DBU-Kooperationsprojekt: UltraSys – AZ 28130-31

Ultraschall-UV-Desinfektionssystem für Kreislaufanlagen

im Rahmen der Förderinitiative „Nachhaltige Aquakultur

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Projektkennblatt einfügen

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Zusammenfassung

In der Aquakultur sind Kreislaufanlagen ein umweltfreundliches und wassersparendes Produktionsverfahren. Hohe Besatzdichten und das Prinzip der Wasserführung im Kreislauf führen jedoch auch zu einem erhöhten Risiko von Infektionskrankheiten. In dem hier beschriebenen Projekt wurde untersucht, wie sich niederfrequenter Ultraschall (nf-US) in Kombination mit der schon in der Aquakultur bewährten UV-C Bestrahlung einsetzen lässt.

Für das Projekt wurde durch BANDELIN ein innovatives Ultraschall-UV-Durchflusssystem entwickelt, in dem erstmals ein hocheffizienter nf-US-Spaltreaktor mit einer einsteckbaren UV-C-Lampe in einem Doppelrohrsystem so kombiniert wurde, dass im Betrieb beide Systeme synergistisch desinfizierend wirken (Kapitel 2). Zwei solche Reaktoren wurden baugleich und anschlussfertig für die Projektpartner IGB und AquaVet aufgebaut und geliefert.

Vergleichende Untersuchungen zur Effizienz von nf-US, UV-C und deren Kombination gegen Modellorganismen (Kapitel 3) zeigten, dass UV-C trotz seiner starken Abhängigkeit von der UV-Durchlässigkeit des behandelten Wassers ein hervorragendes Verfahren zur Kontrolle von heterotrophen Bakterien in Kreislaufanlagen ist. Eine Vorbehandlung des Wassers mit nf-US verringert die mittlere Größe der im Wasser Suspendierten Partikel und kann so die Effektivität von UV-C zur Inaktivierung von Bakterien verbessern. Gegen eukaryotische Organismen wie Ciliaten, Nematoden oder Krebstiere müssen allerdings deutlich höhere als die gegen Bakterien wirksame UV-Intensitäten eingesetzt werden. Alternativ kann zu diesem Zweck nf-US eingesetzt werden. Vergleicht man die energetische Effizienz von nf-US und UV-C gegen Ciliaten oder Nematodenlarven, so hängt es von der UV-Durchlässigkeit des Wassers und dem verwendeten Lampentyp ab, welches der beiden Verfahren besser abschneidet. Gegen große, komplexe Organismen wie die Metanauplien von *Artemia* ist nf-US in jedem Fall das energetisch günstigere Verfahren.

Die Erhöhung der UV-C Intensitäten zur Abtötung eukaryotischer Organismen ist dadurch limitiert, dass bei hohen UV-Dosen in Verbindung mit den in Kreislaufanlagen auftretenden hohen Nitratkonzentrationen durch die photo-induzierte Umwandlung von Nitrat zu Nitrit fischtoxische Nitritkonzentrationen auftreten können (Kapitel 4). Im Gegensatz hierzu ist nf-US auch bei hohen Intensitäten ein anwendungssicheres Verfahren zur Abtötung eukaryotischer Organismen und kann somit eine sinnvolle Ergänzung zu der gegen Bakterien eingesetzten UV-Bestrahlung sein.

Untersuchungen in einer Versuchskreislaufanlage zeigten, dass mit dem kombinierter Ultraschall-UV-Durchflussreaktor die Transmission von Pathogenen zwischen den Becken einer Kreislaufanlage verzögert, aber nicht vollständig verhindert werden kann (Kapitel 5).

Die Möglichkeit zur Installation des Kombireaktors im Bypass wurde ebenfalls in Versuchskreislaufanlagen untersucht (Kapitel 6). Es zeigte sich, dass der UV-Reaktor zur Kontrolle der im Wasser suspendierten Bakterien mit dem gesamten Volumenstrom des Kreislaufs beaufschlagt werden sollte, während nf-US gegen eukariotische Parasiten auch gut im Bypass betrieben werden kann. Somit kann der gegenwärtig verfügbare Kombireaktor praktisch bei kleineren Anlagen direkt in den Filterkreislauf eingebaut werden. Bei größeren Kreislaufanlagen kann es günstiger sein, separate UV-C und nf-US Reaktoren zu verwenden, wobei UV-C mit dem gesamten Volumenstrom des Filterkreislaufes beaufschlagt und nf-US im Bypass betrieben wird.

Kapitel 1

Einleitung

Beschreibung des Umweltproblems

Die Aquakultur, insbesondere die Produktion von Fischen, ist weltweit der am schnellsten wachsende Sektor der Nahrungsmittelindustrie (FOA, 2012). Vor dem Hintergrund rapide sinkender Fischbestände in den Weltmeeren wird der Fischproduktion in der Aquakultur in den kommenden Jahren eine stetig wachsende, kaum zu überschätzende Bedeutung für die Proteinversorgung der Menschheit zukommen. Geschlossene Kreislaufanlagen sind ein umweltfreundliches und wassersparendes Produktionsverfahren, das insbesondere für die Produktion von Fischen eingesetzt wird (Summerfelt *et al*., 2004).

Die intensive Produktion von Fischen in geschlossen Kreislaufanlagen hat allerdings auch einen gravierenden Nachteil. Hohe Besatzdichten und das Prinzip der Wasserführung im Kreislauf führen nicht nur zu einer Anreicherung von Stoffwechselendprodukten und der damit verbundenen Notwendigkeit einer angepassten Wasseraufbereitung, sondern sie erhöhen auch das Risiko von durch Viren, Bakterien, Pilze und Parasiten verursachten Infektionskrankheiten. Diese können hohe Mortalitäten und beachtliche finanzielle Schäden verursachen und sind weltweit der wichtigste limitierende Faktor für den ökonomischen Betrieb geschlossener Kreislaufanlagen (Schnick, 1996). Ein wichtiger Schlüssel zur Verringerung krankheitsbedingter Verluste ist daher die Entwicklung sicherer, effektiver und ökonomischer Verfahren für das Fischgesundheitsmanagement in der Aquakultur.

Traditionell werden Krankheiten in der Aquakultur medikamentös behandelt. Heute besteht allerdings in vielen Ländern, darunter der EU, ein Therapienotstand bei Fischen, da der Einsatz vieler der in der Vergangenheit verwendeten Medikamente nicht mehr für die Produktion von Speisefischen zugelassen ist (Baur *et al*., 2010; Meinelt *et al*., 2009; Schlotfeldt, 1998; Schnick 1996). In anderen Ländern, wo der Einsatz von Medikamenten in der Aquakultur nicht streng geregelt ist, werden Infektionskrankheiten ebenso wie im Zierfisch-Sektor zur Vermeidung von Verlusten nach wie vor medikamentös behandelt, soweit geeignete konventionelle Medikamente zur Verfügung stehen. Dies kann zu erheblichen Umweltbelastungen führen, wenn die verwendeten Chemikalien bzw. Medikamente letztendlich in die Umwelt gelangen. Darüber hinaus kann es z.B. bei der Verwendung von Antibiotika zu Resistenzbildungen kommen.

Stand der Technik

Alternativen zur medikamentösen Bekämpfung pathogener Keime sind die Behandlung des Kreislaufwassers mit Ozon und/oder kurzwelligem ultraviolettem Licht (UV-C) (Timmons und Eberling, 2007). Ozon hat eine stark oxidierende Wirksamkeit und wird in der

Aquakultur sowohl zur Inaktivierung von Pathogenen als auch zur Verbesserung der Wasserqualität eingesetzt (Summerfelt, 2003). Zur Erzielung der desinfizierenden Wirkung muss, abhängig vom Zielorganismus, ein bestimmtes Produkt aus Ozonkonzentration und Einwirkzeit eingehalten werden. In Wasser mit einer hohen organischen Belastung, wie bei der intensiven Fischproduktion in Kreislaufanlagen, verringert sich die Halbwertszeit von Ozon auf wenige Sekunden. Das Einstellen der zur Abtötung von Pathogenen notwendigen Ozonkonzentration gestaltet sich unter solchen Bedingungen schwierig, und es werden deutlich höhere Ozondosen benötigt, als dies lediglich zur Kontrolle der Wasserqualität notwendig wäre (Bullock *et al*., 1997). Aufgrund seiner hohen Toxizität ist Ozon in seiner Anwendung kritisch zu beurteilen. Im Falle einer Fehlfunktion kann es schwerwiegende Gesundheitsschäden beim Anlagenbetreiber und bei den Fischen hervorrufen. Es sind in jedem Falle sicherheitstechnische Lösungen mit hohem technischem Aufwand erforderlich.

UV-Licht hat eine sehr gute Wirkung auf eine große Anzahl von Viren und Bakterien, ist aber weniger wirksam gegen größere Organismen wie Protozoen und parasitische Würmer (Chevrefils *et al*., 2006; Kasai *et al*., 2002). Die desinfizierende Wirkung von UV-Licht beruht auf der Denaturierung von DNA durch Wellenlängen im Bereich um 254 nm. Die Effizienz des Verfahrens hängt von der UV-C Durchlässigkeit des behandelten Wassers ab, welche durch Streuung an Schwebstoffen und Absorption durch gelöste organische Stoffe (DOM) vermindert wird (Liltved, 2002, Gullian *et al*., 2012; U.S. Environmental Protection Agency, 2006; Harris *et al*., 1987). Daher ist für die effiziente Anwendung von UV-C eine niedrige Konzentration an Schwebstoffen und DOM erforderlich, was in intensiv betriebenen Kreislaufanlagen nicht immer wirtschaftlich erreichbar ist (Losordo *et al*., 1999). Weiterhin können Ablagerungen auf dem die UV-Lampe umschließenden Glaszylinder die UV-Dosis verringern (Naddeo *et al*., 2009).

Der Einsatz von Ultraschall (US) ist eine neues und viel versprechendes Verfahren für die Wasserdesinfektion, welches insbesondere hinsichtlich seiner mikrobiziden Wirkung in der Abwasserbehandlung untersucht wurde (Antoniadis *et al*., 2007; Gogate, 2007; Gogate und Kabadi, 2009). An der desinfizierenden Wirkung von Ultraschall, hier insbesondere von niederfrequentem Ultraschall (nf-US), sind verschiedene Mechanismen beteiligt. Die ultraschallinduzierte Kavitation löst starke mechanische (Mikrojets, Scherwellen), thermische (hot spots) und chemische (generieren freier Radikale) Effekte aus. Es konnte gezeigt werden, dass vor allem die mechanischen Effekte ursächlich für die desinfizierende Wirkung sind, während chemische und thermische Effekte zusätzlich unterstützend wirken (Gogate und Karbadi, 2009). Hinzu kommt, dass durch die Ultraschalleinwirkung mikrobielle Cluster

aufgelöst und die Effizienz desinfizierender Maßnahmen dadurch gesteigert wird. Gegenwärtig wird diese Methodik für die Aquakultur noch nicht eingesetzt.

Erst kürzlich wurde die Kombination von nf-US und UV-C für die Abwasserbehandlung untersucht, um die Nachteile einer alleinigen Behandlung mit UV-C auszugleichen (Naddeo *et al*., 2009). Es konnten synergetische Effekte von nf-US und UV-C bei der Inaktivierung von coliformen Bakterien und eine Reduzierung von Ablagerungen auf den UV-Lampen nachgewiesen werden.

Durch Bandelin wurde in einem kürzlich abgeschlossenen BMBF-Projekt[1] (02 WA 0899) die Anwendung von niederfrequentem Leistungsultraschall für die Wasserdesinfektion unter den spezifischen Anforderungen der Aquakultur untersucht. Dieses Projekt hat gezeigt, dass nf-US für die Abtötung größerer Organismen (Ciliaten, Monogenea), aber weniger für die Inaktivierung von Bakterien geeignet ist. Dieses Defizit könnte vorteilhaft durch die Kombination von nf-US mit UV-C ausgeglichen werden. Es kann davon ausgegangen werden, dass die Kombination von US und UV-C ideal geeignet ist, die Anzahl pathogener Keime in Kreislaufanlagen der Aquakultur soweit zu reduzieren, dass der Ausbruch von erregerbedingten Krankheiten wirksam unterbunden wird. Die beiden physikalischen Verfahren sind in ihrer Kombination ein innovativer Ansatz für ein optimiertes Fischgesundheitsmanagement ohne den Einsatz gesundheits- und umweltschädlicher Chemikalien bzw. Medikamente.

Gegenstand und Zielsetzung des Projektes

Ziel des Projektes war die Entwicklung eines effizienten, industriell anwendbaren, marktfähigen und umweltverträglichen Desinfektionssystems für geschlossene Kreislaufanlagen in der Aquakultur. Das Desinfektionssystem soll die gleichzeitige Behandlung des Kreislaufwassers mit nf-US und UV-C im Dauerbetrieb ermöglichen. Der Projektablauf umfasst im Wesentlichen folgende Schritte:

1. Nachweis synergistischer Effekte eines kombinierten US/UV-Desinfektionssystems in geschlossenen Kreislaufanlagen für die Fischproduktion und Definition der wesentlichen Prozessparameter
2. Nachweis, dass mit dem kombinierten US/UV-Desinfektionssystem eine Transmission von Pathogenen mit dem rezirkulierenden Wasser unterbunden wird.

[1] „Entwicklung eines Ultraschall-Desinfektionssystems für die intensive Wasseraufbereitung von Fischzuchtanlagen auf Basis eines neuen Ultraschall-Durchflussreaktors und neu entwickelten chemischen Zusätzen“ – deutsch/israelisches Gemeinschaftsprojekt.

3. Untersuchung einer möglichen Anreicherung von Substanzen im Kreislaufwasser, die durch die Wasserbehandlung mit dem US/UV-Desinfektionssystem entstehen und den Gesundheitszustand der Fische beeinflussen könnten.
4. Einbindung des US/UV-Desinfektionssystems in geschlossene Kreislaufanlagen unterschiedlicher Größe und Nachweis einer Elimination oder Reduktion der Pathogene.

Zielgruppe für die neue Technologie zur Wasserdesinfektion sind alle Aquakulturbetriebe, die in geschlossenen Kreislaufanlagen Fische und andere aquatische Organismen produzieren. Der Mangel an zugelassenen Medikamenten generiert einen dringenden Bedarf für innovative, anwendungssichere und umweltschonende Verfahren für das Gesundheitsmanagement in der Aquakultur.

Kapitel 2

Technische Entwicklung

Rainer Jung

Technische Entwicklung

Um ab Projektstart einen zeitnahen Beginn der Arbeiten bei den wissenschaftlichen Projektpartnern zu ermöglichen, wurde entschieden, dem IGB und AquaVet zunächst jeweils separate Systeme zur Verfügung zu stellen. D. h. jeder Projektpartner erhielt einen Ultraschall-Durchflussreaktor (Spaltreaktor mit Wirbelscheibe) sowie eine separate UV-Einheit für erste Versuchsreihen. Der Ultraschall-Durchflussreaktor aus Edelstahl stellte ein kombiniertes Innen- und Außenrohrsystem mit Zwischenspalt dar. Auf dem äußeren Rohr wurden spezielle 25 kHz-Ultraschall-Schienensysteme mittels einer hochfesten Klebung angebracht. Durch die spezifische Wandlerausführung und den engen Spalt zwischen den Rohren wird ein sehr intensives kavitatives Ultraschallfeld mit hoher Leistungsdichte erzeugt (blau im Bild 1). Ein eingangsseitig fest montiertes Flügelrad (Wirbelscheibe) versetzt das einströmende Medium in eine leichte Drehbewegung für eine sehr homogene Beschallung.

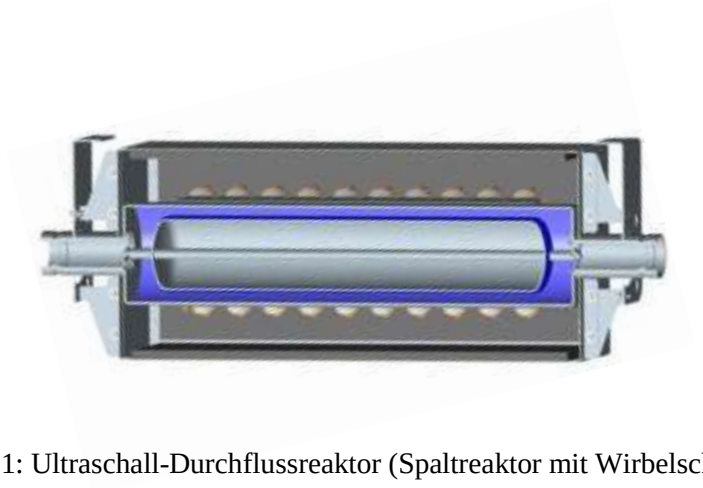

Bild 1: Ultraschall-Durchflussreaktor (Spaltreaktor mit Wirbelscheibe)

Als UV-Einheit wurde das separate UV-C-Rohrsystem „Microlight Basic“ der Firma a.c.k./Karlsruhe eingesetzt, bei der die UV-Lichtquelle, ein Hg-Niederdruckstrahler mit einer Wellenlänge von 254 nm, zentral montiert ist. Diese Wellenlänge hat sich als sehr wirksam gegen Bakterien, Viren und Pilze erwiesen.

Beide Einheiten wurden mit kompletter Anschlusstechnik für einen Durchflussbetrieb in Reihe aufgebaut und auch an die Projektpartner geliefert. Für das IGB wurden auf Wunsch der Ultraschallreaktor und die UV-Einheit zusätzlich in einen Gestellrahmen eingebaut und anschlussfertig geliefert (Bild 2).

