Lancering af ozon på ferskvandsdambrug

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HAV & FISK

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INDHOLD

BILAG 1:
Videnskabelig artikel om protein skimning og ozon behandling af ferskvand fra akvakultur.
de Jesus Gregersen, K.J., Pedersen, L.F., Pedersen, P.B., Syropoulou, E. and Dalsgaard, J., 2021. Foam fractionation and ozonation in freshwater recirculation aquaculture systems. Aquacultural Engineering, 95, p.102195.

BILAG 2:
Videnskabelig artikel om effekter af protein skimning og ozon behandling på bakteriesammensætning i vand og biofiltre fra et ferskvands akvakulturanlæg.
Aalto, S.L., Syropoulou, E., de Jesus Gregersen, K.J., Tiirola, M., Pedersen, P.B. and Pedersen, L.F., 2021. Microbiome response to foam fractionation and ozonation in RAS. Aquaculture, p.737846.

BILAG 3:
Notat om undersøgelser af protein skimmer og ozon på model 3 dambrug
"Commercial fresh water foam fractionation: Preliminary results", de Jesus Gregersen, K.J.

Projekt ”Lancering af ozon på ferskvandsdambrug”

1. INDLEDNING

Udvikling af landbaseret ørredopdræt foregår i stigende grad i recirkulerede anlæg med sikker og stabil vandforsyning og genanvendelse af vandet ved simpel mekanisk og biologisk filtrering. Således kan modeldambrug og fuldt recirkulerede anlæg kombinere en øget produktion med en reduceret miljøbelastning. Som følge af et relativt lavere vandforbrug (stor indfodring i forhold til vandskifte) og dermed en længere opholdstid på anlæggene, er biologisk filtrering særlig vigtigt. Anlæggene kan periodisk have problemer med vandkvaliteten som følge af utilstrækkelig kvælstofomsætning (uønsket ammonium som udskilles af fiskene nedbrydes af bakterier i biofiltrene via nitrit og uskadelig nitrat). Ud over disse kemiske ubalancer (forhøjet ammonium og/eller nitrit) kan der også ophobes bakterier i opdrætsvandet som alt andet lige er uønsket. Disse bakterier, suspenderede stoffer og mikropartikler (< 10 µm) fjernes ikke i tromlefilteret og de kan udelukkende tilbageholdes i varierende grad i dykkede kontaktfiltre (Tabel 1).

Det er endnu ikke tydeligt dokumenteret, at forringet og periodisk svingende vandkvalitet kan medføre øget fiskedødelighed. Men opdrætsvand med et højt indhold af organisk materiale og øget bakteriel aktivitet indebærer en række gener, eksempelvis øget iltforbrug og CO_2 udskillelse, nedsat sigtbarhed, øget risiko for tilslimning af fiskenes gæller og generel belastning af renseenhederne. Rent praktisk kan der opstå gener med periodisk kraftig skumdannelse i forbindelse med vandets passage gennem belufterbrøndene, og flere anlæg døjer med tilsmudsning af gangbroer eller uregerlig skum (Fig. 1). Dette skum er problematisk i produktionsøjemed, da det kan ramme elektriske installationer, tilsvine anlægget og påføre unødig arbejdstid med vedligehold.

Fig. 1. Billeder fra dambrug med skum-gener.

For nuværende er der ingen lette måder at fjerne skummet på ud over at skylle det tilbage i anlægget, benytte afskumnings-middel (mælk, olie) eller at fodre ekstra for at bryde vandets overfladespænding og reducerer luftboblernes ”velcro-effekt” (Fig. 3). Disse løsningsforslag fjerner imidlertid ikke skummet fra anlægget, men udsætter (og måske endda forstærker) problemet. Skummet udgøres af denaturerede proteinstoffer og bakterier og er meget energirigt og bør derfor fjernes fra vandet.



Fig. 2. Skumproduktion fra et model 3 dambrug. Det løse, lyse skum et typisk på sit højeste tidlig morgen og aftager i løbet af dagen, mens det mere klistrede brunlige skum kan være af mere permanent karakter.

Tabel 1. Oversigt over renseforanstaltninger og effekt på bakterier i vandfasen.

Renseforanstaltninger	Virkemåde	Effekt på bakterietrykket i vandfasen		
		Reduktion	Inaktivering	Fjernelse
Slamkegle	Fjernelse af partikulært stof	+ Indirekte ved at fjerne organisk materiale	-	-
Hvirvel separator				
Tromlefilter				
Kontakt filter Membranfiltrering				
Biofilter	Omsætning af opløst og partikulært stof	+ (dykket) - (moving bed)	-	-
Protein skimmer	Fjernelse af opløst og mikro-partikulært stof	+	+	+
Moler	Flokkulering/sedimentation	+	-	(+)
Ozon	Oxidation og flokkulering	+	+	-
UV	Hæmning af celledeling/vækst	-	+	- (forskydning)
Kemiske hjælpestoffer Formalin/pereddikesyre	Oxidation	+	+	- (C-kilde)
Brintoverilte, H_2O_2 kaliumpermang., $KMnO_4$	Beskadigelse af celler strukturer, enzymer	+	+	-
Elektrokemisk oxidation		+	+	-
NaCl	Osmotisk forstyrrelse	-	+	-

Simpel teknisk løsning til fjernelse af mikropartikler og bakterier.

I airliftene sker der en fysisk proces, hvor overfladeaktive stoffer (proteiner og andre organiske stofgrupper) i vandet udsættes for beluftning og indfanges i vand-luft fasen og føres fra vandet til overfladen hvor det koncentreres. Denne hydrofil/hydrofobe adsorptions proces, er styret af boblernes overfladespænding som afgør hvor kraftig velcro effekten er. Jo mindre bobler, desto bedre rensningseffekt.
Processen er kendt som proteinskimning (eller foam fractionation) fra en række industrier (akvarier, fødevarer, olie mv.) og anvendes også inden for saltvandsakvakultur.

Saltvand har en højere ledningsevne og anderledes ionsammensætning sammenlignet med ferskvand og ved beluftning dannes mindre bobler med højere overfladespænding. Af den grund virker proteinskimmere væsentlig bedre i saltvand end i ferskvand, hvor der ikke har været tradition for anvendelse af proteinskimmere.

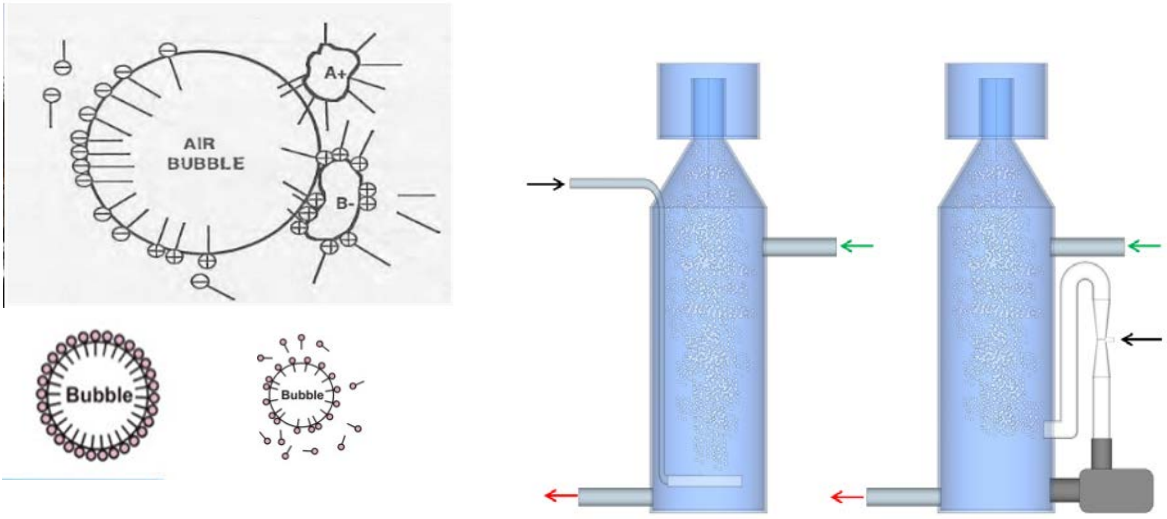

Fig. 3. Princip af den fysiske proces hvor partikler og lavmolekylære stoffer trækkes ud af vandfasen ved hjælp af luftbobler (t.v). Figurer t.h. viser skitse af to forskellige måder hvorpå boblerne dannes i en proteinskimmer. Proteinskimmeren er velkendt fra en række industrier og bygger på et princip om effektiv ladningsbestemt vand-til-luft overførsel af proteinfraktioner ved at have en beholder med mikro-bobler (stor reaktionsoverflade) høj beluftningsgrad og lang kontakttid.

Ozon – et kraftigt oxidationsmiddel
Ozon (O_3), er en kraftig reaktiv og oxiderende gasart og en velkendt desinfektionsmetode, der har en lang række egenskaber der med fordel kan kombineres med proteinskimmere. Herved indløses (opløses) ozonen, og der sker en kraftig inaktivering af bakterier i vandfasen når ozon kan reagere med vandet over en vis periode (kontakttid typisk 1-2 min).
Ozon påvirker boblestørrelsesfordelingen, øger overfladespændingen og oxiderer en række molekyler der ændrer størrelse og ladning og derved lettere kan trækkes ud af vandet via luftbobler til skum.

Ozon har en række fordelagtige egenskaber som kan nyttiggøres ved korrekt design, eksempelvis: Ozon er antimikrobielt (kan erstatte kemiske desinfektionsmidler), ozondoseringen kan tilpasses desinfektionsbehov (kontinuerlig/periodisk), ozon nedbrydes lynhurtigt (ingen miljøpåvirkning), ozon danner ilt under nedbrydning, ozon kan oxidere nitrit til nitrat og forbedre vandets sigtbarhed, og ozon virker flokkulerende og danner små bobler i ferskvand.

Ulemperne ved ozon er blandt andet, at ozon kan danne toksiske produkter ved overdosering (udtalt i saltvand i reaktion med klor, brom og jod), at ozonen skal dannes på stedet (ozon generator med luft eller ilttilledning). Ligeledes at ozondampe er sundhedsskadelig hvortil der kræves særlig sikkerhedsforanstaltninger ved brug indnedørs. Sikker ozondannelse og korrekt overførsel til vandfasen nedsætter disse risici, og ved at lede det ozonerede vand til indløb af anlæggens biofiltre er der ingen risiko for at fiskene eksponeres for overskydende ozon.

Formål

Rationalet med projektet var at afprøve proteinskimmere til at fjerne ophobede mikropartikler i ferskvandsanlæg. Dette skulle undersøges under kontrollerede betingelse ved DTU Aqua i Hirtshals og efterfølgende på et kommercielt dambrug. I undersøgelserne blev effekten af ozon vurderet ved at tilsætte ren ozon til reaktorrør eller en proteinskimmer.

De to vandbehandlingsmetoder forventedes at kunne fjerne bakterier og mikropartikler fra akvakulturvand, og have en yderligere positiv synergetisk effekt.
Der var ved projektets begyndelse ikke lavet undersøgelser af disse kombinerede renseteknikker, og tilsvarende er der først for nyligt kommet brugbare målemetoder til at kvantificere den bakterielle aktivitet og dermed for første gang at kunne måle denne i dambrugsvand.

Projektets formål var således, at *stimulere innovationen i akvakultur erhvervet* ved at *tilpasse ny teknologi med forbedret ozon dannelse*, indløsning i proteinskummer og *monitering af mikrobiel vandkvalitet*. Dette foregik ved en række praktiske afprøvninger og metodeoptimeringer under kontrollerede betingelser.

Formålet med projektet var også at fjerne proteinskum og mikropartikler ved at introducere en ny effektiv, sikker og stabil vandbehandling på danske modeldambrug. Projektets udfordrede paradigmet om at protein skimning i ferskvand ikke er muligt og at ozon ikke kan anvendes på modeldambrug.
Dokumentationen af renseeffektiviteten (proteinskimning med og uden ozon) foregik ved at måle på den tilbageholdte organiske fraktion (skum-fraktionen) og ved at kvantificere eventuelle forskydninger i den resulterende vandkvalitet ud fra en række nye og gængse vandkvalitets-parametre.

Tak til
Der skal lyde en stor tak til personalet på Nørå Dambrug for at indgå i projektet og være en stor hjælp og sparring undervejs i projektet. Fiskemester Jesper AB har ikke alene bidraget til samling og opstilling af skimmeren men har og foretaget justeringer og lavet forbedringer af skimmeren og været meget behjælpelig gennem hele forsøgsperioden.
Ligeledes tak til Svanemøllen for levering af kvælstof på Nørå Dambrug og til AquaPri for velvilligt at lade os tage vandprøver på 3 forskellige typer dambrug (Mosbjerg Dambrug, model 1, Lerkenfeld dambrug, model 3, og sandartanlægget i Gamst). Vi sætter også stor pris på hjælp til analysearbejde udført af laboranterne ved DTU Aqua, Hirtshals.

2. METODER OG FORSØGSOPSTILLINGER

2.1. Ozonbehandling af ferskvands RAS under kontrollerede betingelser.

Samtlige forsøg er blevet udført på akvakulturvand for at afspejle virkeligheden bedst muligt. Der blev lavet en række forsøg med forskellige vandmatricer. Indledningsvis blev der hentet vand fra forskellige dambrug (Model 1 dambrug, Model 3 og fuldt recirkuleret anlæg, hhv. Mosbjerg, Lerkenfeld og Gamst) som efterfølgende blev testet ved DTU Aqua, Hirtshals.

Hovedparten af forsøgene blev herefter lavet med dambrugsvand fra kontrollerede recirkulerede akvakultur systemer ved DTU Aqua, Hirtshals. Vandet afspejlede RAS vand med hensyn til ammonium, nitrit og nitrat og indeholdt opløst og partikulært organisk materiale som alt sammen kom fra fodrede fisk og biologiske processer i biofiltre. På figuren nedenfor ses eksempler på forskellige anvendte forsøgsopstillinger.

Fig. 4. Eksempler på forsøgsopstillinger med test af rensning af dambrugsvand ved hjælp af proteinskimning og ozon.

2.2. Afprøvning af ozon og proteinskimmer på et kommercielt anlæg

I efteråret 2020 blev der i projektet indkøbt en brugt demo proteinskimmer (RATZ Protein skimmer PS (1400) Link) som efterfølgende blev installeret på et Model 3 Dambrug.
Proteinskimmeren blev placeret på en stålplade over kanalen mellem udløbet fra den nederste raceway og indløbet til biofiltersektionen. Skimmeren havde tre 0,75 kW venturipumper der cirkulerede og beluftede vandet i skimmeren. Skimmeren modtag vand fra en propelpumpe (KSC160) ca. 60 m^3/h der var placeret i udløbet fra biofiltersektionerne (Fig. 5).
Det viste sig, at mængden af luft fra venturierne var utilstrækkelig (for få og for store bobler), så skimmeren blev modificeret i februar, 2021. Her monterede fiskemesteren tre styk 1 meter lange gummi-diffusorrør placeret i bunden af skimmeren som blev tilsluttet en kapselblæser.
Forsøgsopstillingen omfattede også en palletank ved siden af skimmeren som blev brugt til opsamling af afskumnings flowet (rejektvand). I juni, 2021, blev der tilkoblet en Gaia ozon generator (Link). Enheden var monteret i et kabinet ved siden af skimmeren; generatoren var vandkølet og tilsluttet en gasflaske med frit kvælstof der via styring blev tilledt i 5 sekunder hver 10 min (Richard Martin, Water ApS, pers. Komm.). Ozon generatoren kunne under disse betingelser producere 50-60 g ozon i timen.

Undersøgelserne af skimmerens renseeffektivitet forgik i perioden fra april til juli, 2021, mens forsøg med ozon forgik i juni og juli, 2021 (Bilag). Undersøgelserne omfattede typisk målinger af ændringer i vandkvaliteten over skimmeren ved at måle en række parametre (Tabel 2) på vandprøver ved indløbet til og udløbet fra skimmeren. Der var ligeledes målt på mængden og koncentrationen af den dannede skum fra skimmeren ved forskellige driftsbetingelser.

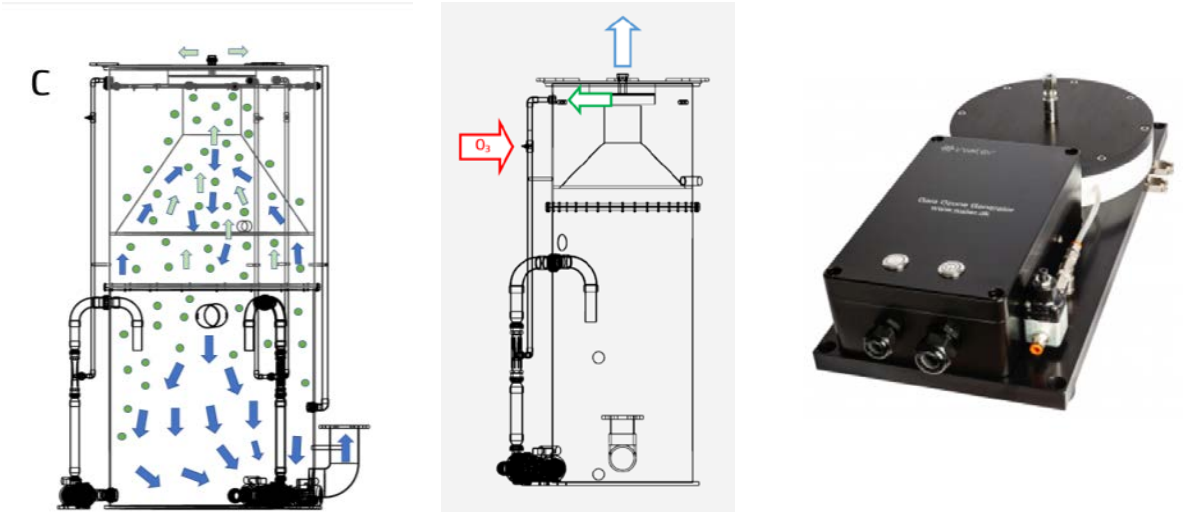

Fig. 4A. Skitse af RATZ skimmer (tv) med venturi med indgang til ozon (midt) og ozon generator (th).

Fig. 5. Test of afprøvning af proteinskimmer på et model 3 dambrug. Skummet i toppen af skimmeren ledes over i en palletank hvorved volumen og koncentration kan bestemmes. De nederste billeder viser kabinet med ozon generator hvor den dannede ozon ledes via en venturi ventil ind i skimmeren.

