

Aus dem Institut für Tierzucht und Tierhaltung
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

**Water treatment
in Recirculating Aquaculture Systems (RAS)
by ultrasonically induced cavitation**

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*„Wissenschaft ohne Religion ist lahm,
Religion ohne Wissenschaft ist blind.“*

Albert Einstein

Für meine Eltern
Kwan Nam Koong und Young-Ok Park

부모님을 위해
관 남궁 영옥 박

Table of Contents

GENERAL INTRODUCTION1

Chapter 1.....21
Removal of the off-flavor compounds geosmin and 2-methylisoborneol from recirculating aquaculture system water by ultrasonically induced cavitation..... 21

Chapter 2.....44
Preliminary test of ultrasonically disinfection efficacy towards selected aquaculture pathogens 44

Chapter 3.....76
Removal of dissolved organic matter and nitrogen compounds from RAS wastewater by ultrasonically induced cavitation..... 76

GENERAL DISCUSSION.....95

SUMMARY 108

ZUSAMMENFASSUNG 112

Appendix A - Analysis of TC, DOC, IC, TN 117

Acknowledgements 118

List of Tables

Table I-1: Physical properties and structural formula of 2-MIB and GSM.	5
Table 1-1: Physical properties of 2-MIB and GSM.	33
Table 2-1: Extract of water temperatures measured during treatment with LFUS and HFUS.	54
Table 2-2: The table shows the specific inactivation rates of all examined microalgae after ultrasound treatment sorted by the size of the microalgae.	57
Table 2-3: The table shows the specific inactivation rates of all tested organisms after ultrasound treatment sorted by the size of the organisms.	59

List of Figures

Figure I-1: Illustration of an exemplary water treatment system in a RAS.	3
Figure I-2: Schematic illustration of transient cavitation. The formation and implosion of a cavity by compression waves in a fluid triggered by sound waves.	9
Figure I-3: Illustration of cavitating bubble caused by ultrasound irradiation	10
Figure I-4: Illustrated comparison of cavitation formation by low-frequency ultrasound (LFUS) and high-frequency ultrasound (HFUS).	10
Figure 1-1: Mean reduction [%] ± confidence interval (p<0.05) of 2-MIB and GSM in tap water, RAS fresh water and RAS marine water after 15 min ultrasound treatment.....	31
Figure 1-2: Mean reduction [%] ± confidence interval (p<0.05) of 2-MIB and GSM in tap water, RAS fresh water and RAS marine water after 15min ultrasound treatment.....	31
Figure 1-3: The mean reduction [%] ± confidence interval (p<0.05) of biogenic 2-MIB and GSM in a trout experimental RAS water (C _{0,2-MIB} =35.5 ng/L; C _{0,GSM} =15.5 ng/L) and in pikeperch RAS water (C _{0,2-MIB} =2.9 ng/L; C _{0,GSM} =3.8 ng/L)	32
Figure 1-4: Mean reduction [%] ± confidence interval (p<0.05) of 2-MIB and GSM in spiked tap water (C ₀ =5µg/L) (a) and a sample taken from a commercial pikeperch RAS (b) containing biogenic off-flavors.	34
Figure 1-5: For each water matrix the mean reduction [%] + confidence interval (p<0.05) of 2-MIB and GSM after 15 min treatment with 20 kHz and 850 kHz ultrasound is illustrated.	36
Figure 1-6: The TRO concentration after 15 min ultrasound treatment determined as equivalent total residual chlorine (TRC).	37
Figure 2-1: Graph a shows the residuals based on the model applied (n=587; r ² = 0.93).	53
Figure 2-2: The logarithmic total viable count (CFU/ml) in relation to the consumed energy during ultrasound treatment at LFUS (20 kHz) and HFUS (850 kHz).	55
Figure 2-3: Comparison of the percentage PAM reduction of different microalgae due to ultrasound treatment at 20 kHz and 850 kHz in relation to the consumed specific energy (kJ/ml).	56
Figure 2-4: Comparison of the decrease of survived zooplankton in percent during ultrasound treatment at 20 kHz and 850 kHz.	58

Figure 2-5: An optical comparison of the physical conditions of <i>Artemia salina</i> after ultrasound treatment.	60
Figure 2-6: The comparison of the physical conditions of <i>Arcatia tonsa</i> after ultrasound treatment.	60
Figure 2-7: Total residual oxidant (TRO) concentration determined as equivalent total residual chlorine of different water samples after 0, 5, 10 and 15 minutes of ultrasound treatment at 20 and 850 kHz (n=3).	61
Figure 3-1: Mean recovery rate [%] ± confidence interval (p < 0.05, n=3) of total carbon (TC), inorganic carbon (IC) and dissolved organic carbon prior and after ultrasound treatment with LFUS (20 kHz) and HFUS (850 kHz).	84
Figure 3-2: Mean recovery rate [%] ± confidence interval (p < 0.05, n=3) of total nitrogen (TN) prior and after ultrasound treatment with LFUS (20 kHz) and HFUS (850 kHz).	86
Figure D-1: Mean reduction [%] ± confidence interval (p<0.05) of 2-MIB and GSM at different volumes (300, 600, 900, 1200 ml) after 15 min treatment time at 850 kHz HFUS (n=4, VB=0.95).	99
Figure D-2: The mean energy consumption [%] ± confidence interval (p<0.05) of the upscaling experiment were exposed to a volume of 1L and a 100% reduction of 2-MIB and GSM to reveal the most efficient treatment volume at 900 ml in the ultrasound reactor (n=4, VB=0.95).....	100
Figure D-3: Ultrasound reactor with a horizontally aligned ultrasound transducer in which the ultrasound waves are emitted in flow direction.	101

List of Equations

Equation 2-1: Estimation of the resonance size of the cavities.	64
Equation 3-1: Calculation of the energy absorption rate E.	82
Equation D-1: Equation for calculating the near-field length (N) with the diameter of the ultrasonic transducer (D=75mm) and the wavelength (λ).	102

GENERAL INTRODUCTION

The global key question of world food supply

The human population is growing and a decrease is not expected in the near future. As a result, the worldwide demand for high-quality proteins will steadily increase. Already today, more people are fleeing worldwide than ever before due to a lack of food security and limited resources (WFP, 2017). In addition, the consequences of unfair trade practices by industrialized countries (Wiewiórowska-Domagalska, 2015; Maciejewski, 2015; Diaz, 2016) and the consequences of global climate change will further exacerbate this situation. Therefore, the question of world food supply and the availability of resources will be one of the most important questions for the peaceful coexistence of humanity in the future.

Aquaculture

In addition to milk, eggs and meat, fish is a very good alternative for high-quality proteins. Consequently, the demand for fish has increased steadily in recent years. Satisfying the high demand from wild catches has led to significant overfishing of the oceans (Pauly et al. 1998; Pauly und Palomares 2005). For this reason and also in view of the growing world population, the ocean as a source of fish proteins cannot be a solution for future food problems. To maintain at least the current per capita consumption of aquatic products of 19.7 kg, further 23 million tonnes of aquatic products would be needed by 2020 (FAO, 2016). This additional supply must come from aquaculture if we want to save the remaining fishing areas from total depletion. The annual growth in aquaculture production of aquatic animals worldwide exceeded 10% between 1990 and 2000 and fell to 4% by 2015. Despite a slowing rate of growth in the recent years, aquaculture is the fastest growing agricultural sector in the world and has the potential to produce high-quality proteins as an alternative to wild-caught fish. Already today, 46.4% of fish for human consumption comes from aquaculture (FAO, 2019). Consequently, the aquaculture sector will play an increasingly important role in the future in terms of food security, poverty reduction and economic development. However, the rapid development and dissemination of aquaculture has led to significant negative local environmental influences (WWF, 2010; BUND, 2013; WWF 2018).

The impacts range from pollution or eutrophication of coastal ecosystems due to uncontrolled wastewater discharges to depletion of water resources. In addition, conflicts arise because of limited access to land and coastal regions. Furthermore, the use of wild fish as feed for fish in aquaculture is not sustainable. For these reasons, legal requirements and the limitation of natural resources limit the expansion of fish production using conventional methods such as pond systems, flow-through or cage systems in coastal areas. The further development of aquaculture requires an extension to alternative and competitive forms of fish production.

RAS

Recirculating aquaculture systems (RAS) can be an ecologically sustainable alternative to common aquaculture systems due to their process technology. RAS can be divided into four categories based on production intensity per water quantity used: extensive, semi-intensive, intensive and super-intensive (Lekang, 2013; Bregnballe, 2015). The rearing water is purified using a high-tech water treatment system and is returned to the water cycle to keep emissions as low as possible (Figure I-1). In this way, the system enables a resource-saving and largely independent production method that has less impact on the environment and allows optimal adaptation of husbandry conditions to the cultivated species through complete control of all environmental factors, e.g. the water temperature, the oxygen concentration or pH. In the last 2 decades RASs have been continuously developed. The limited water supply and the strict control of the discharge of sewage and nutrients into the environment have led to an increasing demand for recirculation systems. The performance and competitiveness of such recirculation systems essentially depends on the investment and maintenance costs and, above all, on the quality of the products.

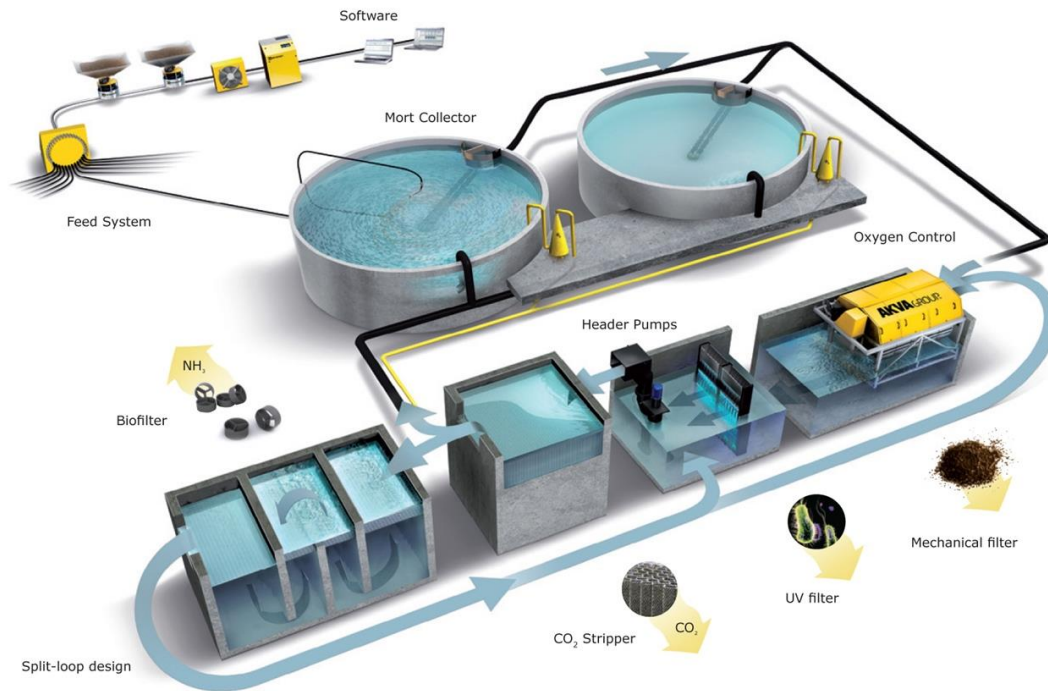


Figure I-1: Illustration of an exemplary water treatment system in a RAS (AKVA Group).

Limiting factors in RAS

The advantages of a RAS are offset by certain economic and technical disadvantages. The fish production in recirculation systems is still comparatively expensive and rarely competitive with fish products of other sources. One reason is often the lack of planning security. With the current infrastructure, a RAS operator has difficulties in reliably producing a profitable output with consistently high quality. A basic requirement could be a guaranteed and constant availability of new fry of the highest quality.

Another reason is the complex and sophisticated water treatment technology in a RAS, which means high investment and operating costs for the operator. In particular, the high energy consumption of the water treatment is a decisive factor for the economic success of a RAS. An optimal operation of such a system requires qualified personnel with the necessary experience and know-how to ensure constant water conditions. Other limiting and decisive factors, which will be discussed in more detail in this study, are the negative impairment of the taste of fish by off-flavor components, the risk of infectious diseases due to high stocking densities and the production and disposal of wastewater.

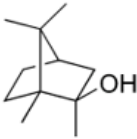
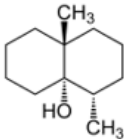
Off-flavor

Decisive for the turnover and the acceptance of fish products produced in a RAS are their quality. A significant impairment of the fish quality due to a negative taste (off-flavor) leads to high financial losses for the producers up to the complete loss of production.

Off-flavor is mainly caused by two chemical compounds: 2-methylisoborneol (2-MIB) and geosmin (GSM). The most important source of these compounds was identified in the aerobic areas of a RAS, such as the drum filter, trigglingfilter or the fishbasin (Guttman & Rijn, 2008). Both components are secondary metabolites, released by different microorganisms, such as cyanobacteria (Izaguirre et al., 1982), actinomycetes (Zaitlin and Watson, 2006) or fungi (Mattheis and Roberts, 1992). Furthermore, the bacterial decomposition of algae can also lead to the release of these compounds (Jenkins et al. 1967, Slater and Blok, 1983). Nevertheless, growth-promoting conditions for these organisms, such as warm, nutrient-rich water, do not necessarily lead to an off-flavor event. Thus, off-flavor events are often sporadic events and difficult to predict and to avoid.

Further complicating factors of 2-MIB and GSM are characterized by a slow rate of biodegradation (Ho et al., 2007) and their lipophilic affinity (Table I-1). Accumulated in the rearing water and absorbed directly via the gills, very low concentrations of GSM and 2-MIB in the range of ng/L in water are sufficient to produce an earthy musty smell and taste in fish (Tucker, 2000; Howgate, 2004).

Table I-1: Physical properties and structural formula of 2-MIB and GSM (Pirbazari et al., 1992).

Parameter	2-MIB	GSM
Boiling point [°C]	196.7	165.1
Vapor pressure [atm]	6.68×10^{-5}	5.49×10^{-5}
Henry's law constant [atm m ³ /mol]	5.76×10^{-5}	6.66×10^{-5}
Aqueous solubility [mg/L]	194.5	150.2
K _{ow}	3.13	3.7
Structural formula		

Several attempts to selectively prevent the growth of off-flavor-producing organisms have usually failed due to a wide range of often unknown organisms which release these components into the water (Wu & Boyd, 1990; Perin et al., 1996; Schrader et al., 1998). As a result, a non-selective treatment to avoid off-flavor producing organisms can also disrupt biological purification stages, such as the biofilter. Furthermore, the direct attack of the organisms can lead to the release of off-flavor components into the water via cell lysis.

So far, experiments with drinking water treatment methods have shown only very low efficiencies in removing off-flavor components. The main problem is the simultaneous presence of organic material. Organic substances, which are usually present in much higher concentrations, have always competed with off-flavor compounds, e.g. during adsorption with powdered activated carbon (PAC) (Chen et al., 1997; Cook et al., 2001) or during chemical cleavage with various oxidants such as ozone or hydroxyl radicals (Schrader et al., 2010; Klausen & Grønberg, 2010). In oxidation processes, there is as well a risk of formation of fish-toxic disinfection by-products (DBPs), especially in marine water due to the higher salinity (Tango & Gagnon, 2003). Until now, the only reliable method to counteract off-flavors in aquaculture is by purging off-flavored fish with clean and odourless water for a certain time prior harvesting. However, purging off-flavored fish leads to the loss of weight due to starvation, as feeding is not allowed during this time. Moreover, the increased consumption of resources and the need for large quantities of clean

water increase the production costs. For this reason, there is a growing interest in researching alternative technologies that prevent or remove off-flavor compounds efficiently.

Infectious diseases

In order to operate a RAS system as profitably as possible, the system is often run with a high stocking density. This increases the risk of an outbreak of infectious diseases by microbes or parasites, which can lead to significant production losses and financial difficulties for the operator of a RAS (Schnick, 1996). The possibilities for a prophylactic protection or the treatment of infectious diseases that have already broken out are very limited in a RAS. The intensive use of antibiotics and other drugs for prophylactic or acute purposes has been heavily criticized in recent years. Especially when using antibiotics, there is always the risk of spreading resistant germs. Furthermore, biological cleaning processes in a biofilter can be considerably disturbed by the use of disinfectants (Pedersen et al., 2009). Disinfection methods with oxidants such as ozone or chlorine can only be used with restrictions under the current rearing conditions in a RAS. The formation and accumulation of fish toxic disinfection by-products (DBP) limits the dosage of such disinfectants (Perrins et al., 2006; Schroeder et al., 2010; Tango and Gagnon, 2003). Furthermore, the efficiency of these processes also depends on the water matrix. Organic and inorganic substances act as scavengers in this process and reduce the oxidizing power by a substantial consumption of oxidizing agents. UV light offers efficient protection against bacteria and viruses and is therefore often used in RAS. Even so, larger organisms such as protozoa or parasitic worms remain unaffected by UV radiation (Chevrefils et al., 1999; Kasai et al., 2002). Furthermore, the effectiveness of UV radiation depends on the penetration depth of the radiation and hence on the turbidity of the water (Hoff, 1978; LeChevallier et al., 1981). In addition, UV radiation can also lead to a photochemical reduction of nitrate to toxic nitrite (Lewis Jr. and Morris, 1986; Goldstein & Rabani, 2007). These facts increase the interest in a treatment system that efficiently disinfects RAS water regardless of the level of water contamination.

Wastewater

In a RAS, especially in an intensive aquaculture system, a considerable amount of wastewater is produced, which either has to be disposed of or treated with a high level of technological effort, for example with a drum filter or a skimmer with additional ozone injection. From an ecological and economic point of view, the efficiency of this wastewater treatment will be one of the most decisive factors for the ecological sustainability and the worldwide expansion of RASs in the future. Organic and inorganic compounds accumulate in the rearing water, consisting mainly of feed residues, faeces and metabolic end products. The aerobic microbiological degradation of the produced organic matter consumes oxygen in the system. This increased oxygen consumption must be compensated by the supply of oxygen to maintain the necessary oxygen concentration in the water. Additionally, finely dispersed organic particles can cause gill damage in the fishes (Adam & Schnervers, 1993) or provide ideal growth substrates for germs and parasites. The finely dispersed or dissolved organic substances are currently removed by the addition of oxidizing agents. Ozone is used as a disinfectant but also for the oxidation of suspended or dissolved organic substances such as proteins or amino acids (Tango & Gagnon, 2003) and inorganic compounds such as nitrite (Rosenthal et al., 1978; Paller & Lewis, 1988). Anyway, the use of ozone as an oxidant is limited because the formation of disinfection by-products (DPBs) can have negative effects on fish health, especially in marine water with numerous reactants (Amy et al., 1994; Hofmann, 2000).

Protein metabolism produces mainly nitrogenous compounds such as ammonium (NH_4^+), which are released through the gills of the fish (Wright and Anderson, 2001). In the biofilter ammonium is further oxidized via nitrification to nitrite (NO_2^-) and then to nitrate (NO_3^-). In certain concentrations nitrate has a negative effect on the health and growth of the fish (Scott and Crunkilton, 2000; Shimura et al., 2004; McGurk et al., 2006; van Bussel et al., 2012; Schram et al., 2014). The most common method of reducing nitrate in the system is to dilute the rearing water by partially exchanging the water with fresh water. Another method is the implementation of a denitrification system. In denitrification, the nitrate is reduced to molecular nitrogen under anoxic conditions (van Rijn et al., 2006; Skiba, 2008). However, anoxic conditions are in contradiction to

the desired husbandry conditions, so that a successful operation of a denitrification system requires extensive technical support and qualified personnel.

Alternative strategies to remove organic or nitrogen compounds in wastewater are technically very complex or inefficient from an energy demand perspective (Matilainen & Sillanpää, 2010). The removal of these inorganic or organic compounds by a more efficient water treatment system would have many advantages.

Ultrasound induced cavitation

In recent years, the topic of ultrasonically induced cavitation in water treatment has become increasingly important. Ultrasound or ultrasonic waves are by definition sound waves between about 16 and 1000 kHz and above the frequency range of the human hearing and below the hypersonic range. Cavitation most frequently occurs with fast moving objects in a liquid, such as water turbines, centrifugal pumps or even when ultrasound-induced pressure waves are sent through the water (Wikipedia, 2020). Here, two forms of cavitation are distinguished: the stable and the transient cavitation (Flynn, 1964; Apfel, 1981). During cavitation, the static pressure falls below the evaporation pressure of the liquid and leads to the formation of vapour bubbles. If these vapour bubbles are carried into an area with higher pressure, the static pressure rises above the vapour pressure again and leads to a sudden condensation in form of an implosion during transient cavitation (Figure I-2). At the stable cavitation, the gas bubbles mainly consist of the gases dissolved in the water, which prevent the implosion.

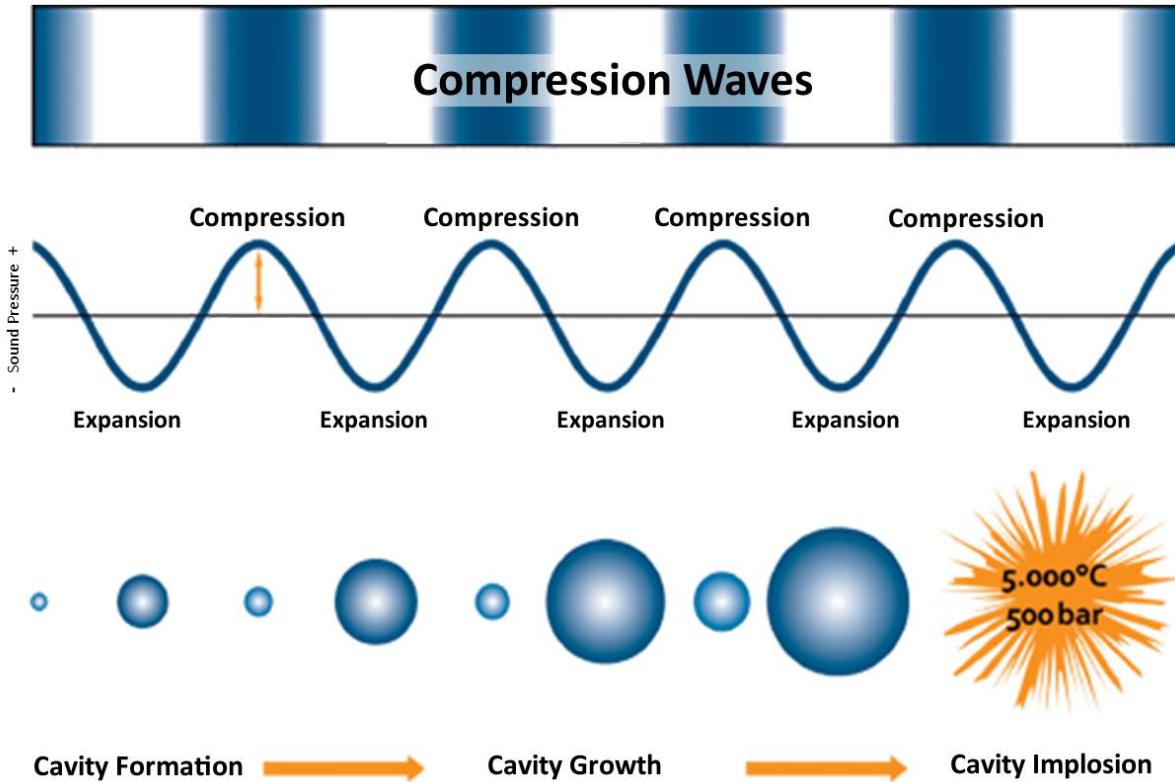


Figure I-2: Schematic illustration of transient cavitation. The formation and implosion of a cavity by compression waves in a fluid triggered by sound waves (ultrawaves.de).

Transient cavitation is particularly important for applications in water treatment. The strong implosion of the steam bubble causes extreme pressure and temperature peaks ($p > 100 \text{ atm}$, $T^\circ\text{C}$ up to 5200 K) due to the adiabatic change of state (Suslick et al., 1986) (Figure I-3). This results in mechanical and sonochemical effects. The mechanical effects are a combination of turbulences, flows and shear forces via shock waves (Piyasena et al., 2003). Sonochemical effects include thermal dissociation (pyrolysis) of volatile compounds within the hot spot and the formation of free radicals or secondary oxidants in the bulk medium. The thermal dissociation of water vapor generates hydrogen atoms and hydroxyl radicals and the recombination of two hydroxyl radicals produces hydrogen peroxide. In addition, a hydrolysis and a low temperature pyrolysis takes place in the hydrophobic gas-liquid interface (supercritical interface) (Riesz, 1992).

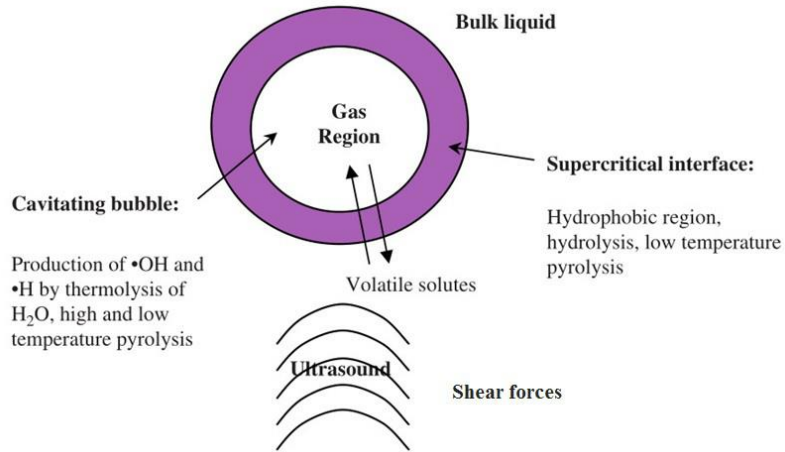


Figure I-3: Illustration of cavitating bubble caused by ultrasound irradiation (Song, W. & O'Shea, 2007).