Bild 2: Ultraschall-Wirbelreaktor und separate UV-C-Einheit – aufgebaut im IGB/Berlin

Aufgrund schon vorliegender Erfahrungen mit dem Ultraschallspaltreaktor mit Wirbelscheibe wurden verschiedene Konstruktionen zur Entwicklung eines neuen kombinierten US/UV-Reaktors nach dem Gegenstromprinzip aufgebaut und getestet. In den praktischen Testbetrieben mit den ersten Prototypen stellte sich heraus, dass die Doppelrohrgeometrie mit Einbindung einer zentralen UV-Lampe den Belastungen nicht standhielt. Es kam zunächst zu unerklärlichem Versagen von Schweißnähten bei maximaler Ultraschallleistung im Dauerbetrieb (Bild 3).

Bild 3: Versagen einer Schweißnaht im Dauerbetrieb

Erst mittels verfeinerter FEM-ANSYS-Schwingungs-Simulationen, konnten Schwachstellen erkannt und durch Geometrie- und Wandleränderungen beseitigt werden (Bilder 4 und 5).

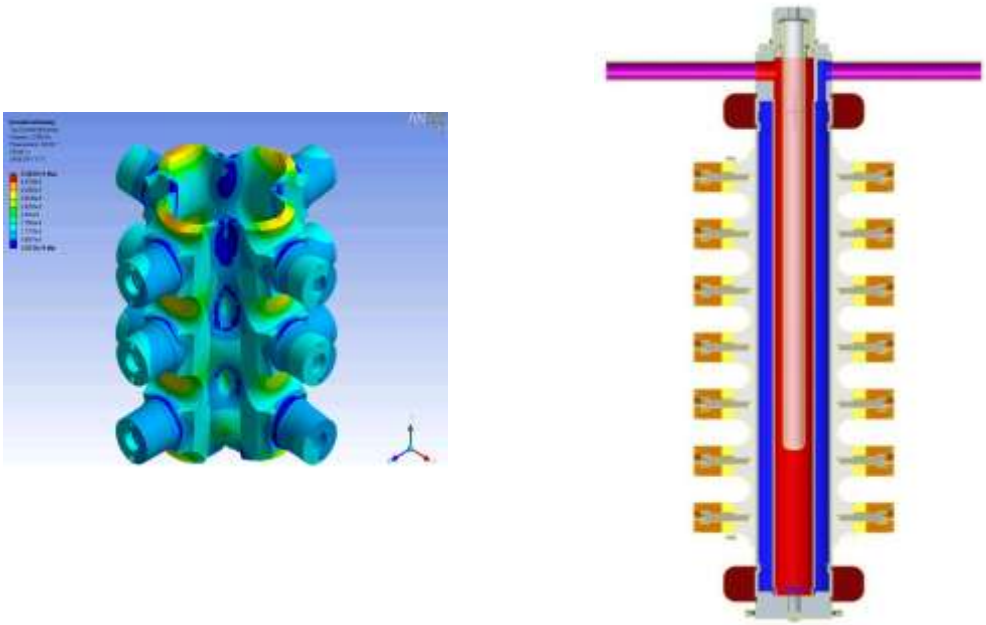

Bild 4: Simulation und Schnittbild des neuen Ultraschall-UV-Gegenstromreaktors

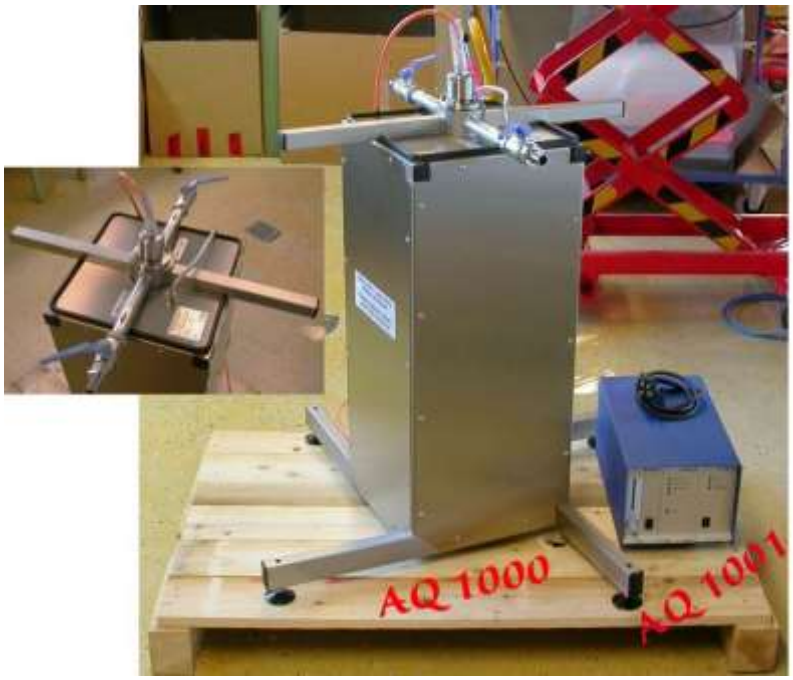

Bild 5: Versandfertiger Reaktor AQ 1000 mit separatem HF/UV-Generator AQ 1001

Im Berichtszeitraum konnten an beide Projektpartner je ein Ultraschall-UV-Gegenstromreaktor mit kompletter Anschlusstechnik für den Durchflussbetrieb ausgeliefert werden (Bild 6). Zusätzlich erhielten die wissenschaftlichen Partner kleinere Gerätetechnik für begleitende Untersuchungen, wie z. B. einen Leistungsmesser und ein kleines Ultraschall-Badgerät.

Bild 6: Eingebauter Gegenstromreaktor im Labor bei AquaVet/Israel

Kapitel 3

Low frequency ultrasound and UV-C for elimination of pathogens in recirculating aquaculture systems

Amir Abbas Bazyar Lakeh, Werner Kloas, Rainer Jung, Ra'anan Ariav, Klaus Knopf

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Abstract

Low frequency ultrasound (LFUS) was evaluated as a novel disinfection technique within recirculating aquaculture systems both individually and combined with UV-C. Dose-dependent inactivation rates were determined for the total viable counts and model organisms representing different taxa of common fish parasites: the ciliate *Paramecium* sp., second larval stage (L2) of the nematode *Anguillicola crassus* and metanauplii of *Artemia* sp. Application of LFUS up to 19 kJ/L did not reduce the number of colony forming units (CFU), whilst UV-C irradiation was highly effective. Pre-treatment with LFUS reduced the mean size of suspended solids in aquaculture water and thus increased the germicidal effect of UV-C by up to 0.6 log units.

LFUS was effective against the eukaryotic organisms, and the dose-dependent inactivation could be well described by functions of an exponential decay. However, the efficiency of LFUS differed greatly between species. A LFUS dose of 1.9 kJ/L (consumed energy) was sufficient to inactivate *Artemia* by 99 %, but a ten times higher dose was necessary to inactivate 95 % and 81 % of *Paramecium* and *Anguillicola* larvae, respectively.

In clear water, the energetic efficiency of UV-C (emitted by a low pressure lamp) against *Paramecium* and *Anguillicola* larvae was higher compared to LFUS, but LFUS was more efficient against *Artemia*. However, the efficiency of LFUS against ciliates or nematode larvae would be similar or even higher than UV-C in highly turbid water or if less efficient medium pressure lamps are used. This study shows that LFUS can be applied safely at energy densities that are effective against a wide range of parasites like ciliates, nematodes and crustaceans. The combination of LFUS and UV-C could provide an appropriate water treatment with regards to all relevant pathogens in recirculating aquaculture systems.

Introduction

Diseases are the most important factor affecting the development of the aquaculture industry. Thus, the attention to the aquatic animal's health and the reduction of losses caused by diseases are key managing factors. Healthy conditions can be achieved by sanitary protection such as water disinfection by means of physical or chemical methods. There is a demand for chemicals to control pathogens in aquaculture, but the lack of approved drugs and chemicals has dramatically reduced the effectiveness and increased the cost of production (Schnick, 1996).

Ultraviolet-C (UV-C) disinfection is a physical method that plays an important role in water treatment (Chevrefils *et al*., 2006). In aquaculture facilities, this technology is used for the prevention of bacterial, viral and fungal diseases (Liltved, 2002; Kasai *et al*., 2002; Gullian *et al*., 2012). UV-C at a wavelength of 254 nm denatures most effectively the DNA of microorganisms, causing their inactivation and death (Liltved, 2002). The efficiency of this technique depends on the UV-C transmittance of the treated water. The UV-C transmittance is adversely affected by a strong absorption by dissolved organic matter (DOM) and scattering by suspended solids (Liltved, 2002, Gullian *et al*., 2012; U.S. Environmental Protection Agency, 2006; Harris *et al*., 1987). Therefore, the efficient application of UV-C requires a low concentration of DOM and suspended solids, conditions which are not always economically achievable in intensive aquaculture systems (Losordo et al., 1999).

Another physical disinfection method, not being impaired by DOM and suspended solids, and with a good potential to kill parasites larger than about 100 µm is low frequency ultrasound (LFUS) (Holm *et al*., 2008). However, LFUS has not yet been applied in aquaculture. Ultrasound with a frequency of 20 kHz or above generates cavitation effects including jet streams, hot spots and free radicals. Yet the exact mechanism of inactivation is still not fully understood. Mason *et al*. (2003) suggested that the disinfecting properties of LFUS are mainly related to the mechanical stress, supported by thermal and chemical effects. The combination of mechanical, thermal, and chemical effects can result in the inactivation of microorganisms (Mason *et al*., 2003; Doulah, 1977; Scherba *et al*., 1991; Thacker, 1973).

Most previous studies on the disinfection efficiency of cavitational reactors have been conducted with a long exposure time up to several minutes, and targeted mainly bacteria, but hardly against eukaryotic pathogens (Gogate, 2007). High flow rates in flow through reactors result in only short exposure times compared to exposure times applied in previous laboratory studies (Gogate, 2007). Furthermore, the method should provide an appropriate reduction rate of all relevant pathogens including protozoan and metazoan parasites. Finally, sonication can

be combined with other disinfection methods. As previously shown, the combination of ultrasound and UV-C has a good potential for the treatment of ballast water (Sassi *et al*., 2005). This study assesses the application of LFUS and UV-C, both individually and in a combined mode, for the reduction of a wide range of pathogens commonly present in recirculating aquaculture systems.

Materials and methods

Technical specification of the LFUS and the UV-C reactors

Technical specifications of the LFUS reactor (Vortex reactor WR 4-1402.03, Bandelin electronic, Berlin, Germany) and the UV-C reactor (Micro light Basic 5; a.c.k. aqua concept, Karlsruhe, Germany) as provided by the manufacturers are summarized in Tables 1 and 2.

The construction design of the LFUS reactor (Fig. 1) is characterized by four rows of transducers that are externally mounted on the reactor tube. Targeted rotary movement of the sonication medium provides cavitation-intensive flow-through sonication in a narrow reaction gap. Low ultrasound frequencies of around 20 kHz, in contrast to higher frequencies, have been shown to be most efficient in killing *Artemia* nauplii (Clasen, 2002). For this reason the present study used a low ultrasound frequency of 25 kHz. The volume-specific power of the LFUS reactor was adjustable in 10 % - steps within a power density ranging from 48 to 480 W/L.

Table 1: Technical specifications of the ultrasound reactor (Vortex reactor WR 4-1402.03, Bandelin electronic, Berlin, Germany)

Technical Data	Vortex reactor WB 4-1402
Filling volume	~ 5 L
Ultrasound volume	2.9 L
Ultrasound distance	500 mm
Flow-through rate	60 – 3600 L/h
Reaction gap	15 mm
Power density max.	480 W/L
Frequency	25 kHz

Table 2: Technical specifications of the UV-C reactor (Micro light Basic 5; a.c.k. aqua concept, Karlsruhe, Germany)

Technical Data	Microlight Basic 5
Filling volume	8 L
Flow rate max.	3000 L/h
Electric power	110 W [a]
Efficiency of UV-lamp	31.8 %
UV-C power	35 W
UV-C dose at SAC_{254} = 22.18 1/m	min. 400 J/m² [b]
UV-C dose at SAC_{254} = 70 1/m	min. 73 J/m² [b]

[a] lamp without electronic ballast

[b] at 3000 L/h; ~ T_{10mm} = 60 % at the end of lamp-life

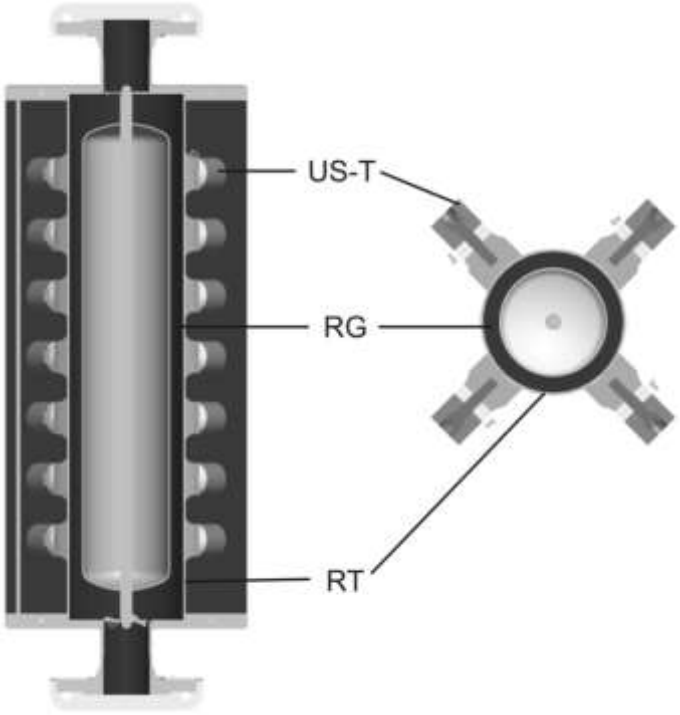

Fig. 1: Assembly of the ultrasound reactor (Vortex reactor WR 4-1402.03, Bandelin electronic, Berlin, Germany), longitudinal section (left) and cross-section (right). US-T, transducers fixed at the reactor pipe; RG, narrow reaction gap; RT, reactor tube (stainless steel, 2.6 mm).

Both reactors were operated in a flow through mode with flow rates ranging from 300 to 3000 L/h. The applied volume-specific energy was adjusted by the variable power (LFUS) and retention time via the flow rate (LFUS and UV-C). When LFUS and UV-C were applied in combination, the water first passed through the LFUS reactor followed by the UV-C treatment. To compare the efficiency of LFUS sonication and UV-C irradiation, dose-dependent inactivation rates for model organisms were related to the volume-specific energy consumption.

Model organisms

As models for taxa containing common fish pathogens, the following organisms were chosen: (1) heterotrophic bacteria naturally occurring in the water of recirculating aquaculture systems, determined as the number of colony forming units (CFU); (2) the ciliate *Paramecium* sp. from hay infusion; (3) second larval stage (L2) of the nematode *Anguillicola crassus* collected from swim bladders of naturally infected eels (*Anguilla anguilla*); (4) 3 days post hatch nauplii of *Artemia* sp. reared from dried cysts (Aquaculture INVE Ltd.).

Determination of inactivation rates

Water taken from recirculating aquaculture systems was used in the experiments with different spectral attenuation coefficients at 254 nm (SAC_{254}). The SAC_{254} was determined by a spectrophotometer (DU 800 UV/Vis Spectrophotometer, Beckmann Coulter, Germany). Afterwards, water with a SAC_{254} ranging from 24 to 35 1/m (27 ± 4 1/m) is called ‘clear water’ and SAC_{254} ranging from 68 to 73 1/m (71 ± 2 1/m) is called ‘turbid water’.

The total viable count (CFU/ml) was quantified by the spread plate technique using a nutrient agar (DEV) (Carl Roth, Germany). Before plate inoculation, all water samples (50 ml each) were dispersed by sonication (20 kHz, 10 s, 70 W; Sonopuls HD 7020, Bandelin). *Paramecium* sp., *A. crassus* larvae or *Artemia* metanauplii were added to the experimental tank and thoroughly mixed for achieving a homogenous distribution immediately before starting the experiment. Water samples containing *Paramecium* sp. were fixed with Lugol’s iodine, 3 ml per well of 24-well microtiter plates and allowed to settle for 60 min. Individuals with an abnormal, spherical shape were considered to be irreversibly harmed. Non-fixed samples containing L2 of *A. crassus* were allowed to settle in 50 ml Utermöhl chambers (HYDRO-BIOS Apparatebau GmbH, Germany) for another 30 min. Immotile nematodes not responding to a physical stimulus applied with a fine needle were considered to be dead.

Viable *Artemia* metanauplii were enumerated using a zooplankton counting chamber (HYDRO-BIOS Apparatebau GmbH).

For each model organism the inactivation rate was calculated as the ratio between viable organisms before and after treatment with 15 replicates for the total viable count (CFU/ml) and five replicates for the eukaryotic model organisms. Lower counts in the treated samples were considered to be eliminated due to the treatment.

Particle size distribution

The effect of sonication on the particle size distribution of total suspended solids (TSS) was measured by laser diffractometry (Mastersizer 2000, Malvern, UK) using aquaculture water with a total suspended solids (TSS) content of 8.5 mg/L. Measurements were performed within 2 h after sampling with six replicates.

Statistical analysis

The non-parametric Kruskal-Wallis test, followed by a *Dunn's multiple comparison* test was used to determine statistically *significant* differences between CFU values obtained from different treatments for each flow rate. Additionally, the effects of water turbidity and LFUS pretreatment on the germicidal effect of UV-C were evaluated using the Mann-Whitney *U*-test.

Mean values of the inactivation rates for *Artemia* metanauplii were compared between different treatments using an analysis of variance (ANOVA), followed by Bonferroni post hoc test. Percentiles of the size distribution of suspended solids in sonicated and non-sonicated samples were compared using the t-test. Significance was accepted when $p \leq 0.05$. Analyses were performed using GraphPad Prism 4.03 (Graph Pad Software, Inc., San Diego, CA). Non-linear regression analysis of the dose-depended inactivation rates by LFUS were performed using OriginPro 8 SR 2 (OriginLab, Northampton, MA).