2.3. Analyser af den resulterende vandkvalitet

Tabel 2. Oversigt over anvendte vandkvalitetsparametre i undersøgelsen

Måleparameter	Bemærkninger og referencer
Ilt-koncentration, pH og temperatur	Målt med Oxyguard Handy og Hach40 D multimeter
Ammonium, nitrit og nitrat konc.	Spektrofotometriske analyser ud fra Dansk Standard 223,224.
UV_T og turbiditet Vandet klarhed, hhv. UV transmission (254 nm)- og vandets indhold af partikler/uklarhed ud fra måling af lysspredning.	UV-spektrofotometrisk analyse og turbiditet bed bruh af Hach håndholdt meter og prober.
COD (Chemical oxygen demand) Måling af total, opløst og partikulært organisk materiale i form af "kemisk" iltforbrug.	Hach cuvette test i forskellige måleområder (aa-bb) - filtrering af råprøver med 0,45 µm filter.
BI_5 (Biokemisk iltforbrug over 5 døgn) Måling af opløst og partikulært organisk materiale som kan bruges af bakterier og indebærer et "biologisk" iltforbrug.	Måling af iltforbrug ved 20,0 °C ifølge dansk standard. Filtrering af råprøver med 0,45 µm filter.
Mikropartikler Fordeling, antal, mængde og samlet overflade af partikler og bakterier (fra 1-30 µm) i vandfasen.	Måling ved brug af Coulter counter og forskellige apertur-størrelser (forbehandling af skumprøver)
Bakteriel aktivitet Måling af den samlede bakterieaktivitet i en vandrøve, ved enten H_2O_2 metode og BactiQuant	Bakteriel aktivitet målt som omsætning af brintoverilte ved en konstant temperatur 22°C eller ved filtrering af kendt volumen og temp. og kvantificering med Bactiquant
Ozon koncentration I vandprøver målt som farvereaktion og spektrofotometrisk bestemt I luft måles ozongas ved brug af flowkuvette og måling af UV absorbans	Spektrofotometrisk målemetoder ved Hach cuvettes test som klor-ækvivalenter (Total residual oxidants). Måling af ozon gas ifølge Ref (Camilla/Spiro)

3. RESULTATER OG DISKUSSION

3.1. Kontrollerede forsøg med proteinskimmer og ozon

Proteinskimning i ferskvand virker
Helt overordnet har forsøgene vist, at proteinskimning virker i ferskvand.
Det vil sige, at lufttilførsel med små bobler er i stand til at trække mikropartikler ud af dambrugsvand i form af skum. Skummet dannes i mange tilfælde umiddelbart efter at proteinskimmeren tændes og fortættes typisk til en brunligt til sort vandig slamfraktion
(Fig. 6). Den synlige mængde der fjernes som skum er ensbetydende med, at vandets indhold af organisk materiale reduceres og at bakterietrykket nedsættes. Og det er særligt de "problematiske" mikropartikler der fjernes hvilket gør metoden særlig interessant.

Fig. 6. Skumdannelse i en proteinskimmer der renser dambrugsvand og eksempler på den vandige fraktion der fjernes fra vandet.

Forsøg, der blev udført i lukkede beholdere med akvakulturvand fra tre forskellige dambrug viste, at der skete tydelige forbedringer på en lang række vandkvalitets parametre. Eksempelsvis blev mængden og antallet af mikropartikler væsentligt reduceret (se Fig. 7), ligeledes vandets indhold af organisk materiale (opløst og partikulært) og vandets bakterielle aktivitet (Fig. 8).

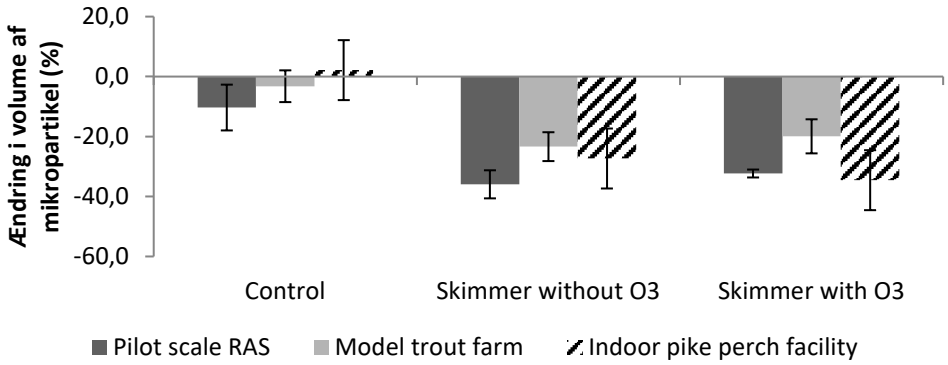

Fig. 7. Effekt af proteinskimmer med og uden ozon i forhold til ubehandlet vand (control) på mikropartikler (volumen) fra 3 forskellige RAS anlæg

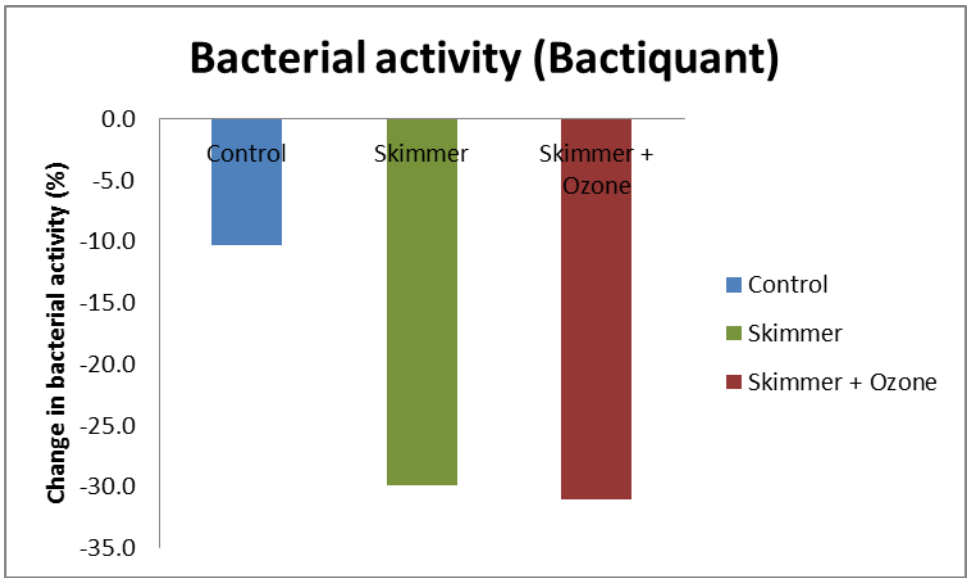

Fig. 8. Effekt af proteinskimmer med og uden ozon i forhold til ubehandlet vand (control) på den bakterielle aktivitet i ferskvand fra 3 forskellige RAS anlæg.

Et andet forsøg undersøgte hvordan saliniteten påvirker afskumningen og vandkvaliteten. Ved at måle på ferskvand fra et recirkuleret anlæg blev det målt, at skimmeren kan reducere vandets indhold af mikropartikler (Fig. 9). I det ubehandlede RAS vand skete en forøgelse i partikel mængden, hvormed skimmeren reducere denne mængde med > 40%. Forsøget viste også, at svag saltholdighed (3 promile salt) har en yderligere forstærkede effekt og resulterede i > 60 % reduktion af partikelantallet.

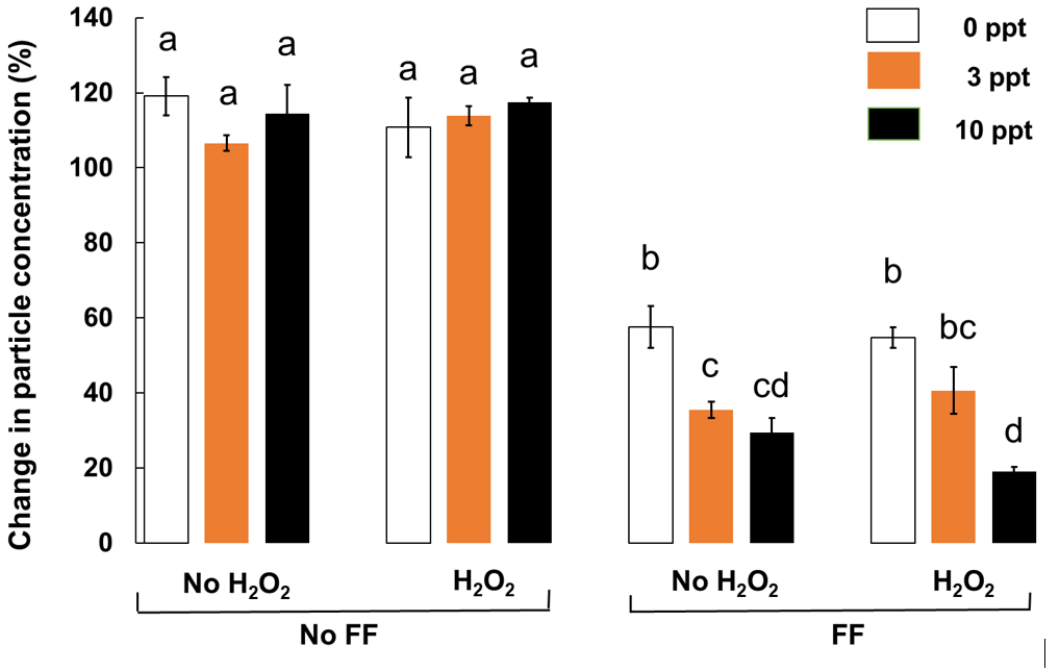

Fig. 9. Effekt af proteinskimmer (foam fractionator, FF) H_2O_2 tilsætning (10 mg/l) og tilsætning af salt hhv. rent ferskvand, og ferskvand tilsat hhv. 3 og 10 g NaCl/l. Værdierne er angivet som relative ændringer efter 6 timers behandling, hvor 100 % betyder at der ikke er sket en ændring (data fra, Jafari, 2020).

Effekt af ozon

I et 8 ugers kontrolleret forsøg med 12 ens recirkulerede systemer med regnbueørreder, blev det vist, at såvel skimmer som ozon har en effekt på den resulterende vandkvalitet.
De umiddelbare synlige effekt af ozon og proteinskimning kan ses som forskel i vandets farve og turbiditet (Fig. 10). Der var ikke forskel i fiskenes tilvækst mellem de 4 forsøgsgrupper og gennem hele perioden var der kun en enkelt død fisk. På en lang række parametre blev der målt tydelige forskelle i vandkvaliteten.

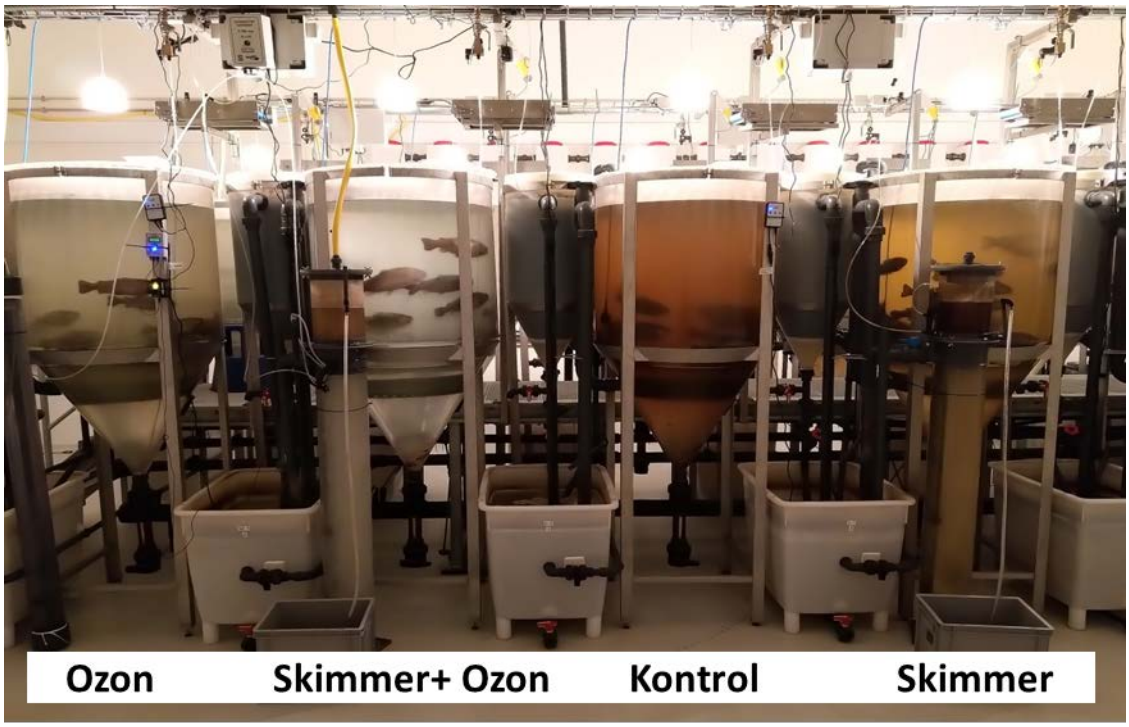

Fig. 10. Billede af forsøgsopstilling efter 8 uger forsøg. Her ses 4 forskellige forsøgsgrupper med akvakulturvand der blev behandlet med enten ozon, protein skimmer eller kombination af begge; hvor kontrol er RAS uden skimming eller ozon behandling (specifikke detaljer kan ses i Bilag 1).

Forsøget viste blandt, at ozon havde en kraftig effekt på vandets sigtbarhed og turbiditet. Når ozon blev tilført direkte i en reaktor forsvandt den brunlige egenfarve som ses i kontrolgrupperne. Ozon kombineret med proteinskimmer førte til det klareste og reneste vand, mens skimmeren resulterede i vand med en lavere turbiditet (Fig. 10) i forhold til de ubehandlede kontroller. I det pågældende forsøg løb anlæggets samlede vandvolumen gennem skimmerne knap 2 gange/time.

Protein skimning alene reducerede antallet af mikropartikler med 58% i ferskvand (Fig. 11A). Effekten af ozon var endnu større (83%) mens kombinationen af de to metoder første til den kraftigste reduktion på 89% sammenlignet med kontrolgruppen.

Kombinationen af protein skimning og ozon reducerede bakterie-aktiviteten i ferskvand RAS med 90%. Proteinskimning havde en isoleret effekt ved at reducere bakterie aktiviteten med 48 %, mens ozonen i sig selv førte til en 61 % reduktion (Fig. 11B)
Tilsvarende mønster blev fundet for reduktion af organisk stof, hvor hhv. proteinskimning og ozon førte til 51 og 43 % mindre BI_5 – mens kombinationen af de to metoder resulterede i 75% reduktion (Fig. 11C).

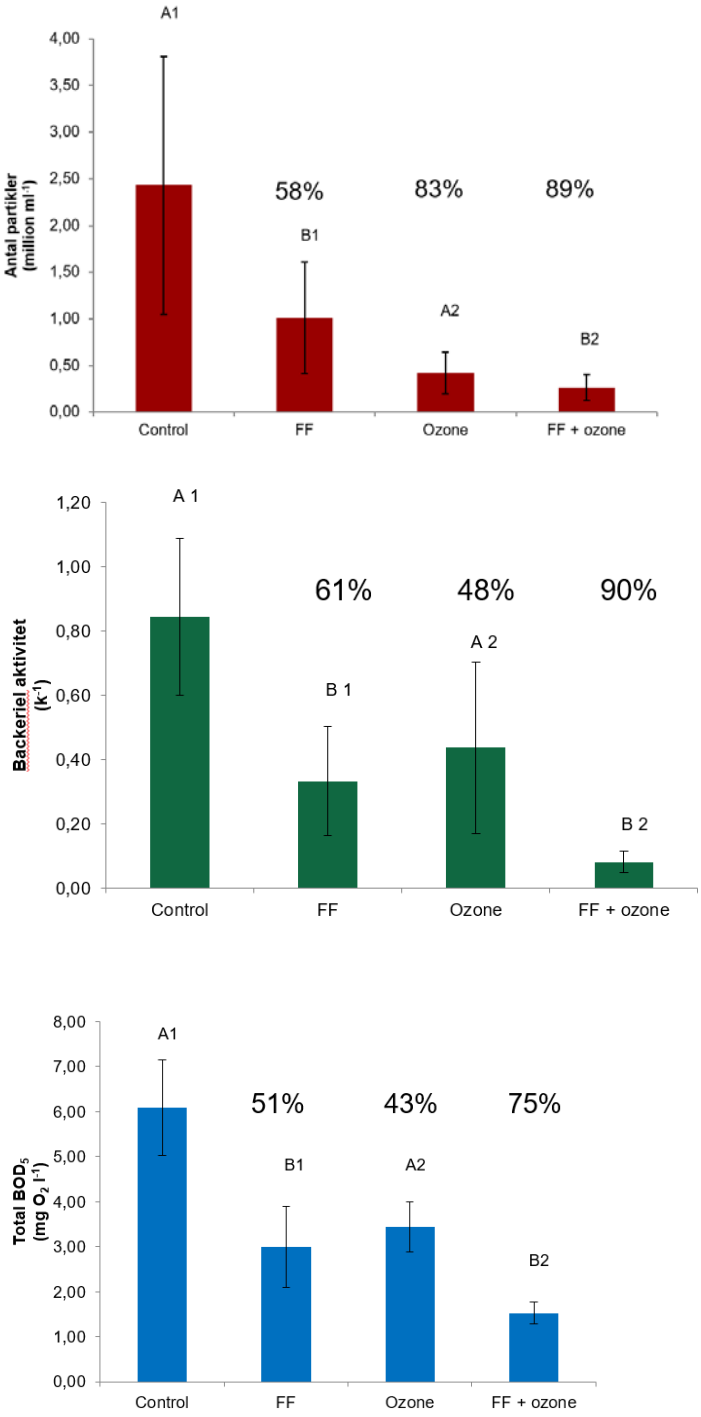

Fig 11. Øverst: Antal mikropartikler (middel ± std. afv.) i RAS ferskvand af de fire behandlingsgrupper. Midt: Bakteriel aktivitet i vandet fra de 4 forsøgsgrupper, og nederst: Organisk materiale målt som biokemisk iltforbrug over 5 døgn. FF= foam fractionator= protein skimmer Control = RAS uden FF eller ozon.

Ovenstående forsøg demonstrerede således tydelige, langvarige positive rense-effekter af såvel proteinskimning som ozon i ferskvand.
Supplerende undersøgelser af bakteriesamfundene i anlæggene (vandfase og i biofiltrene) viste samtidig at der var meget tydelig forskelle i artsammensætning (Bilag 2).
Alle behandlingsgrupper havde tilfredsstillende vandkvalitet med hensyn til ammonium og nitrit, og således ingen umiddelbare negative effekter af behandlingerne. Det blev fundet,
at nitrit indholdet i anlæggene med ozon var lavere end de øvrige grupper. Dette kan enten skyldes, at nitrit oxideres af ozon til nitrat eller forklares ved, at reduktionen i organisk materiale kan have medført mindre vækst af (konkurrerende) heterotrofe bakterier og derved forbedret vilkårene for de nitrificerende bakterier i biofiltrene.

Generelt havde skimmere med og uden ozon en tydelig effekt på vandkvaliteten i ovenstående forsøg med ferskvandsanlæg. Idet der fjernes organisk materiale fra anlægget, vil det kunne give sig udslag i forbedret vandkvalitet eller en mindre belastning i biofiltrene. Det skal nævnes, at mens der tydeligvis fjernes stof fra vandet, er det ikke altid umiddelbart målbart i vandfasen men aflaster de interne renseenheder (pers. observationer). Det er dog ved flere undersøgelser blevet bemærket, at brugen af en skimmer nedsætter biofilm belægninger og fører til en mindre belastning af biofiltrene. Det har den umiddelbare effekt at behovet for returskylning af biofiltrene mindskes, og derved også risikoen for anaerobe forhold og dannelse af svovlbrinte.

