CRANFIELD UNIVERSITY

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Control of Algal Growth in Reservoirs with Ultrasound

SCHOOL OF APPLIED SCIENCES

PhD THESIS

CRANFIELD UNIVERSITY

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Abstract

Algal blooms are a major worldwide water treatment concern due to their potentially harmful effects on humans and livestock. The main cause of algal bloom proliferation is eutrophication of water sources. Anthropogenic effects contribute significantly to the increased levels of nutrients within source waters. With the world's population continuing to grow water demand the likelihood is that algal blooms will become a bigger issue in the future. Algal blooms can cause release of toxins which in high concentrations are a serious health hazard for humans and animals. Cyanobacterial blooms adaptation of rapid cell growth causes higher rates of nutrients and oxygen consumption. A consequence of this bloom type is elevated rates of anoxia increasing the incidences of fish kills and rates of bacterial growth, if conditions are severe then recreational use of a water body may be suspended.

From a water treatment perspective filter blocking can occur due to presence of diatom species such as *Melosira* sp. Green species increase turbidity and chlorophyll a, and cyanobacteria produce taste and odour issues related usually to toxin release. Green algae and cyanobacteria are the two main algal divisions responsible for increased chemical demand required to treat a water supply. Algal bloom control measures presently employed range from nutrient limitation to aeration and destratification. Phosphorus and nitrogen are the two main nutrients that enhance bloom growth therefore if prevention of nutrient accumulation could be implemented this could be considered the best solution for bloom prevention, yet this has proved to be difficult. To find bloom control methods that consume little energy, reduce water treatment costs and

chemical use in water treatment is a challenge. However recent investigations into the use of ultrasound have confirmed it as being one such method. Successful use of ultrasound in numerous other applications including wastewater treatment made it an ideal candidate to test as a green solution to algal bloom control. Ultrasound travels through a liquid medium by a mechanism referred to as acoustic cavitation where the sound wave transfers through the liquid with a series of compression and rarefaction cycles. During the rarefaction phase bubble nucleation occurs which then enlarges up to a point when collapse during compression occurs generating localized pressures of up to 2000 atmospheres (atm) and temperatures of 5000 ^{o}C as the bubble implodes. This study aimed to define which species or algal morphologies were most susceptible to ultrasound and to define what frequency and energy levels would be necessary to control blooms. Results found that filamentous cyanobacteria were the most susceptible group to high frequency ultrasound (862 kHz), and energy of 3.7 kWh.m^{-3}, while at low frequency (20 kHz), using 3.7 kWh.m^{-3} filamentous diatoms were the most susceptible group to ultrasound. Unicellular species i.e. *Microcystis aeruginosa* and *Scenedesmus subspicatus* were resistant to ultrasound at all frequencies tested with >20% cell removal but damage to photosynthetic apparatus in both species produced more promising results, with up to 60% photosynthetic damage incurred. Field trials using natural mixed blooms produced interesting results where all unicellular and filamentous species were susceptible in the first 30 days of a bloom growth but beyond this time point control of either species types were not observed. Comparisons with mechanical agitation found that shear effects produced by low ultrasound frequencies were similar to that produced by mechanical agitation. It was also evident that species considered to have low removal rates at high frequency ultrasound were more susceptible to low

frequency ultrasound and mechanical agitation. These susceptible species physiology composed of rigid cell walls either fully or partly of silica, indicating a different pathway of cell damage than that incurred at high frequency. Other morphological characteristics which added to this susceptibility to ultrasound was the presence of gas vacuoles, which cyanobacteria use for buoyancy and resonate similar to cavitation bubbles produced by ultrasound at high frequency.

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ABBREVIATIONS

ATM – atmospheres

AOP – Advanced oxidative processes

AOM – Algogenic Organic Matter

AF – *Asterionella formosa*

CB – Cyanobacteria

CCAP – Culture Collection for Algae and Protozoa

COCODAFF – Counter Current Dissolved Air Flotation and Filtration

CTAB – cetyltrimethlyammonium bromide

CV – *Chlorella vulgaris*

D - Diatoms

DAF – Dissolved Air Flotation

DDCOD – Degree of Disintegration for Chemical Oxygen Demand

DOC – Dissolved Organic Carbon

DBP – Disinfection By-product

EfOM – Effluent Organic Matter

EPA – Environmental Protection Agency

EPS – Extracellular Polymeric Substances

G – Green algae

GAC – Granular Activated Carbon

HAF – Humic Acid Fraction

HPI – Hydrophilic

HPO – Hydrophobic

IC – inorganic carbon

i.e.p. – isoelectric point

kHz – Kilohertz

L – Litres

ML – Mega Litres

MA – *Microcystis aeruginosa*

MCLR – Microcystin-LR

MIB – Methylisoborneol

MPa – Mega pascals

Msp. – *Melosira* sp.

MW – Molecular Weight

NOM – Natural Organic Matter

NTU – Nephelometric Turbidity Units

PAC – Powder Activated Carbon

PolyDADMAC – polydiallyldimethylammonium chloride

PPM – Parts Per Million

PVSA – Poly (vinylsulphonic acid) sodium salt

Rcf – Relative centrifugal force

Rpm – Revolutions per minute

SCOD – soluble chemical oxygen demand

SMP – Soluble Microbial Products

SUVA – Specific Ultraviolet Absorbance

TC – Total Carbon

THM - Trihalomethane

TOC – Total Organic Carbon

TPI - Transphilic

UK – United Kingdom

W –Watts

WHO – World Health Organisation

WTW – Water Treatment Works

ZP – zeta potential

Chapter 1: Introduction

Chapter 1 Introduction

1.1 Background

Algal are natural photosynthetic organisms that are ubiquitous in their environmental adaptability, as they are found in freshwater, marine water, snow, ice, hot spring runoffs, soil, even within cracks in rocks. Algae are an integral part of an ecosystem within a lake or reservoir system recycling nutrients and in the case of some mixotrophic species consuming bacteria and removing carbon from the water column (Burkholder *et al.*, 2008). Algal species have an extensive variety of shapes and sizes of cells, from unicellular to multicellular and colonial types, and from the smaller protozoan cyanobacteria (blue-green) to the more evolved forms of eukaryotes characterised by the diatoms and green algae (Pearson, 1995; van den Hoek , C. *et al.*, 1995b). An Algal bloom is a natural phenomenon that occurs in eutrophic conditions. Eutrophication is defined as when nutrient accumulation occurs in a water source (Wetzel, 2001). Nitrogen and phosphorus are the main nutrients required for blooming events of cyanobacteria. Silica is additionally required for diatom blooms (Reynolds, 1984; van den Hoek *et al.*, 1995; Lampert and Sommer, 1997). Blooms are a seasonal occurrence with the diatoms out-competing other algal divisions to thrive in late winter early spring at low temperatures (Round *et al.*, 1990; van den Hoek *et al.*, 1995).

Cyanobacteria bloom in late spring early summer as nutrient levels increase with temperature, and they have the added adaptation of prolific speed of cell division ensuring complete dominance once nutrients are available. Green algae are a more resilient species to nutrient deprivation and dropping temperature and so bloom after cyanobacteria in late summer and autumn (van den Hoek, C. *et al.*, 1995a; Henderson *et al.*, 2008a).

Eutrophication of water sources has escalated in recent years due to anthropogenic effects, increasing the levels of phosphorus and nitrogen entering water sources (Ward and Wetzel, 1980; Burrini *et al.*, 2000). Nitrogen is the main source for amino acid synthesis in algae. Wastewater effluents and agriculture are some of the sources of nitrogen and phosphorus, with nitrate also being caused by combustion processes from industry (Knappe *et al.*, 2004). Phosphorus can also enter water bodies through oxygenated sediments, atmospheric precipitation, surface runoff and snow melt (Gburek and Sharpley, 1998; Gburek *et al.*, 2002).

The problems caused by algal blooms in water treatment vary depending on the algal division for example diatoms cause filters to clog requiring backwashing due to their silica cell wall, colony sizes of up to 4,000 µm and elaborate shapes (Bauer *et al.*, 1998; van den Hoek *et al.*, 1995). Green algae cause increased levels of turbidity and chlorophyll a, requiring higher coagulant demand. Cyanobacteria release AOM into their environment during the growth cycle, increasing turbidity, chlorophyll a and requiring additional chemical demand to remove (Henderson *et al.*, 2008b). The cyanobacterial algal division also have toxin (cyanotoxins) producing species, and can taste and odour causing components (Izaguirre *et al.*, 1982). Cyanotoxins are found in

many freshwater cyanobacteria and are generally protein based (cyclic peptides). Their types range from heptotoxins to neurotoxins, dermatoxins and cytotoxins, exposing humans and animals to possible liver damage, muscular paralysis, chromosome loss and allergy reactions of the skin and mucous membranes (Bernhardt, 1984; Hutson, 1987; Carmichael, 1992; Falconer, 1994; Knappe *et al.*, 2004).

Examples of cyanobacteria include *Microcystis* genus produces species with microcystins, a heptotoxin, and *Anabaena* and *Aphanizomenon* contain anatoxins which are neurotoxins (Newcombe, 2002; Knappe *et al.*, 2004). Cyanotoxins are normally present when taste and odour issues occur (Carmichael, 2001). *Aphanizomenon* and *Anabaena* release geosmin which is a compound characterised as an earthy/corn/musty smell (Robarts and Zohary, 1987; Rashash *et al.*, 1995; Crozes *et al.*, 1997; Saadoun *et al.*, 2001; Knappe *et al.*, 2004). Treatment strategies for toxin removal include, activated carbon granular activated carbon (GAC) and powder activated carbon (PAC), ozonation, nanofiltration and combination treatments, (Newcombe, 2002; Knappe *et al.*, 2004).

Algal treatment strategies used in water treatment works WTW in the U.S. include algicides such as copper sulphate and potassium permanganate before re-growth occurs. In general, including the U.K. water companies algal treatment strategies range from covering of basins and filters, PAC, ozonation, coagulation/flocculation, bubble curtains, pulsed sludge blanket clarification, aeration, pre-oxidation using chlorine, dissolved air flotation (DAF), ozoflotation, catalytic processes, advanced oxidation processes (AOP), barley straw and ultrasonication (James, 1992a; James, 1992b; James, 1994; French, 2001; Haarhoff and Edzwald, 2004; Kommineni *et al.*, 2009).

The three best treatment strategies for algal bloom control and mitigating algal growth from 76 utilities surveyed by the EPA in the U.S. in 2009 found that cleaning of basins and mechanical equipment prone to assist algal growth was the best method, secondly chlorination for disinfection and use as an occasional shock treatment, and finally addition of algicides in-situ in reservoirs and within works (Kommineni *et al.*, 2009). Ultrasound use was classified as a non-chemical strategy to control algal growth when documented by a large metropolitan utility company in the U.S. Midwest. Ultrasound transducers were submerged in the settling basins and significantly reduced algal growth on the walls of the basin, and was considered the most successful strategy used at this WTW in 2005, however when ultrasound was applied to the flocculation basins in 2006, no effect on algal control was observed (Kommineni *et al.*, 2009).

Ultrasound works by the phenomenon of acoustic cavitation which occurs after sound waves above the frequency of 20 kHz are passed through a liquid medium. Ultrasound is transmitted via waves which alternately compress and stretch within the liquid medium through which it passes. During each "stretching" phase (rarefaction), provided that the negative pressure is strong enough to overcome intermolecular binding forces and surface tension, tiny cavities (microbubbles) of water vapour are produced. In succeeding cycles these cavities can grow and then collapse violently with the release of large amounts of energy. It has been estimated that temperatures and pressures of the order of five thousand Kelvin and a two thousand atmospheres are produced during this collapse (Suslick, 1988; Suslick, 1990; Mason, 2000). Ultrasonic applications for water treatment include cell disruption, sterilization, dispersion of solids, extraction of plants,

anaerobic digestion, water remediation, and sewage (Shoh, 1988; Suslick, 1988; Shon *et al.*, 2004; Mason, 2007).

Ultrasound has been used within works to aid anaerobic digestion by removing aggregates of bacterial cells (Neis *et al.*, 2001). In another wastewater study reduction in floc sizes from 40 µm to 1 µm, and increasing the soluble chemical oxygen demand (SCOD) were achieved after sonication (Phull *et al.*, 1997; Neis *et al.*, 2001; Kampas *et al.*, 2007; Zhang *et al.*, 2007). A patented ultrasound unit 'Sonoxide' was used within works as a flow through cell, producing bacterial kill rates of 85% (*Bacillus subtillus*), and reduced re-growth of *Scenedesmus capricornutum* by 60% (Mason *et al.*, 2003). Several field studies have used floating transducers working at low frequencies of 28 kHz and had a statistically significant reduction in chlorophyll a (Inman, 2004). Preferential reduction of cyanobacterial species over diatoms was documented in another field study also using low frequency ultrasound 22 kHz and with much higher power of 630 W (Ahn *et al.*, 2007a; Ahn *et al.*, 2007b). Improved water quality occurred in Lake Senba (Mito City, Japan) with increased transparency, reduced chlorophyll a from 200 to 50 $mg.m^{-3}$ and suspended solids from 100 to 20 $g.m^{-3}$ using a flow through unit at a frequency of 400 kHz and 200 W (Lee *et al.*, 2002).

This project aims were to firstly define the algal species that were susceptible to ultrasound, secondly to ascertain what ultrasound frequency was most effective on those species and finally what the minimum energy input was required to control or remove the highest number of algal cells. Laboratory and field studies were carried out to address the project aims. Results found that ultrasound works most effectively at removing species of filamentous algae. Filamentous algal species were damaged more

significantly by low frequency compared to high frequency ultrasound. To explain, *Melosira* sp. a filamentous diatom species had its best cell removal rates of 83% at 20 kHz however at high frequencies of 582, 862 and 1144 kHz, cell removal rates decreased linearly to 50, 11 and -6% respectively. The violent implosion of cavitation bubbles at low frequency compared to high frequency enhances the effectiveness on filamentous diatoms and green algae which have rigid cell walls and large surface area allowing higher rate of interaction between imploding cavitation bubbles and algal cells. Previous ultrasound studies have not included diatoms species even though they and specifically *Melosira* sp. is considered a problematic species for UK Water companies including Thames Water (Bauer *et al.,* 1998).

Cyanobacteria have the contrary response to ultrasound frequency where cell removal increases with frequency. To elaborate *Aphanizomenon fq.,* a highly susceptible filamentous cyanobacterial species with gas vacuoles tested at 862 kHz had cell removal rates of 93%. Two other filamentous cyanobacteria also produced high rates of cell removal with *Anabaena* (wild) 88% and 51% for *Anabaena fq*. In the case of these species the presence of a gas vacuole is critical to the ability of ultrasound to cause severe damage which leads to significantly high cell removal rates. Previous ultrasound studies documented that the collapse of gas vacuoles in cyanobacterial species was caused by ultrasound, (Lee *et al.,* 2002; Ahn *et al.,* 2003). Ultrasound effects these algal species by inducing the bursting of the gas vacuoles due to the size of the vacuoles being in the same order of magnitude as the ultrasound resonance bubbles produced at high frequency. The consequence is that due to the gas vacuoles resonating similarly to the bubbles produced by cavitation at this high frequency a higher rate of gas vacuole

collapse occurs, hence contributing to higher rates of cell removal in this type of algal physiology (Zhang *et al.,* 2006b). The minimum electrical energy input required was to remove the most susceptible species was 3.7 $kWh.m^{-3}$. This energy level makes ultrasound as a sole algal bloom solution not economically feasible as it far exceeds standard energy demand for water treatment and supply at between 0.5-1.0 $kWh.m^{-3}$. However these findings still contribute and extend the number of species which have now been documented to be susceptible to ultrasound treatment. If ultrasound can be used in conjunction with other solutions i.e. chlorine or ozone reducing the chemical volume needed it could still be useful to contribute to greener water treatment solutions to algal bloom control/removal.

1.2 Objectives

It is hypothesised that through a better understanding of the mechanisms by which ultrasound works on algae and the parameters which affect ultrasound, that this technology could be applied as a reservoir scale solution to algal blooms. The aim of this thesis is therefore to identify how ultrasound works with regard to killing algal cells, and identifying which species or morphological types are most susceptible and define the least amount of energy required to kill that maximum number of algal cells. Accordingly, a series of objectives were identified:

1. To assess current knowledge on how ultrasound works on removing bacteria and algae.
2. To characterise the algogenic organic matter (AOM) produced by green algae, diatoms and cyanobacteria during exponential growth phase.
3. To identify algal species that will prove to be susceptible to ultrasound for use in subsequent investigations.
4. To determine whether algal physiology has a role to play in causing susceptibility to ultrasound.
5. To decipher whether ultrasound frequency or power have more effect on algal cell removal.

6. To identify whether ultrasound units in field trials can produce reliable cell removal results, and what parameters influence the ability of ultrasound to successfully treat a bloom at this scale.

1.3 Thesis structure

This thesis takes the form of a series of chapters formatted as papers for publication. The chapter titles are also the papers titles and along with each journal the papers have been submitted to and are listed in Table 1.1. All papers were written by the first author, Diane Purcell and edited by Dr Bruce Jefferson (supervisor). All experimental works was carried out by Diane Purcell except for Chapter 3. Industrial experiences of ultrasound and barley straw, catalogued data from industrial sponsors, Anglian Water (Adam Ellingham, Ella Lamming, Craig Tarft, Barrie Holden, Torril Bigg), Thames Water (Anna Wallen), United Utilities, (Andrew Campbell and Stuart Holden), and Chapter 4 Characterisation of Algogenic Organic Matter (AOM), the experimental analysis included dissolved organic carbon, (DOC), zeta potential (ZP), hydrophobicity, protein and carbohydrate content, molecular weight, specific UV absorbance (SUVA) and charge density were carried out by (Celine Seigle) Placement student, and supervised by Diane Purcell.

Chapter 2 Paper 1 outlines the literature to date on the use of ultrasound to control bacterial and algal species. An integral part of this study was to understand how

ultrasound interacts with biological organisms and their subsequent responses. It was important to define the mechanisms by which ultrasound works and to catalogue the effects on different types of organisms from prokaryotes (bacteria and cyanobacteria) to eukaryotes (green algae and diatoms). *Ultrasound affects on Micro-organisms, a literature review*, by D. Purcell, B. Jefferson, S.A Parsons, E. Joyce, T. Mason. (to be submitted: Environmental Technology).

Chapter 3 Paper 2 - Examines the state of play in industry regarding novel source control solutions such as ultrasound and barley straw. These studies on ultrasound and barley straw were at field scale, using full size reservoirs, to identify whether these solutions can control algal blooms? and if so do so in an economically sustainable rate to be a viable bloom control method. *Experiences of algal bloom control using green solutions barley straw and ultrasound, an industry perspective, D. Purcell, M.* Chipps, Holden, B., T. Bigg., A. Ellingham, E. Lamming, C. Tarft, A. Wallen, A. Wetherill, S.A. Parsons and B. Jefferson. (to be submitted: J. CIWEM).

Chapter 4 Paper 3 – Addresses the consequences of algal blooms within Water treatment works (WTW), where organic matter and particulates expelled from the cells or released from the cells can cause serious removal problems. To rectify this problem, chemical characteristics of these particulates defined what type of solution can be used to remove them from the water. Charge plays a very important role in this process the more charge present the easier it is to treat. *Characterisation of Algogenic Organic Matter (AOM) for Scenedesmus subspicatus, Cyclotella sp., Aphanizomenon flos-aquae*

and Anabaena flos-aquae, D. Purcell, B. Jefferson, S.A. Parson. (submitted to: Environmental Pollution).

Chapter 5 Paper 4 – Outlines the results of treating three divisions of algae using ultrasound frequencies from 20 kHz to 1144 kHz increasing time from 5-500 seconds with a 200 W ultrasound unit. This chapter aims to catalogue the response of species to different frequencies as time increases to decipher what was the most susceptible algal morphological type or species. *The influence of frequency, power input and algal character on the efficacy of algal growth control using ultrasonics* D. Purcell, B. Jefferson, S. A. Parsons. (submitted to: Water Research).

Chapter 6 Paper 5 – Compares the use of ultrasound as a control method to that of mechanical agitation. Susceptible species are defined as filamentous based on results from Chapter 5. Therefore only filamentous species of green algae, diatoms and cyanobacteria were treated with ultrasound and then compared with mechanical agitation to determine how much damage was inflicted normalised to energy input. *The effect of Ultrasound compared to Mechanical Agitation in Susceptible Filamentous species of cyanobacteria, green algae and diatoms*, D. Purcell, B. Jefferson, S. A. Parsons. (to be submitted: Environmental Science and Technology).

Chapter 7 Paper 6 – Investigated the effect that a floating ultrasound transducer had in outdoor tanks volume of (1500L) on two natural field blooms supplied by Thames Water. These blooms were composed of mixed species of green algae and diatoms. The

first bloom was dominated by unicellular green algae, and the second bloom dominated by filamentous green algae. This field set-up aimed on answering two questions, firstly can this type of unit kill algal blooms? Secondly, if so does ultrasound only work on specific morphological types, i.e. filamentous algae? *Effect of sonication on two natural field of predominantly unicellular and filamentous green blooms,* D. Purcell, B. Jefferson, S. A. Parsons. (submitted to: Ultrasonics Sonochemistry).

Chapter 8 – *Discussion,* Addresses the two most important questions from this study, does ultrasound work as a control method for algal blooms? and Is it a financially viable option for water companies in the U.K.?

Chapter 9 - *Conclusions and Further Work,* Lists the key results of the study and makes recommendations on how future investigations can expand current knowledge of ultrasound applications at reservoir scale and within WTW.

Table 1.1 Thesis Structure

Chapter	Objective/s	Focus	Journal	Status
2	1	Literature on Micro-organisms and Ultrasound	*Environmental Technology*	to be submitted
3	1,2,3,4,5,6	Industrial experiences of Ultrasound and Barley Straw	*J CIWEM*	to be submitted
4	2	Characterisation of AOM from Algal species	*Water Research*	submitted
5	3, 4	Treating Algal species with Multi-frequency Ultrasound	*Water Research*	under correction
6	4,5	Comparison of Ultrasound and Mechanical agitation on treating algal species	*Environmental Science and Technology*	to be submitted
7	6	Effect of ultrasound at field scale on natural blooms	*Ultrasonics sonochemistry*	submit elsewhere

1.4 Conference Papers

Purcell D., Parsons S.A. and Jefferson B (2008). Singing algae to sleep: Understanding the use of Ultrasound for Algal Control, Proceedings from the WQTC, Cincinnati, Ohio, U.S.A. 16-20th November 2008.

Purcell D., Parsons S.A. and Jefferson B (2008) Removal of harmful algal bloom species *Microcystis aeruginosa* using ultrasound. Proceedings from the IWA 9th National Young Professionals Conference, Newcastle, U.K. 16-18th April 2008.

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Chapter 2: Ultrasound effects on Micro-organisms, a literature review

Chapter 2: Ultrasound effects on Micro-organisms a literature review

2.1 Abstract

Ultrasound is a well established technology but only recently has it use been applied to controlling algal blooms. Ultrasound works by a phenomenon known as acoustic cavitation which occurs when sound waves of over 20 kHz travel through a liquid medium. Ultrasound is transmitted via waves which alternately go through rarefaction and compression cycles in the liquid through which it passes. During this process small cavities (microbubbles) are created. On the violent implosion of these microbubbles energy is release and up to 5000 ^{o}C and 2000 atm are also produced. Ultrasound causes damage to cell walls and membranes when these microbubbles collapse within the vicinity of the cells. This type of damage applies to bacterial and algal cells. Bacterial cells have been successfully removed by up to 100% using ultrasound. Implementation of ultrasound technology to remove bacterial cells from plant-scale wastewater systems has already been accomplished. The most efficient system for bacterial cell removal uses high frequency (1 MHz) to break up cell aggregates and then low frequency (20 kHz) to kill the cells. Algal cell physiology has specific organelles which make it more

susceptible to ultrasound; these organelles are gas vacuoles used for buoyancy in cyanobacteria. When ultrasound was applied gas vacuoles burst and photosynthetic apparatus was damaged. Cyanobacterial species *Microcystis aeruginosa* had the highest cell removal rate of 65%. Green algae had breakage of cell colonies after treatment with low frequency ultrasound. A comparative study using a plant implemented flow-through ultrasound unit on bacterial species (*Bacillus subtillus*) and green algal species (*Scenedesmus capricornutum*) revealed a higher kill rate for bacterial 85% compared to only 60% for the green alga. At present the knowledge on bacterial responses to ultrasound is extensive but in comparison algal studies have been concentrated on one division the cyanobacteria, and specifically one species *Microcystis aeruginosa.*

2.2 Introduction

2.2.1 What is Ultrasound?

Ultrasound refers to sound energy which typically occurs at a frequency of 20 kHz-100 kHz but extends up to 2MHz (Mason, 2000). The uses of ultrasound are extensive, ranging from plastic welding and cleaning to therapeutic medicine and processing in the form of degassing liquids, emulsification and floc break-down. In addition, very high frequency ultrasound known as diagnostic ultrasound occurs at frequencies beyond 10 MHz leading to further applications including pulse echo, sound velocity and sound attenuation measurement (Suslick, 1988; Mason, 2000). For context, most metal cleaning and large-scale sonochemistry applications such as wastewater treatment occur

between 20-100 kHz (Figure 2.1) (Mason and Lorimer, 1988). In comparison human hearing ranges from 16-20 kHz, but animals and specifically bats have the highest hearing frequency of up to 70 kHz (Mason and Lorimer, 1988). Ultrasonic applications for water treatment include sewage treatment, dispersion of solids, anaerobic digestion, water remediation, cell disruption, sterilization and extraction of plants, (Shoh, 1988; Suslick, 1988; Shon *et al.*, 2004; Mason, 2007).

Ultrasound travels through a liquid medium by a mechanism referred to as acoustic cavitation where the sound wave transfers through the liquid with a series of rarefaction and compressions. During the pull phase bubble nucleation occurs which subsequently enlarges up to a point when collapse occurs generating localised pressures of up to 2000 atmospheres (atm) and temperatures of 5000 ^{o}C (Figure 2.2). The impact of these localised conditions occurring in the vicinity of cells causes damage to cell walls and membranes (Mason *et al.*, 2003; Duckhouse *et al.*, 2004). A secondary effect of ultrasound is the production of free radicals. These are formed when water molecules are broken down producing HO•, H•, O_2^-• ions free in solution which act as an effective oxidising agent (Suslick, 1988; Mason, 2000). The characteristics of ultrasound are frequency dependent such that the distance the wave travels is extended as the frequency decreases. To elucidate, the distance required to halve the energy of the wave is 30 km at 20 kHz compared to 1 km at 118 kHz; explaining why sonar communication in submersibles is carried at low frequencies (Mason, 2000). In addition, high power levels can have a negative effect on cavitation as the produced bubbles remain stable for longer and so create a barrier to the flow of acoustic energy.

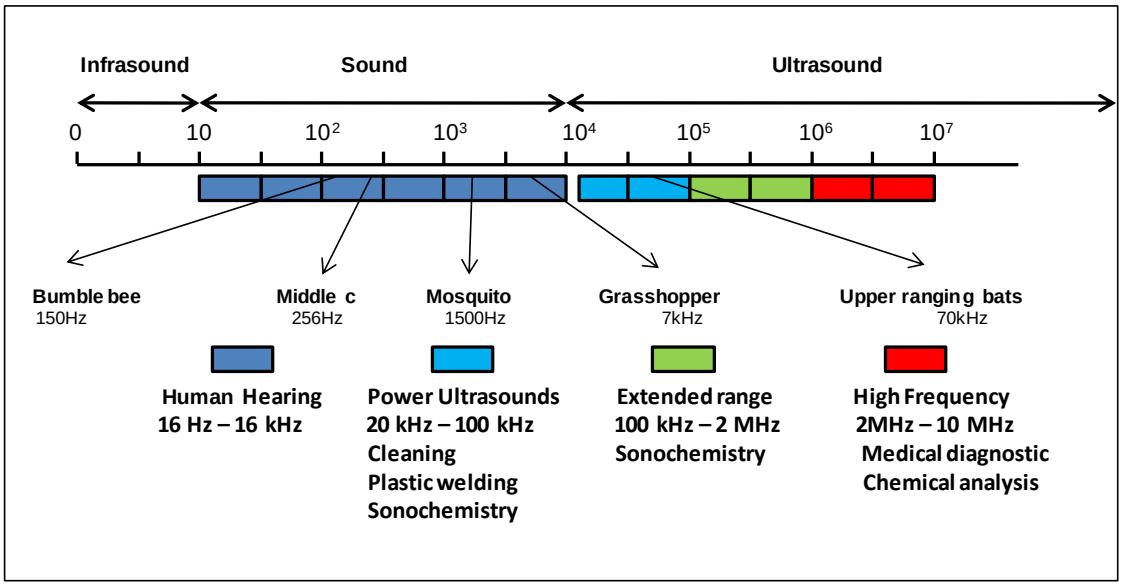

Figure 2.1 Sound frequencies ranging from infrasound to ultrasound adapted from Mason and Lorimer, 1988**.**

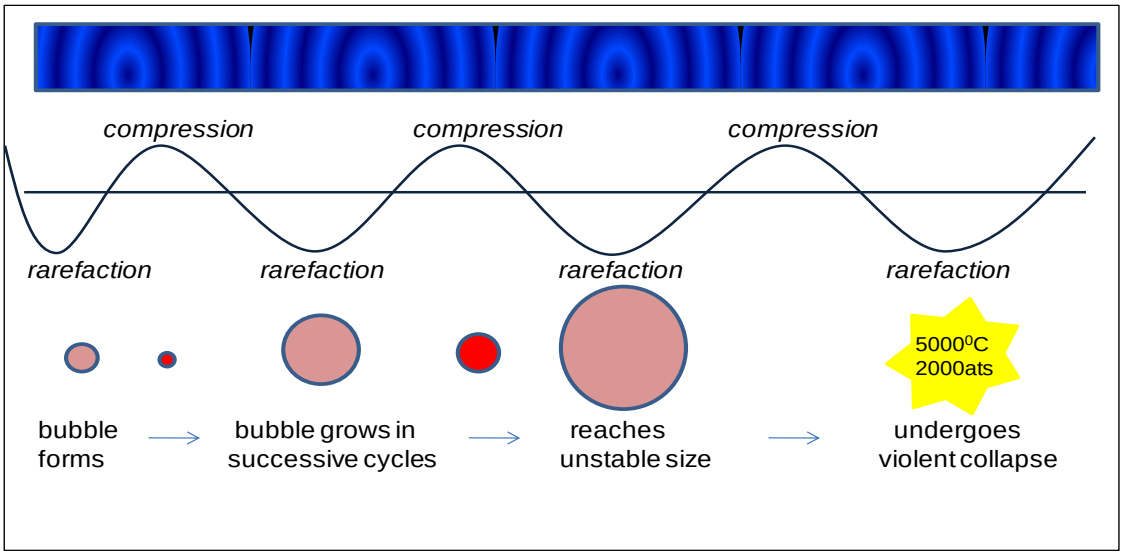

Figure 2.2 The development and collapse of cavitation bubbles adapted from Mason, 2000**.**

Ultrasound has been used very effectively in wastewater treatment. For example within WTW ultrasound aided anaerobic digestion by removing aggregates of bacterial cells (Neis *et al.*, 2001). Other effective studies include a reduction in floc sizes from 40 µm to 1 µm, and increasing the soluble chemical oxygen demand (SCOD) were achieved after sonication (Phull *et al*., 1997; Neis *et al*., 2001; Kampas *et al.*, 2007; Zhang *et al*., 2007). Ultrasound flow-through units are designed to use the least amount of energy by having the ultrasound transducers as close as possible to the culture being sonicated. This is achieved by using a pipe through which the culture is pumped and is then sonicated within the pipe by two ultrasound transducers positioned on both sides of the pipe. One such extremely successful flow through system is the patented ultrasound unit 'Sonoxide' was used within works as a flow through cell, producing bacterial kill rates of 85% (Bacillus subtillus), and reduced re-growth of Scenedesmus capricornutum by 60% (Mason *et al*., 2003). One field study that used 10 flow through units composed of 2X(200 kHz and 100 W) units attached to a pipe with culture pumped through it, Results from the field study saw improved water quality occurred in Lake Senba (Mito City, Japan) with increased transparency, reduced chlorophyll a from 200 to 50 $mg.m^{-3}$ and suspended solids from 100 to 20 $g.m^{-3}$ using a (Lee *et al*., 2002). Field studies have used floating transducers which work at low frequencies such as 28 kHz and caused a statistically significant reduction in chlorophyll a (Inman, 2004). Other effective results as field scale found that a reduction of cyanobacterial species over diatoms using 22 kHz and with much higher power of 630 W (Ahn *et al.*, 2007a; Ahn *et al*., 2007b).

The objective of this literature review was to document what frequencies and power levels of ultrasound have been tested at laboratory and field scale on bacteria and algae and whether morphology had any consequence on that ability of ultrasound to work effectively. It was also intended to decipher whether any trends on the best frequency or power levels was best suited to controlling bacteria and or algal species or morphologies were present. Certain studies had alluded to the theory that the thicker the cell wall of a bacterium was the better it's resistance to ultrasound treatment would be therefore this was investigated. Bacteria have been well studied at laboratory level yet ultrasound studies on algal species and morphologies have been generally limited to a one major problematic cyanobacterial species *Microcystis aeruginosa*.

2.3 Classification of Microorganisms

2.3.1 Bacteria

Bacteria are classified as Prokaryotes and are approximately 3.5 billion years old and inhabit every environment on the planet and are considered the most diverse group on earth (Woese and Gogarten, 1999). Bacteria are unicellular, have no membrane bound nucleus or organelles and have a cell wall which is filled with cytoplasm, ribosomes (protein), plasmids and a nucleoid which contains the DNA of the bacteria. The cell wall is covered in pili (little hairs) and a bacterial flagellum for motility. Bacteria have several different shapes and differ from eukaryotes in size as they are smaller varying from 0.2 µm to 700 µm. The cell wall is made up of peptidoglycan (also called murein) polysaccharide chains cross-linked by peptides containing D-amino acids (van

Heijenoort, 2001). Bacterial cell walls have different composition to the cell walls of plants and fungi which are made of cellulose and chitin, respectively.

2.3.2 Algae

Algae are an extensive and diverse group of simple typically autotrophic organisms including unicellular and multicellular forms. The main groups of true algae include the green algae, the diatoms, the red algae, brown algae and the flagellate algae (van den Hoek , C. *et al.*, 1995b). Algae, by modern definition, are eukaryotes with size ranging from an order magnitude higher than prokaryotes at between 5-20 µm and carry out photosynthesis within membrane-bound organelles called chloroplasts whose structure vary across different types of algae reflecting different endosymbiotic events. Chloroplasts contain circular DNA and are similar in structure to cyanobacteria, presumably representing reduced cyanobacterial endosymbionts. The different DNA structure indicates a different evolutionary source, hence not the same pathway as that of eukaryotic algae who developed their chloroplasts which are completely integrated part their genome unlike the chloroplasts DNA of the cyanobacteria.

Cyanobacteria

Cyanobacteria or blue-green algae are classified as prokaryotes like bacteria. They lack a membrane bound nucleus or internal organelles and include a cell wall made up of peptidoglycan which is usually embedded in a mucilaginous sheath (van den Hoek *et al.*, 1995a). Cyanobacteria include unicellular, colonial and filamentous forms with

movement achieved through use of gas vesicles which act as buoyancy aids facilitating floating and sinking in the water column. Cyanobacteria are commonly considered in parallel to true algae as they produce their own food by photosynthesis. Photosynthesis is achieved by using the activity of photosystem (PS) II and I (Z-scheme) within thylakoids which contain the photosynthetic pigment, chlorophyll a and found equally spaced in the cytoplasm.

Green algae

Green algae are true algae and eukaryotes and so have a membrane bound nucleus and organelles. Green algae have primary chloroplasts surrounded by two membrane lamellas made of 2-6 stacked thylakoids. The chloroplasts contain chlorophyll *a* and *b* and several other accessory pigments xantophylls, lutein, zeaxanthin, violaxanthin, antheraxanthin and neaxanthin (van den Hoek, *et al.*, 1995a). The cell wall is made up of fibrillar and amorphous matrix components which are embedded in a matrix that forms a layer against the plasmalamella. The amorphous part is on the outer side of the wall forming a slimy outer layer but the fibrillar layer does extent out into this. Cell walls of some green species also contain silica. Small vacuoles and lipid droplets are included in the organelles of the green algae.

Diatoms

Diatoms are eukaryotes and are found in the Class *Heterokontophyta* and the Order *Bacillariophyceae*. They are unicellular or colonial with an excessive variety of shapes from the wheel spoke design of *Asterionella* to the tubular filamentous *Melosira*. Cell sizes of diatoms exceed that of most other algal species with colonies reaching several 100 µm in length. Diatoms have a distinct morphological characteristic in their cell wall which is formed of a siliceous box structure with over lapping sides known as a frustule. The silica is polymerised silicic acid and is amorphous with no crystalline structure. There are several different types of diatoms, pennate have a bilaterally symmetrical cell wall and centric which have a radially symmetrical cell wall. Pigments in diatoms include chlorophylls *a* and *c*, and phycobilins. Chloroplasts are golden brown in colour due to the masking by fucoxanthin the accessory pigment, they also contain xanthophyll pigment (van den Hoek *et al.*, 1995b).

2.4 Eutrophication

Eutrophication is the accumulation of increased nutrients within a water source which can occur for numerous reasons from many sources including anthropogenic effects. Eutrophication in lakes and reservoirs and the subsequent formation of algal blooms are a worldwide problem. Blooms are usually caused by several dominant algal species with cell concentrations >20,000 ml^{-1} (Bauer *et al.*, 1998; Albay and Akcaalan, 2003; Hoeger *et al.*, 2004). Spring and late summer/autumn blooms tend to be predominately

diatoms, in the phylum Heterokontophyta, and green algae in the phylum, Chlorophyta. Destructive blooming events by cyanobacteria are enhanced by their resistance to grazing (Lynch and Shapiro, 1981), buoyancy regulation (Ganf and Oliver, 1982; van Rijn and Shilo, 1985), and significant accumulation of nutrients (Allen, 1984) such that summer blooms in eutrophic lakes are usually composed of cyanobacteria as they out compete all other species (van den Hoek *et al.*, 1995a).

The abundance of blooms leads to increased water treatment costs, a degradation of recreational value (Falconer, 1994), taste/odour issues (Izaguirre *et al.*, 1982) and possible toxin accumulation (Carmichael, 1992). Reported specific algae related treatment issues include filter blocking by diatoms, increased coagulant demand from green and cyanobacteria (Johnson *et al.*, 1977; Bernhardt, 1984; Hutson, 1987; Bauer *et al.*, 1998). A range of methods have already been developed and applied to deal with consequences of algal blooms including nutrient diversion, artificial destratification, hypolimnetic aeration/withdrawal, sediment oxidation/removal, phosphorus precipitation, and biomanipulation (Wetzel, 2001), yet blooms continue to occur. Ultimately, this drives a need for alternative methods of algal control of which ultrasound is currently being considered.