The effects vary in intensity depending on the frequency or acoustic intensity (W/m^2). High-frequency ultrasound (HFUS) creates cavities with a smaller maximum diameter due to the shorter wavelength. In contrast, low-frequency ultrasound (LFUS) produces fewer cavities, but offers more time for growth, so that the cavities at LFUS collapse more violently by achieving larger maximum sizes (Portenlänger and Heusinger, 1997) (Figure I-4).

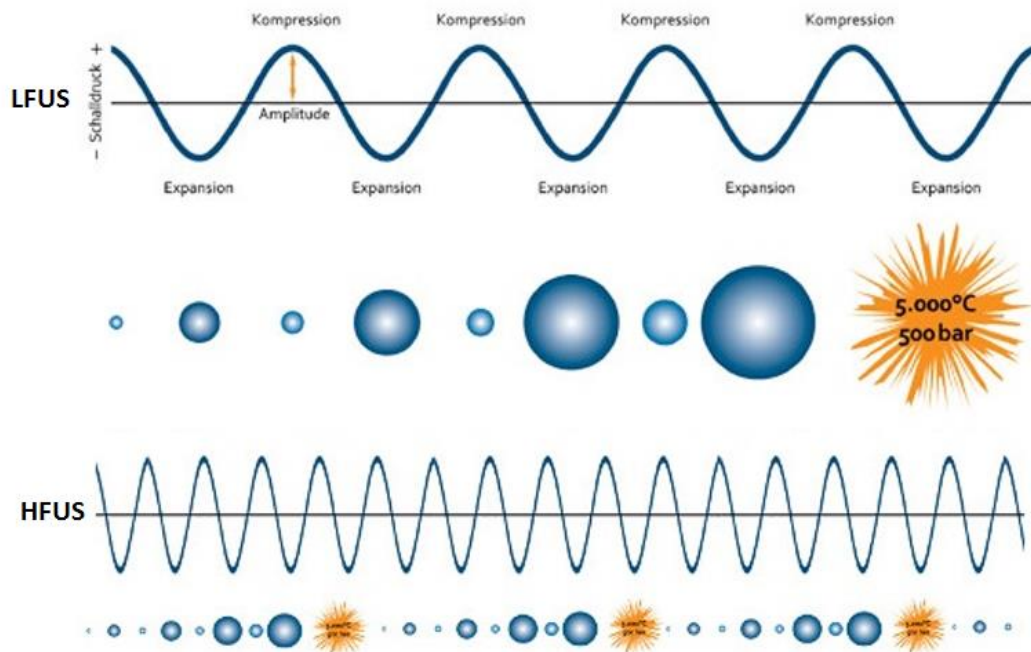


Figure I-4: Illustrated comparison of cavitation formation by low-frequency ultrasound (LFUS) and high-frequency ultrasound (HFUS) (ultrawaves.de).

Consequently, stronger mechanical forces are released via LFUS. On the other hand, HFUS produces more cavities and more hot spots and thus more hydroxyl radicals by increased pyrolysis reactions, which lead to more sonochemical effects (Hua and Hoffmann, 1997). The manifold effects of cavitation and the resulting potential to either act as a biocide or to degrade various chemical compounds have been investigated by several studies in the past. (Thacker, 1973; Scherba et al., 1991; Earnshaw et al., 1995; Nagata et al., 1996; Pétrier & Francony, 1997; Phull et al., 1997; Gonze et al., 1999; Gogate & Pandit I & II, 2000; Gogate et al., 2002; Mason et al., 2003; Neis and Blume, 2003; Hamdaoui & Naffrechoux, 2007; Naddeo et al. 2007; Wang et al., 2008; Joyce and Mason, 2008; Gogate & Kabadi, 2009; Shrestha et al. 2009; Tang & Sivakumar, 2013; Petkovšek et al., 2013; Zupanc et al., 2013; Torabi & Ghiaee, 2015).

Aim and Scope

As demonstrated in several studies, cavitation has a high potential of reducing undesirable substances in water. In particular, the combination of mechanical and sonochemical effects have the advantage of treating several limiting factors of RAS simultaneously with one treatment method. Therefore, the aim of this study is to evaluate ultrasound as a new innovative physico-chemical water treatment process for the prevention of off-flavor, for the prophylactic reduction of germs and for the improvement of the efficiency of wastewater treatment in RAS. Experimental investigations in both fresh and marine water were carried out, taking biological, chemical, technical and economic aspects into consideration.

Chapter 1

Chapter 1 includes the study that examined the ability of ultrasound to reduce the two off-flavor compounds 2-methylisoborneol (2-MIB) and geosmin (GSM) in RAS water.

The following questions have been scientifically investigated:

- How efficiently does ultrasound reduce GSM and 2-MIB in RAS fresh and marine water?
- Which ultrasonic frequency is more efficient: HFUS at 850 kHz or LFUS at 20 kHz?
- What influence does the salinity have on the efficiency of the reduction with regard to the use of the ultrasound in a fresh or marine water RAS?
- To what extent does the water matrix influence the efficiency of the process in terms of organic or inorganic pollution?

Chapter 2

In chapter 2 the potential of ultrasonically induced cavitation for the reduction of germs and parasites in RAS water was investigated using different model organisms representing typical aquaculture pathogens and parasites.

The following questions were examined scientifically:

- What effects does ultrasound have on various model organisms such as bacteria, algae and zooplankton and what is the inactivation rate in relation to the energy consumed?
- Which ultrasonic frequency is more efficient: HFUS at 850 kHz or LFUS at 20 kHz?

- Which specific characteristics of the organisms, such as size or body shape, have an influence on the effectiveness of ultrasound?

Chapter 3

Chapter 3 examines the ultrasound treatment of RAS waste water and investigates whether ultrasound can reduce organic and inorganic compounds in RAS water. Experiments were carried out to answer the following questions:

- Does the concentrations of total carbon (TC), inorganic carbon (IC), dissolved organic carbon (DOC) and total nitrogen (TN) be significantly reduced by ultrasound?
- Which ultrasonic frequency is more efficient: HFUS at 850 kHz or LFUS at 20 kHz?
- What influence does the salinity have on the efficiency of the reduction with regard to the use of the ultrasound in a fresh or marine water RAS?

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Chapter 1

Removal of the off-flavor compounds geosmin and 2-methylisoborneol from recirculating aquaculture system water by ultrasonically induced cavitation

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Abstract

With its high economic impact, off-flavor in fish is still one of the most serious problems in the aquaculture industry worldwide. Until now, the highly cost- and time-intensive, as well as capacity demanding depuration procedure by moving the fish to clean and odor-free water for a certain time prior to harvest is the only reliable way to counteract off-flavors in aquaculture. Alternative strategies and processes for efficient off-flavor prevention are still lacking. Hence, the aim of this study was to investigate the potential of ultrasonic water treatment to decrease the concentration of the relevant off-flavor compounds geosmin (GSM) and 2-methylisoborneol (2-MIB) in aquaculture water. Therefore, different water matrices, varying in their organic and inorganic load and composition (tap water, RAS fresh water, RAS marine water), were spiked with 2-MIB and GSM standard and subsamples of 250 ml were subsequently treated for 15 min using a lab-scale ultrasound transducer at 850 kHz. For verification samples from commercial RAS containing biogenic 2-MIB and GSM were treated equally. The effects of ultrasound frequency and salinity on the removability of 2-MIB and GSM via ultrasonic treatment were investigated by comparing the removal efficiency of high (850 kHz) vs. low (20 kHz) frequency ultrasound and by adding artificial marine salt (10 ppt) to different fresh water samples prior to ultrasound treatment, respectively.

Results have demonstrated that ultrasonically-induced cavitation significantly reduces the tested off-flavor compounds GSM and 2-MIB in all tested water types, seemingly irrespective of the (in)organic load. In general, the reduction of GSM was slightly higher compared to that of 2-MIB. Furthermore, the reduction of tested off-flavor compounds was significantly enhanced at high frequency ultrasound (850 kHz) compared to low-frequency ultrasound (20 kHz). The addition of artificial marine salt to fresh water samples caused an additional improvement in removability of both off-flavor compounds.

Our results evidence high frequency ultrasound as a potential treatment process for significant removal of the relevant off-flavor compounds 2-MIB and GSM from RAS process water. In particular, the seemingly low dependency of the ultrasound-induced removal of GSM and 2-MIB on the organic and inorganic process water load predestines ultrasonically induced cavitation as

a potential strategy for off-flavor prevention in RAS compared to alternative strategies such as advanced oxidation processes or adsorption processes.

1. Introduction

A great deal of attention has been focused on the negative taste and odor of fish apparently associated with off-flavors encountered in aquaculture systems worldwide. Off-flavor in fish is associated with high levels of 2-methylisoborneol (2-MIB) and geosmin (GSM), which are secondary metabolites, released by different microorganisms, such as cyanobacteria (Izaguirre et al., 1982), actinomycetes (Zaitlin & Watson, 2006) or fungi (Mattheis & Roberts, 1992). These compounds are generally characterized by a slow rate of biodegradation (Ho et al., 2007) and their lipophilic affinity. Furthermore, the relevant concentrations of both compounds in the range from ng/L to µg/L might be too low to compensate the metabolic costs associated to their utilization for biodegradation (Arrieta et al. 2015). Therefore, both compounds accumulate in the culture water, where they are directly absorbed through the gills and subsequently stored in the lipid-rich tissue of fish. There are several factors influencing the uptake and depuration rate of these two compounds including the ventilation rate of the gills or fat-content of the tissue (From & Hørlyck, 1984; Martin et al., 1988). Studies have indicated that even very low concentrations of GSM and 2-MIB in the range of ng/L in the surrounding water and a high bio concentration factor (BCF) are sufficient to contribute an earthy-musty smell and taste in fish (Howgate, 2004). Furthermore, the aerobic and organic rich conditions in aquaculture water aggravate the emission of metabolites caused by the stimulation of the growth of off-flavor producing microorganisms (Guttman & van Rijn, 2008). Moreover, off-flavor often appears with extensive seasonal variability or sporadic concentration disparity. As a consequence, the development of strategies to avoid off-flavor events at an early stage proved to be difficult (Tucker, 2000).

The frequent events of off-flavored fish products have adversely affected the economic gains due to a poor product quality (Engle et al., 1995). It has been estimated that in the U.S. catfish production off-flavors add \$15 - \$23 million additional production costs annually due to harvest delays or the expensive application of liquid copper products reducing off-flavor occurrences (Hanson, 2003). Currently there hardly exist effective methods or strategies to avoid off-flavor in practice.

Until now, purging off-flavored fish by moving the fish to clean and odor-free water for a certain time prior to harvest is still the only reliable method to counteract off-flavors in aquaculture.

However, the weight loss of the fish due to starvation, the extended consumption of resources (e.g. energy, holding site) and the large quantity of clean water required lead to a cost intensive and time consuming process. Moreover, the highly wasteful consumption of clean water for aquaculture is a luxury reserved for countries with sufficient water resources. For this reason there is a growing interest to identify cost-effective alternative technologies that prevent or remove off-flavor compounds efficiently.

A number of strategies have been investigated to prevent the growth of cyanobacteria, as one of the main off-flavor producing organisms, e.g. manipulating nutrient availability by phosphorus removal (Wu & Boyd, 1990), biomanipulation by planktivorous fishes (Perin et al., 1996) or algaecides (Schrader et al., 1998). However, the wide range of off-flavor producing and often unknown microorganisms reduces the chance for a selective removal or avoidance of these organisms. Additionally, these strategies restrict biological cleaning applications often used in RAS like bio filtration or result in the lysis of off-flavor producing cells and the release of GSM and 2-MIB into the water.

Accordingly, common methods for treatment of drinking water were conducted with the focus on the reduction of off-flavor compounds in aquaculture water. The biodegradation of GSM or 2-MIB with a biological sand filtration (Ho et al. , 2007) or anaerobic sludge digestion treatment (Guttman & van Rijn, 2009) offered some good results but did not reveal an efficient solution to remove 2-MIB and GSM in an aquaculture system. The adsorption with granular (GAC) or powdered activated carbon (PAC) is restricted by natural organic matter (NOM) due to clogging or competitive adsorption, so that a high economic ineffective dose of activated carbon is required to remove off-flavor compounds to an acceptable concentration (Chen et al., 1997; Cook et al., 2001). Experiments with conventional chemical oxidizing agents showed encouraging removal rates but with regard to further implementation in an aquaculture system, the occurring side effects exclude the applicability of these methods. For example, the applied doses of chlorine or chlorine dioxide to reduce off-flavor substances could produce disinfection by products (DBPs) like trihalomethanes (THMs) or haloacetic acids which are highly toxic to aquatic animals (von Sonntag & von Gunten, 2012). The application of “low-dose” ozone to improve certain water quality parameters did not significantly reduce off-flavor compounds. The addition of higher dosages of O₃ might remove 2-MIB and GSM in RAS water but required ozone-reducing strategies

to avoid the risk of ozone toxicity (Schrader et al., 2010). To improve the oxidation efficiency, advanced oxidation processes (AOPs) like UV/O₃ or UV/H₂O₂ have received considerable attention due to the specific enhanced generation of the more powerful hydroxyl radical (OH·). However, these methods are limited by dissolved or non-dissolved substances of aquaculture water caused by competitive oxidation processes with other organic matter or inorganic radical scavengers (Klausen & Gronborg, 2010) and the production of fish toxic DBPs (Tango & Gagnon, 2003).

In recent years ultrasound has received attention in view of several applications in water treatment. The advantage of this technique is the occurrence of cavitation caused by ultrasonically induced pressure waves. Cavitation is the formation and the sudden implosion of vapor cavities in a local hot spot under extreme conditions ($p > 100$ atm, $T^{\circ}\text{C} \sim 5200$ K) (Suslick et al., 1986). Whereas in the stable cavitation the microbubbles oscillate around a certain radius, in the transient cavitation the bubble radius increases in a couple of acoustic cycles until the bubble implodes. This implosion causes mechanical as well as sonochemical effects. The mechanical effects are a combination of turbulences, liquid circulation currents and shear forces via shock waves (Piyasena, 2003). The sonochemical effects include the thermal dissociation (pyrolysis) inside of the hot spot and the generation of free radicals or secondary oxidants. For example, hydrogen atoms and hydroxyl radicals were generated by the thermal dissociation of water vapor. The formation of secondary oxidants such as hydrogen peroxide derived from the recombination of two hydroxyl radicals (Riesz, 1992). The quantity and the effects of cavitation depend on the intensity (W/m^2), irradiation time and frequency (kHz) of ultrasound as well as from the reactor configuration. High-frequency ultrasound produces more small cavities than low-frequency ultrasound due to the shorter wave length and less time for bubble growth, respectively. Therefore low-frequency ultrasound gives more time for growth, so that bigger cavities collapse more violently by achieving larger maximum sizes. As a consequence, higher shear forces in combination with sonochemical effects are expected at low frequencies while at higher frequencies only sonochemical effects are present (Portenlänger & Heusinger, 1997).

Song & O'Shea (2007) have demonstrated a high potential of high-frequency ultrasound water treatment (640 kHz) for the removal of GSM and 2-MIB in demineralized water. Experiments with radical scavengers have revealed the ultrasound-induced pyrolysis as the dominant degradation process rather than the radical reaction pathway. Furthermore, the ultrasound treatment at 200

kHz has shown higher removal rates of off-flavor compounds than at lower frequencies in pond water (Srisuksomwong *et al.*, 2011).

In RAS the application of ultrasound has hardly been carried out. Hence, the aim of the present study was to investigate the ultrasound-induced degradation of GSM and 2-MIB in different RAS water matrices. Optimization capabilities were examined by investigating the influences of salinity and frequency of the off-flavor degradation.

2. Material & Methods

2.1 Experimental setup

2.1.1 Experiment with standard solution in different water matrices

In order to investigate the ultrasonically-induced degradation of off-flavor in aquaculture water, samples from RAS were spiked with 2-MIB and GSM standard solution and were treated with the 850 kHz ultrasound system. GSM and 2-MIB standard were purchased from Sigma Aldrich (Product Number 47525-U) consisting a solution of 1 ml with 100 µg of both compounds dissolved in methanol. The experiments were examined with tap water (DOC=2.6 mg/L) and 2 different types of aquaculture water taken from the pump sump: aquaculture fresh water from an experimental RAS stocked with juvenile carp (*Cyprinus carpio*) (stocking density 3 kg/m³; DOC=8.6 mg/L) with a salinity of 0.2 ‰ and aquaculture marine water from an experimental RAS stocked with turbot (*Scophthalmus maximus*) (stocking density 19 kg/m³; DOC=8.1 mg/L) with a salinity of 26 ‰. Each type of water sample was spiked with the standard mix solution containing both compounds. The initial concentration of GSM and 2-MIB was adjusted to 5 µg/L and to 100 ng/L, respectively. Samples including tap and aquaculture water without the standard solution were used as control samples. All samples were stored at 6 °C.

In each experiment a sample volume of 250 ml was treated in an open cylindrical glass reactor cooled by cold water surrounding the reactor to maintain the temperature nearly constant. The treatment duration was always 15 min. immediately after the ultrasound treatment the total residual oxidants concentration (TRO) were determined, as described below. GSM and 2-MIB were extracted in 20 ml pentane with a liquid-liquid extraction. Therefore each sample was placed

in a separatory funnel and was firmly shaken twice with 10 ml pentane. The extraction was carefully dried and demulsified with sodium sulfate and then immediately cooled to -18°C . The extract volume was gently reduced by a rotary evaporator at 20 mbar to a volume of about 200 μl and was transferred directly to a glass vial. In this vial the concentrate was finally reduced to a volume between 100 -200 μl with a gentle nitrogen flow. The final volume was exactly determined with a 200 μl syringe. The GSM and 2-MIB concentration was quantified by manually injecting 1-2 μl into the GC/MS. Recoveries from spiked samples ranged from 74 to 134 % for GSM and 81 to 153 % for 2-MIB. In comparison to the initial concentration the percentage degradation were calculated. At the beginning of each experiment only pentane was treated and measured with exactly the same way as the spiked samples used as a blank.

2.1.2 Experiment with biogenic GSM and 2-MIB in different RAS water

To verify the data from the experiment with the standard solution, samples from a commercial RAS containing natural 2-MIB and GSM were examined. The samples were taken from a pikeperch RAS (stocking density 40 kg/m^3) and an experimental RAS stocked with rainbow trout (*Oncorhynchus mykiss*)(stocking density 40 kg/m^3). The samples were treated, extracted and analyzed by the same procedure as for the experiments with the standard solution in different RAS matrices.

2.1.3 Effects of additional added artificial marine salt on the ultrasound-induced degradation

A potential salting out effect to enhance the degradation efficiency was examined adjusting a higher salinity in samples containing 2-MIB and GSM prior to ultrasound treatment. Tap water spiked with both off-flavor compounds ($c_0=5 \mu\text{g}/\text{L}$) and RAS water taken from a pikeperch RAS (stocking density 40 kg/m^3) containing biogenic off-flavor ($c_{0,2\text{-MIB}}=2.9 \text{ ng}/\text{L}$; $c_{0,\text{GSM}}=3.8 \text{ ng}/\text{L}$) were prepared by additionally adding 10 g/L artificial marine salt (Aqua Medic GmbH). All samples underwent the same treatment, extraction and analysis as for the experiments with the standard solution in different RAS water.

2.1.4 Low and high frequency ultrasound-induced degradation of GSM and 2-MIB

To compare the degradation efficiency of high and low frequency ultrasound, 250 ml of tap water, aquaculture water from a marine water RAS stocked with Atlantic salmon (*Salmo salar*) (stocking density 35 kg/m³) and fresh water RAS stocked with rainbow trout (*Oncorhynchus mykiss*)(stocking density 9 kg/m³) were spiked with off-flavor standard (5 µg/L) and treated with 20 kHz and 850 kHz ultrasound. For comparable results the 850 kHz transducer were placed on the top of the cylindrical glass reactor and emitted the ultrasound waves top down like the 20 kHz horn homogenizer. To maintain the temperature nearly constant the reactor was cooled by surrounding cold water. The samples were extracted and analyzed by the same procedure as for the experiments with the standard solution in different RAS water.

2.2 Equipment

2.2.1 Ultrasound systems

The 850 kHz ultrasound was generated with a Meinhardt E/805/T/solo ultrasound transducer with 75 mm diameter powered by a Meinhardt Ultraschall-Leistungs-Generator K8 (200 W) in pulsed mode (on/off ratio 1:5). The low frequency ultrasound of 20 kHz was emitted from a titanium horn homogenizer (13 mm) with a UW 3200 ultrasound transducer and a SH 219 Boosterhorn. The electrical power was generated by a Bandelin GM 3200 HF-Generator (200 W) in pulsed mode (on/off ratio 1:5).

2.2.2 GC/MS-Analysis

The GC (Fisons GC 8000 Series) was operated with a Restek RTX©-5 fused silica capillary column (60m x 0.25 mm ID x 0.25µm, cat. 10226). The detection limit was approximately 2 ng/L. The injector temperature was 200 °C. The split/splitless injector was opened 1 min after the injection. The GC temperature program starts with 40 °C 8 min and increased with a rate of 10 °C/min till

250 °C. The maximum temperature of 250 °C was held for 2 min. Helium was used as the carrier gas at constant flow rate of 1.5 ml/min. The quadrupole mass spectrometer (Fisons Instruments MD800) was operated in single ion mode (SIM). For each compound 3 ions were monitored: 95, 107, 135 for 2-MIB and 111, 112, 125 for GSM. The data were fully acquired and processed with Xcalibur™1.2 software.

2.3 Water analysis

The TROs were determined spectrophotometrically by a DR/2800 Spectrophotometer (Hach Lange GmbH) as equivalent total residual chlorine (TRC) using the colorimetric N,N-diethyl-p-phenylenediamine (DPD) method (DPD Total Chlorine powder pillows, Hach) as recommended by Buchan et al. 2005. TOC was analyzed as non-purgeable organic carbon (NPOC) using a total organic carbon analyzer TOC-V CPH/CPN (Shimadzu corp., Kyoto, Japan). The salinity was determined by a WTW MonoLine Cond 3310 IDS and the temperature was measured using a VOLTCRAFT K204 Type K.

2.4 Statistical analysis

Data are presented as mean \pm confidence interval ($p < 0.05$) of n samples. Statistical analysis of variances was performed using SPSS 20.0 (IBM Inc, Armonk, USA). Data were tested for normality and for equal variances using Levene's test and the Shapiro-Wilk test, respectively. Significant differences in means ($p < 0.05$) were determined by t-Test or one-way ANOVA with Tukey HSD or Dunnet T3post hoc test.

2.5 Energetic evaluation

To estimate the energy absorption rate and the heat transfer from the ultrasound system (850 kHz) to the water, calorimetric measurements were conducted determining the temperature increase of the water during ultrasound treatment without cooling.

3. Results and Discussion

3.1 Ultrasound-induced degradation of GSM and 2-MIB in different water matrices

The results show a significant reduction of GSM and 2-MIB in all spiked water samples (Fig. 1-1 & 1-2). Additionally, the results were verified with water samples from a trout experimental RAS and a commercial pikeperch RAS containing biogenic off-flavors (Fig. 1-3).

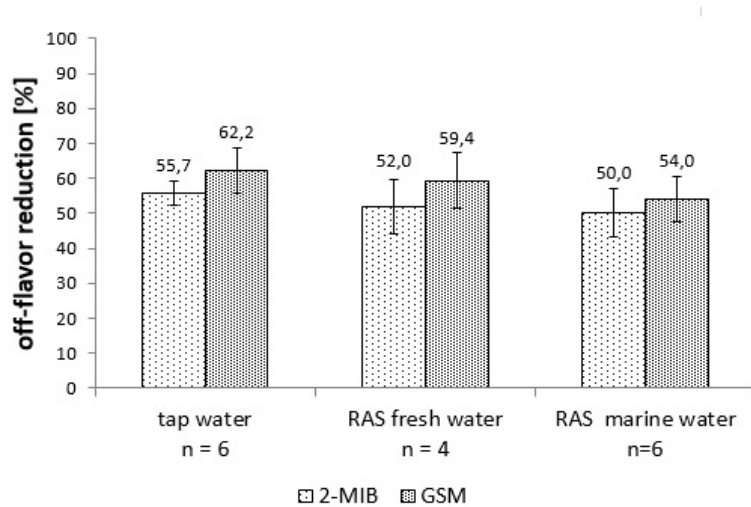


Figure 1-1: Mean reduction [%] ± confidence interval ($p < 0.05$) of 2-MIB and GSM in tap water, RAS fresh water and RAS marine water after 15 min ultrasound treatment. Prior treatment each sample was spiked with both off-flavor compounds ($c_0 = 5 \mu\text{g/L}$).