3.2. Afprøvning af proteinskimmer og ozon på model 3 dambrug

Processen med afprøvning af ozon og en fuld skala proteinskimmer på et kommercielt ferskvandsdambrug forgik over en længere periode og med en række ændringer og forbedringer undervejs. De beskrevne forbedringer (side 8; Bilag 3) med diffusorrør (100 mm perforerede gummirør) og beluftning med trykluft gav væsentlig flere bobler blev anvendt gennem hele perioden sammen med 2 venturipumper.
I vintermånederne var der ikke nævneværdig skum i produktionsanlæggets airlift, og skimmeren var ikke i brug. Dette ændrede sig i begyndelsen af april måned, og protein-skimmeren fungerede øjeblikkeligt med kraftig skumdannelse da den blev tilsluttet.

Fig. 12. Vandprøven ovenfor t.v. er fra anlægget, flasken til højre indeholder en vandprøve fra palletanken der indeholder vandigt skum / rejekt vand fra skimmeren (9. april, 2021).

En enkelt passage gennem skimmeren (ca. 60 m^3/time) førte i gennemsnit i måleperioden til følgende ændringer i vandkvaliteten (Bilag 3):

- Reduktion af vandets biologiske iltindhold (BI_5) på 1,8 %
- Reduktion af vandets kemiske iltindhold (COD) på 1,8 %
- Reduktion af bakterie aktivitet på 1,6-6,0 %
- Reduktion af mikropartikler (antal og volumen) på 8,5-10,2%
- Reduktion af vandets turbiditet med 10,5%
- Forbedret sigtbarhed (UV_T) på 0.5%
- Iltiltning fra 62-65% i indløb til 98% i udløbet fra skimmeren
- Forøgelse i pH på 0,3-0,4 pH enheder

Det bemærkes, at tallene ovenfor er baseret på måling foretaget under varierende produktions- og driftsbetingelser (eksempelvis varierende indfodring, tilsætning af salt eller formalin, tidspunkt på dagen, temperatur m.v.) men at der generelt blev registreret en renseeffekt.
Dette er også sammenholdt med det synlige bevis på skumdannelse og fjernelse af organisk materiale i form af betydelige mængder spildevand fra skimmeren. Mængden af rejektvand fra skimmeren varierede med indstillingerne af skimmer (vandsøjlehøjde) og det blev også bemærket, at tilsætning af salt og ozon gav udslag i øget skumproduktion.
Der blev foretaget målinger af sammenhænge mellem skum-produktionen (flow og volumen) og koncentrationen heraf. Generelt for de målte vandkvalitetsparametre (kemisk og biokemisk iltforbrug, mikropartikler og bakteriel aktivitet) blev det fundet, at netto fjernelsen er stigende med

flowet op til et flow > 4-500 l/time, hvorefter der ikke ses nogen nævneværdig effekt (Bilag 3). Det er således op til den enkelte bruger at finde en volumen (lav volumen- høj koncentrationsfaktor vs. høj volumen-lav koncentration) der passer bedst for den pågældende drift.

Brug af ozon
Der blev lavet målinger af ozon tilkoblet skimmeren ved 2 prøvetagninger (23/6, 2021 og den 30/6, 2021). Her blev der fundet en markant effekt af ozon på hæmning af den bakterielle aktivitet, om end vandkvaliteten den ene dag var atypisk med lavt COD indhold.
Ozonens effekt på bakterieaktiviteten skal ses i lyset af den relative korte opholdstid i skimmeren og det faktum af ozonen ikke blev indløst tilstrækkelig godt. Både ved opstart efter installationen af ozon, og siden hen ved kontinuerlig drift var den karakteristiske ozon lugt meget tydelig i visse vindretninger. Da indløsningen af ozon ikke er optimal er virkningsgraden underestimeret, og det forventes at kommende forbedring vil kunne realisere endnu bedre desinfektionseffekter. Det blev svagt indikeret ved undersøgelsen, at der også var en tidsmæssig effekt af ozonen (18%,34% og 46% målt hhv. 1, 4 og 8 timer efter tilslutning af ozon) hvilket ifølge Richard Martins (Water Aps) er forventeligt.

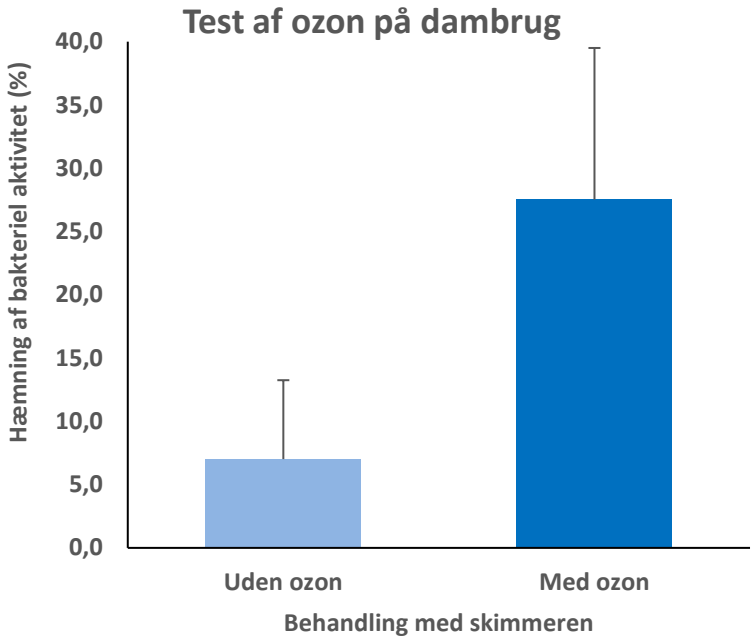

Fig. 13. Resultater af protein skimmerens effekt uden brug af ozon (n=6) og med 50 g O_3/time (n=5). Værdierne er baseret på brintoverilte-aktivitetsmålinger på vandprøver fra skimmerens indløb og udløb den 23/6 og 20/6, 2021 (n=11).

Stikprøvemålinger af ozon i udløbsvandet fra skimmeren blev udført på dambruget (ozon omsættes lynhurtigt) og der blev ikke målt ozon heri (detektionsgrænse på 0,007 mg O_3/l).
Placeringen af skimmeren og udløbet herfra sikrer ligeledes at evt. rest-ozon (såfremt der over tid måtte blive dannet og indløst mere ozon end der omsættes) vil blive øjeblikkelig nedbrudt ved kontakt med organisk materiale i anlæggets biofiltre.

Kommende undersøgelser skal blandt andet afdække,

- hvordan ozonen bedre kan indløses i vandet og dermed bedre udnyttes
- om ozonen evt. kan styres i forhold til desinfektions behovet (organisk materiale)
- om tilsætning af salt til indløbet af skimmeren er fordelagtigt
- om proteinskimmer skal køre kontinuerlig og med ozon
- om kontinuerlig drift af skimmer og ozon har positive effekter på vandkvaliteten
- om kontinuerlig drift af skimmer og ozon giver en bedre omsætning i biofiltrene
- hvor stor en andel af organisk materiale skimmeren fjerner i forhold til øvrige foranstaltninger
- de økonomiske aspekter ved investering i anskaffelse af anlæg + drift

Dette, velvidende at der er en række uforudsigelige faktor og driftsændringer der er uden for kontrol når der laves forsøg på kommercielle anlæg. Det er samtidig vanskeligt at have et sammenlignings-grundlag (kontrol), og derfor er introduktion af ny driftspraksis og rensemetoder ofte vanskelig at evaluere. I projektet har vi været heldige med at den ansvarlige fiskemester brugte megen tid og energi på drift og forbedring af proteinskimmeren. Det er en afgørende forudsætning for yderligere at udvikle og optimere renseteknologien med henblik på at få en bedre vandkvalitet.

Proteinskimning og ozon løser ikke alle problemer
Proteinskimning og ozonering kan vise sig at være et alternativ eller supplement til brugen af hjælpestoffer. Der bruges betydelig summer og tid på kemisk vandbehandling. Såfremt skimmeren og ozon kan bidrage til at holde bakterietrykket nede og måske også være med til at reducere udbrud af f.eks fiskedræbere og generelt mindske gælleproblemer kan teknologien blive en løsning hvor driftsfordelene overstiger udgifter til drift og anskaffelse.

Det vil kræve en række forbedringer af den anvendte teknologi, eksempelvis nye måder at få ozonen bedre opløst på, bedre kendskab til korrekt dimensionering og drift af skimmeren og forhold omkring lugtgener.
Skimning og ozonering er påvirket af vandets beskaffenhed og skal ideelt set ikke bruges til at fjerne organisk materiale, der kunne være fjernet på anden vis. Såfremt der er puljer af organisk materiale i anlægget (slamansamling eller regelmæssige frigivelser fra biofilteret i forbindelse med vedligehold og returskylning) der kontinuerlig skaber gunstige vækstbetingelser for bakterier, vil størrelsen af skimmeren og mængden af ozon være uhensigtsmæssig stor.

4. KONKLUSION

Akvakulturudviklingen bevæger sig i en retning – imod mere recirkulering. Derfor vil der være et fortsat stigende behov for at få løst problematikken med ophobning af bakterier i RAS. Kombineret vanddesinfektion og fjernelse af mikropartikler vil forbedre opvækst betingelser på recirkulerede anlæg. Proteinskimning er en relativ simpel teknologi og har vist sig at kan fungere i ferskvand. Teknologien er afprøvet og dokumenteret i ferskvand fra en række akvakultur anlæg, hvor der er fundet forbedringer i vandkvaliteten (færre mikropartikler, mindre bakteriel aktivitet, nedsat mængde af organisk stof, øget sigtbarhed, øget iltkoncentration m.m.).
Undersøgelsen viste også, at ozon hæmmer bakteriel aktivitet og forstærker renseeffektiviteten i kombination med proteinskimmere.

For at få mest ud af teknologierne skal ozon tilførslen optimeres, og der skal samtidig være øget opmærksomhed på hurtig og effektiv mekanisk fjernelse af partikulært organisk materiale i anlæggene.

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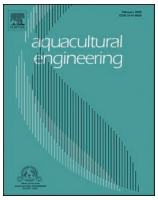

Foam fractionation and ozonation in freshwater recirculation aquaculture systems

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ABSTRACT

Foam fractionation is often considered an ineffective way of removing organic matter from freshwater due to the low surface tension of the water. There is, however, a lack of studies testing foam fractionation efficiency in replicated freshwater recirculating aquaculture systems (RAS). Foam fractionation can be applied with or without ozone. Ozone is a strong oxidiser previously shown to improve water quality and protein skimmer efficiency. To test the efficiency of foam fractionation and ozonation (20 g O_3 kg^{-1} feed) separately and in combination in freshwater RAS, a two-by-two factorial trial was conducted with each main factor at two levels (applied or not applied). Each treatment combination was carried out in triplicates using 12 replicated pilot scale RAS stocked with juvenile rainbow trout (*Oncorhynchus mykiss*) and operated at a feed loading of 1.66 kg feed m^{-3} make-up water. The trial lasted 8 weeks and samples were obtained once a week. Ozone applied by itself significantly reduced the number of particles (83%), bacterial activity (48%) and particulate BOD_5 (5-days biochemical oxygen demand; 54%), and increased ultra violet transmittance (UVT; 43%) compared to the untreated control group. Foam fractionation by itself lead to significant reductions in particle numbers and volume (58% and 62%, respectively), turbidity (62%), bacterial activity (54%) and total BOD_5 (51%). A combination of both treatments resulted in a significant additional improvement of important water quality variables, including a 75% reduction in total BOD_5, 79% reduction in turbidity, 89% reduction in particle numbers and 90% reduction in bacterial activity compared to the control. The removal efficiencies were within the same range as those observed in previous studies conducted with foam fractionators in saltwater systems (with or without ozone), corroborating that foam fractionation may become a useful tool for controlling organic matter build-up and bacterial loads in freshwater RAS.

1. Introduction

The build-up of organic matter in recirculating aquaculture systems (RAS), deriving from fish excretions and feed spill (Schumann and Brinker, 2020), is among the largest challenges in the industry (Martins et al., 2010). Modern aquaculture facilities are typically equipped with primary solids removal technologies based on particle sedimentation (e.g. settling cones) and filtration (e.g. drum filters) (Timmons and Ebeling, 2010). As a result of prolonged retention times in RAS, together with the use of technologies which target mainly larger particles, fine solids and dissolved organic matter accumulate in the system (Chen et al., 1993a; de Jesus Gregersen et al., 2019; Fernandes et al., 2014; Patterson et al., 1999).

Accumulation of fine solids is considered problematic due to their small size and large surface area to volume ratio providing food and space for bacteria growth (Becke et al., 2020; de Jesus Gregersen et al., 2019; Pedersen et al., 2017). Similarly, dissolved nutrients and organic matter provide energy for free-living bacteria. Increased bacterial growth in RAS in turn leads to increased oxygen consumption, clogging of biofilters and potentially reducing nitrification capacity (Chen et al., 2006; Zhang et al., 1994). Organic matter build-up in stagnant areas is also thought to explain recent cases of H_2S driven mortality events (Dalsgaard, 2019; Letelier-Gordo et al., 2020).

A large portion of micro particles is composed of living microorganisms and can therefore be controlled by e.g. ultraviolet radiation (UV) (de Jesus Gregersen et al., 2020). While UV disinfection is

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commercially relevant due to its technological maturity and easiness of application, it does not deal with organic matter build-up which is the underlying cause of microbial growth, causing an increase in system carrying capacity (Blancheton et al., 2013; Vadstein et al., 1993).

Direct removal of fine solids can be achieved using different strategies. Reducing drum filter mesh size is one possibility but rapidly becomes costly (Dolan et al., 2013). Membrane filtration is another option shown to reduce colloidal particles in RAS by 77% and turbidity by 44% (Holan et al., 2014). However, membrane filtration is also costly and a main reason for why it is not implemented in the industry (Viadero and Noblet, 2002). Fossmark et al. (2020) for example estimated that it would increase production costs by 27% to apply membrane filtration to Atlantic salmon (*Salmo salar*) RAS.

An alternative technique for removing fine solids and even dissolved organic matter is foam fractionation (FF). Foam fractionation relies on surfactants in the water generating foam that removes particulate and dissolved organic matter (Timmons and Ebeling, 2010). Foam fractionation has been show to concentrate total suspended solids (TSS) by 17–40 times in the foam condensate (Weeks et al., 1992), and reduce particulate matter and bacteria in saltwater RAS (Barrut et al., 2013; Brambilla et al., 2008). Recently, Ji et al. (2020) tested the combined effects of drum filters followed by FF in saltwater RAS. The results showed similar or better removal efficiently of FF compared to drum filtration when the drum filter was equipped with mesh filters of 120 and 90 μm. Only when the drum filter was equipped with a 40 μm filter did it clearly have superior removal efficiency.

Ozone (O_3) dosage can be coupled to FF. Ozone is a strong oxidising agent that can be used directly for disinfection in RAS, if applied at sufficient concentration and contact time. Ozone addition is often followed by UV for destroying harmful ozone residuals (Gonçalves and Gagnon, 2011; Powell and Scolding, 2016). The strong oxidising properties of O_3 allow it to break down complex molecules and reduce organic matter loads (Davidson et al., 2011; Summerfelt et al., 2009). Applying O_3 together with FF takes advantages of this property, improving foam fractionation efficiency by breaking down complex molecules so that they are more easily removed, and by increasing coalescence of particles (Li et al., 2009; Summerfelt et al., 1997) and altering bubble size distribution and surface tension (Hu and Xia, 2018; Matho et al., 2019). Another benefit of combining low doses of O_3 and FF is a reduced risk of ozone residuals (especially in freshwater) while organic matter to oxidise is present in the system (authors' pers. obs.).

Foam fractionation has traditionally only been applied in saltwater systems due to seawaters high surface tension, whereas its efficiency in freshwater RAS is less clear. A few trials were, however, conducted nearly three decades ago. Chen et al. (1993b) conducted a study using water from fresh water RAS and treated in batch tests, showing a up concentration of total solids in the fomate, especially of organic particles smaller than 30 μm. Weeks et al. (1992) analysed the fomate produced by skimmers attached to pilot scale RAS and determined that the skimmers generated an up concentration of organic particles, total Kjeldahl nitrogen (TKN) and total suspended solids (TSS). However, the effects on water quality of freshwater RAS under operation still remain unknown. The objective of this study was therefore to access the potential of FF and O_3 (separately or in combination) for improving the water quality in freshwater RAS, including effects on organic matter build-up, micro particle accumulation and bacterial activity in the water, as well as organic matter accumulation in the biofilter.

2. Materials and methods

2.1. Experimental setup

A two-by-two factorial experiment with foam fractionation and ozonation as main factors was performed in 12 replicated, 0.8 m^3 pilot scale freshwater RAS (Fig. 1) at DTU Aqua in Hirtshals, Denmark. Four treatment combinations were applied: three control RAS without FF or

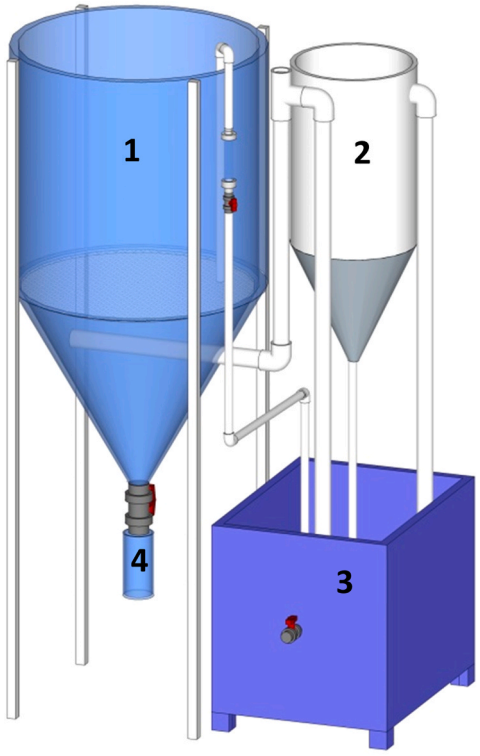

Fig. 1. Pilot scale RAS including a: 1) rearing tank; 2) moving bed biofilter; 3) pump sump; and 4) sludge collector.

O_3, three RAS with FF (FF), three RAS with O_3 dosing (O_3), and three RAS with FF + O_3 dosing combined (FF+O_3). Each RAS was composed of a: 100 L cylindroconical biofilter filled with 40 L RK BioElements (RK BioElements, Denmark) with a specific surface area of 750 m^2 m^{-3} and operated as a moving bed biofilter with an air flow of 4 L min^{-1}; a 200 L pump sump; and a 500 L cylindroconical rearing tank with a metal grid preventing fish from assessing the bottom cone, which contained a 0.8 L waste collector/settling column (Fig. 1). Two DC Runner 5.2 pumps (Aqua Medic GmbH, Bissendorf, Germany) in the pump sump pumped approximately 1500 L h^{-1} to the biofilter and 2000 L h^{-1} to the rearing tank, corresponding to a retention time in the rearing tank of approximately 15 min.