Results

LFUS applied with a mains energy of 1.9 - 19 kJ/L did not result in a significant reduction of the CFU compared to the untreated control, neither in clear water nor in turbid water (Fig. 2A and B). UV-C irradiation with a mains energy of 0.13 - 1.3 kJ/L resulted in a significant reduction of the total viable count compared to the untreated control ($p < 0.001$, Fig. 2A and 2B). In clear water UV-C irradiation was significantly more effective than in turbid water ($p < 0.001$, Fig. 2A and B). Compared to the untreated control, the CFU values were reduced by

2.0 - 2.5 log units in clear water ($p < 0.001$, Fig. 2A), but only by 1.1 - 1.7 log units in turbid water ($p < 0.001$, Fig. 2B). Compared to UV-C irradiation alone, pre-treatment with LFUS did not result in an additional reduction in CFU values in clear water (Fig. 2A), but it increased the germicidal effect of UV-C by up to 0.6 log units in turbid water (Fig. 2B). The pair-wise comparison between CFU-values obtained by UV-C alone and by the combined application of LFUS and UV-C indicated a significant effect of the pre-treatment with LFUS ($p < 0.01$).

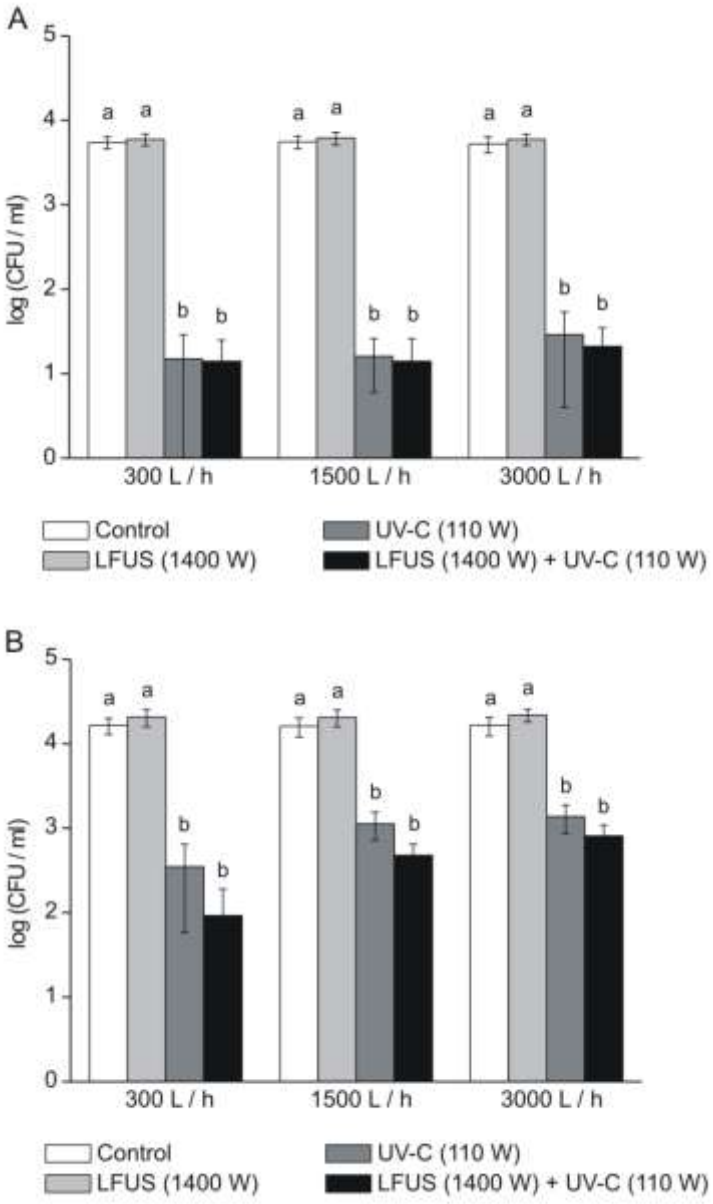

Fig. 2: Effect of low frequency ultrasound, UV-C, and the combination of both treatments on the total viable count (CFU/ml) at different spectral attenuation coefficients at 254 nm (SAC_{254}). (A) SAC_{254} = 27 1/m, (B) SAC_{254} = 71 1/m. Data are presented as mean ± SD (n = 15), different superscripts (a, b) denote significant differences between groups ($p < 0.05$).

A significant reduction of particle size in turbid water resulted from LFUS sonication with a mains energy of 19 kJ/L (Fig. 3). Analysis of the D10, D50, and D90 percentiles indicated significant particle size reduction ($p < 0.05$) with D10 = 21.1 µm, D50 = 72.8 µm and D90 = 262.7 µm before sonication to D10 = 12.3 µm, D50 = 38.8 µm and D90 = 187.6 µm after sonication.

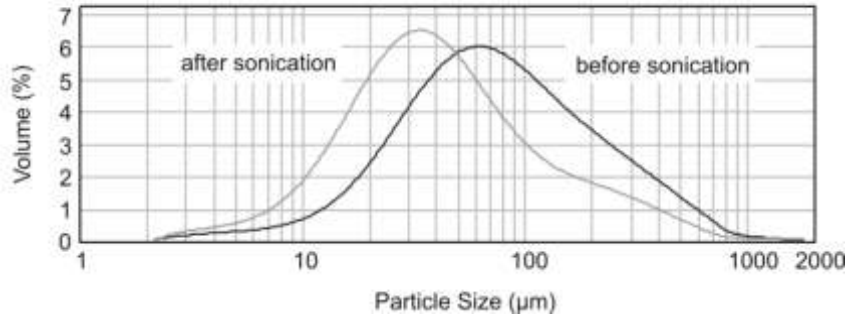

Fig. 3: Effect of low frequency ultrasound applied with 19 kJ/L (480 W/L for 39 Sec) on particle size distribution in water of a recirculating aquaculture system.

For the eukaryotic organisms, in clear water UV-C doses of 0.13 - 1.3 kJ/L (consumed energy) resulted in inactivation rates ranging from 8 - 41 % for *Paramecium*, 37 - 76 % for *Anguillicola* larvae and 8 - 82 % for *Artemia* metanauplii (Fig. 4).

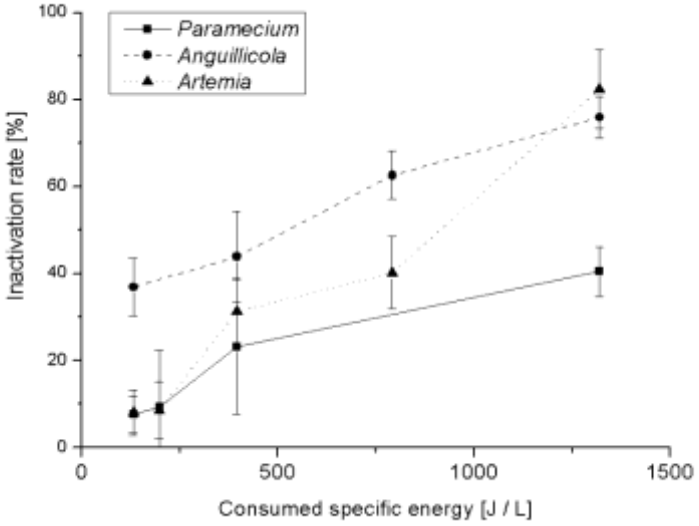

Fig. 4: Dose-dependent inactivation of *Paramecium* sp., second-stage larvae of the nematode *Anguillicola crassus* and metanauplii of *Artemia* sp. by UV-C irradiation. Data are presented as mean ± SD (n = 5).

Sonication by LFUS with 0.2 - 18.8 kJ/L resulted in a dose-dependent inactivation rate of *Paramecium* ranging from 7 to 95 %. For *Anguillicola* larvae, LFUS ranging from 1.9 to 18.8 kJ/L resulted in inactivation rates ranging from 19 - 81 %, and doses ranging from 0.2 to 1.9 kJ/L resulted in 70 to 99 % inactivation rates of *Artemia* metanauplii (Fig. 5). The dose-dependent inactivation of *Paramecium*, *Anguillicola* larvae and *Artemia* metanauplii can be described by functions of an exponential decay. In the interval 0 - 18 kJ/L the best-fit decay functions are:

Paramecium: $y = 104 - 103 \exp(-x / 7.30)$; $r^2 = 0.990$
Anguillicola: $y = 83 - 79 \exp(-x / 5.19)$; $r^2 = 0.979$
Artemia: $y = 100 - 101 \exp(-x / 0.34)$; $r^2 = 0.998$

where y is inactivation rate in % (defined for $0 \leq y \leq 100$) and x is consumed energy in kJ/L. When *Artemia* or *Paramecium* were UV-C irradiated following LFUS sonication, with respect to the consumed energy, the combination of both treatments had a similar efficiency compared to individual application of LFUS.

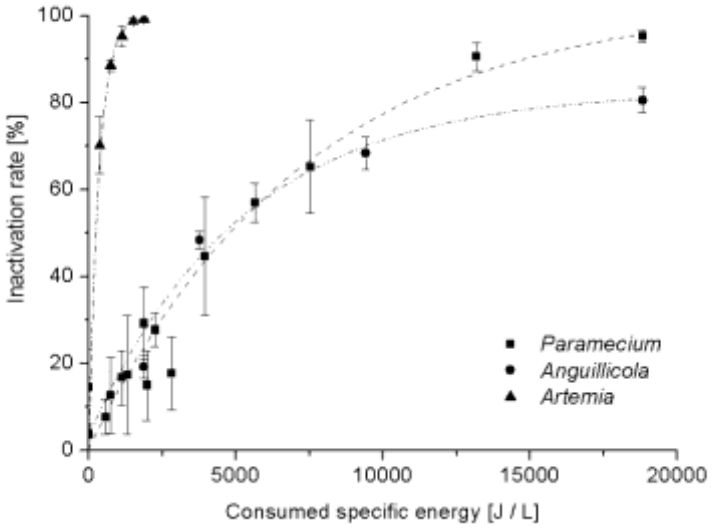

Fig. 5: Dose-dependent inactivation of *Paramecium* sp., second-stage larvae of the nematode *Anguillicola crassus* and metanauplii of *Artemia* sp. by low frequency ultrasound. Data are presented as mean ± SD (n = 5). Regression equations and r^2 values are mentioned in the text.

Alternating LFUS power density (W/L) and exposure times (s) to add up a constant volume-specific energy of 1.9 kJ/L resulted in constant inactivation rate of *Artemia* metanauplii. Only the lowest tested LFUS density (48 W/L) was slightly less effective in clear water ($p < 0.05$; Fig. 6). Under these conditions, LFUS worked irrespective of UV-C attenuation properties of the treated water (Fig. 6).

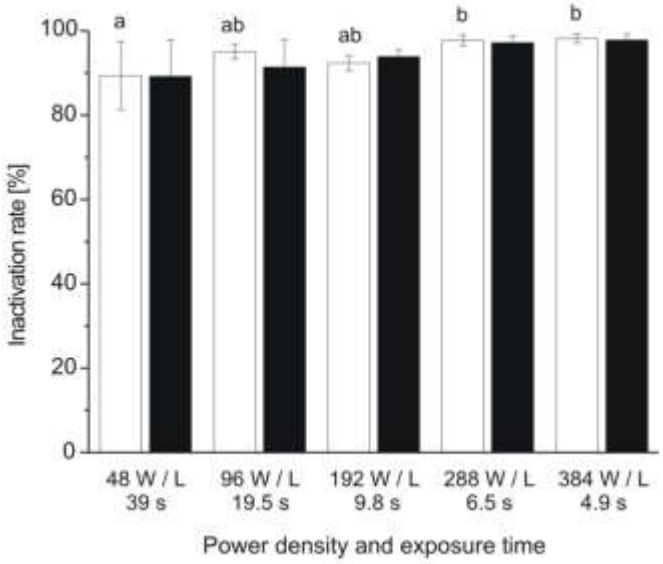

Fig. 6: Effect of low frequency ultrasound at constant applied energy, but variable power on inactivation of *Artemia* metanauplii in water with different spectral attenuation coefficients at 254 nm (SAC_{254}). White bars: SAC_{254} = 27 1/m, black bars: SAC_{254} = 71 1/m. Data are presented as mean ± SD (n = 5), different superscripts (a, b) denote significant differences between treatments in clear water ($p < 0.05$); in turbid water inactivation rates were not significantly different.

Discussion

This study is the first assessment of LFUS as a disinfection technique for aquaculture purposes. Pathogenic bacteria, ciliates, nematodes and crustaceans represent major groups of pathogens causing losses in aquaculture. The organisms used in the present study were chosen to be representatives of each of these groups and cover a size range from a few micrometers to half a millimeter. Heterotrophic bacteria comprise important fish pathogens causing diseases like vibriosis and streptococcosis. The ciliate *Paramecium* was chosen as surrogate for common ectoparasites like *Ichthyophthirius* and *Trichodina*. *A. crassus*, being a parasite itself, was presumed to be representative of other fish nematodes, for example *Camallanus*. Finally, *Artemia* metanauplii represent larvae of parasitic crustaceans such as *Lernaea*.

The effect of the treatments differed for the target organisms. As expected, UV-C proved to be highly effective against bacteria, but its efficiency is significantly impaired by attenuation due to absorption and scattering caused by high amounts of DOM and suspended solids. Consequently, the reduction of bacteria caused by a mains energy of 0.13 kJ/L, was one to two orders of magnitude higher in water with a relatively low UV-C attenuation (SAC_{254} = 27 1/m) compared to water with a high UV-C attenuation (SAC_{254} = 71 1/m). The latter conditions can be found in recirculating aquaculture systems with a high stock density and low water exchange. Even under such high UV-C attenuation conditions CFU counts were still reduced by more than one order of magnitude. Treatment with LFUS alone did not prove effective in reducing the CFU values within the energy range applied in this study (energy consumption up to 19 kJ/L). The successful application of LFUS against bacteria found in previous studies was due to considerably higher energy inputs (Gogate, 2007). Holm et al. (2008) for example, working with LFUS of a frequency of 19 kHz, found that a 90 % inactivation of different bacteria species requires energy densities ranging from 80 to 1240 kJ/L. Compared to the efficiency of UV-C irradiation in reducing bacteria, even under high UV-C attenuation, sonication would require at least 600-times the energy input. Irrespective of the technical and financial constraints of implementing such a powerful sonication device for the high flow rates in aquaculture systems, extensive energy input would lead to an unacceptable water temperature increase (Madge and Jensen, 2002).

Bacteria associated with particles can represent a significant proportion of total bacteria in the water (Simon *et al*., 2002), and these particles protect bacteria from external stressors such as UV-C irradiation (Parker and Darby, 1995; Tang *et al*., 2011). Therefore, de-agglomeration of suspended particles can significantly improve the efficiency of UV-C against bacteria. In the present study, water pretreatment with LFUS improved ultraviolet-C efficacy up to 0.6 log units compared to individual application of UV-C with the same dose of 1.3 kJ/L (consumed energy). Similarly, Blume and Neis (2004) have shown an improved reduction of *Escherichia coli* and fecal streptococci in wastewater by 0.8 log units applying LFUS (20 kHz) pretreatment followed by UV-C irradiation with 1.5 kJ/L (consumed energy). However, in addition to this positive effect, a reduced mean particle size might also impair the mechanical removability of suspended solids in lamella separators or drum filters used in recirculating aquaculture systems. This possible side effect of LFUS needs further attention.

The inactivation of eukaryotic organisms, in our study ciliates, nematodes, and crustaceans, requires a much higher UV-C dose compared to the inactivation of bacteria. Even the highest UV-C dose we could realize in our experiments, being ten-fold higher than the dose reducing

the CFU by 2.3 log units, only killed approximately 80 % of the crustacean and nematode larvae, and 40 % of the ciliates.

LFUS proved to be effective against each of the three eukaryotic model organisms, but the efficiency of sonication differs greatly between species. *Artemia* as the largest target organism with a body length of 500 - 600 µm was the most affected by LFUS. A dose of 1.9 kJ/L was sufficient to achieve a 99 % inactivation of the metanauplii. Compared to ciliates with a cell length of 70 – 80 µm, *Artemia* metanauplii required only one twentieth of the LFUS dose to obtain an inactivation rate of 99 %. Holm *et al*. (2008) also found an inverse relationship between LFUS treatment efficiency and organism size. Accordingly, Wolber and Pietrock (2004) identified a LFUS dose of 6.3 kJ/L to kill 100 % of cercaria of *Bucephalus polymorphus* that have a body length of about 200 µm.

Artemia has already been used as model organism for the evaluation of LFUS as treatment option for ballast water (Holm *et al*., 2008; Sassi *et al*., 2005). Using a laboratory-scale flow-through device consisting of a beaker and a rod-shaped sonotrode operating at a frequency of 19 kHz, Holm *et al*. (2008) determined an energy consumption of 8 kJ/L to obtain a 90 % elimination of *Artemia* nauplii. A similar, but larger device was used by Sassi *et al*. (2005) with a rod-shaped sonotrode mounted inside a steel box serving as flow-through device. With a frequency of 20 kHz they determined an energy consumption of 18 kJ/L for a 100 % elimination of *Artemia* nauplii. In the present study, comparable effects were obtained with ten-time lower energy consumption. These results show that technical optimization can considerably improve the operating efficiency of LFUS sonication for applications requiring high flow rates.