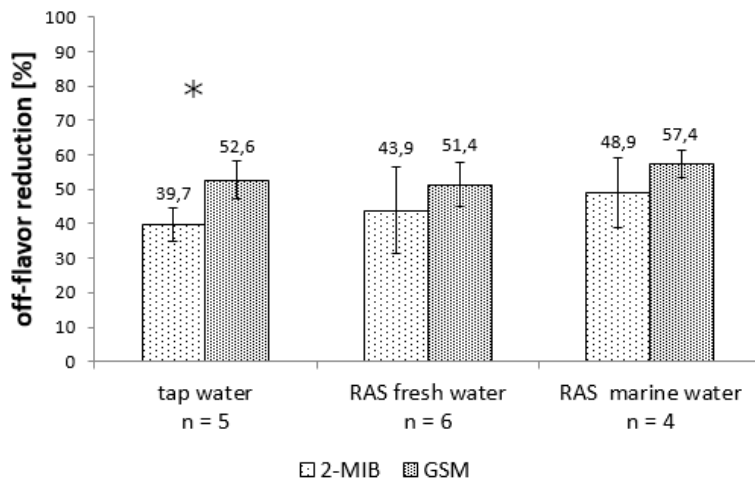


Figure 1-2: Mean reduction [%] ± confidence interval ($p < 0.05$) of 2-MIB and GSM in tap water, RAS fresh water and RAS marine water after 15 min ultrasound treatment. Prior treatment each

sample was spiked with both off-flavor compounds ($c_0=100\text{ng/L}$). * In tap water, significant differences between the 2-MIB and GSM reduction were measured.

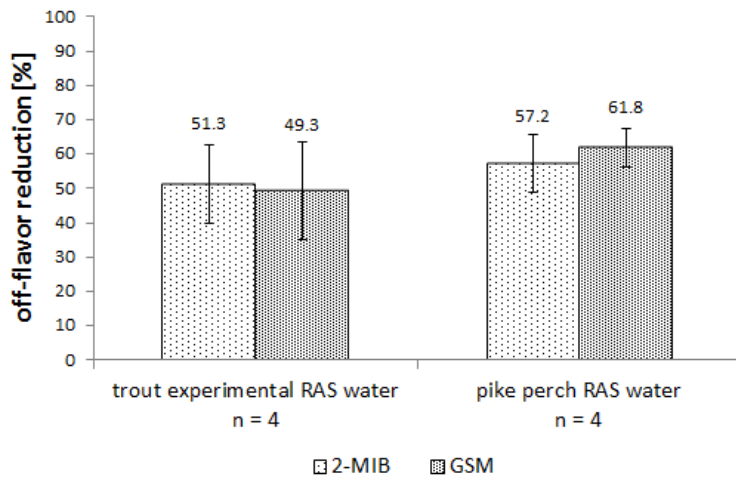


Figure 1-3: The mean reduction [%] \pm confidence interval ($p<0.05$) of biogenic 2-MIB and GSM in a trout experimental RAS water ($c_{0,2\text{-MIB}}=35.5\text{ ng/L}$; $c_{0,\text{GSM}}=15.5\text{ ng/L}$) and in pikeperch RAS water ($c_{0,2\text{-MIB}}=2.9\text{ ng/L}$; $c_{0,\text{GSM}}=3.8\text{ ng/L}$).

It can be assumed that sonochemical effects might be responsible for the degradation of GSM and 2-MIB. The sonochemical reactivity occurs in three regions of the cavities (hot spot): inside the gas region, a thin hydrophobic liquid layer surrounding the gas region (gas-liquid interface) and the bulk liquid (Riesz, 1992). At a critical stage of the cavities the gas region collapses under nearly adiabatic condition and lead to extreme high temperatures ($\sim 5200\text{ K}$) and pressures ($>100\text{ atm}$) (Suslick et al., 1986). Due to these extreme conditions, molecules inside the gas region were destructed by thermal dissociation (pyrolysis) comparable to reactions during a combustion process. It is assumed, that GSM and 2-MIB might be destructed by pyrolysis while diffusing from the liquid into the gas phase. The only 200 nm thick gas-liquid interface acts as a transitional region between the gas phase and the bulk-liquid and provides an additional region of thermal dissociation processes ($\sim 1900\text{ K}$) as well as free radical reactions. Additionally, the destruction of water vapor leads to the formation of hydroxyl radicals and hydrogen atoms (Makino et al., 1983) which can diffuse from the inside of the bubble towards the bulk liquid (Adewuyi, 2005). Therefore, GSM and 2-MIB might be destructed in the hot spot by pyrolysis and/or by oxidation processes via radical reaction. So far, Song & O'Shea (2007) demonstrated that ultrasound

decreases the off-flavor concentration to less than 50 % in Milli-Q water within 10 min ultrasound treatment at 640 kHz. The results are in agreement with the experimental data from Song & O’Shea (2007) and can further expand their research by demonstrating the applicability of ultrasound treatment at 850 kHz to off-flavored tap water, RAS fresh water and RAS marine water.

The comparison of the degradation of both compounds between the higher and the lower initial concentration revealed no significant differences (two-sample t-Test, $p < 0.05$) in the RAS water samples. Consequently, it can be assumed that off-flavor concentrations in the range of ng/L, typical for off-flavor events in aquaculture systems, might have no influences of the degradation efficiency of ultrasound.

Although in most cases the reduction of GSM did not statistically differ from the reduction of 2-MIB within the same water matrix, the results are consistent with the findings of Song & O’Shea (2007) that GSM tended to be more degraded than 2-MIB. This effect could be explained by the slightly higher hydrophobicity (K_{ow}) and less solubility of GSM (Tab. 1-1) which results in an enhanced transport of GSM from the liquid into the gas phase or the interfacial region.

Table 1-1: Physical properties of 2-MIB and GSM (Pirbazari et al., 1992).

Parameter	2-MIB	GSM
Boiling point [°C]	196.7	165.1
Vapor pressure [atm]	6.68×10^{-5}	5.49×10^{-5}
Henry’s law constant [atm m ³ /mol]	5.76×10^{-5}	6.66×10^{-5}
Aqueous solubility [mg/L]	194.5	150.2
K_{ow}	3.13	3.7

These findings verify the assumption that the pyrolysis might be the dominant degradation process rather than the radical reaction pathway. Moreover, the lower boiling point temperature of GSM compared to 2-MIB indicates a higher steam pressure and hence a greater impulse to transfer from the liquid into the gas phase. In addition, former experiments demonstrated that primarily the hydrophobicity controls the transfer process of organic substrate from the bulk liquid to the cavitating bubbles (Wu & Ondruschka, 2005) and that a hydrophobicity effect occurs specifically in the gas-liquid interface (Henglein, 1987).

No significant differences in the reduction of GSM or 2-MIB within the group with the same initial concentration could be measured (ANOVA, $p < 0.05$). Due to the non-selective oxidation ability of hydroxyl radicals, organic or inorganic matter acting as radical scavengers, diminishing the probability of radicals reacting with GSM or 2-MIB. Therefore, the concentration proportion between chloride ions and off-flavor compounds (ng/L), especially in marine water, supports the assumption of Song & O'Shea (2007) that the pyrolysis might be the dominant degradation process. Consequently, the ultrasonically-induced degradation of GSM and 2-MIB seems to be independent of the RAS water matrix containing unavoidable organic or inorganic compounds. Thus, ultrasound water treatment has an important and essential advantage to alternative water treatment methods based on oxidation processes.

3.2 Effects of additional added artificial marine salt on the ultrasound-induced degradation

Data in Fig. 1-4 show that in spiked tap water ($c_0 = 5 \mu\text{g/L}$) containing additionally added artificial marine salt (10 g/L), a significant reduction enhancement of 2-MIB and GSM was achieved.

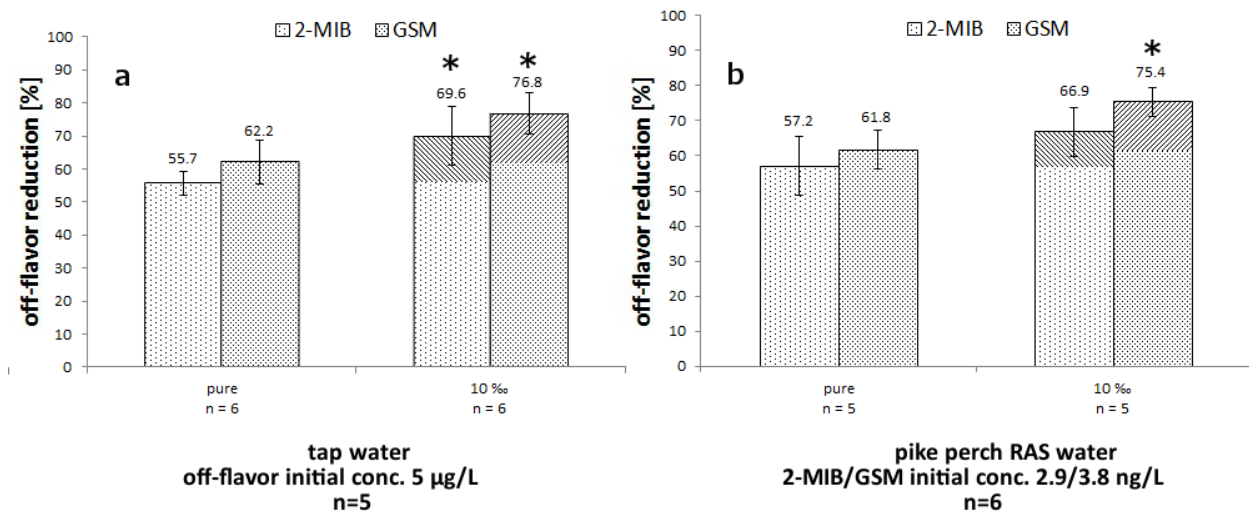


Figure 1-4: Mean reduction [%] ± confidence interval ($p < 0.05$) of 2-MIB and GSM in spiked tap water ($c_0 = 5 \mu\text{g/L}$) (a) and a sample taken from a commercial pikeperch RAS (b) containing biogenic off-flavors. The bars illustrate the reduction of GSM and 2-MIB after 15 min ultrasound treatment in each sample without addition of marine salt compared to the reduction in the same sample

with a higher salinity (10 g/L). Bars marked with * show a significant enhancement of the reduction efficiency with the addition of artificial marine salt.

The degradation was increased of 25 % of 2-MIB and 24 % of GSM, respectively. In the pikeperch RAS sample with biogenic off-flavor and additionally added artificial marine salt of 10 g/L, a significant reduction improvement of GSM of about 21 % was observed. The results can be explained in terms of a salting out effect by increasing the ion strength of the aqueous phase with the addition of salt and promoting the transfer of organic compounds from the water towards the gas region or interfacial region of the cavities (Seymour & Gupta, 1997). Therefore, the salting out effect might lead to a quantitative transfer enhancement of hydrophobic and less soluble compounds like GSM and 2-MIB. However, the approximately equal degradation level of GSM and 2-MIB in different RAS water matrices (Fig. 1-1) demonstrates no significant differences between the off-flavor degradation in marine and fresh water samples. The inconsistency of these results does not imply a reliable and clear relation between the salinity and the reduction potential of GSM and 2-MIB with ultrasound. The fact that it could be achieved a reduction enhancement by increasing the salinity of the same water matrix and no effects by examining samples from different RAS raises the questions which disparate water parameters between these different water types improve or worsen the off-flavor degradation. It is assumed that opposing effects during the ultrasound treatment might be a reason. While the addition of salt drives 2-MIB and GSM towards the cavities, the reduction rate decreases in the attendance of volatile or non-volatile organic compounds by lowering the temperature of the collapsing cavitation bubble due to a lower specific heat ratio (Goel et al., 2004; Visscher et al., 1996). Even the lowest contamination like particulate organic matter containing gas- or vapor-filled microbubbles reduces the tear strength of water and promotes cavitation (Strube, 1970). Finally, under the same technical conditions, the direct comparison of the ultrasonically-induced degradation of GSM and 2-MIB should take the different water matrices into consideration. Further experiments, considering more physical and chemical properties of the RAS water matrix and its ingredients which can potentially influence the efficiency of ultrasonically induced degradation, might be helpful.

3.3 Low and high frequency ultrasound-induced degradation of GSM and 2-MIB

As can be seen from Fig. 1-5 the high frequency ultrasound at 850 kHz always achieved significant higher reduction of GSM and 2-MIB than at low frequency ultrasound at 20 kHz (two-sample t-Test, $p < 0.05$).

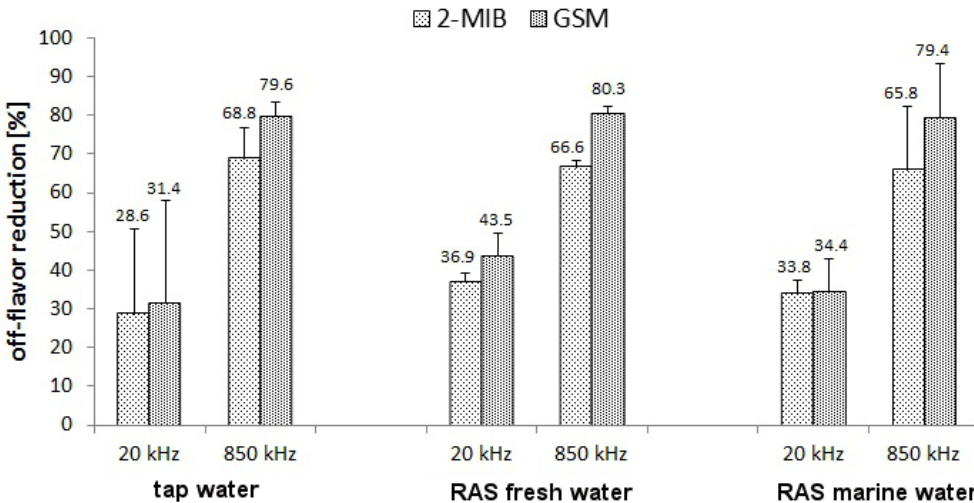


Figure 1-5: For each water matrix the mean reduction [%] + confidence interval ($p < 0.05$) of 2-MIB and GSM after 15 min treatment with 20 kHz and 850 kHz ultrasound is illustrated. Every treatment shows a significant higher reduction of 2-MIB and GSM at 850 kHz than at 20 kHz ultrasound.

This study is compatible with the findings of Srisuksomwong *et al.* (2011) that the irradiation at higher frequencies gave a better removal performance than at lower frequencies. This may be explained by the higher amount of smaller cavities at high frequency ultrasound providing a greater surface availability than less number of greater cavities at low frequency. In consequence, a higher amount of off-flavor molecules were transferred from the liquid into the gas region or the gas-liquid interface and were degraded by thermal dissociation. In addition, the higher amount of cavities consequently applied more sonochemical effects by a higher production of hydroxyl radicals and may support the reduction of 2-MIB and GSM (P tierier & Francony, 1997). In comparison to the lower reduction efficiency of the previous experiments in Fig. 1-1, the higher percentage reduction in this experiment could be explained by the top down direction of the ultrasound waves, inducing reflection effects at the bottom of the reactor and thus a higher production of cavities.

3.4 Total residual oxidant formation by ultrasound in different RAS water

The measured TRO concentrations are illustrated in Fig. 1-6 and were between 0.09 (± 0.01) and 0.23 (± 0.05) mg/L Cl_2 . The results show a non-systematic relation between the treated medium and the amount of oxidants.

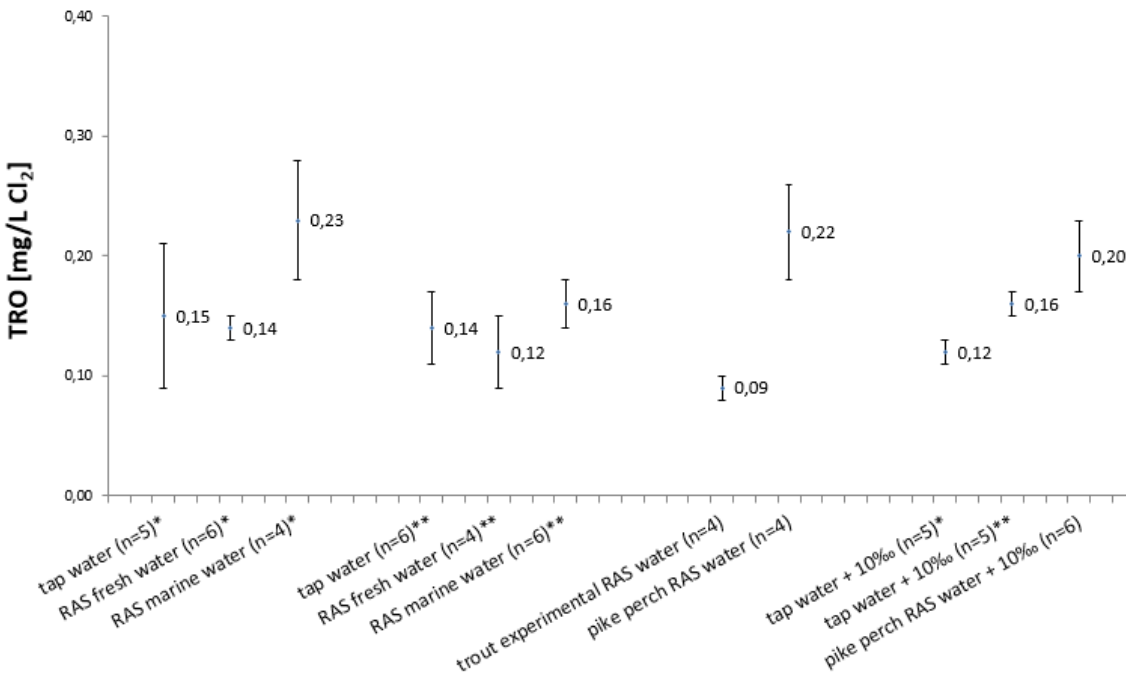


Figure 1-6: The TRO concentration after 15 min ultrasound treatment determined as equivalent total residual chlorine (TRC). The off-flavor initial concentration: * $c_0=5 \mu\text{g/L}$; ** $c_0=100 \text{ ng/L}$; trout experimental RAS water $c_{0_2\text{-MIB}}=35.5 \text{ ng/L}$, $c_{0_GSM}=15.5 \text{ ng/L}$; pike perch RAS water $c_{0_2\text{-MIB}}=2.9 \text{ ng/L}$, $c_{0_GSM}=3.8 \text{ ng/L}$.

The TRO concentration summarizes all substances that oxidize N,N-diethyl-p-phenylenediamine (DPD). Due to the extreme conditions in the hotspot of the cavities, hydrogen atoms, hydroxyl radicals and other radicals are produced (Riesz, 1992). Depending on the composition of the treated water, the non-selective and high reactivity of hydroxyl radicals might form different secondary oxidants containing potential harmful and toxic by-products. In pure water, only reactive secondary oxidants are formed consisting hydrogen or oxygen atoms, such as O^\cdot , O_3 or H_2O_2 . In aquaculture marine water, containing halogen-ions like Cl^- and Br^- and organic matter, the oxidation with hydroxyl radicals leads to formation of free chlorine, bromine or different halogenated compounds like trihalogenated methane (THM), bromate or chlorate (Bergmann &

Rollin, 2007; Echardt & Kornmueller, 2009). Schroeder et al. (2010) determined a safety ozone-produced oxidants (OPO) concentration to juvenile Pacific white shrimp *Litopenaeus vannamei* of 0.06 mg/L Cl₂. However, due to the much faster and non-selective reactivity of hydroxyl radicals compared to ozone, the comparison and its implications of the measured values with the safety value of 0.06 mg/L Cl₂ is difficult. It can be assumed that the increased occurrence of chloride ions in combination of hydroxyl radicals produces more chlorine compounds by treating the water with ultrasound than with ozone. Furthermore, on the basis of the color reaction of the DPD method, only the fast reactive compounds were determined while excluding the oxidants with a lower reactivity. As a consequence, the results express only the oxidation potential of the water equivalent to the corresponding free chlorine concentration at the moment of measuring and not in the aftermath of the ultrasound treatment. For example, stable secondary oxidants like bromate or chlorate with a lower reactivity were not being considered. Nevertheless, due to the relative small sample volume and hence the intensive acoustic irradiation in the near field of the ultrasound transducer, those results are not representative for the TRO formation in an up scaled ultrasound system running as a by-pass system. Future work with ultrasound should continuously monitor the TRO concentration in order to avoid deleterious impacts on animal health in aquaculture.

3.5 Energetic evaluation

The 850 kHz ultrasound treatment of 15 min required a total energy density of 144 kJ/L. The temperature increase of the water indicated that about 43 % (~ 63 kJ/L) of the consumed energy is transferred to heat. It is assumed that the high temperature increase of the treated water results from intensive ultrasound irradiation on a relatively small sample volume.

The consumed energy per nanogram of removed GSM and 2-MIB was about 4.1 kJ/ng for 2-MIB and 3.4 kJ/ng for GSM for the lower initial off-flavor concentration (100 ng/L) and 0.055 kJ/ng for 2-MIB and 0.049 kJ/ng for GSM for the higher initial off-flavor concentration (5 µg/L). Considering that the transfer process from the liquid into the hotspot is most probably an equilibrium reaction and that the amount of cavities was comparable for both experimental setups, these results

demonstrated that a higher concentration of GSM and 2-MIB induces a better energetic reduction efficiency.

4. Conclusion

This study demonstrated that ultrasonic water treatment might provide a new opportunity to effectively remove off-flavor compounds from RAS. The comparison of the efficiency of a low and high frequency ultrasound system revealed a significant enhanced reduction at 850 kHz. The additional improvement in removability of both off-flavor compounds by adding artificial marine salt to fresh water samples could not be confirmed in general. In contrast to AOPs solely producing OH-radicals, the ultrasonically-induced removal of 2-MIB and GSM might be attributed to pyrolysis rather than to OH-radical oxidation and is therefore less susceptible to competing reactions with free radical scavengers such as chloride or organic substances coming from the feed or faeces. Especially for aquaculture, the independence of the ultrasonically-induced degradation of GSM and 2-MIB to organic or inorganic load of the process water might be an important advantage to other alternative water treatment strategies. However, under realistic culture conditions implying a larger flow rate of process water the ultrasound treatment should be operate in a by-pass system distributing the locally produced heat and protecting the cultured species from direct and harming ultrasound irradiation. Keeping in mind that this study was done under laboratory conditions to research basic application opportunities of ultrasound in RAS at first, the transmission of the consumed energy to an up-scaled system would reveal an uneconomic consumption of energy and costs, respectively. Future research should focus on system optimization such as energy efficiency and system architecture considering higher flow rates in a commercial industrial-scale RAS.

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Chapter 2

Preliminary test of ultrasonically disinfection efficacy towards selected aquaculture pathogens

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Abstract

The disinfection potential of ultrasound was evaluated for fish production in aquaculture systems by investigating the sensitivity of a wide range of different model organisms representing different taxa of common fish pathogens such as bacteria or parasites and algae at a size range of a few micrometers to one millimeter. Therefore, dose-dependent inactivation rates depending on consumed energy (kJ/L) at low (20 kHz, LFUS) and high (850 kHz, HFUS) frequency ultrasound were determined in in-vitro tests using a laboratory-scaled set-up for three zooplankton and five algae species as well as for heterotrophic marine and fresh water bacteria separated from recirculating aquaculture system water. LFUS and HFUS were effective against most tested eukaryotic organisms even at low energy input and the dose-dependent inactivation could be well described by functions of an exponential decay. HFUS seemed to be more effective compared to LFUS regarding the inactivation of bigger-sized algae species ($\geq 14 \mu\text{m}$). Within the tested zooplankton species, no systematic correlation between the size of the organisms and the effect of ultrasound frequency on the inactivation efficiency became apparent, suggesting a strong species-dependency likely caused by additional relevant morphological and physiological factors. Our data also suggest ultrasound treatment alone to be insufficient for an efficient bacterial reduction as LFUS and HFUS did not significantly reduced total viable counts even at high energy inputs up to 144 kJ/L. Measurements of total residual oxidants (TRO) revealed a higher formation potential of sonochemically-produced oxidants for HFUS compared to LFUS as well as an increased TRO accumulation at saline waters most likely due to the formation of more persistent halogenated secondary oxidants. Hence, particularly when HFUS is applied in a marine process chain total residual oxidants should be monitored in order to avoid deleterious impacts on animal health.

1. Introduction

Diseases are one of the most limiting factors for aquaculture production and often severely affect profitability (Schnick, 1996). The increasing intensification of fish production in aquaculture systems often leads to suboptimal rearing conditions. Lowered water exchange and increased production intensity result in the accumulation of metabolic by-products coming from excrements of the cultured species and entails the risk of providing ideal growth conditions for germs, parasites or off-flavor producing organisms. Furthermore, microbial degradation processes of the organics lead to a higher oxygen demand in the rearing water.

Various microorganisms may negatively affect the health of the cultured species and often lead to severe disease outbreaks. A lack of therapeutic agents often impedes an effective and successful disease control as the application of common chemotherapeutics and chemical sanitizers is either prohibited by law or limited by its negative impact on biofilter function, e.g. in a recirculating aquaculture system (RAS) (Pedersen et al., 2009).

Beside the minimization of fish stress by optimizing the rearing conditions the prophylactic reduction of pathogen loads should be the preferable method in aquaculture systems to avoid disease outbreaks.

Therefore, growth limitation of heterotrophic bacteria due to an effective mechanical removal of particulate organic matter is a key factor (Ritter and Roeske, 2007; Winward et al., 2008). The biocidal effect of common disinfection methods like ozonation or UV treatment is limited in particle rich water due to an effective protection of microorganisms attached to particles or embedded in biotic or abiotic agglomerations (Hess-Erga et al., 2008). Furthermore, the disinfection efficiency of UV treatment is low towards larger organisms such as protozoa or parasitic worms (Chevrefils et al., 1999; Kasai et al., 2002) and strongly depends on the penetration depth of the UV irradiation and hence decreases with increasing turbidity and coloration of the water in general (Hoff, 1978; LeChevallier et al., 1981; Gullian et al., 2012).