In order to test the effects of FF and O_3, six systems were fitted with foam fractionators (Sander Fresh Skim 200, Erwin Sander Elektroapparatebau GmbH, Germany), three systems were fitted with 1.8 m high bubble columns (same height as the FF) where O_3 was injected and the remaining three systems were kept standard as control systems. Three of the systems fitted with FF were supplied with O_3 as well (injected in the skimmer), while the remaining 3 systems were feed only air to test the effects of FF alone. Three ozone generators (Ozonizer S 500, Erwin Sander Elektroapparatebau GmbH, Germany) were used to supply O_3. Each ozoniser supplied a system fitted with a bubble column and a system fitted with a FF.

Foam fractionators were operated with a water flow rate of 1500 L h^{-1} and an air flow rate of either 1320 L h^{-1} (air alone) or 1200 L h^{-1} (air) plus 120 L h^{-1} ozonized air. Bubble columns were supplied with 120 L h^{-1} ozonized air. Hydraulic retention time within FF and bubble columns was kept equal to ensure equal contact time in both systems. All gas intakes were controlled by flow metres (Key Instruments Variable area flow metre, Key Instruments, USA). Ozone was injected at a dosage of 20 g O_3 kg^{-1} feed per day (83 mg O_3 h^{-1}). Incoming O_3 gas concentrations were measured using a UV spectrophotometer (at 254 nm) and flow through cell as described in Hansen et al. (2010). Furthermore, to estimate the amount of O_3 that reacted in the water, O_3 gas concentrations leaving the foam fractionators and bubble columns outflow air

were measured at regular intervals.

Each system was stocked with 8.05 ± 0.03 kg juvenile rainbow trout (*Oncorhynchus mykiss*) of approximately 200 g each. The fish were fed a fixed amount of 100 g d^{-1} (Efico E 920, Biomar, Denmark), and 60 L of water was replaced each day, resulting in a feed loading of 1.66 kg feed m^{-3}. Oxygen levels were controlled using an OxyGuard Pacific system (OxyGuard International A/S, Denmark) and ranged between 85% and 90% saturation throughout the trial. Sodium bicarbonate was added when needed to keep pH between 7.0 and 7.3. Primary solids were collected in settling columns at the bottom of the tanks. Each day, the conical part of the tanks were cleaned using magnetic cleaners (Tunze care magnet, TUNZE® Aquarientechnik GmbH, Germany) and the settling columns were emptied.

The trial lasted eight weeks and samples were obtained once a week. All 12 RAS had been operated under similar conditions without foam fractionators or ozone for 13 weeks prior to the trial, feed 60 g daily and all biofilters were fully operational. Feeding was increased from 60 to 100 g 3 days prior to the start of the trial, and fish biomasses were weighed at the start and by the end of the trial.

2.2. Water sampling and analysis

Water samples were collected on day 0 prior to starting the foam fractionators and ozonisers. All water samples were collected in the morning before any daily routines. A 5 L water sample was collected from the sump of each RAS and spilt into homogeneous subsamples for individual analysis. pH was measured daily in the sump before daily routines using a Hach HQ40d Portable Multi Meter (Hach Lange, USA), and temperature was logged automatically by the OxyGuard Pacific system (OxyGuard International A/S, Denmark).

Particles between 1 and 168 μm were measured using a Multisizer 4e Coulter Counter (Bechman Coulter, Inc, Indianapolis, USA) with both a 50 μm and 280 μm aperture. Particles were grouped in size classes as described by Patterson et al. (1999). Total particle numbers (PN), total particle volume (PV) and total particle surface area (PSA) for the full range measured (1–168 μm) was calculated by summing the contribution from the different size classes.

To compare systems, particle size distributions were summarised by the β value as described by Patterson et al. (1999). In short, β value is the slope of the log-log transformed relationship between number of particles within size classes and the corresponding size class median diameter. A low β value indicates a system dominated by larger particles whereas a high β value indicates a system dominated by smaller particles.

Turbidity was measured using a Hach 2100Q (Hach Lange, USA), while UVT was measured using a UV spectrophotometer (Beckman DU® 530 Life Science UV/Vis Spectrophotometer, Bechman Coulter Inc, Indianapolis, USA) measuring % transmission in quartz cuvettes at 254 nm. Microbial activity in the water was quantified using a hydrogen peroxide (H_2O_2) decomposition rate assay described in Pedersen et al. (2019), considering the degradation rate constant (k, h^{-1}) as an expression of microbial activity. In short, a 42 ml water sample was placed in a 50 ml centrifuge tube and H_2O_2 was added at a final concentration of 10 mg L^{-1}. The decomposition of H_2O_2 was subsequently measured by collecting samples before addition of H_2O_2 (background level), immediately after H_2O_2 addition and every 15 min thereafter for 1 h. The samples were kept in a water bath at 22 °C for the duration of the assay. The degradation rate constant (k, h^{-1}) was calculated using the data obtained. Additionally, microbial activity was measured using the BactiQuant (Mycometer A/S, Denmark) assay, expressing microbial activity as relative BQ values.

Organic matter was measured as the 5-days biological oxygen demand (BOD_5) and chemical oxygen demand (COD). Both metrics were measured in raw, non-filtered ($BOD_{5\text{-}Tot}$ and COD_{Tot}) and filtered ($BOD_{5\text{-}Diss}$ and COD_{Diss}) water samples using 0.45 μm filters (Advantec® membrane filter, Toyo Roshi Kaisha Ltd, Japan). Corresponding particulate fractions ($BOD_{5\text{-}Part}$ and COD_{Part}) were calculated as the difference between the non-filtered and the filtered sample. BOD_5 was measured following ISO 5815 (1989) modified by adding allylthiourea (ATU) (Fluka Chemika), while COD was measured following ISO 6060 (1989). Nitrate-N, nitrite-N and ammonium-N where measured by spectrophotometry following ISO 7890–1 (1986), DS 223 (1991) and DS 224 (1975), respectively.

Eight bio-elements from each biofilter were collected weekly and placed dry in 50 ml test tubes that were stored at −20 °C prior to COD analysis. To detach the organic matter, 20 ml Milli-Q water was added to each test tube and the tubes sonicated for 10 min using a Bransonic® ultrasonic cleaner (Branson Ultrasonics Corp, USA). The resulting water was transferred to a beaker and analysed for COD_{Tot} as described above. Ozone concentrations in the water were measured using the colorimetric N,N-diethyl-p-phenylenediamine (DPD) method (Buchan et al., 2005; Schroeder et al., 2015) and the indigo method (Ozone AccuVac® Ampules, Hach Lange, USA).

2.3. Data analysis

All data are presented as average ± standard deviation. Statistical analyses were performed in SigmaPlot 13.0 (Systat software Inc., USA). Results of the two main factors (i.e., foam fractionation and ozonation) were compared using data from the last three trial weeks (n = 9) to account for system weekly variability. Data were tested for normality (Shapiro-Wilk test) and equal variance (Brown-Forsythe). Data that did not meet these requirements were log transformed. A two-way ANOVA analysis followed by a Holm-Sidak analysis was conducted in case of significant main effects. Differences were considered significant at $p < 0.05$. As BactiQuant and $BOD_{5\text{-}Diss}$ results did not meet the equal variance assumption either before or after conversion they were not subjected to two-way ANOVA analysis. Removal percentages were calculated relative to the control treatment based on averages of the last three trial weeks as: $\%\ \ removal = \frac{Treatment}{Control} * 100$.

3. Results

One fish died during the trial, and no significant differences were found in biomass growth rates or feed conversion rates (data not shown). Oxygen saturation ranged between 85% and 90%, pH between 7.0 and 7.3, and temperature between 17 and 21 °C throughout the trial due to lack of cooling. There were no observed negative effects of the temperature on the fish, and although undesired, it is not uncommon to reach such high temperatures in Danish commercial facilities during summer. There were no differences in ammonium and nitrate levels by the end of the trial, while nitrite was significantly lower in systems fitted with foam fractionators (Table 1).

3.1. Micro particles

Micro particle numbers declined in the first half of the trial, including control systems (Fig. 2a). Systems treated with ozone displayed rapid declines within the first week (over 80% reduction in numbers) and remained stable at a low level until the end of the trial. Systems fitted with foam fractionators showed a much slower reduction in numbers, resulting in a final reduction of 58% compared to the control. Both factors combined resulted in significantly lower particle numbers in the water.

Particle volumes increased in the control systems and in systems with ozone only, during the trial, albeit at different rates (Fig. 2b). Systems fitted with foam fractionators declined in the start and remained stable at low levels. By the end of the trial, both the use of O_3 and FF had led to significant reductions in particle volume compared to the control. Ozonisers alone resulted in a 32% reduction, foam fractionators reduced particle volume by 62% and the combination of both treatments resulted

Table 1
Average water and biofilter results of the 3 last weeks of sampling (±standard deviation). * indicates statistical significant effects of the main factors (FF and O_3), while [a] indicates interactions between main factors.

Treatment	Control	Foam fractionator	Ozone	Foam fractionator + Ozone	Units
Num. Particles	2.43 ± 1.38	1.01 ± 1.01*	0.42 ± 0.22*	0.27 ± 0.14	million ml^{-1}
Vol. Particles	0.037 ± 0.012	0.014 ± 0.003*	0.025 ± 0.006*	0.009 ± 0.002	mm^3 ml^{-1}
S. A. particles	30.39 ± 8.77	14.32 ± 5.75*	9.84 ± 2.52*	5.23 ± 1.95	mm^2 ml^{-1}
β value	3.74 ± 0.24	3.77 ± 0.28	3.20 ± 0.22*	3.28 ± 0.26	dimensionless
Turbidity	7.02 ± 2.56	2.46 ± 0.83*	4.34 ± 1.07*	1.49 ± 0.43	NTU
UVT	51.72 ± 2.59	59.37 ± 2.01[a]	73.75 ± 4.48[a]	75.94 ± 1.36	% transmission
H_2O_2	0.84 ± 0.24	0.33 ± 0.17*	0.44 ± 027*	0.08 ± 0.03	k^{-1}
Bactiquant	77011 ± 32480	35779 ± 24185	65674 ± 30563	17110 ± 6172	BQV
BOD_{5Total}	6.09 ± 1.05	2.99 ± 0.89*	3.45 ± 0.55*	1.53 ± 024	mg O_2 l^{-1}
$BOD_{5Dissol}$	0.82 ± 0.13	0.67 ± 0.10	1.01 ± 0.33	0.67 ± 0.04	mg l^{-1}
BOD_{5Part}	5.27 ± 0.98	2.33 ± 0.88*	2.44 ± 0.69*	0.86 ± 0.023	mg l^{-1}
COD_{Total}	37.64 ± 5.86	22.84 ± 2.70*	25.21 ± 2.90*	16.01 ± 1.49	mg l^{-1}
COD_{Dissol}	21.36 ± 1.71	17.84 ± 1.01*	14.83 ± 1.05*	12.78 ± 0.78	mg l^{-1}
COD_{Part}	16.29 ± 4.74	5.00 ± 2.91*	10.39 ± 2.93*	3.23 ± 1.94	mg l^{-1}
Ammonium	74.7 ± 30.0	83.8 ± 17.9	88.5 ± 36.7	82.9 ± 11.6	µg NH_4-N l^{-1}
Nitrite	119.3 ± 24.5	77.5 ± 20.6*	104.0 ± 24.3	70.5 ± 24.26	µg NO_2-N l^{-1}
Nitrate	57.5 ± 2.57	56.7 ± 2.70	57.4 ± 2.33	56.6 ± 2.65	mg NO_3-N l^{-1}
Biofilter COD	9.3 ± 2.2	7.2 ± 2.4	7.5 ± 1.9	7.2 ± 1.0	g

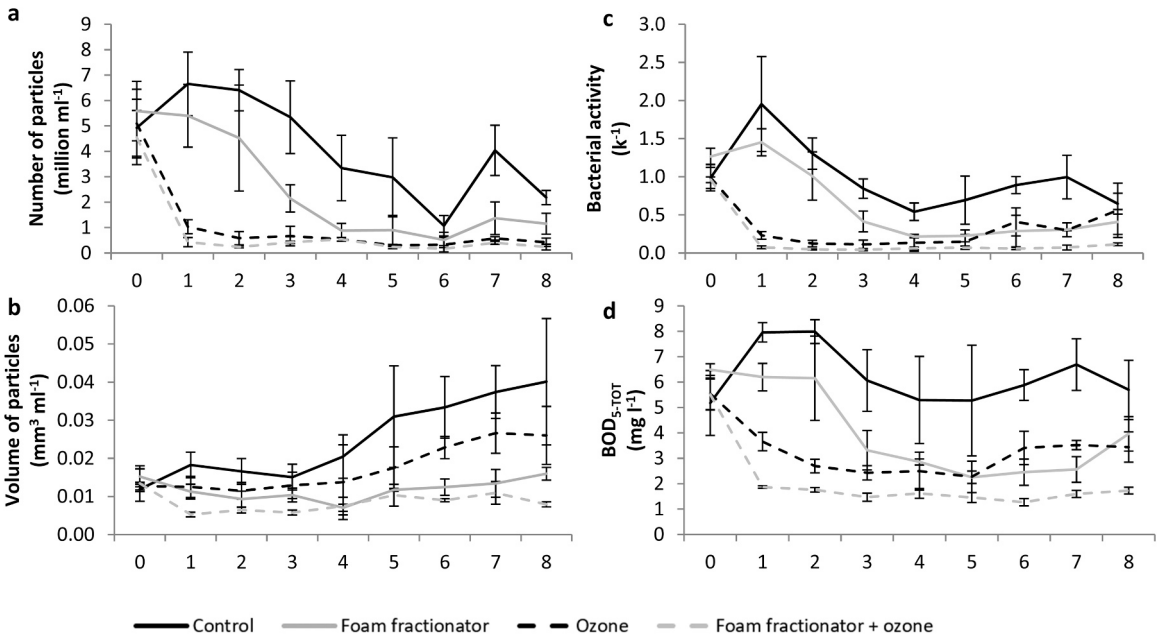

Fig. 2. Variation in selected water quality parameters during the trial. a) Number of particles b) volume of particles c) microbial activity (H_2O_2 degradation) d) BOD_{5TOTAL}. Statistically significant effects are reported in Table 2.

in a 75% reduction compared to the control.

As with the previous two metrics, particle surface area was also affected by the two treatments, while it remained stable at 30.4 ± 8.8 mm^2 ml^{-1} in the control group (Table 2). Foam fractionation resulted in a 53% reduction of total surface area, O_3 treatment in a 68% reduction and a combination of both treatments resulted in a 83% reduction of particle surface area compared to the control with all results being significant.

β values were only affected by the use of ozone. Control systems and systems with foam fractionators had similar β values by the end of the trial (3.74 and 3.77 respectively), while systems treated with O_3 displayed significantly lower β values (3.17 and 3.24 for O_3 and FF+O_3 treatments, respectively).

3.2. Microbial activity

Bacterial activity, measured with the H_2O_2 degradation rate assay, was significantly affected by the two treatment methods (Fig. 2c). Activity declined particularly rapidly in systems treated with ozone, with activity after one week being reduced by 91% in systems with ozonisers only and 96% in systems with FF+O_3 treatments compared to the control. However, activity in systems treated with ozone only appeared to increase again and by the end of the trial were 48% lower than the control. Bacterial activity in systems with foam fractionators was reduced by 61%, while activity in systems with both ozonisers and foam fractionators remained low (90% reduction) compared to the control. Bacterial activity measured using the BactiQuant assay closely followed the H_2O_2 degradation rate constants except that bacterial activity in O_3 treated systems was almost similar to the control by the end of the trial (Table 1). Due to the lack of equal variance of the BactiQuant values, data were not subjected to a statistical analysis.

3.3. Turbidity and UV transmittance (UVT)

Turbidity was significantly improved by both foam fractionation and ozonation (Table 2). By the end of the trial, a 65% improvement in

Table 2
Statistical results of the two-way analysis of variance (ANOVA).

Treatment	Within FF		Within O_3		Interactions	
	F	P	F	P	F	P
Num. Particles	8.25	0.007	41.2	<0.001	0.8	3.660
Vol. Particles	118.5	<0.001	19.1	<0.001	0.03	0.867
S. A. particles	34.4	<0.001	72.2	<0.001	0.2	0.625
β value	0.4	0.524	33.1	<0.001	0.06	0.803
Turbidity	89.5	<0.001	17.3	<0.001	0.03	0.875
UVT	23.9	<0.001	367.8	<0.001	7.4	0.011
H_2O_2	37.0	<0.001	21.4	<0.001	1189	0.284
Bactiquant[a]	–	–	–	–	–	–
$BOD_{5\text{-Tot}}$	107.1	<0.001	65.0	<0.001	0.2	0.635
$BOD_{5\text{-Diss}}$[a]	–	–	–	–	–	–
$BOD_{5\text{-Part}}$	77.8	<0.001	56.8	<0.001	0.5	0.471
COD_{Tot}	114.1	<0.001	71.5	<0.001	0.2	0.630
COD_{Diss}	44.2	<0.001	202.9	<0.001	0.37	0.545
COD_{Part}	60.4	<0.001	10.4	0.003	3.0	0.091
Ammonium	1.7	0.203	0.12	0.731	1.9	0.173
Nitrite	39.1	<0.001	1.4	0.251	0.01	0.911
Nitrate	0.8	0.387	0.03	0.864	0.0007	0.980
Biofilter COD	0.06	3.832	1.1	0.297	1.2	0.286

[a] Statistical analysis not possible due to non-equal variance

turbidity was achieved by foam fractionation compared to the control and 79% when combining both treatments. Ozonation by itself resulted in a 38% improvement by the end compared to the control group. However, as for bacteria activity, turbidity appeared to increase after an initial drop when applying ozone by itself.

Foam fractionation without ozone resulted in a 15% improvement in UVT, while direct ozone or in combination with foam fractionation resulted in 43% and 47% improvement, respectively. Ultraviolet transmittance was the only measurement where there was interaction between treatments (Table 2), and it was therefore not possible to conclude about main effects.

3.4. BOD_5

Total BOD_5 was significantly affected by both foam fractionation and ozonation resulting in reductions of 51%, 43% and 75% for FF, O_3 and FF+O_3, respectively compared to the control (Fig. 2d). The development in $BOD_{5\text{-Part}}$ was similar with that of $BOD_{5\text{-Tot}}$ for all treatment combinations (Table 2). By the end of the trial, foam fractionation alone and direct ozonation had led to similar reductions in $BOD_{5\text{-Part}}$ compared to control of 56% and 54%, respectively, while a combination of the two resulted in an 84% reduction. In contrast to total and particulate BOD_5, the different treatments seemed to have little effect on $BOD_{5\text{-Diss}}$, (Table 2). Lack of equal variance, however, meant that no statistical analysis was performed.

3.5. COD – Water and biofilter

COD was only measured in the last 3 weeks to access final values, so no considerations are made regarding trends.

