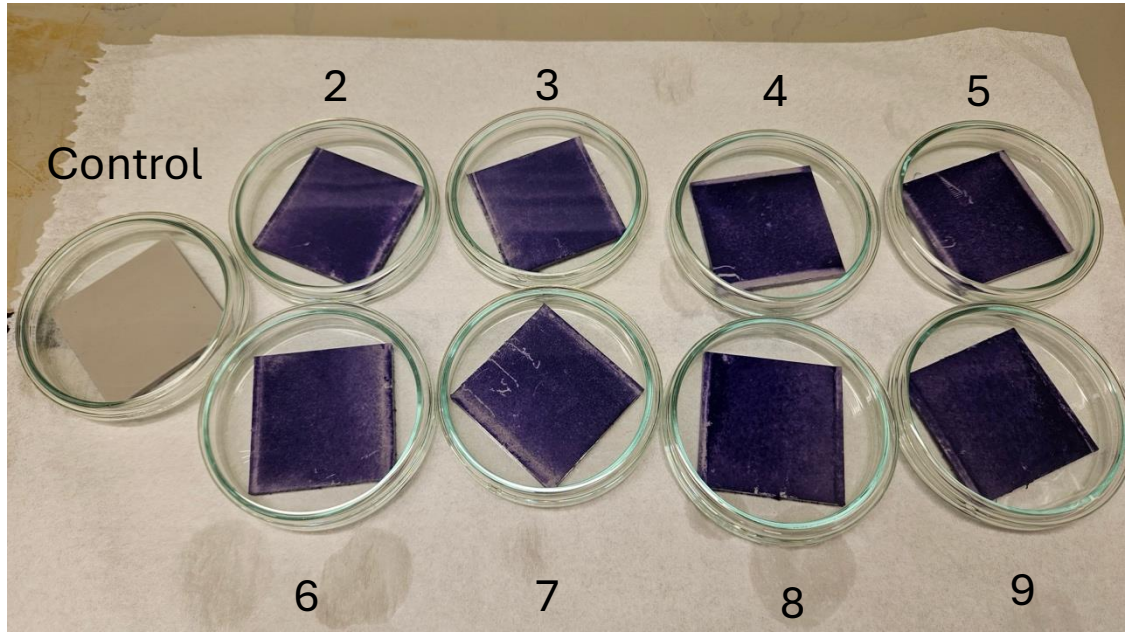
Biofilm carriers were stationed in the biofilter and pump sump