Comparing the energetic efficiency of LFUS and UV-C for 40 % inactivation, LFUS proved to be five times more efficient against *Artemia* than UV-C, whilst UV-C was three and even nine times more efficient against *Paramecium* and *Anguillicola* larvae, respectively. However, comparing the energy efficiency of LFUS sonication and UV-C irradiation, it must be considered that the efficiency of a UV-C device depends on the SAC_{254} of the treated water, the construction of the reactor and type of UV-C lamp. For the UV-C reactor used in our study, increasing the SAC_{254} value from 22 1/m to 70 1/m increases the energy demand by factor 5.5 to obtain the same effective UV-C dose. Furthermore, the gap width of the reaction chamber is crucial, especially at high SAC_{254} values as attenuation increases exponentially with the gap width. Realization of high power densities with medium pressure lamps would decrease the UV-C efficiency by factor 3 because their germicidal UV efficiency is only one third of a low pressure mercury lamp (U.S. Environmental Protection Agency, 2006). Under

such conditions, the efficiency of LFUS and UV-C against ciliates or nematode larvae will be in a similar range, and under most unfavorable conditions for UV-C, irradiation of LFUS would be the most efficient treatment.

The possibility to increase the UV-C dose against eukaryotic parasites is limited by the photo-induced reduction of nitrate to nitrite. In particular, irradiation with medium pressure mercury lamps can lead to a significant formation of nitrite from nitrate, because this type of lamp emits wavelengths down to 200 nm where nitrate absorbs strongly (IJpelaar *et al*., 2005). Nitrite is highly toxic to aquatic animals, e.g. salmonids are affected by concentrations far below 1 mg/L (Roberts and Schlotfeld, 1985). Nitrite concentrations above 1 – 3 mg/L can be reached, if UV-C doses much higher than recommended for bacterial disinfection are applied in combination with high prevalent nitrate concentrations (Sharpless *et al*., 2003). Such high nitrate concentrations of 100 – 1000 mg/L NO_3^-N commonly appear in highly stocked recirculating aquaculture systems (Van Rijn, 2010).

In contrast to UV-C, LFUS is robust against differences in water quality and can safely be applied at energy densities that are effective against a wide range of parasites like ciliates, nematodes and crustaceans. Thus, a combination of UV-C (against viruses and bacteria) and LFUS (against eukaryotic parasites) could provide an appropriate water treatment with regards to all relevant pathogens in recirculating aquaculture systems.

As described, for the inactivation of *Artemia* metanauplii, the destructive effect of LFUS depends on the applied volume-specific energy that can be realized by any constant product of the specific power and retention time. Only the inactivation rates determined for the lowest power density (48 W/L) showed a slightly reduced efficiency. The findings of this aspect of the experiment might be helpful for further adjustment of ultrasound reactors, since specific applications have different needs concerning inactivation rates of certain species, as well as technically feasible flow rate and power.

Acknowledgements

We thank Dr. Gustav Csik (a.c.k. aqua concept GmbH, Karlsruhe, Germany) for technical advice and information. We also thank Prof. Dr. Cornelia M. Keck and Run Chen who made possible the laser diffraction analysis. Also we appreciate the support of Prof. H.-P. Grossart, Dr. Elke Zwirnmann and Claudia Theel for advice, and Josefin Stiller who provided writing assistance. This work was supported through a grant by Deutsche Bundesstiftung Umwelt (DBU).

Kapitel 4

Evaluation of the combined ultrasound/ultraviolet flow through method for disinfection of water in recirculating aquaculture systems

Ra’anan Ariav

Introduction

Recirculating aquaculture systems have become more and more popular. They are commonly found in aquaculture facilities, wholesale and retail tropical fish facilities, and public aquaria. However, water quality fluctuations in these systems (such as temporary increases in ammonia or nitrite) will result in disease or significant losses. These environmental fluctuations will often lead to suppressed immune systems and greater susceptibility to pathogens (i.e., disease-causing organisms, such as bacteria, parasites, fungi, and viruses) and disease outbreaks.

Recirculating systems favor the growth of many disease-causing organisms and spread of disease. There are a number of reasons for this tendency, including higher densities of fish when compared to other culture systems; buildup of sediment and subsequently pathogens in tanks, sumps, or filtration components (especially mechanical and biological filters); and slower turnover of water.

Over time, pathogens can become concentrated (i.e., present in high numbers). Most pathogens are considered opportunistic, causing disease only in fish with suppressed immune systems. However, if pathogens become sufficiently numerous they can also cause disease in healthy fish. In addition, the continuous flow of water throughout a system can spread pathogens rapidly, especially in a system lacking adequate disinfection protocols or components.

System description

The testing infrastructure in this experiment included the use of the new ULTRASCHALL U.V. REACTOR (AQ 1000 which was supplied by the ULTRASCHALL UV GENERATOR (AQ 1001).

Clinical trial protocol

Three Lg. Tanks (10 cubic Meters each) were re-circulated for a period of 120 Days in the Aquavet Wet – Lab facility.

Tank I (reservoir) was stocked with 500 Hybrid *Tilapia* characterized by heavy presence of bacterial and parasitic infection. Affected fish in Tank I showed corneal opacity, exophthalmia, erratic swimming, sunken body, ulceration and inflammation along the lateral line.

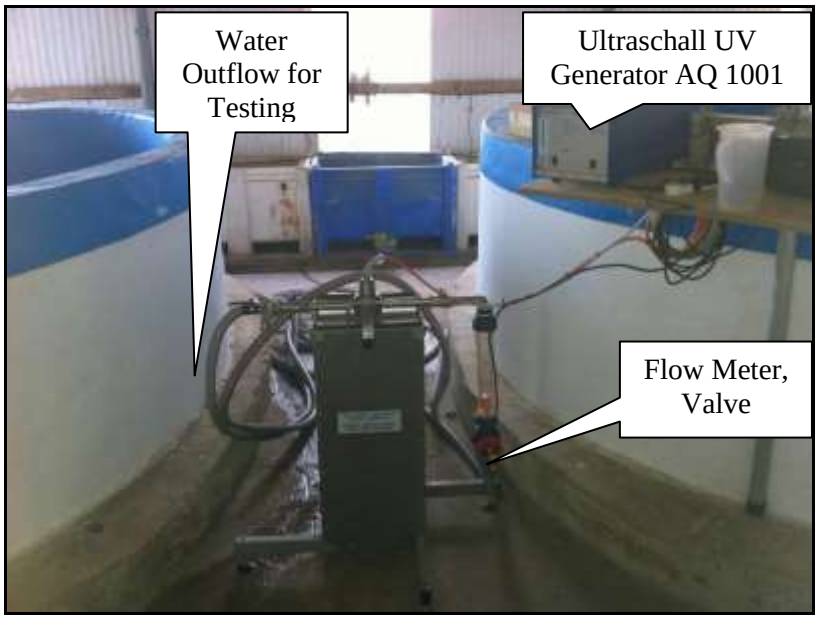

Fig. 1: The Ultraschall AQ 1000 US/UV reactor in the AquaVet wet – lab facility

Fig. 2: The US/UV experimental tank in the AquaVet wet – lab facility

Fig. 3: Severe ulceration in Hybrid *Tilapia* – TANK I (Reservoir)

Fig. 4: Severe clinical symptoms of Gram (+) *Streptocoucus* spp. infection - TANK I (Reservoir)

Isolation of Bacterial disease causing agents

Presence of Gram (-) *Aeromonas* spp. in these fish was established following standard bacteriological isolation procedures:

Bacteriological samples were collected from internal organs and external ulcers of clinically symptomatic fish. Samples were collected by a sterile inoculation loop, and inoculated on plates with brain heart infusion (BHI) agar. The inoculated plates were incubated at 25ºC for 24-48 h.

Bacteria growing in all plates were identified as *Aeromonas* spp. using the following criteria:

- Morphological features: The colonies appeared circular, raised, and translucent. They produced a brown pigment that diffused into the medium, surrounding the colonies.
- Gram stain: The bacterium is stained red and is therefore Gram negative.

Presence of Gram (+) *Streptococcus* in these fish was established following standard bacteriological isolation procedures:

The selected tilapias were killed before swabs from brains, kidneys and eyes were collected and immediately streaked onto blood agar. The agar plates were then incubated at 30°C for 24 hours. Colonies suspected of *S. agalactiae* were further tested and confirmed using the API 20 STREP Detection Kit.

Tank II (treatment) and Tank III (control) were stocked with 500 Hybrid *Tilapia* (each), free of infectious disease. Both tanks were treated with two consecutive treatments of formalin at the recommended dose of 30 ppm (for 6 hours) in order to eliminate any presence of parasitic pathogens prior to the beginning of recirculation.

In addition, Tank II (treatment) and Tank III (control) were treated with medicated feed (O.T.C. at 100 mg per kg of body weight x 15 days) between 1/11/2011 – 15/11/2011 in order to eliminate any presence of bacterial pathogens and/or bacterial disease symptoms in this population prior to the beginning of recirculation. Fish in both tanks were found free from infectious disease on 15/11/2011 (Day 1 of the clinical trial).

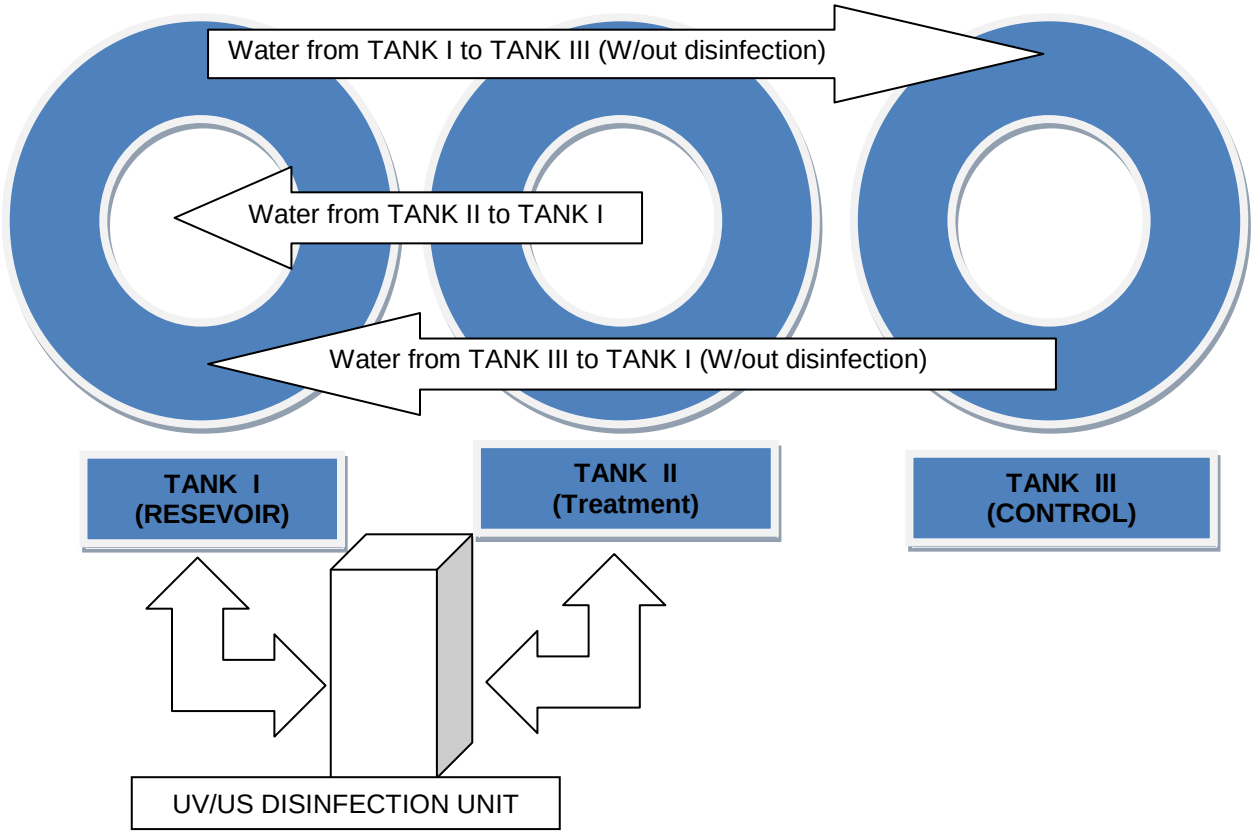

Fig. 5: Schematic view of experimental design

All incoming water from Tank I (reservoir) to Tank II (treatment) was treated by the combined Ultrasonic/Ultraviolet disinfection system. Water from Tank I (reservoir) was not treated prior to its transfer to Tank III (control).

Fish in all three tanks were monitored for a period of 120 days. Presence of mortality in all three tanks was monitored on daily bases. Presence of parasitic and bacterial infection in TANKS I, II and III was monitored every 30 days.

Water quality parameters in all three tanks were monitored on daily bases. These parameters included: ammonia, nitrite, pH, temprature, oxygen.

Bacteriology

We evaluated the effect of the UV/US disinfection system on total bacterial counts in TANK I, II and III.

Total bacterial contamination of incoming and out flowing water was evaluated by utilization of the hy-giene monitoring system. The hy-giene monitor line of dip slides provides the solution for reliable monitoring kits of bacteria. The hy-giene monitor incorporates a double-sided paddle poured with specially formulated growth media. The paddle's design allows dip testing and the hermetically sealed vial ensures a long shelf life.

Fig. 6: The Hy-Giene monitoring system

Parasitology

Tilapia spp. in Tank I were characterized by heavy loads of external parasites. Microscopic examination of gill and skin biopsies revealed the presence of *Trichodina*, *Costia* (*Ichthyobodo necatrix*) and *Glossatella* spp.

Trichodina is a genus of ciliate protists that is ectocommensal or parasitic on aquatic animals, particularly fish. They are characterized by the presence of a ring of interlocking cytoskeletal denitcles, which provide support for the cell and allow for adhesion to surfaces including fish tissue. Trichodinids have a simple direct life cycle. That is, they have a single host and do not use alternation of generations or mass asexual replication off the host. They reproduce by binary fission, literally cell-splitting. As such, presence of *Trichodina* spp. in all 3 fish populations was regarded as a clear indicator as to the possible effect of the U.V./U.S. disinfection system.

Taxonomy

Trichodinids are members of the peritrichous ciliates, a paraphyletic group within the Oligohymenophorea. Specifically, they are mobiline peritrichs because they are capable of locomotion, as opposed to sessiline peritrichs such as *Vorticella* and *Epistylis*, which adhere to the substrate via a stalk or lorica. There are over 150 species in the genus *Trichodina*. *Trichodinella*, *Tripartiella*, *Hemitrichodina*, *Paratrichodina* and *Vauchomia* are similar genera.

Life history

Trichodinids are typically found on the gills, skin and fins of fishes, though some species parasitize the urogenital system. A range of invertebrates is also host to trichodinid infections, including the surfaces of copepods and the mantle cavity of molluscs. Transmission occurs by direct contact of infected and uninfected hosts, and also by active swimming of trichodinids from one host to another. *Trichodina* cells swim with the adoral surface facing forwards. On surfaces, they move laterally, with the adoral surface facing the substrate.

Pathogenesis

Most trichodinids are ectocommensal in that they use the fish only as a substrate for attachment, while they feed on suspended bacteria. Some species are certainly primary pathogens, however, since they occur in sterile sites (e.g. urinary system), or provoke pronounced responses on the part of the host (e.g. *Tripartiella* on gills).

Parasitic loads of *Trichodina* spp. on the fish in TANKS I, II and III (gill and skin) were evaluated microscopically, and were graded as follows:

1 – Very low parasitic load.

2 – Moderate parasitic load.

3 – High parasitic load.

4 – Very high parasitic load

5 – Parasitimia: Extremely high parasitic load

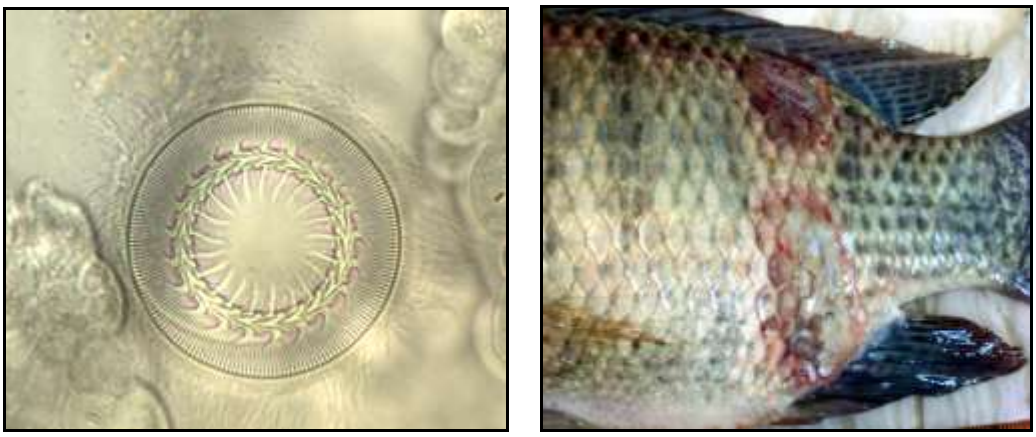

Fig. 7: *Trichodina* spp. infection in Tilapia

Results

Water quality parameters

The water quality parameters during the experiment are shown in Fig. 8.

Fig. 8: Water quality parameters during the experiment

Fish mortality

Fish mortality was monitored on daily bases in all 3 tanks. Mortality was observed in both, the control (Tank III) and the UV/US-treated tank (Tank II). However, the mortality in the tank connected to the UV/US reactor was about half of the mortality in the non-treated control (Tank III) (Tab. 4-1, Fig. 9).