COD_{Tot} was significantly affected by both foam fractionation and ozonation with a combination of the two resulting in the largest decrease compared to the control (58% reduction). Foam fractionation and ozonation by themselves resulted in similar reductions of 39% and 33%, respectively. Both treatment types affected COD_{Part} significantly, with reductions of 69%, 36% and 80%, respectively in systems with either foam fractionation, ozonation or a combination of the two (Table 2). Dissolved COD was also significantly affected by the different treatments. As with every other metric, the combination of foam fractionation and ozonation had the largest effect reducing COD_{Diss} by 40%. Foam fractionation by itself reduced COD_{Diss} by 16%, while ozonation reduced it by 31%.

Although all treatments seemingly lowered COD_{Tot} levels in the biofilters compared to the control group, there were no significant differences ($p > 0.05$) by the end of the trial in total COD in the biofilter elements (approximately 17% lower value in systems with ozonation only, and 23% lower values in systems with foam fractionation).

4. Discussion

The different treatments had clear visual effects on the water colour and transparency as seen in Fig. 3. The systems fitted with ozone lost most of the "yellow" colour, while the overall turbidity was reduced in system fitted with FF. The loss of yellow colour was likely caused by oxidation of humic substances as seen in previous studies (Davidson et al., 2011; Schroeder et al., 2011; Spiliotopoulou et al., 2018).

The systems were operated for 13 weeks prior to the start of the trial with a lower feed loading (1 kg m^{-3}). This was changed a few days prior to the start of the trial when daily feed allocation was increased from 60 to 100 g d^{-1}. It is likely that this change resulted in the increase of some of the metrics, which could explain some of the initial variation in the control group (initial increase in numbers followed by a re-stabilisation).

4.1. Foam fractionation

Foam fractionation has been shown to reduce organic matter loads in RAS (Barrut et al., 2013; Brambilla et al., 2008; Ji et al., 2020; Weeks et al., 1992). Most of the previous studies were conducted in saltwater as foam fractionation is anticipated to have minimal effect in freshwater RAS due to lower surface tension (Timmons and Ebeling, 2010). However, the current trial showed that foam fractionation also works well in freshwater with positive effects on all measured metrics. The positive impact of foam fractionation appeared to manifest at a slower pace than that of direct ozonation, with a steady removal of organic matter over the course of 3–4 weeks (Fig. 2). The foam fractionator seemed particularly effective at controlling particulate organic loads and particle volume (both $BOD_{5\text{-Part}}$ and COD_{Part}). These results are similar to those obtained by Barrut et al. (2013) using a vacuum airlift foam fractionator in seawater RAS and obtaining an approximate 80% removal of particulate organic matter measured as dry matter. Brambilla et al. (2008), testing foam fractionation for removing organic matter and heterotrophic bacteria from seawater RAS with seabass (*Dicentrarchus labrax*), obtained removal rates of total suspend solids (TSS) between 12% and 40% over a single pass. The treatment in that study affected both the smallest (0.22–1.22 μm) and largest (>60 μm) size fractions measured. In the current study, a uniform β value suggests that particles of all sizes were affected.

Bacterial activity was also strongly affected by foam fractionation. The approximately 60% reduction obtained in the current trial is similar to that obtained by Brambilla et al. (2008) in a seawater RAS, achieving 55–90% removal depending on operational conditions, using count of viable heterotrophic bacteria in agar plates. Likewise, Rahman et al. (2012) achieved 2.6 times lower bacterial levels compared to a control in seawater hybrid abalone (*Haliotis discus hannai X H. sieboldii*) pilot scale RAS fitted with foam fractionation. In the current study, a lower level of nitrite was found in the systems fitted with foam fractionators. A possible explanation for this could be an improved biofiltration process, resulting from a reduced competition from heterotrophic bacteria caused by lower levels of organic matter present in the system (Zhang et al., 1994).

The simultaneous reduction in both organic matter and bacterial activity observed in the current study suggests a direct removal of bacteria by foam fractionation in freshwater, similarly to that observed in seawater. In addition, the reduction in organic matter reduces a systems overall carrying capacity (Vadstein et al., 1993) making it less prone to potentially harmful bacteria blooms.

Fig. 3. Visible effects of the different treatments on water clarity. From left to right: Ozone, Ozone + foam fractionator, control and finally foam fractionator.

4.2. Ozone

Unlike systems fitted with only foam fractionators, which showed progressive reduction in all metrics in the first half of the trial, systems dosed with ozone showed immediate responses and most metrics reached their lowest levels within the first few weeks. This development was most likely a result of ozone's oxidising effect on bacteria and a resulting self-perpetuating pattern leading to a cumulative improvement in water quality, as corroborated by the rapid decline in bacterial activity and particle numbers compared to the control. Part of the effect was also likely caused by improved solids removal as ozone is known to improve solids removal efficiency. Park et al. (2013) for example found that ozone improved solids removal in a radial flow settler, while Summerfelt et al. (1997) found that ozone improved microscreen filtration. It is likely that ozone had similar effects in the current trial as the reduction in particle volume, $BOD_{5\text{-Part}}$ and COD_{Part} was similar to that observed in previous trials (Good et al., 2011; Park et al., 2013; Rueter and Johnson, 1995; Summerfelt et al., 1997). Examining the effects of ozone by itself in replicated RAS, Davidson et al. (2011) found that ozonation lead to a reduction in BOD_5, total organic carbon (TOC), dissolved organic carbon (DOC), TSS, and heterotrophic bacteria abundance, while UVT increased.

Systems treated with ozonation only displayed an increase over time in most metrics. We speculate that this increase was caused by a too low realised O_3 dose. While a nominal dose of 20 g O_3 kg^{-1} feed was applied, measurements of air leaving the treatment units suggested that an ozone transfer rate to the water of approximately 35% was achieved (in both the bubble columns and FF), corresponding to an actual dose of about 7 g kg^{-1} feed. It is possible that this lower dose allowed bacteria with higher O_3 tolerance to proliferate. The hypothesis is supported by the observed increase in bacterial activity accompanied by a similar increase in particle volume, suggesting that bacteria were aggregating. At the same time, particle numbers did not increase suggesting that free swimming bacteria were removed or eliminated. Bacteria in biofilms and bacteria associated with particles are generally more resistant to disinfection, including O_3, than free living bacteria (Hess-Erga et al., 2008).

One of the issues arising when using ozone in a system is its potential toxicity to the fish (Gonçalves and Gagnon, 2011; Powell and Scolding, 2016; Stiller et al., 2020). This risk seems minimal in the current study as no ozone was detected in the water measured both via the DPD and indigo method. Ozone presumably reacted immediately with the organic matter available as seen in a previous study on the combined use of O_3 and foam fractionators (Guilherme et al., 2020).

4.3. Combined effects

Combining foam fractionation with ozonation lead to additional improvements in the water quality parameters. Ozone is typically applied together with foam fractionation (Attramadal et al., 2012; Park et al., 2011, 2013; Schroeder et al., 2011) as it is an efficient way of transferring ozone. Similarly, ozone improves foam fractionation removal efficiency by degrading complex molecules and improving particle flocculation by formation of smaller bubbles (Li et al., 2009; Rueter and Johnson, 1995). In the current study, ozone primary affected micro particle numbers and UVT presumably by killing free swimming bacteria (resulting in a decline in particle numbers) and oxidising dissolved substances (e.g. humic substances) that would otherwise absorb and refract light. On the other hand, by removing solids foam fractionation led to a reduction in particulate volume, particulate COD and turbidity. Combined, this presumably led to a reduction in system carrying capacity, aggravating the conditions for bacterial growth. Furthermore, the combined use of foam fractionation and ozonation may potentially reduce the risk of unwanted biofilm formation and reduce the consumption of oxygen by heterotrophic bacteria degrading organic matter.

4.4. Effects on biofilters

Few studies have addressed the potential implications of different treatments on biofilters in RAS and their role in storing and releasing organic matter (de Oliveira et al., 2019). As discussed in a previous study (de Jesus Gregersen et al., 2020), a decline in organic matter in the water might be accompanied by translocation of organic matter to the biofilter. To resolve this, the current study examined the organic matter (total COD) associated with biofilter elements. Although not significant, a lower organic matter build-up was observed in all treated systems compared to the control, suggesting that the applied treatments not only improved water quality directly but also overall "system quality". This is further supported by a lower level of nitrite in systems treated with foam

fractionation. A mass balance analysis of the organic matter present in the system (measured as COD_{total}) showed that despite only making up 5% of the total volume of the system and being operated as a moving bed biofilter, the bioelements in the biofilter contained between 23% and 36% of all organic matter present in the system (depending on treatment), reinforcing the need to better understand organic matter processes within biofilters.

5. Summary and future perspectives

The current study provided new knowledge about the effects of foam fractionation and ozone on the water quality in freshwater RAS, and demonstrated the potential benefits on biofiltration by reducing the amount of organic matter in the system. The study demonstrated that using foam fractionation in freshwater RAS may lead to similar reductions in organic matter as that observed in saltwater RAS. Furthermore, the study confirmed the positive effects of ozone on overall RAS water quality. Organic matter removal efficiency from foam fractionation was further improved by simultaneous application of ozone. While ozone is already used in both freshwater and saltwater RAS and foam fractionation is used in saltwater RAS, foam fractionation is to our best knowledge not yet commonly applied in commercial freshwater RAS. As demonstrated here, foam fractionation may have large potentials in freshwater RAS as well, either by itself or in combination with ozone for improving rearing conditions and maintaining high water quality standards. The large reductions in organic matter in the systems, accompanied by a reduced level of bacterial activity and an apparent increase in biofilter nitrification efficiency, can lead to a decrease in the use of disinfectants as well as an improvement in overall production quality. To resolve the most optimal use of foam fractionation in freshwater RAS and make specific recommendations to the industry on best application of the technology, supplementary studies of fish performance are needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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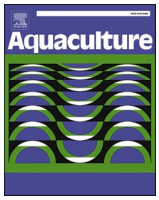

Microbiome response to foam fractionation and ozonation in RAS

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ABSTRACT

Efficient water treatment is required to maintain high water quality and control microbial growth in recirculating aquaculture systems (RAS). Here, we examined the effects of two treatment methods, ozonation and foam fractionation, separately and combined, on the microbiology in twelve identical experimental RAS with rainbow trout (*Oncorhyncus mykiss*) during 8 weeks. Microbes suspended in water and growing in biofilter biofilms were examined using flow cytometry analysis and high throughput sequencing of the 16S rRNA gene. The results showed that foam fractionation did not cause large changes in abundance or overall community composition of free-living microbes. Instead, through decreasing the organic matter availability in water, it targeted specific microbial taxa, leading to e.g. decreased potential for off-flavor production. In contrast, ozonation was found to have a profound impact on the system microbiology, by reducing the overall cell abundance, increasing microbial dead/live ratio, and changing the community composition of both free-living and biofilm microbes. Ozonation increased the abundance of certain key microbial taxa adapted to low carbon conditions, which might form a stable and more abundant community under a prolonged ozone dosing. Combining the two treatment methods did not provide any additional benefits as compared to ozonation solely, corroborating the high disinfection potential of ozone. However, ozone had only a minor impact on biofilter microbial communities, which were, in general, more resistant to water treatment than water communities. Water treatment had no effect on the overall genetic nitrification potential in the biofilter biofilms. However, foam fractionation led to changes in the nitrifying microbial community in biofilter, increasing the abundance of *Nitrospira* conducting complete ammonia oxidation to nitrate (comammox). Altogether, the results obtained indicate that although these two water treatment methods have similar outcomes on physico-chemical water quality and microbial activity, their underlying mechanisms are different, potentially leading to different outcomes under the long-term application.

1. Introduction

The concept of recirculating aquaculture system (RAS) is based on high water recirculation rate (Martins et al., 2010). A central treatment unit is a biofilter, where nitrifying microbes maintain good water quality for fish by converting toxic ammonium into less harmful nitrate (Hagopian and Riley, 1998). Biofilters host a diverse microbial community (Hüpeden et al., 2020; Schreier et al., 2010), including a high amount of heterotrophic microbes degrading organic matter. Furthermore, the presence of microbes in RAS is not limited to the biofilter, but they inhabit all RAS compartments, floating as flocs or free-living cells in the water phase or forming biofilms on the surfaces e.g. tank walls and pipes (Bartelme et al., 2019). Although a majority of these microbes is harmless or even beneficial for maintaining stable water quality conditions, RAS microbial communities can also involve harmful microbes, such as opportunistic pathogens, hydrogen sulfide or off-flavor producers (e.g. Fossmark et al., 2020; Lukassen et al., 2017). In high intensity RAS with high levels of feed loading and long retention times, high organic matter concentrations in the system promote the abundance and activity of heterotrophic microbes (e.g. Michaud et al., 2006). This can increase the need for aeration and degassing and associated operational costs of the system, as heterotrophic microbes consume high amounts of oxygen and release CO_2.

To maintain sufficient system water quality as well as to hinder

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blooms of potential harmful microbes (e.g. Moestrup et al., 2014), water treatment methods to remove microbes and other organic matter are currently searched and developed for RAS. Two potential water treatment methods for this are foam fractionation and ozonation. Foam fractionation, often termed as protein skimming, is based surface-active particles (e.g. organic matter) adsorbing to the surface of fine air bubbles injected to water, generating foam that is then removed (Timmons and Ebeling, 2010). Foam fractionators have primarily been applied in marine RAS, where they have been found to remove microbes but also reduce the overall availability of organic matter (Barrut et al., 2013). In marine RAS with abalone, applying foam fractionators led to 2.6 times lower amount of heterotrophic bacteria (Rahman et al., 2012), leading to 7% higher oxygen concentrations in system water. In marine seabass RAS, foam fractionation reduced the abundance of both large (>60 μm) and small (0.22–1.2 μm) particles, but not of the intermediate ones, and reduced the abundance of heterotrophic microbes in water by 32–88% depending on operation time and pre-filtration of water (Brambilla et al., 2008). In addition to targeting microbes and organic matter, foam fractionation can also decrease the concentrations of dissolved inorganic nitrogen (DIN), as 13–35% lower DIN concentrations were recorded after applying foam fractionator in marine abalone RAS, due to increased nitrification activity after decreased abundance of heterotrophic microbes (Rahman et al., 2012).

Ozone oxidizes organic matter, decreasing chemical oxygen demand (COD) of the water in general (Spiliotopoulou et al., 2018), but also destroys and inactivates microbes through damaging cell walls (Ramseier et al., 2011). Ozone has been found to decrease the abundance of heterotrophic bacteria in RAS water (Davidson et al., 2021; Davidson et al., 2011), while bacteria embedded in biofilm or attached to particles are expected to be less susceptible. Furthermore, a high abundance of particles reduces the oxidative effect of ozone on microbes, as ozone is consumed by other particles before attacking microbes (Hess-Erga et al., 2008). This means that the effect of ozone on the overall microbial abundance can be moderate in intensive RAS with high organic loading or when using low ozone dosage, as has been observed in marine larval RAS (Attramadal et al., 2012). However, ozone can cause significant changes in the microbial community. Previously, ozonation has been found to shift the bacterial community growing as biofilms on tank walls from Alphaproteobacteria-dominated to Gammaproteobacteria-dominated, through altering water chemistry (bacterial habitat conditions), and oxidizing complex organic molecules into more bioavailable forms (Wietz et al., 2009). When selecting for certain microbial taxa, ozonation can also open niches for potentially harmful opportunistic microbes (Dahle et al., 2020). Furthermore, in seawater, a moderate ozone dosage (≤0.15 mg/L OPO) has been found to have either no effect or even to slightly improve biofilter nitrification performance through removing organic matter and heterotrophic bacteria commonly present in the biofilter and/or by indirect liberation of oxygen (Schroeder et al., 2015). However, a detailed knowledge on the response of microbial communities in both water and biofilms to these two water treatment methods is still lacking.

In this study, we examined the effect of foam fractionation and/or ozonation on microbial communities in RAS water and biofilter biofilms in replicated freshwater RAS with rainbow trout. We hypothesized that when applied alone, foam fractionation would affect microbial abundance in water and potentially change the microbial community composition through reduced organic matter concentrations. Furthermore, ozonation alone or together with foam fractionation was expected to have a more profound effect on the microbial community composition than foam fractionation alone.

2. Materials and methods

2.1. Experimental setup

The experiment was conducted in 12 replicated, 0.8 m^3 pilot-scale freshwater RAS (Suppl. Fig. 1) at DTU Aqua in Hirtshals, Denmark. Each system was stocked with 8.05 ± 0.03 kg juvenile rainbow trout (*Oncorhynchus mykiss*). For 13 weeks before the trial, all 12 RAS were acclimatized by daily feeding of 60 g d^{-1} Efico E 920 (Biomar, Denmark), which was increased to the final feed amount 100 g d^{-1} three days before the trial started, the final feed loading being 1.66 kg feed m^{-3} make-up water. Each RAS had a 100 L biofilter filled with 40 L of new RK BioElements (injection-molded polypropylene with surface specific area of 750 m^2/m^3; Dania Plast, Skive, Denmark) operated as a moving bed biofilter with an airflow of 4 L min^{-1}. All biofilters were fully operational after the pre-acclimatization period. After taking week 0 samples, four treatments were applied in triplicate: 1) three control RAS, 2) three RAS with foam fractionator (ff), 3) three RAS with ozone (oz), 4) and three RAS with ozone and foam fractionator (oz + ff). Foam fractionators were operated with a water flow rate of 1500 L h^{-1} and an airflow rate of either 1320 L h^{-1} (ff) or 1200 L h^{-1} plus 120 L h^{-1} of ozonized air (oz + ff). Bubble columns were supplied with 120 L h^{-1} ozonized air (oz). Ozone was injected at a dosage of 20 g O_3 kg^{-1} feed, the estimated true dosage applied being appr. 7 g O_3 kg^{-1} feed, which can be considered as a low dosage level. The trial lasted eight weeks. Temperature in the system ranged between 17 and 21 °C (Table 1), due to the lack of cooling in the experimental facility. Despite being a high temperature, it is commonly achieved on commercial trout farms during summer and no negative impacts were seen on the fish during the trial.

2.2. Sampling and water quality conditions

The water quality characteristics during the last three experimental weeks are described in Table 1, and the details for sampling are given in de Jesus Gregersen et al. (2021). For microbial abundance measurements, water was collected weekly from the sump of each RAS. For the microbial community analysis, sump water was collected using syringe filters (0.22 μm Millipore Express® PLUS PES membrane) before feeding at the beginning of the experiment (week 0) and at weeks 1, 3, and 7. In addition, at week 7, eight bioelements from each MBBR were collected and microbial biofilm was detached from them by sonication of 4 min (Branson 1510). Microbiological samples were stored at −20 °C before DNA extraction.

2.3. Microbial abundance using flow cytometry

Immediately after the sampling, 10 mL of water from each system was prefiltered through a cell strainer (40 μm FisherBrand, Thermo Fisher Scientific), and 500 μL of filtrate was labelled with 5 μL of SYBR Green (100×, MilliporeSigma, Germany) and 5 μL of propidium iodide (PI, 600 μM, MilliporeSigma, Germany) for incubating at 37 °C for 10 min, after which the total abundance of cells (cells mL^{-1}) and the proportion of dead cells (%Dead) was measured with BD Accuri C6 Plus flow cytometer (Becton, Dickinson and Company, NJ, US).