The application of oxidants is successfully used in aquaculture (Verner–Jeffreys et al., 2009) but leads to the production and accumulation of potential harmful and fish toxic residual oxidants or disinfection by-products (DBPs) (Perrins et al., 2006; Schroeder et al., 2010; Tango and Gagnon, 2003). Furthermore, in aquaculture process water the disinfection power of reactive oxidants is

restricted due to their consumption by abundant organic or inorganic oxidant scavengers such as easily degradable organic matter or halogen ions in competitive oxidation reactions. Due to the lack of approved chemical agents and oxidation processes for disinfection purposes in turbid and (in)organically loaded water, there is a demand for alternative non-hazardous processes which are less susceptible to the water matrix.

Ultrasound represents a potential alternative disinfection process which might meet these demands (Joyce and Mason, 2008; Phull et al., 1997).

Cavitation is the ultrasonically induced formation and the sudden implosion of vapor cavities in a local hot spot under extreme conditions ($p > 100$ atm, Temp. ~ 5200 K) (Suslick et al., 1986). Cavitation generates sonochemical and mechanical effects which are little impaired by high amounts of dissolved or particulate matter (Nam-Koong et al., 2015; Song and O'Shea, 2007). The sonochemical effects are caused by different pathways such as the oxidation by hydroxyl radicals produced by the thermal dissociation of water, the pyrolytic decomposition inside the hotspots or the supercritical water oxidation (Hoffmann et al., 1996). The mechanical effects arise from strong shear forces due to acoustic shockwaves induced by transient and stable cavitation (Doulah, 1977).

Although the exact mechanism of inactivation is still not fully understood, it is assumed that shear forces generate mechanical shear stress to the organisms resulting in their inactivation supported by the chemical effects and high temperatures (Earnshaw et al., 1995; Scherba et al., 1991; Thacker, 1973). The application of 20 kHz ultrasound against sea lice has already been successfully tested in an open aquaculture system (Prado, 2014).

However, most of the studies have examined the disinfection efficiency of low frequency ultrasound (LFUS) mainly against bacteria but hardly against eukaryotic pathogens or microalgae. Furthermore, the combination of LFUS with UV-irradiation was examined demonstrating that LFUS supports the UV-based disinfection in wastewater by declumping bacteria flocs (Blume and Neis, 2005; Gogate, 2007). Until now the application of ultrasound for pathogen control in aquaculture production systems is nearly unexplored. Lakeh et al. (2013) investigated the combined application of LFUS and UV-irradiation in RAS and demonstrated that LFUS (25 kHz) is effective against a wide range of larger organisms like ciliates, nematodes and crustaceans

whereas the UV irradiation provide an efficient reduction of bacteria. Nevertheless, the efficiency of LFUS differed strongly between the eukaryotic species.

A standardized comparison of the inactivation efficiency of low (LFUS) vs. high frequency ultrasound (HFUS) for a comprehensive selection of aquaculture relevant model organisms is still lacking.

Therefore, the aim of this study was to investigate the potential of LFUS and HFUS for disinfection specifically in aquaculture. For this purpose, laboratory-scaled in-vitro tests were performed, mainly to investigate the sensitivity of a wide range of different model organisms representing different taxa of common fish pathogens and parasites towards low (LFUS / 20 kHz) and high frequency ultrasound (HFUS / 850 kHz). LFUS was generated with a horn homogenizer (\varnothing 13 mm) and HFUS with an ultrasound transducer (\varnothing 75 mm). Both applications were used in pulsed mode (1:5) and at 200 W. On the basis of these data, it will be estimated whether the use of ultrasound for aquaculture is worthwhile. Additionally, total residual oxidants (TRO) were measured in order to examine the formation and accumulation potential of sonochemically-produced oxidants.

2. Material & Methods

2.1 Experimental set-up

Various model organisms representing different taxa and covering a size range from a few micrometers to one millimeter were treated with both, low frequency ultrasound (LFUS / 20 kHz) and high frequency ultrasound (HFUS / 850 kHz) in a laboratory-scaled set-up. Beside the unspecific quantification of heterotrophic marine and fresh water bacteria, zooplankton *Paramecium sp.*, *Artemia salina*, *Brachionus plicatilis* and *Arcatia tonsa* were chosen as model organisms. Furthermore, four marine (*Nanochloropsis salina*, *Pavlova viridis*, *Tetraselmis chui*, *Rhodomonas baltica*) and one fresh water microalgae (*Haematococcus pluvialis*) were tested in order to investigate the effect of ultrasound on microalgae of different size classes. At different exposure times total viable bacteria counts (CFU/ml), viable algae and viable zooplankton were quantified using the spread plate technique, Pulse-Amplitude-Modulation (PAM) fluorometry and the counting chamber method, respectively. Finally, dose-dependent inactivation rates were determined and plotted. The individual test series were always started from a total sample, so

that it could already be seen at the beginning of the subsequent repetition that in the short time in which the previous sample was treated, the activity or the number of living cells did not change. Thus the untreated samples at time 0 were simultaneously regarded as control samples during the experiments.

2.1.1 Experiments with bacteria

Marine water samples were taken directly from the rearing tank of an experimental RAS stocked with Atlantic salmon (*Salmo salar*) (stocking density in tank 34 kg/m³) at a salinity of 26 ‰ (17.0 ± 1.3 °C temperature, 0.38 ± 0.17 mg/L NH₄-N, 0.63 ± 0.29 mg/L NO₂-N, 7.4 ± 0.1 pH). The fresh water samples were taken directly from the pump sump of an experimental RAS stocked with juvenile carp (*Cyprinus carpio*) (stocking density in tank 19 kg/m³, 3.5 ± 0.2 °C temperature, 0.8 ± 1.3 mg/L NH₄-N, 0.3 ± 0.1 mg/L NO₂-N, 7.2 ± 0.1 pH). The total viable counts (CFU/ml) were quantified by the spread plate technique using nutrient agar. Samples from the marine water were plated in a series of dilution on BD Difco™ Marine Agar 2216 and samples from the fresh water were plated in a series of dilution on unspecific BD BBL™ Trypticase™ Soy Agar (Soybean-Casein Digest Agar) 221283. Colony forming units (CFU/ml) per plate were recorded after 48 h of incubation at 37°C. A plate was prepared from the total sample at the beginning of each repetition, which was not treated with ultrasound. These plates were used as the starting concentration (t=0) and as the control group. The inactivation rate was calculated based on the ratio between the viable organisms prior and after treatment in relation to the consumed energy (kJ/L).

2.1.2 Experiments with algae

Marine microalgae (*Nanochloropsis salina*, *Pavlova viridis*, *Tetraselmis chui*, *Rhodomonas baltica*) and fresh water microalgae (*Haematococcus pluvialis*, Cyst of *Haematococcus pluvialis*) were all purchased from BlueBiotech International GmbH (Büsum, Germany). The algae were grown in optimal nutrient conditions. The marine water algae were grown in F Medium and the fresh water algae were grown in BG11 medium (marine algae: 24.0 ± 1.1 °C temperature, 8.3 ± 0.1 pH, 32 ± 0.8

PSU salinity; fresh water algae: 24.0 ± 1.0 °C temperature, 7.8 ± 0.1 pH). The sizes of the algae cells were between 6 and 60 μm . For each microalgae suspension the total biomass was adjusted approximately to 0.1 g/L (dry weight). The algaecide effects were measured with a portable mini-PAM (Pulse-Amplitude-Modulation) instrument (Walz, Effeltrich, Germany), which measured the photosynthetic yield (chlorophyll fluorescence) as an indication for the photosynthetic vitality of the phytoplankton. It is assumed that constraints of the photosynthetic vitality indicate a disturbance of the activity of the microalgae. The photosynthetic yield Φ_{PSII} , known as the quantum yield of PSII electron from the photosystem II electron transport chain, was calculated according to Genty et al. (1989): $\Phi_{\text{PSII}} = (F_m - F_0)/F_m$, where F_0 and F_m describes the minimum and the maximum fluorescence, respectively.

Before each measurement, the sample was homogenized by stirring. After each treatment, the samples were incubated at room temperature for 12 h under light in order to exclude short-term effects of ultrasound to the tested species. The photosynthesis activity of algae was always measured at the beginning of each experiment before the sample was treated with ultrasound. At the next repeat, the sample was taken from the total sample and the photosynthesis activity was measured again without ultrasound treatment to demonstrate that the starting activity had not changed over the short period of the previous experiment. The inactivation rate was calculated based on the ratio between the photosynthetic vitality prior and after treatment in relation to the consumed energy (kJ/L).

2.1.3 Experiments with zooplankton

Different zooplankton organisms were purchased and tested: the ciliate *Paramecium caudatum* (Interaquaristik, Germany), the rotifer *Brachionus plicatilis* (e-nema, Germany) and the arthropods *Artemia salina* in the stage of metanauplius (Ocean Nutrition, America) and the marine copepod *Acartia tonsa* (University Hamburg, Germany). All organisms were quantified under a binocular microscope prior and after treatment. Zooplankton with abnormal spherical shape and not responding to physical stimulus applied with a needle was assumed to be dead. *Paramecium caudatum* were fixed with Lugol's iodine to count the individuals before treatment (n=4). After

the treatment only the surviving individuals showing vital signals were counted and were set in relation to an averaged initial number of *Paramecium caudatum*.

The total sample of each organism was divided into n samples. At the beginning of every subsequent repetition it could be seen that in the treatment time which the previous sample was treated, the number of organisms did not change.

The inactivation rate was calculated based on the ratio between the viable organisms prior and after treatment in relation to the consumed energy (kJ/L).

2.1.4 Oxidant formation experiment

In order to examine the formation and accumulation potential of sonochemically-produced oxidants for LFUS and HFUS in dependence of the water matrix composition, samples taken from the experiments with bacteria (s. 2.1.1) representative for marine and fresh RAS water, distilled water and artificial marine water (32 psu) were treated with LFUS and HFUS and total residual oxidants (TRO) were measured at 0, 5, 10 and 15 min of ultrasound treatment.

2.2 Ultrasound equipment

The high frequency ultrasound at 850 kHz was generated using a Meinhardt E/805/T/solo ultrasound transducer with 75 mm diameter powered by a Meinhardt Ultraschall-Leistungs-Generator K8 (200 W) in pulsed mode (on/off ratio 1:5). The low frequency ultrasound at 20 kHz was emitted from a titanium horn homogenizer with a UW 3200 ultrasound transducer and a SH 219 Boosterhorn with 13 mm diameter (BANDELIN electronic GmbH & Co. KG). The electrical power was generated by a Bandelin GM 3200 HF-Generator (200 W) in pulsed mode (on/off ratio 1:5). The samples were exposed to ultrasound in a 250 ml glass cylinder sonicated from the bottom and cooled by cold water surrounding the cylinder to maintain the temperature nearly constant.

2.3 Water analysis

Total residual oxidants (TRO) were determined spectrophotometrically by a DR/2800 Spectrophotometer (Hach Lange GmbH) as equivalent total residual chlorine (TRC) using the colorimetric N,N-diethyl-phenylenediamine (DPD) method (DPD Total Chlorine powder pillows, Hach) as recommended by Buchan et al. (2005). The salinity and water temperature were measured by a WTW MonoLine Cond 3310 IDS and a VOLTcraft K204 Type K, respectively. The pH was determined with a GMH 3530 (Greisinger, Germany). Ammonia and nitrite were determined with a Microquant test kit for NH_4 and NO_2 (Merck KGaA, Darmstadt, Germany).

2.4 Energetic evaluation

The consumed specific energy (kJ/L) was calculated from the treatment duration (t), the treated volume (L) and the electrical power (W) of the ultrasound systems.

2.5 Statistical analysis

The statistical software R (Version 3.1.2, 2014) was used to evaluate the data. An exponential relation between the measurement values y_i and the consumed energy E was modeled. Therefore, the usual pseudo-linear model $\ln(y_i + 0.01) = k_i E + b_{ij}$ was applied for each combination of organism (bacteria, algae and zooplankton) and system (20 kHz, 850 kHz). A graphical residual analysis was performed revealing the residuals b_{ij} to be normally distributed and homoscedastic (Fig. 2-1a). Although the Shapiro-Wilk-Test is significant for these data ($p < 0.001$) the residual plot analysis clearly illustrates a normal distribution of the data (Fig. 2-1 b) and should be preferred for checking prerequisites according to Kozak and Piepho (2018). An analysis of covariances (ANCOVA) was conducted to identify the general effects of organism, system and consumed energy (Cochran, 1957). Subsequently, multiple contrast tests for general parametric models (Bretz et al., 2011; Hothorn et al., 2008) were conducted in order to compare the resulting curves with regard to the several organisms and systems.

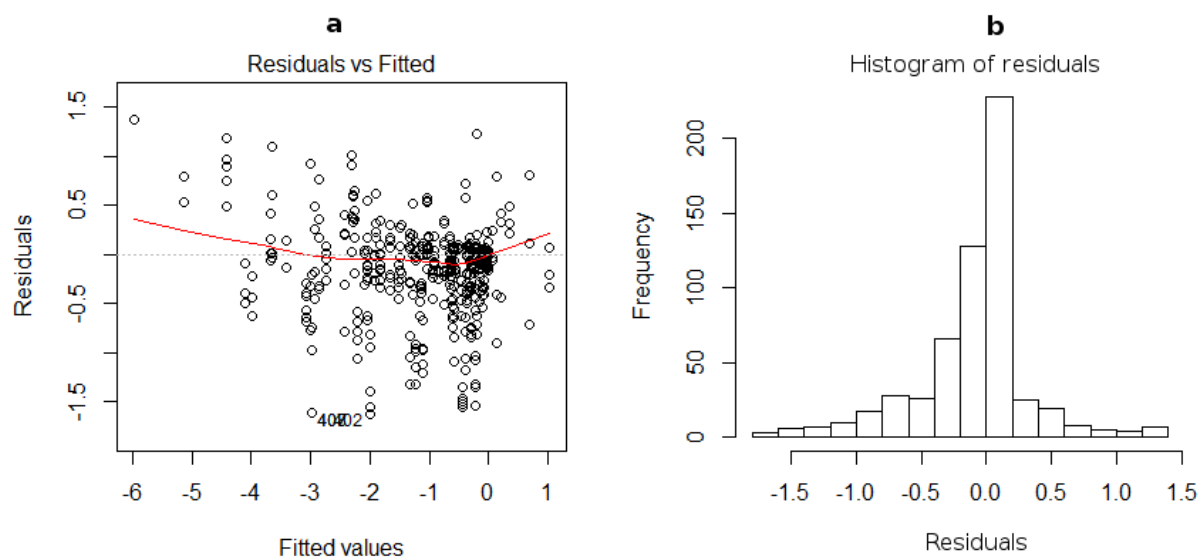


Figure 2-1: Graph a shows the residuals based on the model applied (n=587; $r^2=0.93$). Additionally, the corresponding histogram of the residuals is shown in diagram b to illustrate the assumption of a normal distribution. In the lowest measuring range, the measured values are underestimated by the assumed model, as can be seen on the left side of the graph a from the positive residuals.

3. Results

Assuming that the degradation follows first-order reaction kinetic, the inactivation rate k of each organism was calculated by $y_E = y_0 * e^{-k * E}$ with $y_0 = 100\%$. In most cases, the applied kinetic model gave a sufficient approximation offering a statistical comparability of the inactivation rate with ANCOVA with a p-value of <0.05 . The overall residual standard error was 0.4638 with $r^2=0.9279$.

The temperatures have remained relatively constant during the treatment time with LFUS and HFUS, so that temperature effects can be excluded (Tab. 2-1).

Table 2-1: Extract of water temperatures measured during treatment with LFUS and HFUS. The organisms were selected for which the reduction was significant and the treatment duration the longest (n=5).

	Time [s]	Temperature [°C]
20 kHz <i>Nanochloropsis salina</i>	0	21.2 ± 0.6
	30	20.6 ± 0.3
	60	20.0 ± 0.6
	120	20.2 ± 0.8
	300	22.9 ± 0.8
	480	22.9 ± 0.6
850 kHz <i>Haematococcus pluvialis</i>	0	20.3 ± 0.7
	30	20.9 ± 0.6
	60	20.9 ± 0.3
	120	21.4 ± 0.4
	240	22.8 ± 0.3
	360	23.9 ± 0.9

3.1 Experiments with bacteria

In the sample from the experimental RAS stocked with juvenile carp (*Cyprinus carpio*) an initial bacteria population of about 10^3 per ml was determined (Fig. 2-2a). At the beginning of LFUS and HFUS treatment CFUs started to increase for both frequencies. During further sonication at 20 kHz the total viable count was reduced while at 850 kHz the CFUs continuously increased. Finally, at a consumed energy of 144 kJ/L the cell concentration was significantly reduced by about 50% at 20 kHz, whereas at 850 kHz the CFUs increased significantly about twice. The sample from the experimental RAS stocked with Atlantic salmon (*Salmo salar*) contained about 10^3 - 10^4 cells per ml (Fig. 2-2b). For both frequencies no significant changes over time and used energy could be observed.

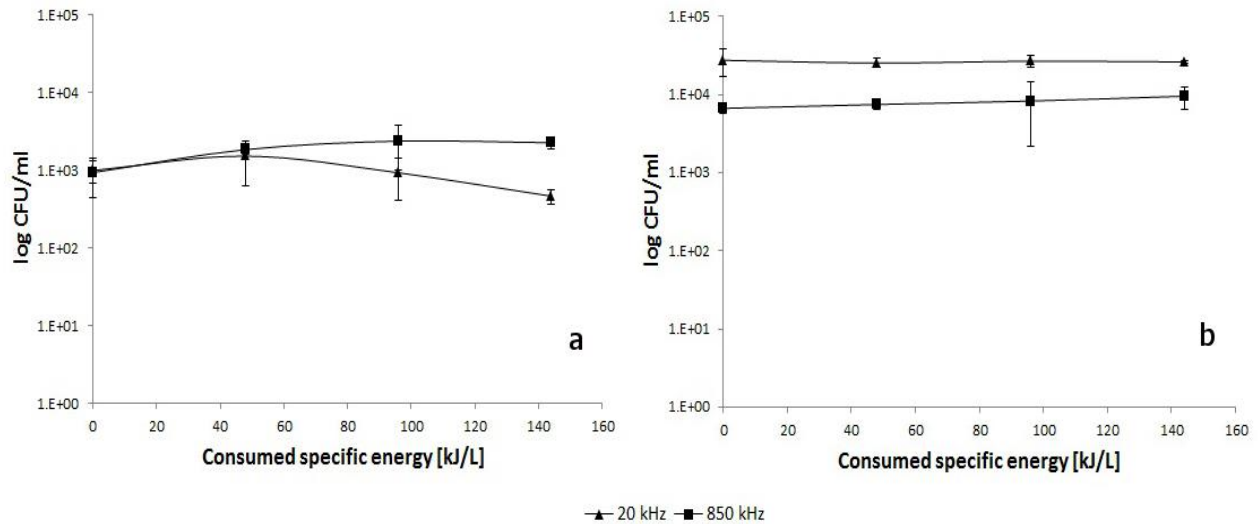


Figure 2-2: The logarithmic total viable count (CFU/ml) in relation to the consumed energy during ultrasound treatment at LFUS (20 kHz) and HFUS (850 kHz). The samples were taken directly (a) from the pump sump of an experimental RAS stocked with juvenile carp (*Cyprinus carpio*) (stocking density in tank 19 kg/m³) and (b) from an experimental RAS stocked with Atlantic salmon (*Salmo salar*) (stocking density in tank 34 kg/m³) with a salinity of 26 ‰. Data are presented as mean ± SD (n=3).

3.2. Experiments with algae

The experiments revealed that the ultrasound treatment at both frequencies induced a significant reduction of the photosynthetic yield of all tested microalgae with the exception of *Nannochloropsis salina*, (Fig. 2-3).

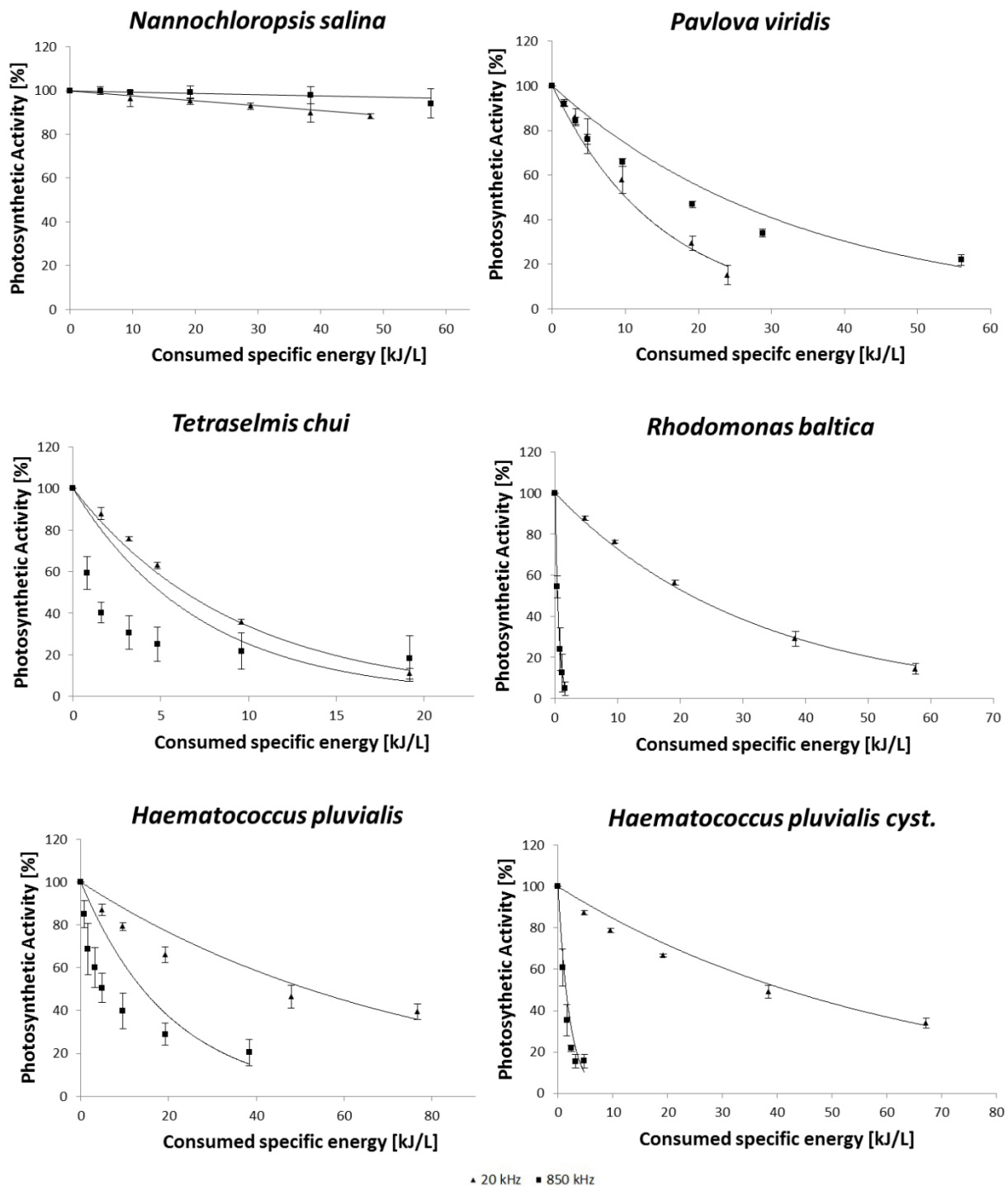


Figure 2-3: Comparison of the percentage PAM reduction of different microalgae due to ultrasound treatment at 20 kHz and 850 kHz in relation to the consumed specific energy (kJ/ml). The curves illustrate the inactivation calculated on the basis of the measured PAM values before and after the treatment. The data are presented in mean \pm SD (n=4). It was assumed that the reduction follows a first-order reaction kinetic.

Even after 12 h of incubation at room temperature no recovery of the photosynthetic activity could be observed. The specific inactivation rates of each microalgae are shown in table 2-2.

Table 2-2: The table shows the specific inactivation rates of all examined microalgae after ultrasound treatment sorted by the size of the microalgae. It was assumed that the inactivation follows first-order reaction kinetic calculated by $y_E = y_0 * e^{-k * E}$ starting with $y_0=100$ %. The highlighted values indicate the significantly higher inactivation rates ($p < 0.05$). The organisms marked with * show significant ($p < 0.05$) differences between the 20 kHz and 850 kHz ultrasound treatment.⁵⁴

Microalgae	Size [μm]	Inactivation rate k x 10^{-2} [% per kJ/L]	
		20 kHz	850 kHz
<i>Nannochloropsis salina</i>	4	0.24	0.06
<i>Pavlova viridis</i> *	6	6.89	2.89
<i>Tetraselmis chui</i>	14	10.86	12.01
<i>Rhodomonas baltica</i> *	18	3.18	182.75
<i>Haematococcus pluvialis</i> *	30	1.33	4.92
<i>Haematococcus pluvialis Cyst.</i> *	50	1.66	47.43

Within the tested algae species *Nannochloropsis salina* was most resistant against ultrasound almost irrespective of the frequency. Whereas LFUS was shown to be more effective against *Pavlova viridis*, HFUS treatment resulted in higher inactivation rates for the rest of the tested algae.