Table 1: Effect of water treatment with the UV/US disinfection system on total fish mortality

Date	Tank I RESEVOIR	Tank II TREATMENT	Tank III CONTROL
15-30/11/2011	39	2	2
1-31/12/2011	47	9	22
1-31/1/2012	79	13	39
1-29/2/2012	91	18	39
1-15/3/2012	75	22	34
TOTAL MORTALITY	331	64	136

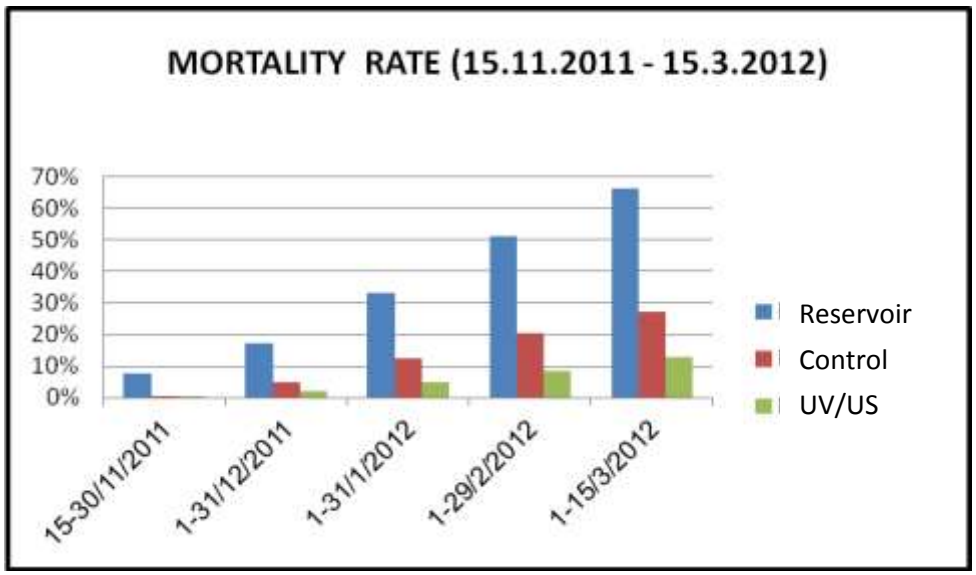

Fig. 9: Effect of water treatment with the UV/US disinfection system on the fish mortality rate.

Bacteriology

Treatment with the UV/US disinfection system resulted in a significant reduction of the heterotrophic bacteria suspended in the fish tanks. Compared to the reservoir (Tank I) and the non-treated control (Tank III), the CFU value in the tank connected to the UV/US reactor was reduced by 9 and 8 log units, respectively (Tab. 4-2).

Table 2: Effect of water treatment with the UV/US disinfection system on total bacterial counts

Date	Tank I RESEVOIR	Tank II TREATMENT	Tank III CONTROL
01/12/2011	1.00E+11	1.00E+05	1.00E+11
01/01/2012	1.00E+12	1.00E+04	1.00E+12
01/02/2012	1.00E+13	1.00E+04	1.00E+12
01/03/2012	1.00E+13	1.00E+04	1.00E+12
AVERAGE	1.00E+13	1.00E+04	1.00E+12

Parasitology

In the reservoir (Tank I), fish constantly showed an extremely high load with the ciliate fish parasite *Trichodina.* In the control (Tank II), the infection level became high two and a half months after the experiment started. In contrast, until that time, no *Trichodina* were found in the UV/US treated tank. However, until end of the experiment, moderate parasitic loads were also found in the UV/US treated tank (Fig. 10).

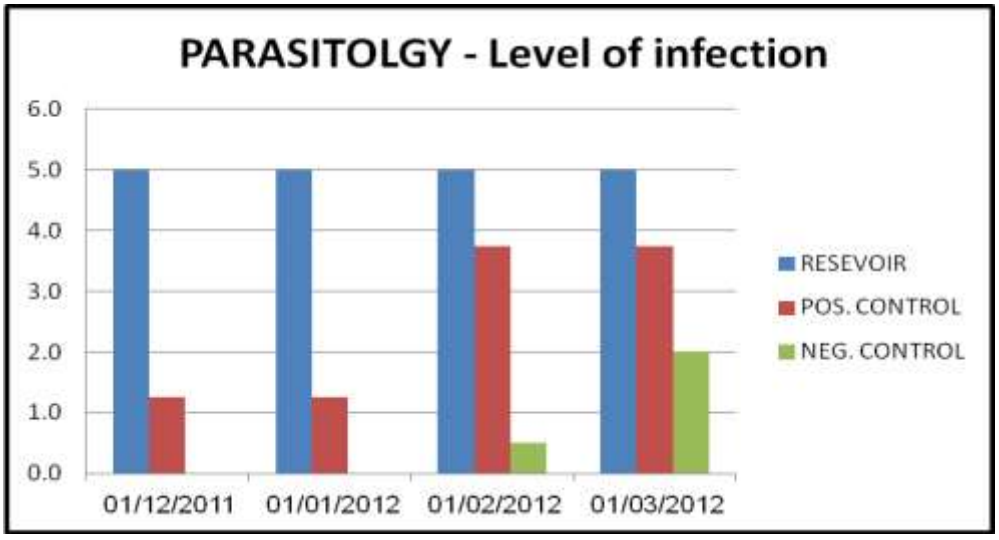

Fig. 10: Effect of water treatment with the UV/US disinfection system on *Trichodina* infection

Conclusions

Following four months of observation during the winter period of 2011 – 2012 , we may conclude that the combined effect of the combined U.V. / U.S. system was highly effective in preventing the transfer of infectious disease agents between re-circulated tanks.

This positive effect was clearly expressed by the following parameters:

- Decreased level of infection in the treated tanks (Parasitological and Bacteriology) Decreased morbidity rate in the treated tank.
- Decreased mortality rate in the treated tank.
- Overall health status of the fish in the treated tank.

These results are evident following comparison of morbidity and mortality in TANK II (treated water) to those obtained from Tank III (untreated water, control). Detailed evaluation of morbidity and mortality patterns in Tank III (control) revealed a steady increase in the frequency of disease, bacterial counts, morbidity and mortality. These results are in accordance to those obtained during our first year of operation of the U.V/U.S. system.
Based on these results, we clearly recommend further development and use of this technology in recirculating aquaculture systems.

The development of the combined U.V./U.S disinfection system results in numerous benefits:

1) Higher yields due to decreased losses from morbidity and mortality
2) Higher yields due to increased feed conversion efficiency
3) Reduced utilization of medications & chemicals
4) Higher prices due to enhanced public perception of quality (both specific markets and aquaculture in general)
5) Value-added products for penetration of growing niche markets (e.g., "organically" grown)
6) Reduced trade barriers and enhanced ability to export products
7) Reduced regulatory pressure due to lessened environmental impacts and concerns

While there will certainly be economic costs for using this technology, there is considerable potential for a large, positive, cost/benefit ratio.

Kapitel 5

Application of low frequency ultrasound and UV-C in recirculating aquaculture systems

Amir Abbas Bazyar Lakeh, Klaus Knopf

Abstract

Low frequency ultrasound (LFUS) and UV-C light were evaluated individually and in a combined mode as disinfection measures for recirculating aquaculture systems. During a single passage of the flow through reactor, LFUS (25 kHz) with a dose of 1.8 -18 kJ L^{-1} significantly reduced the ciliated protozoan parasite *Trichodina* by 28 - 99%, respectively. The least LFUS dose for the elimination of this fish parasite was estimated with a mathematical model, assuming exponential growth and linear elimination. Operating LFUS in a bypass, treating 40 % of the total volume of the system per hour, lead to a 96% reduction of the free-floating *Trichodina* sp. within 96 h. In contrast, the reduction of suspended bacteria requires a higher flow rate through the reactor. UV-C applied to the entire flow of the system significantly reduced the bacteria density in fish tanks, but the same UV-C dose in a 50 % bypass could not achieve this goal. Toxicological tests, including the fish egg test and the luminescent bacteria test, gave no indication that UV-C irradiation or LFUS sonication or the combination of both techniques lead to the formation toxic byproducts. The results of this study proved the suitability of LFUS for the reduction of eukaryotic parasites from the water. The combined application of UV-C and LFUS can be a useful disinfection strategy in recirculating aquaculture system. In order to reduce the bacterial load of the water, UV-C should be applied continuously on the total water flow of the system, whilst LFUS against eukaryotic parasites could also be applied temporarily and in a bypass mode.

Introduction

The application of UV-C light for water disinfection is one of the most important disinfection strategies in the aquaculture industry (Kasai *et al*., 2002). Recently, the application of low frequency ultrasound (LFUS) showed a promising disinfection potential as a novel water disinfection method against eukaryotic pathogens in recirculating aquaculture systems (RAS) (Bazyar Lakeh *et al*., 2013). In this chapter, we describe the evaluation of LFUS individually and in combination with UV-C in research recirculating aquaculture systems. Toxicological tests were performed in order to investigate if the treated water contains toxic by-products.
Since LFUS requires a relatively high energy input, we tested whether a LFUS flow through reactor can efficiently operate against parasites in a bypass flow. Subsequently, with respect to the combined LFUS / UV-C reactor, the same question was examined for the efficiency of UV-C against bacteria.

Materials and methods

Toxicological tests

The fish egg test (DIN EN ISO 15088) and luminescent bacteria test (DIN EN ISO 11348) to evaluate possible formation of toxic by-products by UV-C, LFUS and their combination. Zebrafish (*Danio rerio*) eggs (Fig. 1) and luminescence bacteria, *Vibrio fischeri* (Fig. 1) were exposed to LFUS sonicated water (25 kHz, consumed energy 18 kJ L^{-1}), UV-C irradiated water (consumed energy 0.42 kJ L^{-1} and 6.4 kJ L^{-1}) and the combination of LFUS (18 kJ L^{-1}) and UV-C (0.42 kJ L^{-1}). The water for these tests was taken from a RAS stocked with 21 kg fish per m^3.

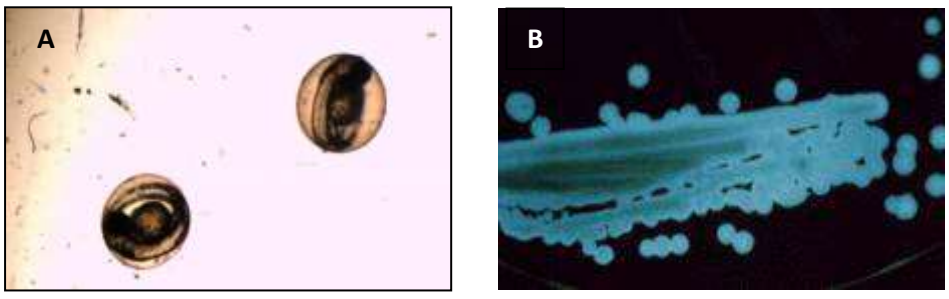

Fig. 1: Zebrafish (*Danio rario*) egg (A) and luminescent bacteria (*Vibrio fischeri*) (B)

Four toxicological endpoints (observed after 24h and 48 h) were used in the fish egg test: coagulation of eggs and embryos, failure to develop somites, lack of heart-beat, and non-detachment of the tail from the yolk. For luminescent bacteria test the inhibitory effect of water samples on the light emission of *Vibrio fischeri* was determined.

Evaluation of LFUS against parasites

The efficiency of LFUS against parasites was examined in a RAS with a total volume of 16 m^3, stocked with European sturgeon, *Acipenser sturio* (6.2 kg m^{-3}). Clinical symptoms of the fishes such as jumping, gathering at the water inflow and rapid gasping indicated an infection with ectoparasites. Parasitological examination confirmed the infection of the fish with *Trichodina* sp. (Fig. 2).

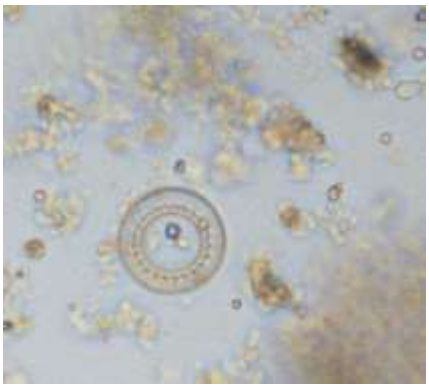

Fig. 2: *Trichodina* sp. in the water of a recirculating aquaculture system

First, the amount of free-floating *Trichodina* sp. and the dose-dependent elimination rate of *Trichodina* achieved by a single pass through the LFUS reactor were determined. The least LFUS intensity required was estimated with a mathematical model assuming exponential growth and a linear elimination of the parasites:

$$N_t = N_0 \times e^{\left(\ln\left(\frac{2}{t_g}\right) - p \times \frac{Q}{V}\right)} \times t$$

with

N_t = number of parasites at time t

N_0 = initial number of parasites

t_g = generation time of the parasite

p = elimination rate

Q = flow rate

V = total volume of the RAS

t = time

For t_g = 24 h (Feng, 1985), V = 16 m³ and Q = 6 m³ h^{-1}, the model reveals that an elimination rate of 25 % could result in a reduction of the parasites by 90 % within 2 days. Consequently, two LFUS reactors, each of them operated at 1.4 kW (25 kHz) and 3 m^3 h^{-1}, were installed in parallel to sonicate 40 % of total water volume per hour.

The duration of this experiment was 96 h and the water was sampled daily to check the elimination rate of *Trichodina*. Therefore, water samples were fixed with Lugol's iodine and allowed to settle for 24 h in 50 ml Utermöhl chambers (HYDRO-BIOS Apparatebau GmbH, Germany). The parasites were enumerated using an inverted microscope. *Trichodina* with an abnormal and broken shape were considered to be irreversibly harmed and dead (Fig. 3).

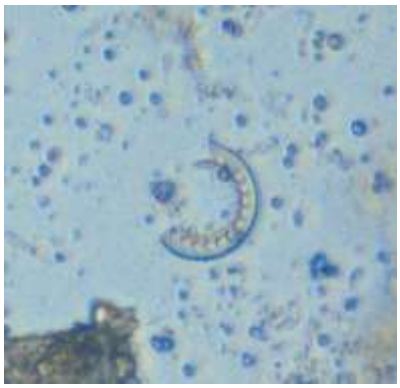

Fig. 3: *Trichodina* sp. harmed by application of LFUS

Evaluation of UV-C irradiation in a bypass mode

Studies on the effectiveness of an UV-reactor operating in a bypass mode were performed in two RAS with a total water volume of 12 m^3, each, stocked with Nile tilapia, *Oreochromis niloticous* (12.5 kg m^{-3}). In one RAS, a 110 W UV-C reactor (Fig. 4) was operated either with the total water flow of the system (16 m³ h^{-1}) or in a bypass mode with 8 m³ h^{-1}. Finally, a 110 W UV-C reactor was operated following a pretreatment with LFUS (1.4 kW) in a bypass mode at a flow rate of 3 m³ h^{-1}. The second RAS, not treated with UV-C, was used as control.

The duration of all experiments was 4 d, and each day the total viable count (CFU ml^{-1}) was determined by the spread-plate technique using a nutrient agar (DEV) (Carl Roth, Germany). Additionally, the total bacterial number was determined by DAPI staining by means of an epifluorescence microscope (Fig. 5).

Fig. 4: UV-C reactor (1), optional connection to external reactors (2)

Fig. 5: Bacteria stained with DAPI, representing the total bacterial count of the water sample

Results

Toxicological tests

The toxicological tests revealed no evidence of the formation of toxic by-products during UV-C irradiation and/or LFUS sonication of water from a RAS. Neither negative effects on the viability of the zebra fish egg (Table 1) nor on the light emission of *Vibrio fischeri* were observed (Table 2).

Evaluation of LFUS against parasites

The dose-dependent elimination rate of *Trichodina* achieved by a single pass through the LFUS reactor matched to the results we have previously determined for the model organism *Paramecium* sp. reported by Bazyar Lakeh *et al*. (2013). With increasing LFUS energy, the

inactivation rate of *Trichodina* also increased and reached up to 99 % when the LFUS was operated at 18 kJ L^{-1} (Fig. 6).

During continuous operation of LFUS in the RAS, within 4 d the number of free-floating *Trichodina* decreased by 96 %. The course of the measured elimination of the parasites followed the theoretical mathematical model, but was slower than predicted (Fig. 7).

Table 1: Fish egg test (DIN EN ISO 15088) with water from a RAS after UV-C irradiation and/or sonication. 3,4-Dichloroaniline (3,4-DCA) was used as positive control.

Treatment	**Egg mortality (%)**		**Coagulation**	**Formation of somites**	**Heart-beat**	**Tail detachment**
	24 h	48 h				
Positive control	80	80	yes	no	no	no
Negative control	0	0	No	yes	yes	yes
LFUS (18 kJ L^{-1})	0	0	No	yes	yes	yes
UV-C (1.3 kJ L^{-1})	0	0	No	yes	yes	yes
UV-C (6.4 kJ L^{-1})	0	0	No	yes	yes	yes
LFUS (18 kJ L^{-1}) + UV-C (1.3 kJ L^{-1})	0	0	No	yes	yes	yes

Table 2: Luminescent bacteria test (DIN EN ISO 11348) with water from a RAS after UV-C irradiation and/or sonication.

Treatment	**Inhibitory effect (%)**
Reference substance (Zinc sulfate)	82.1
Reference substance (Potassium dichromate)	46.3
Reference substance (3,5-Dichlorphenol)	33.2
Input water	-9.87
LFUS (18 kJ L-1)	-11.37
UV-C (1.3 kJ L-1)	-11.9
UV-C (6.4 kJ L-1)	-10.7
LFUS (18 kJ L-1) +UV-C (1.3 kJ L-1)	-10.4

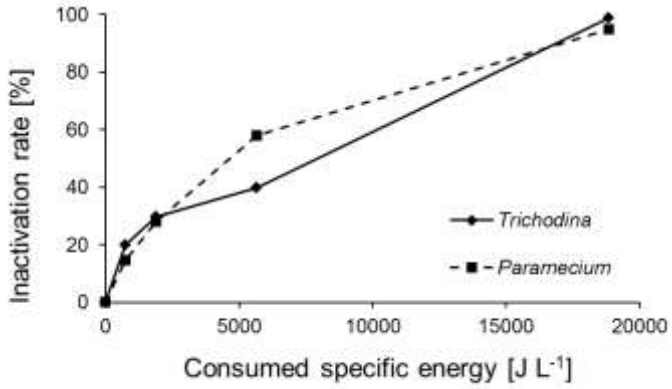

Fig. 6: Dose-dependent inactivation of *Trichodina* sp. and *Paramecium* sp. (data from Bazyar Lakeh *et al*., 2013) by LFUS (25 kHz). Data are presented as mean ± SD (n = 5).