Biofilm formation – pump sump

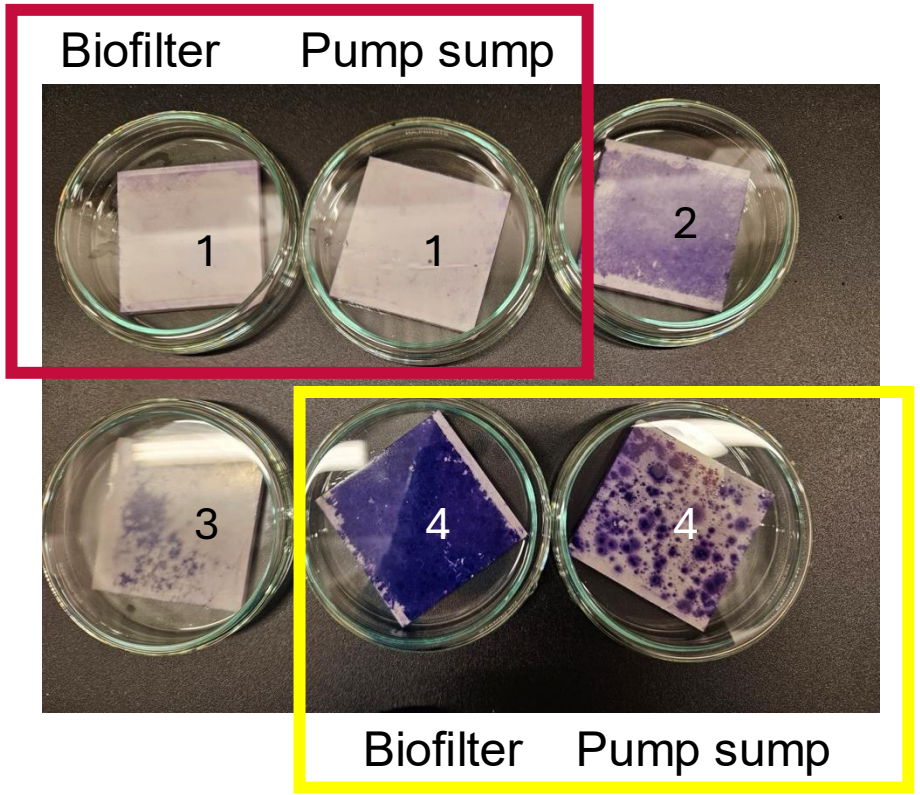


Biofilms

Before breach



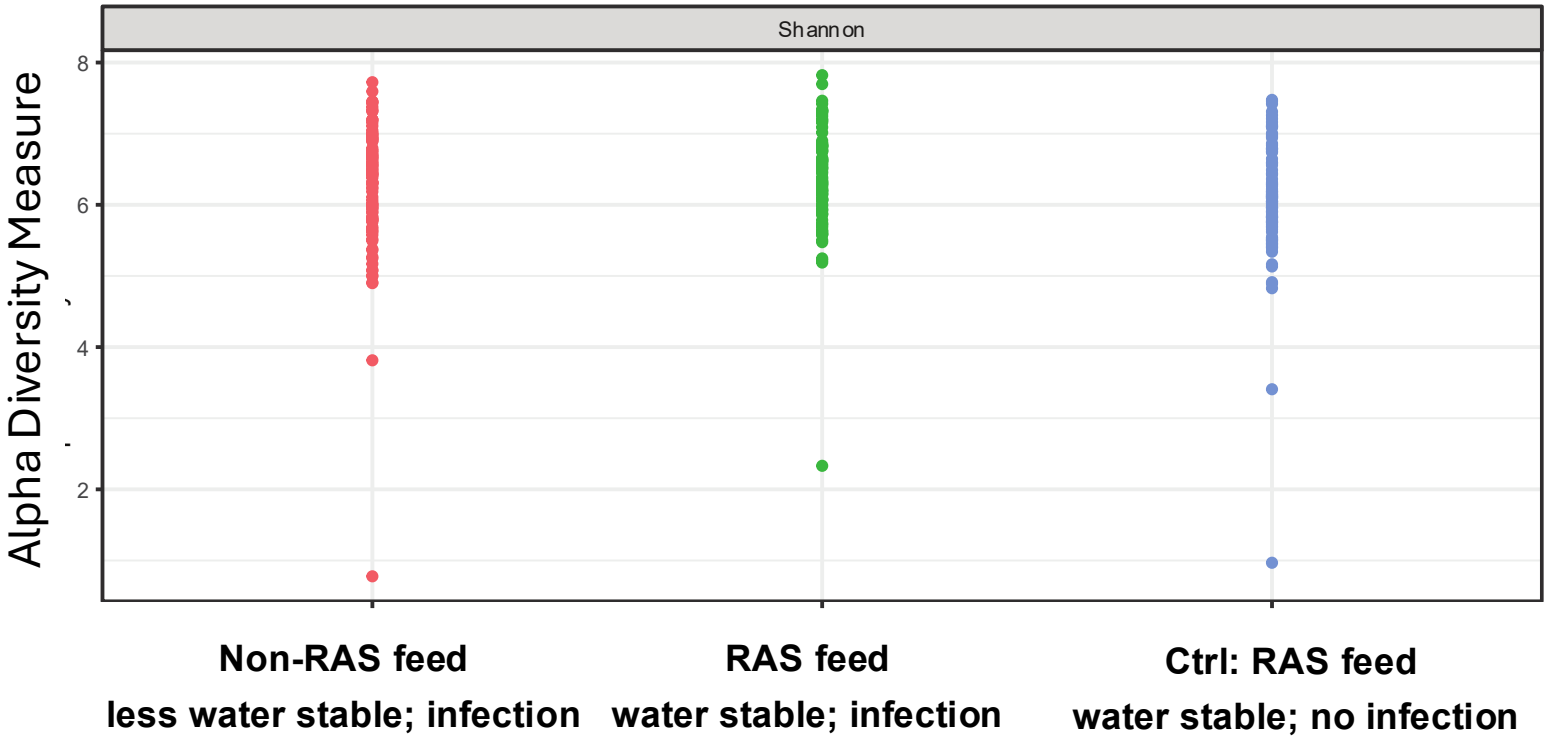
After disinfection



Disinfection of the system was achieved by manipulating the pH

Diversity of microbial communities

Alpha diversity (Shannon Index) is a measure of the diversity **within a single sample or community**.