It has been ascertain that in 3 of 6 cases the 850 kHz frequency implicated a significant higher inactivation rate than 20 kHz. The approximated curve shapes of the photosynthetic vitality reduction of *Tetraselmis chui* and *Haematococcus pluvialis* pointed out the limitation of the first-order reaction kinetic model. The calculated inactivation rates of *Tetraselmis chui* showed no significant differences between the treatment with 20 kHz or 850 kHz, respectively. However, based on the measured photosynthetic yield it can be assumed that the treatment with 850 kHz led to a faster decrease in the photosynthetic vitality of *Tetraselmis chui*. An overall comparison of the reduction rates revealed no systematic correlations between the size of the microalgae and

the examined ultrasound frequency, although microalgae with a size greater than 14 μm seemed to be more vulnerable at 850 kHz.

3.3 Experiments with zooplankton

For all larger eukaryotic organisms a significant reduction of living individuals after ultrasound treatment with both frequencies could be observed (Fig. 2-4). Furthermore, significant differences of the inactivation efficiency between the 20 kHz and the 850 kHz ultrasound system were detected.

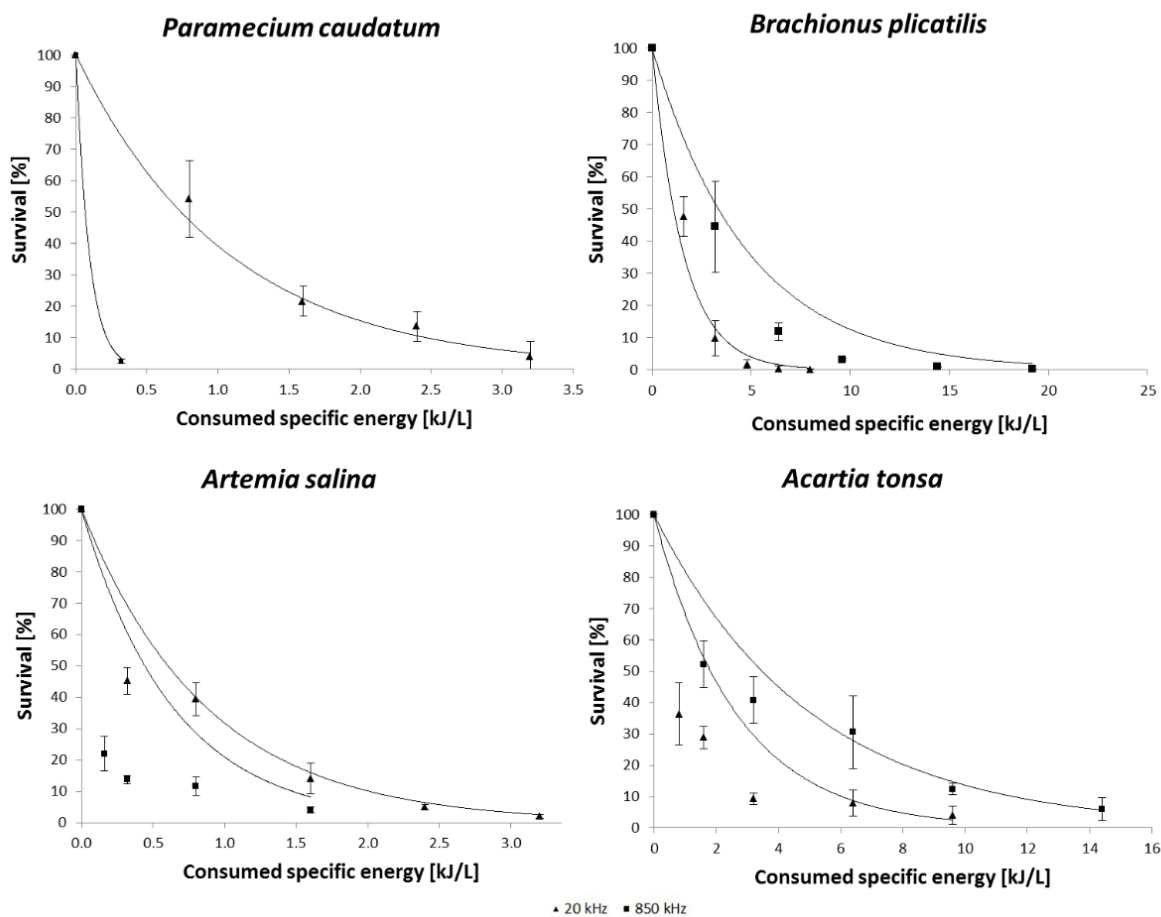


Figure 2-4: Comparison of the decrease of survived zooplankton in percent during ultrasound treatment at 20 kHz and 850 kHz. The curves describe the reduction of the survivals after a certain treatment time and consumed energy assuming that the reduction follows first-order reaction kinetic. The initial amount of organisms: *Paramecium caudatum* (8 individuals per ml), *Artemia salina* (2 individuals per ml), *Brachionus plicatilis* (25 individuals per ml), *Acartia tonsa* (3 individuals per 10 ml). The data are presented in mean \pm SD (n=5-6).

With respect to the tested zooplankton species, LFUS was more efficient against *Brachionus plicatilis* and *Acartia tonsa*, HFUS treatment resulted in faster inactivation rates for *Paramecium caudatum* and *Artemia salina* (Tab. 2-3).

With an 11-fold higher inactivation rate at 850 kHz compared to 20 kHz, the strongest frequency effect was detected for *Paramecium*. Following a consumed energy of 0.3 kJ/L no countable individuals could be observed in the treated sample.

Table 2-3: The table shows the specific inactivation rates of all tested organisms after ultrasound treatment sorted by the size of the organisms. It was assumed that the inactivation follows first-order reaction kinetic calculated by $y_E = y_0 * e^{-k * E}$ starting with $y_0=100$ %. The highlighted values indicate significant higher inactivation rates ($p < 0.05$).

Organism	Size [μm]	Inactivation rate $\times 10^{-1}$ [% per kJ/L]	
		20 kHz	850 kHz
<i>Paramecium caudatum</i>	180	9.39	106.44
<i>Brachionus plicatilis</i>	265	6.43	2.07
<i>Artemia salina</i>	430	11.46	15.63
<i>Acartia tonsa</i>	1000	3.81	1.99

Moreover, the comparison of the physical conditions of the treated zooplankton after sonication at different frequencies revealed that apparently different effects led to the inactivation of *Artemia salina* and *Acartia tonsa*. After the treatment at 850 kHz the organisms remained nearly unscathed but showed no response to physical stimulation applied with a needle (Fig. 2-5a & 2-6a). In comparison to 20 kHz, immense physical damages of the organisms could be observed after sonication (Fig. 2-5b & 2-6b).

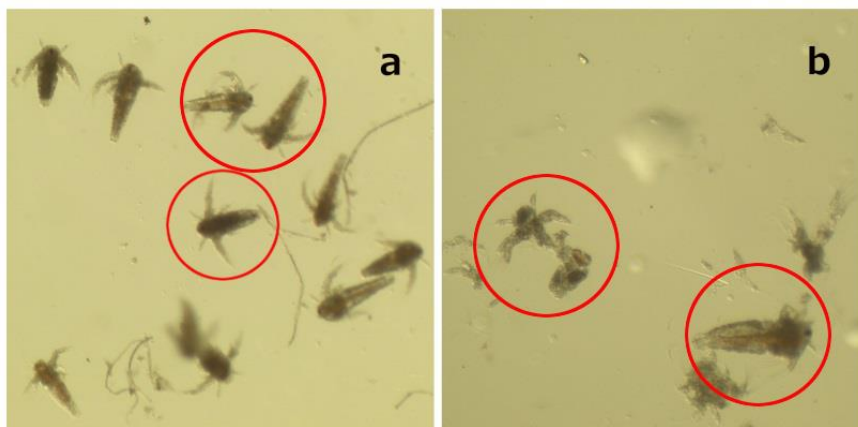


Figure 2-5: An optical comparison of the physical conditions of *Artemia salina* after ultrasound treatment. After sonication at 850 kHz and an energy consumption of 1.6 kJ/L the organisms showed no external damages of the outer shell but did not react to physical stimulation (a). On the other hand extreme physical damages of the organisms could be observed at 20 kHz ultrasound (energy consumption 2.4 kJ/L) (b).

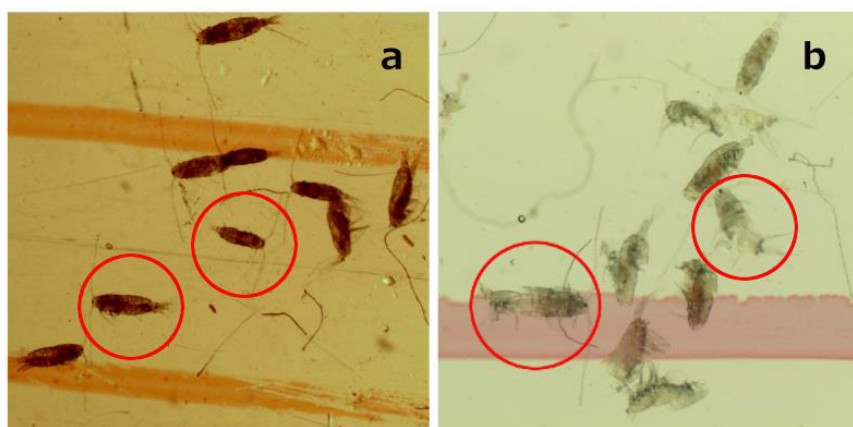


Figure 2-6: The comparison of the physical conditions of *Arcatia tonsa* after ultrasound treatment. After sonication at 850 kHz and total consumed energy of 14.4 kJ/L the organisms revealed no physical harms of the outer shell but did not response to physical stimulation (a). However, extreme physical damages of the organisms could be observed at 20 kHz ultrasound and an energy consumption of 9.6 kJ/L (b).

3.4 Total residual oxidant formation

After 15 min of ultrasound treatment measured TRO concentrations of the treated samples varied between 0.02 and 0.26 mg/L Cl_2 depending on the water matrix composition and the applied frequency (Fig. 2-7).

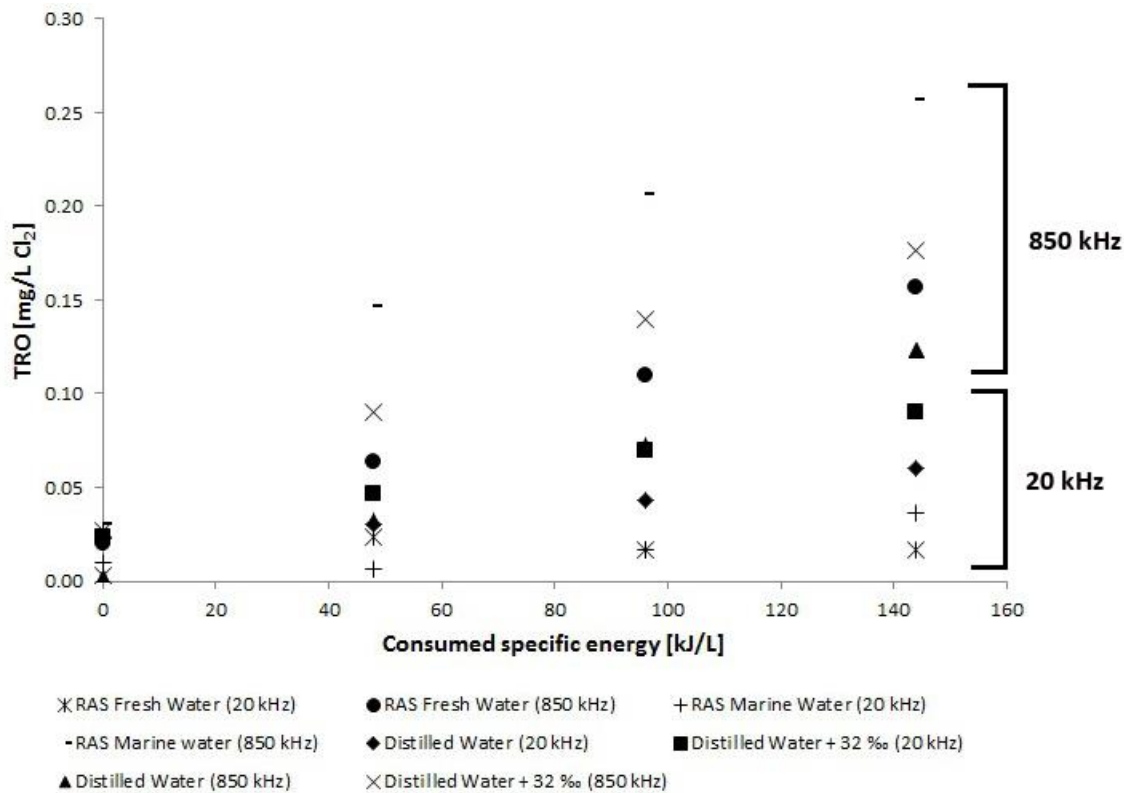


Figure 2-7: Total residual oxidant (TRO) concentration determined as equivalent total residual chlorine of different water samples after 0, 5, 10 and 15 minutes of ultrasound treatment at 20 and 850 kHz (n=3). The RAS marine water sample was taken from an experimental RAS stocked with Atlantic salmon (*Salmo salar*) (stocking density in tank 34 kg/m³) with a salinity of 26 ‰. The RAS fresh water sample was taken from an experimental RAS stocked with juvenile carp (*Cyprinus carpio*) (stocking density in tank 19 kg/m³).

Particularly for sonication at 850 kHz, our data showed a positive correlation between TRO formation and the (in)organic load of the sample with highest TRO formation in saline waters. However, TRO formation was always higher at HFUS compared to LFUS irrespective of the water matrix. Moreover, sonication of distilled water at 850 kHz resulted in even higher TRO accumulation than sonication of saline waters at 20 kHz.

4. Discussion

In the present study the potential of ultrasound treatment at 20 kHz and 850 kHz for disinfection purposes in an aquaculture system was investigated. Shock waves and shear forces coming from

ultrasonically-induced cavitation are capable to apply high physical forces in a short period of time. Additionally, extreme local pressure and temperature conditions due to ultrasonically-induced hotspots can cause chemical-oxidative forces (Suslick et al., 1986). Therefore, the effect of disinfection might be a consequence of very high force effects per time in a very small area due to the combination of simultaneously acting chemical and mechanical effects. Cell walls or membranes are exposed to mechanisms such as high energetic turbulences, the generation of active free radicals, heat effects, high velocity jets, energetic turbulences, micro eddies and flow gradients (Doulah, 1977; Mason et al. 2003; Thacker, 1973). Hence, the inactivation efficiency of ultrasound depends on the development and the timing of cavitation. Therefore the inactivation efficiency is dependent from parameters like ultrasound frequency or acoustic intensity (W/m^2), irradiation time, but also from external conditions such as temperature or surface tension. Ultrasound at 850 kHz produces cavities with smaller maximum diameter due to the shorter wavelength and thus collapsing less violently than under LFUS. Consequently, ultrasound at 20 kHz produces fewer cavities but provides more time for growth, so that the cavities collapse more violently by finally achieving larger maximum sizes. Thus, it can be assumed that 20 kHz produces stronger mechanical forces than 850 kHz (Portenlänger and Heusinger, 1997). On the other hand the 850 kHz produces a higher amount of cavities and consequently more hydroxyl radicals which might apply more sonochemical effects (Hua and Hoffmann, 1997).

4.1 Experiments with bacteria

The results demonstrated that in fresh water the treatment with both frequencies seemingly increased the cell number at the beginning of each treatment. This could be explained by strong pressure waves coming from ultrasonically-induced shockwaves caused a disintegration of bacterial agglomerates (Doulah, 1977). The following significant reduction of bacteria only observed at 20 kHz might be the result of the more violent collapse of larger cavities initiating powerful jet streams with strong shear forces. In contrast, at 850 kHz the cell number increased twice after the complete treatment. This could be the consequence of smaller cavities at 850 kHz not capable to significantly reduce the bacteria but rather to continue the deagglomeration process. These results are consistent with the suggestion that mechanical forces are mainly

responsible for a disinfection process by a substantially higher reduction rate than sonochemical effects. It is assumed that the major effect of 850 kHz is the declumping of bacterial agglomerates with little deactivation or the support of other disinfectants in a combined treatment while 20 kHz are characterized by destructive influences of shear forces within bacterial cells or near the bacterial cell walls (Carrère et al., 2010; Furuta et al., 2004; Joyce et al., 2003; Mason et al., 2003; Neis and Blume, 2003). A significant inactivation of bacteria cells due to free and highly active radicals or secondary oxidizing agents attacking the chemical structure of bacterial cell walls till disintegration (Oyane et al., 2009) could not be confirmed. Although the results under marine conditions are statistically non-significant, the cell numbers tended to increase at 850 kHz and decrease at 20 kHz and confirms the findings under fresh water conditions.

4.2 Experiments with algae

Algae growth could become to an incalculable risk for RAS operators due to the production of fish toxins (Moestrup et al., 2014; Repavich et al., 1990; Rodgers, 2008) or off-flavor (Tucker, 2000). The microalgae used in these experiments are unicellular species typically found in fresh water and marine systems. Their sizes range from a few to several micrometers. The data indicated that in most cases both frequencies could sustainably reduce the photosynthetic vitality of algae cells. However, the inactivation efficiency of 20 kHz or 850 kHz ultrasound considerably varied depending on the algae species or sizes. It was assumed that ultrasound affects algae cells by collapsing internal gas vesicles during cavitation, inhibiting the cell division or disturbing the photosynthetic activity (Ahn et al., 2003; Hao et al., 2004; Lee et al. 2002; Zhang et al., 2006). For the tested algae, in particular the larger algae, ultrasound at 850 kHz became more efficient than at 20 kHz and is in agreement with previous studies demonstrating that higher ultrasonic frequencies led to faster removal rates than lower frequencies. The observed results could be explained by the size of algae gas vacuoles and the resonance size of the ultrasonically-induced cavities. The closer the sizes of the gas vacuoles are in the magnitude of the resonance size of the cavities, the more likely gas vacuoles resonate and collapse. The resonance size of cavities at given ultrasound frequency can be estimated by (Equa. 2-1):

$$f = 1/2\pi R \sqrt{3 \gamma P / \rho}$$

Equation 2-1: Estimation of the resonance size of the cavities. Calculated with the parameters f =resonant frequency, R =radius of the cavities, P =ambient pressure, γ =ratio of heat capacities of the gas at constant pressure and volume, ρ = density of the surrounding medium (Anderson and Hampton, 1980).

Assuming a heat capacity of 1.4 for air and a density of 1.0 g/cm³, the resonance size of a free air bubble in water is approximately 163 μm at 20 kHz and 3.8 μm at 850 kHz. In consequence, the higher the frequency of ultrasound the smaller the cavities will be. This may provide a partial explanation why no significant inactivation could be observed for *Nannochloropsis salina*. Considering their small size of about 4 μm , *Nannochloropsis salina* is not in the order of magnitude to be negatively affected by ultrasonically-induced cavities. Additionally, *Nannochloropsis salina* is known as a resistant microalgae very likely to resist strong shear forces by buffering the external forces with its flexible and spherical shape and cell wall. In fact that the treatment with 850 kHz indicated a higher efficiency of inactivation for the tested organisms, sonochemical effects cannot be excluded entirely, particularly the radical production increases at higher frequencies (P  trier and Francony, 1997). An explanation for the discontinuously increasing reduction rate with the size of the algae might be the dependency of the ultrasonically inactivation on other different parameters like the shape or the conditions of the cell walls. This could be at least the reason why the inactivation of the cysts of *Haematococcus pluvialis* was nearly 10-fold higher than of the life stage. The cell wall of the cysts might be more inflexible against external forces like microjets or shearforces coming from pulsating or collapsing cavities.

In conclusion, it is evident that ultrasound causes sustained damage to the photosynthetic activity of microalgae. Furthermore, the experiment revealed that in the cases where 850 kHz was more efficient, the efficiency was considerably higher than at 20 kHz. Therefore the treatment with high frequency ultrasound might be an alternative method to prohibit algae bloom for algae larger than 14 μm in aquaculture systems.

4.3 Experiments with zooplankton

In this experiment different model organisms were chosen to surrogate certain types of fish parasites. *Paramecium caudatum* represented common ectoparasites like *Ichthyophthirius* and *Trichodina*, *Artemia salina* as the larvae of parasitic crustaceans such as *Lernaea* and *Acartia tonsa* as the sea lice *Lepeophtheirus salmonis* and *Caligus sp.*. It was commonly observed that under laboratory conditions ultrasound at 20 kHz and 850 kHz are capable to inactivate eukaryotic organisms. However, it could not be clearly established whether the low or the high frequency ultrasound treatment was the most efficient method. In consideration of the determined values, it can be further suggested that *Artemia salina* and *Paramecium caudatum* were faster inactivated at 850 kHz and *Acartia tonsa* and *Brachionus plicatilis* were more efficiently inactivated at 20 kHz. In the case of the more efficient frequency for the reduction of *Artemia salina* and *Acartia tonsa* the calculated inactivation curve follows not approximately the real measured values, so that the assumed first-order reaction model must be interpreted carefully. The observed physical damages illustrated as well how the different frequencies of ultrasound affected the tested organisms. Due to the more violent implosion of cavities at 20 kHz, strong mechanical forces like pressure waves and shear forces may be responsible for the massive destruction of the zooplankton and would be in agreement with the findings of Holm et al. (2008). However, the results did not provide sufficient evidence that there exist an inverse relationship between the treatment efficiency of ultrasound and the size of the examined organisms. The apparently intact zooplankton bodies at 850 kHz allow the assumption that the implosions of smaller cavities were not strong enough to harm the outer shell of the organisms like at 20 kHz but caused most probably internal destructive processes leading to death. It is suggested that endogenous cavities affected negatively interior organs of the organisms due to shock waves or sonochemical effects in terms of the production of radicals (Sawant, 2008). Additionally, it is assumed that not only the size of the examined organisms are primarily responsible for the inactivation via ultrasonic but also the common structure of the zooplankton. The efficiency of the ultrasonically induced shear forces is potentially addicted to species-dependencies like morphological and physiological factors. External structures of the organism like the development of the hard-shelled chitin or the location of the extremities with potentially higher sensitivities

against shear forces than other parts of the organisms may have essential influences of the efficiency of ultrasound. Within the tested zooplankton species, no systematic correlation between the size of the organisms and the effect of frequency on the inactivation efficiency of ultrasound became apparent, suggesting a strong species-dependency likely caused by additional relevant morphological and physiological factors.

4.4 Energetic evaluation

In common, the results are in general agreement with previous disinfection studies. Holm et al. (2008) reported the energy consumption of 6 – 19 kJ/L at 19 kHz to reduce 90% of the zooplankton. In comparison, we consumed 4 kJ/L and 12.1 kJ/L at 20 kHz to inactivate 99% of *Artemia salina* and *Acartia tonsa*, respectively. The experiments of Lakeh et al. (2013) examined a frequency of 25 kHz and used 1.9 kJ/L to reduce 99% of *Artemia salina*. In the case of bacteria, it could also be demonstrated that a high energy consumption of over 140 kJ/L at both frequencies was not sufficient to reduce the prevalent population to a relevant order of magnitude. The comparison of the reduction efficiency to reduce 90% of the algae activity revealed a higher efficiency of high frequency ultrasound by consuming less energy than the low frequency ultrasound (20 kHz: 21 -172 kJ/L ; 850 kHz: 1.3 – 77.3 kJ/L). Additionally, only 0.4 kJ/L at 850 kHz was sufficient to inactivate 99% of the ciliates. In contrast, at 20 kHz an 11-fold higher energy was required to achieve the same effect. Currently, from an energetic point of view a standalone ultrasound system does not provide a solution to avoid infection diseases or algae blooms in an industrial-scaled aquaculture. Nevertheless, the combination of ultrasound with existing disinfection methods might be helpful to enhance the disinfection efficiency, e.g. supporting the UV radiation with the disintegration of algae agglomerates via ultrasound (Joyce et al., 2003; Lakeh et al. 2013).

4.5 Residual oxidants

Ultrasound-induced cavitation causes extreme temperature and pressure conditions which result in the formation of hydrogen atoms, hydroxyl radicals and other radicals (Riesz, 1992). The high and non-selective reactivity of hydroxyl radicals might form various fish-toxic secondary oxidants and potentially harmful persistent by-products depending on the composition of the water matrix. In natural or artificial marine water, the oxidation of halogen-ions like Cl^- and Br^- by hydroxyl radicals leads to the formation of free chlorine, bromine or different halogenated compounds such as trihalomethanes (THM), bromate or chlorate (Bergmann and Rollin, 2007; Echardt and Kornmueller, 2009).

Accordingly, our results reveal a higher accumulation of total residual oxidants (TRO) at saline waters most likely due to the formation of more persistent halogenated secondary oxidants. This correlation became most obvious at HFUS at 850 kHz. Furthermore, TRO formation was always higher at HFUS compared to LFUS irrespective of the water matrix. Moreover, sonication of distilled water at 850 kHz resulted in even higher TRO accumulation than sonication of saline waters at 20 kHz, suggesting the impact of frequency to be even more relevant for TRO accumulation than the impact of the water matrix composition. Our results clearly reveal a higher formation potential of sonochemically-produced oxidants for HFUS compared to LFUS caused by a shorter wavelength producing more small cavities and in consequence of a surface enlargement a higher production of hydroxyl radicals (Hua and Hoffmann, 1997).