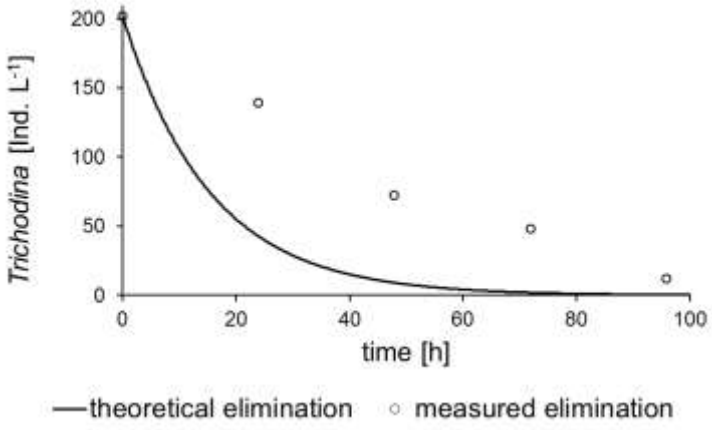

Fig. 7: Elimination of *Trichodina* from a recirculating aquaculture system by LFUS (25 kHz).

Evaluation of UV-C irradiation in a bypass mode

UV-C irradiation with a 110 W UV-C reactor significantly reduced the amount of suspended bacteria when the total water flow of the system was treated. After 96 h, the reduction determined by the spread-plate technique was 80 %, whilst evaluation of DAPI stained samples revealed an 89 % reduction of suspended bacteria. The same UV-C dose had no effect on the bacterial counts when the UV-C reactor was operated in a bypass mode. During

the observation periods, the bacteria numbers did not significantly change in the control system.

Discussion

In aquaculture industry especially intensive recirculating aquaculture systems, attention to the aquatic animal's health and the reduction of losses caused by diseases are key managing factors. Protection of cultured animal can be achieved by sanitary procedures and one of the most important elements is water disinfection and removing the undesired pathogens from water body. Protozoan parasites are one of the most important pathogenic group that cause an important economic loss in aquaculture (Durborrow, 2003).

In the first part of this project we have shown that a combination of UV-C (against viruses and bacteria) and LFUS (against eukaryotic parasites) could provide an appropriate water treatment with regards to all relevant pathogens in recirculating aquaculture systems (Chapter 3 and 4). At this point one needs information on the ideal dimensions of both techniques in order to guarantee an effective and economic operation. This is especially true for the combined reactor where the operating parameters for UV-C and LFUS have to be coordinated.

With respect to possible toxic byproducts, both, the fish egg test and the luminescence bacteria test revealed negative results, even if UV-C or LFUS were applied with the highest doses we could realize with our equipment. Obviously, the aquaculture water even had a nourishing effect on *V. fischeri*. However, it should be noted that all our experiments were conducted with a LP UV-lamp, and when using a MP lamp other results could emerge (compare IJpelaar *et al*., 2005). Furthermore, both tests did not detect the problem of photo-induced formation of nitrite from nitrate, which we became aware at a later time during the project (chapter 6). This might be explained by a certain nitrite tolerance of the test organisms. The toxicity of nitrite to bacteria is attributed to NO (Zhang *et al*., 2013), and *V. fischeri* uses an alternative oxidase, which plays a role in NO resistance (Dunn *et al*., 2010). Concerning the fish egg test, Meinelt *et al*. (2010) have shown that zebrafish eggs possess a high nitrite tolerance.

A simple mathematical model can be used to estimate the least elimination rate and flow rate that are required to achieve a reduction of a certain organism in the RAS. However, our model uses assumptions that are not met in the reality. The most critical points are that the pathogens would not show an exponential growth under limiting conditions, and they are not uniformly distributed in the water, but they also live in or on the fish. Thus, the model is suitable to get

an idea about the feasibility of a certain treatment, but finally the process parameters must be determined by empirical data.

The similarity of the dose-effect relationships for the inactivation of *Trichodina* sp. and *Paramecium* sp. by LFUS confirmed the suitability of *Paramecium* sp. as model organism for ciliated protozoan parasites (Bazyar Lakeh *et al*., 2013).

In accordance with the prediction based on the mathematical model, free-floating *Trichodina* sp. where efficiently reduced. However, the measured reduction was slower compared to the theoretical prediction. This observation can easily be explained by the fact that the parasites are not only free-floating in the water as assumed in the model, but of course also live on the fish.

UV-C irradiation can efficiently reduce the amount of suspended bacteria in the fish tanks. Assuming a mean generation time of 2 h 50 min for all the free bacteria (Leonard *et al*., 2000) and an inactivation by only one log unit, the mathematical model predicts that the bacteria in the RAS could be reduced when the UV reactor is operated with a least 30 % of the system volume per hour. However, our empiric results showed that the bacterial growth overcompensate the reduction by UV-C irradiation even when the UV reactor is operated with 70 % of the total system volume per hour.

In conclusion, the reduction of suspended bacteria requires a high turnover rate in order to compensate their short generation time. In contrast, free-floating parasites can also be reduced with a flow-through device operated in a bypass mode. Thus, in a combined UV / LFUS reactor, the UV-unit determines the necessary flow rate. Then, the UV-unit should operate continuously at a low power to reduce the bacterial load, whilst the more energy-consuming inactivation of parasites can be achieved with LFUS when required.

Acknowledgements

We thank Dr. Hans-Jürgen Pluta, Dr. Frank Brauer, Mrs. Martina Gutsche and Dr. Thomas Meinelt for their support in the implementation of the toxicological tests.

Kapitel 6

Photo-induced formation of nitrite from nitrate by low-pressure UV lamp irradiation

Amir Abbas Bazyar Lakeh, Daniel Graeber, Klaus Knopf

Submitted to *Aquaculture*

Abstract

During the disinfection of water by ultraviolet-C (UV-C) light, photo-induced formation of nitrite from nitrate may occur, and the amount of nitrite formed increases with the ambient nitrate concentration. The importance of nitrite as undesired disinfection by-product during irradiation of natural waters with low-pressure or medium-pressure UV lamps for drinking water quality has been the subject of previous works. However, UV technology is also applied for the treatment of water with considerably higher nitrate concentrations than those relevant in the drinking water industry. Recirculating aquaculture systems (RAS) are commonly characterized by high concentrations of nitrate (several 100 mg L^{-1}) as the end product of the nitrification process. Under these conditions, intensive UV-C irradiation might result in nitrite concentrations that are critical for the cultured animals. In the present study, the photo-induced formation of nitrite by irradiation with a low-pressure UV lamp was evaluated for UV doses ranging from 42 to 6300 J L^{-1} and nitrate concentrations ranging from 100 to 1200 mg L^{-1}. The results indicate that irradiation high UV doses at high ambient nitrate concentrations can lead in a nitrite formation that can reach lethal or sub-lethal concentrations for some cultured fish species.

Introduction

One of the most important factors in recirculating aquaculture systems (RAS) is the water quality management as it directly affects the fish health status and the production cycle (Nicholson *et al*., 1990). Water quality is closely related to the water treatment process within the system and if this is not controlled properly, low water quality can negatively affect fish growth, increase the risk of infectious disease and stress, and consequently result in loss of production (Timmons *et al*., 2002).

The application of ultraviolet (UV) light is a common, powerful disinfection method to reduce the microbial load of the water and providing better health conditions for fish (Chevrefils *et al*., 2006; Masser *et al*., 1999). The production of undesired by-products during UV irradiation has been matter of concern of several studies, as these by-products may violate safety requirements and corresponding drinking water regulations (Buchanan *et al*., 2006; IJpelaar *et al*., 2005; Lu *et al*., 2009; Sharpless *et al*., 2003; Sharpless and Linden, 2001; Sonntag and Schuhmann, 1992). The photo-induced formation of nitrite, NO_2^-, from NO_3^- by UV irradiation might pose a serious health risk because NO_2^- adversely affects the oxygen-carrying capacity of blood by changing hemoglobin to methemoglobin (Jensen, 2003; Tomasso, 1994). The photochemistry of NO_2^- and NO_3^- is well described (Mack and Bolton, 1999). In aqueous solutions, NO_3^- has a strong absorption in the lower UV spectrum below 230 nm with a maximum at 200 nm, and a weak absorption with a maximum at 300 nm. NO_3^- photolysis leads to the formation of NO_2^- and oxygen, O_2 (Mack and Bolton, 1999; Takeda and Fujiwara, 1993). The level of NO_2^- formation depends strongly on the UV dose and the ambient NO_3^- concentration (Mack and Bolton, 1999; IJpelaar *et al*., 2005; Lu *et al*., 2009; Sharpless *et al*., 2003) and rises with increasing pH (Mack and Bolton, 1999; Lu *et al*., 2009).

With respect to the formation of byproducts such as NO_2^- it is important to consider the different emission properties of low-pressure (LP) and medium-pressure (MP) mercury lamps, both of which are used as UV light source (Sharpless *et al*. 2003). Due to the high germicidal efficiency and relatively low operation costs, LP lamps, characterized by an essentially monochromatic light output in the germicidal range at 253.7 nm (UV-C), are commonly used in RAS (Summerfelt, 2003). As alternative UV light source, MP lamps also have the ability to disinfect water in RAS. These lamps emit polychromatic light ranging from 200-600 nm and allow the application of a higher power density, but are less efficient compared to LP and thus are rarely used (Summerfelt, 2003). In drinking water industry, NO_2^- formation is insignificant when LP lamps are used (IJpelaar *et al*., 2005) but MP lamps can cause a much higher NO_2^- formation as these have a much stronger emission at wavelengths between 200

and 240 nm, where NO_3^- absorbs strongly (IJpelaar *et al*., 2005; Sharpless *et al*. 2003, Summerfelt, 2003). However, even for LP lamps, the application of hydrogen peroxide as well as alkaline conditions and uncommonly high UV doses can result in a NO_2^- yield that exceeds the common drinking water standard of 1 mg L^{-1} (Lu *et al*. 2009, Sharpless *et al*., 2003).

In RAS, NO_3^- concentrations can easily reach several hundred milligrams per litre as it accumulates as the end product of the nitrification process (Pillay and Kutty 2005). This is much higher than in natural waters used in the drinking water industry and under these high NO_3^- concentrations, UV-C irradiation by LP lamps could result in critical NO_2^- yields, as NO_3^- absorbs light also in the range of UV-C emitted by LP lamps when it occurs in very high concentrations. Furthermore, the photo-induced formation of NO_2^- might limit the use of high UV doses that are required for the elimination of protozoan and metazoan parasites (Bazyar Lakeh *et al*., 2013; Kasai *et al*., 2002).

The risk of NO_2^- formation by UV irradiation is known in the aquarium hobby, where LP lamps are used. However, there are no published studies on whether the use of LP lamps in RAS might result in a critical, toxic NO_2^- formation. The objective of this study was to evaluate whether NO_2^- is produced in critical levels with respect to a range of realistic NO_3^- concentrations and different UV doses. In these tests, a flow-through reactor equipped with a LP lamp was used. Such reactors are typically used in RAS and thus, together with the realistic NO_3^- concentrations and high UV doses, our study will clarify the NO_2^- production potential of LP lamps in RAS.

Materials and methods

Experimental design

Tests on NO_2^- formation were performed with a flow-through reactor equipped with a LP lamp (Micro light Basic 5; a.c.k. aqua concept, Karlsruhe, Germany). Technical specifications of the UV-C reactor are summarized in Table 1. Since the effective UV-C dose strongly depends on the spectral absorbance coefficient at 254 nm (SAC_{254}) of the treated water, the applied UV-C doses are expressed as the volume-specific UV-C energy input, where 42 J L^{-1} corresponds to 40 mJ cm^{-2} for SAC_{254} = 22.18 m^{-1}. The employed UV-C doses ranged from 42 J L^{-1} to 6300 J L^{-1} after adjustment to the retention time of the flow-through reactor.

For the tests water with different NO_3^- concentrations (3, 100, 300, 800 and 1200 mg L^{-1}) was prepared by adding sodium nitrate, $NaNO_3$ (Karl Roth, Germany) to tap water with 3 mg L^{-1} NO_3^- and < 0.03 mg L^{-1} NO_2^-. All measurements were carried out at 13°C and at 27°C with 8

replicates, and the pH value was always 7.6 (measured with WTW, Weilheim, Germany). Water samples were taken from the outlet of the ultraviolet reactor and NO_2^- was immediately determined using the Griess' reaction analog to DIN EN 26777 (1993). The transformation rate of NO_3^- to NO_2^- was calculated as the percentage of the initial NO_3^--N that was transferred to NO_2^--N.

Table 1: Technical specifications of the UV-C reactor (Micro light Basic 5; a.c.k. aqua concept, Karlsruhe, Germany)

Technical Data	Microlight Basic 5
Filling volume	8 L
Flow rate max.	3000 L h^{-1}
Electric power	110 W [a]
Efficiency of UV-lamp	31.8 %
UV-C power	35 W
UV-C dose at SAC_{254} = 22.18 m^{-1}	min. 40 mJ cm^{-2} [b]

[a] lamp without electronic ballast
[b] at 3000 L h^{-1}; ~ T_{10mm} = 60 % at the end of lamp-life

For each NO_3^- concentration the absorbance and transmittance spectrum from 200 nm to 400 nm was determined in a 1 cm quartz cuvette (Shimadzu UV/Vis-2401). Additionally, according to the estimated gap width of the UV reactor used in this study, the transmittance was also determined with a 5 cm cuvette. Ultrapure water (Milli-Q, Millipore, Germany) was used as negative control.

Statistical analysis

Data were analyzed for normal distribution by Kolmogorov–Smirnov test (passed if $p < 0.05$). Spearmann rank correlation was used to test the relationship between photo-induced NO_2^- formation with nitrate concentration and UV dose, as well as the transformation rate with nitrate concentration and UV dose. All data were analyzed using IBM SPSS 20.0 (IBM, Armonk, NY).

Results

The photo-induced formation of NO_2^- from NO_3^- was correlated with the UV-C dose (rho = 0.693, $p \leq 0.001$ at 13°C and rho = 0.683, $p \leq 0.001$ at 27°C) and the NO_3^- concentration (rho = 0.484, $p \leq 0.001$ at 13°C and rho = 0.531, $p \leq 0.001$ at 27°C) (Fig. 1). At a water temperature of 27°C the NO_2^- formation was approximately 40 % higher compared to the NO_2^- formation at 13°C (Fig. 1).

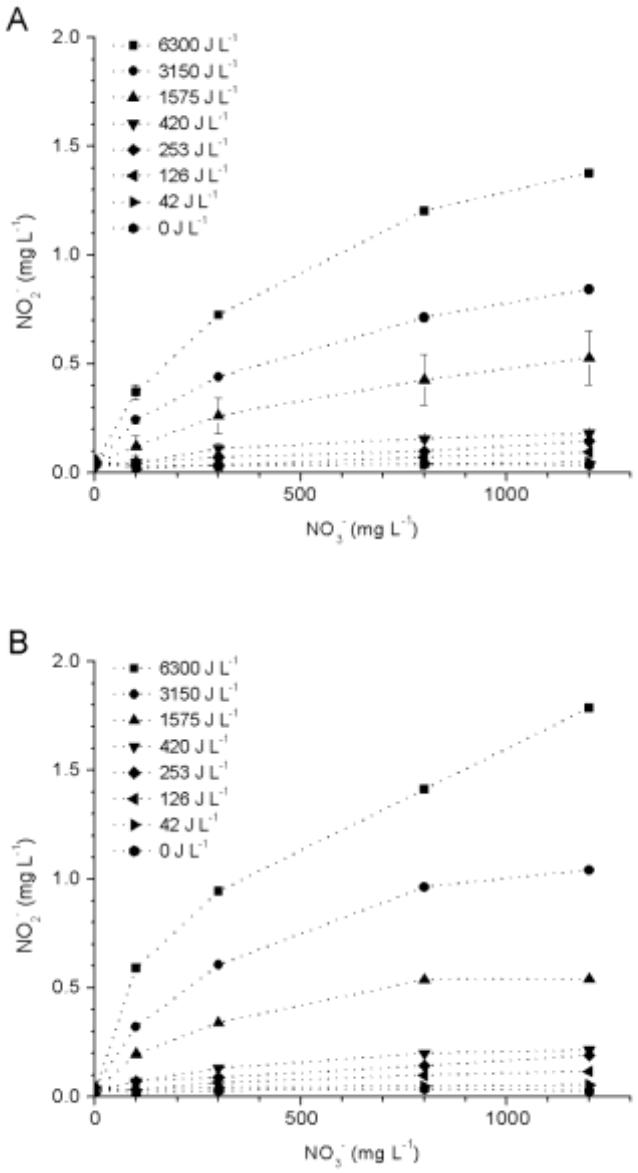

Fig. 1: Photo-induced formation of NO_2^- from NO_3^- at different NO_3^- concentrations, UV-C doses and two different water temperatures, (A) 13°C and (B) 27°C.

The transformation rate of NO_3^- to NO_2^- also showed a positive correlation with the UV-C dose (rho =590, $p \leq 0.001$ at 13°C and rho = 0.584, $p \leq 0.01$ at 27°C) and showed a negative correlation with the NO_3^- concentration (rho =0.267, $p \leq 0.01$ at 13°C and rho =0.268, $p \leq 0.001$ at 27°C) (Fig. 2).