2.5 Effects of Ultrasound from Bacteria

Ultrasound is used at two frequencies to treat bacteria effectively: high frequency (1 MHz) to break-up the cell aggregates and once liberated low frequency (20 kHz) is

applied to kill monoculture bacterial cells (Mason *et al.*, 2003; Duckhouse *et al.*, 2004). To explain, the treatment of *Bacillus subtillus* showed only a slight drop in colony forming units (CFU) when low frequency (20-38 kHz) was applied but a sharp increase of CFU'S was observed when applying high frequencies 512-850 kHz which was shown to relate to de-clumping of the aggregates (Joyce *et al.*, 2003). Similarly results from mixed bacterial species observed bacteria in raw wastewater had increased in cell numbers due to de-clumping of cell aggregates when treated with 20 kHz of ultrasound and energy input increasing to <80 $kJ.L^{-1}$ (Foladori *et al.*, 2007). Size measurements have supported this with a reduction from 100 µm to 5 µm seen in ultrasonic treatment of waste activated sludge with a 20 kHz unit (Chu *et al.*, 2001). Similarly, the use of ultrasound to effectively de-clump wastewater aggregates at plant scale was tested at Severn Trent WTW at Finham, Coventry, where particulates of 40 µm were reduced to 1 µm after 8 minutes of sonication (Phull *et al.*, 1997). Other reported changes that occur include a corresponding increase in turbidity and an increase of >70% in the (degree of disintegration for chemical oxygen demand) DDCOD when using at 41 kHz for 60 minutes (Tiehm *et al.*, 2001). The DDCOD was shown to be related to frequency through the corresponding change in bubble size formed such that a linear correlation with an R^2 of 0.99 was reported. Another study on activated sludge reported similar results using 25 kHz with a reduction in floc size of 30%, and an increase in DDCOD (>30%) and a reduction in solid mass of (24%) (Zhang *et al.*, 2007). Effective application of such effects have been reported with regards to anaerobic digestion, water remediation, cell disruption, sterilization, solids dispersion, plant extraction and sewage breakdown (Suslick, 1988; Mason, 2007). For instance, power ultrasound has been

shown to remove filamentous material responsible for sludge buoyancy leading to improvements in sludge settle-ability significantly (Neis *et al.*, 2001). Other examples include disintegration of waste activated sludge to provide the necessary carbon for biological nutrient removal and excess sludge reduction (Minervini, 2008) as well as fortification of sewage flows enabling the application of anaerobic processes (Lester *et al*., 2009). Other reported effects include temporary cell membrane permeability during ultrasonic treatment of *Pseudomonas aeruginosa* (Rapport *et al*., 1997), haemoglobin release was increased from erythrocytes (Hughes and Nyborg, 1962) and bacterial spores of *Bacillus stearothermophilus* released intracellular calcium (Palacios *et al.*, 1991). These studies clearly demonstrate the effectiveness of low frequency ultrasound for wastewater applications where the shear forces created through microstreaming of cavitation bubbles and large cavitation bubble collapse maximised the de-aggregation of the wastewater flocs.

The alternative application of ultrasound is in disinfection through the direct kill of bacteria in wastewater. Successful cell removal for the bacteria *E-coli* of a 99.9% *was* reported when exposed to 20 kHz and 140 W (Hua and Thompson, 2000). Similarly, cell removal rate of 85% for bacterial species (*B. subtillus*) using the Sonoxide flow-through system (a Undatim company patented system) has been implemented at full scale (Mason *et al.*, 2003) demonstrating its overall efficacy.

A comparative study on bacterial kill rates using 1 ppm chlorine as a benchmark to ultrasound found that chlorine alone produced 43% kill in 5 minutes and 86% kill in 20 minutes whereas an ultrasound bath of 38 kHz caused 19% kill in 5 minutes and 49% kill in 20 minutes. Combining the two resulted in 86% kill in 5 minutes and 100% in 20

minutes. Sequencing the two components revealed that better removal was observed when ultrasound was applied prior to chlorination indicating that ultrasound enhanced the effect of chlorination by increasing the cell removal rate using less chlorine (Phull *et al.*, 1997). Comparative studies on the efficiency of ultrasound on bacterial species (*B. subtillus*) and algal species (*Scenedesmus capricornutum*) have revealed higher levels of kill in the case of bacteria at 85% compared to 60% for algae (Mason *et al.*, 2003). These results highlight the need for optimisation of these techniques specifically using algal species as the bacterial data does provide directly translatable insights into how the mechanism of ultrasound works across the numerous types of bacteria.

Analysis of the reported data reveals no clear relationship between frequency and cell removal rates (Figure 2.3) although only a limited amount of data has been reported outside the 10-100 kHz range. Outside this range both increases in cell number due to de-clumping (Joyce *et al.*, 2003) and decreases (Limaye *et al.*, 1996) have been reported. For instance, after treatment using a frequency of 3 MHz 72% decrease for bacteria *E-coli* and 97% removal for the eukaryote, yeast (*S. cerevisiae*) was observed.

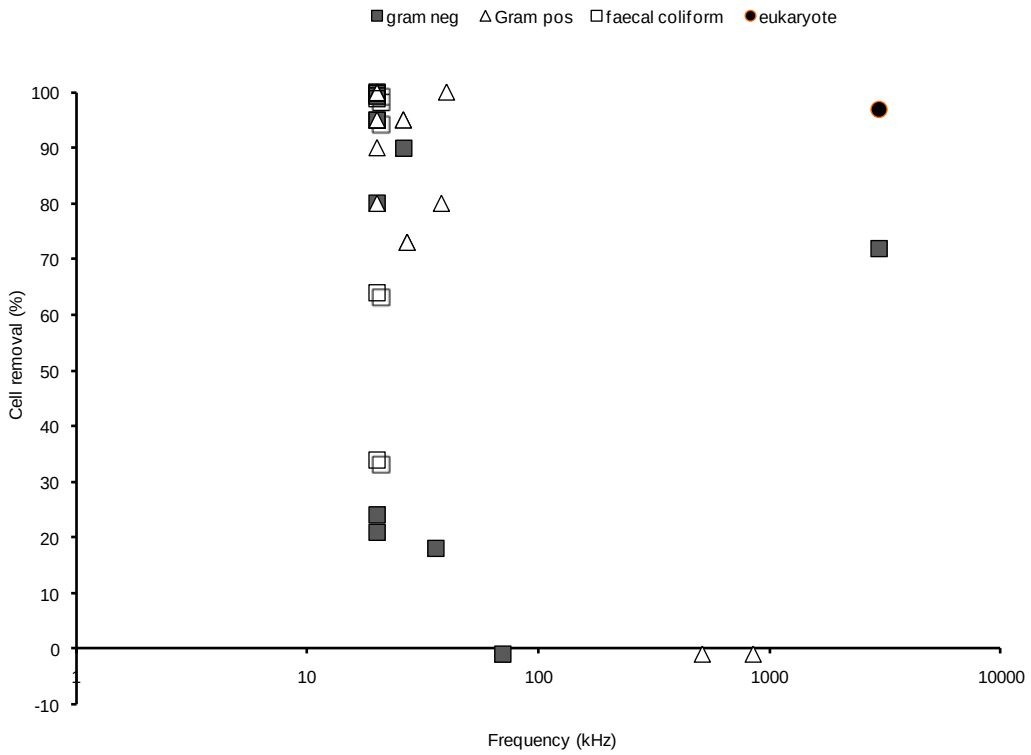

Figure 2.3 Bacterial cell removal data graphed against frequency from ultrasound studies published in the literature to date. Frequency range most tested within was 10-100kHz with varying results from 0-100% cell removal no obvious trend on an optimal frequency for best cell removal was visible.

No significant difference in resistance of gram negative over gram positive species is evident which contradicts suggested theories that as bacterial cell wall thickness increased so would resistance to ultrasound. To demonstrate, ultrasound studies on four bacterial species (*B. sub. E-coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*) at a frequency of 26 kHz found that cell removal ranged from 40-80% with no significant differences based on cell wall morphology (Figure 2.3) (Scherba *et al.*, 1991). No direct correlation could also be observed in relation to power with both high

and low removal rates observed for gram negative and gram positive bacteria across the reported power level (Figure 2.4). Results with respect to faecal coliform show consistently >80% removal but experiments at high powers have only been reported. The data set appears to separate into two groups: one reaching just under 30% kill using just over 100 W and the second group using the same power but with kill rates in excess of 70%. Both groups contain gram negative and gram positive species with a few outliers of faecal coliforms and spores (Figure 2.4) indicating no clear link between ultrasound operation and removal suggesting the nature of the suspension is the critical factor.

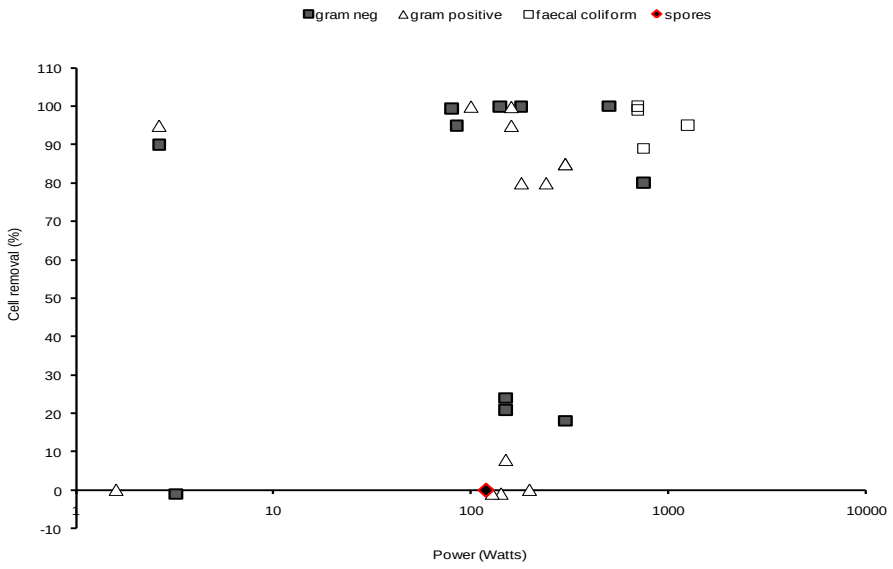

Figure 2.4 Studies from literature on the response of bacteria to ultrasound. Literature data has been graphed via increasing power level. The only trend noticeable was the higher number of studies with 10% when over 100 W of power was applied.

The literature data from bacterial species which have been exposed to ultrasound at a range of ultrasound frequencies and power levels have not found any obvious based on

frequency or power. It had been assumed that bacterial species with thicker more robust cell walls would be more resistant to the effects of ultrasound. This however was not the case. In general all studies found that gram negative and gram positive bacteria as well as bacterial spores and faecal coliforms had similar cell removal levels with a frequency range or 10-100kHz. There was a visible trend with the limited data set for bacterial responses to ultrasound with increasing power levels with higher rates of 100% as power levels were increased from 100 to 1000W.

2.6 Ultrasound and Algae

To date the majority of studies on the use of ultrasound to control algal blooms have focussed on cyanobacterial species at bench scale, with concerted interest on *Microcystis aeruginosa*. Kill rates of 90% using ultrasound frequencies between 20 kHz and 1.7 MHz are commonly reported (Tang *et al.*, 2003; Hao *et al.*, 2004a; Hao *et al.*, 2004b; Tang *et al.*, 2004). Other cyanobacterial species studied include filamentous cyanobacterial species, *Spirulina* (*Arthrospira*) *platensis*, which had a decrease of 60% in biomass concentration after exposure to high frequency ultrasound of 1700 kHz (Tang *et al.*, 2003). In a separate study growth inhibition decreased according to frequency in the following order 200 kHz >1700 kHz >20 kHz. In this case increased energy did not significantly affect biomass reductions with 20, 40, 60 and 80 W units removing 43, 43 48 and 48% respectively (Hao *et al.*, 2004a). Comparison of the experimental conditions in the studies reveals extensive variance in terms of the ultrasound exposure time, frequency and power and sample volume.

Growth has also been reported to occur after ultrasound treatment (Tang *et al.*, 2003; Inman, 2004). For instance *Microcystis* sp. increased by 67% during continuous application of ultrasound at a frequency of 28 kHz and power 20 W (Inman, 2004). Similarly, *Spirulina platensis* increased by 60% after a pulse of 12 minutes every 11 days of 1.7 MHz ultrasound frequency (Tang *et al.*, 2003) as well as a 5% increase in growth of the filamentous cyanobacteria *Spirulina maxima* (Al-Hamdani *et al.*, 1998). Another study confirming the previous findings, on filamentous cyanobacteria *Anabaena flos-aquae* (Francko *et al.*, 1990) found cell growth increased subsequent to low frequency (20 kHz) ultrasound. *Chlorella* a unicellular green species also experienced growth of cells after ultrasound of 20 kHz at laboratory scale (Zhang and Chen, 2001). In some cases no effect is observed such as with *Synechococcus* (PCC 7942) which grew at the same rate as the control after 5 minutes of 1.7 MHz; *Synechococcus* is a non-vacuole, marine species of cyanobacteria (Tang *et al.*, 2004).

The mechanisms by which ultrasound influences algal growth are reported to be linked to the extreme temperatures and pressures which occur when nucleated cavitation bubbles collapse (Suslick, 1988). Specific mechanisms reported for algal control include: disruption of gas vesicles (Jong Lee *et al.*, 2000; Lee *et al.*, 2001), inhibition of photosynthesis by damaging photosystem II as a consequence of gas vacuole ruptured due to algal pigments being in proximity to gas vacuoles after exposure to high intensity ultrasonic radiation (Lee *et al.*, 2001; Mason *et al.*, 2003) and free radical production which occurs as a secondary effect of acoustic cavitation (Mason *et al.*, 2003). Although, no proposed mechanisms have been implemented based on the observed variations in results, there is a need to incorporate specific characteristics which make

them susceptible to ultrasound such as the presence of gas vacuoles in the species being targeted.

Control of algal blooms at reservoir, field and pilot scale has been tested with limited success. Commercially available units used in studies to date work at frequency ranges of 20-40 kHz and power of 20-40 W (Inman, 2004; Warnock, 2005). One unit used in several field and pilot studies, is the Dynamco model SS-5000, which is available in the U.K. from the manufacturer Dynamco. Mixed species of green algae, diatoms and cyanobacteria were tested at pilot scale using a treatment and control pond with a volume of 700 L, and an ultrasound unit of 28 kHz and 20 W composed of a floating transducer attached to the side of the pond. Results found a reduction in growth of diatoms of 60% and 41% for green algae but an increase of unicellular cyanobacterial species *Microcystis* sp. of 67% (Inman, 2004). A second study using the same unit at pilot scale but at a reducing the volume from 700 L to 250 L with the same experimental set-up with mixed algal species of green algae, diatoms and cyanobacteria except using smaller pond containers, found a growth reduction of 49% for filamentous cyanobacteria *Aphanizomenon* sp. and a 60% reduction for *Scenedesmus* sp. *a* colonial oval green algae (Warnock, 2005). Field scale trial using a 28 kHz 40 W floating transducer was tested on a lagoon of 5000 L in volume in the UK and a significant reduction in chlorophyll a was observed (Inman, 2004). Preferential kill of cyanobacteria as opposed to diatoms and green algae has been reported suggesting different levels of susceptibility as a function of species type. For instance, a field study in Korea using a submerged unit of 22 kHz and 630 W reported that only 13% of all algal species were cyanobacterial species within the field test enclosure in comparison

to 47% in the control area (Ahn *et al.*, 2003). Further, a Japanese study used Lake Senba, in an urban location with a volume of 365,000 L as a study site for three years. 10 flow-through cell units were attached to a pipe beneath a float and composed of by 2X (100 W, and 200 kHz) ultrasound transducers. Initial positive results were observed in the first two years but the flow rate was then reduced at the lake opening and in the final year blooms returned suggesting issues around long term susceptibility to ultrasound may be a concern, (Nakano *et al.*, 2001; Lee *et al.*, 2002).

Comparison of the number of studies, specifically on the use of ultrasound at laboratory and field scale to investigate algae and bacteria, highlights that the majority of studies were focussed on bacteria (30) and cyanobacteria (25), with green algae (2), diatoms (2), wastewater (6) and stream-water (3), and spores (1) making up the final 14 studies (Figure 2.4). As mentioned previously two bacterial studies and three cyanobacterial studies produced cell growth in response to ultrasound. Bacterial species were successfully treated with ultrasound producing >80% cell removal for 66% of studies, with cyanobacteria producing >80% cell removal for only 24% of studies. Green algae and diatoms based on the very few studies have proved to be challenging algal divisions to treat, but are in need of further work before trends based on susceptibility can be established (Figure 2.5).

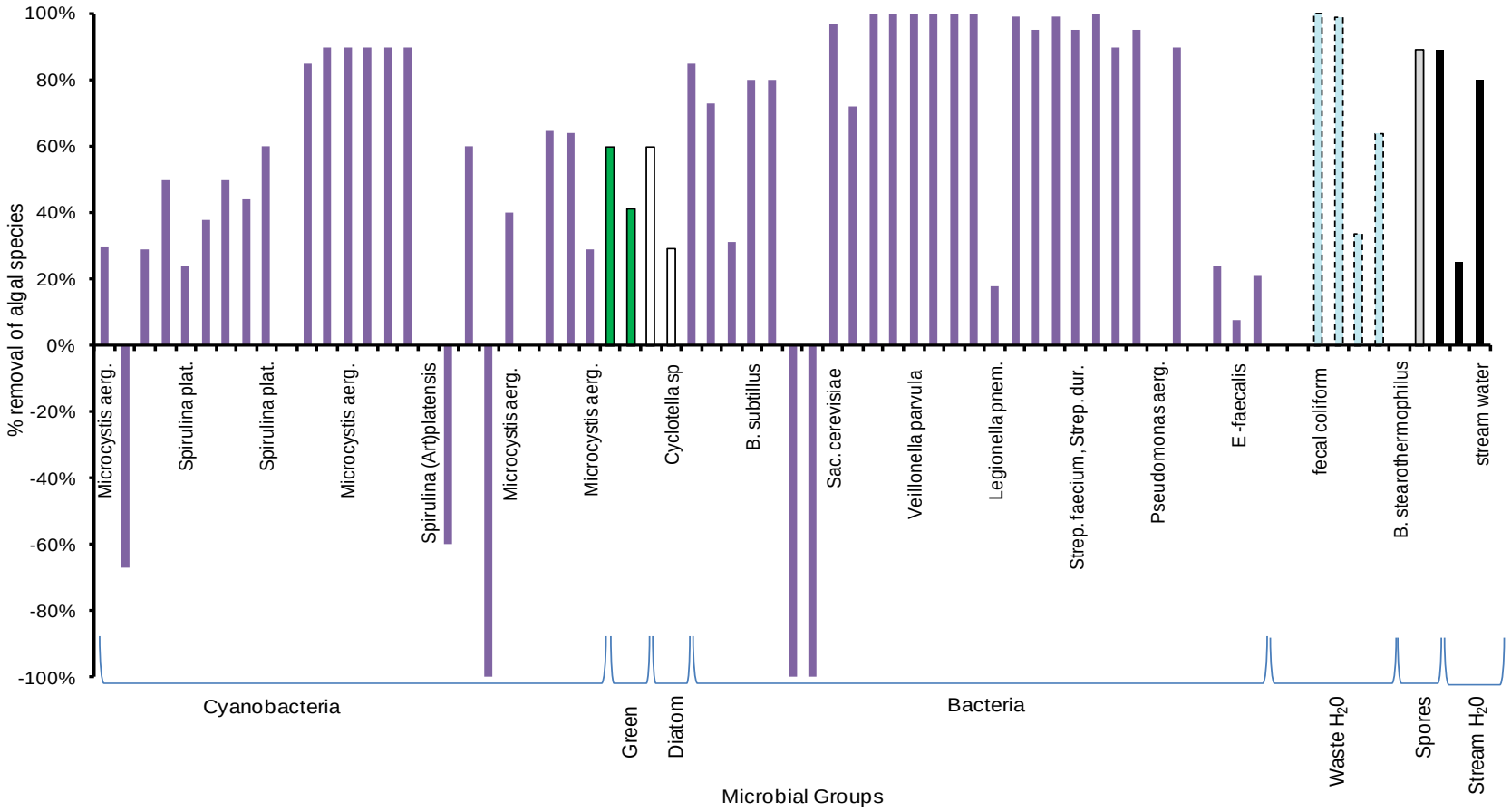

Figure 2.5 Ultrasound experiments on all microbial studies from the published literature to date

Bench scale was the most researched with most experiments (59) out of 69, with only 10 at pilot and field experiments, with bacteria (41) and cyanobacteria (17) the two main divisions being researched (Figure 2.6).

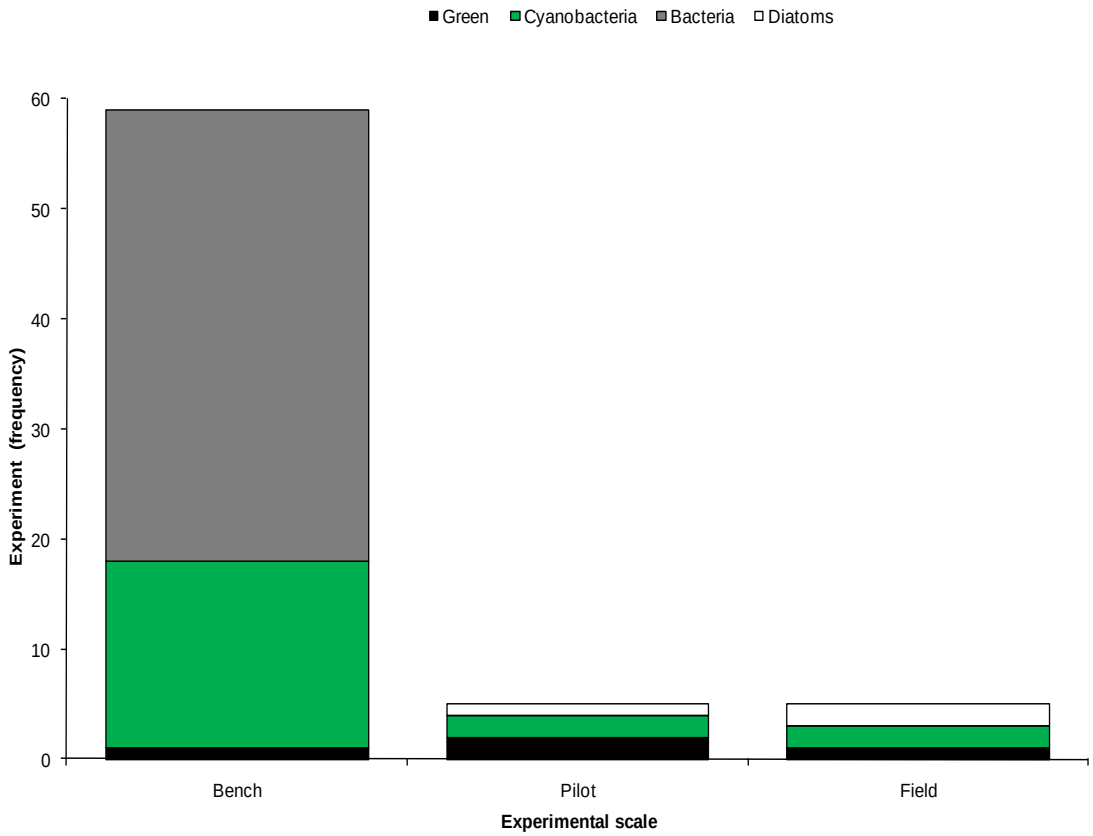

Figure 2.6 Ultrasound experiments from the literature data at bench, pilot and field scale sub-divided by studies on microbial divisions, green algae, diatoms, cyanobacteria and bacteria.

Ultrasound studies vary in relation to two variables: frequency and power. Power levels below 1 W have been shown to be effective for *Spirulina* (*Arthrospira*) *platensis*, a

filamentous cyanobacteria. To explain, using a very high frequency of 1.7 MHz between 50% and 60% cell removal of *Spirulina* was observed compared to 40% with *Microcystis*. In one specific case, a rate of -100% was reported for a non-gas vacuolated unicellular colonial cyanobacterial marine species *Synechococcus* which grew at the same rate as the control (Figure 7), (Tang *et al.*, 2003; Tang *et al.*, 2004). As power input increased from 14-600 W, cell removal increased from 4-65%. Frequency remained low throughout the higher powered experiments except for 1.7 MHz using 14 W produced 38 and 50% removal for *Spirulina platensis*, a filamentous cyanobacteria. *Microcystis* was the species used in all the higher powered, low frequency experiments and it proved to be a difficult species to kill, with the highest removal reaching 65%. Over 60% of *Microcystis* population must be removed to avoid re-generation of the species, otherwise gas-vacuoles can re-generate in a short time period, and resume growth (Jong Lee *et al.*, 2000) and so most reported cases can be termed unsuccessful. Explanation for the resistance of *Microcystis* is provided by the fact that it contains narrow gas-vesicles which exhibit a high critical pressure rate of up to 1 MPa higher than that of filamentous cyanobacteria (Walsby, 1992). These characteristics make the gas vacuoles in *Microcystis* difficult to burst as it can withstand the conditions produced during low frequency ultrasound (Zhang *et al.*, 2006). Power and frequency appear to have consistent effects on susceptible filamentous cyanobacterial species *Spirulina* (*Arthrospira*) *platensis* (Tang *et al.*, 2003) however with resistant unicellular cyanobacterial species i.e. *Microcystis*, it is evident that high frequency and high power are required to achieve significant cell removal >60% to avoid cell re-growth (Zhang *et al.*, 2006).

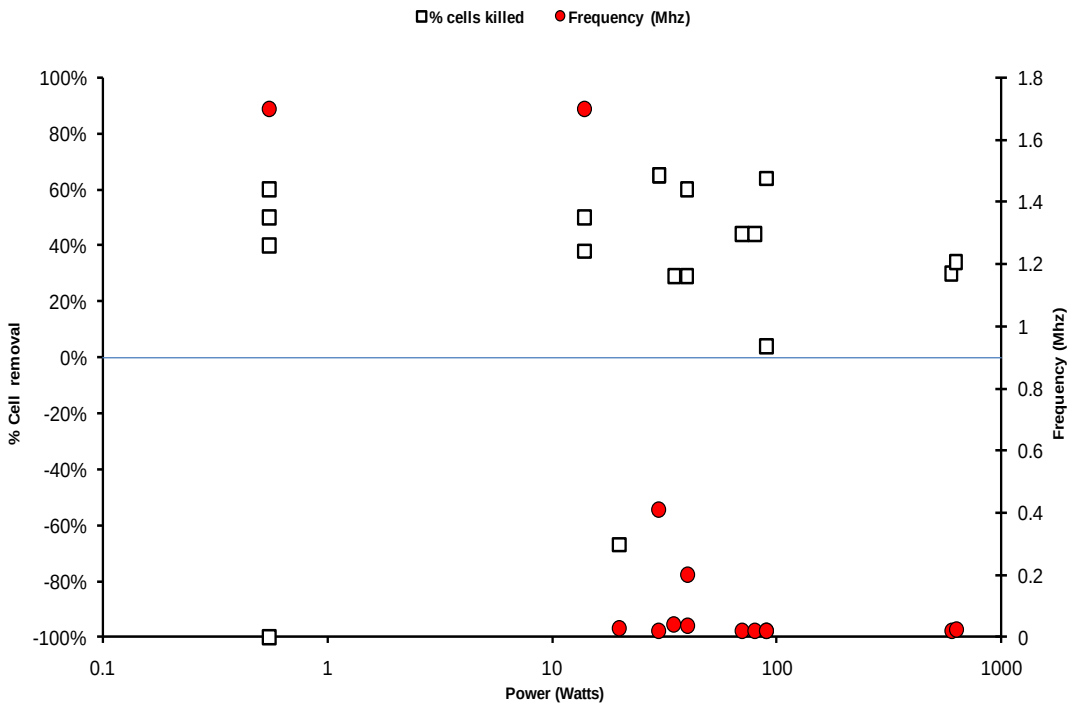

Figure 2.7 Literature studies on ultrasound cyanobacterial experimental data where cell removal rates were graphed via increasing power and frequency. Low frequency studies caused cell growth rather than cell removal, with power of between 10-100 W producing significant cell removal rates between 20-65% cell removal.

Ultrasound has produced the highest kill of 100% consistently among monocultures of bacterial species and one study on cyanobacterial *Microcystis* found between 90-100% kill. However other studies on *Microcystis* did not achieve the same high levels of kill with the next most successful study reaching only 60% kill approximately. The scale of experiments was concentrated almost completely at bench scale, thereby negatively influencing whether these results could be reproduced at larger scales from a financial

and equipment design perspectives. Literature studies on ultrasound and algal have produced no clear trends based on the optimal frequency for enhanced cell removal on any specific division of algae. However regarding power usage in previous ultrasound studies, the greater the power levels in Watts (W) the greater the negative effect on the algal species being sonication with higher rates of cell removal.

2.8 Conclusions

In regard to control of growth of microbial species a lack of consistency in the reported findings suggests that species' morphological characteristics may be a factor yet cell wall thickness in relation to bacteria is not one of these. Bacterial species appear to be quite susceptible to ultrasound but what characteristics cause this response has still to be defined. Previous suggestions concerning cell wall thickness do not appear to be supported by the literature data but that requires further investigation. Algal studies on ultrasound have seen no trend regarding the best frequency to be used to highest number of algal cells, but regarding power the higher the power being used does appear to influence the cell removal rate with increased cell removal with increased power usage.

Algal pilot and field studies are extremely under represented and more studies are necessary to understand how large scale potable water reservoirs will respond to algal bloom control using ultrasound. Overall, the reported work from the literature to date indicates that investigations into a link between species character and treatment are required to further elucidate the potential for ultrasonic treatment.

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Chapter 3: Experiences of algal bloom control using green solutions barley straw and ultrasound, an industry perspective

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3.1 Abstract

Water companies in the U.K. have been dealing with algal blooms for many decades. Due to seasonal upwelling and subsequent increased nutrient availability increases the likelihood of blooms from early summer until late autumn. Costs of tackling this problem have escalated as bloom occurrences have increased markedly due to elevated demand on water supply. Environmentally friendly or green solutions are defined as those that use less energy and reduce chemical use in water treatment. Ultrasound and barley straw are two such solutions. Barley straw has been used quite extensively by certain U.K. water companies since the 1960's and results are generally positive based on data I have collated from across the U.K. water companies, with work still on-going. Results were compared across the five case study sites found that both barley straw and ultrasound are selectively inhibitory towards specific algal groups with both positive and negative results. In the case of barley straw, cyanobacteria appear to be the preferentially influenced division irrespective of morphological features such that cell counts of both unicellular/colonial species such as *Microcystis* and filamentous species such as *Aphanizomenon flos-aquae* were successfully reduced. More mixed and

generally negative results were reported for other divisions such as green algae and diatoms where increases were observed. Ultrasound has only recently been used as a treatment for algal removal, and so data from industry is less abundant, to date results have not been as encouraging as barley straw. Several successful trials have found cyanobacteria to be the most susceptible algal division to ultrasound at field scale however from a financial perceptive implementation as an in reservoir is not considered a viable option however as an in-works two-tier system used with chlorination it could considered effective algal bloom solution.

3.2 Introduction

Algal blooms occur in any water body once the environmental conditions are right and then grow quickly becoming difficult to treat (Reynolds, 1972; Reynolds, 1973; Reynolds, 1975; Reynolds, 1984). When bloom concentrations of algae enter the treatment works a range of problems exist including filter blocking, increased coagulant demand and treatability, and toxin release (Henderson *et al.*, 2008a). Estimates of the costs of such events in the UK are £75-114 million per year to remove algal toxins and £19 million to remove particulates emitted from algal cells during growth. In addition, a further £55 million is necessary to deal with the consequences of eutrophication (Pretty *et al.*, 2003). Consequently there is a need for source control to ensure such high levels of algae do not enter the treatment works. A range of methods currently used include nutrient diversion, destratification, aeration, and phosphorus precipitation (Wetzel,

2001). However, the efficacy of each is inconsistent leading to the desire for alternative solutions. Perhaps the two most commonly considered alternatives are barley straw (Waybright *et al.*, 2009) and ultrasound.

Barley straw has been used in the UK since the 1970s at a range of scales from applications at household ponds to larger systems such as canals, streams and reservoirs. The process is driven by a fungal degradation pathway which takes between 6 and 12 weeks to become effective (Murray *et al.*, 2009a). Doses of barley straw vary between 5 gm^{-3} (Barrett, 1994) to 40 kgm^{-3} (Gibson *et al.*, 1990) and have been shown to be most effective when treating algae within their exponential growth phase (James, 1994; Murray *et al.*, 2009). The mechanism of degradation that occurs in barley straw is attributed to the slow release of the phenolic compounds present in the lignin of the barley straw that has an inhibitory effect on the growth of the algal species (Barrett, 1994). Consequently, in many cases partially degraded straw is used although a time lag remains requiring careful planning of when the straw is added to ensure effective treatment. Reported studies on the use of straw have shown mixed results against cyanobacteria, green algae and diatoms (Ball *et al.*, 2001; Murray *et al.*, 2009; Murray *et al.*, 2010). To demonstrate, reported removal levels of 50% for *Microcystis aeruginosa* and 16% for *Cladophora glomerata* (James, 1992a; James, 1992b) indicate that susceptibility may be species dependent.

Ultrasound functions by initially physical pathways destroying the algae causing impacts that are relatively instantaneous. The physical action is generated by cavitation as sound waves propagate through the water. A number of mechanisms have been proposed to describe its action on algae including disruption of gas vesicles in

cyanobacteria (Jong Lee *et al.*, 2000; Lee *et al.*, 2001), bursting of gas vacuoles causing cells to sink (Walsby, 1968.; Nyborg, 1982), breakage of filaments and direct shattering of cell walls (Purcell *et al.*, 2008). Reports on its effectiveness are mixed with reductions of up to 90% (Hao *et al.*, 2004), no change (Tang *et al.*, 2004), and even increases (Inman, 2004) in cell concentration being reported. Analysis across all the reported trials shows no direct link to power input or frequency of operation, indicating that a number of complex factors require consideration (Chapter 2). Critically, morphological factors (Purcell *et al.*, 2008) and growth conditions which are known to change algal characteristics (Henderson *et al.*, 2008b) are reported to have an influence. The current paper aims to clarify such issues by examining the current state of practice in the UK through a series of case studies and includes a comparison of the performance of barley straw and ultrasound to establish practical differences in operation.

3.3 Methods

Data was collected from 5 case study sites within the following UK water companies: United Utilities, Thames Water and Anglian Water (Figure 3.1). Ultrasound was employed at locations A, B and D and barley straw at sites C and E from between 2000 and 2008. Specific location information on the test sites is excluded in response to a request from the participating water companies.

Case study site A was based around a 1200 ML reservoir for test and control trials. Dynamco model SS-5000 ultrasound units rated as 40 W at 28 kHz were used to control a dominant population of diatoms. Case study site B utilised 995,000-800,000 m^3 reservoirs with a depth of 5.9-6 m and a total surface area of 13.8-17.5 ha^3 for both test and control trials. Floating 40W, 40-50 kHz transducers were installed on the 1st June 2004. Inspection of the algal population indicated the observed blooms were dominated by filamentous species of undetermined species. Case study site D used Dynamco model SS-500 ultrasound units in a 300 m^2 reservoir for 27 weeks starting from April 2004. The units were fixed to one of the banks of the reservoir in order to emit the wave across the site.

Case study site C included a 0.5 km^2 test and a 1 km^2 control reservoir. Barley straw was dosed in four floating lines situated near the river inlet at internals such that approximately 33% of the reservoir was covered. Across the three year trial straw was dosed at different rates such that 290 bales were used in 2007 and 330 bales in 2008. Converted to a surface area dose rate this equated to 4.5-5 $g.m^{-2}$. Case study site E was based around a 1,882,000m^3 reservoir with a max depth of 8.2 m and total surface area of 0.5 km^2. Barley straw was dosed at a rate of 5-7 g/m^2 using 7.5 kg bags attached at 8 m intervals across the lake in strategic positions determined by bathymetric studies. The first dose was added at the beginning of April 2008 and the second dose in July 2008, with the trial lasting two years.

In all cases data was determined within the water company's laboratories and included cell counts for microbial groups such as including diatoms, green algae and cyanobacteria, species diversity and mean chlorophyll a concentrations.

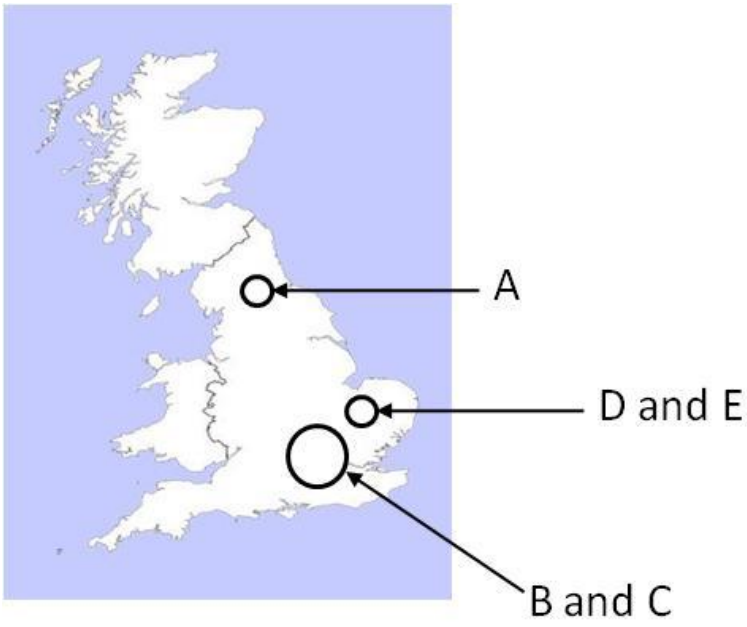

Figure 3.1 Locations of each of the case study sites

3.4 Results

3.4.1. Barley Straw

Algal blooms of *Microcystis* sp. were recorded in both test and control reservoirs in site C during the summer of 2008 (Figure 3.2). Baseline chlorophyll a remained below 10 μgL^{-1} outside of the bloom period which is approximately the threshold value where winter coagulation levels are used (WTW C, Site Operating Manual) indicating that the site had no algal related issues outside of the peak bloom periods. Application of the barley straw resulted in a decrease in both chlorophyll a and turbidity when dosed in April and July 2008. To describe, chlorophyll a levels of 15.7-22.8 ug/L pre barley

straw treatment decreased to >10 ug/L of chlorophyll a post the application of barley straw in April 2008 (Figure 3.2).