2.4. Microbial community composition

DNA was extracted using the DNeasy PowerLyzer™PowerSoil DNA Isolation Kit (Qiagen, Germany) from water and biofilm samples, and the DNA quantity was measured with Qubit™ dsDNA HS assay and Qubit 2.0 Fluorometer (Thermo Fischer Scientific). Microbial community composition was studied using Ion Torrent PGM next-generation sequencing targeting the V4 region of the 16S rRNA gene with primers 515F—Y (Parada et al., 2016) and 806R (Caporaso et al., 2011). The analysis of gene sequences was done using mothur (version 1.44.3; Schloss et al., 2009) to remove sequences shorter than 200 bp, low-quality sequences, barcodes and primer sequences. The sequences were aligned using Silva reference alignment (Release 132), chimeric sequences were identified and removed (Edgar et al., 2011), and a preclustering algorithm was used to reduce the effect of sequencing errors (Huse et al., 2010). Sequences were assigned to taxonomies with a

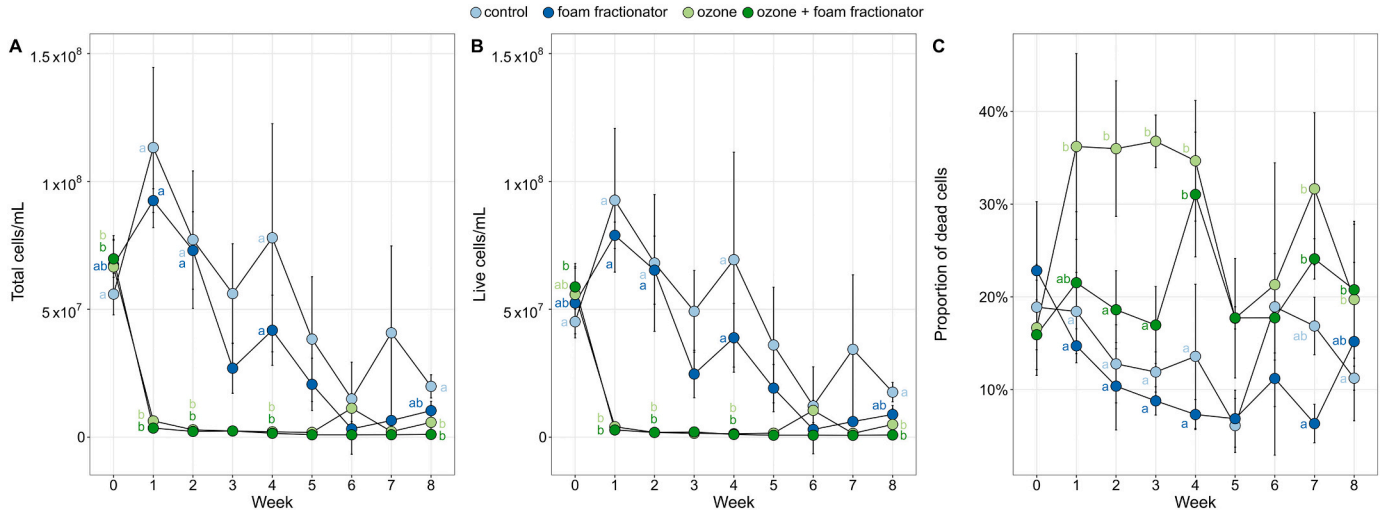

Fig. 1. The abundance of A) total and B) live cells (cells/mL), and C) the proportion of dead cells in four triplicate treatments during 8 weeks experiment. Values are reported as mean ± SD (n = 3). The letters denote for post-hoc test results within sampling time.

Table 1
Water quality characteristics in the pilot-scale recirculating aquaculture system units with either foam fractionator, ozone, ozone + foam fractionator, or unexposed control. Values are given as mean ± SD over experimental weeks 6–8 (n = 9/treatment). Modified from de Jesus Gregersen et al. (2021).

	Control			Foam fractionator			Ozone			Ozone + Foam fractionator		
Particle abundance (10^6/mL)	2.43	±	1.38	1.01	±	1.01	0.42	±	0.22	0.27	±	0.14
Turbidity (NTU)	7.02	±	2.56	2.46	±	0.83	4.34	±	1.07	1.49	±	0.43
Microbial activity k (h^{-1})[1]	0.84	±	0.24	0.33	±	0.17	0.44	±	0.27	0.08	±	0.03
BOD5 (mg O_2/L)[2]	6.09	±	1.05	2.99	±	0.89	3.45	±	0.55	1.53	±	0.24
COD (mg O_2/L	37.6	±	5.86	22.8	±	2.70	25.2	±	2.90	16.0	±	1.49
TAN (μg NH_4-N/L)[3]	74.7	±	30.0	83.8	±	17.9	88.5	±	36.7	82.9	±	11.6
Nitrite (μg NO_2-N/L)	119	±	24.5	77.5	±	20.6	104	±	24.3	70.5	±	24.3
Nitrate (mg NO_3-N/L)	57.5	±	2.57	56.7	±	2.70	57.4	±	2.33	56.6	±	2.65

[1] see Pedersen et al. (2019).
[2] BOD5 = 5 day Biological Oxygen Demand.
[3] TAN = Total Ammonia Nitrogen.

naïve Bayesian classifier (bootstrap cutoff = 80%) (Wang et al., 2007), using the Silva 132 database, and sequences classified as chloroplast, mitochondria, and eukaryota were removed. Sequences were divided into operational taxonomic units (OTUs) at a 97% similarity level, and singleton OTUs were removed. The total amount of sequences obtained was 4,531,155. For calculating alpha and beta diversities, each sample was subsampled to 25,772 sequences. To identify OTUs *Nitrospira*, we analyzed these OTU sequences using MiDAS 4.8.1 taxonomic database (Dueholm et al., 2021) and separated them into strictly nitrite-oxidizers and comammox Nitrospira (Pinto et al., 2016). Sequences have been submitted to NCBI Sequence Read Archive under BioProject PRJNA695118.

2.5. Statistical testing

The data analysis was conducted using R (version 3.6.3; R Core Team, 2020) using packages "vegan" (Oksanen et al., 2019), "phyloseq" (McMurdie and Holmes, 2013), and"ggplot2" (Wickham, 2016). The differences in the abundance of cells (alive cells) and the proportion of dead cells (%Dead) between treatments and weeks were tested with non-parametric Aligned Ranks Transformation ANOVA (ART ANOVA; Wobbrock et al., 2011), since the normality assumptions were not met. The differences in the microbial community composition between treatments were assessed with principal coordinates analysis (PCoA) and PERMANOVA based on Bray-Curtis similarities. The four main OTUs explaining the differences between treatments or between water and biofilm communities were determined with SIMPER function. The differences in the similarities (based on Bray-Curtis dissimilarity), diversity, OTU richness, and the abundance of ammonia/nitrite-oxidizing or off-flavor producing bacteria between treatments and different weeks in water samples, or between treatments and sample types (water, biofilm) in week 7 were tested with two-way ANOVA. The differences in the similarities in time within treatments were tested with one-way ANOVA.

3. Results

3.1. Microbial abundance in water

At the beginning of the experiment, the abundance of total cells ranged from 5.6×10^7 to 7.0×10^7 mL^{-1} and the abundance of live cells ranged from 4.5×10^7 to 5.9×10^7 mL^{-1} with limited variation within and between treatments (Fig. 1A, B). The abundance of both total cells and live cells decreased towards the end of the experiment, also in control RAS units, however, being still significantly affected by the water treatments (ART, Total cells: Treatment × Week, $F_{24,72} = 8.8$, $P < 0.001$, Live cells: Treatment × Week, $F_{24,72} = 8.1$, $P < 0.001$; Fig. 1A, B). Over time, large within-treatment variation was found in the control and foam fractionation treatment groups, while the ozonated units were more similar to each other. In weeks 1, 2, and 4, ozonated units had a significantly lower amount of alive cells than the control and foam fractionator units, and in week 8, control units had a higher amount of alive cells than the ozonated units (post-hoc comparisons). In week 0, the proportion of dead cells ranged from 13 to 28%. Similarly to the live-cell abundance, treatments affected the proportion of dead cells

(Treatment × Week, $F_{24,72} = 3.0$, $P < 0.001$, Fig. 1C), with a significant difference only in a few weeks, as the proportion of dead cells was higher in ozonated units in weeks 2–4, the latter including also units with both ozone and foam fractionator.

3.2. Microbial community composition

The microbial communities suspended in water and growing as biofilms in biofilters (Fig. 2) were significantly different (pseudo-$F_{1,23} = 8.4$, $P = 0.001$). Although the microbial communities evolved over time, water treatment had a significant effect on the microbial community composition both in water samples (PERMANOVA: Treatment × Week, pseudo-$F_{3,35} = 2.18$, $P = 0.002$) and biofilm samples (Treatment, pseudo-$F_{1,11} = 3.0$, $P = 0.001$) (Fig. 2A). In water samples, the communities sampled from ozone-treated units were distinct from the non-ozone-treated units in all sampling times ($P < 0.05$; Fig. 2A), and treatment explained 49–69% of the variation in the community composition. In biofilms, water treatment explained 53% of variation, control unit communities being different from the treatment unit communities. The communities suspended in the water in the ozone-treated units evolved significantly in time, as the similarity with week 0 communities decreased from 33 ± 8% in week 1 to 7 ± 1% in week 7 (Fig. 2B; Supp. Table 1). This was not observed in either control or foam fractionator units, where communities in week 7 were 41 ± 9% similar to the original week 0 communities. When comparing the similarities between treatments within sampling time (Suppl. Fig. 2), control communities were more similar to the communities from foam fractionator units than from ozone-treated units, except in week 3, when foam fractionator communities were dissimilar from the other three treatments (Suppl. Table 2). In biofilm samples, the overall similarity between treatments was higher than in water samples. There, the composition of the foam fractionator communities overlapped with both control and ozone-treated unit communities, while control communities were separated from the ozonated units (Suppl. Fig. 2, Suppl. Table 2). The similarity between water and biofilm community in week 7 was significantly lower in the foam fractionator units (15 ± 2%) than in the other units (Fig. 2A; Suppl. Table 3). Furthermore, the communities in water and biofilm were less similar to each other in control (31 ± 7%) and ozone + foam fractionator units (26 ± 6%) than in ozone units (40 ± 8%; Suppl. Table 3).

In water samples, treatment had a significant effect on the OTU richness and diversity (Fig. 3; Suppl. Table 4), while there was no significant effect of sampling time or interaction between treatment and time. The units with ozonation hosted the highest richness (4468 ± 573) and diversity (4.6 ± 0.4; Suppl. Table 4). When comparing units with ozone to the units with ozone and foam fractionation, the richness was similar (ozone + foam fractionation: 3992 ± 716), but diversity was significantly lower in the latter (ozone + foam fractionation: 3.9 ± 0.7). Furthermore, richness (3043 ± 443) and diversity (3.1 ± 0.2) were lowest in the foam fractionation units. The richness (3537 ± 635) and diversity (3.4 ± 0.4) of the control units were similar to the two latter treatments. In biofilm samples, treatment also affected both richness and diversity (Suppl. Table 4), both being significantly higher in the units with foam fractionator (5387 ± 361, 5.5 ± 0.4) or with ozone and foam fractionator (5351 ± 153, 5.6 ± 0.1) than in control units (4572 ± 409, 5.2 ± 0.1), while ozone units did not differ from the other units (5046 ± 173, 5.4 ± 0.2). Furthermore, when comparing biofilm and water samples taken in week 7, the richness was higher in biofilm than in water in the units with foam fractionator or with ozone + foam fractionator and the diversity was higher in biofilm than in water within all the treatments (Suppl. Table 5).

Throughout the experiment, the most abundant microbial class was Alphaproteobacteria (Fig. 4). When comparing the development of the microbial communities suspended in water in the ozonated units to that of the ones in the non-ozonated units, Actinobacteria disappeared and the relative abundance of Alphaproteobacteria decreased, whereas Bacteroidia and Deltaproteobacteria became more abundant towards the end of the experiment. Furthermore, class Verrucimicrobiae disappeared from the non-ozonated units before week 7. All biofilter biofilm communities had a higher relative abundance of classes Gemmatimonadetes and Nitrospira than the communities suspended in the water (Fig. 4).

Of the main ten OTUs (Table 2) explaining the differences between non-ozonated and ozonated units (46% of difference explained), OTUs assigned to alphaprotebacterial genera *Hyphomicrobium* (OTU1) and *Tabrizicola* (OTU7), gammaproteobacterial *Comamonas* (OTU6), actinobacterial *Aurantimicrobium* (OTU9), *Candidatus* Planktophila (OTU21), and bacteroidial *Lacihabitans* (OTU13) had a higher abundance in the non-ozonated units, whereas deltaproteobacterial *Haliangium* (OTU4), alphaproteobacterial *Gemmobacter* (OTU2), and bacteroidial *Flectobacillus* (OTU5) were more abundant in the ozonated units.

The main ammonia-oxidizing bacterial (AOB) genus in biofilter biofilm samples was *Nitrosomonas* (85 ± 8% of AOB sequences), while *Nitrospira* was the only nitrite-oxidizer (NOB) found (Table 3). The relative abundance of all *Nitrospira* was higher than of AOB, and the

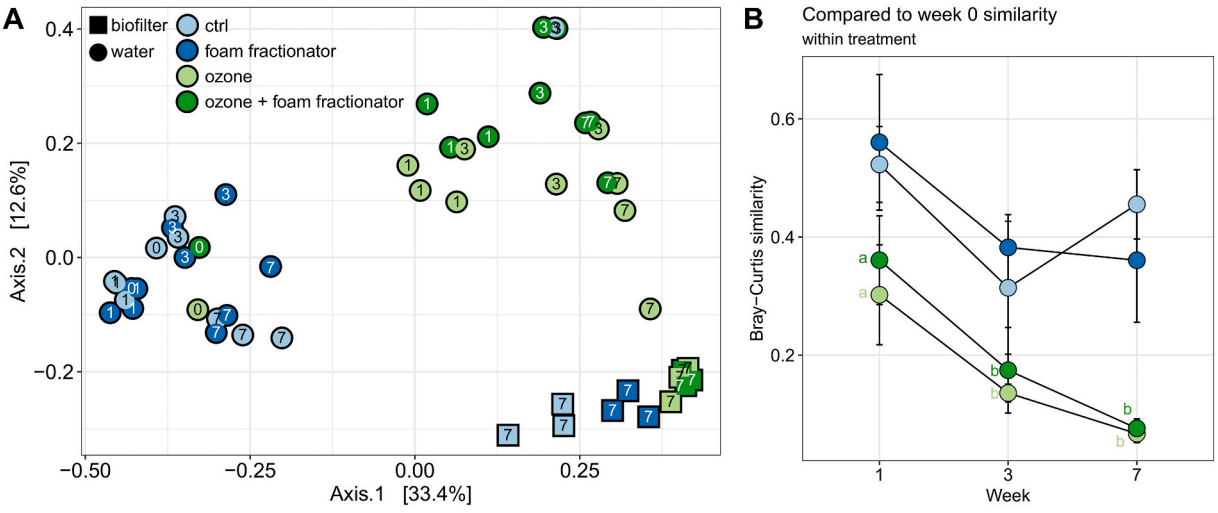

Fig. 2. A) PCoA of water samples based on Bray-Curtis similarities and B) similarities as compared to week 0 communities within treatment. Values are reported as mean ± SD. The letters denote for significant differences in the Tukey post-hoc test results between sampling times.

Table 2
The average relative abundance of the ten main OTUs of 16S rRNA genes differentiating non-ozonated (control, foam fractionator) and ozonated (ozone, ozone + foam fractionator) RAS units, their contribution to differences in the microbial community structures, and the accession number, taxonomy, isolation habitat and proposed physiology of their closest matching organisms represented in the SILVA 132 database.

OTU	Relative abundance		Contribution	Accession number	Identity percentage	Taxonomy (Genus)	Isolation habitat	Physiology
	Non-ozone	Ozone						
OTU1	14.3%	1.8%	7.8%	HM124367.1	98%	*Hyphomicrobium*	Lake sediment	Heterotroph, uses simple carbon compounds (Oren and Xu, 2014)
OTU7	11.1%	0.1%	6.5%	KU360709.1	98%	*Tabrizicola*	Lake water	Heterotroph, some strains are aerobic anoxygenic phototrophs (Tarhriz et al., 2019)
OTU4	1.9%	9.4%	5.4%	CP001804.1	91%	*Haliangium*	Coastal sand	Heterotroph, degrades biomacromolecules, lyse microbial cells (Garcia and Müller, 2014)
OTU6	9.5%	1.6%	5.3%	MT323131.1	99%	*Comamonas*	Rainbow trout	Heterotroph, degrades complex aromatic compounds (Willems, 2014)
OTU2	2.2%	8.0%	4.9%	CP028918.1	99%	*Gemmobacter*	River water	Heterotroph (Chen et al., 2013; Kang et al., 2017a)
OTU5	0.0%	7.1%	4.2%	MK402935.2	99%	*Flectobacillus*	Groundwater	Heterotroph (Sheu et al., 2017)
OTU9	5.9%	0.2%	3.4%	NR_145615.1	99%	*Aurantimicrobium*	River water	Heterotroph, ultra-micro sized (Nakai et al., 2015)
OTU16	5.2%	0.1%	3.1%	NR_136787.1	99%	*Emticicia*	Stream sediment	Heterotroph, abundant in high C:N (Yu et al., 2016)
OTU13	5.1%	0.7%	3.0%	MG780349.1	99%	*Lacihabitans*	Lake sediment	Heterotroph, degrades biomacromolecules (Kang et al., 2017b)
OTU21	4.5%	0.1%	2.7%	CP016773.1	97%	*Candidatus* Planktophila	Freshwater lake	Heterotroph (Neuenschwander et al., 2018)

Table 3
The relative (% of sequences) and absolute abundance (amount of reads) of total ammonia-oxidizing (AOB) bacteria and *Nitrospira,* the absolute abundances of AOB genera, nitrite-oxidizing (NOB) *Nitrospira* and comammox-*Nitrospira*, and the proportion of comammox of all *Nitrospira* reads (mean ± SD) in biofilter biofilms in the four treatments in week 7.The letters denote for significant differences in the Tukey post-hoc test results between treatments

	Control			Foam fractionator			Ozone			Ozone + foam fractionator		
All AOB	0.35%	±	0.13%	0.83%	±	0.17%	0.54%	±	0.31%	0.80%	±	0.22%
	89	±	34	210	±	44	138	±	77	204	±	56
Nitrosomonas	72	±	37	181	±	36	125	±	72	174	±	66
Other Nitrosomonadaceae	5	±	2	7	±	5	4	±	5	6	±	2
Nitrosomonadaceae unclassified	11	±	7	21	±	4	9	±	2	23	±	10
All *Nitrospira*	1.78%	±	0.64%	4.29%	±	0.71%	3.26%	±	1.21%	4.62%	±	1.64%
	452	±	164	1089	±	179	827	±	308	1173	±	416
Strictly NOB *Nitrospira*	357	±	97	652	±	246	585	±	213	646	±	301
Comammox *Nitrospira*	95	±	85^{a}	437	±	90^{bc}	242	±	103^{ab}	527	±	117^{c}
Comammox of all *Nitrospira*	19%	±	$12\%^{a}$	42%	±	$14\%^{ab}$	29%	±	$4\%^{ab}$	46%	±	$6\%^{b}$

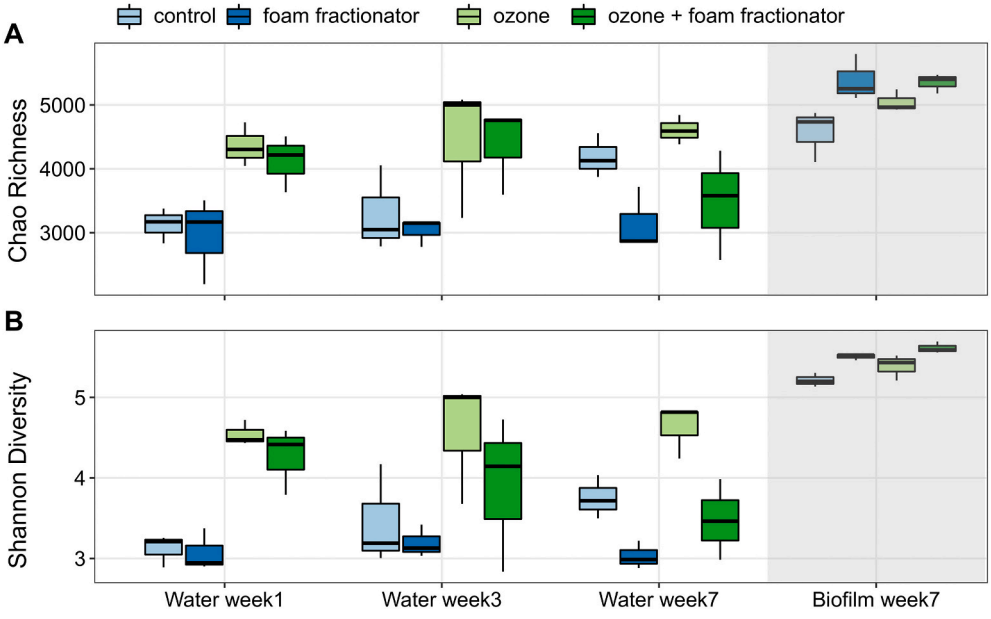

Fig. 3. A) OTU richness (chao) and B) Shannon diversity index in water and biofilm samples in four treatments in weeks 1, 3, and 7.