Hence, besides the biocidal effect on the target species the TRO formation potential is a further factor that should be considered choosing the optimum frequency for ultrasound application. Until now, there exist no safety levels for sonochemically-produced oxidants and little information is available on the toxicity of residual oxidants formed via the radical pathway at comparable advanced oxidation processes. However, until respective information is available, TRO concentrations should be kept below the established safety level of 0.06 mg/L Cl_2 for ozone-produced oxidants (OPO) recommended for RAS production of selected marine fish and shrimp species (Schroeder et al., 2010; Reiser et al., 2010). Although the composition of sonochemically-produced secondary oxidants is assumed to be dominated by different chlorine species, while

ozone-produced oxidants mostly consist of bromine and iodine species, the acute toxicity of respective residual oxidants detected by the DPD-method is assumed to be comparable. However, less reactive oxidation by-products like bromate, chlorate and THM, which are not detectable by the DPD-method, are suggested to accumulate during sonication of saline waters as well. These by-products are generally less acute toxic to the aquaculture species but might be harmful at high concentrations and long exposure times. Further research is needed on the formation and toxicity of sonochemically-produced by-products being one of the relevant limiting factors of ultrasound treatment in aquaculture.

4.6 Future perspective regarding scalability

Due to the relatively high energy requirement of ultrasonic treatment, it is already clear that the simple scaling of the ultrasonic system used here to an industrial scale has an enormous energy consumption and therefore makes no economic sense. Nevertheless, from a purely technical point of view, the challenge arises how a significantly higher volume of process water can be treated with ultrasound. The aim must be to optimize this process in order to bring as much water volume as possible into the near field of the ultrasound and at the same time promote the formation of cavitations.

Studies indicate that these conditions are best achieved with a flow-through reactor. Already Mason et al. (2003) points out that a flow system could be advantageous and in the study of Lakeh et al. (2013) a flow through reactor with the combination of LFUS and UV-C radiation was successfully tested against eukaryotic organisms. Several ultrasonic transducers were installed in the reactor radially to the flow direction and the UV lamp was positioned in the middle of the reactor.

A direct exposure of ultrasound with the tested adjustments in the rearing tank is not possible without harming the organisms. For further investigations, different settings could be investigated. Since the influence of direct ultrasound exposure on the cultivated species cannot yet be definitively excluded, a first implementation of an ultrasound reactor in an aquaculture system could be realized such as a firewall. For example, the make-up water could be disinfected before it is fed into the system. This would be feasible for systems that are relatively closed, such

as larval rearing system or a RAS. Due to the relatively strong heat emission in the ultrasonic reactor, a cooling system for the treated water would most probably have to be implemented. It could also be conceivable to carry out a permanent ultrasound treatment of the rearing water. For example, water from the pump sump could be fed continuously to an ultrasound reactor. In this bypass mode with concern to heat generation, it would be necessary to check whether a cooling system is also required or whether the treated water volume can be adjusted so that dilution with the untreated water is sufficient.

In the start-up stage, the TRO concentration should also be permanently controlled, as it is not currently possible to say which TRO concentration the organisms will ultimately be exposed to as a result of this treatment.

5. Conclusion

Our study demonstrated that LF and HF ultrasound alone for disinfection in aquaculture cannot offer an all-round solution. However, on one side our results indicate a high potential of ultrasound treatment for the control of parasites and microalgae in aquaculture. Both, LFUS and HFUS were effective against most tested eukaryotic organisms. As the inactivation effect of LFUS and HFUS is highly species-dependant, the optimum ultrasound frequency varies with the respective target species.

On the other side our data also suggest ultrasound treatment alone to be insufficient for an efficient reduction of small-sized microorganisms such as bacteria or small microalgae. Therefore, a combination of ultrasound with other methods that have already been used successfully is recommended. In particular, when HFUS is applied in marine process water stream total residual oxidants should be monitored in order to avoid deleterious impacts on animal health. Further studies should focus on the up-scaling and economic optimization of ultrasound treatment as a potential disinfection technique in aquaculture systems.

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Chapter 3

Removal of dissolved organic matter and nitrogen compounds from RAS wastewater by ultrasonically induced cavitation

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Abstract

In recent years, ultrasonically induced cavitation has aroused interest for use in water treatment. Due to the formation and the subsequent implosion of vapor bubbles (cavities) in a local hotspot under extreme conditions ($p > 100$ atm, $T^{\circ}\text{C} \sim 5200$ K), mechanical and sonochemical effects were induced into the water. Especially the sonochemical effects generate hydrogen atoms and hydroxyl radicals triggered by the thermal dissociation of water. Therefore, the high and unspecific reactivity of the radicals indicates a high potential of oxidizing organic and nitrogen compounds in RAS water.

The aim of this study was to investigate the potential of low- and high-frequency ultrasound to reduce the concentration of dissolved organic carbons and nitrogen compounds. For this, water samples were taken from an experimental marine and fresh water RAS and were treated with ultrasound. The reduction efficiency of the treatment was rated by the ratio of the concentration prior and after ultrasound treatment in percent. Total carbon (TC), inorganic carbon (IC), dissolved organic carbon (DOC) and total nitrogen (TN) were analyzed.

The results show that low-frequency ultrasound at 20 kHz was not capable to reduce the investigated parameters in RAS wastewater. Only high-frequency ultrasound at 850 kHz could slightly reduce the DOC concentration in RAS fresh water and the TN concentration in RAS marine water.

However, the various results between low- and high-frequency ultrasound suggest that the production of cavities and its sonochemical effects were markedly affected by the frequency. Furthermore, the different data between fresh- and marine water samples demonstrated that the water matrix may have substantial influences on the efficiency of the sonochemical oxidation.

In conclusion, the investigated treatment could not reveal an alternative treatment method to reduce dissolved organic or nitrogen compounds in RAS wastewater.

1. Introduction

Intensive fish husbandry produces wastewater that needs to be treated before reuse in recirculating aquaculture systems (RAS). Consequently, the efficiency of wastewater treatment will be one of the most decisive factors in terms of environmental sustainability and the global expansion of RAS in the future.

Metabolic by-products coming from excrements of the cultured species or feed residues accumulate in the culture water and impede efficient production, e.g. a higher oxygen demand due to the bacterial degradation processes of organics. Furthermore, dissolved or finely-dispersed organic particles can cause gill damage (Adam & Schnervers, 1993) and provide a new and ideal substrate for germs and parasites and increase the risk of infectious diseases. In addition, metabolites, especially ammonia nitrogen (NH_4^+), accumulates in the water as a metabolic end product of the protein digestion (Fiedler, 1991; Wright and Anderson, 2001). In aqueous media ammonium is in chemical balance with the undissociated ammonia depending on pH, dissolved oxygen, temperature and salinity (Trussell 1972; Bower 1978). Even very low concentrations of ammonia are highly toxic to fishes (Colt und Armstrong 1979; Russo und Thurston 1991; Hargreaves 1998). Thus, the removal of these inorganic or organic compounds by a water treatment system generates multifold benefits.

Until today, few conventional water treatment techniques have been established to remove these compounds with varying efficiencies. The bio-filter is the most common technique to remove ammonium from the system water by nitrifying bacteria oxidizing ammonium to nitrate which remains as the final degradation product in the water. However, nitrate at species-specific concentrations could impede fish health and growth performance (Scott and Crunkilton, 2000; Shimura et al., 2004; McGurk et al., 2006; van Bussel et al., 2012; Schram et al., 2014). The common practice to remove nitrate from the system is to exchange a part of the contaminated water with fresh water or to denitrify by facultative anaerobic heterotrophic bacteria. In the denitrification process nitrate is reduced as an oxidizing agent to molecular nitrogen by oxidizing organic matter (van Rijn et al., 2006; Skiba, 2008). It seems that denitrification is an ideal complement to the bio-filter but the anaerobic conditions and the presence of the required

organic matter are in contrast to the needed conditions of nitrification and can have a negative impact on the performance of the bio-filter. A high concentration of organic matter leads to a higher degradation of organic compounds by heterotrophic bacteria and in consequence a higher consumption of oxygen which will not be available for the nitrification process (Ranjan et al., 2014). Although, diffuse denitrification occurs in small sedimentary depositions within the system, it is not sufficient to avoid the accumulation of nitrate in the rearing water (Eding et al. 2009). Hence, denitrification needs an extensive technical support.

Due to its high reactivity, ozone is used in water treatment for disinfection but also for the oxidation of suspended or dissolved organic matter like proteins or amino acids (Tango & Gagnon, 2003) and inorganic compounds like nitrite (Rosenthal, 1981; Paller & Lewis, 1988). The additional application of ozone enhances the efficiency of the skimmer due to a faster decomposition of larger hydrocarbon molecules to smaller molecules (Rosenthal & Sander, 1975; Rosenthal et al., 1978). However, the usage of ozone in marine-based RAS is limited by the formation of bromate known as a carcinogen. Therefore ozone is under suspicion to have a chronic impact on fish health (Amy et al., 1994; Health Canada, 1999; Hofmann, 2000).

An alternative approach is the electrochemical treatment in bio-electrochemical reactors (BERs). This advanced oxidation processes (AOPs) combines the reduction of organic matter by the Electro-Fenton process and nitrate by a cathodic nitrate reduction (Mook, 2012; Pushpalatha & Krishna, 2017). An industrial application of electrochemical treatment has to be finally tested due to the enormous volume of wastewater. However, alternative strategies to remove organic or nitrogen compounds in wastewater are technically very complex or are inefficient from an energy demand point of view (Matilainen & Sillanpää, 2010).

In the last few years the advantages of cavitation caused by ultrasonically induced pressure waves has moved increasingly into focus by several applications in water treatment. The phenomenon of cavitation is based on the formation and the subsequent implosion of vapor bubbles in a local hot spot under extreme conditions ($p > 100$ atm, $T^{\circ}\text{C} \sim 5200$ K) (Suslick et al., 1986). Cavitation can be distinguished in two types: The stable cavitation generates microbubbles oscillating around a definite radius, and the transient cavitation, where the radius of the cavities increases in a couple

of acoustic cycles until the bubble implodes in a hotspot. In the case of the transient cavitation, the sudden implosion produces mechanical and sonochemical effects. While the mechanical effects induce turbulences, liquid circulation currents and shear forces via shock waves (Piyasena, 2003), the sonochemical effects induce the production of free radicals or secondary oxidants caused by the thermal dissociation (pyrolysis) of compounds inside the hot spot, e.g. the thermal dissociation of water vapor generates hydrogen atoms and hydroxyl radicals. Secondary oxidants, such as hydrogen peroxide, can arise from the recombination of two hydroxyl radicals (Riesz, 1992). The magnitude of the effects by ultrasonically induced cavitation depends on the setting like intensity (W/m^2), irradiation time, ultrasound frequency (kHz) and the reactor architecture. High-frequency ultrasound (HFUS) produces mainly small cavities due to the shorter wave length and in consequence less time for bubble growth. Low-frequency ultrasound (LFUS) provides more time for bubble growth due to the longer wave length and leads to greater cavities collapsing more violently by achieving larger maximum sizes (Portenlänger & Heusinger, 1997). There exist diverse investigations evaluating cavitation effects for wastewater treatment or chemical engineering with regard to the degradation of dissolved organic compounds like toxics, medicine residue, pesticides (Nagata et al., 1996; Pétrier & Francony, 1997; Gonze et al., 1999; Gogate & Pandit I & II, 2000; Gogate et al., 2002; Hamdaoui & Naffrechoux, 2007; Naddeo et al. 2007; Gogate & Kabadi, 2009; Shrestha et al. 2009; Tang & Sivakumar, 2013; Petkovšek et al., 2013; Zupanc et al., 2013; Wu et al., 2014; Torabi & Ghiaee, 2015;) or the degradation of ammonia-nitrogen (Wang et al., 2008). The results show that both the direct effect of ultrasonically induced cavitation and its associated side-effects such as the deagglomeration of pollutant particles have led to improved purification. The resulting combination of cavitation with well-established methods such as ozonation or UV disinfection has shown promising outcomes for degradation of organic pollutants (Goncharuk et al., 2008; Gogate & Patil, 2015; Barik & Gogate, 2016; Prajapat & Gogate, 2016; Rajoriya et al., 2017).

Therefore, the potential of ultrasonically-induced cavitation as an alternative or additional water treatment system in a RAS is of high interest. Thus, the aim of presented study was to examine the reduction of organic, inorganic and nitrogen compounds in RAS water via HFUS (850 kHz) und LFUS (20 kHz).

2. Material & Methods

2.1 Experimental set-up

Water samples were taken from an experimental RAS stocked with turbot (*Scophthalmus maximus*) (stocking density 19 kg/m³) and with a salinity of 26‰. In addition to the marine water RAS fresh water samples from an experimental RAS stocked with juvenile carp (*Cyprinus carpio*) (stocking density 3 kg/m³) at a salinity of 0.2‰ were analyzed. In all experiments a sample volume of 250 ml were exposed to ultrasound for 15 min. The temperature of the sonicated samples was kept nearly constant at approximately 16 °C by remaining the glass reactor submerged in an ice water bath. All samples were analyzed prior and after ultrasound treatment. The following parameters were examined: Total carbon (TC), inorganic carbon (IC), dissolved carbon (DOC) and total nitrogen (TN).

The efficiency of the treatment is determined on the basis of the mean recovery rate [%] ± confidence interval (p < 0.05, n=3). The value indicates the ratio of the concentrations of the substance investigated before and after treatment.

2.2 Ultrasound system

The HFUS (850 kHz) was performed using a MeinhardtE/805/T/solo ultrasound transducer (Ø 75 mm) energized by a Meinhardt Ultraschall-Leistungs-Generator K8 (200 W) in pulsed mode (on/off ratio 1:5). The LFUS (20 kHz) was carried out using a titanium horn homogenizer (Ø 13 mm) with a UW 3200 ultrasound transducer and a SH 219 Boosterhorn. The electrical power was applied by a Bandelin GM 3200 HF-Generator (200 W) in pulsed mode (on/off ratio 1:5).

2.3. Analysis of TC, DOC, IC, TN

The samples were analyzed with a total organic carbon analyzer model TOC-L CPH/CPN with the high-salt sample combustion tube kit (Shimadzu Corp., Kyoto, Japan). To determine the dissolved organic compounds the samples were pretreated by filtration with a syringe (0.45 µm). TC, IC and DOC were determined with the NPOC (non-purgeable organic carbon) method which takes into

consideration that there is a significantly higher concentrations of inorganic than organic carbons. The acidification was performed with hydrochloric acid (1.5%). TN was analyzed by the same analyzer and was determined with organic carbons at the same time.

2.4 Energetic evaluation

The consumed specific energy (kJ/L) was calculated from the treatment duration (t), the treated volume (L) and the electrical power (W) of the ultrasound systems. The energy absorption rate and the heat transfer of the ultrasound system were estimated by calorimetric measurements determining the increase of the water temperature during treatment without cooling (Equa.3-1).

$$E = \frac{mC_p \Delta T}{t}$$

Equation 3-1: Calculation of the energy absorption rate E. It was calculated from the temperature difference (ΔT), the treatment duration (t) and the heat capacity (C_p) (Chisti, 2003).

The consumed specific energy for both frequencies was approximately 5.84 kJ/min.

2.5 Statistical Analysis

All data are shown as mean recovery rate [%] \pm confidence interval ($p < 0.05$, $n=3$). Statistical analysis of variances was carried out using SPSS 20.0 (IBM Inc., Armonk, USA). The Levene's test and the Shapiro–Wilktest were used to test the data for normality and for equal variances, respectively. Significant differences in means ($p < 0.05$) were confirmed by t-test or one-way ANOVA with Tukey HSD or Dunnett3 post hoc test.

3. Results & Discussion

In RAS fresh water a total carbon concentrations between 20.0 and 25.3 mg/L was determined. The concentration of dissolved organic carbon was approximately 4.6 mg/L. Hence, the major part of the analyzed carbon originated from inorganic forms with approx. 15.4 – 20.7 mg/L. The samples taken from the RAS marine water showed a similar distribution. The total carbon concentration was between 24.1 and 30.4 mg/L and the dissolved organic carbon concentration 7.5 – 7.7 mg/L. Here as well, the major part of carbon originated from inorganic carbon with 16.6 – 22.7 mg/L. At a pH level of about 7.2 for both fresh water and marine water, it can be considered that hydrogen carbonate was the predominant part of the inorganic carbon. The dissolved organic carbon consists mainly of feed residues or metabolic end products e.g. proteins or carbohydrates. Figure 3-1 illustrates the mean recovery rate [%] \pm confidence interval ($p < 0.05$, $n=3$) of total carbon (TC), inorganic carbon (IC) and dissolved organic carbon.

Sonochemical effects are arising from the oxidation power of free radicals, e.g. hydroxyl radicals produced by the thermal dissociation of water or the pyrolytic decomposition of compounds inside or nearby the hotspots or supercritical water oxidation (Makino et al., 1983; Hoffmann et al., 1996). Regarding this, three regions of the cavities can be distinguished: 1. inside the gas region, 2. a thin hydrophobic liquid layer surrounding the gas region (gas–liquid interface) and 3. the bulk liquid. Extreme conditions ($p > 100$ atm, $T^{\circ}\text{C} \sim 5200$ K) inside the gas region are capable to destroy molecules by thermal dissociation (pyrolysis) comparable to reactions during a combustion process. A 200 nm thick transitional region between the gas phase and the bulk-liquid phase (gas–liquid interface) provides an additional region of thermal dissociation processes (~ 1900 K) as well as free radical reactions (Riesz, 1992). In addition, hydroxyl radicals and hydrogen atoms can diffuse from the inside of the bubble towards the bulk liquid (Adewuyi, 2005). Mechanical effects are coming from strong shear forces due to acoustic shockwaves coming from oscillating (stable cavitation) or imploding cavities (transient cavitation) (Doulah, 1977; Piyasena, 2003).

In general, the reduction of DOC can be achieved by two different pathways: Due to the extreme conditions inside or nearby the cavities, the dissolved hydrocarbons will be cracked to short-chain

hydrocarbons and/or finally oxidized to CO₂ (Hart et al., 1990; Hoffmann et al., 1996). Thus, short-chain hydrocarbons as well as carbon dioxide can still remain in the water and will induce an increase in the IC concentration while the TC concentration remains constant or the short-chain hydrocarbons remain in DOC. If the carbon dioxide can be stripped out through the boundary water-air layer the TC concentration will be reduced in the same proportion than DOC.

The presented results demonstrated that LFUS at 20 kHz could not significantly reduce the concentration of TC, IC and DOC in both sample types (Fig. 3-1). Due to several studies confirming the degradation capability of ultrasound at 20 kHz (Petrier et al., 1992; Pétrier, C., & Francony, A., 1997; Goel et al, 2004), it is excluded that the applied ultrasound treatment had no effects of the investigated compounds. In consequence, it is assumed that most probably the organics were converted to other degradation products and that the final degradation of the compounds to CO₂ was only achieved in such small quantities undetectable by the carbon analysis in use.

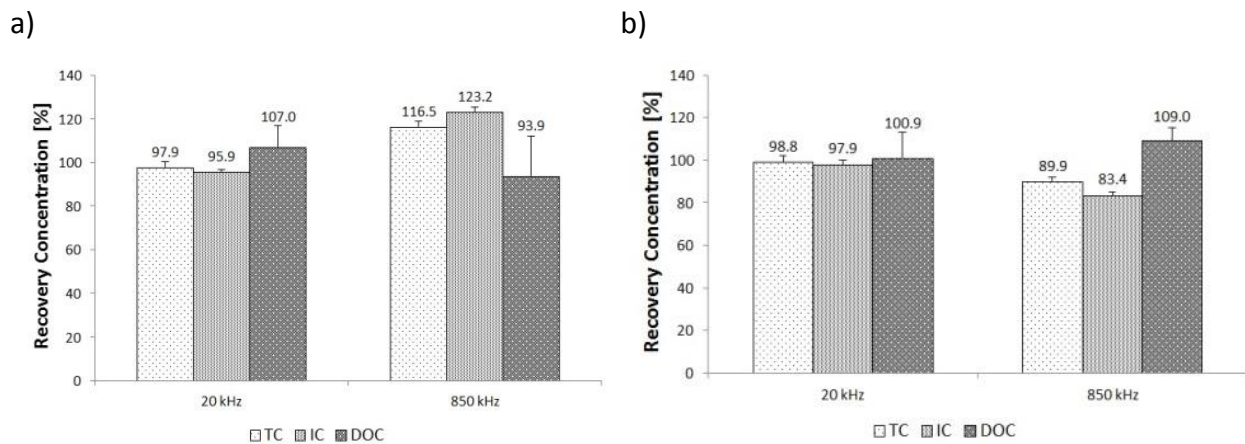


Figure 3-1: Mean recovery rate [%] ± confidence interval ($p < 0.05$, $n=3$) of total carbon (TC), inorganic carbon (IC) and dissolved organic carbon prior and after ultrasound treatment with LFUS (20 kHz) and HFUS (850 kHz). Samples were taken from an experimental RAS stocked with juvenile carp (*Cyprinus carpio*) (stocking density 3 kg/m³) representing RAS fresh water with a salinity of 0.2‰ (a) and from an experimental RAS stocked with turbot (*Scophthalmus maximus*) (stocking density 19 kg/m³) representing RAS marine water with a salinity of 26‰ (b).

In contrast, the experiments with HFUS at 850 kHz showed only in the marine RAS water sample a significant IC reduction of approx. 16.6 % ($p < 0.05$) (Fig. 3-1b). Here the TC concentration was not significantly reduced but tended to decrease in an equal ratio as IC while the DOC concentration remained unchanged). It can be assumed that ultrasound at 850 kHz applies more

sonochemical effects in marine water than 20 kHz resulting in a higher amount of cavities and consequently more hydroxyl radicals (Hua and Hoffmann, 1997). It cannot be totally excluded that the unchanged concentrations of DOC are caused by an incomplete degradation of hydrocarbons to CO₂, e.g. by depolymerization (Gogate & Prapajat, 2015). Therefore, the derived carbons from the sonochemical reactions might be still measurable as DOC as a cumulative parameter. A reason for the reduction of IC could be stripping of dissolved CO₂ from the liquid phase into the atmosphere at the boundary layer. The crucial factors for the gas exchange of CO₂ at the boundary layer are its concentration, solubility and chemical reactivity in water depending on parameters like temperature and salinity of the water. Thus, the observed decrease of IC in the marine samples compared to that of fresh water might be the result of the higher salinity, lowering the solubility of CO₂ and thus supporting its deaeration (Seymour and Gupta, 1997). Furthermore, the downward trend of TC concentration could be the result of CO₂ stripping.

It was observed that the experiments with RAS fresh water (Fig. 3-1a) were different as compared to the results of the experiments with RAS marine water (Fig. 3-1b). At 850 kHz the DOC concentration did not significantly decrease but showed a slight tendency of reduction (6.1 %). In comparison, several studies have described the removal of TOC in water by ozonation and determined removal rates from 8 – 15 % (Veenstra et al., 1983; Hirayama et al., 1988; Chang and Singer, 1991; Tango and Gagnon, 2003). It should be noted that the IC and TC concentration increased inversely to the DOC reduction. The final degradation or oxidation of organics to CO₂, remaining mainly in the water as hydrocarbonate, or the transfer of organics to inorganic carbons could be a reason for a concentration shift from organic to inorganic carbon while maintaining TC nearly constant. However, the increased IC concentration cannot be entirely explained by the decomposition of organics considering the significant lower initial concentration of DOC. Based on the fact that the temperature remained nearly constant and no gas exchange near to the water surface were initialized, the possibility that the water samples may act as a sink for CO₂ coming from the atmosphere was excluded. Nevertheless, further research is required to examine such an increase in TC concentration in RAS fresh water.

The determination of nitrogen compounds includes ammonia, ammonium, nitrite, nitrate and organics which can be oxidized to nitrogen dioxide under the applied analysis conditions.

Dissolved gaseous nitrogen has not been considered by this method. In the experimental fresh water RAS and marine water RAS the total nitrogen concentration were between 15.9 – 17.4 mg/L and 110.5 – 111.0 mg/L, respectively. Significant differences of total nitrogen between fresh- and marine water were measured (Fig. 3-2). Approximately a 10-fold higher concentration in RAS marine water was determined, which could most probably be attributed to the higher stocking density.

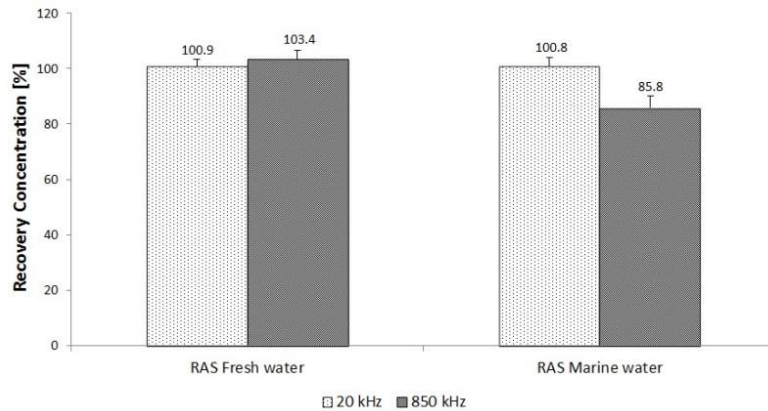


Figure 3-2: Mean recovery rate [%] ± confidence interval ($p < 0.05$, $n = 3$) of total nitrogen (TN) prior and after ultrasound treatment with LFUS (20 kHz) and HFUS (850 kHz). Samples were taken from an experimental RAS stocked with turbot (*Scophthalmus maximus*) (stocking density 19 kg/m³) representing RAS marine water with a salinity of 26‰ and from an experimental RAS stocked with juvenile carp (*Cyprinus carpio*) (stocking density 3 kg/m³) representing RAS fresh water with a salinity of 0.2‰.