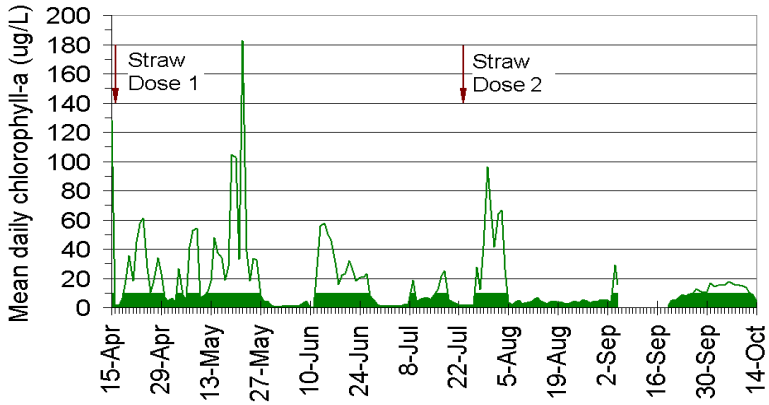

Figure 3.2 Chlorophyll a results for barley straw treated reservoirs C1 and C2, 2008.

Algal count data in the reservoirs used in case study site E revealed large differences in the observed bloom concentrations such that apart from 2005 and 2006 the peak concentration remained below 74,000 cells.mL^{-1} of cyanobacteria (Figure 3.3(A). Whereas two significant blooming events occurred between July and September in 2005 and 2006 with peak total cell concentrations of 253,077 cells.mL^{-1} on the 1/9/05 and 285,610 cells mL^{-1} on the 18/8/06 (Figure 3.3(A). Species identification revealed the blooms to consist predominantly cyanobacteria with a secondary group made up of green algal species. To explain, peak concentrations prior to addition of barley straw were 48,968 cells.mL^{-1} and 3,802 cells.mL^{-1} for cyanobacteria and green algae respectively (Figure 3.3B). Addition of barley straw generated reduction levels of 50% in cyanobacteria but increases of 63% and 88% for green algae and brown algae respectively (Figure 3.3B) suggesting a potential heightened susceptibility towards cyanobacteria and consequently algal succession within the reservoir; a usual

occurrence in field studies when the dominant species is removed (Henderson *et al.*, 2008a).

Previous studies confirming the results from these two case study sites have found algal populations including cyanobacteria (*Aphanizomenon flos-aquae*) suffered significant cell number reduction of (50%) after treatment with decomposing barely straw dosed at a concentration of 25 $g.m^{-3}$. (Everall and Lees, 1997). For example, a 10-fold increase in inhibition of *Scenedesmus*, *Microcystis* sp. and chlorophyll a has been reported when using decomposed barley straw extract in a dilute concentration of (0.005%) (Ball *et al.*, 2001). Similarly, barley straw extract of 7.2 $kg.m^{-3}$ tested at laboratory scale showed significant growth inhibition of cyanobacteria *Microcystis* and green algae *Synura* within several weeks but no effect on growth of colonial green *Scenedesmus* was detected (Ferrier *et al.*, 2005).

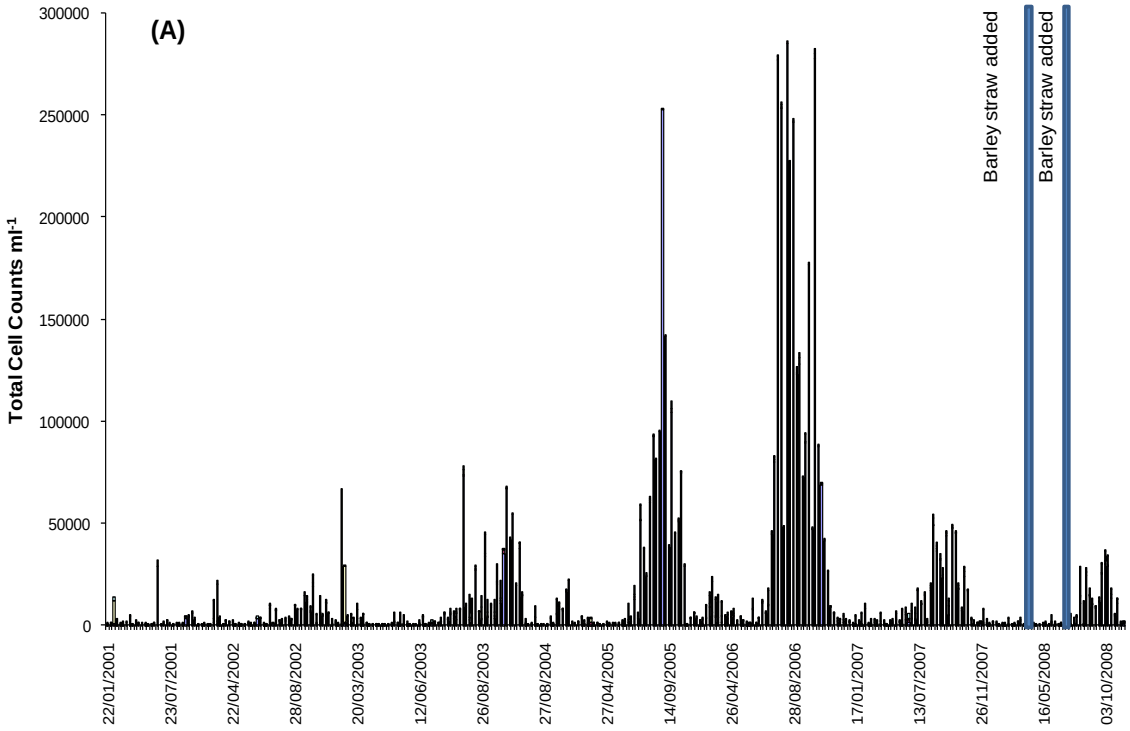

Figure 3.3 (A) Total cell counts for all algal species from 2001-2008, (Reservoir E).

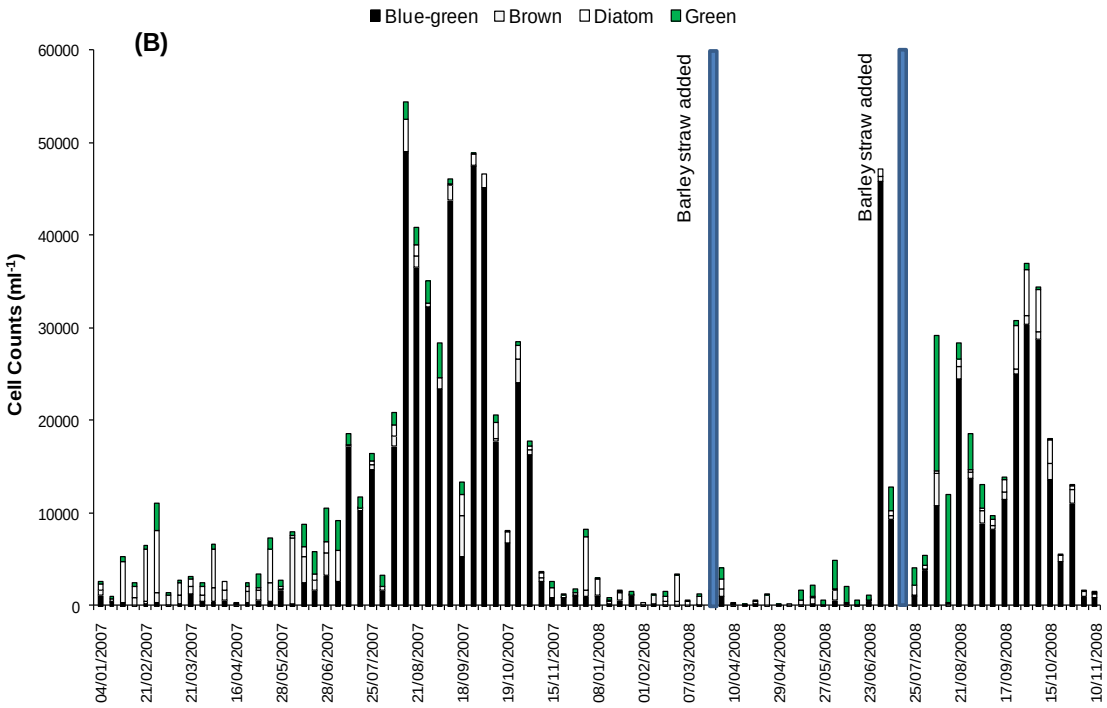

Figure 3.3 (B) Cell counts for barley straw treated versus control reservoirs defined by 4 algal types blue-green algae, brown algae, diatoms and green algae for 2007-2008 in Reservoir E Blue-green algae were the most dominant group.

3.4.2 Ultrasound

Case study site A includes data on algae levels dating back from 2001 with the ultrasound trial commencing in 2004 enabling benchmarking of any imposed change against the natural variations in the algal population. Overall no difference was observed between test and control in terms of mean chlorophyll a values for the control

and test reservoir in 2004 (Figure 3.4(A). To explain, in 2004 control values varied between 19 and 22 μgL^{-1} compared to 15-48 μgL^{-1} for the test system.

Analysis at a microbial division level observed a difference between test and control for all three divisions although a significant difference was only observed in the case of cyanobacteria. To clarify, cyanobacterial cell counts were reduced from 40% in the control system to 18% in the test systems where ultrasound was applied. Equivalent changes in the other divisions were from 19% and 15% for green algae and from 32% to 29% for diatoms respectively. However, when compared against the natural variation observed during the previous three years no significant differences were evident between the test and control reservoirs (Figure 3.4). Selective inhibition of cyanobacteria in a mixed bloom including green algae and diatoms at field scale has been previously observed, with the treated reservoir decreasing to only 7% of control, a 93% removal rate for cyanobacteria (Ahn *et al.*, 2007).

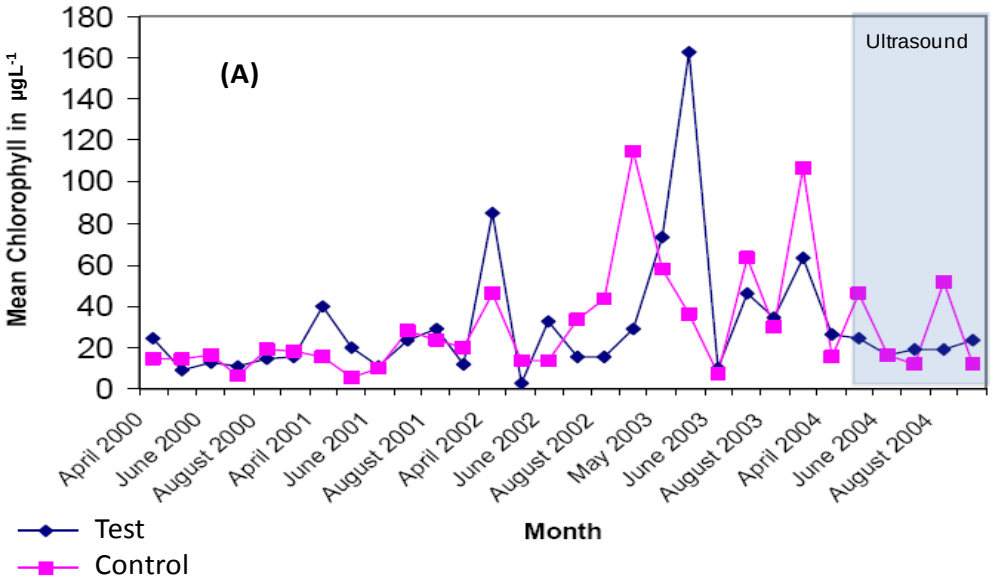

Figure 3.4 (A) Mean chlorophyll a concentrations treated and control reservoir from April 2000 to August 2004, with ultrasound treatment only from June 2004.

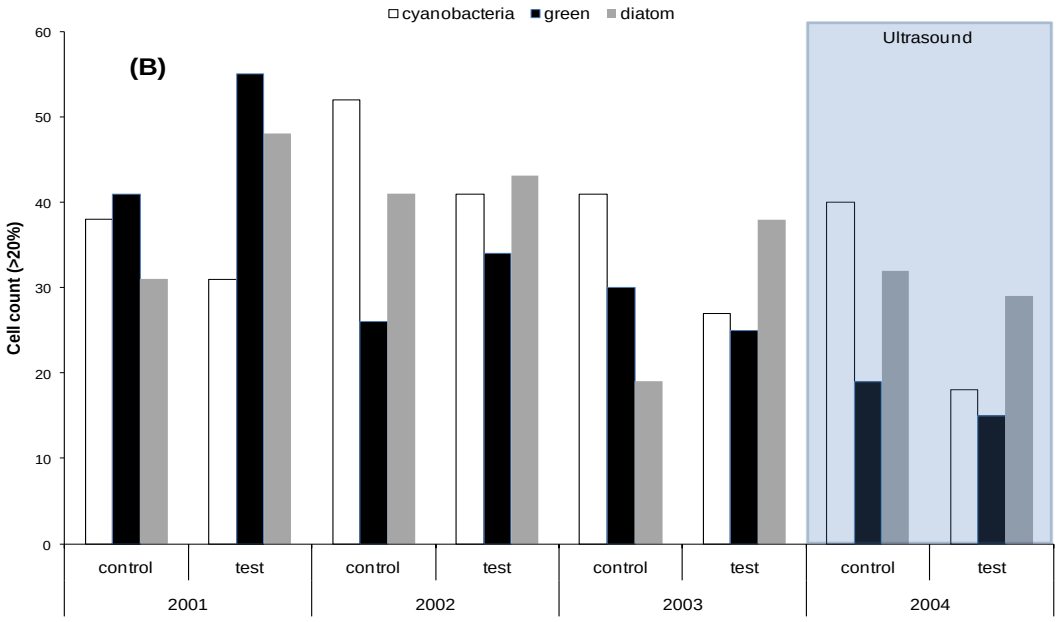

Figure 3.4 (B) Microbial divisions based on cell count frequency >20% for ultrasound treated and control reservoir, from April 2000 to August 2004, both results from reservoir (B).

Results from case study site D support the observations of no significant difference in terms of total algal concentration between test and control reservoirs with both following a fluctuating pattern across the whole trial (Figure 3.5). In contrast, the test systems showed an overall reduction of 55% in chlorophyll a compared to the control. Further analysis in terms of selective counting of cyanobacteria and diatoms revealed similar responses (Figure 3.5). %Co is the defined as the percentage cell counts normalised based on the cell counts of each reservoir before treatment began. For instance, peak concentrations of cyanobacteria between 2707-3084 %Co were observed in the control systems compared to 1694 %Co in the test system. Overall, across the trial, application of ultrasound generated a 36% decrease in cyanobacterial concentration. An even greater difference of 60% was observed in relation to diatoms suggesting the system was most effective for such species. The lower relative levels of diatoms throughout the trials are consistent with their growth kinetics as they grow slower than the other algal groups and their cells are much larger (van den Hoek *et al.*, 1995). Pilot studies agree with such findings where reductions in diatoms of 60%, green algae 41%, filamentous cyanobacteria, *Aphanizomenon* sp. of 49% and unicellular green, *Scenedesmus* sp. of 60% have been reported (Inman, 2004; Warnock, 2005).

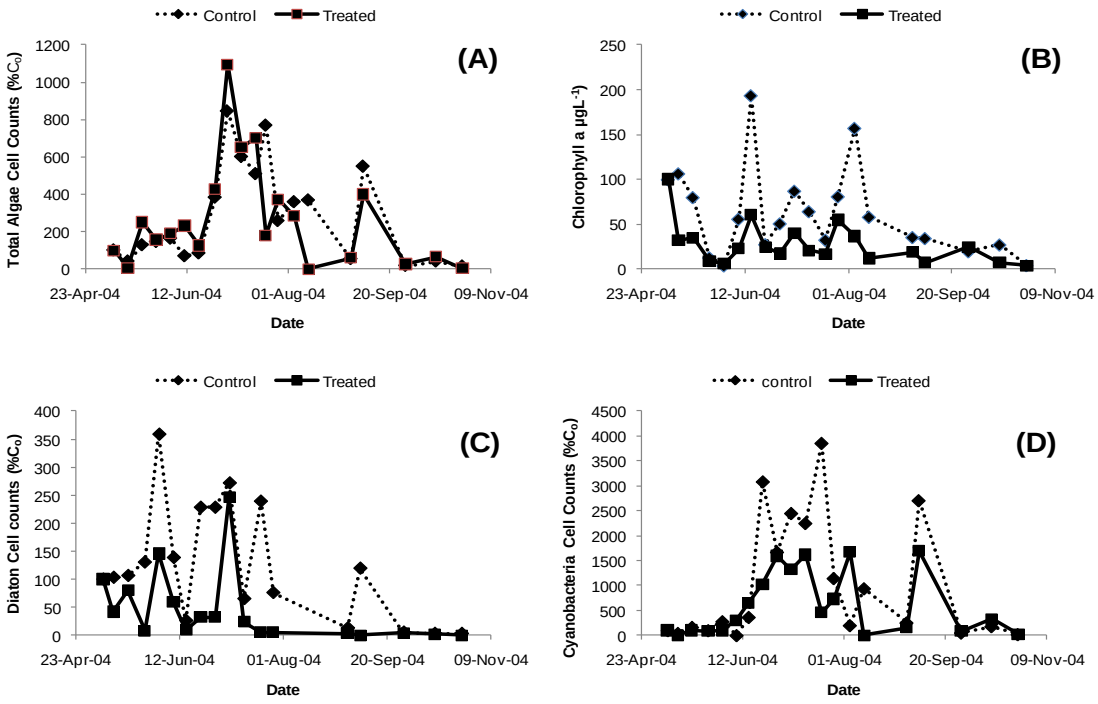

Figure 3.5 (A) Total algal cell counts after ultrasound treatment, (B) chlorophyll a quantified in ugL^{-1} after ultrasound, (C) Diatom cell counts after ultrasound treatment, (D) Cyanobacterial cell counts after ultrasound treatment in reservoir (D).

3.5 Discussion

Comparison across the five case study sites has indicated that both barley straw and ultrasound are selectively inhibitory towards specific algal groups with both positive and negative results. In the case of barley straw, cyanobacteria appear to be the preferentially influenced division irrespective of morphological features such that cell

counts of both unicellular/colonial species such as *Microcystis* and filamentous species such as *Aphanizomenon flos-aquae* were successfully reduced. More mixed results were reported for other divisions such as green algae and diatoms where increases were observed. Confirmation of this reduced efficacy is shown with only 16% removal reported for the filamentous green algae *Cladophora glomerata* (James, 1992a; James, 1992b). In contrast, laboratory chemostat trials have shown a 65% reduction in the colonial green algae *Scenedesmus* sp. after 4 weeks of exposure to pre rotted straw (Murray *et al.*, 2009), suggesting barley straw can be effective against some species. Whilst mixed results are observed for other divisions, literature data supports the main finding in that cyanobacteria appear consistently controlled with barley straw. The heightened susceptibility of cyanobacteria compared to other algal divisions infers the importance of a biological characteristic. Cyanobacteria are different from all other algae divisions in that they are bacteria and as such they have no membrane bound nucleus or organelles and a cell wall made up of peptidoglycan which is polysaccharide chains cross-linked by peptides containing D-amino acids rather than from cellulose and chitin (van Heijenoort, 2001). Whilst the exact mechanism by which barley straw works is still under debate the most likely mechanism is thought to be centred on the release of phenolic based algistatic compounds during the degradation of the straw (Pillinger *et al.*, 1994; Murray *et al.*, 2009). The plant phenols present in the lignin of the barley straw is slowly released as degradation occurs, these phenols can cause algal cells to reduce or stop cell growth (Murray *et al.*, 2009). For instance, 50% inhibition of *Microcystis aeruginosa* was reported from plant produced polyphenols (Nakai *et al.*, 2001). Consequently, the process is mass transfer controlled where the algistatic

compounds must first adsorb onto the surface of the algae and then subsequently diffuse through the cell wall. In such circumstances differences in the biological make up of the wall will influence the resistance to mass transfer and so control the efficacy of the process. Such suggestions are consistent with the need to pre-treat the straw prior to use so that sufficient algistatic compounds are present to adsorb onto the concentration of algae to be treated. For instance, field trials conducted on the Royal Canal in Ireland showed that the straw was effective at controlling a filamentous bloom whilst rotted straw was still visible (Caffrey and Monahan, 1999).

In the case of ultrasonic sonication, data from the field case study sites was less consistent with reductions across all algal divisions in some cases but no overall effect in others. This is consistent with laboratory and pilot trials where reductions, no change and even increases in algal counts have been observed. For instance, growth reduction rates of 60% for diatoms, 41% for green algae were reported whilst a corresponding increase of 67% in cyanobacteria occurred. However, in a second trial on the same system reductions of 49% in the filamentous cyanobacteria *Aphanizomenon* sp. was observed (Inman, 2004; Warnock, 2005). Reports from a three year trial in Japan show changes in efficacy as a function of time where effective bloom control was observed during the first two years followed by the bloom re-establishing itself in year three (Nakano *et al.*, 2001; Lee *et al.*, 2002). Overall, susceptibility to sonication has been suggested to relate to morphological factors such as filamentous structures such that unicellular species are less likely to be treated (Ball *et al.*, 2001; Brownlee *et al.*, 2003; Hayes *et al.*, 2005; Purcell *et al.*, 2008; Murray *et al.*, 2009). Such a selection feature

will cross all algal divisions and so provides a distinctly different basis for selection compared to barley straw.

3.6 Conclusions

Case study comparison has revealed that both barley straw and ultrasonic sonication can be effective control methods against algal blooms. The two approaches appear selective to different characteristics of algae. In the case of barley straw algal division is key, such that heightened susceptibility is observed against cyanobacteria due to differences in their biological make up compared to other algae. In contrast, susceptibility to ultrasound based on these field studies has produced inconsistent results clearly highlighting the need for further field studies. To focus new field study efforts previous studies from the literature at laboratory scale have indicated that ultrasound has had more detrimental effects on filamentous rather than unicellular species.

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Chapter 4: Characterisation of Algogenic Organic Matter (AOM) for *Scenedesmus subspicatus*, *Cyclotella* sp., *Aphanizomenon flos-aquae* and *Anabaena flos-aquae*

Chapter 4: Characterisation of Algogenic Organic Matter (AOM) for *Scenedesmus subspicatus, Cyclotella* sp., *Aphanizomenon flos-aquae* and *Anabaena flos-aquae*.

4.1 Abstract

Algal species lose particulates and organic compounds from inside their cells throughout their life cycle to their external environment and the concentration increases significantly towards the end of their life. These emissions are called algogenic organic matter (AOM), and lead to increased use of coagulants in the water treatment process. AOM was characterized from species of green algae *Scenedesmus subspicatus*, diatoms *Cyclotella* sp., and cyanobacteria *Aphanizomenon flos-aquae* and *Anabaena flos-aquae.* Parameters tested for included dissolved organic carbon (DOC), zeta potential (ZP), hydrophobicity, protein and carbohydrate content, molecular weight, specific UV absorbance (SUVA) and charge density.

Low SUVA and negative zeta potential (ZP) values were characteristic of these algal species. Charge density was found to be absent in all the species tested during the exponential growth phase. The AOM from all the species tested was dominated by

hydrophilic polysaccharides and hydrophobic proteins. The lack of charge density in AOM is attributable to hydrophilic (water loving), polysaccharides and carboxylic acids with neutral charge and low MW which is contrary to natural organic matter (NOM), but similar characteristics to effluent organic matter (EfOM).

4.2 Introduction

Algal blooms are a naturally occurring microbial population in lakes and reservoirs worldwide. Increased nutrient levels cause eutrophication which consequently facilitates the formation of blooms (Wetzel, 2001). Eutrophication causes biological activity to increase rapidly, the consequence of this is a substantial increase in dissolved and particulate organic and inorganic matter in the water body (Cooke *et al.*, 1993). For instance, 4.45 billion kg of organic matter was produced by algal blooms in one year in lake Eerie, 18 times the overall contribution of wastewater effluent to the lake per year (Harlow, 1968).

Early investigations into the character of organic matter derived from algae (AOM)) has shown that molecular weight, DOC, carbohydrate and uronic acid concentrations vary considerably between species and age of the population (Bernhardt *et al.*, 1985; Hoyer *et al.*, 1985). The latter occurs because as the cells grow from exponential to stationary phase the AOM increases as the cell surface charge decreases therefore allowing the cell surface mucous to be worn off (Konno, 1993). The impact of AOM can lead to increased water treatment costs, a degradation of recreational value (Falconer, 1994),

and possible toxin accumulation (Carmichael, 1992). Removal of high concentrations of AOM is known to be difficult as it has been shown to interfere with coagulation and cause severe membrane fouling (Her *et al.*, 2004).

The current chapter aims to characterise AOM originating from algae that are problematic blooming species found in water bodies using techniques used in algal studies and also those used to characterise NOM in clean water. Specifically, the AOM character from four algae the green alga, *Scenedesmus subspicatus*, the cyanobacteria *Aphanizomenon flos-aquae*, and *Anabaena flos-aquae* and the diatom *Cyclotella* sp. are compared as they are known to be common species found in UK reservoirs. Furthermore, the overall character is compared to well studied and understood NOM and biomass systems, this therefore allows the implications of the AOM character with respect to treatment to be assessed.

4.3 Methods

4.3.1 Algae cultivation procedure

The freshwater algae cultures of *Scenedesmus subspicatus* (276/20), *Aphanizomenon flos-aquae* (1401/3), *Anabaena flos-aquae* (1403/13B) *and Cyclotella* sp. (1070/4) were obtained from the Culture Collection of Algae and Protozoa, (Oban, Scotland). All algae were grown in 1, 2 and 5 L conical flasks at 20°C (+/-2^{o}C) in 16/8 h light/dark

cycle and constantly mixed on an orbital shaker except *Cyclotella* sp. which was mixed by hand daily. *Scenedesmus subspicatus, Aphanizomenon flos-aquae* were grown using Jaworski media (JM); *Cyclotella* sp. was grown using diatom media (DM) and *Anabaena flos-aquae* was grown in blue-green media without nitrogen (BG-11-N). One sun-glo and one aqua-glo 30 W aquatic lights were used for lighting. Neutral density filters were added to lights for all species except Scenedesmus subspicatus which required higher UV light intensity to grow. Each algal species grew at various rates, reaching different cell concentrations to hit exponential phase of growth. To describe, maximum concentrations of 1.8 $x10^6$, 1.8 $x10^6$, 8.8 $x10^5$, 7.48 $x10^5$, were achieved on days 14, 28, 30 and 35 for *Scenedesmus subspicatus*(SS), *Aphanizomenon flos-aquae*(AP), *Anabaena flos-aquae*(AN) and *Cyclotella* sp.(Csp.) respectively. AOM was extracted from all algal species at this point. To avoid contamination, checks were undertaken daily and to determine cell concentrations. Cell population numbers were measured by counting cells in triplicate using a light microscope and a haemocytometer.

4.3.2 AOM extraction procedure

AOM was extracted by centrifuging 5 L of algal cell suspension at 4,000 rcf (relative centrifugal force) for 15-30 minutes with the duration of the centrifugation depending on the algal species, i.e., *Anabaena fq.* pelleted faster than *Scenedesmus sub*. The supernatant was then filtered using 0.7μm Whatman GF/F glass micro-fibre. Samples were checked under a light microscope to confirm that the majority of cells were still

intact and had not been lysed during centrifugation. AOM extracted from *Scenedesmus subspicatus, Aphanizomenon flos-aquae, Anabaena flos-aquae* & *Cyclotella sp*., will be denoted as SS-AOM, AP-AOM, AN-AOM and Csp.-AOM respectively from henceforth. All characterisation was undertaken within 4 days of extraction and conducted at pH 7 unless stated otherwise.

DOC was measured using a Shimadzu TOC-5000A, (Shimaduz, Milton Keynes, U.K.) The Shimadzu analyser detects the difference between total carbon and inorganic carbon. Each sample was analysed in triplicate with errors less than 5%. UV_{254} absorbance was measured using a Jenway 6505 UV/Vis spectrophotometer, Patterson Scientific Luton, U.K. and SUVA was calculated as UV254/DOC.

Zeta potential (ZP) measures electrical potential movement around particles. The value of the zeta potential gives information about the potential stability of the colloidal system. A low zeta potential value makes flocculating particles difficult which can be a problem within WTW to get rid of particulates from water sources. The measurement was carried out using a Malvern ZetaSizer 2000 (Malvern, UK) which measures the electrophoretic mobility and converts it to zeta potential using the Smoluchowski Approximation which is valid when ka>1. F(ka): Henry's function which is taken at 1.5 in the Smoluchowski approximation (particles larger than 0.2 microns and dispersed in electrolytes containing more than 10^{-3} molar salts) ZP analyses were carried out over a pH range from 0-10 in order to determine the isoelectric point. Each sample was analysed in triplicate.

The charge density method was that used by (Kam and Gregory, 2001). An AOM solution of known concentration was added to 1mM NaH_2PO_4/Na_2HPO_4 pH 7 buffer, excess 6.2 meq L^{-1} low-molecular-weight PolyDADMAC (Sigma, UK) and the indicator ortho-Toluidene blue was back-titrated against 1 meq L^{-1} poly(vinylsulphate) sodium salt (Sigma, UK) to give a measure of charge density. Standardisation of solutions was done using 1 meq L^{-1} cationic cetyl-trimethylammonium bromide (Sigma, UK). The point of neutralisation was attained by measuring UV635 nm absorbance using a Jenway 6505 UV/Vis spectrophotometer which coincided with a colorimetric change from blue to purple.

The carbohydrate content of each species was determined using the phenol–sulphuric acid method (Dubois *et al.*, 1956; Zhang *et al.*, 1999). Protein analysis was performed using the modified Lowry method was used for protein analysis (Frølund *et al.*, 1995.). Glucose and bovine serum albumin (BSA) were used for calibration respectively at UV480 and UV750 absorbance using a Jenway 6505 UV/Vis spectrophotometer. Carbohydrate and protein measurements were taken in triplicate.

Resin fractionation was performed using 2 columns; XAD-7HP & XAD-4. These columns fractionated AOM into hydrophobic and hydrophilic components as outlined by Malcolm and MacCarthy, (1992.). An AOM sample of 2 L and approximately 10 mg L^{-1} of DOC was acidified to pH 2, and then passed consecutively through the XAD-7HP and XAD-4 resins (resin volume was 60 ml in each 15 mm column). HPI is the hydrophilic fraction, which is the non-retained sample. To isolate the hydrophobic fraction (HPO) and transphilic fraction (TPI), each fraction column was back-eluted with NaOH (0.1 M, 120 mL). The HPO fraction was desorbed from the XAD-7HP resin

the TPI fraction was desorbed from the XAD-4 resin. DOC and carbohydrate content of all fractions was measured after fractionation as previously described.

This method of molecular weight fractionation was based on that reported by (Henderson *et al.*, 2008b), which used nitrogen gas at a constant pressure of 1 bar to push the AOM solution through polyestersulfone, 500, 100, 30 and 10 kDa and kDa3 and kDa1 (Millipore) regenerated cellulose membranes using the Amicon Stirred Cell (Model 8400) in a linear series so that AOM was fractionated into the following >500, 100–500, 30–100, 10–30, 3–10, 1–3 and <1 kDa fractions. The Amicon stirred cell was operated at 75 Rpm and 60% permeate, with the exception of initial filtration through the 500 kDa membrane for which only 40% permeate was obtained over two runs as a mucilaginous layer developed easily on this membrane. This experiment was performed in duplicate.

4.4 Results

4.4.1 DOC

Exponential phase DOC averaged 0.6±0.03, 9.2±0.1, 5.7±0.34 and 12.8±0.06 $mg.L^{-1}$ for SS-AOM, AP-AOM, Csp-AOM and AN-AOM respectively. Normalising DOC against cell number indicates a more significant difference between species with levels of 0.0031, 0.0051, 0.0076, 0.0146 $ng.cell^{-1}$ for SS-AOM, AP-AOM, Csp-AOM and AN-AOM respectively (Figure 4.1), representing an increase of 164, 245 and 470% for SS-

AOM, AP-AOM, Csp-AOM respectively compared to the DOC released per cell from the lowest species, AN. Comparison to previous studies suggests an ever greater variability with levels for *Microcystis aeruginosa* of 0.00071 ng.cell^{-1} in the exponential phase and 0.00095 ng.cell^{-1} in the stationary phase of growth (Henderson *et al.*, 2008b). Comparison across all species indicates a maximum variation of 2056% suggesting that DOC release as a consequence of algae blooms must be considered on a species specific basis. Converting to bloom cell numbers indicates that AOM could potentially range from 5.4 to 27 mg.L^{-1} depending on the species dominating the bloom and its phase of growth. Such levels are higher than those normally reported for high DOC contents in raw waters from catchments dominated by natural organic matter where DOC levels are typically in the range 4.5-14.5 mg.L^{-1} (Sharp *et al.*, 2006) indicating the significance of AOM. The reduced AOM levels reported for some of species in the current study compared to previous work in part relates to the phase of growth. All the current work was conducted in the exponential phase where much of the produced organic matter is known to remain bound to the cell surface until the stationary phase where it is more easily sheared off (Konno, 1993).

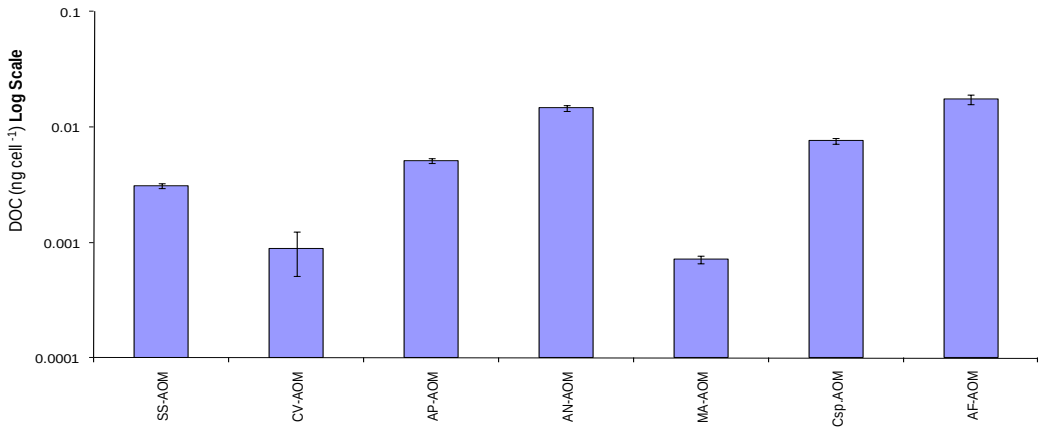

Figure 4.1 The DOC concentration for SS-AOM, AP-AOM, Csp- AOM, AN-AOM & with CV-AOM, MA-AOM and AF-AOM from Henderson *et al.*, 2008) all normalised by cell number.

Table 4.1 Summary table of zeta potential and charge density for each species.

	Zeta Potential		
Species	**Isoelectric pt**	**Plateau value (Mv)**	**Charge Density**
SS	3.1	-23.7	neg.
AP	3.2	-10.1	neg.
AN	2.4	- 6.0	neg.
Csp.	1.7	-13.3	neg.

Neg=negligible(<0.0001)

4.4.2 Zeta potential and charge density

The zeta potential of the AOM for all species was shown to decrease sharply between pH's of 1 and 4 and then remain stable thereafter. Correspondingly, isoelectric points for the four species were 3.1, 3.2, 2.4 and 1.7 with stabilised zeta potential values were of -23.7±2.0, -10.1±2.0, -6.0±1.3, -13.3±2.6, mV for SS-, AP-, AN and Csp-AOM, (Table 4.1), respectively indicating that significantly different levels of electrostatic repulsion could be expected when two algae cells approached one another. For instance, previous work on the coagulation of algae has shown that aggregation occurs once the zeta potential is less that -15 mV beyond which stable particles are observed (Henderson *et al.*, 2009). Charge density levels of the AOM for all 4 species was below the levels of detection indicating the released DOC was predominately made up of uncharged molecules and that the AOM would exert only minimal coagulant demand. Contradictory findings have been reported in the literature with values of up to 3.2 meq g^{-1} of C (C is Carbon, per mg (or g) for charge density (Henderson *et al.*, 2008b). The specific levels differ between species with reported values of 3.1 meq g C^{-1} for the AOM of *Dictyosphaerium*, 2.3 meq g C^{-1} for *Pseudanabaena* (Bernhardt *et al.*, 1985), 0.5-1.8 meq g C^{-1} for *C. vulgaris, Scenedesmus quadricauda* and *Cyclotella* sp. (Paralkar and Edzwald, 1996). Similar results to the non detectable levels reported here have also been found for *Melosira* sp. (Henderson *et al.*, 2008b) with AOM charge densities typically increasing from exponential growth to stationary phase conditions

consistent with the release of previously bound materials when the cells enter stationary growth phase of their cycle. The levels reported are equivalent to those of a typically highly coloured natural organic matter source water at 2.7-3.8 meq g C^{-1} and indicates that AOM can exert a significant coagulant demand. In fact previous attempts to find coagulant dose correlations have identified a strong relationship between the charge density of the AOM and the demand for coagulant (Henderson *et al.*, 2008b).

4.4.3 Hydrophobicity—XAD resin fractionation and SUVA

Characterisation by resin fractionation revealed the AOM to be predominantly hydrophilic in nature. To elucidate, the HPI fraction represents 54%, 63%, 62% and 81% of the DOC for SS, AP, Csp. and AN respectively (Figure 4.2). Percentage sample recovery was between 83-113%. Consequently, hydrophobic organic matter made up between 10-30% of the total DOC which is significantly lower than experienced with NOM where up to 80 % of the total DOC can be found in the hydrophobic fractions. This current data provides equivalent data to that reported for other algal species where HPI levels were, 57.3% for AOM from blue-green algae which also had a SUVA of 1

m^{-1} mg^{-1}, (Her *et al.*, 2003; Her *et al.*, 2004). Hydrophilic compounds are described as neutral polysaccharides, low-MW mono- and di-carboxylic acids and acidic sugars (Edzwald, 1993), thus supporting observations in this study. The high hydrophilicity of the AOM when measured using XAD-resin fractionation was supported by SUVA numbers. SUVA values were 1.18, 0.79,0.34 and 0.67 l m^{-1} mg^{-1} for SS-, AP-, AN and Csp-AOM, respectively, compared with 1.29, 1.65 and 1.7 l m^{-1} mg^{-1} for *Chlorella vulgaris*, *Microcystis aeruginosa* and *Asterionella formosa* respectively (Table 4.3), (Henderson *et al.*, 2008b). For context, the current SUVA values compare to values of 6.59 and 1.5 l m^{-1} mg^{-1} for typical surface and ground water samples respectively indicating that AOM is more akin to the organics found in ground waters than those experienced from source waters. The low SUVA values occur due to the low aromicity of the AOM in that this has very low absorbance ability (Hoyer *et al.*, 1985). This concurs with larger-scale studies where escalating eutrophication had elevated DOC concentration which coincided with a decrease in the SUVA (Cheng and Chi, 2003.).

