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The effects of ultrasonic treatment on cyanobacteria in surface waters

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The effects of ultrasonic treatment on cyanobacteria in surface waters

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ABSTRACT

The effect of power ultrasound on algae blooms (*Microcystis aeruginosa*) over a 30 minute period was assessed using 200 and 400 mL suspensions of optical density of 2.0 at 680 nm. The frequencies employed were 20, 40, 580 (40%, 80%, and maximum intensity), 864 (40%, 80% and maximum intensity) and 1146 kHz (40%, 80% and maximum intensity). Ultrasound can induce two different effects on algal cells; inactivation at high power (≥ 0.0022 Wcm^{-3}) and de-agglomeration at low power (≤ 0.0042 Wcm^{-3}). Ultrasonic effects were observed using haemocytometer, optical density, UV-visible spectrometer, fluorospectrometer and flow cytometry. Using a 40 kHz bath (0.0214 Wcm^{-3}) led to de-agglomeration resulting in an overall increase in algae of -0.28% by haemocytometer and -4.20% by optical density. The highest inactivation achieved was 91.54% (haemocytometer) and 44.63% (optical density) using 1146 kHz (maximum intensity, 0.0248 Wcm^{-3}) and 200 mL suspension. In terms of efficiency to achieve inactivation (i.e. inactivation % / power) the best result was observed at 864 kHz (40% power setting, 0.0042 Wcm^{-3}) with 200 mL suspension giving 8226.19 by haemocytometer and 5011.90 by optical density. This initial part of the study allowed a comparison to be made of the ultrasonic parameters that would lead to optimum algae removal in terms of acoustic energy input. The haemocytometer results for cells number were generally higher than those indicated by optical density which is probably due to the fact that the former records only cell numbers remaining whereas the latter is an overall measure of algae concentration (ruptured cells will still register, because their contents remain in suspension).

Studies on de-agglomeration and inactivation were also undertaken using small or medium-scale ultrasonic equipment that were models for industrial scale systems. The following volumes of algae suspension and equipment were employed: Sonolator (Sonic Corporation, 5L flow), 16 kHz and 20 kHz Dual Frequency Reactor (DFR, Advanced Sonics LLC, 1L static and 3.5 L flow), 20 kHz Vibrating Tray (Advanced Sonics LLC, 1.5L static) and 20 kHz ultrasonic probe (made at Southeast University, 4L static). The most effective inactivation effects were obtained with the DFR reactor in static mode and 60% power setting for 10 minutes which achieved reductions calculated at 79.25% using haemocytometry and 60.44% by optical density.

The third part of this study was to gain a greater understanding of the basic mechanisms of the action of ultrasound on algae and to interpret this in terms of its potential for algal cell removal and control. Algal cell activity was assessed by three methods: using a UV-visible spectrometer (Shimazu, 2450PC), a fluorometer (Shimazu, RF5301) and a flow cytometer (BD FACS Calibur). Ultrasonic damage to Chlorophyll A was revealed through observation of the loss in UV-Vis spectrophotometer peaks around 600 nm together with the decrease in fluorometer results for peaks around 500 and 680 nm. Flow cytometer results were able to identify the number of both intact cells and damaged/ruptured cells thus giving greater insight into the mechanism of ultrasonic inactivation. The direct rupture of cells by power ultrasound was prevalent at low frequencies ≤ 40 kHz due to the mechanical effects of cavitation collapse and inactivation of algal cells by free radicals occurred at high frequencies ≥ 100 kHz and medium powers where mechanical effects are much reduced.

In conclusion, this work has shown that power ultrasound can provide a suitable method to control algal growth in small and medium laboratory scales. Scale-up beyond this point is the subject of further research but the results herein clearly demonstrate the importance of choosing the correct ultrasonic parameters in terms of frequency, power and exposure time.

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Content

1.0 Introduction: Drinking water and health

Drinking (potable) water refers to water of high quality, which will not cause immediate or long term harm to consumers. It is well known that water is vital for supporting life. 70% of the earth surface is covered by water and 70% of the mass of a human body is composed of water. Humans can survive without food for several weeks, but for only a few days without water. Drinking water is essential to sustain human life and is one of the primary considerations for maintaining public health. Furthermore, many modern industries also require water of drinking water quality. Therefore, safe drinking water is important for human health, economic development and the overall stability of society.

Concerns about the quality of drinking water are not only focused on the aesthetic qualities such as transparency or odour. There are many contaminants in drinking water, which cause adverse effects on human health. In this chapter, water resources, contamination in drinking water and drinking water treatment are reviewed.