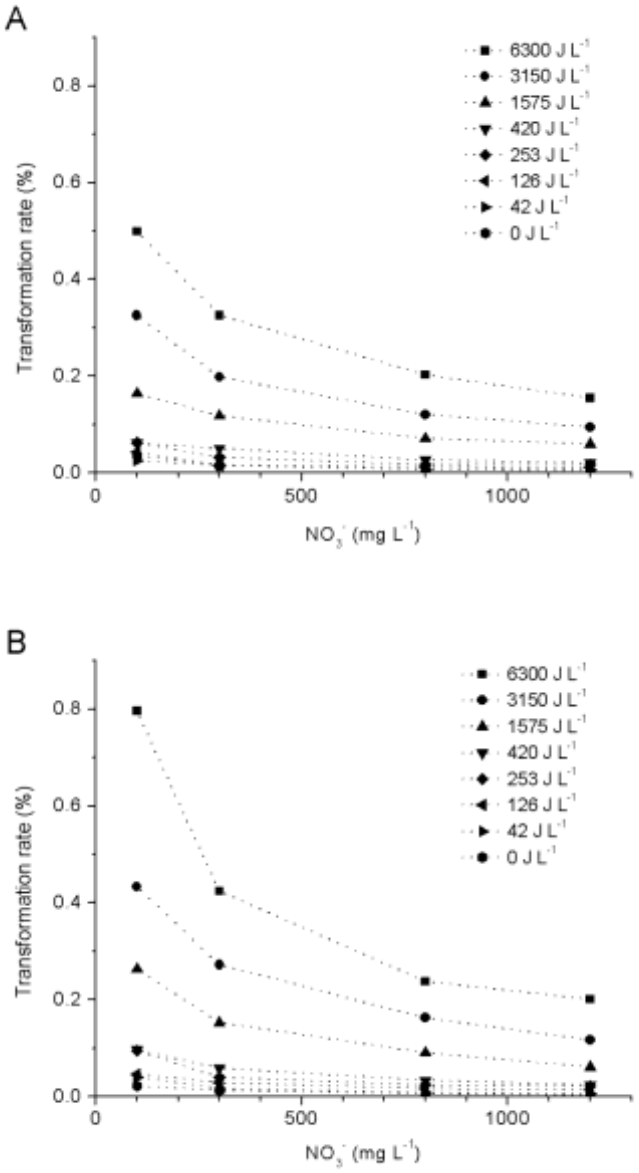

Fig. 2: Transformation rate of NO_3^- to NO_2^- at different ambient NO_3^- concentrations, UV-C doses and two different water temperatures, (A) 27°C and (B) 13°C.

At a water temperature of at 27°C , the irradiation with 42 J L^{-1} (corresponding to 40 mJ cm^{-1} at SAC_{254}= 22.18 m^{-1}), the UV dose recommended for bacterial inactivation in drinking water (DVGW Technical Standard W 294), did not lead to a measurable NO_2^- formation at an

ambient NO_3^- concentration of 100 mg L^{-1}. However, with the same NO_3^- concentration a tenfold higher UV dose of 420 J L^{-1} increased the NO_2^- concentration to above 0.1 mg L^{-1}, and a further increase of the UV dose to 6300 J L^{-1} resulted in a NO_2^- concentration of 0.6 mg L^{-1}.

The effect of high NO_3^- concentrations on the NO_2^- yield was relatively small when the irradiation dose was low. At 27°C, irradiation of water with a NO_3^- concentration of 1200 mg L^{-1} with 42 J L^{-1} resulted in a NO_2^- concentration of 0.05 mg L^{-1}. However, the effect of an increased UV dose on the NO_2^- formation was considerably boosted by increased NO_3^- concentrations. For example, at a NO_3^- concentration of 300 mg L^{-1} irradiation with 420 J L^{-1} lead to a NO_2^- yield of 0.13 mg L^{-1}, and at 1200 mg NO_3^- L^{-1}, irradiation with 420 J L^{-1} and 6300 J L^{-1} resulted in NO_2^- concentrations of 0.22 and 1.79 mg L^{-1}, respectively.

Figure 3 shows the absorbance spectrum of NO_3^-, measured for all NO_3^- concentrations used in this study, with two absorbance peaks: a very strong peak around 200 nm extending its shoulder beyond 250 nm and a weak peak around 300 nm. Accordingly, there was almost no transmittance across the 5 cm light path around 200 nm and a depression in the transmission curve at the second peak of the NO_3^- absorbance spectrum around 300 nm. At 253.7 nm, the wavelength mainly responsible for the inactivation of microorganisms, NO_3^- concentrations of 100 mg L^{-1} and 1200 mg L^{-1} reduced the transmittance of the aqueous medium to 31.3 % and 17.9%, respectively (Fig. 4).

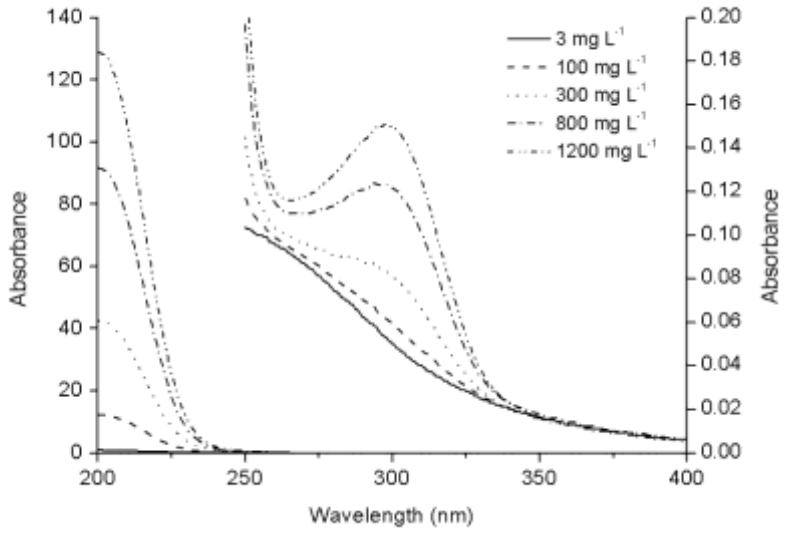

Fig. 3: Absorption spectrum of water with different NO_3^- concentrations.

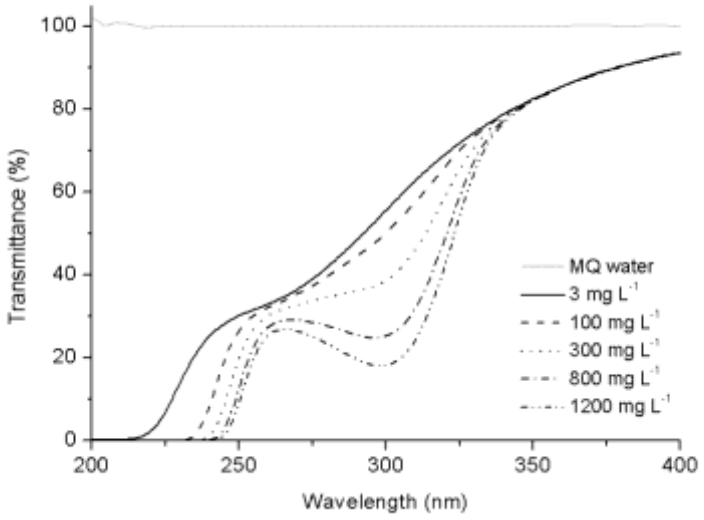

Fig. 4: Transmittance spectrum of water with different NO_3^- concentrations, measured in a 5 cm cuvette.

Discussion

The photo-induced transformation of NO_3^- to NO_2^- is based on the absorption of UV light by NO_3^- (Mack and Bolton, 1999; Takeda and Fujiwara, 1993). LP lamps mainly emit light at 253.7 nm, the wavelength that accounts for the high germicidal effect (EPA, 2006). However, LP lamps possess further emission lines at 184.9, 313.1, 365.0, 404.7 and 435.8 nm, altogether contributing about 10 % to the total emitted energy (Roig *et al*., 1999). Radiation situated below 240 nm has a pronounced photochemical effect and is therefore prohibited from use in UV radiation sources for drinking-water disinfection according to DVGW Technical Standard W 294 or OENORM M5873-1. In ozone-free lamps, the use of doped quartz glass filters out the 185 nm UV radiation that is responsible for ozone production, but it cannot prevent the emission of wavelengths above 240 nm. Considering the absorption spectrum of NO_3^-, it becomes obvious that the excitation of NO_3^- at 253.7 nm and 313.1 nm, both wavelengths emitted by LP lamps, could promote the reduction of NO_3^- to NO_2^- (Mack and Bolton, 1999; Takeda and Fujiwara, 1993).

As expected, the NO_2^- yield rose with increasing UV doses as well asNO_3^- concentrations and with water temperature. Similarly, the transformation rate of NO_3^- to NO_2^- was positively correlated with the UV dose, but it decreased with increasing NO_3^- concentrations. A likely explanation for the latter can be found in the subsequent reactions during NO_3^- photolysis (Mack and Bolton, 1999; Wagner *et al*., 1980). The intermediate NO_2• radical can react with

the •OH radical to NO_3^- (Mack and Bolton, 1999). The back transformation to NO_3^- is increasing with the NO_2• concentration and is finally inhibiting the NO_2^- production (Wagner *et al*., 1980; Mack and Bolton, 1999). Parts of this chain reaction have considerably slow reaction rates and thus, with a too short time-frame, the chain of reactions mentioned above does not reach equilibrium between nitrate and nitrite molecules (Wagner *et al*., 1980). Furthermore, the high optical densities of the NO_3^- solutions used in this study result in an inhomogeneous exposure of NO_3^- to UV light within the reactor. Consequently, not all potentially transformable NO_3^- could be transformed to NO_2^- (Wagner *et al*., 1980). Since the treatments with nitrate concentrations > 100 mg L^{-1} used in our study were optically very dense in the UV range and the residence time of the water in the UV-C reactor was relatively short, this, together with the self-inhibition of the nitrite production, probably has led to the lower nitrate transformation rates at higher nitrate concentrations.

When UV-C irradiation is used to improve the rearing conditions for fish in aquaculture or in aquaria, there are several scenarios in which the photo-induced formation of NO_2^- by LP lamps is a matter of concern: When UV doses exceed by far those regularly used for disinfection; the NO_2^- concentrations in the outflow of the UV reactor are significantly increased. This can be the case when greatly oversized UV units are operated in relatively small systems such as aquaria, or when UV irradiation is used to control eukaryotic pathogens, which requires considerable more energy compared to bacterial inactivation (Bazyar Lakeh *et al*., 2013; Kasai *et al*., 2002). Then the synergistic application of UV with another disinfection strategy such as low frequency ultrasound, as it was already suggested for ballast water treatment (Sassi *et al*., 2005), may be a good alternative for aquaculture purposes (Bazyar Lakeh *et al*., 2013).

In addition, high ambient NO_3^- levels promote the photo-induced NO_2^- formation. Consequently, a high UV dose to remove eukaryotic pathogens in combination with high NO_3^- levels of several 100 mg that are typical for intensive RAS can lead to a significantly increased NO_2^- load in the outflow of the UV reactor even when a LP lamp is used. An alkaline pH further increases the risk of a critical NO_2^- formation (Lu *et al*., 2009).

In RAS or aquaria with a well-functioning biofilter, nitrifying bacteria will quickly transform the NO_2^- formed by photolysis of NO_3^- back to NO_3^-. However, it should be considered that UV irradiation is commonly used as the final step of the water treatment, and thus fish are continuously exposed to the NO_2^- concentration that leaves the UV reactor. In systems without a biofilter, NO_2^- could accumulate. Depending on UV dose, recirculation time, and

total water volume of the system, NO_2^- could raise to a critical concentration within a short time.

Whether a certain NO_2^- concentration is critical for the cultured fish cannot easily be answered. Fish species differ greatly in their susceptibility to NO_2^- (Lewis *et al*., 1986; Kroupova *et al*., 2005; Wuertz *et al*., 2013), and NO_2^- toxicity depends on a large number of factors (Kroupova *et al*., 2005). Especially the presence of chloride, Cl^-, significantly decreases NO_2^- toxicity because both ions compete for the branchial Cl^- uptake mechanism (Jensen, 2003; Kroupova *et al*., 2005; Tomasso and Grosell, 2005; Wuertz *et al*., 2013). Published acute toxicity data for NO_2^-, measured as LC50 values, range from 0.6 to 2.9 mg L^{-1} for salmonids to 460 mg L^{-1} for largemouth bass, *Micropterus salmoides* (Wuertz *et al*., 2013). Thus, under common conditions, fish mortality due to the photo-induced NO_2^- formation by a LP lamp is unlikely when biofilters are employed. However, if e.g. water with a NO_3^- concentration of 300 mg L^{-1} is irradiated with the UV dose of 1680 J L^{-1} (equivalent to 1600 mJ cm^{-2} for SAC_{254} = 22.18 m^-1, 40 times more than the dose recommended for the treatment of drinking water), a single flow through the UV reactor could already lead to a NO_2^- concentration that are lethal for some susceptible species such as salmonids.

In addition to the acute toxicity, sublethal effects of NO_2^- on the fish must be considered. Subchronic intoxication with NO_2^- concentrations far below the LD50 value can cause physiological disturbances, tissue damage and reduced growth (Alcaraz and Espina, 1997; Das *et al*., 2004; Frances *et al*., 1998; Kroupova *et al*., 2008; Wuertz *et al*., 2013). Only little is known about safe NO_2^- concentrations that cause no pathological changes in different fish species. For rainbow trout, *Oncorhynchus mykiss*, a representative of the most susceptible fish species, Kroupova *et al*. (2008) estimated a NOEC (28d LC_0) at 0.01 mg L^{-1} NO_2^- the basis of growth rate inhibition. For pike-perch, *Sander lucioperca*, a moderately sensitive species, Wuertz *et al*. (2013) considered a NO_2^- concentration < 0.2 mg L^{-1} as safe. Therefore, it is both from the point of view of animal welfare as well as for economic reasons, a good idea to keep the NO_2^- concentration in the fish tanks as low as possible. Although LP lamps are commonly assumed to be harmless in terms of the formation of by-products, the potential of low-pressure UV lamp irradiation to induce photolysis of NO_3^- to NO_2^- should be carefully considered; especially when a high level of NO_3^- is present and high UV doses are to be applied.

Kapitel 7

Zusammenfassende Diskussion

In der Aquakultur kann in Kreislaufanlagen mit sehr hohen Besatzdichten bei einem geringen Wasserverbrauch eine sehr hohe Produktivität erzielt werden. Eine wichtige Voraussetzung für den Betrieb von Kreislaufanlagen ist eine an den Besatz angepasste Wasseraufbereitung. Hierbei handelt es sich mindestens um eine Einrichtung zur Entfernung von suspendierten Partikeln und einen Biofilter, in dem nitrifizierende Bakterien Ammonium zu Nitrat umwandeln.

Hohe Besatzdichten und die Führung des Wassers im Kreislauf verursachen ein erhöhtes Risiko für die Ausbreitung von Infektionskrankheiten. Die Praxis zeigt, dass sich auch im geschlossen System einer Kreislaufanlage trotz Einhaltung von Hygienemaßnahmen kaum vollständig pathogenfreie Bedingungen realisieren lassen. Daher kann in der Wasseraufbereitung ein weiterer Schritt zur Abtötung von Pathogenen zum Einsatz kommen. Bisher werden hierzu in der Aquakultur Ozon oder die Bestrahlung mit UV-C Licht verwendet. In intensiv bewirtschafteten Kreislaufanlagen mit geringem Wasseraustausch wird allerdings ein großer Teil des zugegebenen Ozons für die Oxidation der im Wasser gelösten organischen Substanzen verbraucht. Zur Gewährleistung einer für die desinfizierende Wirkung ausreichenden Ozonkonzentration und Einhaltung der Sicherheitsgrenzwerte ist ein erheblicher verfahrenstechnischer Aufwand nötig (Summerfelt, 2003). Auch die Effizienz der Bestrahlung mit UV-C Licht wird beim Einsatz in Kreislaufanlagen erheblich aufgrund Absorption durch im Wasser gelöster Stoffe vermindert (Gullian *et al*., 2012; Summerfelt, 2003). Am effektivsten wirkt UV-C gegen Bakterien und viele Viren. Die Abtötung eukaryotischer Organismen (Pilze, parasitische Einzeller und Würmer, u.a.) erfordert jedoch eine deutlich höhere als die üblicherweise zur Inaktivierung von Mikroorganismen verwendete UV-C Dosis (Kasai *et al*., 2002).

Niederfrequenter Ultraschall (nf-US) ist ein alternatives physikalisches Verfahren mit einem guten Potential zur Abtötung von eukaryotischen Parasiten. Bislang wurde die Wirkung von nf-US auf *Artemia* Nauplien im Hinblick auf die Behandlung von Ballastwasser mit kleinskaligen Laborexperimenten untersucht (Sassi *et al*., 2005, Holm *et al*., 2008). Gegenstand des hier beschriebenen Projektes war es, die Wirksamkeit von nf-US in Kombination mit UV-C zur Behandlung von Wasser in Kreislaufanlagen in der Aquakultur zu untersuchen. Als praktische technische Lösung für den Anwender wurde im Projekt ein neuer Durchflussreaktor für die kombinierte Anwendung von nf-US und UV-C entwickelt, konstruiert und aufgebaut. Nach entsprechenden Vorversuchen im Labor wurde das Gerät auch in mehrwöchigen Feldtests im Technikum am IGB als auch bei AquaVet in Israel getestet.