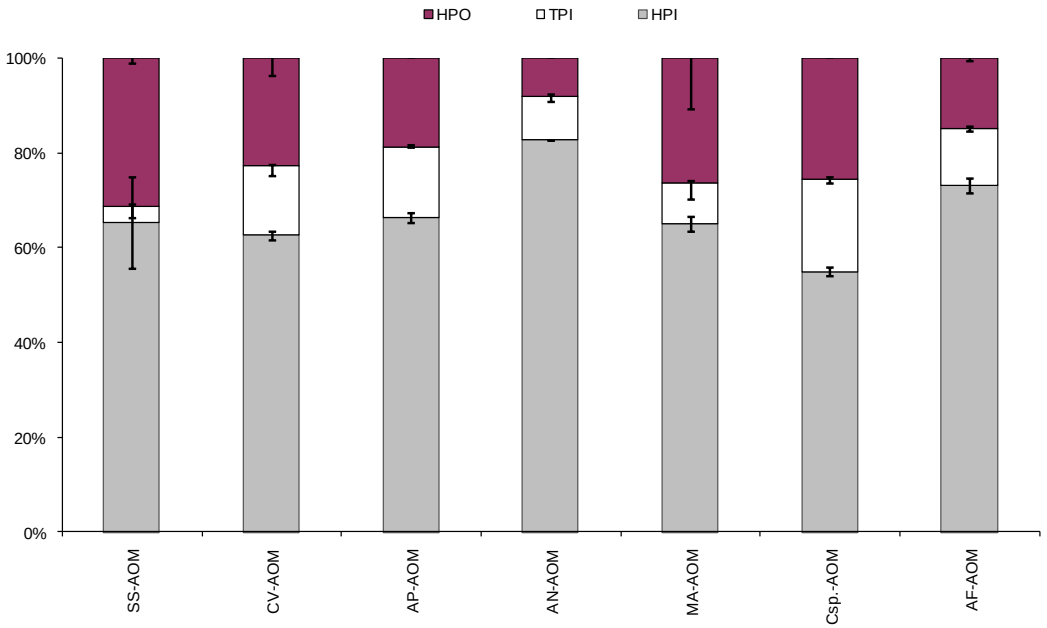

Figure 4.2 The proportion of AOM contained within hydrophilic (HPI), transphilic (TPI) and hydrophobic (HPO) fractions for SS-AOM, AP-AOM, Csp.-AOM., AN-AOM, with CV-AOM, MA-AOM and AF-AOM from Henderson *et al.*, 2008).

4.4.4 Carbohydrates and proteins

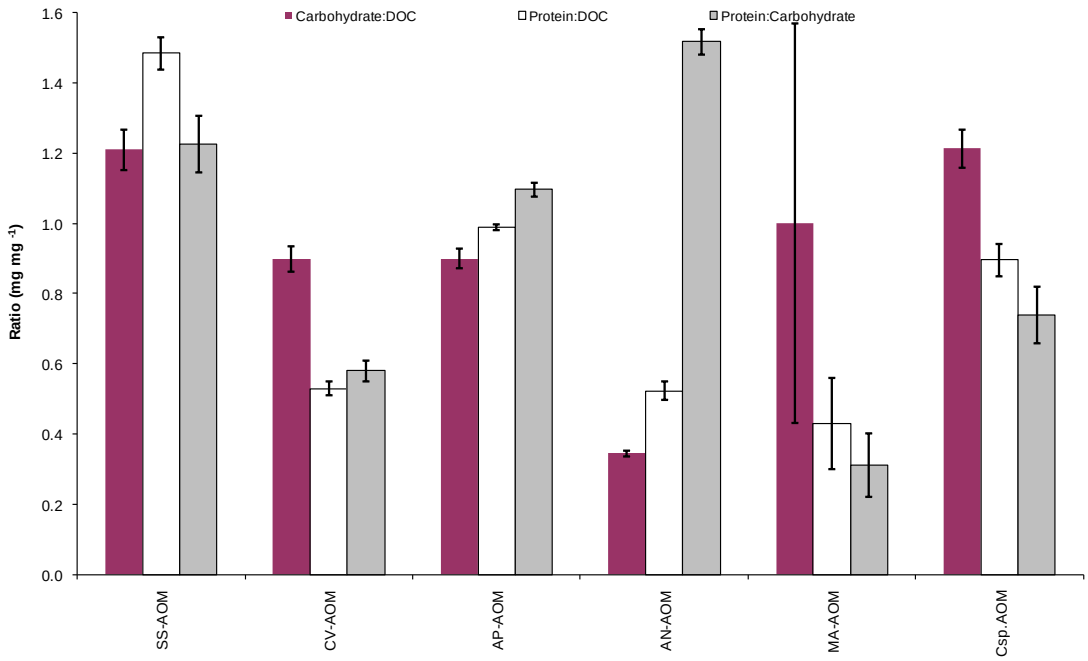

Figure 4.3 Carbohydrate:DOC, protein:DOC and protein:carbohydrate ratios for exponential phase SS-, AP-, Csp.-, and AN-AOM compared against MA-AOM aeruginosa & CV-AOM from Henderson *et al.*, 2008.

The carbohydrate:DOC weight ratios varied across the tested species (Figure 4.3) at 1.21, 0.9, 1.21 and 0.34, for SS-, AP-, Csp- and AN -AOM mg/mg^{-1} as glucose:C, respectively (Figure 4.3) showing much greater variation than previously reported for *Microcystis aeruginosa* and *Chlorella* in either stationary or exponential growth phases at 1 and 0.9 respectively (Henderson *et al.*, 2008b). Slightly higher variations were observed in relation to the Protein:DOC ratio with values of 1.48, 0.99, 0.9 & 0.52 for

SS-, AP-, Csp and AN -AOM as mg/mg^{-1} BSA:C (Figure 4.3). Combined, this variation was reduced with protein to carbohydrate ratios of 1.23, 1.10, 0.74 and 1.52 mg/mg^{-1} as BSA:glucose for SS-, AP-, Csp and AN –AOM indicating that the AOM is dominated by proteins. For context, the Protein:carbohydrate ratios for activated sludge have been determined as 0.59 and 0.85 for a full-scale and lab processes, respectively (Morgan *et al.*, 1990) indicating that AOM is significantly more dominated by proteins. AN had the lowest Protein:C mass ratio at 0.52mg/mg^{-1} which is similar to previous reported value for *Chlorella* and *Microcystis* at 0.43 and 0.53 mg/mg^{-1} respectively, (Henderson *et al.*, 2008b). Analysis of the locations of the carbohydrates within the hydrophobicity fractions revealed the material to be predominantly hydrophilic in nature. To demonstrate this, the HPI faction represented 86±2, 84±1, 65±1, 51±2%, of the carbohydrate material for AP, AN, Csp and SS–AOM, respectively (Figure 4.4) indicating that significant differences can be expected in AOM between species. Csp. and SS compare to previous studies which found 61, 52 and 49% HPI for *Microcystis aeruginosa*, *Chlorella vulgaris* and *Asterionella formosa* respectively (Henderson *et al.*, 2008b) and this is consistent with the suggestion that hydrophilic compounds are described as neutral polysaccharides, low-MW mono- and di-carboxylic acids and acidic sugars (Edzwald, 1993).

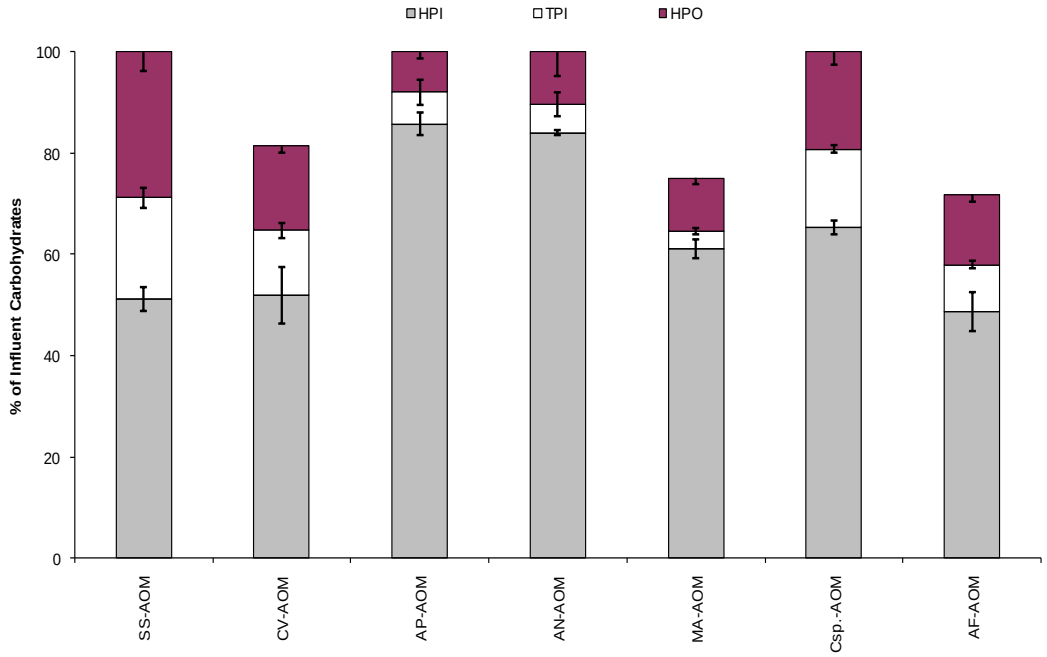

Figure 4.4 Percentage of total carbohydrates in the AOM present in the HPO, TPI and HPI fractions for SS-, AP-, Csp.-, and AN-AOM compared against MA-AOM aeruginosa & CV-AOM from Henderson *et al*., 2008.

These results also indicate that some loss of carbohydrate to the resin as indicated by recovery numbers between 51-84%. Similar irreversible carbohydrate binding onto the resin has been reported when analysing stationary phase AOM but not exponential growth AOM. Overall such recovery losses have direct relevance when considering the application of ion exchange technology for coagulant load reduction (Mergen *et al.*, 2008).

4.4.5 Molecular weight fractionation

Molecular weight fractionation of the exponential phase AOM revealed the proteins, carbohydrates and DOC to be predominantly found in the larger molecular weight fractions. For instance, in the case of proteins, the largest fraction (>100 kDa) contained 23, 27, 27, 39% of the total for AP, SS, Csp and AN respectively (Figure 4.5C). In all cases a progressive reduction in contribution to the total mass was seen as the molecular weight cut off became smaller. For instance, less than 10% of the DOC was found in the less than 10 kDa fractions for all species. The pattern was similar for all three components although a slightly greater spread of values was observed in terms of proteins and carbohydrates compared to DOC.

This study was performed exclusively in exponential phase which allows observation of a contrasting fractionation structure with stationary phase results reported in previous studies (Hoyer *et al.*, 1985; Lusse *et al.*, 1985; Henderson *et al.*, 2008b). Different molecular fractional distribution occurs during different growth phases and this observation is confirmed by different species of algae also releasing different amounts and types of carbohydrates and proteins depending on their growth phase, *Microcystis aeruginosa* and *Chlorella vuglari*s had bimodal distributions in stationary phase with 55% and 62% in >30 kDa and 38% and 30% in <1 kDa, respectively. A bimodal distribution was also evident in stationary phase for *Chlorella* sp. and *Scenedesmus obliqus* and *Dictyosphaerium* sp. and cyanobacteria *Pseudanabaena catenata*, for 0.2 µm and <1.1 nm (2 kDa) sized membranes (Konno, 1993; Henderson *et al.*, 2008b).

Similar distributions with decreasing molecular size fractions have been observed for AOM derived in the exponential phase for the green algae *Chlorella* sp. and *Scenedesmus obliqus* and the diatoms *Melosira granulate* and *Achnanthes exigua* (Lusse *et al.*, 1985). Consequently, growth phase rather than species appears to be the major influence on the MW size distribution of the AOM.

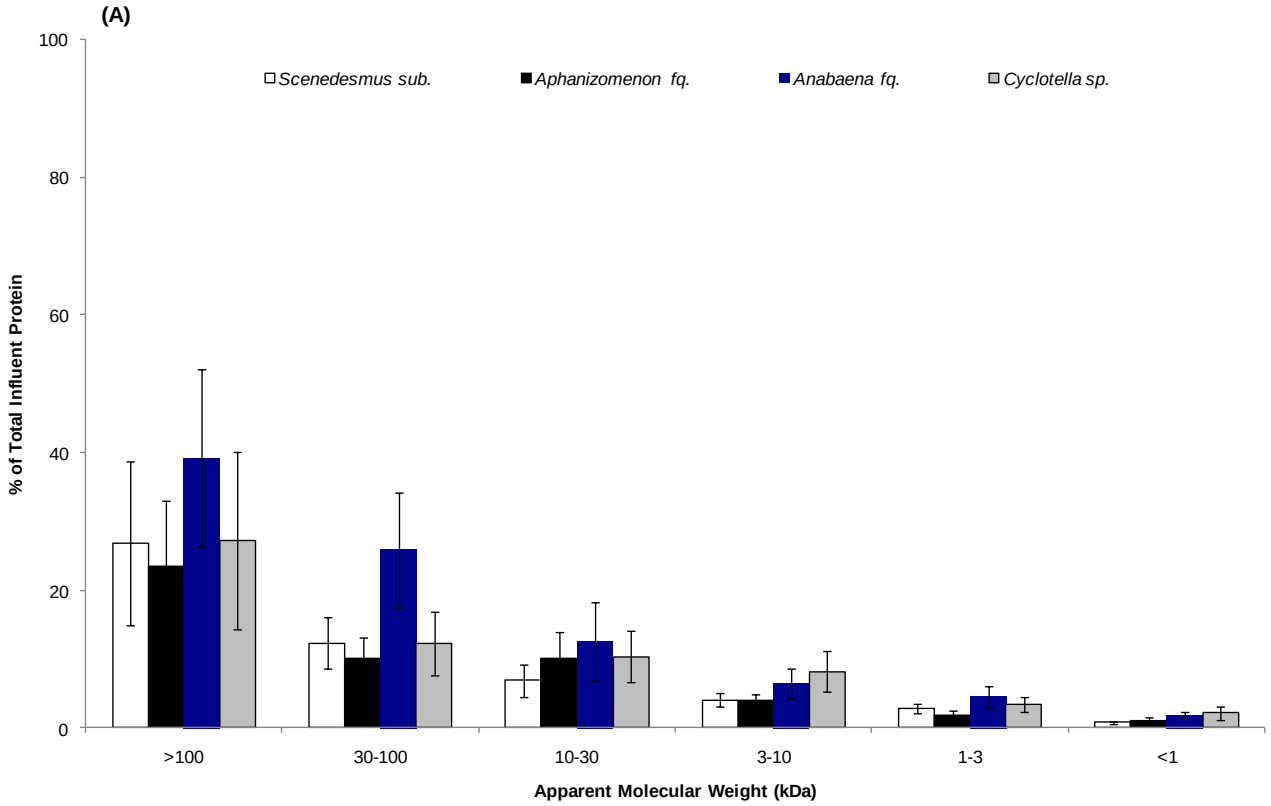

Figure 4.5 (A) Molecular weight UF membrane fractionation results for protein for SS-, AP, AN & Csp. -AOM.

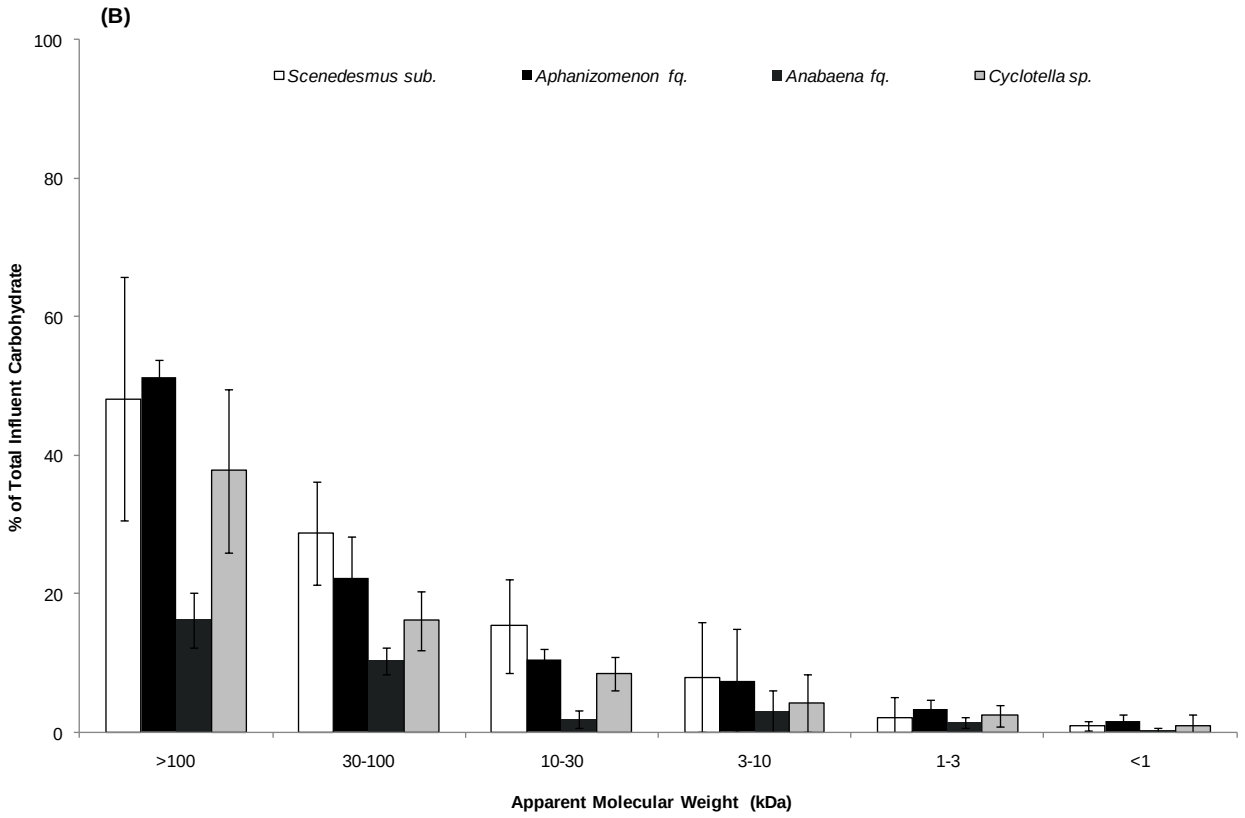

Figure 4.5 (B) Molecular weight UF membrane fractionation results for carbohydrate of SS-, AP, AN & Csp. -AOM.

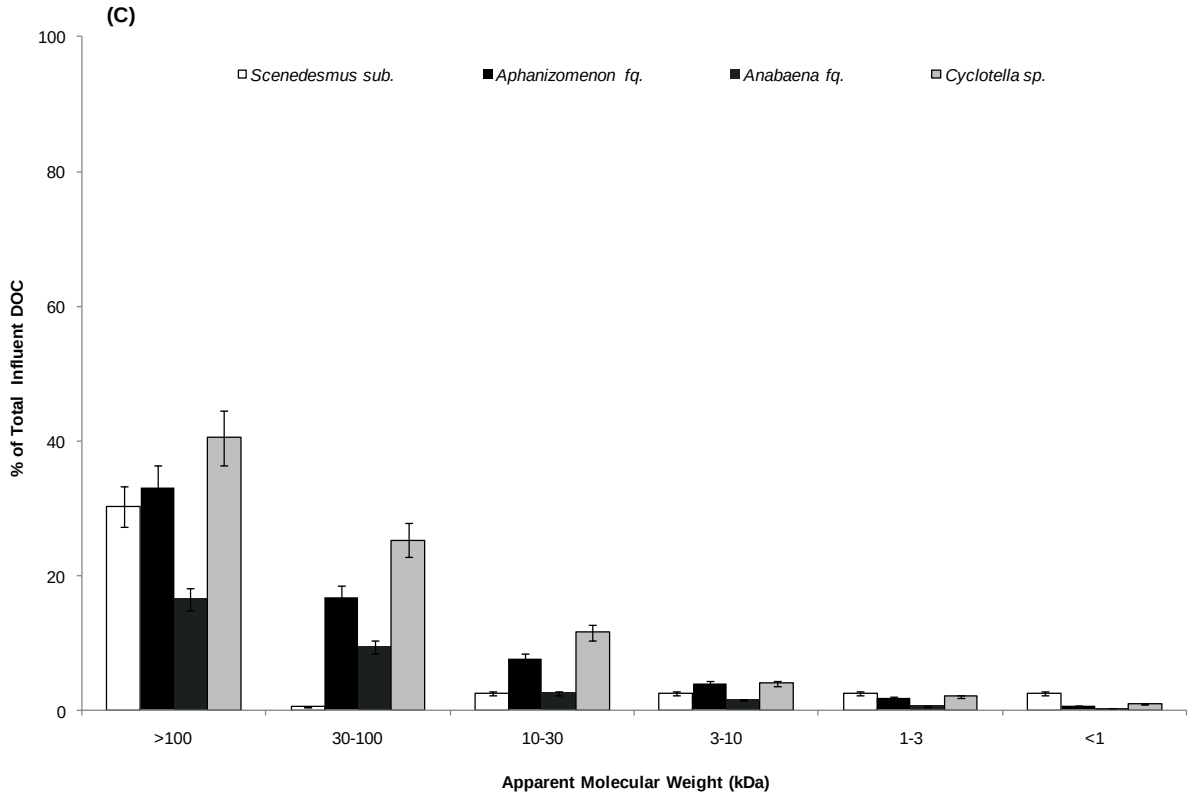

Figure 4.5 (C) Molecular weight UF membrane fractionation results for (c) DOC of SS-, AP, AN & Csp. -AOM. Results are presented as a percentage of the total influent respective parameter.

Table 4.2 Summary table of characterisation for each species in exponential growth phase.

	SS-AOM	AP-AOM	Csp-AOM	AN-AOM	*Chlorella vulgaris*, Henderson et al. 2008	*Microcystis aeruginosa*, Henderson et al. 2008	*Asterionella formosa*, Henderson et al. 2008
SUVA(l/m/mg)	1.18	0.79	0.67	0.34	1.29	1.65	1.7
Isoelectric point	3.1	3.2	1.7	2.4	1	1.9	0.9
Hydrophylicity (%)	54	63	62	81	60	59	73
Hydrophiobicity(%)	26	18	29	8	22	24	15
charge density (meq/g)	neg	neg	neg	neg	0.9	0.2	neg
carbohydrates/DOC (mg/mg)	1.21	0.9	1.21	0.34	0.9	1	
Trans-/hydrophilic carbohydrates (%)	57	77	84	90	65	64	58
Proteins/DOC (mg/mg)	1.48	0.99	0.9	0.52	0.53	0.4	
Proteins/carbohydrates (mg/mg)	1.23	1.1	0.74	1.52	0.58	0.3	
>500 kDa proteins/carbohydrates(mg/mg)	0.569	0.584	0.337	0.082			
>500kDa proteins/carbohydrates/hydrophobicity	0.018	0.031	0.013	0.01			
AOM >30 kDa	47	85	77	95			
AOM < 1 kDa	24	1	2	0.6			

Neg = negligible (<0.00001)

4.5 Discussion

Irrespective of species, AOM produced during exponential growth was seen to be made up of predominately large MW hydrophilic compounds that exerted no measurable charge demand (Table 4.2). Comparison across species and growth phase with other works indicate that growth cycle rather than species has the greatest influence of the overall properties of AOM. Comparison of exponential to stationary phase AOM reveals an increase in mass, charge and a decrease in MW as the age of the algae increases consistent with the concept that organic matter is shed off the surface of the algae during its life cycle (Hoyer *et al.*, 1985). The change is observed not by a removal of the large MW organics but by the generation of a bimodal distribution where both large and small MW compounds exist. The high molecular weight substances include sugars and uronic acids which break down with age. To clarify, sugar contents within the large MW sizes decreased from 26.9 to 2.2% for *Scenedesmus*, 13.5 to 9% for Chlorella and 19.1 to 12.0% for the cyanobacteria *Pseudoanabaena*. Respective reduction in uronic acids were 2.2 to <0.5%, 3.2 to <0.5% and 22.6 to 4.0% (Hoyer *et al.*, 1985; Henderson *et al.*, 2008a). This is also consistent with the lack of charge density in the AOM samples in the current study as charge in AOM is thought to be derived principally from charged polysaccharides such as uronic acids as they break down (Leppard, 1995).

Comparison of the AOM from the different algal species revealed the major differences in character to be associated with hydrophobicity, protein content and its associated characterisation. This is consistent with suggestion that in algal systems proteins govern

the hydrophobicity of AOM. Comparison can be made to the HPO of exo-polymeric substances (EPS) found in effluent organic matter which have been shown to be comprised predominately of protein, and not carbohydrate compounds such as amino acids with hydrophobic side groups (Jorand *et al.*, 1998). In such cases a link between decreasing protein:carbohydrate ratio in EPS, increasing charge density and decreasing hydrophobicity have been proposed (Morgan *et al.*, 1990). Unfortunately, the current data does not support this even in the case of the two cyanobacteria which again may link to community age where traditional EPS systems are equivalent to stationary phase rather than exponential growth phase algae. This is supported as equivalent data in stationary phase AOM of cyanobacteria supports the suggestion above and showed a similar link between protein to carbohydrate ratio and hydrophobicity (Henderson *et al.*, 2008b). The differences observed in the current study are significant in comparison to those reported between AOM from algae whilst in the stationary phase of growth where major differences were seen in charge density, hydrophobicity, protein:carbohydrate ratio and MW fractionation confirming that growth cycle is a critical factor in determining AOM character.

Analysis of this data in relation to water treatment suggests a number of features. The predominately high MW, low charge density of the AOM indicates that charge demand should be low (Edzwald, 1993). Although, coagulation efficiency may be limited given the lack of charged species, the increasing protein concentration is also known to impede coagulation (Tirado-Miranda *et al.*, 2003), and the fact that charge neutralisation is known to be more effective for AOM derived in stationary and endogeny growth phases (Konno, 1993). Further, the large MW compounds are likely to aid flocculation

(Bernhardt *et al.*, 1985) and so significant differences in floc properties should be expected as the age of the algae community changes.

The DOC concentrations of the AOM from all the algal species ranged from 5-12 $mg.L^{-1}$ which exceeds the standard 2 $mg.L^{-1}$ commonly reported during blooms (Henderson *et al.*, 2008a). When compared with previous studies on algal treatment it suggests that SS- AP- and Csp.-AOM should produce higher Trihalomethane (THM) levels during chlorination due to the higher protein contents (Scully *et al.*, 1988; Henderson *et al.*, 2008b) as well as producing much greater proportions of haloacetic acids and emerging nitrogen based DBPs. Overall, the varying character of AOM from different algae and in different growth phases means that each bloom will cause a unique set of challenges restricting the ability to infer generic algal derived issues.

4.6 Conclusions

The character of AOM derived from algae in their exponential growth phase can be described as being dominated by large MW hydrophilic polysaccharides and hydrophobic proteins that contain negligible charge. Key differences between species were observed in terms of hydrophobicity, protein content and it associated characterisation. However, greater difference was observed between AOM derived during exponential and stationary phase growth. Combining the different features indicates principle impacts are likely to be seen in relation to coagulation efficiency and the formation of DBPs.

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Chapter 5: The influence of frequency, power input and algal character on the efficacy of algal growth control using ultrasonics

Chapter 5: The influence of frequency, power input and algal character on the efficacy of algal growth control using ultrasonics

5.1 Abstract

This results in this chapter reports on the efficacy of ultrasonics on the control of four morphologically different problematic algal species from three algal divisions. *Microcystis aeruginosa* (unicellular) and *Aphanizomenon flos-aquae* (filamentous) are both cyanobacteria and *Scenedesmus subspicatus* (colonial) green algae with *Melosira* sp. a (filamentous) diatom were subjected to ultrasound of selected frequencies from 20-1144 kHz. *Microcystis aeruginosa* and *Scenedesmus subspicatus* best cell removal rates were 16±2% and 20±3% when treated with ultrasound of 862 kHz and energy of 133 and 67 kWh.m^{-3} respectively. *Aphanizomenon flos-aquae* best removal rate was 99±1% after 862 kHz and 133 kWh.m^{-3} of energy. *Melosira* sp. had it's best cell removal of 83% subsequent to ultrasound of 20 kHz and 19 kWh.m^{-3}. *Microcystis aeruginosa* and *Scenedesmus subspicatus* are considered non-susceptible species to ultrasound with *Aphanizomenon flos-aquae* and *Melosira* sp. being highly susceptible species to ultrasound. Morphological differences in shape and cell wall structure are possible sources for these differing levels of susceptibility to ultrasound.

5.2 Introduction

Algal blooms are a natural phenomenon defined by one or two principal algal species within a water body at concentrations above 20,000 cells.mL^{-1} (Bauer *et al.*, 1998; Albay and Akcaalan, 2003; Hoeger *et al.*, 2004). The specific blooming species vary considerably due to a complex set of environmental and biological drivers (Wetzel, 2001). However, in general cyanobacterial blooms predominate in summer due to their faster growth kinetics enabling them to out-compete the other species. However, in the colder spring months diatoms are more common and in autumn green species tend to dominate (Walsby, 1992; Henderson *et al.*, 2008a). Algal blooms cause serious deterioration in water quality resulting in an increase of water treatment costs, degradation of its recreational value, taste and odour issues and potential release of harmful algal toxins (Izaguirre *et al.*, 1982; Falconer, 1994). The physical and chemical characteristics of the different species varies across all major phyla and as such biological classification does not inform on treatment issues (Henderson *et al.*, 2008b). Examples of reported algae related treatment problems include: filter blockage and growth requiring extensive cleaning (Renton *et al.*, 1994; Bauer *et al.*, 1998), increased dissolved organic carbon (DOC) and pH resulting in coagulation problems (Johnson *et al.*, 1977) and an increase in disinfection by product formation (Bernhardt, 1984; Graham *et al.*, 1998). The overall impact is either a reduction in water production capacity and/or an increase in treatment costs with estimates suggesting an increase of around 25% in a UK water utility company.

Improvements in the understanding of how the physical character of algae affect treatment can offset some of these problems and enable the development of new processes (Henderson *et al.*, 2008b; Henderson *et al.*, 2008c). However, source control remains the most likely method to be effective in preventing the issues surrounding algal blooms. Several methods have been developed and implemented to achieve this including: nutrient diversion, artificial destratification, hypolimnetic aeration/withdrawal, sediment oxidation/removal, phosphorus precipitation, and biomanipulation (Wetzel, 2001). In addition techniques such as barley straw which produces polyphenols which have a detrimental effect on algal growth but ultrasonics act directly on the algae inhibiting growth (Lee *et al.*, 2001; Murray *et al.*, 2010).

Ultrasonic systems work by concentrating the energy of sound waves through acoustic cavitation causing the compression, rarefaction, and finally implosive collapse of bubbles leading to intense but short-lived local heat (5000 °C) and pressure (2000 atm) (Suslick, 1990). To date, these characteristics have been successfully utilized in a number of applications including: cleaning, cell disruption, emulsification, medical surgery and dispersion of solids (Mason, 2007). The parameters which influence the efficacy of ultrasound are frequency, power and liquid characteristics (Mason, 2000). When frequency is increased the rarefaction of the wave is decreased such that to maintain the same amount of cavitation effect amplitude/power must be increased leading to a preference for low frequency systems in water applications to reduce costs. Interest in utilising ultrasonic systems in the water industry has considered both water and wastewater applications. For example, in the case of wastewater treatment a 20 kHz, 1 kW unit has been shown to successfully reduce excess sludge production by

88% (Minervini, 2008) and release dissolved organic materials which can be used to enhance biological phosphorous removal (Kampas *et al.*, 2007) and anaerobic treatment (Lester *et al.*, 2009). Similarly, investigations into the application of power ultrasound for bacterial control have shown kill rates of up to 85% for the gram positive *Bacillus subtillus* (Mason *et al.*, 2003). In such cases the ultrasound operates in flow loops with rates around 10 $L.min^{-1}$ per unit. Treatment has been shown to follow a two stage pathway where initial de-aggregation occurs prior to high kill rates being observed (Mason *et al.*, 2003; Mason, 2007).

In relation to algae, reported control mechanisms include: disruption of gas vesicles, inhibition of photosynthesis and production of free radicals (Lee *et al.*, 2001). To date, promising results have been reported for field case studies where application of a 40 W device operating at a frequency of 28 kHz produced a statistically significant reduction in total algal growth measured in terms of a reduction in chlorophyll a (Inman, 2004). This is complimented by results at bench scale which have shown a reduction of 60% in the re-growth of *Scenedesmus capricornutum* (Mason *et al.*, 2003) and a 90% reduction in the cell numbers of *Microcystis aeruginosa* for both 20 kHz and 1.7 MHz (Tang *et al.*, 2003; Hao *et al.*, 2004a; Hao *et al.*, 2004b; Tang *et al.*, 2004).

Whilst the potential for ultrasonic control of algae has been demonstrated a number of questions remain. The vast majority, over 90%, of all reported studies have focussed on *Microcystis* but the widespread application of ultrasound for other species is unclear. Further, impact of operating conditions and in particular the energy input required remains largely unreported. Consequently, the investigation reported in this chapter addresses these questions by investigating the efficacy of ultrasonic control of algae at

different frequencies and power input levels for four common blooming species which covers a cross section of the physical types of algae that can occur.

5.3 Materials & Methods

5.3.1 Laboratory experimental conditions

Microcystis aeruginosa (CCAP 1450/15), *Aphanizomenon flos-aquae* (CCAP 1401/1) and *Scenedesmus subspicatus* (CCAP 276/20) culture was obtained from Culture Collection of Algae and Protozoa (CCAP), Oban, Scotland. The specific strain of *Microcystis* is non toxin forming which was required to comply with health and safety guidelines within the laboratories. *Melosira* sp. (JA72) was obtained from Sciento, Manchester. *Microcystis aerg. Aphanizomenon fq. & Scenedesmus sub.* were grown under sterile conditions in Jaworski media, (JM) with *Melosira* sp. grown in diatom media, (DM) and all species were shaken during culturing except *Melosira* sp. All cultures were grown in Erlenmeyer flasks 1-3 L at 18-21 ºC on a Patterson Scientific Bibby Stuart SO1 shaker. All species were grown under 16-8hr light/dark cycle radiation provided by two bulbs: 1 Sun-Glo and 1 Aqua glow 30 W fluorescent light. The light was dimmed slightly using neutral density filters to encourage higher density of growth. Cell concentration for experiments was $4\text{-}6\times10^{6}$ cells.mL^{-1}. Cultures were maintained for 10-30 days before the experiments depending on the species. The cultures were maintained in exponential growth phase during all experiments.

Laboratory experiments were performed using an Ultrasonic Probe, Model Virsonic Digital 600 and Ultrasound Multi-frequency unit, Model MGFL Meinhardt. The Meinhardt multi-frequency unit emits three frequencies, 1144 kHz, 862 kHz & 582 kHz, with power of up to 200 W. Refer to Appendix 5 for detailed calorimetric calculations of for the 20 kHz Ultrasonic Probe and the Ultrasound Multi-frequency unit, Model MGFL Meinhardt for frequencies 582,862 and 1144 kHz. The probe emits a frequency of 20 kHz and power of up to 600 W. Sample volumes of 1500 mL were used for *Microcystis aerg*., *Scenedesmus sub*., *Aphanizomenon fq*. and *Melosira* sp. Exposure time ranged from 5 to 500 seconds at each frequency already listed and up to 1 hr exposures at 862 kHz and 200 W of power was used for all experiments.

Temperature control was maintained within 2^{o}C of the normal growth conditions of the species ~23^{0}C throughout the experiments using a temperature controlled chiller unit when necessary. Normal growth conditions of the sonicated samples were maintained subsequent to the ultrasound treatment except for the addition of shaking the culture once a day for 1-12 days. Samples were taken every 24-48 hrs. Analysis of samples was performed using a haemocytometer for cell counts, and also a spectrophotometer model SpectraMax Plus 384 with a UV/visible detector 190-1000 nm, to detect cell number at 684 nm (Zhang *et al.*, 2006a; Zhang *et al.*, 2006b). Chlorophyll fluorescence measurements were performed using a fluorometer model Gemini EM at emission 645-665 nm and excitation of 635 nm, which varied based on the natural chlorophyll fluorescence of the species in question. Specifically defined hand counts were performed for *Melosira* sp. and *Aphanizomenon fq*. to quantify for levels of cell damage Firstly intact cells i.e. 10 cells or more in a filament, secondly broken cells were defined

as a filament with <10 cells and damaged, the third category was lysed cells, for *Melosira* sp. which were empty of cell contents but pieces of silica exoskeleton were still visible. Results were normalised and expressed cell number as percentage. Olympus confocal transmission microscope was used with an Olympus DP20 (2 mega-pixel) digital camera to take microscope images at magnification of 40x. Samples were taken directly after every time point, all time points were performed in triplicate with all samples including controls also in triplicate. Electrical energy calculations were performed using the following conversion, time in seconds (t), power in watts (W), volume in litres (v). (t×W)/v = J/L (Joules/Litre). 3600 Joules = 1 kWh, 3600/L=0.000278, (m^{-3}) = Litre (J/l)×0.000278 = $kWh.m^{-3}$.

For calorimetric calculations Refer to (Appendix 5) for the 20 kHz Ultrasonic Probe and the Ultrasound Multi-frequency unit, Model MGFL Meinhardt for frequencies 582,862 and 1144 kHz.