1.1 Water resource without human and animal contamination

Without human activity water sources are naturally affected by the dissolution of minerals from soil, rock, biosynthesis, and biodegradation of organic matter. When water is in contact with soil/rock (aquifer bed) it will naturally contain some of dissolved elements such as calcium, sodium, magnesium and potassium. The atmosphere is another factor because it influences the pH of natural water. When water absorbs carbon dioxide from the air it becomes slightly acidic. Some specific rock types may also contain radioactive elements which leach into the water. For organic matter, many compounds found in natural water are the result of biosynthesis and biodegradation. Chemical contamination in natural water is dominated by bicarbonate and calcium ions (Sullivan, 2005: 2−15).

1.2 Contamination in drinking water

The majority of contamination in water resources is a direct consequence of man-made or related to human activities (e.g. domestic sewage and industrial wastewater). Annually more than five million people die from illnesses linked to

contaminated drinking water (Gleick, 2002). It is vital to have a brief understanding of contaminants present prior to deciding on the appropriate treatment. To determine if drinking water is safe for consumption the World Health Organization (WHO) has defined the key issues. These are international water quality standards which must be adhered to. Contamination in drinking water is basically derived from two types; microbial and chemical.

1.2.1 Water quality standards of WHO

When identifying if drinking water is safe for consumption, it is vital to define all key issues related to safe drinking water. International water quality standards are set by the World Health Organization guidelines (WHO). The guidelines help governments and local communities set national or local standards as well as researchers working on related issues to water quality. The Guidelines for Drinking−Water Quality (WHO, 3rd edition, 2004) publication is designed as an advisory tool regarding impacts relating to human health and contains quality standards for microbial, chemical and radiological aspects (WHO, 2004: xv−2).

These WHO guidelines define safe drinking water as water which does not pose a significant risk to health over a lifetime of consumption. No pathogenic organisms, compounds causing offensive tastes or odours, or corrosive chemicals should be present. Concentrations of compounds that are acutely toxic or that have serious long-term effects should be low (e.g. arsenic, lead, mercury, etc.). Safe drinking water should also be clear/transparent and not contain harmful chemicals (WHO, 2005: 1−3).

WHO has set acceptable standards and chemical and microbial contaminants are outlined below in Table1.1 and Table 1.2. Major pathogens have been reviewed for the selection criteria which are based on adverse effects on human health, magnitude, frequency and duration of exposure to contamination, population numbers exposed to hazardous substances and international concerns. The WHO recommendations for water quality are presented using Guideline Values (GVs) which are intended to help set national requirements and situations to set limits and standards. GVs are set for indicator bacteria and operational parameters such as turbidity and residual chlorine. For microbial contamination human health risk is the basis of the GV and a particularly risk for faecal contamination and the likely potential

of disease incidents. Most chemical GV are set for the health risks associated with lifetime consumption. GVs are not lower than the detection limits achievable under routine laboratory operating conditions. Guidelines are continuously updated as knowledge increases (WHO, 2004: xv−2, WHO, 2005:1−3, and Meybeck, 1989:1−7).

1.2.2 Microbial contaminants

Based on the 'WHO Guidelines for drinking−water quality' pathogens are divided into a number of categories (WHO, 2004: 221− 295):

- Bacteria, viruses, protozoa and helminthes
- Potentially emerging pathogens
- *Bacillus* (food−borne pathogenic species *Bacillus cereus*)
- Hazardous cyanobacteria (*Microcystis aeruginosa*)

Microbial contamination in drinking water mainly leads to human infections. High levels of pollution may result in public health disease outbreaks (WHO, 2004: 221− 295). As illustrated in Table 1.1, microbial pathogens may survive and reproduce in the drinking water distribution system or occur naturally in water bodies. Each respective pathogen is defined using examples, occurrence and significance to human health. Biological hazards usually cause human infection/disease and in some cases they can result in liver damage or tumour development. Most biological contamination originates from human and animal faecal material. It is almost impossible to completely remove all microbial contamination during treatment of drinking water, so a safe level of biological hazard is required. WHO "Guidelines for drinking-water quality" have listed safe levels for most microbial contamination.

Microbial treatment includes processes such as: pre-treatment, coagulation/flocculation/sedimentation, filtration and disinfection. Reductions are targeted to bacteria, viruses and protozoa using different treatment measures.