A significant reduction of total nitrogen in RAS marine water by HFUS at 850 kHz were observed. These findings are similar to the results of the experiments with organics showing little effects in RAS marine water but no effects in RAS fresh water. The central question then is, what had happened with these compounds and where did they remain? Denitrification can be excluded under the applied oxidizing conditions and treatment duration. The study by Wang et al. (2008) demonstrated the removal of ammonia-nitrogen at 20 kHz and a treatment time over 180 min. The authors suggested that ammonia-nitrogen were removed by entering the cavitation bubble and were transformed into nitrogen and hydrogen molecules via pyrolysis due to its high volatility. Despite the fact that Wang et al. (2008) used a longer treatment time and energy, the reduction of TN at 850 kHz could be the consequence of the higher amount of cavities occurring at HFUS. Furthermore, there might be an influence of the salinity. Due to the counteracting results

of the treatment of RAS fresh water and RAS marine water with 850 kHz, the effects of salinity cannot be excluded. A higher amount of salt in water leads to a higher ion strength of the aqueous phase, enhancing the transfer of hydrophobic and volatile compounds from the water toward the gas region or interfacial region of the cavities. Another reaction pathway is the reaction of ammonium nitrate and sodium nitrite at 70 °C. In this process ammonia nitrite is produced and splitted into nitrogen and water (Falbe, J. and Regitz, M., 2014). Due to the extreme conditions in and nearby the local hotspots of the cavities, this reaction pathway is quite possible but could not be comprehensively investigated in this study. Furthermore, it should be considered that the analysis method used may have some limitations. In RAS fresh water and RAS marine water the DOC concentrations were 4.6 mg/L and 7.7 mg/L, respectively. Thus, the relative high concentration of organics in the samples may have affected the analysis. In addition, not all nitrogen compounds were quantitatively converted to nitrogen dioxide and the determination of compounds with double and triple bonds can lead to a poor recovery rate.

4. Conclusion

Overall, in the study the results of the various experiments showed no significant effects of ultrasonically induced cavitation on carbon or nitrogen compounds in RAS waters. In comparison to other treatment methods, neither ultrasound at the frequency of 20 kHz nor at 850 kHz was able to reduce the concentration of DOC or TN in RAS wastewater efficiently. We observed a tendentially reduced DOC content in RAS fresh water following the 850 kHz treatment. This reduction could only be observed in RAS marine water probably caused by sonochemical effects producing secondary oxidizing agents such as hydroxyl radicals. This assumption is supported by the results at 20 kHz, inducing less sonochemical effects and therefore less oxidizing power for the degradation of carbons or nitrogen compounds. Due to the measurement of only cumulative parameters, a more differentiated analysis of which substances are degraded, modified or newly formed is not possible at this stage. Therefore, future studies are important to gain a better understanding of the effects of ultrasound on the substances examined here.

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GENERAL DISCUSSION

In the general discussion, the results of the different studies are examined under the aspect of necessary considerations when developing an ultrasound system for an industrial scale RAS application. Aspects such as salinity, frequency, reactor architecture and recommendations for further investigations are discussed in order to make a final evaluation of ultrasound treatment in a RAS.

The study demonstrated that ultrasound is able to significantly reduce geosmin (GSM) and 2-methylisoborneol (2-MIB) in RAS marine and fresh water (I). The removal of GSM and 2-MIB was more effective at high frequency (HFUS, 850 kHz) than at low frequency ultrasound (LFUS, 20 kHz) and was not significantly affected by dissolved organic or inorganic substances. Furthermore, the study revealed that HFUS and LFUS can efficiently reduce various model organisms representing typical parasites or algae species in an aquaculture system (II). However, under the tested conditions no relevant reduction of bacteria or small microalgae (< 4 µm) were observed. In the last study, it has been shown that the treatment, neither at 20 kHz nor at 850 kHz, led to a significant reduction of the dissolved organic carbon (DOC) concentration in RAS wastewater (III). Only a significant reduction of total nitrogen (TN) in RAS marine wastewater by HFUS was observed.

In conclusion, when treating water with ultrasound, 2 different effects occur, which can be used for different applications in water treatment:

- **Sonochemical effects**, such as the formation of radicals or pyrolysis within the cavities, which are mainly responsible for the removal of organic substances such as off-flavor compounds.
- **Mechanical effects**, such as shear forces from shock waves, which are mainly responsible for disinfection effects.

Which frequency should be preferred?

The frequency of the ultrasound has a decisive influence on the properties of the various cavitation effects in the treated water. In contrast to LFUS, the shorter wavelength of HFUS results in a larger number of smaller cavitations and generates more hotspots in the treated water. As a result, sonochemical effects (radical formation, pyrolysis, etc.) are more intense at HFUS. Due to the longer wavelength at LFUS, fewer but larger cavitation nuclei are formed, which can produce stronger implosions and finally lead to stronger mechanical forces (shear forces, shock waves, etc.) (Hua and Hoffmann, 1997; Pétrier and Francony, 1997; Portenlänger and Heusinger, 1997).

In principle, both frequencies would be suitable for the removal of off-flavor compounds and disinfection, because HFUS and LFUS showed significant reductions in both applications in the experiments. There are only significant differences in efficiency. For the off-flavor removal, HFUS was at least twice as efficient as LFUS. During disinfection, HFUS was several times more efficient in removing algae than LFUS. The result for zooplankton was more differentiated. There were no significant differences between the frequencies. For wastewater treatment only HFUS showed reducing effects.

A further aspect is the wear of the technology and the associated operating and maintenance costs. In case of the 20 kHz ultrasonic transducer, increased material wear on the diaphragm in form of flaked material was observed due to the direct effect of mechanical forces of cavitation. In contrast, no comparable material wear occurred with the 850 kHz diaphragm.

In addition, the production of DPBs should also not be underestimated. At 850 kHz, the highest concentrations of 0.26 mg/L Cl₂ always occurred at high salinities in a marine environment (26 ‰). Even if a strong dilution of the treated water with the already existing water in an industrial scaled RAS is expected, the formation of fish toxic substances (DPBs) should be observed.

Consequently, the treatment with HFUS would offer the most efficient solution, as reducing effects could be shown in all 3 studies. Depending on the aim of the application, parallel or alternating applications with ultrasound could be a solution as well. For example, the rearing water could be permanently treated with HFUS to achieve a low concentration of off-flavor

compounds or to prevent an algae bloom, while LFUS could act as a firewall to prevent parasitic organisms from entering the system during water exchange.

Concluding from that, the most efficient mode should always be used for the required application.

What should be considered regarding salinity: Marine vs. Fresh water RAS

The salinity of the treated water has a significant influence on the cleaning efficiency of the ultrasonic treatment. In consequence, the salinity must be considered when configuring an ultrasonic system in a commercial aquaculture facility. A higher salt content creates an environment in the water that favours the sonochemical effects of cavitation, especially those of pyrolysis. The transfer of hydrophobic organic compounds such as GSM or 2-MIB from the water into the gaseous or interfacial region of the cavities is promoted due to the salting effect by increasing the ionic strength of the aqueous phase through the addition of salt (Seymour and Gupta, 1997). So, when planning and designing an ultrasound system in a RAS, the question of whether the plant is a fresh water or a marine RAS must also be taken into consideration in order to take the most efficient configuration of the ultrasound system. Off-flavor could be removed approx. 20 - 25% more efficiently in marine water, hence it can be considered whether the animals should always be kept in the highest possible salinity to avoid off-flavor. A significant removal of nitrogen-containing compounds could only be measured in marine water (26‰). As a result, a significant reduction of the nitrogen content and most probably also of dissolved organics with ultrasound can only be achieved with a salinity around 26‰. In consequence, a fresh water aquaculture facility requires a higher energy consumption as a marine aquaculture facility to achieve the same efficiency for removing water constituents.

For the disinfection it seemed to be irrelevant at which salinity the treatment was performed. This corresponds with our results that mainly the mechanical effects (shock waves) are responsible for disinfection.

Another limitation of the use of ultrasound is the development of DPBs. The samples with the highest salt content were always those with the highest DPB concentrations. In our investigations the DBP concentrations were about 0.26 mg/L Cl₂ and therefore significantly above the safety

level of 0.06 mg/L Cl₂ for ozone-produced oxidants (OPO), which is recommended for the RAS production of selected marine fish and shrimp species (Schroeder et al., 2010; Reiser et al., 2010). This should at least be considered when dimensioning the flow rate of the water to be treated in an ultrasonic reactor.

It can be concluded that the ultrasound has removed off-flavor compounds in both RAS water types. The disinfection effect could also be proven for different salinities. Consequently, an ultrasonic system would be suitable for a fresh water and a marine RAS. However, it should always be ensured that a balance is maintained between the applied ultrasound and the concentration of DPBs below the safety level of 0.06 mg/L Cl₂.

Reactor architecture under process engineering aspects

After the general investigation, whether ultrasound is useful for the treatment of aquaculture water, the positive results lead to the question, how these results can be transferred from laboratory scale to a pilot scale system. A simple enlargement of the ultrasound system is very complex and technically extremely difficult to implement due to the difficult calculation of acoustic fields and the problematic construction of large-sized generators and transducers. In order to get a first impression of the efficiency and the feasibility of this treatment for larger volumes, first unpublished upscaling experiments were carried out. To investigate the volume-related reduction of off-flavor compounds with ultrasound, first experiments at 850 kHz were performed with larger water volumes (Fig. D-1).

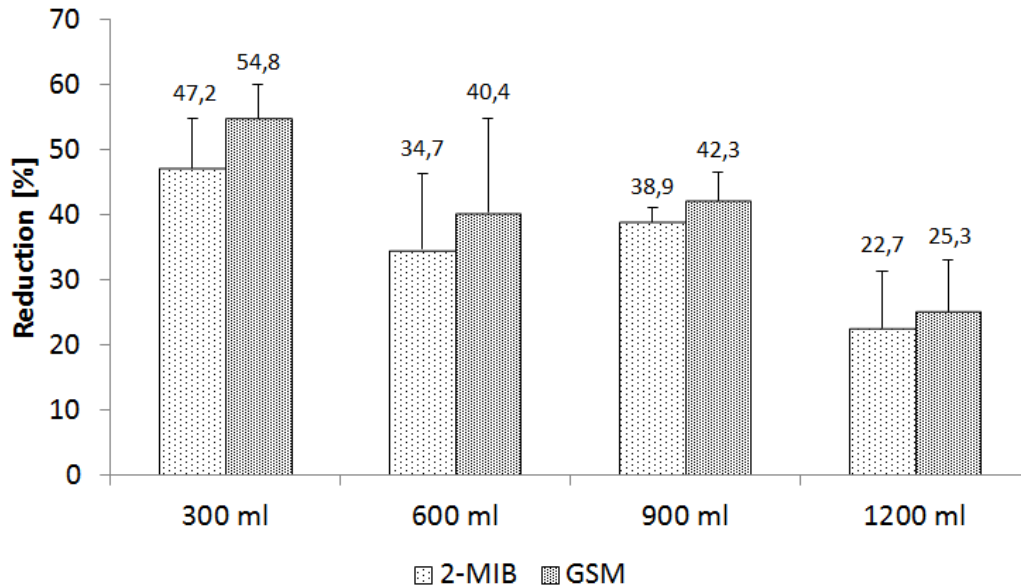


Figure D-1: Mean reduction [%] ± confidence interval ($p < 0.05$) of 2-MIB and GSM at different volumes (300, 600, 900, 1200 ml) after 15 min treatment time at 850 kHz HFUS ($n=4$, $VB=0.95$). Samples were taken from the rearing tank of an experimental RAS stocked with Atlantic salmon (*Salmo salar*) (stocking density in tank 34 kg/m^3) at a salinity of 26 ‰.

Samples of 300, 600, 900, 1200 ml were taken from the rearing tank of an experimental RAS containing Atlantic salmon (*Salmo salar*) (stocking density in tank 34 kg/m^3) at a salinity of 26 ‰ (17.0 ± 1.3 °C temperature, 0.38 ± 0.17 mg/L $\text{NH}_4\text{-N}$, 0.63 ± 0.29 mg/L $\text{NO}_2\text{-N}$, $\text{pH } 7.4 \pm 0.1$). The results show that the degradation occurs mainly in descending order to the higher volume. Hereby, it is remarkable that the reduction is not proportional to the volume. Significant differences in reduction exist between 300 ml and 1200 ml and between 900 ml and 1200 ml.

For a better comparability, the results were exposed to a treatment of 1L water volume and a 100% removal of both off-flavor components (Fig. D-2).

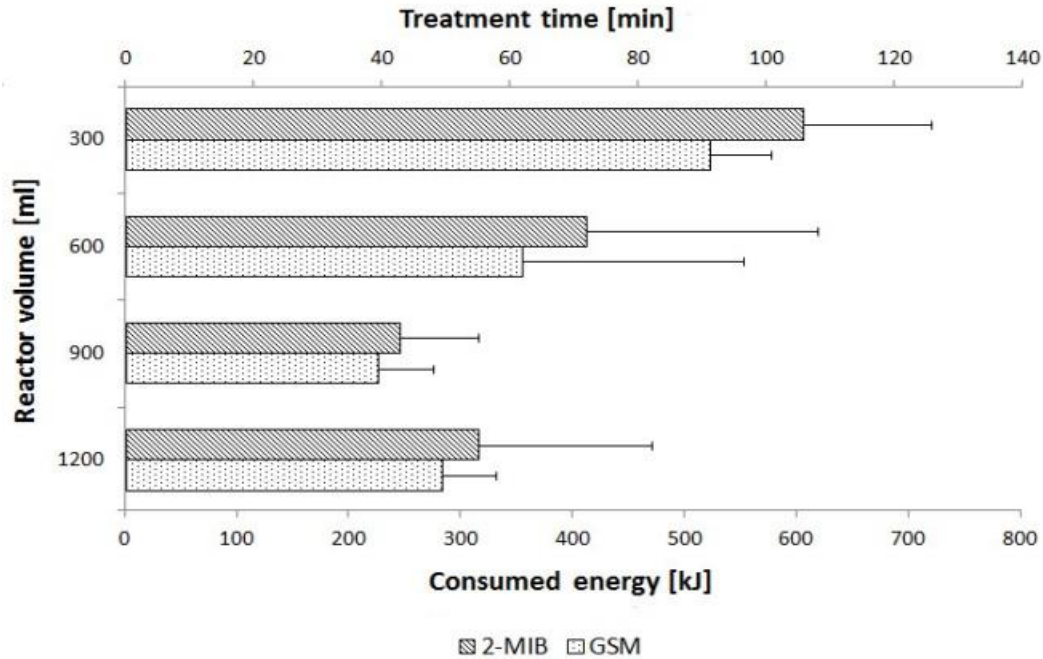


Figure D-2: The mean energy consumption [%] ± confidence interval (p<0.05) of the upscaling experiment were exposed to a volume of 1L and a 100% reduction of 2-MIB and GSM to reveal the most efficient treatment volume at 900 ml in the ultrasound reactor (n=4, VB=0.95).

It could be shown that the least energy was consumed with a volume of 900 ml. Ergo the smallest volume of 300 ml and the largest volume of 1200 ml are not the most efficient volumes. This could be due to the fact that with the increasing water column new pressure conditions have developed in the water body. It also seems that at 300 ml the near field of the ultrasound could not develop to its maximum due to a too low water column. On the other hand, the intensity of the formation of cavities decreases faster with the distance from the membrane, so that at 1200 ml a dead zone has formed at the upper water layer of the higher water column (Maslak, 2010). Consequently, upscaling the reactor by simply increasing the water column could be inefficient. Depending on the number and arrangement of the ultrasonic transducers, there seems to be a very individual optimum for a preferred volume.

What does this mean for the technical equipment?

Depending on the dimension or architecture of the ultrasonic source, it should always be determined at which volume or hydrostatic pressure conditions the maximum formation of cavities occurs. Because different frequencies and applications have shown different efficiencies,

a combination of HFUS and LFUS in one reactor could be an advantage. Depending on the required treatment, it could be switched to the appropriate frequency. Regarding the question of whether a batch system or a flow system would be more practical, studies have already indicated that a flow system may be the better solution for treating a larger volume of water and avoiding unused dead zones (Mason et al., 2003; Lakeh et al., 2013).

In contrast to the study by Lakeh et al (2013), in which several ultrasonic reactors were arranged radially to the flow direction and additionally a UV reactor in the middle of the flow chamber, a flow tube with the ultrasound transducer arranged horizontally and in the flow direction would be conceivable. Here the ultrasound waves are emitted in flow direction (Figure D-3).

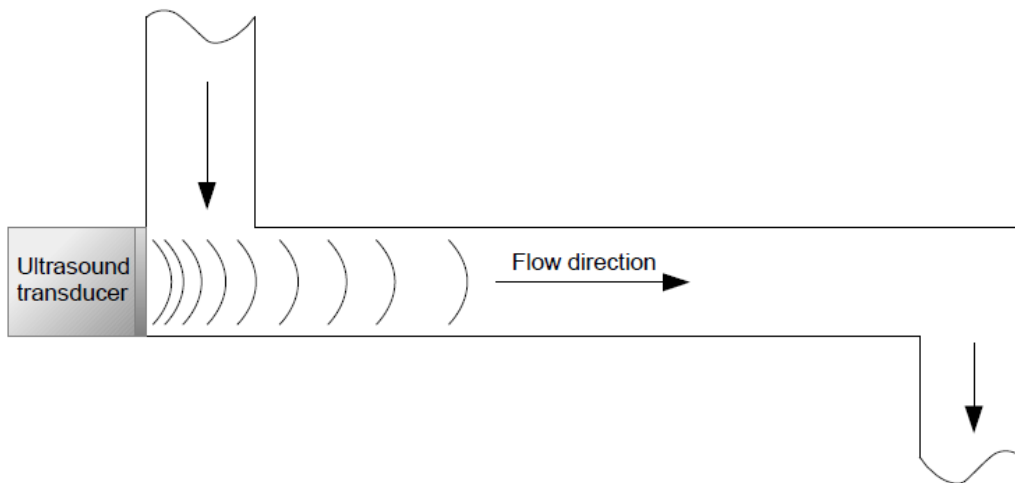


Figure D-3: Ultrasound reactor with a horizontally aligned ultrasound transducer in which the ultrasound waves are emitted in flow direction.

Several conditions in such a flow reactor would have a positive effect on the formation of cavitation: The increased flow velocity leads to a pressure reduction in the medium and so to optimal conditions for the formation of cavities. In addition, the water always flows in the near field of the ultrasound. By using the formula for the near-field length of ultrasound, the maximum length (N) of such a flow reactor can be calculated (Equa. D-1):

$$N = \frac{D^2 - \lambda^2}{4\lambda}$$

Equation D-1: Equation for calculating the near-field length (N) with the diameter of the ultrasonic transducer (D=75mm) and the wavelength (λ). The wavelength λ is calculated from the quotient of the sound velocity in the water (1,500 m/s) and the frequency used (f=850,000 1/s).

The ultrasound transducer used in this study would generate a near-field length of 79 cm. This would also be the maximum recommended length of the flow reactor with a volume of 3.5 L. The flow rate and in consequence the resulting retention time of the medium in such a reactor would depend on the concentrations of the off-flavor compounds or the amount of pathogens to be removed. To increase the volume of water and to extend the treatment time, several reactors could operate simultaneously.

Finally, the wear caused by corrosion, especially by salt water, as well as the material removal due to cavitation must also be taken into consideration when selecting the material. At least a titanium coating would be useful.

It should also be noted that the energy required for the ultrasonic system is mainly converted into kinetic and thermal energy. Finally, the kinetic energy is also converted into heat, so that it must be expected that the temperature of the treated water will increase considerably. Depending on the implementation of the ultrasonic system in a RAS, whether as a bypass or as a firewall, cooling of the treated water must be considered. In bypass operation it should be controlled whether the strong dilution of the treated water during the return into the existing system water is sufficient. If this is not the case, active cooling must be implemented. It can be assumed that if ultrasound is used as a firewall, active cooling would be required. How active cooling is technically implemented must be specifically decided for each RAS.

Can ultrasound be an alternative to the usual water treatment methods?

The following advantages over established methods can be concluded from our investigations:

Ultrasound can be used very efficiently against zooplankton parasites, which cannot be removed sufficiently by the UV method. Furthermore, the treatment can promote the disinfection efficiency of UV light by deagglomerating particles due to the mechanical cavitation forces and thus improving the accessibility of UV radiation to organisms attached to these particles.

One of the most advantageous aspects of the ultrasonic technology is the removal of off-flavor components in the rearing water. The independence of the degradation of GSM and 2-MIB from the organic or inorganic load of the RAS water matrix could be an important advantage to other alternative water treatment strategies that use oxidants. Compared to AOPs (Advanced Oxidation Processes), which mainly produce OH radicals as oxidants, the ultrasonically induced removal of 2-MIB and GSM is mainly promoted by pyrolysis due to the chemical degradation of these compounds at high temperatures in the hot spots. Therefore, the treatment with ultrasound is less affected by competing reactions with free radical scavengers such as chlorides or organic substances derived from feed or faeces.

On the other hand, our investigations could also reveal the following limitations:

The results from chapters 1 & 3 suggest that ultrasound is apparently only capable to significantly remove trace substances in the range from ng/L to µg/L in water. Higher concentrations of organic or nitrogenous compounds could not be significantly reduced. In addition, no antibacterial effects could be demonstrated in all water types tested. Consequently, an alternative method such as UV radiation or chemical disinfection (e.g. ClO₂) would have to be added to an ultrasound system to ensure a comprehensive protection. It should be considered whether UV treatment is implemented after the ultrasound reactor or in combination with ultrasound treatment as in Lakeh et al. (2013).

The production of DPBs or other toxic by-products known as TROs by ultrasound should not be underestimated and should always be controlled due to the negative effects on the cultivated

organisms. Here it would be necessary to determine and to observe the concentrations of TROs in the rearing water after the treated water has been recirculated.

One of the major limitations of an ultrasound system is the high energy consumption, which has already been confirmed by other studies (Adewuyi, 2005; Kotronarou, 1992). In our studies, the energy consumption was between 1.3 - 172 kJ/L to achieve a sufficient effect for the relevant application. Currently, from an energy point of view, a standalone ultrasound system cannot be an alternative to established water treatment technologies, such as a UV disinfection or a skimmer in combination with ozone.

The use of an ultrasonic system in a RAS would be conceivable if energy costs are reduced by optimizing the energy consumption or by using renewable energy. Finally, the investment, operating and maintenance costs of such a system must also be considered.

There are currently no adequate solutions for ultrasound systems that can treat water volumes reliably and efficiently on an industrial scale. Ultrasound at this stage of development is neither an alternative nor a supplement to current water treatment methods in a RAS. From a purely energetic point of view, the permanent use of an ultrasound reactor would not make economic sense. For this reason, the use of ultrasound would possibly only be conceivable in an emergency, e.g. an off-flavor event or a parasitic infection. The use of ultrasound as a purely prophylactic application would be rather uneconomic at this stage of technology.

Also, it must be considered that this study was primarily done under the aspects of basic research. An economic assessment with regard to an industrial scale ultrasound system, in particular the investment and operating costs (life cycle costs), can only be transferred to a limited extent.

Recommendations for future investigations

The efficiency of ultrasound to remove off-flavor or for disinfection in RAS water depends on the formation of cavitation. Further efforts can be initiated to optimize the sonolytic formation of cavitations. The aim is to increase the number of cavitation nuclei in order to improve the efficiency of the ultrasound process by producing additional hot spots, e.g. by actively injecting additional small gas bubbles into the medium directly above the ultrasonic transducer.

Furthermore, at the same time the tensile stress of the water could be reduced with the increasing gas content (Barth, 2004). Another method can be the direct addition of hydrophilic cavitation nuclei into the medium, such as gypsum powder, which also lead to an increase in cavitation (Eisenmenger et al., 2010). The influence of these additives on animal health or on the rearing water should be analyzed in advance.

Additionally, studies would be necessary to achieve a better understanding of possible contradictory effects. For example, it would be important to research whether the deagglomeration of particles by ultrasound supports the UV process, due to the better accessibility of the UV radiation but on the other hand the deagglomeration leads to a more inefficient water treatment, because larger particles in the water are fragmented and have to be removed from the water more effortfully.

Due to the negative energy balance of ultrasonically induced cavitation, alternative methods for the generation of cavitation could be very helpful. In recent years, hydrodynamic cavitation has become increasingly important for the generation of a large number of powerful cavities (Badve et al. 2013; Amin et al., 2010; Arrojo et al. 2007; Capocelli et al. 2014; Dular et al., 2015; Kumar et al., 2000; Sivakumar et al. 2002). In this method, a rotor in the reactor mechanically generates a flow in the medium. The cavitations are generated due to the alteration of flow conditions and subjected to turbulent pressure field oscillations. The rotor blades are usually provided with notches, so that cavitation nuclei are generated both, on the surface and in the notches of the rotor blades. The great advantage of this method compared to ultrasound is the easier technical implementation of upscaling, because flows in pipe systems can be described with dimensionless parameters. Compared to ultrasonically induced cavitation, hydrodynamic cavitation causes more cavitation, but the collapse conditions are much weaker than with ultrasound (Arrojo et al., 2007). Thus, even with hydrodynamic cavitation in a standalone reactor, no effective disinfection could be achieved (Badve et al. 2015; Schüler et al., 2008).