5.4 Results

5.4.1 Algal Morphology

Two unicellular: *Scenedesmus sub*. and *Microcystis aerg*. and two filamentous: *Melosira* sp. and *Aphanizomenon fq*. species of algae were used throughout the trials (Figure 5.1). *Scenedesmus sub.* (Figure 5.1a) can be unicellular or colonial but typically remains unicellular in laboratory conditions as observed during the current

investigation. The cells were oval shaped with a long axis length of 8-10 µm. *Scenedesmus sub.* is a typical species of the green division of algae which are well known as a bloom forming species particularly during the summer months, causing increased chlorophyll a and coagulant demand (van den Hoek, *et al.*, 1995). *Microcystis aerg*. (Figure 5.1b) is a unicellular and colonial cyanobacteria which is circular in shape with a diameter of between 5-10 µm within which gas vacuoles exist to enable vertical movement within any water column. *Melosira* sp. (Figure 5.1c) is a pennate filamentous diatom with a measured cell length between 10-30 µm made up of 5-100 cells. The cell wall is known as a frustule and is composed of silica (Round *et al.*, 1990; van den Hoek *et al.*, 1995b). *Melosira sp.* tends to predominate during colder periods of the year and is known to be a substantial problem at filtration plants during blooms as dissolved air flotation appears relatively ineffective and the filaments blind the surface of the filter bed (van den Hoek *et al.*, 1995b; Bauer *et al.*, 1998). *Aphanizomenon fq*. (Figure 5.1d) is a filamentous multicellular colonial cyanobacteria that is non toxin forming and contains gas vacuoles for buoyancy. Individual cells lengths were measured between 5-8 µm with colonies of up to 160 µm containing between 4 to 80 cells per filament (Canter-Lund *et al.*, 1995; van den Hoek *et al.*, 1995a).

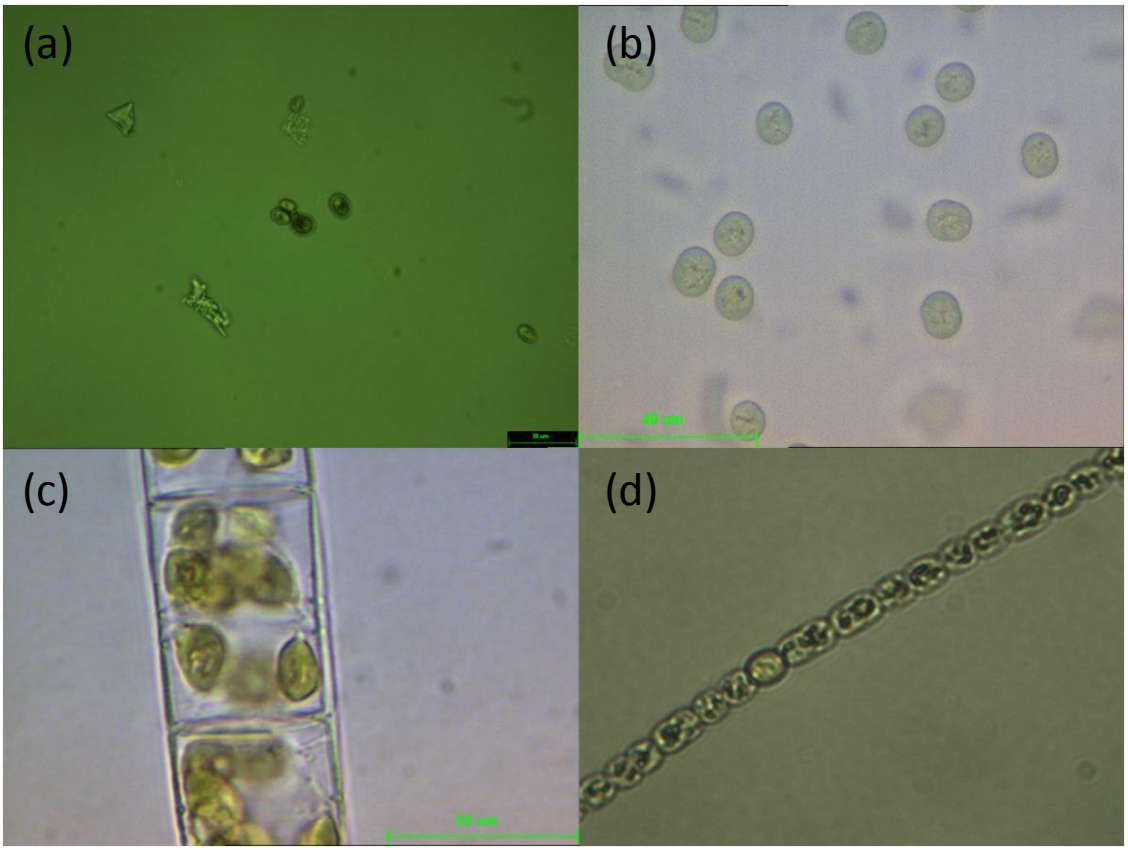

Figure 5.1 Algal species tested: *Scenedesmus sub.*(a), *Microcystis aerg.(*b), *Melosira* sp.(c), *Aphanizomenon fq.*(d).

5.4.2 Removal

Two significantly different responses were observed across the four algae types as the energy input was increased from 0.19 to 133 kWh.m^{-3} at frequencies of 20-1144 kHz (Figure 5.2). In the case of the unicellular species, maximum removal of 16±2% and 20±3% were observed for *Microcystis aerg*. and *Scenedesmus sub*. respectively at corresponding energy inputs 133 and 67 kWh.m^{-3}. Removal was observed to increase in relation to the energy input as shown in the case of *Microcystis aerg*. with removal rates

of -1.3±5.6, 0.8±7.4, 5±7.9 and 15.7±2% at energy inputs of 0.19, 1.9, 19 and 133 $kWh.m^{-3}$. Cell removal rates of below 20% are considered in this study to be classed as un-effective as they will have no impact in the treatment works and as such both species are defined as non susceptible to ultrasound. In contrast in the case of the two filamentous species a large increase in removal was observed as the energy input increased (Figure 5.2). To explain, in the case of *Aphanizomenon fq.*, removal rates of -6±5, 35±5, 56±5, 91±3, 88±6, 96±3, 98±3, 99±1% were observed as the energy input increased from 0.19, 1.9, 11, 19, 22 ,44, 67, 133 $kWh.m^{-3}$. The results indicate a threshold value beyond which limited additional removal occurs. In the case of *Aphanizomenon fq.*, this was observed to be around 19 $kWh.m^{-3}$. Previous studies using laboratory grown bacterial species generally found a high susceptibility to ultrasound. In most studies they achieve over 80% cell removal using energy of 0.001-10000 $kWh.m^{-3}$ using monocultures of bacteria (Figure 5.2). Comparison with field samples of heterogeneous species of coliforms, *E-coli*, and heterotrophic bacteria indicates greatly reduced impact with observed removal of only between 7.8% and 34% with the application of energy rates ranging between 750-800 $kWh.m^{-3}$, indicating that mixed cultures of bacteria are more difficult to kill using ultrasound.

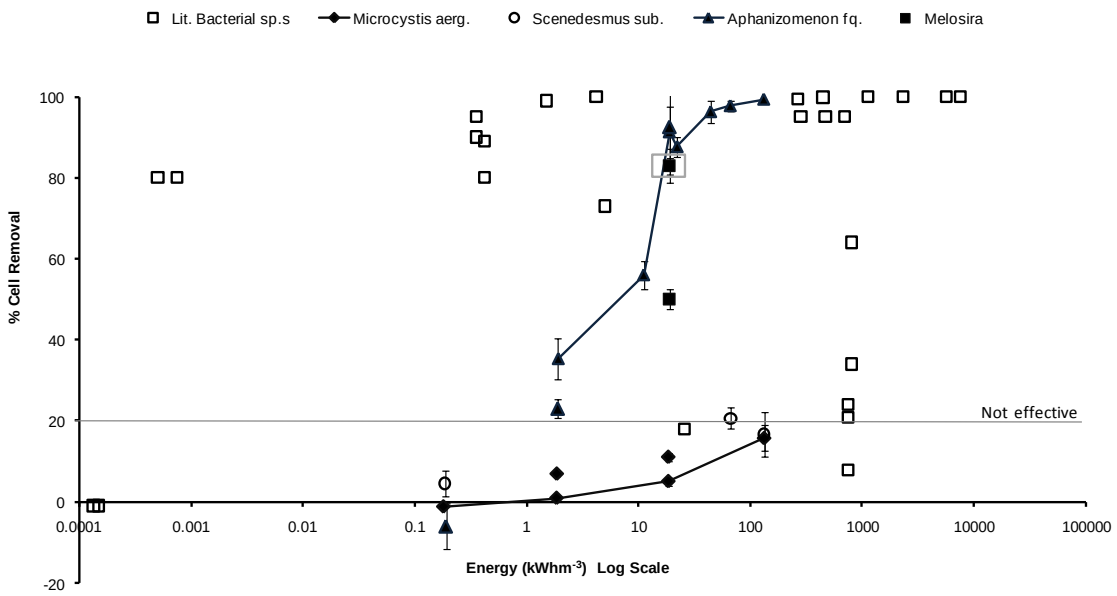

Figure 5.2 Cell removal as a function of energy during ultrasonic irradiation of *Microcystis aerg.*, *Scenedesmus sub.*, *Aphanizomenon fq.*, *Melosira* sp. and literature data on bacterial species frequencies ranged from 20-1144kHz.

Increase of ultrasound frequency beginning at 20 and increased to 582 and then 862 with the final frequency of 1144 kHz, produced three different types of response in the algal species tested. In the case of *Microcystis aerg.* no statistical difference in removal could be observed when comparing operation at the different frequencies. To demonstrate, removal rates of 11±4%, 11±4%, 16±7% and 10±7% were observed at frequencies of 20, 582, 862 and 1144 kHz respectively (Figure 5.3). This confirms previous trials (Hao *et al.*, 2004b; Zhang *et al.*, 2006a) and indicates that removal can be insensitive to changes in frequency for some species. In comparison, a slight but not significant increase in cell removal (due to results falling with the error margin of 10%)

was observed for *Scenedesmus sub*. with removal rates of 2.5±6, 8±5, 20±3 and 14±8% at frequencies of 20, 582 862 and 1144 kHz, respectively. In contrast, frequency was observed to have a large impact on the removal of the filamentous algae although the trends were in opposite directions (Figure 5.3). In the case of *Aphanizomenon fq*. removal rates increased from 48±5%, 72±4%, 91±3% to 93±7% as the frequency increased from 20, 582, 862 to 1144 kHz respectively. Whereas removal of *Melosira* sp. followed the opposite trend whereby removal was greatest at the lowest frequency, with removal rates of 83, 50, 11 and -6% at frequencies of 20, 582, 862 and 1144 kHz respectively (Figure 5.3). In comparison, removal of the algae *Spirulina* (*Arthrospira*) *platensis* was shown to follow a frequency ranking of 200 kHz > 1700 kHz >20 kHz Removal was reported to be unchanged at around 43-48% with power levels that ranged from 20-80 W suggesting that frequency was more important than power (Hao *et al.*, 2004a). However, when converted to energy input these power levels equate to 2.78-8.34 $kWh.m^{-3}$ which is consistent with the curves reported here. Further, Tang *et al.* (2003) reported a 60% loss of biomass when applying 1700 kHz ultrasound to the system again suggesting with filamentous cyanobacteria that higher frequencies are more effective. In the case of *Melosira* sp. treated with an ultrasonic input of 19 $kWh.m^{-3}$ more physiological damage was observed to occur at the lower frequencies of 20 and 582 kHz compared with the higher frequencies of 862 and 1144 kHz (Figure 5.4). At the lower frequencies severe cell damage in the form of cell wall rupture and lysis can be observed whereas at the higher frequencies cell joint breakage was more commonly seen. Quantification of the percentage change in cell physiology compared to the control revealed cell breakage levels of 21±2, 10±2, 4±0.5 and 9±1% and cell lysis

levels of 71±5, 57±6, 48±5 and 9±4% as the frequency increased from 20, 582, 862 to 1144 kHz respectively (Figure 5.5).

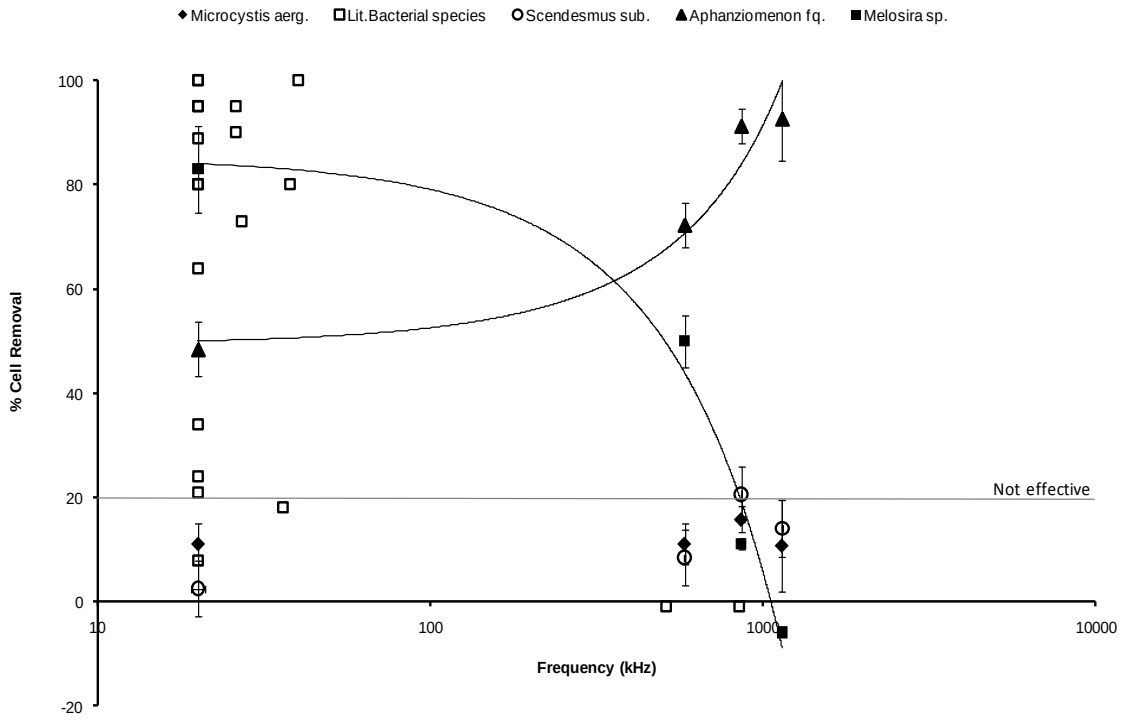

Figure 5.3 Cell removal expressed in percentage as a function of frequency during ultrasonic irradiation of *Microcystis aerg*., *Scenedesmus sub*., *Aphanizomenon fq*., *Melosira* sp. and bacterial species from the literature.

In contrast, in the case of *Aphanizomenon fq.* more cell lysis was observed at frequencies of 862 and 1144 kHz compared to that at 20 or 582 kHz. To show, cell lysis rates increased from 48±5, 72±4, 91±3 and 93±7% as frequency increased from 20, 582, 862 and 1144 kHz respectively, while the intact cells decreased from 52±5, 28±3, 9±2 and 7±1% respectively at the same frequencies (Figure 5.5).

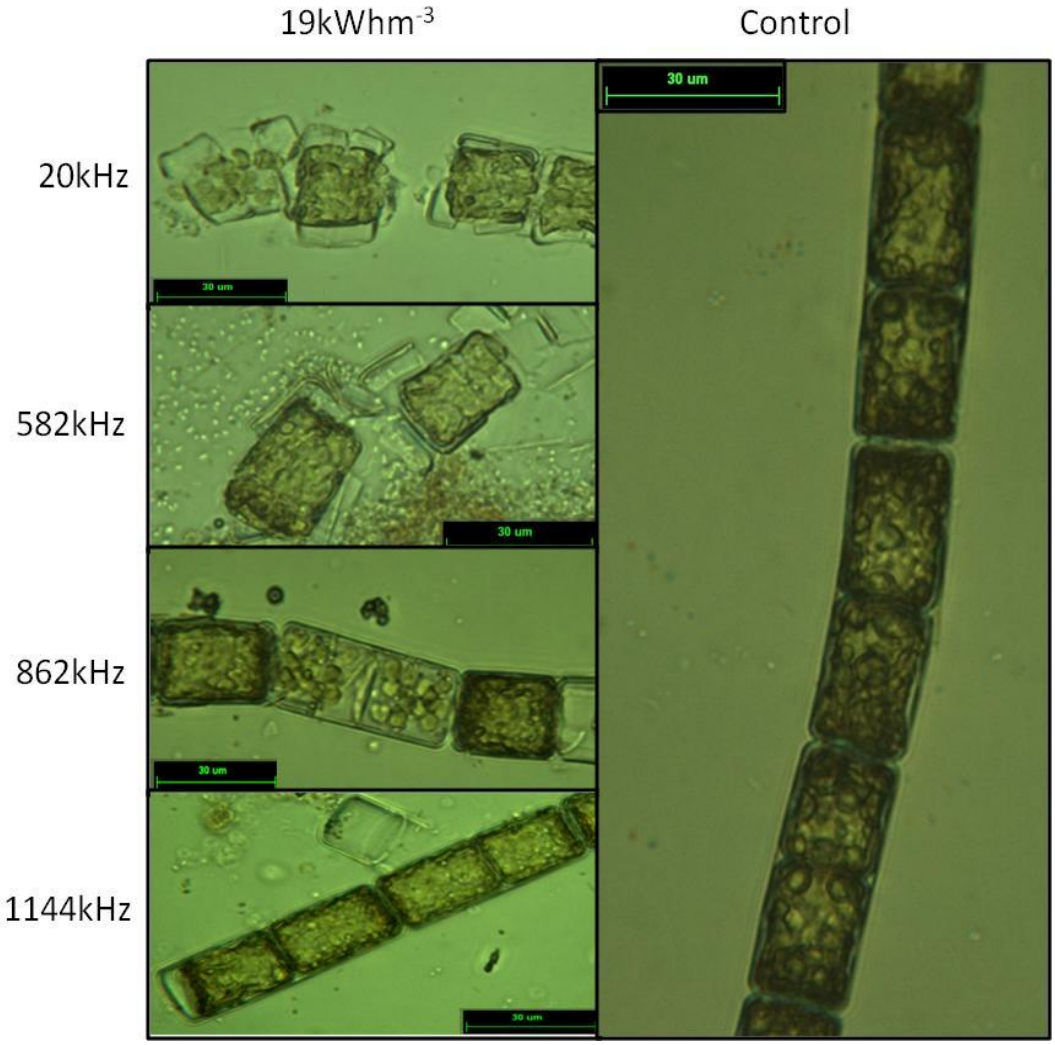

Figure 5.4 *Melosira* sp. after treatment using 19 kWh.m^3 and frequencies of 20, 582, 862 and 1144 kHz in sequence with control image on right for comparison.

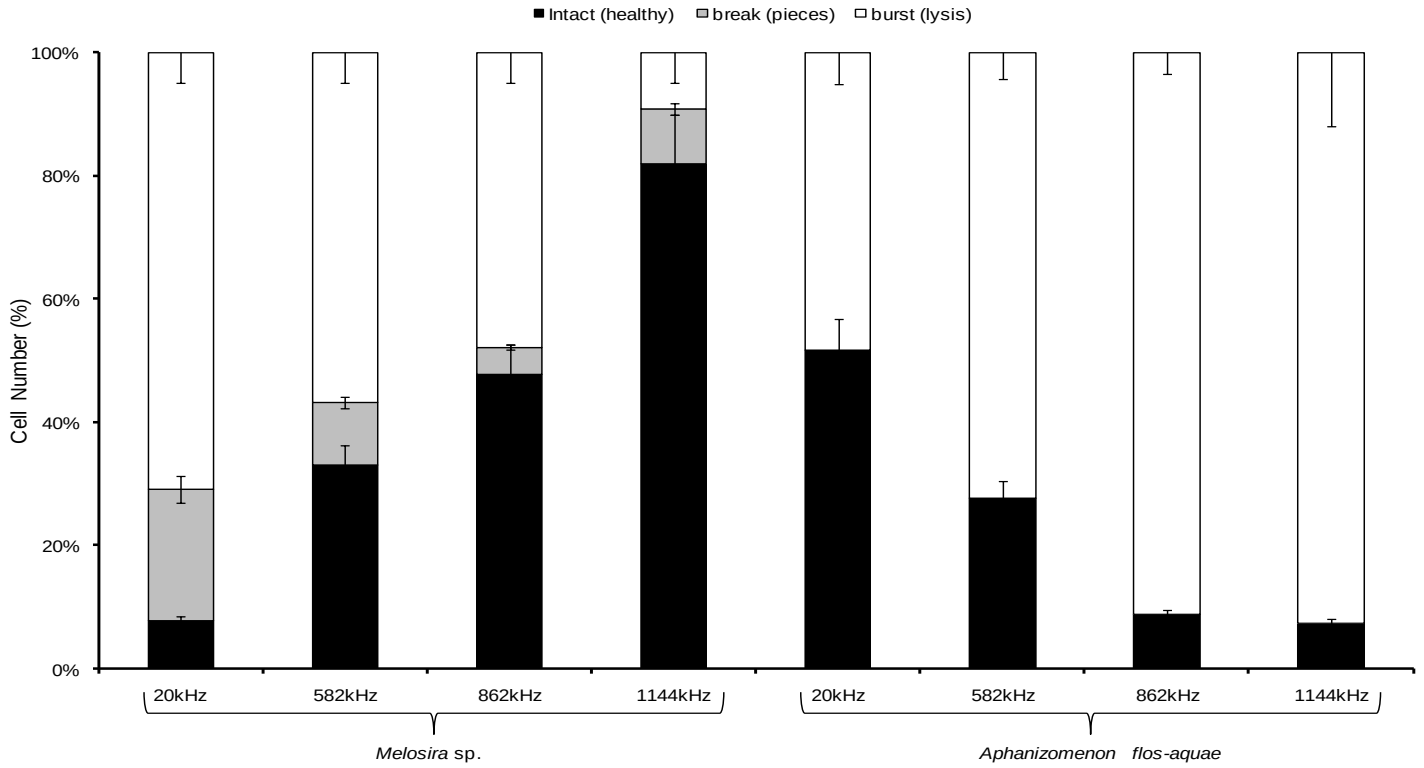

Figure 5.5 Cell damage expressed as % damage of control against all frequencies tested (20,582, 862 and 1144kHz) using energy of 19 kWh.m^3 for *Melosira* sp. and *Aphanizomenon fq*.

5.4.3 Photosynthetic activity

Ultrasonic irradiation caused more significant impact to photosynthetic activity compared to cell removal for the colonial/unicellular algal species tested (Figure 5.6). To quantify, maximum reductions were observed at 133.4 kWh.m^{-3} and a frequency of 862 kHz which resulted in a reduction of chlorophyll fluorescence of 37±3% and 60±5% for *Scenedesmus sub*. and *Microcystis aerg.* respectively. The corresponding cell removal levels were 17±5% and 16±3% respectively indicating that ultrasound is more effective at damaging photosynthetic apparatus than killing the cell overall. The impact of such photosynthetic damage is growth retardation slowing down the development of the bloom. Similar results have been observed in terms of photosynthetic inhibition with levels around 40-50% commonly reported (Lee *et al.*, 2001; Zhang *et al.*, 2006b), although these have occurred at both low and high frequency. In the case of *Aphanizomenon fq*. maximum impact was observed at 1144 kHz using 19 kWh.m^{-3} energy which resulted in a 93±7% reduction in cells and a 100% loss of chlorophyll fluorescence. In all other regards the trends were similar to the cell numbers but with a larger reduction in chlorophyll fluorescence observed up to the point where near complete cell removal was recorded. The difference between cell count and chlorophyll fluorescence measurements reported here also explains some of the discrepancies in reported removal with values based on chlorophyll fluorescence always providing an overestimate of performance. Previous studies have shown complimentary findings where the photosynethic activity of the filamentous cyanobacteria, *Spirulina platensis*, was reported to be reduced by 50% at 1700 kHz compared to 20 kHz (Hao *et*

al., 2004b). Research on *Microcystis aeruginosa* has shown that the impact may be even more specific corresponding to reductions of 21.3% chlorophyll a and 44.8% phycocyanin upon application of 20 kHz and using 6.7 $kWh.m^{-3}$ (Zhang *et al.*, 2006a).

5.5 Discussion

The current paper has shown that ultrasound is only effective on only certain species of algae. In particular *Aphanizomenon fq.* (filamentous cyanobacteria) and *Melosira* sp. (filamentous diatom) were significantly affected by ultrasound whilst *Microcystis aerg.* (unicellular cyanobacteria) and *Scenedesmus sub*. (unicellular green) were resistant to ultrasound indicating that traditional biological classification of algae is not a good indication of algae susceptibility to ultrasonic control. Instead, morphological factors appear to be the dominant factor where filamentous species are more susceptible to ultrasound than unicellular species. The key mechanism appears to be breakage of filament structure and/or the cell wall but this occurs at different frequencies for two algal species suggesting different pathways.

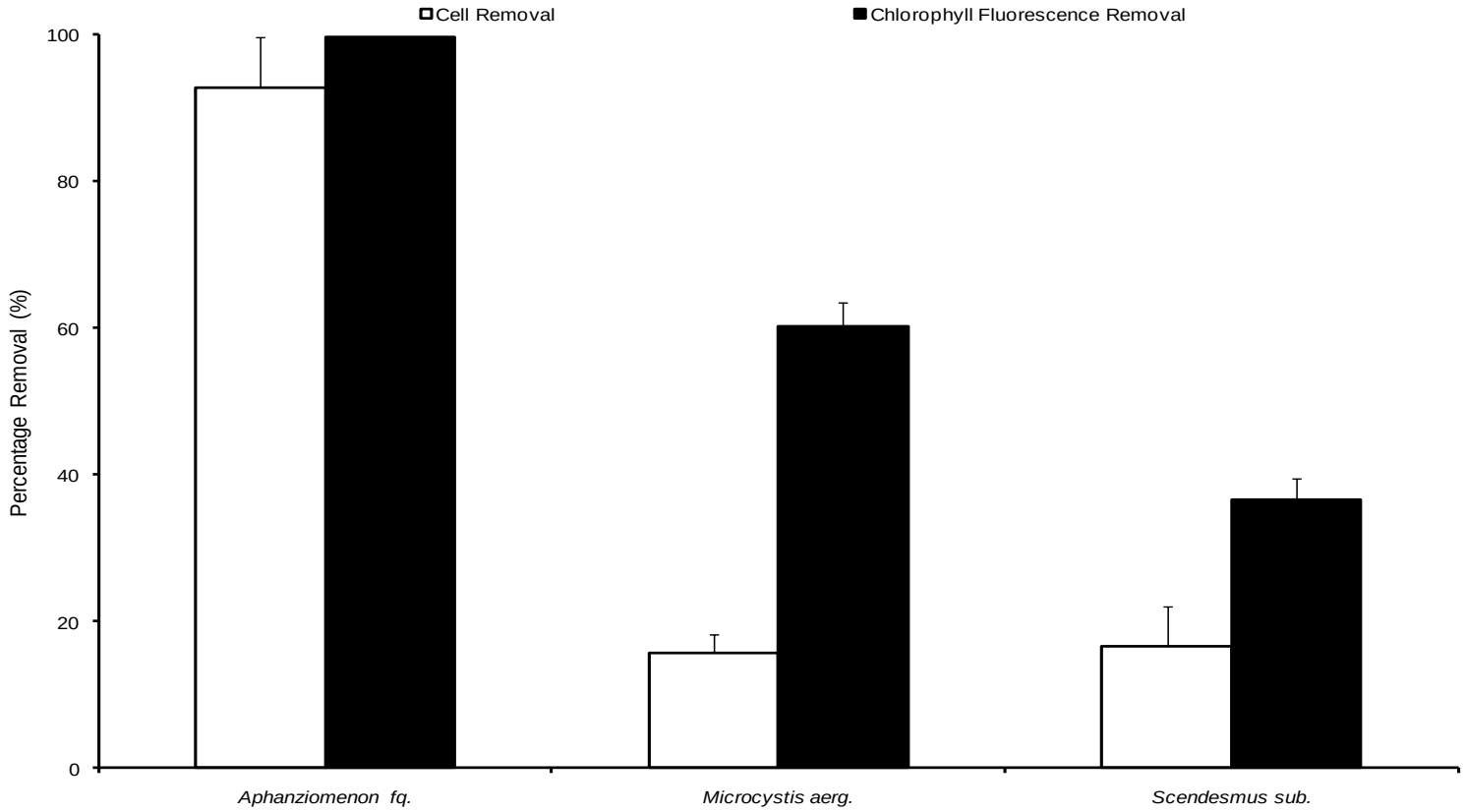

Figure 5.6 Best cell removal and chlorophyll fluorescence removal percentages for *Aphanizomenon fq*. (filamentous cyanobacteria) *Microcystis aerg.* (unicellular cyanobacteria) and *Scenedesmus sub*. (unicellular green alga).

In the case of *Melosira* sp. maximum effectiveness was observed at low frequencies where cavitation is maximized for any given energy input suggesting that the mechanism is more destructive compared to the mechanism for the other algal species. Visual observation of the algal filaments suggested that for lysis to occur the silica frustules of the diatom had to be shattered. Direct measurement of the force required to break the exoskeleton of other diatoms ranges include 750 μN (100–700 tonnes.m^{-2}) for *Fragiariopsis kerguelensis* and 90 μN *Coscinodiscus granii*. The observed range is accounted for by the silica content and differing structure between centric and pennate diatoms within the cell walls with the higher forces required for *Fragiariopsis kerguelensis,* linked to an evolutionary need for protection from predation (Hamm *et al.*, 2003). In contrast, maximum effectiveness in the case of *Aphanizomenon fq.* was observed at higher frequencies where the rarefaction and compression cycles become much shorter reducing the impact of cavitation. However, the size of the formed bubbles is such that they vibrate at the same resonance frequency as the gas vacuoles within the cells leading to bursting of the gas vacuoles with subsequent damage to photosynthetic apparatus (Lee *et al.*, 2001; Tang *et al.*, 2003). Additionally, the microbubble streaming action caused by the acoustic cavitation has a detrimental effect on the links between the cyanobacterial cells thereby increasing the likelihood of filament breakage (Nyborg, 1982; Suslick, 1988; Suslick, 1990).

The major differences in terms of the two species are that the cell wall of *Aphanizomenon fq.* is a four layer peptidoglycan embedded in a mucilaginous sheath compared to a more rigid silica based exoskeleton and that *Aphanizomenon fq.* contains

gas vacuoles (Round *et al.*, 1990; van den Hoek *et al.*, 1995a; van den Hoek *et al.*, 1995b). Consequently, the more flexible cell wall of the cyanobacteria appears less susceptible to being broken and that in this case, breakage of the inter-cellular joints appears more important. Such internal cell disruption will be enhanced by the implosion/damage of gas vacuoles within the cells which is known to occur at higher frequencies due to its closer resonance frequency to the gas vesicles (Lee *et al.*, 2001; Tang *et al.*, 2003; Hao *et al.*, 2004b; Zhang *et al.*, 2006a). This would be consistent with the large differences seen in reduction of photosynthetic activity compared to cell numbers in the case of *Microcystis aerg*. However, if damage is confined to just bursting gas vacuoles then growth recovery in cyanobacteria is inevitable, as disrupted gas vesicles regenerate in a short time (Jong Lee *et al.*, 2000). This has been observed in a number of studies where reduction in *Spirulina platensis* of 70% at 1700 kHz (Tang *et al.*, 2003), and 44% at 20 kHz (Hao *et al.*, 2004b), recovered within a week. At present there has been no establishment of how many times gas vacuoles can re-generate post-ultrasound treatment. Therefore based on the present knowledge cell removal requires more than just loss of photosynthetic activity but requires breakage of the filaments so that the intracellular material can no longer be contained to cause complete cell lysis. Overall, whilst ultrasound appears suitable for the control of filamentous species scale up for the experiments here suggest that its application is unlikely to be financially viable. Significant reduction in cell numbers started at approximately an energy input of 10 $kWh.m^{-3}$. Given that typically total water treatment and supply energy consumption is less than 1 $kWh.m^{-3}$. 7 pence is the cost of 1 $kWh.m^{-3}$ in the U.K. (Fletcher, 2009) so therefore ultrasound at present requires energy levels which are too high. However,

given an economy of scale, such that larger the volume of water being treated the lower the overall cost of treatment, if only a bloom occurs on part of the reservoir then the cost may be far less, which is likely (Suzenet, 2009), then the application of ultrasound in reservoirs may be suitable for the most susceptible species like *Melosira* sp. and *Aphanizomenon fq*. In such cases it would appear preferential to employ ultrasonics in a short term capacity during the very early stages of the bloom when cell numbers are below $4x10^5$ cells.mL^{-1} as the cells are dividing rapidly and are most sensitive to sonication (Ahn *et al.*, 2003; Zhang *et al.*, 2006b). This approach would keep the population number within a controllable level avoiding a full scale bloom event thereby offsetting excessive energy or chemical costs necessary for algal removal within a water treatment works (WTW). However, economic application of ultrasound appears likely for only filamentous algae and that different frequencies are required depending on the blooming species application require careful consideration of the likely blooms.

5.6 Conclusions

Laboratory trials of ultrasonic treatment of algal blooms has revealed that the technology is only effective against filamentous species and will not significantly impact on any unicellular/colonial species such as *Microcystis aerg*. Cell removal levels of up to 99% for *Aphanizomenon fq*. and 83%±5 for *Melosira* sp. at 19 and 133 kWh.m^{-3} were observed, respectively. The two susceptible species were most effectively removed at different ends of the frequency range indicating different control mechanisms. In the case of *Melosira* sp., removal was most effective at lower

frequencies and this was attributed to the rigid silica exoskeleton requiring cell wall rupture to achieve removal. Whereas in the case of *Aphanizomenon fq*. removal was best at high frequencies due to the need to break the filament structures which was aided by gas vacuole bursting which occurs at higher frequency.

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Appendix: Chapter 5

Appendix 5.0 Calorimetry Readings and Power Calculations for Ultrasonic Equipment

Appendix 5.1 Results from experiment: Ultrasonic 20 kHz Probe Model Virsonic Digital 600 using 1500ml of de-ionised water in the 2L Pyrex Beaker. (Power level 4).

Time (sec)	Temp °C	Temp °C	Temp °C	Average	Standard deviation	Standard error
0	20.8	20.8	20.7	20.7667	0.0577	0.0333
10	20.8	20.9	20.7	20.8000	0.1000	0.0577
20	20.8	20.9	20.8	20.8333	0.0577	0.0333
30	20.8	20.9	20.8	20.8333	0.0577	0.0333
40	20.8	20.9	20.9	20.8667	0.0577	0.0333
50	20.8	20.9	20.9	20.8667	0.0577	0.0333
60	20.9	21.0	20.9	20.9333	0.0577	0.0333
70	21.0	21.0	21.0	21.0000	0.0000	0.0000
80	21.0	21.0	21.0	21.0000	0.0000	0.0000
90	21.1	21.1	21.1	21.1000	0.0000	0.0000
100	21.1	21.1	21.1	21.1000	0.0000	0.0000
110	21.2	21.1	21.1	21.1333	0.0577	0.0333
120	21.3	21.2	22.2	21.5667	0.5508	0.3180
130	21.3	21.2	22.2	21.5667	0.5508	0.3180
140	21.3	21.2	22.2	21.5667	0.5508	0.3180
150	21.3	21.3	22.3	21.6333	0.5774	0.3333
160	21.3	21.3	22.3	21.6333	0.5774	0.3333
170	21.3	21.4	22.3	21.6667	0.5508	0.3180
180	21.4	21.4	22.4	21.7333	0.5774	0.3333

Power = solvent mass x specific heat capacity x temperature rise per second

Specific heat capacity of water = 4.19J/(g x ºC)

Volume 1500ml (1500g)

P = 0.2336 ºC x 4.19J/(g x ºC) x 1500g

P = 'c' x cp x m

P = 1468.4486

I = W/cm²

I = 1468.44/ 1000

I = 1.46844 W / cm³

Appendix 5.2 Results from experiment: 582 kHz – (power setting 200W) in 1500ml of de-ionised water in the 2L Pyrex custom made vessel Unit and Glass reaction vessel of the Ultrasound Multi-frequency unit, Model MGFL Meinhardt.

Time (sec)	Temp ^{o}C	Temp ^{o}C	Temp ^{o}C	Average	Standard deviation	Standard error
0	21.5	21.6	21.1	21.4000	0.2646	0.1528
10	21.7	21.6	21.3	21.5333	0.2082	0.1202
20	21.7	21.8	21.3	21.6000	0.2646	0.1528
30	21.9	21.9	21.4	21.7333	0.2887	0.1667
40	21.9	21.9	21.4	21.7333	0.2887	0.1667
50	22.1	22	21.6	21.9000	0.2646	0.1528
60	22.1	22.1	21.8	22.0000	0.1732	0.1000
70	22.2	22.2	21.8	22.0667	0.2309	0.1333
80	22.3	22.2	21.9	22.1333	0.2082	0.1202
90	22.3	22.2	21.9	22.1333	0.2082	0.1202
100	22.4	22.3	22	22.2333	0.2082	0.1202
110	22.4	22.3	22.1	22.2667	0.1528	0.0882
120	22.5	22.5	22.2	22.4000	0.1732	0.1000
130	22.5	22.6	22.2	22.4333	0.2082	0.1202
140	22.5	22.6	22.2	22.4333	0.2082	0.1202
150	22.5	22.6	22.3	22.4667	0.1528	0.0882
160	22.6	22.7	22.4	22.5667	0.1528	0.0882
170	22.8	22.8	22.5	22.7000	0.1732	0.1000
180	22.8	22.8	22.5	22.7000	0.1732	0.1000

Power = solvent mass x specific heat capacity x temperature rise per second

Specific heat capacity of water = 4.19J/(g x ºC)
Volume = 1500ml (1500g)
P = 'c' x cp x m
P = 0.2106 ºC x 4.19J/(g x ºC) x 1500g
P=1323.85

I = W/cm²
I = 1323.85/ 1000
I = 1.3238 W / cm³

Appendix 5.3 Results from experiment: 862 kHz – (power setting 200W) in 1500ml of de-ionised water in the 2L Pyrex custom made vessel Unit and Glass reaction vessel of the Ultrasound Multi-frequency unit, Model MGFL Meinhardt.