Table 1.1 Microbial contaminants (WHO, 2004: 221− 295)

Pathogen	General examples	Human health significance	Source and occurrence
Bacteria	*Escherichia coli* *Pseudomonas aeruginosa* *Vibrio cholerae*	Infection Dehydration	Soil Water Sewage People Animals Food
Viruses	*Adenoviruses* *Hepatiti* *Rotavirus*	Infections Liver damage Viral hepatitis	Soil Water Sewage People Animals Food
Protozoa	*Cryptosporidium parvum* *Giardia* *Cyclospora* *Toxoplasma gondii*	Asymptomatic infections Fatal disease Diarrhoea	Soil Water Sewage People Animals Food
Helminths	*Dracunculus medinensis* *Schistosoma spp.*	Infections Abscess formation Hepatic lesions	Low quality drinking water system
Potentially emerging pathogens	*Helicobacter pylori* *Tsukamurella* *Isospora belli*	Asymptomatic infections Organ diseases Immune suppression	Water Humans
Bacillus	*Bacillus cereus*	Food poisoning	Soil Water Water supply System
Hazardous cyanobacteria	*Anabaenopsis millenii* *Cylindrospermun spp.* *Microcystis sp.*	Liver damage Neurotoxicity and tumour promotion	Soil Seawater Fresh water

1.2.3 Chemical contaminants

Based on the WHO 'Guidelines for drinking-water quality' (2004: 296−460) and Fawell's (2003) paper 'Contaminants in drinking water', the most important chemical contaminants are outlined in Table 1.2 (arsenic, fluoride, selenium and uranium, iron and manganese, agricultural chemicals, urban pollution, and by-products of water disinfection). Some chemicals are not immediately toxic to human health and some are essential elements in human nutrition such as iron and manganese. However, long term exposure or intakes can cause adverse effect on human health (cancer).

Negative health effects from chemicals are usually caused by prolonged periods of exposure. This is the main difference between microbial and chemical contamination. The priority of monitoring and remedial action is to ensure that water resources are safe for consumption (WHO, 2004: 6). Hazards may arise intermittently or result from seasonal activities, so seasonal monitoring is required. For example, it is vital to monitor and control cyanobacterial blooms each summer in some eutrophic lakes (WHO, 2004: 30).

Approaches to control chemical hazards in drinking water (WHO, 2004:166) included chlorination, ozonation, filtration, aeration, chemical coagulation, activated carbon adsorption and ion exchange.

Table 1.2 Chemical contaminants in drinking water (WHO, 2004: 221− 295)

Chemicals	**Human health significance**	**Occurrence**	**Comments**
Arsenic	Cancer Hyperkeratosis disease	Natural waters	An important drinking-water contaminant
Fluoride	Skeletal tissues Morbidity	Groundwater	Need to consider the intake of water and the fluoride from other source
Selenium & Uranium	Manifested in nails Hair and liver Nephritis	Drinking water	Only long term exposure would cause toxic effect
Iron & manganese	Long term exposure may cause problems of nervous system	Anaerobic source water	Cause discolouration and turbidity
Agricultural chemicals	Infants health problems Grow toxic algae in water	Surface water drinking water	Pesticide may cause illness, nutrients grow toxic algae
Urban pollution	Infection without safe drinking water treatment	Groundwater and hydrocarbons	May cause odour problems
By-products of water treatment	Cancer Adverse birth outcomes	Drinking water	Evidence is inconsistent and inconclusive
Endocrine disrupters (EDC's)	Cancer Adverse health effects	Surface water	Interfere with endocrine system
Microcystins	Liver damage Cancer promotion	Surface water drinking water	Most common and toxic toxin: Microcytin−LR

1.3 Drinking water treatment processes

The aim of drinking water treatment is to purify water so it is fit for human consumption and to ensure that water quality is safe for human health over long-term exposure (Sullivan, 2005:103).

General drinking water treatment processes includes: pre-treatment (screening, aeration, pre-settlement and oxidation), coagulation, clarification (settlement,

flotation), filtration, disinfection, stabilisation and safe supply to consumers. By comparison to wastewater treatment, drinking water treatment is mainly physico-chemical whereas wastewater treatment is basically biological (Stevenson, 1997:11). The flow chart (figure 1.1) below describes the process of basic drinking water treatment. For each process, main objects and applied treatment methods are listed. Beyond basic water treatment, there are a number of advanced technologies employed in modern water treatment plants. These technologies are required to optimize the quality of water within operations (Sullivan, 2005:99). Typical advanced technologies are discussed below and they include granulated activated carbon (GAC), ion exchange, ozonation, ultraviolet light and the emerging technology of ultrasound.

Figure 1.1 Flow chart of basic drinking water treatment (Stevenson, 1997)

1.3.1 Basic water treatment

1.3.1.1 Pre-treatment

Pre-treatment refers to processes prior to coagulation and separation (Solt, 1991: 12−13). Pre-treatment includes screening, aeration, pre-settlement and oxidation.

Screening removes large solids and oils before raw water enters the treatment system (Stevenson, 1998:17). The main purpose of screening is to protect primary pumps. Screens are equipped with bars spaced at different distances (Twort, 2000:271). The growth of algae in backwash water may cause blockage problems. Ultraviolet lamps or washing with sodium hypochlorite is often applied as a preventive or control measure (Stevenson, 1998:101−103).