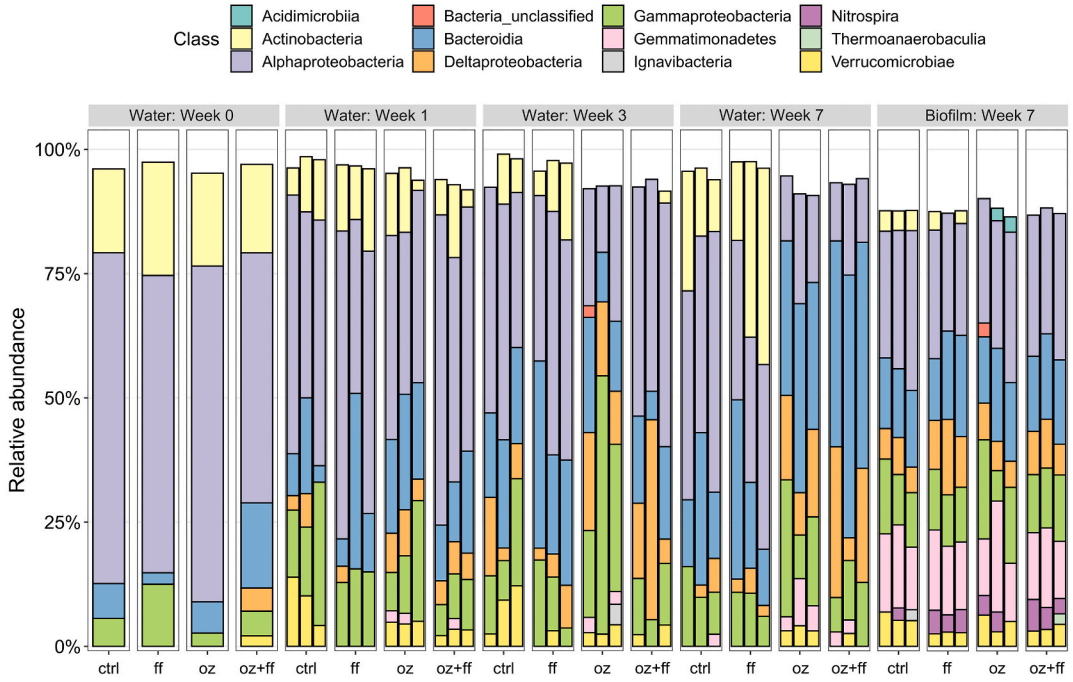

Fig. 4. The relative abundance of microbial classes in water and biofilm samples in four treatments (ctrl = control, ff = foam fractionator, oz. = ozone, oz. + ff = ozone and foam fractionator) in the beginning (week 0), and in weeks 1, 3, and 7. Only classes with an abundance of >1% are included.

relative abundance of both groups seem to be higher, yet statistically insignificant (ANOVA, AOB: $P = 0.08$, NOB: $P = 0.06$), in the units with foam fractionators. When dividing *Nitrospira* into strictly NOB-*Nitrospira* and comammox *Nitrospira*, the absolute abundance of the latter one was significantly higher in the units with foam fractionation and foam fractionation + ozonation (Suppl. Table 6). Furthermore, the relative abundance of comammox *Nitrospira* of all *Nitrospira* sequences was higher in the foam fractionation + ozonation units than in the control units.

The only potential off-flavor (geosmin or MIB) producers found were assigned to deltaproteobacterial genus *Nannocystis* and actinobacterial *Nocardia*. Treatment had a significant effect on the potential geosmin-producer abundance, as units exposed only to ozone had a significantly higher relative abundance of geosmin producers in water (0.37 ± 0.18%) than units without ozonation in all the weeks (control: 0.13 ± 0.26%, foam fractionator: 0.04 ± 0.02%; Suppl. Tables 7, 8). When comparing the relative abundances between water and biofilter in week 7, biofilters hosted a significantly higher relative abundance of potential geosmin-producers (0.69 ± 0.38%) than water samples (0.20 ± 0.24%), independently of the treatment (Suppl. Tables 7, 8).

4. Discussion

In this study, we quantified the separate or combined effects of two water treatment methods, ozonation and foam fractionation, on freshwater RAS microbiology. Each treatment was found to have different effects on and mechanisms to affect water and biofilm microbial communities. Foam fractionation had only a limited effect on the microbial abundance and community composition, while ozone caused dramatic microbiological changes recorded after only one week of the experiment. Even though we demonstrated that biofilter biofilm communities are less vulnerable to the water treatment than the communities suspended in the system water, we saw differences in the relative abundances of key microbial groups in biofilter biofilm between control and different water treatment units.

The water quality data collected from the experiment showed that ozonation and foam fractionation each improved the water quality by decreasing the amount of particles, microbial activity, and biological oxygen demand in water, and these effects were pronounced when these two treatments were combined (de Jesus Gregersen et al., 2021). Foam fractionation, when implemented solely, decreased turbidity and particle volume, but the microbiological results presented here indicate that the effect of foam fractionation on the system microbiology is less substantial and indirect. Based on the flow cytometry data (Fig. 1), foam fractionation did not lower the abundance of free-living microbes as compared to the control units, suggesting that the lower microbial activity observed under foam fractionation is not due to the reduced microbial abundance as such, but rather due to the lower amount of organic matter i.e. substrate being available for heterotrophic microbes. However, we acknowledge that samples were prefiltered to remove particles larger than 40 μm, so it is possible that foam fractionation could still have reduced the amount of large microbial flocs or larger eukaryotic micro-organisms, which are covered in the microbial activity measurements (Pedersen et al., 2019). No information on the effect of foam fractionation on total microbial abundance in RAS exists before this study, but it has previously been found to decrease the abundance of viable heterotrophic microbes (Brambilla et al., 2008; Rahman et al., 2012) and to target both small (microbial cells) and large (organic matter aggregates) particles, but not of medium-sized ones (Brambilla et al., 2008). These previous findings support our conclusion on foam fractionation controlling microbial abundance through decreasing organic matter content. Furthermore, the microbial community composition was not significantly different between the control and foam fractionation units (Fig. 2), while OTU diversity and richness were lower under foam fractionation in week 7 (Fig. 3). This indicates that the organic matter removal through foam fractionation affects the abundance of specific rare microbial taxa, not being visible in the overall abundance patterns, but potentially having a functional significance. For example, the microbial genus with very small cell size, *Aurantimicrobium* (Nakai et al., 2015), was more abundant in foam fractionation units (data not shown), indicating that the communities can adapt to the foam fractionation treatment. Furthermore, the potential geosmin producers, such as genera *Nocardia* and *Nannocystis* (Azaria and van Rijn, 2018), which abundance is known to be connected with the organic matter

availability in RAS (Guttman and van Rijn, 2008), were found to have lower relative abundance in the water of the RAS units with foam fractionation than in the other units. Even low relative abundances (<1%) of off-flavor producers can lead to significant accumulation of the produced compounds in water and fish (Lukassen et al., 2017), so this result on foam fractionation reducing their relative abundance is encouraging and needs further investigation.

Unlike foam fractionation, ozone was observed to have a strong effect on the microbial communities suspended in water already one week after application (Figs. 1-4). Ozone attacks the microbial cells directly, so even the moderate dosing used in this experiment was enough to significantly lower cell abundance and increase microbial mortality. Combining foam fractionation with ozonation did not seem to have an additional effect and the variation in the cell abundance was much lower in ozonated units than in non-ozonated ones (Fig. 1), highlighting the high disinfection efficiency of ozone. Ozonation led to significant changes in the microbial community composition, making them to deviate more and more from the communities in the beginning and from the control communities during the experiment (Figs. 2, 4). After two weeks of applying ozone, the microbial abundance had dropped by 97%, but it started to rise slightly towards the end of the experiment, indicating that the remaining microbial taxa had adapted to tolerate ozone and grow. Indeed, when inspecting the ten main OTUs explaining the differences between non-ozonated and ozonated units, the genera being previously isolated from very clean water with low carbon availability (spring, artificial fountain; *Gemmobacter*, *Flectobacillus;* Chen et al., 2013; Kang et al., 2017) or being capable to produce spores to survive through harsh conditions (*Haliangium;* Garcia and Müller, 2014) were substantially more abundant in ozonated than in non-ozonated (control, foam fractionator) units (Table 2). In contrast, the main taxa that were more abundant in non-ozonated units (control and/or foam fractionator) are known to thrive in carbon-rich conditions (Emticicia; Yu et al., 2016), degrade complex organic molecules (*Comamonas*, *Lacihabitans;* Kang et al., 2017a; Willems, 2014) or inhabit lake environments (*Hyphomicrobium*, *Tabrizicola)* with presumably variable organic matter and nutrient concentrations. Overall, these results suggest that continuing with a moderate ozone dosing could eventually lead to the increase in the microbial abundance with community consisting of the adapted key taxa. Interestingly, the relative abundance of potential geosmin producers was highest in the ozonated units, suggesting them to benefit from the higher abundance of bioavailable molecules. Ozonation has already previously shown to be ineffective in reducing the off-flavor compounds in water or fish flesh (Schrader et al., 2010), so this result indicates the low potential of ozonation in targeted control of off-flavor production. However, the overall cell abundance was very low in ozonated units (17 ± 29% of control cell abundance), so the relative increase may not have a true biological impact.

Our results corroborate the previous findings on biofilms being microbial richness and diversity hotspots (Hüpeden et al., 2020), both values being higher in biofilms than in water communities. Although the major part of the biofilter community consisted of non-nitrifying microbes, both ammonia-oxidizers (AOB) and nitrite-oxidizers (NOB) were found to be present. Nitrite-oxidizing *Nitrospira* was more abundant than any AOB in all the biofilters, as has been previously observed in freshwater and marine RAS biofilter samples (Bartelme et al., 2017; Fossmark et al., 2021; Suurnäkki et al., 2020). When inspecting *Nitrospira* sequences, 19–46% of them were affiliated with comammox Nitrospira, which conducts complete nitrification (Daims et al., 2015), suggesting that the higher abundance of *Nitrospira* than AOBs can be explained by a large proportion of them conducting complete nitrification rather than only nitrite oxidation. Only singleton sequences assigned to ammonia-oxidizing Archaea (AOA; *Candidatus* Nitrocosmicus) were found in two biofilter samples (one from the system with foam fractionator, one from ozone + foam fractionator). Since AOA have also not been found in RAS biofilters in the previous RAS studies (Hüpeden et al., 2020; Keuter et al., 2017; Suurnäkki et al., 2020), they may have low importance for nitrification in the system. Biofilm communities responded differently than water communities to the treatments applied. In general, biofilm communities were more resistant to the water treatment, exhibiting rather high similarity (50–56%) between control and treatment units. Since the effect of foam fractionation on microbes seem to be indirect, and ozone is known to have a weaker effect on particle-attached than free-living microbes (Hess-Erga et al., 2008) and in general, disappear fast when applied to freshwater (Bullock et al., 1997), this outcome could be expected. However, such slight changes in the community composition in all treatment units, also in foam fractionation units, are in contrast with the results obtained in the communities suspended in water. Interestingly, the OTU richness was higher in the biofilter biofilms of the foam fractionator units, which was an opposite trend as compared to water communities. This could be related to organic matter removal decreasing the proportion of heterotrophs and opening more niches for autotrophs e.g. nitrifiers in the biofilms. Indeed, the absolute abundance of comammox *Nitrospira* was higher and relative abundance of comammox *Nitrospira* of all *Nitrospira* sequences in the foam fractionation units, and the accumulation of nitrite was lower. These results suggest that foam fractionation potentially promotes nitrification in the biofilters by decreasing the activity of heterotrophic microbes, allowing higher abundance of nitrite oxidizers but also a shift in the nitrifying community from canonical two-step process into the complete nitrification. Previously, foam fractionation has been shown to promote DIN removal (Rahman et al., 2012), and our results seem to explain the underlying reasons. Ozone did not alter the genetic nitrification potential i.e. the abundance of nitrifiers in the bioreactors, as has already been previously observed (Schroeder et al., 2015).

5. Conclusions

Altogether, the results obtained in this study demonstrate that both foam fractionation and ozonation affect the microbial abundance, microbial activity in the water, and/or community composition in the freshwater RAS, but with different mechanisms. Foam fractionation caused only slight changes in the overall microbiology but has a targeted effect on the biofilter biofilm microbial community, suggesting that it may reduce unwanted heterotrophic growth and activity through decreasing organic matter in the system, thus promoting more stable nitrification in the biofilters. In contrast, ozonation poses a strong selection pressure by attacking the microbes directly, shaping the microbial communities in water, which may potentially open niches for specific ozone-tolerant taxa. However, more information on the long-term development of RAS microbial communities under ozonation is still needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2021.737846.

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Commercial fresh water foam fractionation: Preliminary results

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1. Introduction

The build-up of organic matter in production systems is one of aquacultures largest challenges (Martins *et al.,* 2010). Due to high stocking densities and feed loadings, RAS experience large amounts of organic matter build-ups, in both particulate and dissolved state. In the case of particulate organic matter, this is mostly made up of micro particles of very small dimensions (below 30 µm) (Chen et al., 1993; Fernandes et al., 2014; Patterson et al., 1999). This build-up of micro particles has been linked to the bacterial activity in these systems. Pedersen *et al.* (2017) first reported a link between the amount of particulate surface area and bacterial activity in samples collected from experimental RAS and constructed wetlands receiving effluents from model trout farms (MTF). More recently de Jesus Gregersen *et al.* (2019) found similar results when sampling 7 different MTF and sampling 20 individual RAS, with as much as 92% relationship between micro particle surface area and bacterial activity.

Unfortunately both micro particles and dissolved organic matter are extremely difficult to remove from the production units as there small size allows them to evade most treatment units. Typically, MTFs rely on settling of particles and drum filters for removal of organic matter. While this methods are effective at removing larger particles, there effect is very limited on particles below 50 µm (Timmons and Ebeling, 2010).

In order to control the increase levels of microbial activity and in an attempt to prevent impacts on fish performance, producers will routinely use disinfectants to lower microbial activity (Noble and Summerfelt, 1997; Pedersen et al., 2013) . While this helps control bacterial activity, it's has limited to no effect on the root cause of the problem.

One of the few alternatives available in aquaculture for the capture and removal of micro particles and dissolved organic matter is foam fractionation (de Jesus Gregersen et al., 2021; Timmons and Ebeling, 2010). Foam fractionation is a technic where water and air are mixed together and through a process of adhesion of surfactants to the air bubbles foam is generated. The foam rises to the top of the collection cup and exits the water phase. Foam fraction has mostly been used in salt water due to the increase surface tension, resulting in the production of much smaller air bubbles, which results in a much higher amount of surface area. However, recent studies have shown a large potential for the applicability of foam fractionation in fresh water. de Jesus Gregersen *et al.* (2021), in a pilot scaled study, obtained over 50% reduction in organic matter build-up and 60% reduction in bacterial activity in a systems fitted with foam fraction compared to control systems.

Foam fractionation is typically used in conjunction with ozone. Ozone is a strong oxidizer that helps break down complex molecules in to simpler ones and it also increases coagulation of particles (Good et al., 2011; Rueter and Johnson, 1995; Summerfelt et al., 1997). If used in high enough quantities, ozone is also a powerful disinfectant (Summerfelt et al., 2009).

In the previous mentioned trial (de Jesus Gregersen et al. 2021), the addition of ozone to the foam fractionation resulted in an 80% removal of organic matter and over 90% reduction in bacterial activity, suggesting a strong potential for applicability in fresh water systems.

In order to study the applicability of using foam fractionation in commercial fresh water systems, a commercial foam fractionator was installed in Nørå Dambrug and monitored of the course of multiple sampling rounds in order to determine its impact of different water quality parameters.

2. Methods

2.1. Setup

A Galaxy 1400 foam fractionator (FF) (CM Aqua Technologies ApS, Denamrk) was installed at a MTF (Nørå dambrug, Billund, Denmark) during winter, 2020. The FF was modified with the addition of 3 diffusers on the bottom of the main body, which were supplied with air from the farm's compressors, in order to increase the amount of bubbles generated. The FF was installed over the production unit with the inlet pump placed after the end the biofilters and the discharge pipe placed before the biofilters. This installation reduced the potential hazard of discharging ozone back into the system. A lift pump supplied the FF with approx. 70m^3 h^{-1} of water, resulting in a retention time of approximately 160 seconds.

A drain pipe was installed in the collection cup in order to allow for the retrieval of the removed fomate. This configuration also allowed for the measurement of the volume of fomate produced at any given moment.

A valve on the outlet of the FF regulated the height of the water within the FF and allowed for changes on the volume of foam produced.

Two parameters were tested on the FF to determine changes in efficiency: 1) changes in the removal efficiency based on water height within the FF and 2) addition of ozone.