Time (sec)	Temp °C	Temp °C	Temp °C	Average	Standard deviation	Standard error
0	21.4	21.5	21.4	21.4333	0.0577	0.0333
10	21.5	21.8	21.8	21.7000	0.1732	0.1000
20	21.7	21.9	21.9	21.8333	0.1155	0.0667
30	21.8	21.9	21.9	21.8667	0.0577	0.0333
40	21.8	21.9	21.9	21.8667	0.0577	0.0333
50	22.1	22	21.9	22.0000	0.1000	0.0577
60	22.1	22	22	22.0333	0.0577	0.0333
70	22.2	22.1	22.1	22.1333	0.0577	0.0333
80	22.2	22.1	22.2	22.1667	0.0577	0.0333
90	22.3	22.2	22.2	22.2333	0.0577	0.0333
100	22.3	22.3	22.2	22.2667	0.0577	0.0333
110	22.5	22.3	22.3	22.3667	0.1155	0.0667
120	22.5	22.4	22.3	22.4000	0.1000	0.0577
130	22.4	22.4	22.4	22.4000	0.0000	0.0000
140	22.5	22.5	22.5	22.5000	0.0000	0.0000
150	22.5	22.6	22.6	22.5667	0.0577	0.0333
160	22.7	22.7	22.7	22.7000	0.0000	0.0000
170	22.7	22.8	22.7	22.7333	0.0577	0.0333
180	22.7	22.8	22.8	22.7667	0.0577	0.0333

Power = solvent mass x specific heat capacity x temperature rise per second

Specific heat capacity of water = 4.19J/(g x ºC)
Volume = 1500ml (1500g)
P = 'c' x cp x m
P = 0.0652 ºC x 4.19J/(g x ºC) x 1500g
P = 409.92 W

I = W/cm²
I = 409.92/ 1000
I = 0.409 W / cm³

Appendix 5.4 Results from experiment: 1144 kHz – (power setting 200W) in 1500ml of de-ionised water in the 2L Pyrex custom made vessel Unit and Glass reaction vessel of the Ultrasound Multi-frequency unit, Model MGFL Meinhardt.

Time (sec)	Temp ^{o}C	Temp ^{o}C	Temp ^{o}C	Average	Standard deviation	Standard error
0	21.7	21.8	21.9	21.8000	0.1000	0.0577
10	21.9	22.3	21.9	22.0333	0.2309	0.1333
20	22.1	22.3	21.2	21.8667	0.5859	0.3383
30	22.2	22.3	21.2	21.9000	0.6083	0.3512
40	22.2	22.3	22.3	22.2667	0.0577	0.0333
50	22.2	22.3	22.3	22.2667	0.0577	0.0333
60	22.3	22.4	22.3	22.3333	0.0577	0.0333
70	22.3	22.5	22.3	22.3667	0.1155	0.0667
80	22.3	22.5	22.3	22.3667	0.1155	0.0667
90	22.5	22.6	22.5	22.5333	0.0577	0.0333
100	22.5	22.6	22.6	22.5667	0.0577	0.0333
110	22.5	22.7	22.6	22.6000	0.1000	0.0577
120	22.5	22.8	22.7	22.6667	0.1528	0.0882
130	22.6	22.9	22.8	22.7667	0.1528	0.0882
140	22.6	22.9	22.8	22.7667	0.1528	0.0882
150	22.7	23.0	22.8	22.8333	0.1528	0.0882
160	22.8	23.0	22.8	22.8667	0.1155	0.0667
170	22.8	23.0	22.9	22.9000	0.1000	0.0577
180	22.8	23.0	22.9	22.9000	0.1000	0.0577

Power = solvent mass x specific heat capacity x temperature rise per second

Specific heat capacity of water = 4.19J/(g x ºC)
Volume = 1500ml (1500g)

P = 'c' x cp x m
P = 0.1616 ºC x 4.19J/(g x ºC) x 1500g
P=1015.93

I = W/cm²
I = 1015.93/ 1000
I = 1.015 W / cm³

Chapter 6: The effect of ultrasound compared to mechanical agitation in susceptible filamentous species of cyanobacteria, green algae and diatoms.

Chapter 6: The effect of ultrasound compared to mechanical agitation in susceptible filamentous species of cyanobacteria, green algae and diatoms.

6.1 Abstract

Control of algal blooms requires energy. Algal morphology varies significantly between divisions for example many cyanobacterial species contain gas vacuoles for buoyancy, while diatoms are protected by a rigid silica based cell wall to combat predation. Mechanical agitation and ultrasound, two bloom control methods were compared based on their capacity to cause physiological damage and remove cells of six filamentous algal species. After sonication with 862 kHz and 3.7 $kWh.m^{-3}$ the highest cell removal rates were 98% and 88% for cyanobacterial species *Aphanizomenon flos-aquae* and *Anabaena* (wild) respectively, however the lowest cell removal rates were 28% for green alga *Tribonema viride* and 9% for diatom *Melosira* sp. However when treated with 20 kHz and 3.7 $kWh.m^{-3}$ *Melosira* sp. had 62% removal. Mechanical agitation using energy of 4.2 $kWh.m^{-3}$ produced 70% and 51% cell removal for *Melosira* sp. and *Tribonema viride*, both species had the lowest removal after sonication at 862 kHz, and have rigid cells walls. The shear forces caused during low frequency ultrasound and mechanical agitation appear to be similar and may explain why filamentous species of

green algae and diatoms are susceptible to low frequency ultrasound or mechanical agitation, with filamentous gas vacuolate cyanobacteria being susceptible to high frequency ultrasound.

6.2 Introduction

Algal blooms are a natural phenomenon which occur when nutrients accumulate in stagnant water bodies (Wetzel, 2001). When the environmental conditions are conducive the concentration of cells can significantly elevate resulting in a bloom. A number of problems are associated with such periods including: elevated chlorophyll a content, increased turbidity, filter blocking (Bauer *et al.*, 1998), production of harmful toxins (Carmichael, 2001), and emission of algogenic organic matter (AOM) (Henderson *et al.*, 2008b). Consequently, there is a need to prevent blooms from forming with reported methods including: manipulated destratification of lakes, hypolimnetic aeration to remove water stagnation, oxidation of sediment to avoid anoxia, nutrient diversion, biomanipulation and phosphorus precipitation (Wetzel, 2001). The efficacy of each is inconsistent with the majority of water treatment works still experiencing issues during bloom season and as such research is ongoing into alterative solutions such as barley straw and ultrasound.

Barley straw works through a fungal derived degradation pathway that ultimately leads to the release of an algicide that controls bloom populations (Murray *et al.*, 2009). For Barley straw to be effective it must be sufficiently degraded at the point the bloom is starting to form and so can only be used as part of a well structured management plan.

In contrast ultrasound works by physically damaging cells due to the influence of cavitation bubbles and hence requires no preparation time. Previous studies on the efficacy of ultrasound (Chapter 5) has revealed that only filamentous algae morphologies are susceptible to ultrasound at sufficiently low energy inputs to be considered economically viable. The mechanistic pathway has been shown to depend on species and frequency. In the case of cyanobacteria ultrasound appears most effective at higher frequencies where microbubble streaming is maximised and the resonance frequency of the cavitation bubble is similar to that of the gas vesicles (Nyborg, 1982; Mason, 2000; Zhang *et al.*, 2006a; Wu and Nyborg, 2008). Whereas for non cyanobacterial species a more direct shear impact has been postulated which shatters the structure of the cell wall (Purcell *et al.*, 2008). Overall, further definition of the impact of algal morphological character is required to understand what drives susceptibility to ultrasonic treatment and whether alternative lower energy options can be considered. For instance, where shear breakage is the key mechanism can alternative methods of generating shear be effective. Analogy can be made to conventional water treatment where high shear zones are known to break flocs up generating problematic fines for downstream treatment (Jarvis *et al.*, 2006) and so suggests mechanical agitation may be a suitable alternative.

The aim of this study is to explore this by assessing the susceptibility of a range of filamentous algal species to both ultrasound and mechanical agitation thereby assessing the opportunity for algae control.

6.3 Materials and Methods

6.3.1 Laboratory experimental conditions

Aphanizomenon flos-aquae (CCAP 1401/3) was obtained from Culture Collection of Algae and Protozoa (CCAP), Oban, Scotland. *Melosira* sp. (JA72) and *Tribonema viride* (JA 436) were obtained from Sciento (Manchester). (Wild) *Anabaena* (SD-051) was isolated from Scots Dyke, Yorkshire in (2007) and (Wild) *Tribonema* sp. (mixed species) was sampled from Thames Water's Grimsbury Reservoir, Banbury (Apr 2009). All species except the (wild) *Tribonema* sp. were grown under sterile conditions in the laboratory, using the following media types; for *Aphanizomenon flos-aquae* Jaworski media (JM), blue-green (BG)-11-N media for *Anabaena flos-aquae*, BG-11 for *Anabaena* (wild), diatom (DM) media for *Melosira* sp. (JA72) and bold basal's (BB) media for *Tribonema viride.* The (wild) *Tribonema* was grown as part of a predominantly green and diatom bloom in outdoor tanks at ambient temperatures ranging from 5-7 ^{o}C overnight to 8-12 ^{o}C during daylight from April-May '09. All species except wild *Tribonema* were incubated in laboratory conditions using 1-3 L Erlenmeyer flasks at 18-21 ºC on a Patterson Scientific Bibby Stuart SO1 shaker under 16-8hr light/dark cycle radiation provided by two bulbs: 1 Sun-Glo and 1 Aqua glow 30 W fluorescent light. *Tribonema viride* and *Melosira* sp. were not shaken constantly during culturing as it is not necessary to facilitate growth of these species. The light was dimmed slightly using neutral density filters to encourage higher density of growth. Cell

concentration for experiments were set at 1-4×10^5 cells.mL^{-1} for all species except (wild) *Anabaena* & *Anabaena fq*. were 1-3×10^6 cells.mL^{-1}. Cultures were for maintained for 10-30 days before the experiments depending on the species. The cultures were maintained in exponential growth phase during all experiments.

Laboratory experiments were performed using an Ultrasonic Probe, Model Virsonic Digital 600 and Ultrasound Multi-frequency unit, Model MGFL Meinhardt. The meinhardt multi-frequency unit emits 582, 862 kHz and 1144 kHz with power of up to 200 W. The probe emits a frequency of 20 kHz and power of up to 600 W. Refer to (Appendix 5) for detailed calorimetric calculations of for the 20 kHz Ultrasonic Probe and the Ultrasound Multi-frequency unit, Model MGFL Meinhardt for frequencies 582,862 and 1144 kHz.

Mechanical agitation was provided by a Stuart stirrer model SS10, with impeller dimensions 17.5×2.5 cm. Sample volumes of 1500 ml in 2 L circular Pyrex beakers were used for all experiments. Ultrasound exposure times ranged from 5 to 500 seconds at a frequency of 862 kHz and 200 W for all species and *Melosira* sp. was also treated at 20 kHz and 200 W. Mechanical agitation experiment exposure time was set at 30 minutes and the speed of the impeller was measure in revolutions per minute (Rpm). Rpm was increased incrementally from 380-1240 rpm throughout the experiments at each time point accumulatively. Rpm as a unit measurement is used throughout water treatment processes, as agitation is necessary to mix and homogenise water with chemicals and other substances during the purification of reservoir water within the WTW, hence the reason for Rpm use in this study. Rpm's were independently monitored using an Amp meter, to confirm the correct readings, and the electrical

energy input from the Stuart stirrer model SS10 was also tested separately to confirm the correct electrical energy conversion to $kWh.m^{-3}$ which is the typical unit used in the water industry to calculate energy costs for water treatment processes. The purpose of this type of agitation test is to define at what rate of Rpm would be required to break the algal filaments, and to compare this electrical energy consumption with the electrical energy of the ultrasound tests define the lowest rate of electrical energy consumption with the highest algal cell damage rate. Rpm was converted to $kWh.m^{-3}$ using the following equation,

Energy/ Volume = $kWh.m^{-3}$

Energy = W (Watts) X Time (Seconds)

Volume = Volume (Litres)

Watts per Joule Second= Voltage X Current

(Watts per Joule Second)*0.000278= $kWh.m^{-3}$

This test was performed using the same paddle size and unit as well as the energy conversion calculations were independently performed by a Stuart Company Applications Specialist (David Corrigan) using the Stuart stirrer model SS10 to confirm the above calculations (Table 6.1).

Table 6.1 Rpm conversions to (Joules per Watt second) based on testing of Stuart stirrer model SS10 carried out by Applications Specialist David Corrigan at Stuart.

RPM	Current (mA)	Voltage	Watts / Joules per second
0	50	230	11.5
250	61	230	14
500	64	230	14.7
750	71	230	16.3
1000	77	230	17.7

Temperature control was maintained throughout the experiments to within 2^{o}C of the normal growth conditions of the species ~23^{0}C throughout the experiments with the use of a thermostatically controlled chiller unit. Normal growth conditions of the sonicated samples were maintained subsequent to the ultrasound treatment except for the additional step of shaking the culture once a day for 1-12 days. Samples were taken directly after every time point, all samples cell for each part of the analysis were taken in triplicate, and then analysed, so each data point is an average of three replicates. Analysis of samples was performed directly after the experiments using a spectrophotometer model SpectraMax Plus 384 with a UV/visible detector 190-1000 nm, to detect cell number at 635-684 nm (Zhang *et al.*, 2006a; Zhang *et al.*, 2006b) and hand counts were also performed using a haemocytometer for cell counts, and preserved in Lugol's iodine until counted. Cell counts for this chapter were re-defined to describe cell count damage on >4 cells rather than >10 cells as previously stated in chapter 5 due to the addition of species which were more susceptible to natural cell filament breakage

during natural growth conditions prior to ultrasound or mechanical agitation treatment. Specifically defined hand counts were performed for all species to define the levels of cell damage, intact cells i.e. 4 cells or more in a filament, broken cells were defined as a filament with >4 cells and damaged, the last category was lysed cells, for *Melosira* sp., *Tribonema viride*, and *Tribonema* (wild) this was cells which were empty of cell contents but pieces of silica exoskeleton were still visible, for the other species lysed cells were counted where only heterocysts were visible. Results were normalised and expressed as cell number, percentage cell count and percentage cell removal with standard deviation.

6.4 Results

Four different filamentous algal species were tested during the investigations including two species where both culture collection and wild sources were tested (Figure 6.1). *Anabaena* (wild) (SD-051) was isolated from the Scouts Dyke reservoir, Yorkshire (Figure 6.1a) and is a filamentous multicellular cyanobacteria with a similar size and physiology to the laboratory strain *Anabaena fq.* (CCAP 1401/13B) (Figure 6.1b). Individual 6-10 µm barrel shaped cells contain heterocysts (nitrogen fixing cells) and gas vacuoles with cells combining into filament lengths ranging up to 100 µm (Figure 6.1a). The filament configuration is a combination of straight, coiled and tangled chains which tend to bloom in early spring and late summer and are a common species of concern for water treatment (Henderson *et al.*, 2008a). In the case of the field strain, the filaments formed mat like clumps with mucilaginous consistency that was not observed

in the case of the culture collection strain but are typical of the species and visible in *Anabaena* (wild) (Bellinger, 1992). *Tribonema* (wild) was part of a mixed field bloom dominated by species of *Tribonema* sourced from Thames Water's Grimsbury Reservoir, Banbury (Figure 6.1c). The field sample contained a combination of predominantly green *Tribonema* species, including species such as *Tribonema affina* and *Ulothrix zonata* which were physiologically similar to the *Tribonema* culture collection strain. *Tribonema v.* (JA 436) (Figure 6.1d) is a filamentous green unbranched colonial species from the class *Xanphytophycopsida* made up of 5-7 µm cylindrical shaped cells combining to form filaments with an average diameter of 200 µm. The cell wall is made up of two h-shaped bi-partite structure composed of pectic materials, silicates and cellulose. *Melosira sp*. (JA72) (Figure 6.1e) is a filamentous pennate diatom made up of a cylindrical cell with a maximum axial length of 10-40 µm and an end diameter of 8-20 µm. The cell wall (Frustule) is a siliceous box shape with an overlapping lid providing a relatively rigid structure compared to the other species. Typical *Melosira sp.* blooms occur in early spring and late summer when water temperatures decrease and form filaments of between 5 and 100 cells (Bellinger, 1992; van den Hoek *et al.*, 1995).

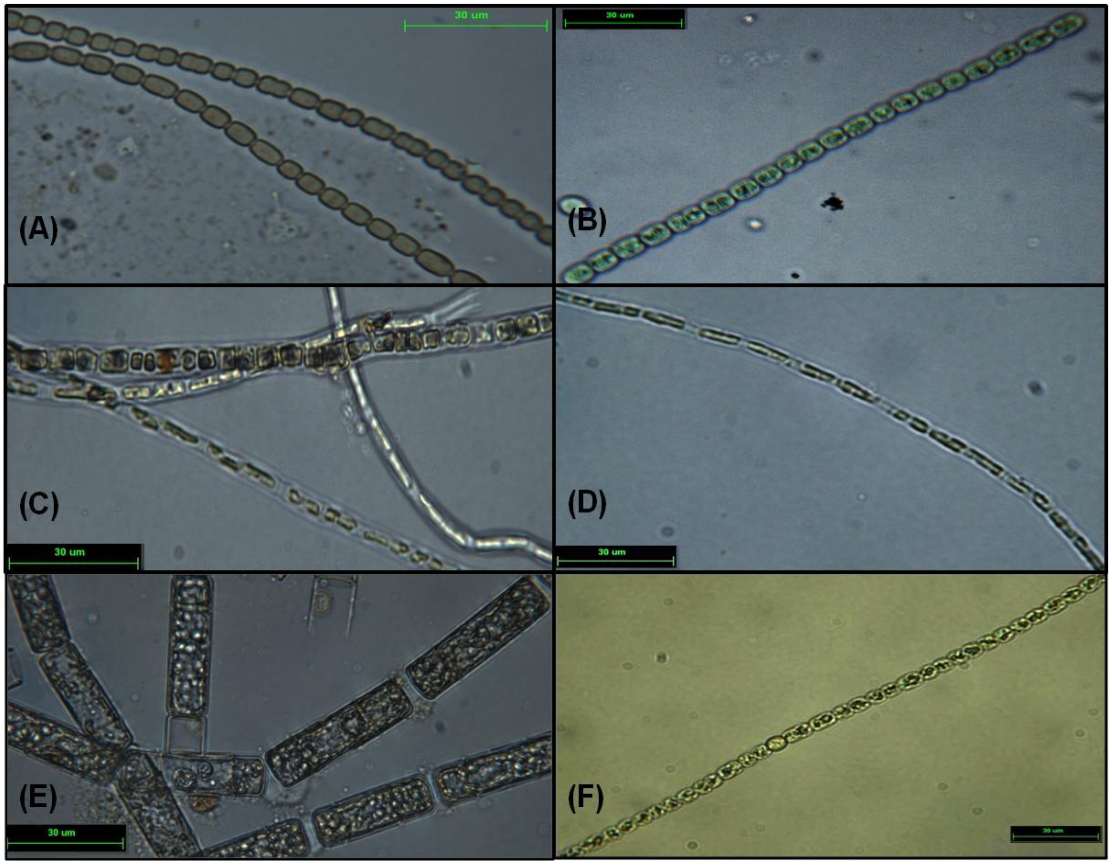

Figure 6.1 (A) *Anabaena wild,* (B) *Anabaena flos-aquae,* (C) *Tribonema,* (wild) mixed species, (D) *Tribonema viride,* (E) *Melosira* sp., (F) *Aphanizomenon flos-aquae.*

Aphanizomenon fq. (CCAP 1401/3) (Figure 6.1f) is a filamentous multicellular colonial cyanobacteria with rectangular 8-12 µm cells combined to form trichomes of between 4 and 80 cells. The cells contain heterocysts and gas vacuoles with the trichomes formed in raft like parallel configurations to form dense mat like structures. *Aphanizomenon fq*. typically blooms in late spring and early summer and is frequently found in bloom surface scum associated with *Microcystis*, and are far more common in hard water conditions (Bellinger, 1992) .

6.4.1 Sonication

Sonication produced a similar response in all species with an initial decrease beyond which higher energy inputs caused no further reduction in cell numbers (Figure 6.2). The energy required to reach the plateau and its concentration varied between species. For instance, sonication was most effective against the two cyanobacteria species with almost complete cell removal at 98% for *Aphanizomenon fq*., 88% for *Anabaena* (wild) and 51% for the culture collection strain *Anabaena fq*. The order of removal based on the other species at an energy input of 3.7 $kWh.m^{-3}$ was 9% for the *Melosira* sp., 28% for culture collection *Tribonema v*. and 72% for the *Tribonema* (wild), differences between laboratory and field strains can be attributed to differing environmental conditions but further investigation is necessary to confirm this (Figure 6.2). The response of *Melosira* sp. was different from the other trials in that the residual cell concentration initially dropped from 96,666 to 34,444 $cells.mL^{-1}$ at 0.2 $kWh.m^{-3}$ but then increased back to the original levels as higher energy inputs were applied before decreasing once the energy input had exceeded 11.1 $kWh.m^{-3}$ beyond which a plateau region existed at a concentration of 11,000 $cells.mL^{-1}$. In terms of residual cell concentration in the plateau region, *Anabaena* (wild), *Aphanizomenon fq.* and *Melosira* sp. were all reduced to a level such that re-growth did not occur with no residual for *Anabaena* (wild) and *Aphanizomenon fq.* and 23,333 $cells.mL^{-1}$ for *Melosira* sp. In contrast, plateau concentrations between 148,889-225,000 $cells.mL^{-1}$ were observed for

Tribonema v. compared to ranges of 56,667-138,889 cells.mL^{-1} and 397,778-437,889 cells.mL^{-1} for *Tribonema* (wild) and *Anabaena fq.* respectively indicating that sonication was only partially effective at controlling these species. Analysis of the threshold energy input required to reach the plateau showed an opposite sequence to the residual level with *Tribonema v.* requiring the least energy at 0.2 kWh.m^{-3} as it had reached its maximum removal of only 28% followed by *Tribonema* (wild) at 1.9 kWh.m^{-3} with it's best removal of 72%, *Aphanizomenon fq*. at 3.7 kWh.m^{-3} and then *Melosira* sp. at 11.1 kWh.m^{-3} (Figure 6.2). The two *Anabaena* strains exhibited a different pattern where a more exponential style response was observed and hence a gradual change with energy input of beyond 3.7 kWh.m^{-3}.

The impact of sonication on the filamentous algae tested in this investigation is much greater than that previously reported for the more commonly tested unicellular/colonial algae such as *Microcystis aerg*. and *Scenedesmus sub*. In such cases removal levels are reported to be below 20% with energy inputs as high as 133 kWh.m^{-3} demonstrating that sonication is only effective for filamentous strains of algae (Chapter 5). The majority of the trials were conducted at 862 kHz as previous work had indicated that sonication is most effective at higher frequencies as demonstrated for *Aphanizomenon fq*. and *Spirulina* (*Arthrospira*) *platensis* (Tang *et al.*, 2003; Hao *et al.*, 2004 ; Chapter 5). However, in the case of *Melosira* sp. previous work has also indicated that lower frequencies are more effective as the cavitation effect is maximised (Purcell *et al.*, 2008). Experiments during the current investigation support this in that at 20 kHz removal of *Melosira* sp. was more consistent reaching a maximum level of 84.5% at an energy input of 18.5 kWh.m^{-3} (Appendix 6.0).

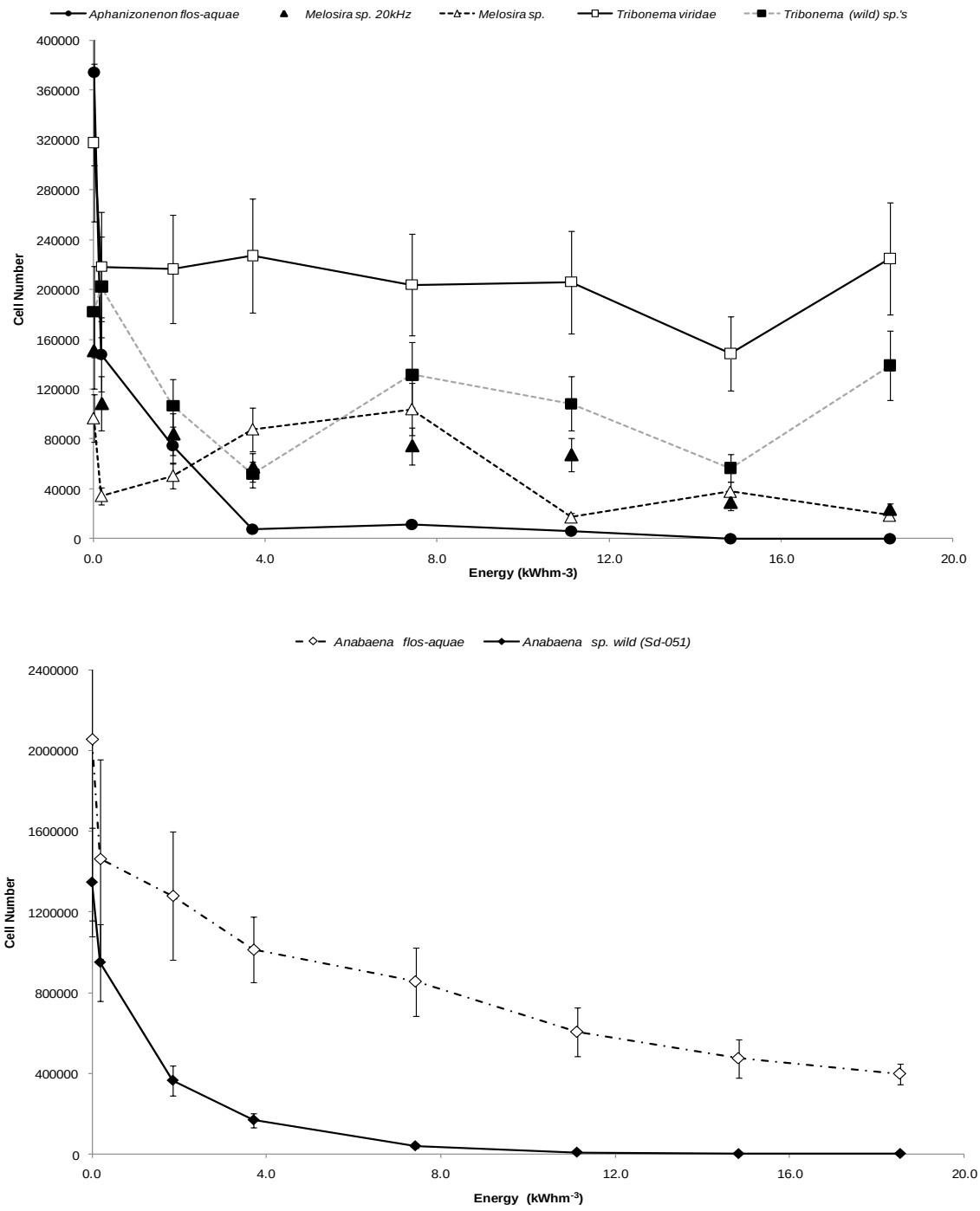

Figure 6.2 Impact of increasing energy input of sonication at a fixed frequency of 862 kHz against (A) *Aphanizomenon flos-aquae, Melosira* sp., *Tribonema* (wild) mixed species, *Tribonema viride* and (B) *Anabaena* (wild), *Anabaena flos-aquae.*

Photographic inspection of the algal systems during the trials indicated that the impact of ultrasound could be described in two ways: (1) Cell filament breakage which is where the joints between cells had been broken, this damage is termed as broken cells for the purposes of this study and (2) Severe cell damage in the form of rupture of the cell wall liberating cell contents and presence of only heterocysts; this is termed cell lysis for the purpose of this study. At very low energy inputs of 0.2 $kWh.m^{-3}$, the action of the ultrasound is principally filament shortening by breakage with only a limited amount of cell lysis (Figure 6.3). At higher energy inputs more severe breakage occurs and is accompanied by increasing amounts of cell lysis where the nitrogen fixing cell subsists. For instance in the case of *Anabaena* (wild), at 3.7 $kWh.m^{-3}$ filaments were limited to two cell in a chain indicating that near complete breakage had occurred.

Quantification of the percentage change in cell physiology compared to the control revealed two different responses (Figure 6.4). In the case of *Tribonema (wild), Tribonema v.* and *Melosira* sp. no specific sequence of change could be observed with fluctuations by stable balance of healthy, broken and lysed cells across all energy input levels. For instance, in the case of *Tribonema v*. an initial reduction of healthy cells was observed from 57±6% to 40±4% upon application of 0.2 $kWh.m^{-3}$. Thereafter the percentage of healthy cells remained within 5% of this level across all further energy input levels. Similar responses were observed for both *Tribonema (wild)* and *Melosira* sp. although the level of fluctuation was greater than seen for *Tribonema v*.

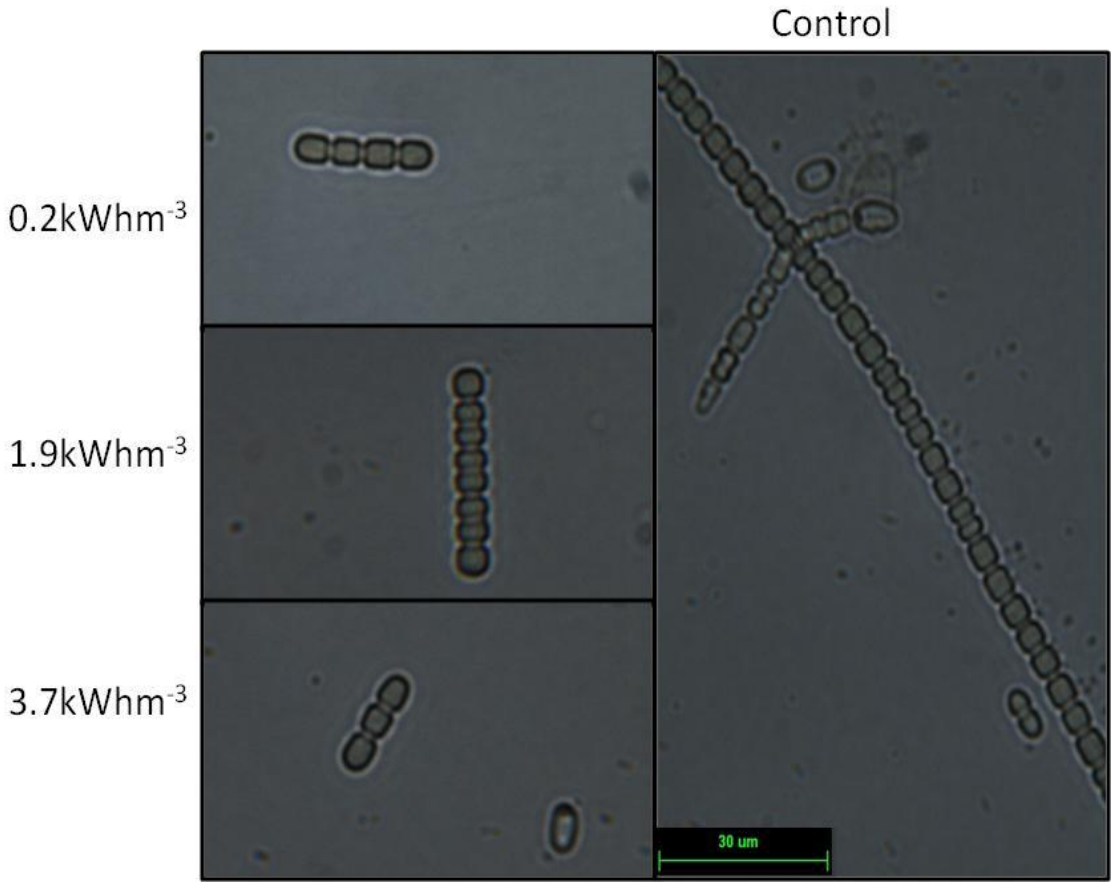

Figure 6.3 Illustration of visible impact of sonication on the (wild) *Anabaena* samples.

In contrast, in the case of *Anabaena* (wild), *Anabaena fq.* and *Aphanizomenon fq.*, a transition from healthy to lysed cells was observed as the energy input increased. To demonstrate, in the case of *Anabaena* (wild) the percentage of cells defined as healthy decreased from 82% before application of ultrasound to 69%, 40%, 22%, 7% and 2% as the energy input increased to 0.2, 1.9, 3.7, 7.4 and 11.1 $kWh.m^{-3}$ respectively. Application of low levels of ultrasonic energy resulted in the remaining cells being defined as broken to an energy input of 1.9 $kWh.m^{-3}$ beyond which healthy, broken and

lysed cells co-existed. The percentage of cells identified as lysed increased from 46% at 3.7 kWh.m^{-3} to 83%, 77%, 100% and 100% at 7.4, 11.1, 14.8 and 18.5 kWh.m^{-3} respectively demonstrating that once lysis was observed it became the dominate fraction (Figure 6.4).

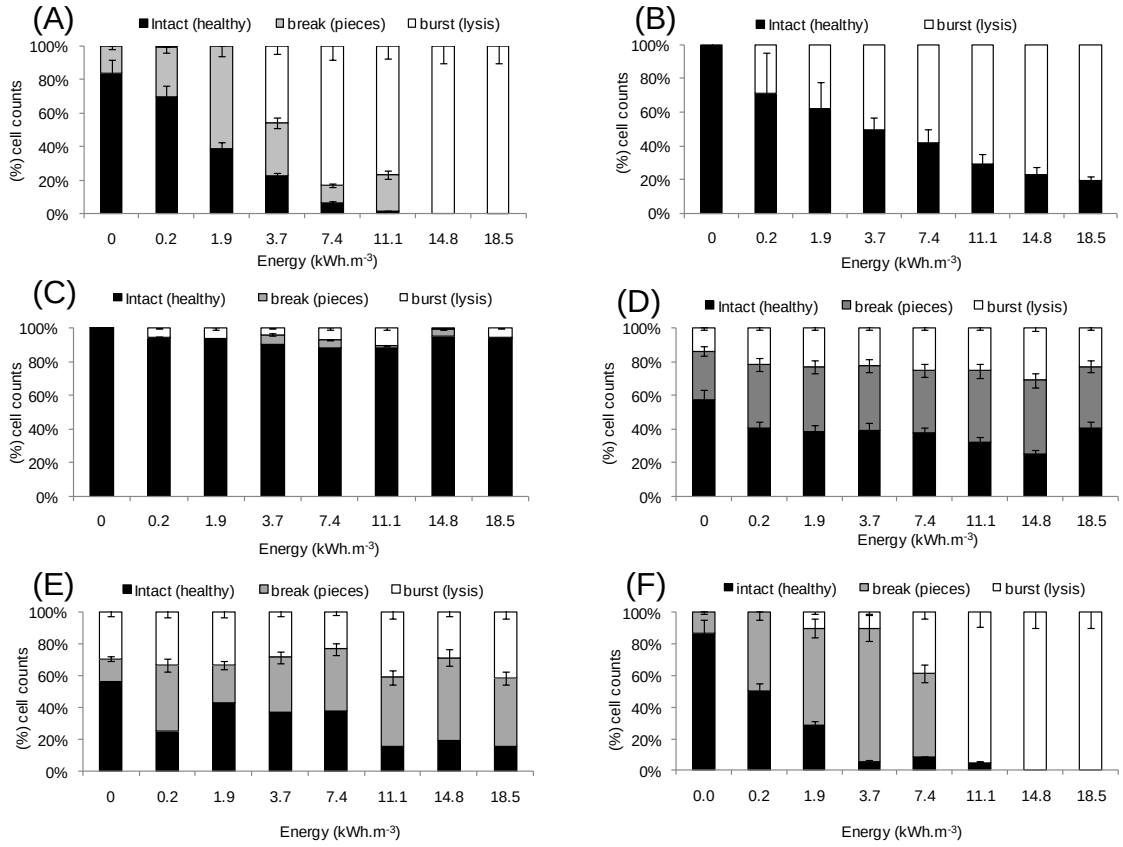

Figure 6.4 862 kHz frequency effects on cell number expressed as percentage on (A), *Anabaena wild*, (B), *Anabaena flos-aquae*, (C), *Tribonema*, (wild) mixed species, (D), *Tribonema viride*, (E), *Melosira* sp. (F), *Aphanizomenon flos-aquae*.

The response of the *Anabaena fq.* and *Aphanizomenon fq.* was similar in general to the above strain with two specific differences (Figure 6.4). In the case of the laboratory strain *Anabaena fq.* no broken cells were observed with only healthy and lysed cells

present at all energy levels. Overall the strain was more resistant to ultrasound with the percentage of cells identified as healthy decreasing progressively from 71% at 0.2 $kWh.m^{-3}$ to 19% at 18.5 $kWh.m^{-3}$ such that the reduction in the percentage of cells identified as healthy decreased less significantly than in the *Anabaena* (wild) case with healthy cell still visible even at the highest energy input. In the case of *Aphanizomenon fq*. filament breakage was a more significant fraction of the total cell count. To explain, the percentage of cells identified as broken accounted for 50, 61, 84, 53% at energy inputs of 0.2, 1.9, 3.7 and 7.4 $kWh.m^{-3}$ resulting in lysed cells becoming the dominant fraction only once the energy input reached 11.1 $kWh.m^{-3}$ compared to 3.7 $kWh.m^{-3}$ for *Anabaena* (wild).

6.4.2 Efficacy of mechanical agitation

The impact of mechanical agitation on the algae was observed to follow three patterns (Figure 6.5). In the case of the two field samples: *Tribonema* (wild) and *Anabaena* (wild), no significant change in cell numbers was observed due to the application of mechanical energy although the cell counts fluctuated considerably across the trial. Similarly, no distinct change in cell numbers could be observed in the case of *Anabaena fq*. up to an energy input of 29.2 $kWh.m^{-3}$ where after the cell concentration dropped as increased energy inputs were applied leading to a reduction of 49% at an energy input of 40.7 $kWh.m^{-3}$ (1240 rpm).