In conclusion, it has not been proven which water treatment method is more suitable and hence leaves enough potential for further research.

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SUMMARY

Over the last 20 years, the volume of fishery products produced by aquaculture has increased significantly worldwide. Until today, it is one of the fastest growing sectors in the agricultural industry. The global stagnation in the fishing industry and the steadily growing world population suggest that this development will not change in the near future. However, aquaculture using conventional methods, e.g. cage or ponds systems, has been repeatedly criticized due to the increasing eutrophication of the environment or the transmission of fish diseases to wild stocks. An alternative could be a recirculating aquaculture system (RAS). This technology has the advantage of keeping emissions as low as possible due to a closed water cycle with an integrated water treatment system. In addition, a complete control of the rearing conditions such as temperature, pH, conductivity and feed quantity is possible and can help to optimize the growth conditions for the animals. However, the water treatment is very resource-intensive and cannot currently offer optimal solutions for all system-specific problems. The reuse of process water leads to the accumulation of metabolic end products from fish and microorganisms that are not removed sufficiently by conventional water treatment technologies, resulting in negative effects on fish health or product quality. On the one hand, there is the formation and accumulation of non-desired flavor compounds (off-flavor) in the rearing water and their absorption, particularly in the fatty tissue of the fish. Even in the lowest concentrations (ppb), the two well-known off-flavor substances geosmin and 2-methylisoborneol cause a musty and earthy taste in fish. On the other hand, especially in intensive RASs with high stocking densities, there is an increased risk of infectious diseases spreading rapidly and leading to a partial or complete loss of production. In addition, large quantities of organic and inorganic waste materials accumulated in the process water, must be removed and disposed of with a high technical effort. Therefore, the efficiency of the water treatment is one of the most decisive factors for a long-term economically successful operation of a RAS. Consequently, the optimization of the water treatment should always be a key objective of a plant operator to ensure the economic efficiency of his/her RAS.

The aim of the present work was the evaluation of ultrasound for the optimization of water treatment in a RAS with regard to product quality (Chapter 1: Removal of the off-flavor

compounds geosmin and 2-methylisoborneol from recirculating aquaculture system water by ultrasonically induced cavitation), fish health (Chapter 2: Preliminary test of ultrasonically disinfection efficacy towards selected aquaculture pathogens) and wastewater treatment (Chapter 3: Removal of dissolved organic matter and nitrogen compounds from RAS wastewater by ultrasonically induced cavitation).

In recent years, the treatment of process water with ultrasound has received a lot of attention in water treatment and biotechnology. The ultrasound waves cause cavitation (small vapor-filled cavity formation) in the water. Cavitation is classified into two behavioral types: inertial (transient) cavitation and non-inertial cavitation. Especially during the transient cavitation, extreme pressures and temperatures ($p > 100 \text{ atm}$, $T^\circ\text{C} < 5200 \text{ K}$) result from the implosion in the core of the cavities. This generates sonochemical effects such as the formation and release of hydroxyl radicals by the dissociation of water in combination with pyrolysis within the gas phase. At a non-inertial cavitation, in contrast, dissolved gases penetrate into the cavities and reduce or prevent an implosion (stable cavitation). Additionally, mechanical effects are released by shear forces of the generated pressure waves before and after the implosion. The intensity of these effects depends on a number of factors such as frequency, intensity, water temperature and salinity. While at high-frequency ultrasound (HFUS) the sonochemical effects are more dominant, at low-frequency ultrasound (LFUS) the mechanical effects are more prevalent.

In the first part of this study (Chapter 1) it was investigated whether it is possible to reduce the undesired off-flavor compounds geosmin (GSM) and 2-methylisoborneol (2-MIB) in the rearing water of a RAS by ultrasonically induced cavitation. Water samples from a salt water and fresh water RAS (250 ml) were prepared with GSM and 2-MIB standard ($5 \mu\text{g/L}$ and 100 ng/L) and were treated with low-frequency (LFUS, 20 kHz) and high-frequency ultrasound (HFUS, 850 kHz) for 15 min. Additionally, water samples were treated with biogenic GSM and 2-MIB from a RAS for pike-perch (*Sander lucioperca*), and the influence of the salinity on the reduction of the two substances was investigated. The experiments could demonstrate that there were significant reductions of both off-flavor compounds in all samples. In most cases, a significant reduction of about 50 - 60% could be measured. The reduction at 850 kHz was always greater than at 20 kHz, suggesting that mainly the sonochemical effects of ultrasound were responsible for this result. The addition of

salt (10 ppt) in a tap water sample (5 µg/L) with off-flavor standard and a sample from a fresh water RAS with biogenic off-flavor improved the reduction by approx. 10%. Thus, in this study it could be demonstrated that ultrasound is capable to significantly reduce the off-flavor compounds GSM and 2-MIB in RAS water.

The second part of the study (Chapter 2) examined the potential of ultrasound for disinfection of process water. For this purpose, water samples (250 ml) with different model organisms representing different taxa of typical pathogenic or parasitic organisms were treated with ultrasound. In total, 3 zooplankton organisms (*Paramecium caudatum*, *Brachionus plicatilis*, *Artemia salina*), 5 algal species (*Nanochloropsis salina*, *Pavlova viridis*, *Tetraselmis chui*, *Rhodomonas baltica*) and bacteria from both, marine and fresh water RAS were investigated. Different frequencies (850 kHz, 20 kHz) were also tested. For a better comparability of the disinfection effectiveness of ultrasound, an inactivation rate as a function of the consumed energy (kJ/L) was determined for each tested organism. The results showed that although ultrasound was very effective in reducing the number of living cells or photosynthesis activity in most of the tested organisms, such as parasites and microalgae, but did not show significant effects for the smallest microalgae (4 µm) and bacteria. While the photosynthesis activity of algae decreased more efficiently at 850 kHz with the increasing organism size, this performance could not be observed for zooplankton. Here, no frequency could provide significantly better results over all tested zooplankton. The extremely destroyed zooplankton organisms during treatment with LFUS indicate strongly developed mechanical forces of cavitation. In contrast, at 850 kHz the lifeless but externally intact bodies of the zooplankton when treated with HFUS indicate rather sonochemical effects within the organisms. In summary, it can be determined that ultrasound can be used very efficiently against zooplankton, but as a standalone system in a RAS, it does not provide sufficient protection against pathogenic germs.

In the third and last part of the study (Chapter 3) it was investigated whether ultrasound supports the removal of organic and nitrogenous compounds in process water. In this study samples were taken from a marine and fresh water RAS and the parameters total carbon (TC - Total Carbon), inorganic carbon (IC - Inorganic Carbon), dissolved organic carbon (DOC - Dissolved Organic Carbon) and total nitrogen (TN - Total Nitrogen) were determined. Then these samples were

treated at 20 kHz and 850 kHz for 15 minutes to determine the recovery rate of the previously determined parameter. In all samples treated with 20 kHz no significant reductions of the investigated parameters could be measured. Based on previous scientific studies which demonstrated the degradation of both inorganic and organic substances by ultrasound, we assume that this treatment had an effect on the investigated substances, but the analysis of the parameters could not sufficiently reveal this change. For example, it is possible that the sonochemical effects led to a transformation of long-chain substances into short-chain substances, but they were grouped under the same sum parameter and therefore no measurable change occurred. At high-frequency ultrasound at 850 kHz a significant reduction of IC of approx. 16.6% could be measured in marine water, as well as a decreasing trend of TC. Nevertheless, the DOC content remained unchanged. We assume that especially the sonochemical effects at 850 kHz and the increased salinity led to a conversion of the inorganic carbon to CO₂, which could be partially released into the atmosphere and thus out of the system. Furthermore, a significant reduction of TN by about 14.6 % could be measured in fresh water at 850 kHz, but not in marine water, which leaves a lot of potential for further investigations. In general, the results clearly indicate that both frequencies could not significantly reduce the investigated substances. Thus, under these examined conditions, ultrasound does not support the treatment of aquaculture waste water.

The results of the present studies could clearly show the effectiveness, but also the limitations of the ultrasound technology. In principle, ultrasound is able to reduce GSM and 2-MIB and pathogens in the process water of a RAS. A conclusive and clear assessment of whether ultrasound can be used against off-flavor and pathogens in an industrial RAS is not possible at this stage of knowledge, especially as the study was done exclusively with in-vitro tests on a laboratory scale. The upscaling of the ultrasound system to industrial scale could only be partially investigated in this study. It was revealed that simply scaling up the sample volume or the ultrasound system did not produce the desired effects and thus, further investigations are absolutely necessary. In this context, aspects such as the formation of undesirable by-products due to sonochemical effects and the energy consumption of the system should be considered.

ZUSAMMENFASSUNG

In den letzten 20 Jahren ist die Menge der in Aquakultur produzierten Fischereiprodukte weltweit stark gestiegen und zählt bis heute zu den am schnellsten wachsenden Sektor in der Agrarwirtschaft. Der weltweit stagnierende Fischfang und die stetig wachsende Weltbevölkerung lassen in absehbarer Zukunft keine Veränderung dieser Entwicklung erkennen. Allerdings ist die Aquakultur mit konventionellen Verfahren, wie z.B. Netzkäfigen an den Küstenregionen oder Teichsysteme, immer wieder durch den erhöhten Nährstoffeintrag in die Umwelt oder die Übertragung von Fischkrankheiten in die Kritik geraten. Eine Alternative könnten hier geschlossene Kreislaufanlagen (KLA) sein. Sie bieten den Vorteil, durch einen geschlossenen Wasserkreislauf mit integrierter Wasseraufbereitung, die Emissionen so gering wie möglich zu halten und zusätzlich eine vollständige Kontrolle der Haltungsbedingungen und damit für einen optimalen Aufwuchs der zu kultivierenden Art zu gewährleisten. Allerdings ist die Wasseraufbereitung sehr ressourcenintensiv und kann derzeit nicht für alle systemspezifischen Probleme optimale Lösungen bieten. Durch die Wiederverwendung des Haltungswassers können sich in dem System Stoffwechselprodukte von Fischen und Mikroorganismen anreichern, die nicht durch die üblichen Wasseraufbereitungstechnologien ausreichend oder effizient genug entfernt werden und damit negative Auswirkungen auf die Fischgesundheit oder auf die Produktqualität haben. Da wäre zum einen die Entstehung und Akkumulation von unerwünschten Geschmacksstoffen, sogenannte Off-flavor Verbindungen, im Haltungswasser und deren Absorption vor allem im Fettgewebe der Fische. Die zwei bekanntesten Geschmacksstoffe Geosmin (GSM) und 2-Methylisoborneol (2-MIB) verursachen bereits in geringsten Konzentrationen (ppb) einem modrig-erdigen Geschmack im Fisch. Zum anderen besteht vor allem in Kreislaufanlagen mit hohen Besatzdichten ein erhöhtes Risiko, dass sich Infektionskrankheiten schnell verbreiten und es damit zu einem Teil- oder Totalausfall der Produktion kommen kann. Des Weiteren fallen im Haltungswasser vor allem große Mengen an organischen und anorganischen Abfallstoffen an, die mit einem hohen technischen Aufwand entfernt und entsorgt werden müssen. Für einen wirtschaftlich erfolgreichen Betrieb einer KLA ist somit die Effizienz der Wasseraufbereitung von entscheidender Bedeutung. Folglich sollte die

Optimierung der Wasseraufbereitung immer ein zentrales Ziel des Anlagenbetreibers sein, um die Produktionsrentabilität seines Kreislaufsystems sicherzustellen.

Ziel der vorliegenden Arbeit war die Evaluierung eines Ultraschallverfahrens zur Optimierung der Wasseraufbereitung in einem Kreislaufsystem in Hinblick auf Produktqualität (Kapitel 1: Entfernung der Geschmacksstoffe GSM und 2-MIB aus dem Wasser eines Kreislaufsystems durch ultraschallinduzierte Kavitation), Fischgesundheitsmanagement (Kapitel 2: Erste Vorversuche zur Desinfektionswirkung von Ultraschall gegen typische Pathogene in der Aquakultur) und Abwasserbehandlung (Kapitel 3: Entfernung von organischen und stickstoffhaltigen Verbindungen in KLA Abwasser).

Die Behandlung von Prozesswasser mit Ultraschall hat in den letzten Jahren eine hohe Aufmerksamkeit in der Wasseraufbereitungs- und in der Biotechnologie bekommen. Durch die Ultraschallwellen kommt es im Wasser zu sogenannten Kavitationen (Bildung und Auflösung von dampfgefüllten Hohlräumen). Hierbei unterscheidet man zwischen einer weichen und harten Kavitation. Insbesondere bei der harten (transienten) Kavitation entstehen durch die plötzliche Implosion im Kern der Hohlräume extreme Drücke und Temperaturen ($p > 100 \text{ atm}$, $T^{\circ}\text{C} < 5200 \text{ K}$). Die Folge sind sonochemische Effekte wie die Bildung und Freisetzung von Hydroxylradikalen durch die Spaltung von Wasser in Kombination mit einer Pyrolyse innerhalb der Gasphase. Bei der weichen Kavitation dagegen dringen gelöste Gase in die Hohlräume ein und mildern oder verhindern eine Implosion (stabile Gaskavitation). Zusätzlich werden mechanische Effekte durch Scherkräfte der entstandenen Druckwellen vor oder während der Implosion im Wasser freigesetzt. In Abhängigkeit von Parametern wie Frequenz, Intensität, Salzgehalt oder Temperatur sind diese Effekte unterschiedlich stark ausgeprägt. Bei hochfrequentem Ultraschall (HFUS) sind eher die sonochemischen Effekte und beim niederfrequentem Ultraschall (NFUS) die mechanischen Effekte, die zur Geltung kommen.

Im ersten Teil der Arbeit (Kapitel 1) wurde untersucht, ob es mit Hilfe von ultraschallinduzierter Kavitation möglich ist, die unerwünschten Geschmacksstoffe GSM und 2-MIB im Haltungswasser zu reduzieren. Dazu wurden Wasserproben aus einer Salzwasser- und Süßwasserkreislaufanlage (250 ml) mit einem GSM und 2-MIB Standard ($5 \mu\text{g/L}$ und 100 ng/L) versetzt und mit

niederfrequenten (LFUS, 20 kHz) bzw. hochfrequenten Ultraschall (HFUS, 850 kHz) 15 min lang behandelt. Zusätzlich wurden Wasserproben mit biogenem GSM und 2-MIB aus einer Kreislaufanlage für Zander (*Sander lucioperca*) behandelt, sowie der Einfluss des Salzgehaltes auf die Reduzierung der beiden Stoffe untersucht. Die Experimente konnten zeigen, dass es in allen untersuchten Proben zu signifikanten Reduzierungen der beiden Geschmacksstoffe gekommen ist. In den meisten Fällen konnte eine Verminderung von ca. 50 - 60 % bei beiden Substanzen gemessen werden. Im direkten Vergleich der eingesetzten Frequenzen war die Reduzierung bei 850 kHz stets größer als bei 20 kHz, was ein Indiz dafür sein kann, dass hauptsächlich die sonochemischen Effekte des Ultraschalls für den Abbau verantwortlich waren. Eine zusätzliche Zugabe von Salz (10 ppt) in einer mit Standard (5 µg/L) versetzten Leitungswasserprobe und einer Probe aus einer Süßwasserkreislaufanlage mit biogenen Geschmacksstoffen verbesserte die Reduktion um ca. 10%. Somit konnte allgemein gezeigt werden, dass Ultraschall in der Lage ist, die beiden Geschmacksstoffe GSM und 2-MIB in Kreislaufanlagenwasser zu reduzieren.

Im zweiten Teil (Kapitel 2) ist das Potenzial von Ultraschall zur prophylaktischen Keimreduktion des Haltungswassers untersucht worden. Hierfür wurden Wasserproben (250 ml) mit verschiedenen Modellorganismen, stellvertretend für die verschiedenen Klassen typischer pathogener bzw. parasitärer Organismen, mit Ultraschall behandelt. Es wurden insgesamt 3 Zooplanktonorganismen (*Paramecium caudatum*, *Brachionus plicatilis*, *Artemia salina*), 5 Algenarten (*Nanochloropsis salina*, *Pavlova viridis*, *Tetraselmis chui*, *Rhodomonas baltica*) und Bakterien sowohl aus einer marinen als auch aus einer Süßwasser Kreislaufanlage untersucht. Auch hier wurden verschiedene Frequenzen (850 kHz, 20 kHz) getestet. Zur besseren Vergleichbarkeit der Wirksamkeit von Ultraschall wurde eine Inaktivierungsrate in Abhängigkeit der verbrauchten Energie (kJ/L) für jeden getesteten Organismus bestimmt. Die Ergebnisse konnten zeigen, dass Ultraschall zwar bei den meisten getesteten Organismen wie Parasiten und Mikroalgen sehr wirksam die Lebendzellzahl bzw. die Photosyntheseaktivität reduzierte, jedoch keine signifikanten Effekte bei den kleinsten Mikroalgen (4 µm) und Bakterien zu beobachten waren. Während bei den Algen mit zunehmender Größe bei 850 kHz auch parallel die Photosyntheseaktivität effizienter reduziert werden konnte, ließ sich dieses beim Zooplankton nicht beobachten. Hier konnte keine Frequenz über alle getesteten Organismen signifikant

bessere Ergebnisse liefern. Die stark zerstörten Zooplankton Organismen bei der Behandlung mit NFUS weisen auf stark ausgeprägte mechanische Kräfte der Kavitationen hin. Im Gegensatz dazu, weisen die leblosen aber äußerlich intakten Körper des Zooplanktons bei der Behandlung mit HFUS daraufhin, dass eher die sonochemischen Effekte innerhalb der Organismen zu deren Tod geführt haben. Zusammenfassend kann man also festhalten, dass Ultraschall zwar sehr effizient gegen Algen und Zooplankton eingesetzt werden kann, aber als alleiniges einzelnes System, keinen ausreichenden und allumfassenden Schutz vor pathogenen Keimen in einer KLA bieten kann.

Im dritten und letzten Teil der Arbeit (Kapitel 3) wurde untersucht, inwieweit Ultraschall die Entfernung von organischen und stickstoffhaltigen Verbindungen im Prozesswasser begünstigen kann. Bei dieser Untersuchung wurden wiederum Proben aus einer marinen und Süßwasser Aquakulturanlage genommen und anschließend die Parameter Gesamtkohlenstoff (TC – Total Carbon), anorganischer Kohlenstoff (IC – Inorganic Carbon), gelöster organischer Kohlenstoff (DOC – Dissolved Organic Carbon) und Gesamtstickstoffgehalt (TN – Total Nitrogen) bestimmt. Anschließend wurden diese Proben 15 min lang bei 20 kHz bzw. 850 kHz behandelt, um dann die Wiederfindungsrate der untersuchten Stoffgruppen zu ermitteln. Bei allen Proben, die mit 20 kHz behandelt worden sind, konnten keine signifikanten Reduzierungen der untersuchten Parameter gemessen werden. Auf Grund von vorherigen wissenschaftlichen Untersuchungen, die den Abbau von anorganischen wie organischen Substanzen mittels Ultraschall nachweisen konnten, gehen wir aber davon aus, dass diese Behandlung zwar Auswirkungen auf die hier untersuchten Substanzen hatte, die Analyse der Parameter jedoch diese Veränderung nicht genügend aufzeigen konnte. Möglich wäre beispielweise eine Konvertierung langkettiger Substanzen zu kurzkettigen Substanzen, die aber unter dem gleichen Summenparameter zusammengefasst worden sind und es deshalb zu keiner messbaren Veränderung der hier untersuchten Parameter gekommen ist. Beim hochfrequenten Ultraschall von 850 kHz konnte eine signifikante Reduzierung des IC Wertes von ca. 16.6% im marinen Aquakulturwasser gemessen werden. Sowie eine abnehmende Tendenz des TC-Gehaltes. Der DOC Gehalt blieb jedoch unverändert. Wir interpretieren dieses Ergebnis so, dass insbesondere die sonochemische Effekte bei 850 kHz und die erhöhte Salinität eine Umwandlung des anorganischen Kohlenstoffs zu CO₂ zur Folge hatte,

der aber nur teilweise in die Atmosphäre und damit aus dem System abgegeben werden konnte. Des Weiteren konnte im Aquakultursüßwasser bei 850 kHz eine signifikante Reduzierung des TN Gehaltes um 14.6 % gemessen werden, jedoch nicht im marinen Aquakulturwasser, was viel Spielraum für weitere Untersuchungen lässt. Zusammenfassend betrachtet, zeigen die Ergebnisse aber deutlich, dass beide Frequenzen nicht in der Lage waren die untersuchten Parameter signifikant zu reduzieren. Somit begünstigt Ultraschall unter den getesteten Bedingungen nicht die Behandlung von Aquakulturabwasser.

Die Ergebnisse der vorliegenden Arbeit konnten deutlich die Wirksamkeit aber auch die Grenzen des Ultraschallverfahrens aufzeigen. Grundsätzlich ist Ultraschall in der Lage die beiden Geschmacksstoffe GSM und 2-MIB und Pathogene im Prozesswasser einer KLA zu reduzieren. Eine abschließende Bewertung, ob Ultraschall gegen geschmacksbeeinträchtigende Substanzen und Krankheitserreger in einer industriell geführten KLA eingesetzt werden kann, ist bei dem derzeitigen Wissensstand nur schwer zu treffen, zumal in dieser Arbeit ausschließlich mit in-vitro Tests im Labormaßstab gearbeitet worden ist. Die Frage des Upscalings des Ultraschallsystems auf einen Industriemaßstab konnte in dieser Arbeit nur ansatzweise untersucht werden, mit dem Ergebnis, dass eine einfache Skalierung des Volumens bzw. des Ultraschallsystems nicht den gewünschten Effekt brachte. Es bleibt also viel Potenzial für weitere Untersuchungen. Hierbei sollten dann auch Aspekte wie die Bildung unerwünschter Nebenprodukte durch die sonochemischen Effekte und der Energieverbrauch des Systems berücksichtigt werden.

Appendix A - Analysis of TC, DOC, IC, TN

In order to get a general overview of the composition of the RAS wastewater with regard to carbon and nitrogen compounds, the sum parameters total carbon (TC), dissolved organic carbon (DOC), inorganic carbon and total nitrogen (TN) were determined. In each case 250 ml of water samples were taken from an experimental RAS stocked with turbot (*Scophthalmus maximus*) (stocking density 19 kg/m³) with a salinity of 26‰. RAS fresh water samples were taken from an experimental RAS stocked with juvenile carp (*Cyprinus carpio*) (stocking density 3 kg/m³) at a salinity of 0.2‰. At the beginning the initial concentrations of all sum parameters were determined using a total carbon analyzer model TOC-L CPH/CPN with the High-salt sample combustion tube kit (Shimadzu Corp., Kyoto, Japan). For the analysis of the dissolved organic compounds the samples were filtered (0.45 µm) prior analysis. The determination of TC, IC and DOC were calculated using the NPOC (non-purgeable organic carbon) method, which takes into consideration that inorganic carbons are significantly higher dissolved than organic carbons. The acidification was carried out with hydrochloric acid (1.5%). TN was analyzed with the same analyzer and simultaneously determined with organic carbons. In all experiments the samples were then treated with high-frequency (HFUS - 850 kHz) or low-frequency ultrasound (LFUS - 20 kHz) for 15 minutes. The HFUS (850 kHz) was generated with a MeinhardtE/805/T/solo ultrasonic transducer (75 mm), which was energized by a Meinhardt ultrasonic power generator K8 (200 W) in pulse mode (on/off ratio 1:5). The LFUS (20 kHz) was performed with a titanium horn homogenizer (13 mm) with an ultrasonic transducer UW 3200 and a booster horn SH 219. The electrical power was applied by a Bandelin GM 3200 HF generator (200 W) in pulse mode (on/off ratio 1:5). The sample temperature was kept almost constant at about 16 °C by keeping the glass reactor in a bath of ice water. The treatment efficiency was calculated on the basis of the mean recovery rate [%] ± confidence interval (p < 0.05, n=3) of the sum parameters. The value indicates the ratio of the concentrations of the analyzed substance before and after treatment.

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마지막으로 저는 제 부모님께 특별히 감사드립니다. 당신들께서는 제 길을 인도해 주셨고 제가 오늘날에 어떤 사람인지 만들어 주셨습니다. 당신들께서 항상 저를 위해 모든 시간들을 희생과 힘으로 함께해 주셨던 것을 잊지 않겠습니다

Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.

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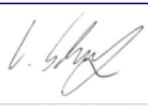
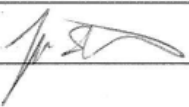
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|--|
| <p>1. Removal of the off-flavor compounds geosmin and 2-methylisoborneol from recirculating aquaculture system water by ultrasonically induced cavitation</p> |
| <p>2. Preliminary test of ultrasonically disinfection efficacy towards selected aquaculture pathogens</p> |
| <p>3. Removal of dissolved organic matter and nitrogen compounds from RAS wastewater by ultrasonically induced cavitation</p> |

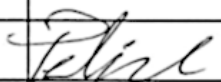
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3. Declaration on the individual phases of the scientific work (A,B,C)	Extent
<p>Concept: Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summary of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations</p>	B
<p>Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered</p>	B
<p>Execution: Involvement in the analysis or the concrete experiments/investgation</p>	C
<p>Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form</p>	C

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