2.2. Sample collection

In order to study the effect of water height within the FF and establish best practices for the application of the FF, the valve on the outlet of the FF was adjusted manually over 4 approximate set points. 10

minute intervals were conducted between adjusting the height and collecting the samples in order to allow for the stabilization of the foam production. At each set point water was collected before and after the FF for measuring potential changes in water quality parameters. The fomate volume was measured by allowing the fomate to drain into a 15l container and timing the amount of time needed to collect 15 litters of fomate. A 5 litter subsample from this fomate was collected for analysis.

Samples were collected over 5 visits to the farm. For purposes of this report, 10 samples were collected before the FF, 10 samples after the FF and 16 fomate samples without ozone. Only samples collected between 9 and 11 am where used for assessing FF efficiency in order to have similar operational conditions on the farm. Each sample consisted of a 5 litter sample that was subsequently subdivide in the individual measurements.

In order to study the effect of ozone on the efficiency of the FF, samples were collected from the FF while being operated without ozone. Afterwards the ozone generator (Gaia, Water Aps, Denmark) was turned on and allow to produce ozone for 30 minutes. After this, extra samples were collected. Samples collected included water before and after the FF and a fomate sample. This process was repeated in two different visits to the farm, and a total of 5 sets of samples (in, out and fomate) with ozone and 6 sets without ozone where collected.

2.3. Sample measurement

Oxygen, pH and temperature were measured at the intake and outflow of the FF using a Hach HQ40d Portable Multi Meter (Hach Lange, USA).

Organic matter removal was analysed by measuring total biological oxygen demand after 5 days (BOD_5) following ISO 5815 (1989) modified by adding allylthiourea (ATU) and chemical oxygen demand (COD), following ISO 6060 (1989). COD was measured as both total (COD_{Tot}) and dissolved (COD_{Diss}), where COD_{Tot} samples where ran unfiltered and COD_{Diss} samples were pre-filtered using 0.45 µm filters (Advantec® membrane filter, Toyo Roshi Kaisha Ltd, Japan).

Micro particles in the water samples were measured using a Multisizer 4e Coulter Counter (Bechman Coulter, Inc, Indianapolis, USA), between 1 and 30 µm using a 50 µm aperture.

Bacterial activity in the water phase was quantified using 1) hydrogen peroxide (H_2O_2) decomposition rate assay described in Pedersen et al. (2019), considering the degradation rate constant (k, h^{-1}) as an expression of microbial activity and 2) commercial BactiQuant (Mycometer A/S, Denmark) assay.

UVT was measured in a UV spectrophotometer (Beckman DU® 530 Life Science UV/Vis Spectrophotometer, Bechman Coulter Inc, Indianapolis, USA) measuring % transmission using quartz cuvettes, at 254 nm, while turbidity was measured using a Hach 2100Q (Hach Lange, USA).

2.4. Data analysis

In order to account for changes in water quality during the duration of the trial, all data was normalized. When looking at the effects of the foam fractionator with and without ozone, the data was normalized using the formula:

$$x = \left(\frac{C_{Out}}{C_{In}} - 1\right) * 100$$

Where x is the % of change in a certain water quality parameter over the FF, C_{Out} is the concentration out of the FF and C_{In} is the concentration in the intake water to the FF. Negative values represent reduction (improvement) in the concentration of each parameter.

When assessing the effects of water height on the removal efficiency of FF, a mass balance of the water going in to the FF and the fomate collected was conducted.

The increase water level within the FF resulted in more water being removed through the collection cup of the FF, which resulted in a higher amount removed. In order to account for the extra removal of water, the concentration of fomate was normalized, by removing the concentration of the water going in to the FF from the concentration measured in the fomate, using the formula:

$$C_{FN} = C_{fomate} - C_{In}$$

where C_{FN} represents the normalized fomate concentration, C_{fomate} is the concentration measured on the fomate samples and C_{In} is the concentration in the intake water to the FF.

The percentage collected in the fomate was calculated as:

$$\text{Percentage collected in the fomate} = \frac{C_{FN} * Volume\ of\ fomate}{C_{In} * Volume\ of\ water\ FF} * 100$$

The volume of water through the FF was (set at) approx. 70 $m^3\ h^{-1}$ and the volume of fomate was measured for each sample.

3. Results

Oxygen levels before the FF averaged 62,0% saturation , while on the outflow of the FF the value increased to 97,9%. Water pH on the intake water was 7,14, while pH on the outflow measured 7.52.

One pass through the FF resulted in a 1.8 % reduction in the amount of BOD (table 1), a 3.9 % reduction of COD_{Tot} and a 10.4 % reduction in COD_{Diss}.
Turbidity was reduced by 10.0 % and UVT improved 0.45 %.

Bacterial activity showed only minor improvements (1.6 %) when activity was measured using Bactiquant. However, when the activity was measured using H2O2 degradation assay, FF alone reduced bacterial activity by 6.0 %.
The effect of FF on micro particles was in general consistent across the different metrics. Number of particles were reduced by 8.5%, volume of particles by 10.2 % and surface area of particles was reduced by 9.9 %.

The application of ozone to the FF resulted in some changes to the effect of the FF. Turbidity values were similar (9.7 % reduction), while there was an increased effect in UVT (2.1 % improvement).
The net change in CODTot over the FF increased (9.6 % reduction), while BOD removed remained similar (1.6 %).

Micro particles were also reduced during a passage of the FF and with the number of micro particles removed increasing to 14 % and the total surface area reduced by 8 % . The volume of micro particles in the measured size range increase by 10.9% over one pass in the FF.

Table 1. Changes in concentration of different water quality parameters before and after the FF, with and without ozone. Negative changes indicate improvement (reduced levels) in the outlet of the FF compared to the concentration at the inlet.

	No ozone (n = 11)		Ozone (n = 3)	
	Variation (%)	Standard deviation	Variation (%)	Standard deviation
Turbidity	-10.0	4.6	-9.7	6.6
UVT	-0,45	0,3	-2.1	1.4
Bacterial Activity (H_2O_2)	-6.0	3.6	-19.4	1.5
Bacterial Activity (Bactiquant)	-1.6	16.6	-38.9	15.2
COD_{Tot}	-3.9	5.5	-9.6	3.5
COD_{Dis}	-10.4	3.9	*	*
BOD	-1.8	5.0	-1.6	15.4
Number of particles	-8.5	14.7	-14.0	8.9
Volume of particles	-10.2	7.8	10.9	28.5
Surface area of particles	-9.9	12.2	-8.0	10.9

***interference caused by the ozone did not allow for accurate readings.**

The largest changes caused by the ozone were related to bacterial activity. Bacterial activity was reduced by 19.4% (when measured as H_2O_2) and by 38.9% when measured as Bactiquant. Furthermore, 2 sampling runs where the FF was operated with and without ozone (figure 1) showed a clear improvement on the FF to reduce bacterial activity. Bacterial activity reduction without ozone was on average 4.7% (measured using H_2O_2 assay) during these dates, while the inclusion of ozone reduced bacterial activity by 27.5 % on average and up to 45.6% on the last sampling event.

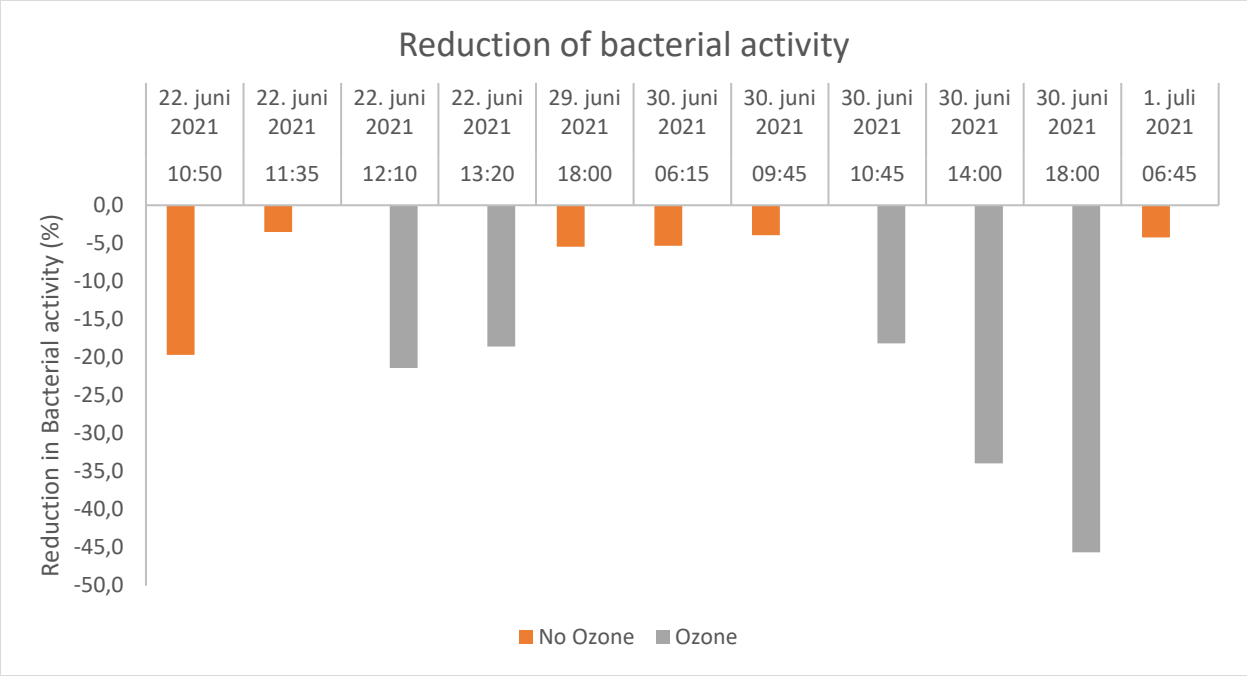

Figure 1. Reduction in bacterial activity over one pass of the FF, with and without ozone on two different sampling dates. Orange bars represent the percentage of reduction caused by one pass of the FF with ozone, while the grey bars represent the reduction of bacterial activity when ozone was applied.

The adjustment of the water height in the FF resulted in large changes in the volume of fomate produced, with fomate volumes varying from 110l h^{-1} to as much as 2250l h^{-1}.
This resulted in large different in the concentration of the fomate collected.
When the values where adjusted to account for extra water removed (figure 2) removal efficiency remained relatively stable across the wide range of fomates collected regarding CODTot. Number of particles, Volume of particles and BOD_5 seem to benefit from an increase in fomate production in the lower range of fomate production. Above 700 l h^{-1} the values stabilized.

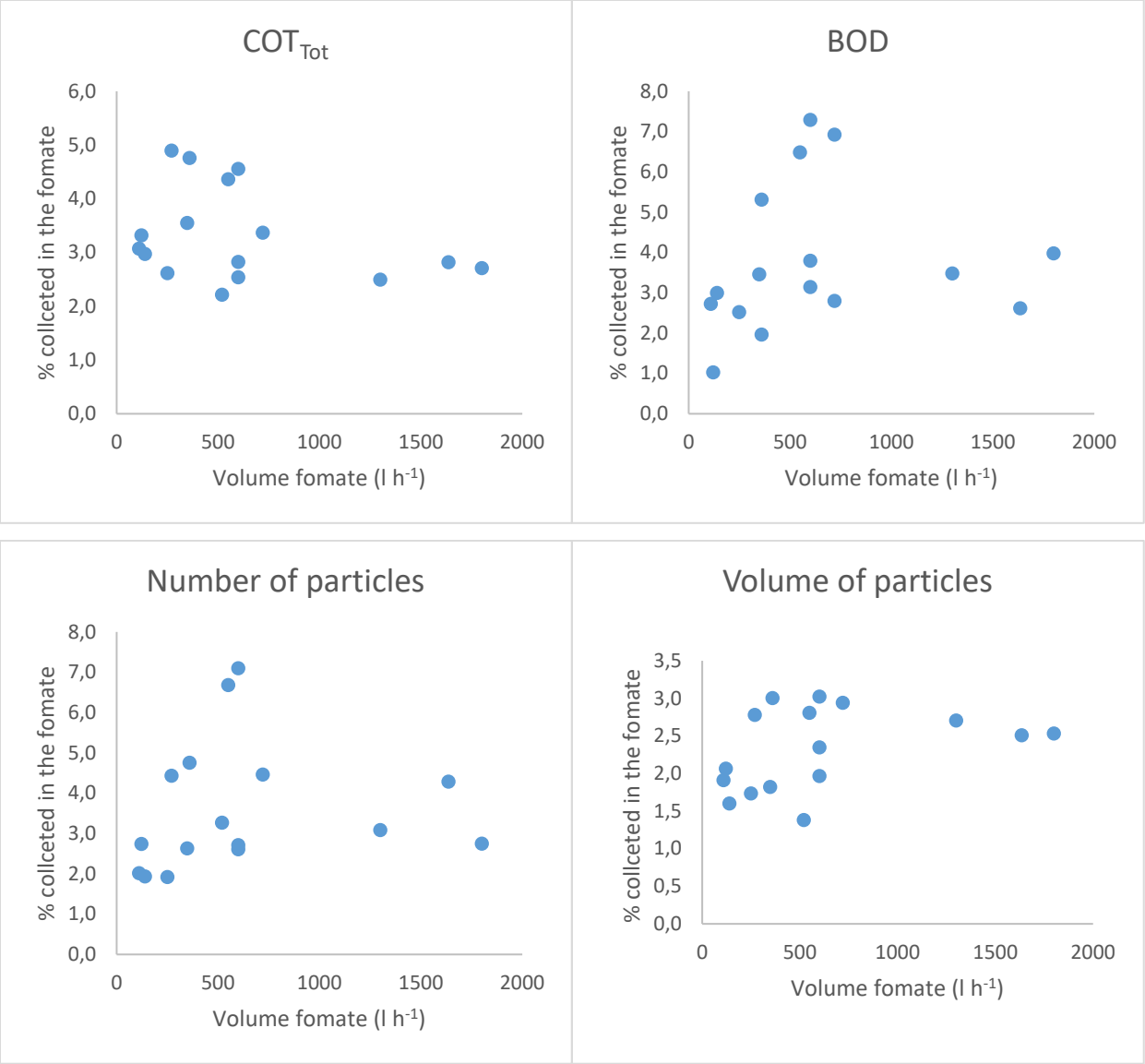

Figure 2. Effect of fomate volume on the removal rate of selected water quality parameters. Add more info about the units (netto removal (conc x V) – and % in relation to the inlet water concentration). Some outliers where found in the last day of measurement, due to clear water conditions in the farm, resulting in a higher % of removal.

4. Discussion

Build-up of organic matter in RAS facilities as a major impact on the overall water quality of RAS facilities. Current treatment units are in general geared towards removing large particulate organic matter, but are in general inefficient with micro particles and dissolved organic matter.

The application of foam fractionation in fresh water has typically been consider to be inefficient due to low surface tension of fresh water (Timmons and Ebeling, 2010). However, newer research as shown that if applied properly, freshwater foam fractionation can have large and positive effects in fresh water systems (de Jesus Gregersen et al., 2021).

As far as the authors are aware, this is the first time a large FF is applied to a MTF. Despite very large variations caused by normal operation of the commercial facility, FF resulted in the improvement of all parameters measured in the facility.

As seen in previous studies (Barrut et al., 2013; Brambilla et al., 2008; de Jesus Gregersen et al., 2021), foam fractionation reduced particulate matter, seen both by approximately 10% reduction in all micro particle variables (numbers, volume and surface area), as well as 10% reduction in overall turbidity. The similar changes in all 3 micro particle variables suggest that the FF remove particles of all measured sizes similarly. Furthermore, all particles measured in this study where below 30um, which are the dominant form of particles in RAS (Chen et al., 1993; Fernandes et al., 2014). Normally this type of particles are unaffected by screen filters, as has been show before by Fernandes *et al.* (2015), where the use of increasing smaller filters did not change the overall amount of micro particles. In the current study, there was a clear effect on this size of particles. Besides the difficulties in removing this type of particles, micro particles have been shown to be in have an impact on the amount of bacteria present in MTF's (de Jesus Gregersen et al., 2019; Pedersen et al., 2017). Likewise, the use of mechanical filters tends to have small, to no effects on dissolved organic matter (Fernandes et al., 2015). In this case, the FF reduced dissolved organic matter (measured as COD) by 10.4% at each pass.

It is likely that the removal of micro particles and dissolved organic matter could have a large impact on the system carrying capacity. Together with the direct impact on bacteria, it is possible that FF could provide a tool for the control of bacterial activity in MTF, not only by removing bacteria, but also by addressing the core issue of organic matter build up.

The addition of ozone to the FF resulted in some changes on the effects on water quality. The most striking difference was an even larger decrease in bacterial activity. Bacterial activity reduction measured as H_2O_2 was reduced by 20%, while bacterial activity measured as Bactiquant reduced by almost 40% in a single pass. Ozone has been shown to have a disinfectant activity when applied in high enough concentrations (Figueiras Guilherme et al., 2020; Summerfelt et al., 2009). This results are clearly seen in the two sampling days where ozone was ran off and on (figure 1), with large decreases in

bacterial activity while the ozone generator was on. At the same time, total organic matter removed (COD_{Tot}) was also improved to approximately 10% per pass. The effects on micro particles indicate a stronger reduction of the smaller size classes. This could, in part be the result of a more efficient removal of micro particles, but also a result of the destruction of bacteria.

Interestingly, micro particle volume apparently increased, suggesting a coagulation of particles, as previously seen in other studies (Rueter and Johnson, 1995; Summerfelt et al., 1997). This could potentially lead to an improvement in removal efficiency of the drum filters as seen in previous studies (Summerfelt et al., 1997).

While the effects seem at first small, typical filtration units in MTFs will treat the full volume of water several times an hour. It is likely that a proper dimension FF, capable of treating all water at least 5 to 10 times a day should provide much higher benefits in terms of water quality by removing a small percentage at every pass, resulting in cumulative benefits over the day.

The results obtained during this trial also showed that the FF works as a good aeration device, leading to large increases in oxygen levels in the water. Also, the significant increase in pH indicates a significant degassing effect on CO_2 in the system, which could have further positive effects.

The adjustment of water level within the FF provided some benefits on organic matter and micro particle removal. However, this benefit seemed to only be present in the lower end of the removal volume. Freshwater FF generates foam that has less consistency then saltwater FF (Pers. observation). This is likely the result of the smaller surface tension of the water. This seems to results in the need to have the water level higher within the FF compared to saltwater FF (Pers. observation) in order to push the foam into the collection cup. In the current FF setup, a volume between 750 and 1000 l/h of fomate seem to provide the best results. Passed this point there did not seem to be any increase in removal efficiency, while the concentration of the fomate became smaller and smaller, which results in a much more diluted fomate that would make end of pipe treatment more complicated.

5. Conclusion

Newer research has shown that, when properly applied, freshwater FF can be a powerful tool for water quality improvement. However, its applicability in commercial settings is still unknown. This trial provides a first look into the application of FF in MTFs. And while the large variations found in this

preliminary study make drawing conclusions difficult, a positive impact on all parameters tested, including clear impacts on some of the more challenging parameters to control (dissolved organic matter, micro particles and bacterial activity) clear indicates that FF could be used in commercial settings to improve overall water and system quality. The use of ozone helped to improve some of effects of the FF. Additionally, FF had clear additional benefits in terms of aeration and degassing of the water.

Further studies are necessary to further optimise FF operation and also to evaluate effects on the full flow.

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