- Similar microbial diversity across treatments.
- Most samples showed high Shannon diversity (5–7).
- Limited treatment effects on overall community diversity.

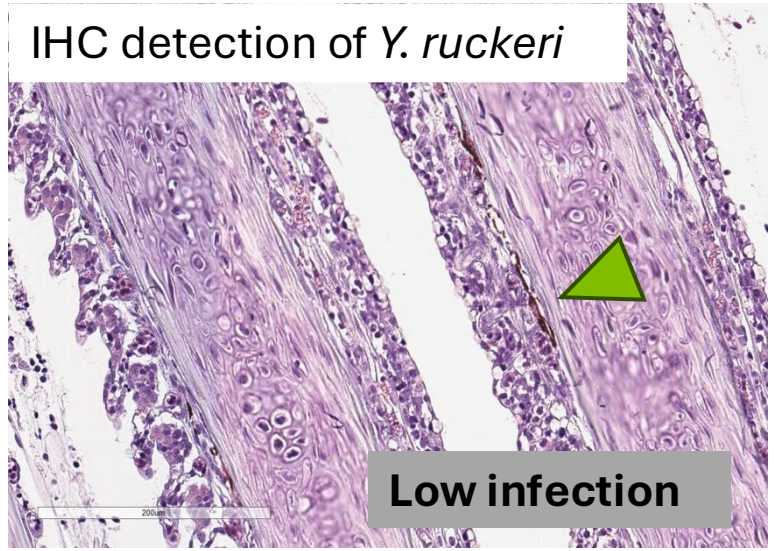
Did the pathogen establish in the system?