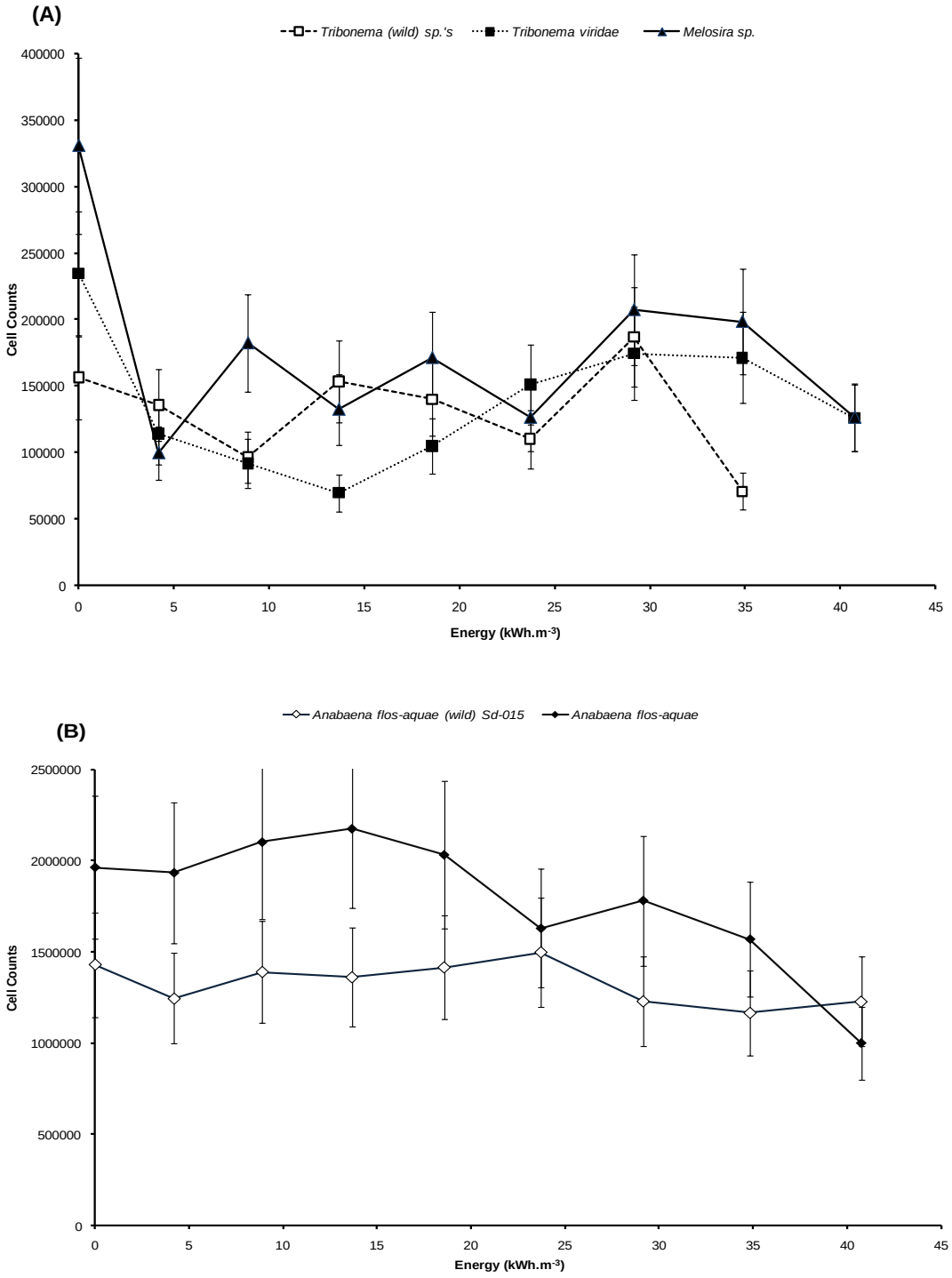

Figure 6.5 Impact of increasing energy input due to mechanical agitation on (A) *Melosira* sp., *Tribonema* (wild) mixed species, *Tribonema viride* and (B) *Anabaena* (wild), *Anabaena flos-aquae.*

In contrast, in the case of *Tribonema viride* and *Melosira* sp. there was an initial decrease in cell concentration after the lowest energy input of 4.2 $kWh.m^{-3}$ (380 rpm) leading to a reduction of 51% and 70% respectively (Figure 6.5). Thereafter no consistent trend was observed with high levels of fluctuations across the trials although the concentration remained below that of an un-agitated system throughout. The last two species are morphologically distinct in that they have relatively rigid structures containing silica and their natural culturing conditions do not include any agitation indicating a natural susceptibility to agitation.

Photographic inspection of the impacts of mechanical agitation supports the overall cell numbers. In the case of *Melosira* sp. both breakage and lysis can be observed for all energy inputs with visible cell emptying and broken joints (Appendix 6.1). Images at increased energy levels show no discernable difference with both breakage and lysis visible in all cases. A similar situation is evident with *Tribonema v.* where distinct cell damage and breakage can be observed for all the energy levels tested. Again no discernable difference can be consistently observed as the energy level is increased although the filaments appear shorter once the energy input exceeds 18.6 $kWh.m^{-3}$. Such observations are consistent with a traditional breakage pattern observed in flocculated systems when additional energy is applied to pre-formed flocs (Jarvis *et al.*, 2006).

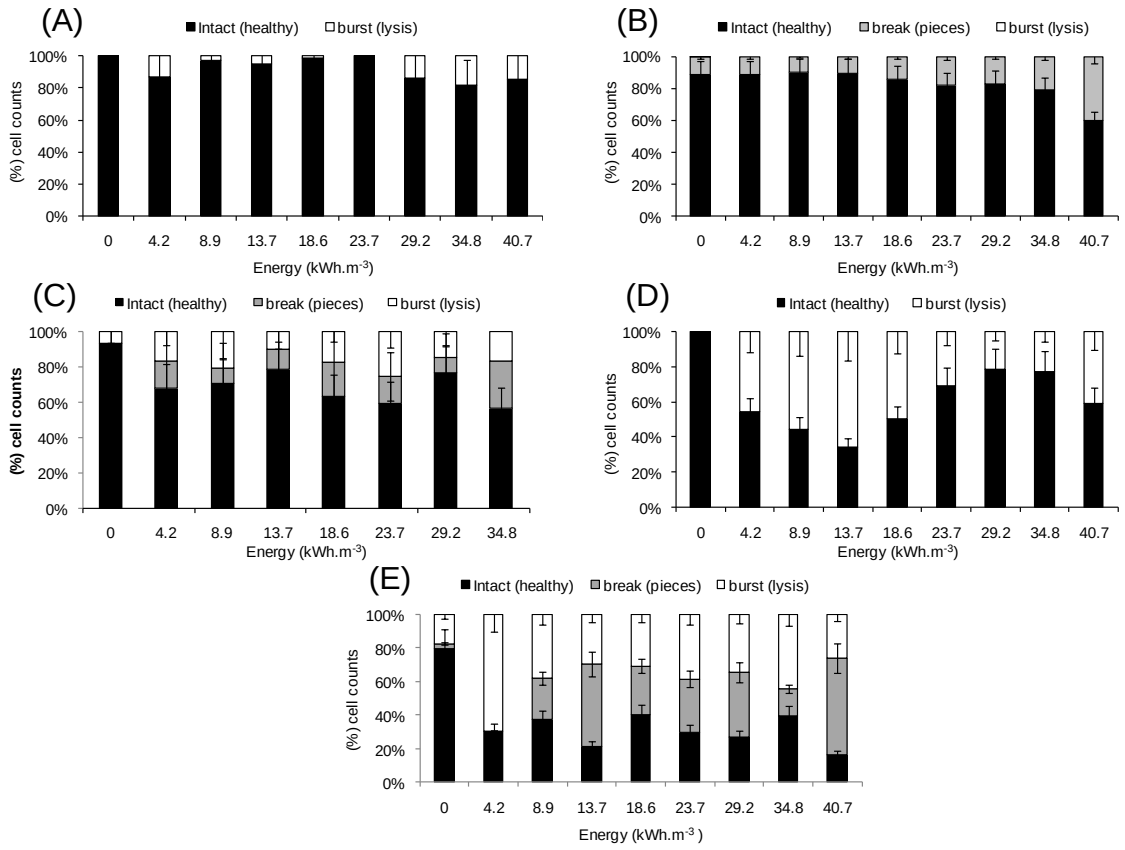

Figure 6.6 Mechanical agitation effects on cell physiology categorised by healthy filaments, filaments broken in pieces and burst or lysed filamentous cells for the following species (A), *Anabaena* wild, (B), *Anabaena flos-aquae*, (C), *Tribonema*, (wild) mixed species, (D), *Tribonema viride*, (E), *Melosira* sp.

Quantification of the proportion of healthy, broken and lysed cells in comparison to the control revealed two different patterns. In the case of the two *Anabaena* samples a very different response was observed to the equivalent measurement when ultrasound was applied. In the case of mechanical agitation, only small changes in cell health were observed supporting the suggestion that this species of algae is not susceptible to mechanical agitation. The increased removal at high energy inputs observed for

Anabaena fq. was identified to be due to filament breakage. To define, the percentage of cell identified as broken increased from 11% when no agitation was applied to 14% and 18% when 18.6 and 23.7 $kWh.m^{-3}$ (770 and 870 rpm) was applied respectively (Figure 6.6). In the case of *Tribonema* v. and *Melosira* sp. agitation caused a reduction in the percentage of cell identified as healthy at the lowest energy input confirming a susceptibility of these species to agitation. To describe, application of 4.2 $kWh.m^{-3}$ (380 rpm) reduced the percentage of cells identified as healthy from pre-agitation level of 79% and 100% to 30% and 49% respectively for *Melosira* sp. and *Tribonema v*. Additional energy input then caused no further observable change with a high level of fluctuation observed between samples with the percentage of cells identified as healthy varying between 15-41% for *Melosira* sp. and 39% and 73% for *Tribonema* v. suggesting that *Melosira* sp. was more susceptible to agitation.

6.5 Discussion

Previous studies had shown that filamentous species are more susceptible to treatment by sonication compared to unicellular/colonial algae as the ultrasound influences the weaker connection points between cells in the filaments (Tang *et al.*, 2003; Purcell *et al.*, 2008). Results from the current study extends this understanding by identifying that across all the filamentous algae tested the cyanobacterial strains of algae are the most susceptible (*Aphanizomenon fq*.; *Anabaena fq*.). Links between the observed heightened susceptibility and algae physiology relates to the presence of gas vacuoles within the

filamentous structure. Support for this suggestion is provided by a study which tested two cyanobacterial strains with (*Microcystis*) and without (*Synechococcus* sp.) gas vacuole and found that only the strain with vacuoles was influenced by ultrasound (Tang *et al.*, 2004). The susceptibility to ultrasound can thus be related to the combination of size and structure of the algae and the size and strength of the gas vesicles. The pressure required to burst a vesicle is related to both the strength of the wall and the overall width of the vesicle. Reported vesicle widths are 78 nm and 84 nm for *Aphanizomenon fq*. and *Anabaena fq*. respectively which compares to 67 nm for *Microcystis aeruginosa* (Walsby and Bleything, 1988). Additionally the cell wall structure is different between the filamentous and colonial cyanobacterial species (Flores *et al.*, 2006). In the case of the filamentous species, the cell wall is made up of peptidoglycan with an outside continuous membrane interlinking the cells within the filament. In comparison, in the case of *Microcystis*, the cell wall is made up of a different amino acid sequence without an outer membrane connecting multiple cells (Walsby, 1994). Measured critical pressure ranges support such observations with ranges between 0.45-0.85 MPa for *Aphanizomenon fq*. and *Anabaena fq*. and 0.6-1 MPa for *Microcystis aeruginosa* (Walsby, 1992). Overall, the combination of the physical size of the filamentous structures combined with a weaker vesicle makes such species the easiest to influence by ultrasound.

In both cases where field and laboratory samples of the same species were tested field strains were more influenced by ultrasound with between 37-43% more removal observed for the field samples. This is consistent with the fact that environmental conditions are known to influence the strength of the vesicles (Walsby, 1992) and the

rate of growth. In the current case the transfer of the field samples into laboratory conditions appears to have prompted an acceleration in growth which is known to be the optimum time to influence the algae with ultrasonic treatment (Purcell *et al.*, 2008; Chapter 7). Conversely, the use of laboratory strains appears to provide a conservative assessment framework for investigating the efficacy of ultrasound and so acts as a worst case scenario.

The work presented here showed a reduced efficacy of ultrasonic treatment to the algae with no gas vacuoles such as *Melosira* sp. and *Tribonema* sp. when operating at high frequency. However, at a low frequency of 20 kHz, the diatom *Melosira* sp. removal was enhanced from 9% to 62% at an energy input of 3.7 $kWh.m^{-3}$. Previous work has postulated an alternative treatment pathway whereby removal occurs due to a shattering of the rigid wall structure (Purcell *et al.*, 2008). In such cases the importance of the ultrasound is in producing large shear forces during cavitation which is related to the size of the bubbles formed reported bubbles sizes are 166 μm at 20 kHz and 2.5 μm at 1320 kHz (Zhang *et al.*, 2006a). Previous work has shown these forces are sufficient to rupture polymer chains and increase the rate of hydrolysis (Price, 1990; Mason *et al.*, 1985) Support for this pathway is provided from the mechanical agitation experiments where the algae with the more rigid filament chain like structures (*Melosira* sp.; *Tribonema v.)* were more effectively treated. In such cases the minimum energy input trialled (4.2 $kWh.m^{-3}$) was sufficient to achieve the maximum level of removal with no further treatment occurring as the rotational speed of the mixer was increased up to a maximum of 40.7 $kWh.m^{-3}$ (1250 rpm) indicating threshold damage point had been reached and that even lower shear rates are possibly sufficient. Further, mechanical

agitation had minimal influence on the cyanobacterial species supporting the idea of two distinct pathways for treatment. The implications of the work are that different systems will be effective for different species of algae dependent upon whether they are (a) filamentous, (b) contain a gas vacuole and (c) have a rigid wall structure. Ultimately this suggests that no one treatment method will be universally appropriate and selection must be tailored to match physiological characteristics of the algae to be treated.

6.6 Conclusions

The susceptibility of algae to ultrasonic treatment is species dependent such that the filamentous cyanobacteria *Anabaena fq*. and *Aphanizomenon fq*. were identified as the most susceptible species to high frequency ultrasound which was linked to a treatment pathway related to gas vacuole bursting. In contrast, more rigid wall structured algae such as *Melosira* sp. and *Tribonema v*. were susceptible to exposure to shear effects which was seen to be effective irrespective of the method of delivery. Overall, the presented work suggests a distinct treatment selection based on specific algal morphological features where non gas vacuolated species are better treated by low frequency ultrasound or mechanical agitation and cyanobacterial species with gas vacuoles are better treated by high frequency ultrasound.

6.7 References

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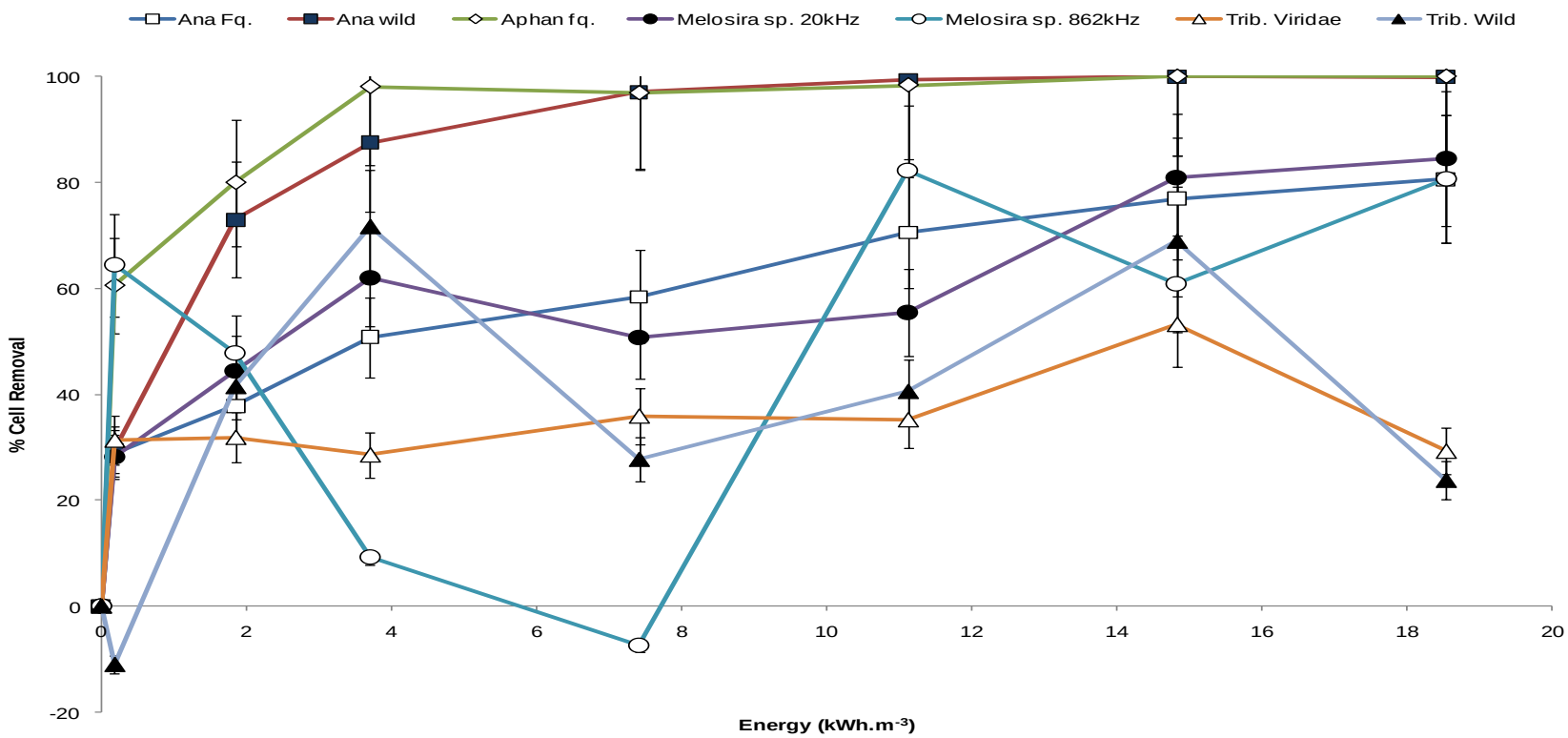
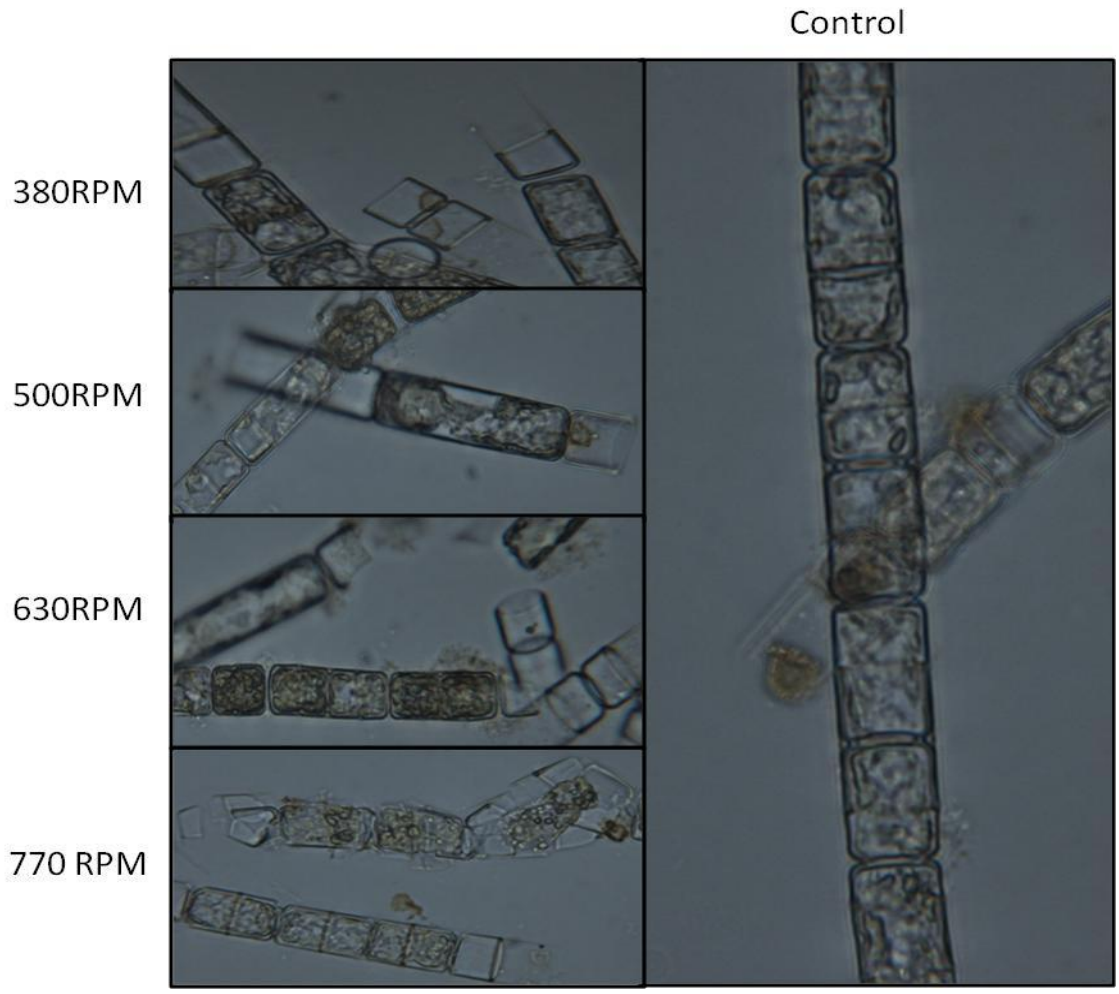
Appendix: Chapter 6



Appendix 6.0 Cell removal percentages of all species after ultrasound treatment using 862 kHz for all species expect *Melosira* sp. which was treated additionally using 20 kHz.

Cell removal rates for *Melosira* sp. were significantly high when treated with low frequency ultrasound 20 kHz. Previous tests in Chapter 5, noted *Melosira* had higher susceptibility to low frequency, with removal of 71% cell removal at 20 kHz, and 9% at 1144 kHz. *Melosira* sp. had 62% cell removal after 3.7kWh.m^{-3} at 20 kHz, comparing to 9% using the same energy at 862 kHz (Appendix 6.0). As sonication increased 20 kHz results increased gradually with cell removal rates of 51, 55, 81, 85% at energy levels of 7.4, 11.1 14.8, and 18.5 kWh.m^{-3}. The results for 862 kHz fluctuated significantly until the final time point with the following removal rates, -7, 82, 61, 81% using energy of 7.4, 11.1 14.8, and 18.5 kWh.m^{-3}(Appendix 6.0). These results confirm that 20 kHz is the most energy efficient and consistent cell removal frequency for the filamentous diatom *Melosira* sp.



Appendix 6.1 *Melosira* sp. after mechanical agitation at increasing rpm, microscopic images (mag.40x).

Chapter 7: Effect of sonication on two natural algal field blooms of predominantly unicellular and filamentous green algae

Chapter 7: Effect of sonication on two natural algal field blooms of predominantly unicellular and filamentous green algae

7.1 Abstract

Natural algal blooms are prolific throughout water sources worldwide but they vary in size and toxicity. Field blooms have been documented to block filters, increasing chlorophyll a and turbidity increasing backwashing and chemical demand in water treatment works. Two natural blooms from the south of England were treated with 20 kHz and 28 kHz of ultrasound at laboratory and pilot scale. The first bloom was composed of mixed species dominated by unicellular green species *Sphaerocystis* sp. and *Scenedesmus* sp. The second bloom which underwent sonication in this study was also composed of mixed species but with dominance of filamentous green species including *Tribonema* sp. Results from the first trial observed complete kill of the dominant unicellular green species *Sphaerocystis* sp. and *Scenedesmus* sp. in the last 8 days of the trial, conversely the second trial using the same bloom but was now 30 days older saw *Scenedesmus* sp. the dominant species have growth rates which were

significantly higher than the control by 100,000 cells.mL^{-1} or 25%. In the third trial a second natural bloom dominated by filamentous green species was sonicated and resulted in cell removal rates of >90% by the final day of the trial. The fourth and final trial used the same filamentous green algal bloom as trial three except it was 30 days older which was the same age as the algae in trial two. A similar trend was also observed in this trial where enhanced growth of the filamentous bloom in the test exceeding the control growth levels for the majority of the trial by over 100,000 cells.mL^{-1}. Laboratory ultrasound experiments using 20 kHz ultrasound on the filamentous bloom at 60 days old post both field trials, resulted in insignificant decrease in cell numbers. Chlorophyll fluorescence results confirmed the cell count results over the majority of the trials. These results indicate that the age of the bloom has a significant input on effectiveness of ultrasound, the older the bloom the lower the effectiveness of ultrasound, these trials did not observe any significant difference in response based on morphological type with unicellular or filamentous green species having similar responses throughout all 4 trials..

7.2 Introduction

Algal blooms are usually caused by one or two dominant algal species with cell concentrations beyond 20,000 cells.mL^{-1} (Bauer *et al.*, 1998; Albay and Akcaalan, 2003; Hoeger *et al.*, 2004). A range of source control methods exist to prevent bloom concentrations from forming such as nutrient diversion, destratification, aeration,

oxidation of sediment and phosphorus precipitation (Wetzel, 2001). None of the solutions work consistently and so research into alternative methods remains ongoing including the application of barley straw (Murray *et al*., 2010) and ultrasound (Purcell *et al.*, 2008) to provide more effective short term solutions. The commercially available ultrasonic units commonly utilised work over a frequency range of 20-40 kHz with power levels of 20-40 W. To date, research has focussed on the impact of ultrasound for control of cyanobacteria blooms and have been conducted at bench scale. For instance, 90% of all reported studies have been based on species of *Microcystis*, highlighting the lack of knowledge at field scale on green algae or diatoms. Analysis across all the reported trials indicates a range of responses with both positive and detrimental effects. For instance, trials using a floating transducer of 28 kHz and 20 W treating 250 litres and culture collection species found growth reduction of 49% for *Aphanizomenon* sp. and 60% reduction in *Scenedesmus* sp. after 24 days (Warnock, 2005). In a similar trial, a reduced growth rate of 60% of *Cyclotella* sp. and 41% of *Scenedesmus* sp. were observed over 14 days (Inman, 2004). Scaled up experiments have shown more mixed responses. To demonstrate, (Ahn *et al.*, 2003; 2007) reported on a comparison of a 200 L pilot trial with a field test on a 9000 m^2 pond at a mean depth of 2 m next to a control pond of 7000 m^2 with a pulsed 630 W, 22 kHz ultrasound unit. A 34% reduction in cyanobacteria was found at pilot scale but during the field trials chlorophyll a levels in both ponds remained the same which was confirmed by algae speciation which showed a switch in succession from cyanobacteria to diatoms in both tanks consistent with the seasonal norm. Further, a full scale study over three years using 10 ultrasonic flow through units consisting of 2X (100 W & 200 kHz transducers attached to a pipe

through which the algae are pumped) made by Honda electronics Co., LTD, Toyohashi, Japan) in a 365,000 L reservoir found that blooms were removed for the first two years but once the flow rate into the lake was slowed down for the third year the blooms returned (Nakano *et al.*, 2001). Previous studies have also reported that in certain cases cell growth can occur. For example continuous irradiation of *Microcystis* sp. caused an increase in cell numbers of 67% in 14 days (Inman, 2004). Similarly, pulsed ultrasound at laboratory scale caused *Spirulina platensis* and *Spirulina maxima* to increase by 60% and 5% respectively (Al-Hamdani *et al.*, 1998; Tang *et al.*, 2003; Inman, 2004). Two other studies on the green algae *Chlorella* (Zhang and Chen, 2001) experienced increased growth after low power ultrasonic irradiation using a frequency of 20 kHz at laboratory scale.

The overall picture is difficult to interpret as algae represent a broad classification of species, the properties of which are known to change with average age of the population (Henderson et al, 2008; Chapter 7). Previous work has shown that filamentous algae and especially cyanobacteria species have a heightened susceptibility to ultrasound in laboratory conditions (Chapter 5 & Chapter 6) yet this does not completely reflect reported pilot and field trials indicating the overall picture is very complex. The current chapter reports on pilot scale investigation on the efficacy of ultrasound to treat common algae species found in UK source waters with specific reference to the age and species of the algal population in an attempt to further refine our understanding of the efficacy of ultrasound for algal control.

7.3 Methods

7.3.1 Pilot and laboratory experimental conditions

Two field blooms were supplied by Thames Water. The first bloom was from Farmoor Reservoir, Oxford with the first trial running from the 11th of August to 8th of September '08 and the second trial from the 19th of September to the 20th of October '08. The second bloom was sampled from Grimsbury Reservoir, Banbury with trial dates of 18th of March-10th of April '09 for the third trial and 17th of April-19th of May '09 for the fourth trial.

Two 1500 L tanks of dimensions 104×208 cm were set-up outdoor as test and control tank. Ultrasound was provided by a 40 W, floating transducer with a frequency of 28 kHz (Dynamco), and distance range of 1000 m. Further trials were conducted on 1.5 L sub samples on the second bloom using a 600 W, 20 kHz ultrasound probe (Model Virsonic Digital 600) and a 200 W, 20 kHz Ultrawaves (GmbH) Probe, connected to flow through cell which operated at 10 L.s^{-1} (See Appendix 7.0-7.1 for image and schematic of the Ultrawaves probe and the purpose built flow cell). Temperature control was maintained within 2 ^{0}C of the normal growth conditions of the species ~24 ^{0}C throughout the experiments.

Analysis of samples was performed using a haemocytometer for cell counts, and an Olympus confocal transmission microscope. Microscope pictures were taken using an Olympus DP20 (2 megapixel) digital camera and chlorophyll fluorescence

measurements were taken using a fluorometer model Gemini EM at emission 645-665 nm and excitation of 635 nm, which varied based on the natural chlorophyll fluorescence of the species in question.

To ensure quality control the following measures were taken. All field samples had one control and one test tank outdoor fed by the same bloom source and set up so that cell concentrations in both tanks were as uniform as possible and air supply was the same for both tanks. Field samples were taken in triplicate from each tank at approximately the same time of day every 24-48 hrs. Temperature was taken at this time for the first bloom trials and pH and temperature were taken for the second bloom trials. All laboratory experiments, including all time points were performed in triplicate from homogenised samples, temperature was monitored throughout the experiments and controlled if necessary, to allow the effect of ultrasound to be monitored without the assistance of heat. Digital Cannon Shot Camera (4 megapixel) was used to take pictures of the tanks throughout the trials.

7.4 Results

7.4.1 Trials 1 and 2

The dominant algal species observed during the first two trials were *Sphaerocystis* sp. and *Scenedesmus* sp. (Figure 7.1). The test tank had lost both of these dominant species after 21 days of the trial while a healthy dominant population of these species was maintained by the control tank (Figure 7.1(A). For example, cell concentrations in the control tank fluctuated with a peak concentration of 260,000 cells.mL^{-1} and 340,000 cells.mL^{-1} for *Sphaerocystis* sp. and *Scenedesmus* sp. before stabilising to a concentration of around 100,000 cells.mL^{-1} for both species. In comparison, operation of the ultrasound unit resulted in no discernable change in cell concentration over the first 10 days with a fluctuating concentration between 160,000 and 300,000 cells.mL^{-1} for *Sphaerocystis* sp. and around 135,000 cells.mL^{-1} for *Scenedesmus* sp. (Figure 7.1). On day 15 the concentration of *Scenedesmus* sp. began to decrease at a rate of 6,065 cells.d^{-1} until day 23 when the concentration was <4000 cells.mL^{-1}. An equivalent decrease occurred for *Sphaerocystis* sp. which started on day 18 and resulted in a decline to 39,000 cells.mL^{-1} within 2 days.

The second trial used the control tank from trial 1 and so operated on a bloom 30 days older than during trial 1 which indicates a switch from exponential to stationary phase growth. The concentration of *Scenedesmus* sp. increased in both test and control tanks

although the rate of increase was greatest in the test tank. To clarify, cell concentration increased from 85,800 to 437,000 cells.mL^{-1} within 15 days and then stabilised compared to an increase from 66,000 to 191,000 in 15 days in the case of the control tank. In contrast, *Sphaerocystis* sp. concentrations did not increase as dramatically and remained below 10,000 cells.mL^{-1} throughout the second trial. Diatom presence was low in the test tank throughout the second trial with a slight increase of just over 20,000 cells.mL^{1} towards the end of the trial yet this may not be statistically significant (Figure 7.1(A). Similar findings has been reported in previous trials where elevated rates of cell growth of *Spirulina maxima* and *Anabaena flos-aquae* (Al-Hamdani *et al.*, 1998; Francko *et al.*, 1990) and *Chlorella* (Zhang and Chen, 2001) have been observed when low power ultrasonic irradiation (20 kHz) had been applied to a bloom.

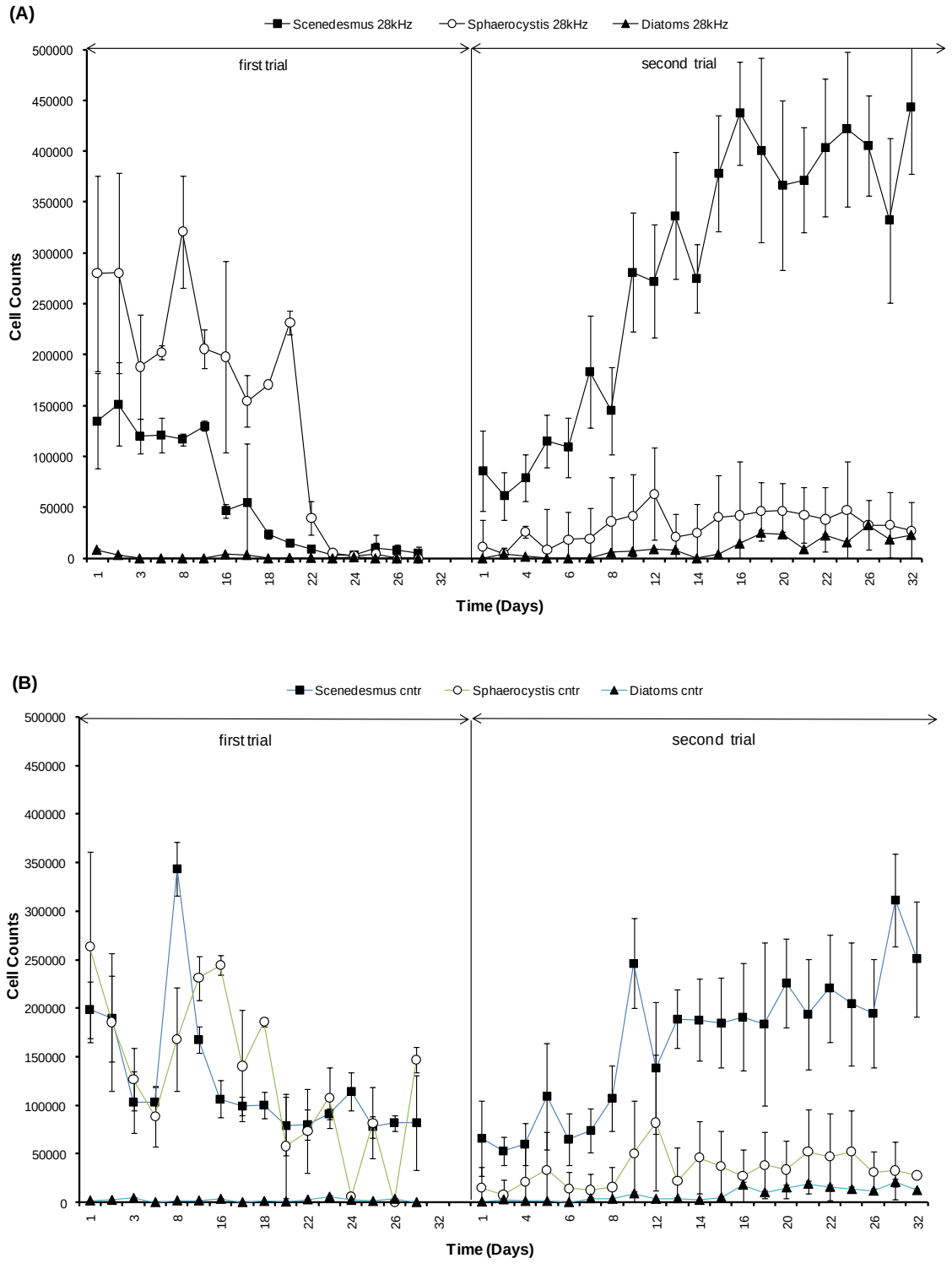


Figure 7.1 Cell counts for *Sphaerocystis* sp., *Scenedesmus* sp. and diatoms during the first and second ultrasound trials (A) test tank (B) control tank.

The health of the bloom was measured using chlorophyll fluorescence as an indication of photosynthetic activity of all the algal species combined. During the first trial the photosynthetic activity was constant in the control tank but dropped rapidly in the test tank to 8.5 from 26 relative fluorescence units (RFU) by day 17. Photosynthetic activity continued to decrease reaching 2 RFU by the final day of the trial. This is consistent with previous findings where a 71% reduction in photosynthetic activity of *Spirulina platensis* using 200 kHz ultrasound and a 40% reduction for *Microcystis aeruginosa* at 25 kHz have been reported (Lee *et al.*, 2001; Hao *et al.*, 2004). In contrast, the second trial produced the opposite trend in line with the cell count data in that the recorded levels of photosynthetic activity were higher in the test tank than the control tank (Figure 7.2). In fact photosynthetic activity in the test tank exceeded that of the control by 10 RFU within the first 11 days of the trial, an increase of 25% over the activity of the control.

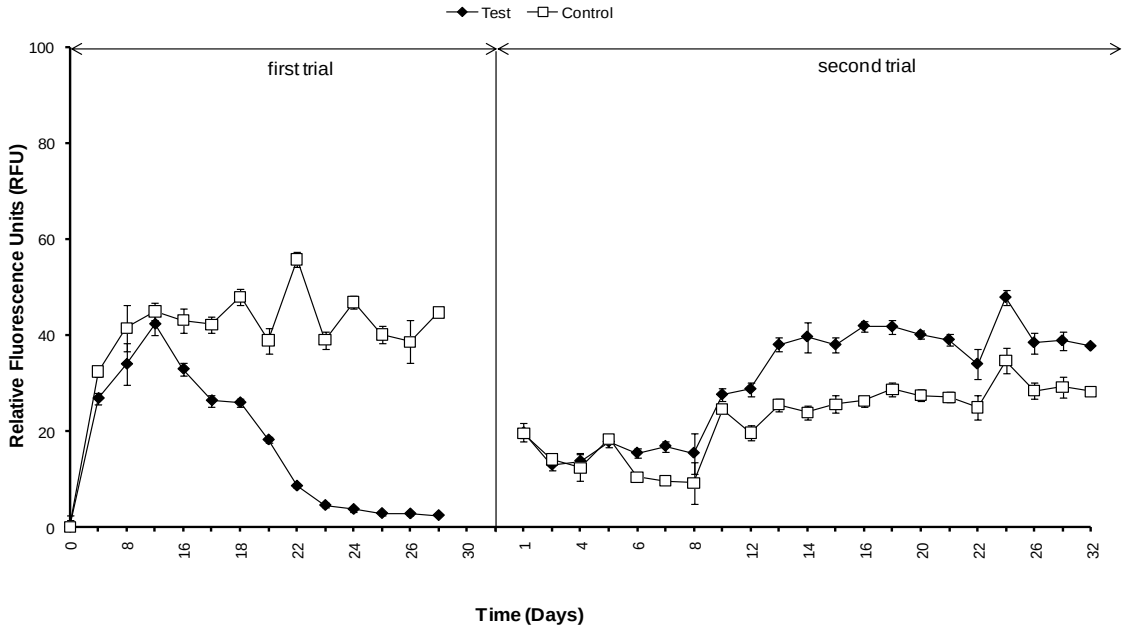


Figure 7.2 Relative fluorescence units (RFU) measure the chlorophyll fluorescence of the natural blooms of filamentous algal species in the test and control tanks throughout the duration of ultrasound treatment during the first and second trials.