Desinfizierende Wirksamkeit von nf-US, UV-C und deren Kombination

Vergleichende Untersuchungen zur Wirksamkeit von nf-US (25 kHz), UV-C und der Kombination beider Verfahren wurden mit heterotrophen Bakterien (Gesamtkeimzahl), dem Ciliaten *Paramecium* (Pantoffeltierchen), Larven des Schwimmblasennematoden *Anguillicola crassus* und Metanauplien des Krebstieres *Artemia* durchgeführt. Das Größenspektrum der untersuchten Organismen umfasste einen Bereich von wenigen 1-2 µm (Bakterien) bis zu etwa 500 µm (*Artemia* Nauplien). Abgesehen von *A. crassus* handelt es sich bei den Modellorganismen nicht um Fischpathogene, sie repräsentieren jedoch Organismengruppen, aus denen in der Aquakultur wichtige Fischpathogene stammen. Die Auswahl dieser Zielorganismen erwies sich als vorteilhaft, da sie problemlos in der erforderlichen Menge für die Versuche bereitgestellt werden konnten und keine Tierversuche notwendig waren. Die vergleichbare, Dosis-abhängige Wirkung von nf-US auf *Paramecium* und den Fischparasiten *Trichodina* belegt die Eignung von *Paramecium* als Modellorganismus für fischpathogene Ciliaten.

Erwartungsgemäß erwies sich UV-C als sehr wirksam gegen Bakterien, wobei die Effizienz der Bestrahlung maßgeblich von der Strahlungsdurchlässigkeit des Wassers bestimmt wurde. Selbst bei dem hohen spektralen Abschwächungskoeffizienten des Wassers aus einer intensiv betriebenen Kreislaufanlage zur Produktion von Tilapia (SAK_{254} = 71 m^{-1}) konnte mit einem Energieverbrauch von 0,13 kJ L^{-1} (dies entspricht nach Angaben des Herstellers des UV-Reaktors nominell 400 J m² bei SAK_{254} = 22,18 m^{-1}) bei einer einmaligen Passage des UV-Reaktors noch eine Verringerung der Gesamtkeimzahl um mehr als eine Größenordnung festgestellt werden.

Im Gegensatz zu UV-C zeigte nf-US mit einer Dosis bis zu 19 kJ L^{-1} in seiner alleinigen Anwendung keine nachweisbare Wirkung auf die Gesamtkeimzahl. Die Inaktivierung von Bakterien würde eine deutlich höhere nf-US Energie erfordern (Holm *et al*., 2008), was im Vergleich zu UV-C für Kreislaufanlagen nicht ökonomisch und aufgrund der mit dem Energieeintrag verbundenen Erhöhung der Wassertemperatur nicht praxistauglich ist.

Bei der kombinierten Anwendung von nf-US und UV-C zeigte sich, dass die Vorbehandlung des Wassers mit nf-US die Effizienz von UV-C um bis zu 0,6 log Einheiten verbessern kann. Dieser Effekt wurde auch von Blume und Neis (2004) für die Behandlung von kommunalem Abwasser beschrieben und kann damit erklärt werden, dass im Wasser suspendierte Partikel, die Bakterien vor der UV-C Strahlung schützen (Parker und Darby, 1995; Tang *et al*., 2011), durch nf-US zerschlagen werden. Inwiefern die erhöhte Effizienz der UV-C Bestrahlung den

erforderlichen energetischen und technischen Aufwand für eine nf-US Vorbehandlung rechtfertigt, sollte für die jeweils gegebenen Anwendungsbedingungen geprüft werden.

Die Untersuchungen zur Wirkung von UV-C auf die größeren, eukaryotischen Modellorganismen bestätigen, dass zu deren Abtötung eine deutlich höhere als die üblicherweise zur Kontrolle von Bakterien verwendete Strahlendosis erforderlich ist. So ist mit einer gegen Bakterien sehr gut wirksamen Strahlendosis zum Beispiel gegen die häufig auftretenden Fischparasiten aus der Gruppe der Ciliaten (*Trichodina*, *Ichthyophthirius*) kaum eine Wirkung zu erwarten. Dies ist wohl einer der Gründe, warum die Anwendung von UV-C von Praktikern in der Aquakultur nicht durchgängig als hilfreich beurteilt wird.

Alternativ kann nf-US zur Abtötung eukaryotischer Parasiten verwendet werden. Die Dosis-abhängige Wirkung von nf-US auf die untersuchten Modellorganismen kann sehr gut mit Funktionen einer exponentiellen Abnahme beschrieben werden. Da die Wirksamkeit von nf-US weitgehend unabhängig von der Wasserqualität ist, lässt sich so die von einer bestimmten nf-US Intensität bewirkte Abtötungsrate leicht abschätzen.

Die energetische Effizienz von nf-US im Vergleich zu UV-C ist abhängig von der UV-C Transmissibilität des Wassers, dem eingesetzten Lampentyp und konstruktiven Eigenschaften des UV-Reaktors sowie dem Zielorganismus. So verringert die Erhöhung des SAK_{254} von 22 m^{-1} auf 70 m^{-1} die Effizienz des von uns verwendeten UV-Reaktors (Micro light Basic 5; a.c.k. aqua concept, Karlsruhe) um den Faktor 5,5. Wird zur Realisierung hoher Strahlungsintensitäten anstelle einer UV-Niederdrucklampe eine UV-Mitteldrucklampe eingesetzt, verringert sich die energetische Effizienz der keimtötenden Bestrahlung nochmals etwa um Faktor drei. Während sich nf-US unter allen Bedingungen das effizientere Verfahren gegen relativ große, komplexe Organismen wie die hier untersuchten *Artemia* Metanauplien erwies, war UV-C bei Verwendung einer Niederdrucklampe und Wasser mit einem geringen SAK_{254} die effizientere Technik zur Abtötung von Ciliaten und Nematodenlarven. Mit zunehmend schlechteren Randbedingungen für den Einsatz von UV-C wird jedoch nf-US auch gegen diese Organismen das effizientere Verfahren. Grundsätzlich ist jedoch zu berücksichtigen, dass eine Steigerung der UV-C Dosis zur Abtötung von Parasiten um das mindestens zehnfache gegenüber der üblichen UV-C Dosis zur Kontrolle von Bakterien durch das erhöhte Risiko einer photo-induzierten Bildung von Nitrit limitiert wird.

Im Gegensatz zu UV-C funktioniert nf-US weitgehend unabhängig von der Wasserqualität und kann mit Energiedichten, die gegen ein breites Spektrum von Parasiten wie Ciliaten, Nematoden und Krustentiere wirksam sind, sicher angewendet werden. Somit könnte eine Kombination von UV-C (gegen Viren und Bakterien) und nf-US (gegen eukaryontische

Parasiten) ein geeignetes Verfahren zur Kontrolle eines weiten Spektrums relevanter Pathogene in Kreislaufanlagen.

Untersuchung auf toxische Nebenprodukte

Eine mögliche Bildung toxischer Nebenprodukte bei der Behandlung von Kreislaufwasser mit nf-US, UV-C und deren Kombination wurde mit dem Fischembryo-Toxizitätstest (FET) gemäß DIN 38415-T6 mit *Danio rerio* sowie dem Leuchtbakterientest nach DIN EN *ISO 11348*-3 mit *Vibrio fischeri* untersucht. Beide Tests gaben keine Hinweise auf toxische Substanzen im Auslauf des Durchflussreaktors. Anhand dieser Ergebnisse sind beide Verfahren als sicher für die Anwendung in der Aquakultur zu beurteilen.

Die Bildung des fischtoxischen Nitrits durch die bei den Untersuchungen verwendete hohe UV-C Dosis von 420 J L^{-1} konnte mit dem FET-Test nicht gezeigt werden, obwohl hier entsprechend unserer Untersuchungen zur photo-induzierten Bildung von Nitrit aus Nitrat eine Nitritkonzentration von 0,6 mg L^{-1} zu erwarten gewesen wäre. Dies kann damit erklärt werden, dass die nicht geschlüpften Embryos von *D. rerio* offensichtlich sehr unempfindlich gegen Nitrit sind (Meinelt *et al*., 2010).

Photo-induzierte Bildung von Nitrit aus Nitrat

Anlässlich einer Studienreise nach Israel wurden wir vom Betreiber einer Kreislaufanlage darauf aufmerksam gemacht, dass die intensive Bestrahlung des Prozesswassers mit UV-C zu einer kritischen Nitritkonzentration führt. Infolgedessen untersuchten wir, ob hierdurch die Anwendbarkeit von UV-C in hohen Intensitäten limitiert wird.

Die Absorption von UV-Licht durch Nitrat führt zur photo-induzierten Bildung von Nitrit (Mack und Bolton, 1999; Takeda und Fujiwara, 1993). Die Menge des gebildeten Nitrits ist abhängig von der Nitratkonzentration und der UV-Dosis (Mack und Bolton, 1999; IJpelaar *et al*., 2005; Lu *et al*., 2009; Sharpless *et al*., 2003). Bezüglich des Potentials zur Bildung von Nitrit muss zwischen UV-Niederdruck- und UV-Mitteldrucklampen, die beide in der Aquakultur verwendet werden (Sharpless *et al*., 2003), unterschieden werden. UV-Mitteldrucklampen verursachen eine deutlich stärkere Bildung von Nitrit als UV-Niederdrucklampen, weil sie viel stärker im Wellenlängenbereich zwischen 200 und 240 nm emittieren, wo Nitrat stark absorbiert (IJpelaar *et al.*, 2005; Sharpless *et al*. 2003). UV-Niederdrucklampen hingegen emittieren nahezu monochromatisches Licht einer Wellenlänge von 253,7 nm, die kaum von Nitrat absorbiert wird. Infolgedessen wird die photo-induzierte

Bildung von Nitrit als vernachlässigbar eingeschätzt, wenn UV-Niederdrucklampen in der Trinkwasserindustrie zur Desinfektion verwendet werden (IJpelaar *et al*., 2005).

In der Aquakultur stellt die Bildung von Nitrit als Nebenprodukt der UV-Bestrahlung ein potenzielles Risiko dar, weil Nitrit für Fische stark toxisch ist (Kroupova *et al*., 2005). In Kreislaufanlagen reichert sich Nitrat als Endprodukt der Nitrifikation stark an und seine Konzentrationen sind häufig viel höher als in natürlichen Gewässern. In Verbindung mit einer hohen UV-Dosis kann dann die Nitritkonzentration im Auslauf des UV-Reaktors selbst bei Verwendung von UV-Niederdrucklampen deutlich erhöht sein.

Ob eine bestimmte Nitritkonzentration kritisch für die kultivierten Fische ist, kann nicht pauschal beantwortet werden. Zum einen unterscheiden sich verschiedene Fischarten sehr stark in ihrer Toleranz gegenüber Nitrit (Lewis *et al*., 1986; Kroupova *et al*., 2005; Wuertz *et al*., 2013), zum anderen ist die Toxizität von Nitrit von weiteren Faktoren wie z.B. dem Chloridgehalt des Wassers abhängig (Kroupova *et al*., 2005). Die subchronische Intoxikation mit Nitrit weit unterhalb der letalen Dosis (LD50) kann zu physiologischen Störungen, Gewebeschäden und einem verminderten Wachstum führen (Alcaraz und Espina, 1997; Das *et al*., 2004; Frances *et al*., 1998; Kroupova *et al*., 2005, 2008; Wuertz *et al*., 2013). Es liegen allerdings kaum Informationen vor, welche Nitritkonzentrationen bei verschiedenen Fischarten als sicher angesehen werden können, also keine pathologischen Veränderungen verursachen. Einen Anhaltspunkt geben für die Regenbogenforelle (*Oncorhynchus mykiss*), einem sehr empfindlich auf Nitrit reagierenden Salmoniden, und den Zander (*Sander lucioperca*), einer moderat empfindlichen Art, als sicher ermittelten Konzentrationen vom 0,01 mg L^{-1} bzw. < 0,2 mg L^{-1} (Kroupova et al., 2008; Wuertz *et al*., 2013). Daher ist es sowohl unter dem Gesichtspunkt des Tierschutzes als auch aus wirtschaftlichen Gründen anzustreben, die Nitritkonzentration in den Fischbecken so niedrig wie möglich zu halten.

Insbesondere bei den in Kreislaufanlagen hohen Nitratkonzentrationen sollte die UV-C Strahlung nicht auf die zur Abtötung von Parasiten erforderliche Intensität erhöht werden, ohne deren Potential zur photo-induzierten Bildung von Nitrit unter Berücksichtigung der anderen Wasserparameter und der gepflegten Fischart sorgfältig zu prüfen. Unsere Untersuchungen zeigen, dass selbst bei der Verwendung von UV-Niederdrucklampen-Lampen, die diesbezüglich gemeinhin als unproblematisch gelten, fischtoxische Nitritkonzentrationen auftreten können. Somit kann UV-C in hohen Dosen nicht vorbehaltlos als sicheres Verfahren zur Kontrolle von Parasiten in der Aquakultur empfohlen werden.

Einsatz des nf-US / UV-C -Kombireaktors in Versuchs-Kreislaufanlagen

Nach den grundlegenden Untersuchungen zur Wirksamkeit des von nf-US, UV-C und deren Kombination war zu klären, wie der US/UV–Kombireaktor für die Praxis zu dimensionieren und einzusetzen ist.

Bei Aquavet in Israel wurde in einer 30 m^3 fassenden Anlage unter praxisnahen Bedingungen geprüft, ob sich mit dem US-UV-Kombireaktor eine Transmission von Fischpathogenen zwischen den Becken einer Produktionseinheit kontrollieren lässt. Bei einer Durchflussrate von 3 $m^3\ h^{-1}$ war gemäß unserer Wirksamkeitsuntersuchungen eine weitgehende Inaktivierung von Bakterien und maximaler nf-US Leistung eine Abtötung von etwa 25 % des Ciliaten *Trichodina* sp. zu erwarten. In dem Experiment konnte die Übertragung der Pathogene so nicht verhindert, aber das Krankheitsgeschehen deutlich hinausgezögert werden.

Um die Transmisson von *Trichodina* in einer Kreislaufanlage zu unterbinden, wären eine sehr viel höhere nf-US Intensität notwendig, was bei den üblichen Durchflussraten aufgrund des Energieeintrages unvermeidbar zu einer deutlichen Temperaturerhöhung führen würde.

Durchflussreaktoren werden jedoch in Kreislaufanlagen auch eingesetzt, um den Infektionsdruck auf die Fische im gesamten System niedrig zu halten. Das Ziel einer solchen Anwendung ist nicht die vollständige Elimination von Pathogenen, was auch kaum möglich wäre, da ja nur das im Kreislauf befindliche Wasser, aber nicht die Fische behandelt werden.

Die theoretisch zur Reduktion von Pathogenen in einer Kreislaufanlage notwenige Abtötungsrate bei einmaliger Passage durch den Reaktor kann mit einem vereinfachenden mathematischen Modell bei Annahme eines exponentiellen Wachstums und einer gleichmäßigen Verteilung der Pathogene im Wasser abgeschätzt werden. Beide Voraussetzungen sind in der Realität so zwar nicht gegeben, sodass anhand des Modells eine zu optimistische Abschätzung zu erwarten ist. Die Stärke des Modells liegt darin, dass es zeigt, bei welchen Randbedingungen überhaupt eine Reduktion der betrachteten Organismen im Kreislaufwasser zu erwarten ist. So zeigt sich, dass es bei der Behandlung des Kreislaufwassers vor allem darauf ankommt, dass der Anteil des pro Zeit behandelten Gesamtvolumens der Anlage an die Generationszeit der zu bekämpfenden Organismen angepasst ist.

Unter Praxisbedingungen einer Kreislaufanlage mit einem Gesamtvolumen von 12 m^3 hat sich gezeigt, dass die Zahl der im Wasser suspendierten heterotrophen Bakterien mit einer geringen UV-Dosis (UV-Niederdruck-Lampe, 2 x 55 W Anschlussleistung) wirksam reduziert werden kann, wenn das Anlagenvolumen 1,3 mal pro Stunde behandelt wird. Beim Betrieb desselben UV-Reaktors im Bypass blieb die UV-C Bestrahlung jedoch wirkungslos.

Die Kontrolle der im Wasser suspendierten Bakterien durch UV-C ist also mit einem vergleichsweise geringen Energieverbrauch realisierbar, wenn der UV-Reaktor mit dem gesamten Volumenstrom des Filterkreislaufes beaufschlagt wird.
Am Beispiel des Ciliaten *Trichodina* konnten wir zeigen, dass die Bekämpfung eukaryotischer Parasiten im Kreislaufwasser mit nf-US auch im Bypass erfolgen kann. Die durch eine nf-US Behandlung erzielte Elimination frei im Wasser schwimmender *Trichodina* folgte den mit dem o.g. mathematischem Modell, verlief allerdings etwas langsamer. Das kann einfach damit erklärt werden, dass die Parasiten nicht nur, wie im Modell angenommen, frei im Wasser suspendiert sind, sondern natürlich auch auf den Fischen leben.

Schlussfolgerung

Für Kreislaufanlagen in der Aquakultur ist die Bestrahlung mit UV-C ein hervorragendes Verfahren zur Kontrolle von frei im Wasser suspendierten Bakterien mit einem relativ geringen Energieaufwand. Eine Erhöhung der UV-C Dosis zur Bekämpfung eukaryotischer Parasiten ist insbesondere bei hohen Nitratkonzentrationen durch die photo-induzierte Bildung von Nitrit limitiert. Niederfrequenter Ultraschall (nf-US) ist ein geeignetes, anwendungssicheres Verfahren zur Abtötung eukaryotischer Organismen und kann somit eine sinnvolle Ergänzung zu der gegen Bakterien eingesetzten UV-Bestrahlung sein.
Praktisch kann die Kombination von UV-C und nf-US mit dem in diesem Projekt entwickelten Kombireaktor angewendet werden. In kleineren Anlagen kann der Reaktor direkt in den Filterkreislauf eingebaut werden. Bei hohen Volumenströmen kann es aus technischer und energetischer Sicht sinnvoller sein, separate UV-C und nf-US Reaktoren zu verwenden. Während der UV-C Reaktor mit dem vollen Volumenstrom des Kreislaufes beaufschlagt werden sollte, kann der nf-US Reaktor dann im Bypass betrieben werden.

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