No mortality

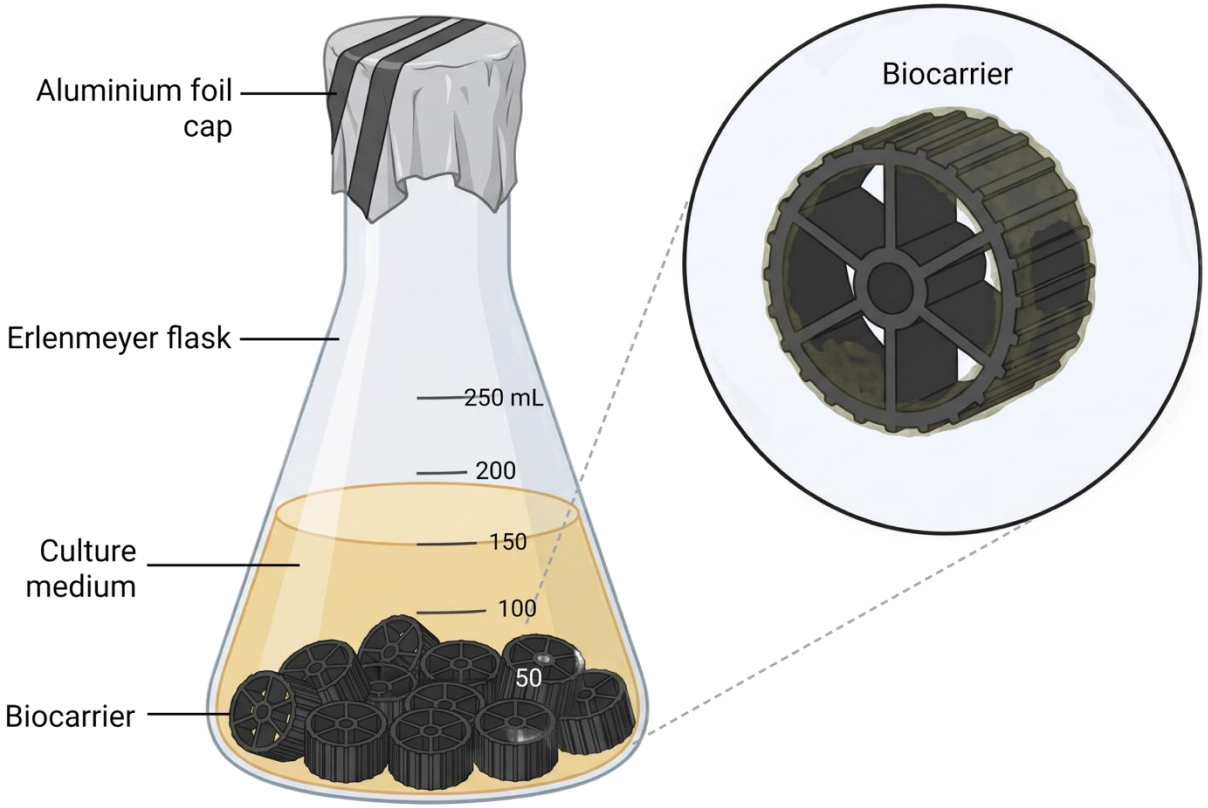


IHC detection of *Y. ruckeri*



Yersinia ruckeri did not manage to establish in the system

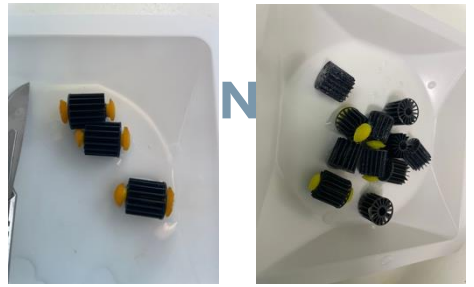
Can *Yersinia ruckeri* attach to biocarriers?



- Autoclaved biocarriers were incubated in marine broth containing *Yersinia ruckeri* for 24 h and 3 days.
- Biofilters were then rinsed with PBS, and excess liquid was removed before detaching bacteria into marine broth by vigorous shaking.
- The bacterial suspension was serially diluted and plated on marine agar and *Yersinia*-selective agar supplemented with a *Yersinia* selective supplement.

A 24 h exposure period is sufficient for *Yersinia ruckeri* to attach to sterile biocarriers.

Can *Yersinia ruckeri* attach better to sterile or biocarriers with biofilm?





10^7 cfu/ml



24-incubated

Sterile biocarriers
Biocarriers with biofilm

Control, uninfected

Sterile biocarriers
Biocarriers with biofilm

Infected, **water** as a vector

Sterile biocarriers
Biocarriers with biofilm

Infected, **biocarrier** as a vector

12°C, 12ppt

Yersinia ruckeri was followed for 3 weeks

Y ruckeri persisted longer on biocarriers with biofilms.

Biofilms enhanced *Y ruckeri* attachment and persistence.

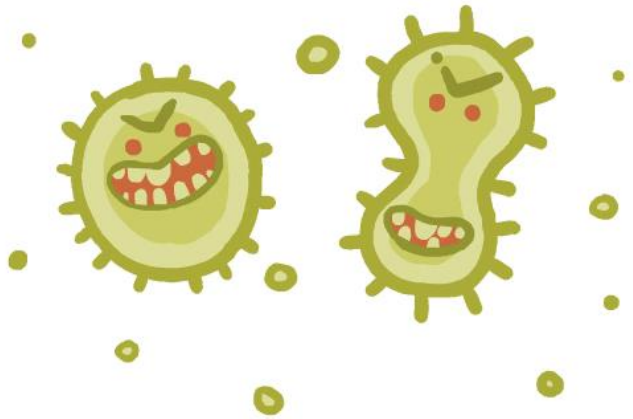
Y ruckeri was difficult to detect after 2 weeks.

No detectable *Y ruckeri* remained after 3 weeks.

Take-home messages

- Effective disinfection = balancing pathogen control, biofilter function, fish welfare, and operational sustainability.
- Most disinfectants tested were highly effective against fish pathogens in suspension, but biofilms can reduce disinfection efficacy.
- Biofilms promote attachment and prolonged persistence of *Yersinia ruckeri* on biocarriers.
- *Y. ruckeri* did not successfully establish in the experimental RAS despite deliberate introduction.
- Biosecurity strategies should be evidence-based and consider both planktonic and biofilm-associated microorganisms.

Takk!



FHF
THE NORWEGIAN SEAFOOD RESEARCH FUND

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Partners

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Norwegian University of Life Sciences