7.4.2 Trials 3 and 4

In the third and fourth trials the populations were principally made up of filamentous green algae, unicellular green algae and diatoms (Figure 7.3). During the third trial, the cell numbers of all three species increased in the control tank although the filamentous green showed the largest increase reaching a peak of 455,000 cells.mL^{-1} on day 14 before decreasing back to 156,000 cells.mL^{-1} by the end of the trial. Similarly,

unicellular green algae and diatoms increased from around 8,800 cells.mL^{-1} at the start of the trial to a peak of 100,000 cells.mL^{-1} for the diatom and 81,000 cells.mL^{-1} for the unicellular green algae. In comparison, no growth of the filamentous green was observed in the test tank remaining relatively stable between 100,000 and 150,000 cells.mL^{-1} until day 15 indicating that significant growth inhibition had occurred. Thereafter, the cell concentration decreased from 101,000 cells.mL^{-1} on day 21 to 6,600 cells.mL^{-1} by day 24. During the same period diatom numbers increased consistently from 6,600 cells.mL^{-1} to a peak of just under 100,000 cells.mL^{-1} on day 16 before reducing to a final level of 51,600 cells.mL^{-1} on day 24. The same was also observed in the case of the unicellular green algae indicating a succession in the dominant population during the course of the experiment. Significantly different responses were observed during the fourth trial which was conducted on the control population from trial 3 and hence 30 days older than during trial 3. Specifically, no discernable difference could be observed between the test and control tank in terms of the unicellular green (Figure 7.3) with average cell concentrations of 159,000 and 164,000 cells.mL^{-1} for the test and control respectively.

Similarly, changes in the concentration of diatoms was not discernibly different between the test and control tanks with an initial stable period for 7-8 days followed by a decline leading to a non detectable level in both test and control by day 21 (Figure 7.3). In contrast, the response of the filamentous green was different between test and control with the growth rate in the test tank significantly faster than in the control. To describe, in the test tank, cell concentrations remained between 100,000 and 200,000 cells.mL^{-1} for the first 6 days and then increased to between 469,000 and 482,000 over

the next 12 days before increasing again to between 724,000 and 589,000 cells.mL^{-1} for the remainder of the trial (Figure 7.3B). Whereas, in the control tank the initial cell concentration of 185,000 fluctuated slightly for 6 days before increasing rapidly to 490,000 cells.mL^{-1} where after it fluctuated without additional consistent growth.

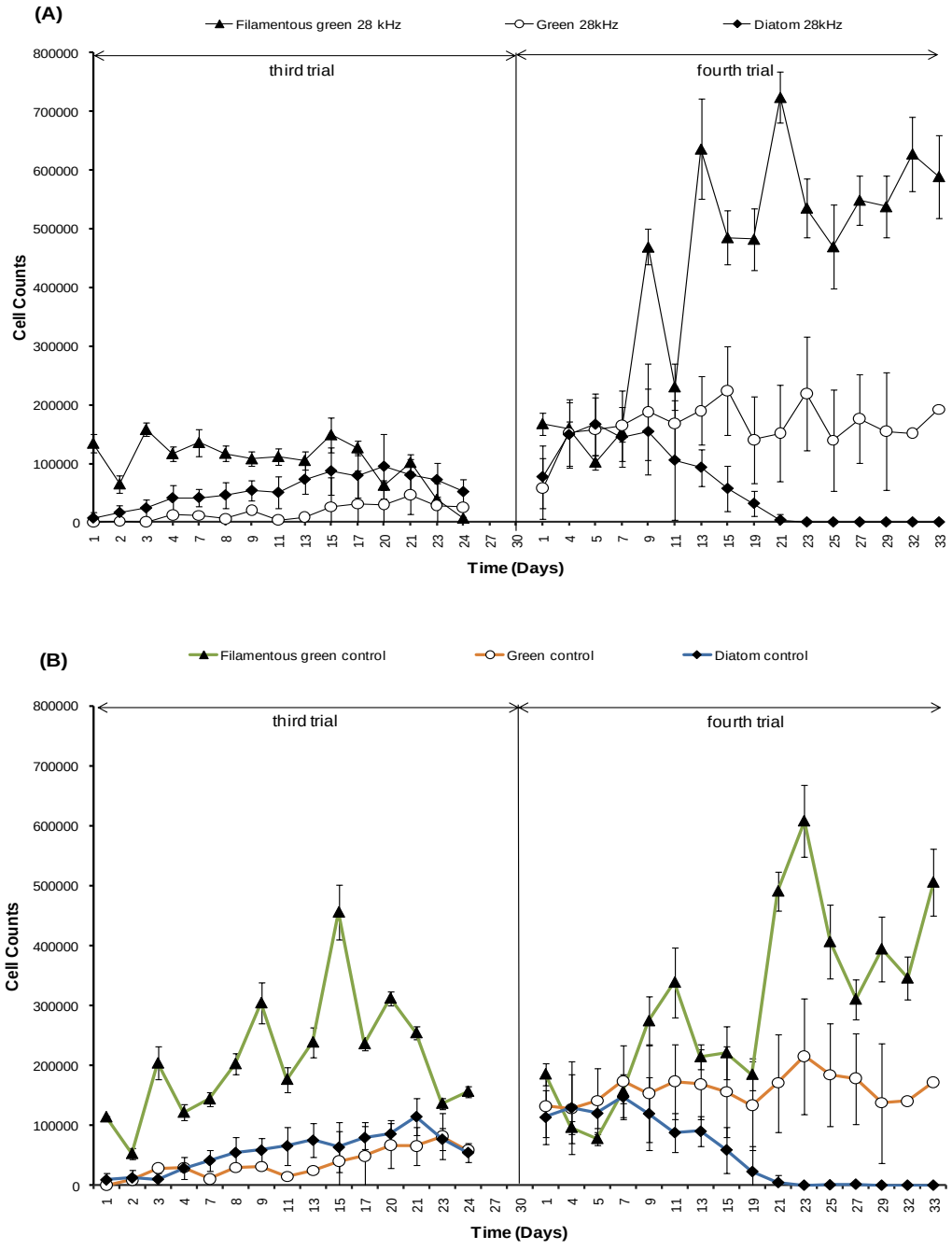


Figure 7.3 Cell counts for the natural blooms dominant species of filamentous green species in the test and control tanks during the third and fourth ultrasound trials (A) test tank (B) control tank.

Chlorophyll fluorescence confirmed the observations with increased photosynthetic activity in the control tank during trial 3 and no statistical difference in activity between the test and control during trial 4. For example, during trial 3 chlorophyll fluorescence within the test tank increased from 25±5 RFU to 31±2 RFU during the trial while the control tank began at 23±2 and finished at 76±4 RFU maintaining a healthy increase in chlorophyll content (Figure 7.4). In contrast, during trial 4 the chlorophyll fluorescence in the test tank increased from 48±2 to 127±8 RFU compared to an increase from 58±3 to 132±8 RFU in the case of the control tank.

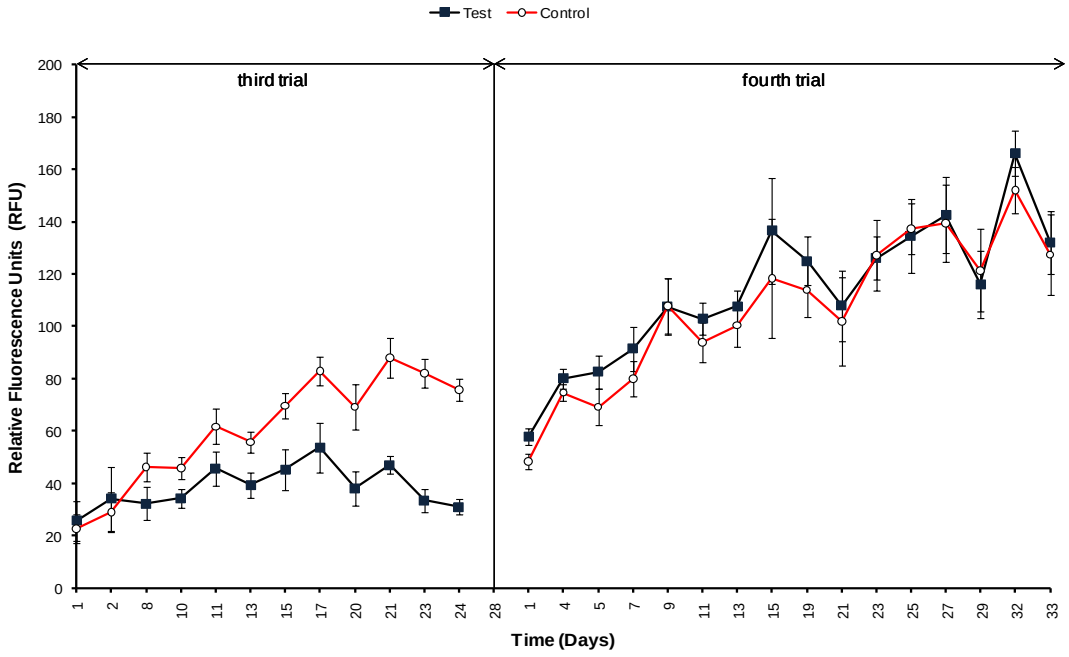


Figure 7.4 Relative fluorescence units (RFU) measure the chlorophyll fluorescence of all filamentous algal bloom species in the test and control tanks throughout the duration of ultrasound treatment during the third and fourth trials.

7.4.3 Batch experiments

Verification of the change in susceptibility was observed in two batch experiments where no appreciable change in cell counts was observed for any species when up to 19 $kWh.m^{-3}$ of ultrasonic energy was applied at a frequency of 28 kHz (Figure 7.5-7.6). The first test involved a sample from the control tank after completion of trial 3 using an ultrasonic probe (Figure 7.5). Cell counts remained stable between 167,500 and 246,600 $cells.mL^{-1}$ for the filamentous green algae and between 162,000 and 126,000 $cells.mL^{-1}$ for the unicellular green algae and diatoms. Additional experiments with a flow through unit, acting on the algae that were 60 days old, confirmed the observation with no significant change in either cell count or photosynthetic activity when up to 22 $kWh.m^{-3}$ of ultrasonic energy was applied. Initial cell counts for the different species were 256,000 $cells.mL^{-1}$, 70,000 and 33,000 $cells.mL^{-1}$ for the filamentous green, unicellular green and diatom respectively. After the first pass through the 20 kHz unit, equivalent to 11 $kWh.m^{-3}$, the cell numbers reduced to 220,000; 89,000 and 37,700 $cells.mL^{-1}$ respectively and after a second pass recovered to levels of 276,000; 104,000 and 52,700 $cells.mL^{-1}$ respectively. These results indicate ultrasonic treatment had no effect on either cell count or photosynthetic activity. However, during the course of the experiments consistent de-clumping was observed with both filamentous and unicellular green algal species. This is consistent with previous reported trials were de-clumping of field *Microcystis aeruginosa* was observed with a flow through cell unit with a higher frequency of 200 kHz (Nakano., *et al* 2001).

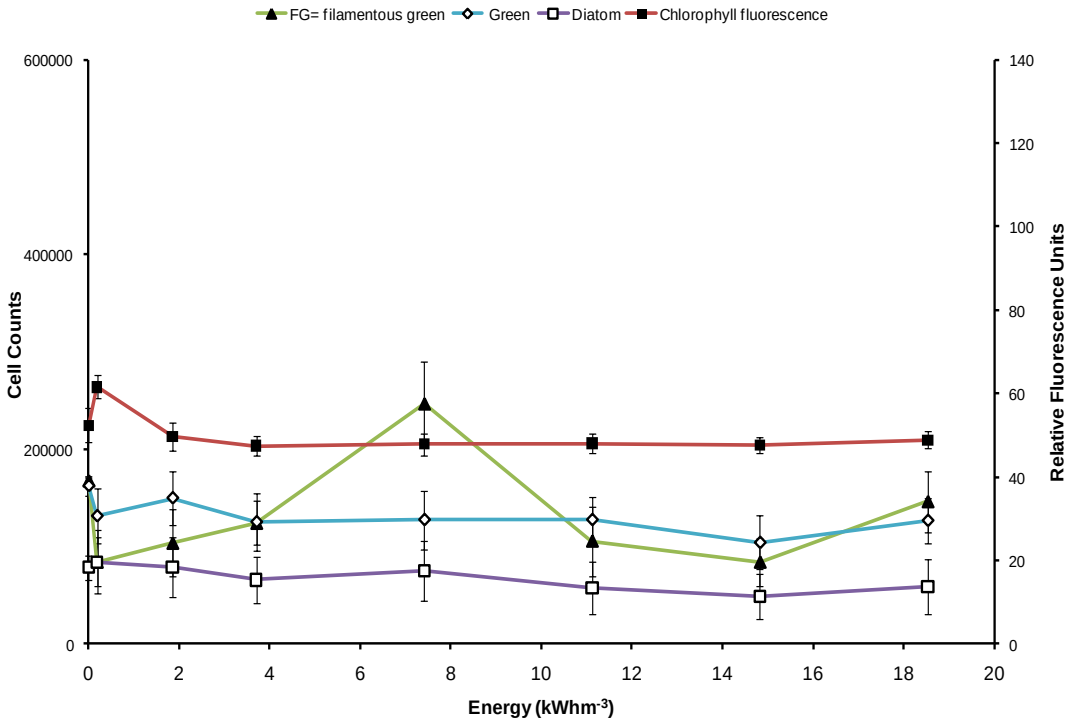


Figure 7.5 Natural bloom of mixed species dominated by filamentous green species after treatment with the Model Virsonic Digital 600 using up to 19kWhm^{-3} of energy.

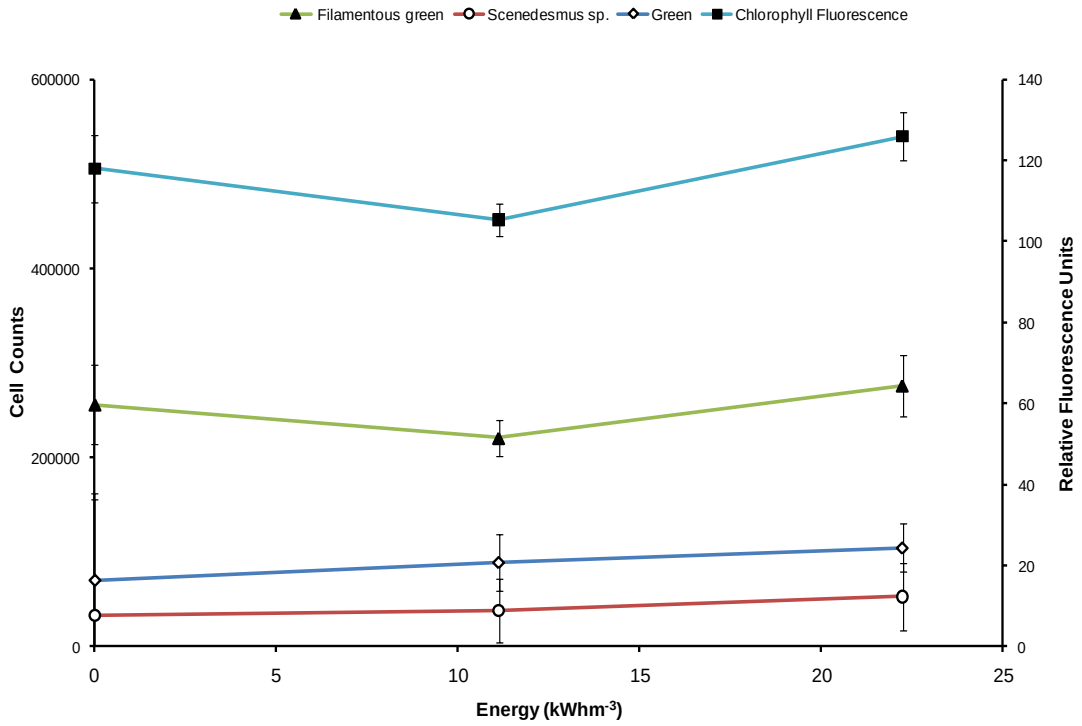


Figure 7.6 Natural algal bloom of mixed species dominated by filamentous green species after treatment with a Ultrawaves probe 20kHz in a custom made flow-through cell using 11 and 22 kWhm^{-3} of energy.

7.5 Discussion

The work presented in this paper has identified that the efficacy of ultrasonic treatment of algae is highly variable which is consistent with the findings across the literature (Nakano *et al.*, 2001; Ahn *et al.*, 2003; Inman, 2004; Warnock, 2005; Ahn *et al.*, 2007). The observed differences show that the age of the algal bloom in relation to its growth

cycle effects the success of ultrasonic treatment. Once growth has been established beyond the exponential growth phase, irrespective of species, algae lose any susceptibility to ultrasound. Thus the key factor to efficiently treating and controlling algal blooms with ultrasound is to carry out the treatment early in the bloom's life cycle preferably during exponential growth. This overall picture is consistent with other studies which have identified a sensitivity to ultrasound early in the bloom's exponential growth when cells were dividing rapidly (Ahn *et al.*, 2003; Zhang *et al.*, 2006). For instance a 30% drop in growth rate of *Microcystis aeruginosa* was observed when cells had just divided (Ahn *et al.*, 2003). Exponential growth phase is defined as where the highest rate of growth or cell division occurs during a life cycle (Walsby, 1992; Konno, 1993) and so the cells are likely to be at their most vulnerable. In fact, equivalent practical advice is given when using chemical treatments where application is suggested before cell numbers exceed 4×10^5 cells.mL^{-1} to slow down exponential growth (Ratajczyk, 2008). Such an overall picture is consistent with a mechanism known as age related resistance (ARR) (Kus *et al.*, 2002), for example reduced membrane damage in *E-coli* occurred during stationary rather than exponential growth (Pagán and Mackey, 2000). Similar findings have also been reported for algae with greater preservation of *Stauroneis anceps* diatoms for examination in older populations (Hostetter and Hoshaw, 1970). Similarly, induced chemical resistance has been observed in the second year of growth of *Ascophyllum Nodosum* in response to herbivory of the fronds in the first year (Pavia and Toth, 2000). Laboratory trials have shown selective inhibition of filamentous algae over unicellular algae (Chapter 5). This is mirrored in pilot scale experiments where 49% cell removal of the filamentous

cyanobacteria *Aphanizomenon* sp. was observed after 30 days of 28 kHz ultrasonic treatment. However the longer term, >30 days monitoring, at pilot and field experiments have shown little success at removing the dominant species, or killing the bloom, finding no difference in cell growth between the control and test tank/pond (Nakano *et al.*, 2001; Inman, 2004; Warnock, 2005; Ahn *et al.*, 2007). Our results are confirming these previous findings that blooms must be treated in the exponential growth phase, when the blooms are most susceptible to ultrasound (Ahn *et al.*, 2003). The contradictory results which are reported in the literature therefore reflect the complexity of treatment of a living population in terms of both its relative age and the specific species involved. Further, variation is however observed due to the type of measurement used to inform on algal population. Specifically, chlorophyll a measurements used in several studies can be a useful indication of the amount algal biomass present but it gives no indication of the changes in cell number occurring between algal divisions or species due to sonication. To delineate, field scale trials using natural blooms were carried out using the same floating units as this study with a 40 W, 28 kHz unit applied to identical 300 m^2 lagoons over a three month period. Results showed a statistically significant reduction in total algal growth when measured by chlorophyll a, but total cell counts, and cyanobacterial cell counts showed no difference in control and test lagoons (Inman, 2004).

7.6 Conclusions

The application of ultrasound for the control of algal blooms' has been shown to only be effective when applied during exponential growth phases of the algae. Typically this means ultrasound will only be effective within the first 30 days of the blooms life. Beyond such time ultrasound will potentially become detrimental to reducing or controlling cell numbers and so its application must be carefully considered. Comparison with previously reported studies must consider measurement methods as use of chlorophyll a can provide a falsely positive result which in reality indicates reduced activity rather than cell reduction. Field tests however are essential to the understanding of a very complex ecological system where slight environmental changes can have quite significant effects on population dynamics within algal blooms. The findings of this study have produced informative insights into the different behaviour of species dependent on whether they are in exponential or stationary phase of their growth cycle.

7.7 References

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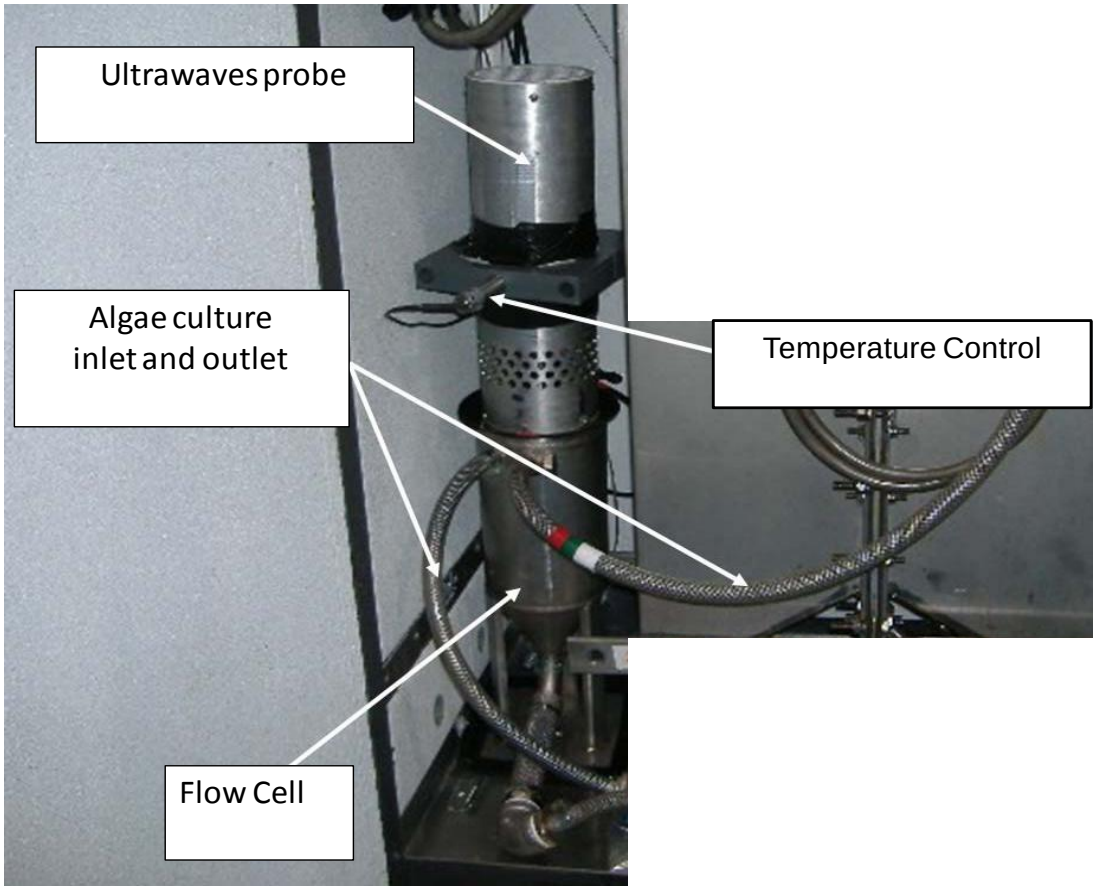
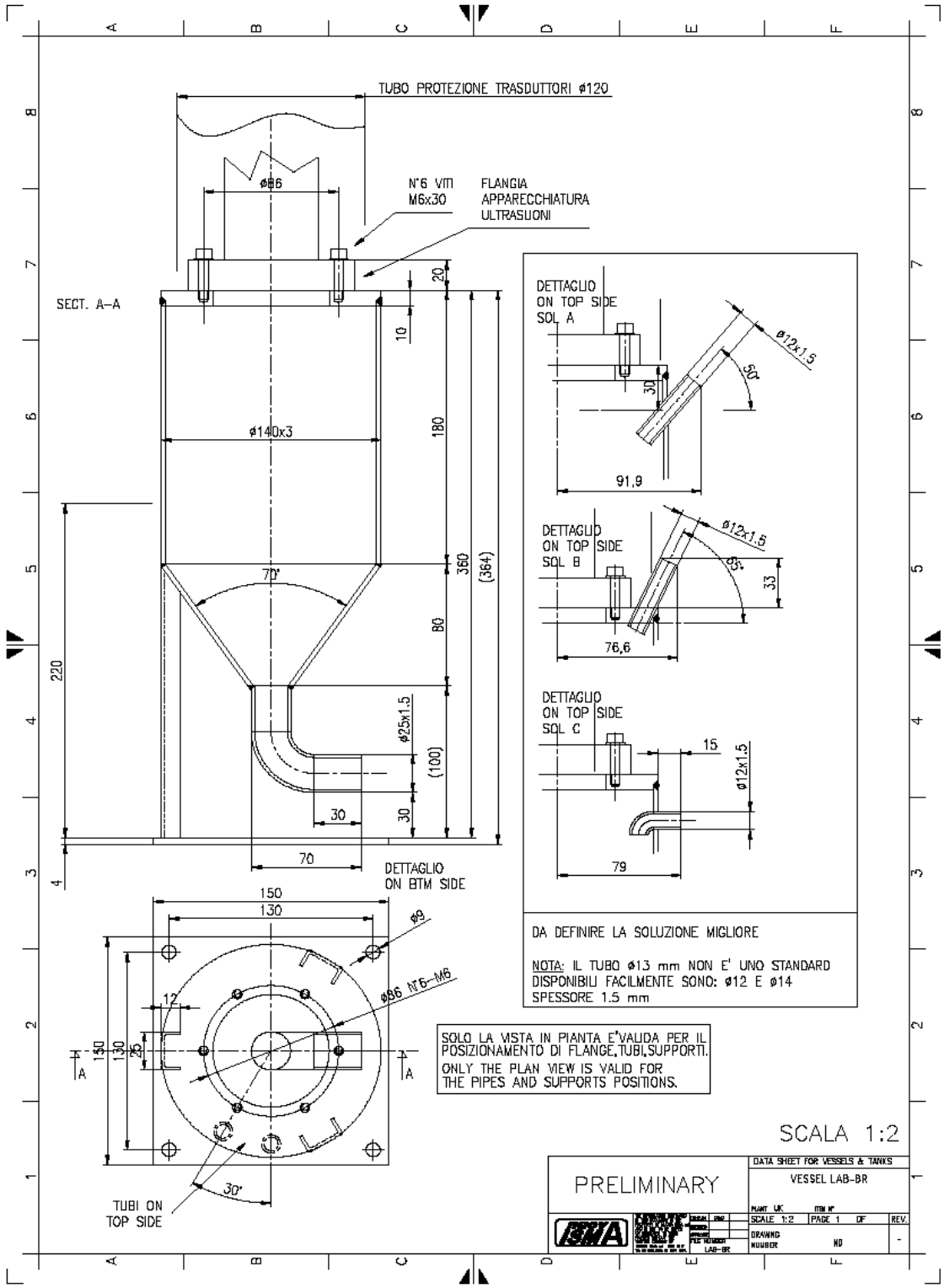
Appendix: Chapter 7

(A)

(**B**)



Appendix 7.0 (A) Ultrawaves Probe manufactured by GmbH. (B) Integrated flow through cell and Ultrawaves Probe Unit with temperature control and inlet and outlet for delivery and removal of the algal cells.



Appendix 7.1 Flow cell schematic diagram

Chapter 8: Discussion

Chapter 8: Discussion

The major finding of this thesis is that ultrasound works most effectively at removing species of filamentous algae (Table 8.1). Two distinct pathways have been postulated which indicates a link between specific algal characteristics and the optimum ultrasonic frequency to use. In the case of filamentous diatoms and green algae with rigid cell walls, removal proceeds via a direct physical action on the joints between individual cells in the filaments. The ultrasonic action is to break the cell wall and release the contents (Images in Chapter 5: Figure 5.4). In this case low frequency ultrasound is the most effective as it produces the most intense energy dissipation (Chapter 5 & 6). To clarify, *Melosira* sp., the diatom species tested had its best cell removal rates of 83% at 20 kHz. When compared with other higher frequencies of 582, 862 and 1144 kHz, cell removal rates decreased linearly to 50, 11 and -6% respectively (Chapter 5). Previous ultrasound studies have not concentrated on diatoms even though they and specifically *Melosira* sp. is considered a problematic filter blocking species for WTW (Bauer *et al.*, 1998). Industrial ultrasound units successfully implemented in treatment works function only at low frequency (20 kHz), indicating implementation possibilities for such species.

An opposite effect is seen with cyanobacteria where cell removal increases with frequency (Chapter 5). Highly susceptible filamentous species with gas vacuoles tested at 862 kHz had cell removal rates of 93% for *Aphanizomenon fq.*, 88% for *Anabaena* (wild) and 51% for and *Anabaena fq*. In these cases, the gas vacuole is critical to the

ability of ultrasound to inflict severe damage which leads to significantly high cell removal rates. Ultrasound has been documented to cause the collapse of gas vacuoles in cyanobacterial species, (Lee *et al.,* 2002; Ahn *et al.,* 2003). Ultrasound affects these algal species by inducing the bursting of the gas vacuoles due to the size of the vacuoles being in the same order of magnitude as the resonance bubbles at high frequency, thereby causing the gas vacuoles to resonate similarly to the bubbles produced by cavitation causing a higher rate of gas vacuole collapse, hence contributing to higher rates of cell removal in this type of algal physiology (Zhang *et al.,* 2006a).

For the non-susceptible cyanobacteria species, *Microcystis aeruginosa*, and colonial green algae *Scenedesmus subspicatus*, the best treatment method using ultrasound was seen to be growth restriction by reducing photosynthetic ability. Results from (Chapter 5) revealed that using 133.4 $kWh.m^{-3}$ at 862 kHz reduced chlorophyll fluorescence of 37±3% and 60±5% for *Scenedesmus sub*. and *Microcystis aerg*. respectively incurring reductions of two and three times the cell removal rates for these species which were >20% at 17±5% and 16±3% respectively. Overall this indicates that sonication is more effective at damaging photosynthetic apparatus than killing the cells. These results were confirmed by several other studies with photosynthetic inhibition levels of approximately 40-50% commonly reported (Lee *et al.*, 2001; Zhang *et al.*, 2006b) yet these removal rates were at both low and high frequency.

Table 8.1: Summary of algal susceptibility based on the exposure of species of green algae, diatoms and cyanobacteria to ultrasound treatment using frequencies ranging from 20-1144 kHz and electrical power input of ranging from 0.2-133 (kWh.m^{-3}).

Species	Division	Key property	Best removal (%)	Energy required for plateau (kWh.m^{-3})	Frequency (kHz)
Aphanizomenon fq	Cyanobacteria	Filamentous, gas vacuole	98	3.7	1144
Anabaena wild	Cyanobacteria	Filamentous, gas vacuole	88	3.7	*(862)
Melosira sp	Diatom	Filamentous, rigid wall	83	11.1	20
Tribonema mixed	Green	Filamentous, semi rigid wall	72	1.9	*(862)
Anabaena fq	Cyanobacteria	Filamentous, gas vacuole	51	exponential	*(862)
Tribonema v	Green	Filamentous, semi rigid wall	28	0.2	*(862)
Scenedesmus sub.	Green	Unicellular	20	67	No difference
Microcystis aerg	Cyanobacteria	Unicellular, gas vacuole	16	133	No difference

"*(862) indicates only one frequency tested"

A number of other observations made during the thesis complicate the overall picture, heightened susceptibility and enhanced removals were observed in the field samples (*Anabaena* wild; *Tribonema* mixed) compared to the culture collection equivalents (Chapter 6). It is suggested that moving the field samples into laboratory conditions stimulated their growth during which time algae are shown to be most susceptible

(Chapter 7). This is confirmed with the observation in the field trials and the case study sites that application of ultrasound to established blooms enhanced growth rather than reduced cell numbers and so ultrasound only appears effective during exponential growth phases (Chapter 7). However, even on established blooms ultrasound was seen to de-clump their interwoven filaments (Chapter 7) which is known to be very effective at enhancing grazing pressure on the cells post-sonication (Nakano *et al.*, 2001). Breaking up of the cell aggregates to >70µm encourages predation by zooplankton, which otherwise would not be able to feed on the bloom mass (Lee *et al.*, 2001; Nakano *et al.*, 2001; Lee *et al.*, 2002; Ahn *et al.*, 2003; Tang *et al.*, 2003; Hao *et al.*, 2004; Tang *et al.*, 2004; Zhang *et al.*, 2006b; Mason, 2007a; Chapter 5, 6, 7). These effects were not apparent in the laboratory trials as no ecologically balanced systems were utilized.

Threshold energy levels for plateau cell removal were reached at over 1.9 $kWh.m^{-3}$ for all species where significant cell reduction was observed (Table 8.1). This far exceeds the operational levels which are between 0.4-1.0 $kWh.m^{-3}$ for all treatment and supply of water in the U.K.. For instance, the field unit tested in the current study (Chapter 7) was also used by Anglian Water at full scale and produced positive results but once implementation costs were calculated based on the actual volume of the reservoir and if used constantly, the cost projections were too high to be financially viable. However in light of the results from (Chapter 5-7), with more specific guidelines for implementation and removing the necessity for constant use, the operating cost may decrease sufficiently to be considered. Key features of any successful application will include using ultrasound in the correct circumstance: early in algae growth cycle, not infested

by floating plants which reflect the ultrasonic waves decreasing the effect of ultrasound (Inman, 2004; Warnock, 2005), only at the bloom source and not the entire reservoir volume (Ahn *et al*., 2003), and only if the species are susceptible (Chapters 5 & 6).

8.2 References

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Chapter 9: Conclusions and Further Work

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9.1 Conclusions

The key finding of the work has been that ultrasound is only effective against filamentous species. Two distinct mechanisms have been identified which determines a link between algal properties and the frequency at which the ultrasound should be employed. In the case of filamentous cyanobacteria (*Aphanizomenon fq., Anabaena* (wild) and *Anabaena fq*.) the presence of gas vacuoles is critical. In such cases high frequency ultrasound is most effective as the cavitation effects, including the microstreaming of bubbles around the resonance frequency of the gas vacuoles, are maximised. In the case of non cyanobacterial species, low frequency ultrasound is most effective (Chapter 5 & 6). In such cases, rigid wall structures (*Melosira* sp., *Tribonema viride)* due to the presence of silica is key as the cavitating gas bubbles can directly influence the physical structure of the filament causing breakage at the joints releasing the cell contents (Chapter 5 & 6).

A secondary effect of ultrasound was also observed in terms of damage to the photosystem II and this applied to all the species tested. This is important for non susceptible species (*Microcystis aeruginosa* and *Scenedesmus subspicatus*) where reduction in chlorophyll fluorescence indicates that ultrasound could control bloom growth rather than bloom removal (Chapter 5). Care is also required when comparing previous studies as some report only in terms of chlorophyll fluorescence which provides an overly positive result compared to cell counts.

The energy required to remove the most susceptible species was 3.7 $kWh.m^{-3}$. At this energy level ultrasound is not economically feasible as it far exceeds standard energy demand for water treatment and supply at between 0.5-1.0 $kWh.m^{-3}$. Given an economy of scale and improvements in delivery it is possible that ultrasound could be considered an option for treatment of specific blooms which are highly susceptible (filamentous cyanobacteria) to ultrasound, but only if treated early in their growth in the source areas of the reservoir, when their cell numbers are below 400,000 $cells.mL^{-1}$ (Ratajczyk, 2009; Chapter 3 & 7). Additionally, application of ultrasound outside of such conditions may cause growth rather than reduction (Chapter 7) and so its use must be carefully considered. Overall, the combination of requiring different frequencies for different species, it only worked on some species and the fact it may make the bloom more active, means that ultrasonic treatment of algae is unlikely to become standard practice.

9.2 Further Work

- A more extensive list of ultrasound susceptible algal species needs to be developed to allow treatment procedures to be formulated for WTW in the U.K.
- Further testing is required on specific parts of the algal cells which appear most susceptible, to determine whether these components are the correct locations within the algal cells where ultrasound can attack. For example, extensive work has already been carried out on gas vacuoles susceptibility to ultrasound (Walsby and Bleything, 1988; Walsby, 1992; Walsby, 1994). Isolation and testing of silica's response to ultrasound, (similar to the Walsby work of gas vacuoles) would definitely improve the knowledge base required to treat all problematic diatom and green cell types which have silica as their cell wall material in the case of diatoms or is partial component of the cell wall of filamentous green algae .
- Further testing on the specific damage caused by ultrasound to non-susceptible species in the form of chlorophyll fluorescence is necessary. *Microcystis aeruginosa* and *Scenedesmus subspicatus* and other unicellular problematic algal species i.e. *Chlorella* could be controlled if photosynthesis could be stopped or slowed down, therefore more specific methods to quantify this physiological damage could be very useful to correlate the damage with decreased growth levels to attain the optimal damage level needed to control bloom growth.

- Quantification of phycocyanins the signature pigments of cyanobacteria (Ahn *et al.*, 2007a) may be a useful addition to the chlorophyll fluorescence data to decipher the level and type of damage occurring in non-susceptible samples, and compare responses to non-gas vacuolate cyanobacteria allowing damage levels to be defined via the least amount of energy required to create the most significant damage.
- To increase the speed of counting cells and determining healthy versus dead cells, flow cytometry and or fluorescein diacetate could be used. These methods can also detect membrane permeability and determine whether gas vacuoles are still intact (Jong Lee *et al.*, 2000; Mason *et al.*, 2003). Basic cell counts can also be performed on a Coulter counter to increase the speed of the process.
- Detection of free radicals in samples during and post-sonication would be most valuable in determining the secondary effects of ultrasound i.e. the production of free radicals is taking place (Loreto *et al.*, 2005).
- Cyanotoxin detection is essential in determining how ultrasound affects the release of algal toxins from cells during sonication and also if toxins are released; the effect sonication has on the toxins themselves. Some preliminary studies have found that ultrasound can in fact breakdown pure microcystin toxin by up to 70% after 20 minutes sonication using 150 kHz and 30 W (Ma *et al.*, 2005), yet further investigation would be required to confirm this.
- Further field trials on natural blooms from all types of algal species are essential to determine the results attainable at full scale. To date with only 7 studies

including those carried out here, too little information is available to clearly determine whether ultrasound can function efficiently at full scale.

- Ultrasound unit types vary greatly depending on their application. Those tested so far at field scale include flow through cells units, submersible transducers and floating transducers (Nakano *et al.*, 2001; Ahn *et al.*, 2003; Inman, 2004; Warnock, 2005; Ahn *et al.*, 2007b). Most units are custom made therefore removing the ability to compare removal rates accurately. Therefore commercially available ultrasound units other than those tested here need to be tested at field scale under initially optimal conditions using a monoculture to decipher their optimal cell removal rates, and then tested on different natural algal field blooms types including cyanobacteria, diatom and green algae.

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