Aeration is a process of dispersing air in water; increasing levels of dissolved oxygen in water and removing gases/volatile compounds. The benefits of aeration include (Stevenson, 1998:17):

- Meets requirements for dissolved oxygen set in water quality guidelines or by customers
- Removal of carbon dioxide and increases pH
- Removal of harmful gases (hydrogen sulphide) or traces of volatile organics

Pre-settlement is used to reduce suspended solids usually by chemical dosing. Normally, suspended solid concentrations should be controlled to less than 100−200 mg/L following pre-settlement (Stevenson, 1997:18).

Oxidation is divided into chemical oxidation and biological treatments. These processes prevent living organisms growing within the pre-treatment plant (Solt, 1991: 13). Chemical oxidation precipitates iron and manganese via pre-chlorination or dosing with potassium permanganate. Biological oxidation oxidizes manganese (from soluble Mn^{2+} to insoluble Mn^{4+}), ammonia and improves taste using natural bacteria or by adding fine sand (Stevenson, 1997:18).

1.3.1.2 Coagulation

Coagulation is used to destabilise suspensions of suspended solids, in preparation for further treatment. Coagulants (aluminium and ferric salts) are mixed with water making soluble contaminants separable (Stevenson, 1997:19). At high alkalinity, insoluble hydroxides of aluminium or iron are formed, shown below:

$$Al_2(SO_4)_3 + 3Ca(HCO_3)_2 \rightleftharpoons 2Al(OH)_3 + 3CaSO_4 + 6CO_2$$

In this way, soluble contaminants become insoluble particles, which can be removed. Raw water quality, coagulants dosage and pH can affect efficiency. A “Jar test” is used to test coagulation efficiency on a small scale/batch by assessing the removal rate of colour and turbidity (Solt, 1991: 37).

1.3.1.3 Clarification (settlement, flotation)

Clarification reduces the concentration of solids prior to conventional filtration. Clarification processes consist of settlement and flotation systems which remove solids that are heavier than water (Stevenson: 1997: 19). In a settlement system, flocculents coalesce small particles into a larger particle which can be removed by filtration (Twort, 2000: 277). Water is mixed with flocculents and placed in a sedimentation basin for settlement and then transferred to a flotation system. During this step, gas bubbles are forced into water increasing the buoyancy of suspended solids, which float to water surface where they are removed (Solt, 1991: 27−36).

1.3.1.4 Filtration

Filtration removes suspended and colloidal materials using sand beds. Treated water from clarification processes is passed through sand which acts as a filter. The efficiency of the process is determined by the particle size of sand, depth of sand bed and filtration flow rates (Stevenson: 1997: 387).

1.3.1.5 Disinfection

Disinfection inactivates bacteria and protects the drinking water distribution system from re-growth of bacteria during supply to consumers. A typical river water source can have up to 10^4 *E. coli*/100 mL, which is considered unfit for human consumption.

Chlorination or treatment with chlorine dioxide are used to destroy biological contaminants. However, some disinfection by-products may be produced during disinfection processes, which can be harmful to human health (Stevenson: 1997: 33). In large scale applications, there are four common disinfectants: chlorine, chloramines, chlorine dioxide and ozone (Twort, 2000: 429).

1.3.1.6 Stabilisation

Following the above treatment processes water is usually acidic, which makes it unsuitable for consumption, so alkali is often added to raise the final pH. For water with low alkalinity, sodium carbonate can be used to correct the pH. Carbon dioxide and phosphates are also added to soften water (Stevenson: 1997: 37).

1.3.2 Advanced water treatment technologies

The use of advanced water treatment technologies reduces both the chemical loading and labour input during treatment processes and improves the final water quality. However, the application of these technologies increases capital costs for water companies. Despite this certain advanced water treatment technologies are effective and necessary for environmental protection.

1.3.2.1 Granulated activated carbon (GAC)

Granulated activated carbon is used to removing organic chemicals from water (Sullivan, 2005: 99). GAC is composed of a carbon media usually with 12×40 mesh (maximum and minimum size for the bulk of materials), resulting in efficient filtration. GAC filters can also remove solids and absorb colour/turbidity. However, regular back washing is required to maintain filtration rates. GAC is more efficient than any other filter media (Twort, 2000: 333−335). GAC is applied widely in large water companies for economic reason. The main disadvantage is that GAC must be cleaned or replaced when the majority of the GAC surface is covered with contaminants which can occur after a few months but they can up to years last.

1.3.2.2 Ion exchange

Ion exchange technology is used to remove metal salts by employing resin beds to reduce the hardness of water. When a resin bed is exhausted, reverse exchange is required prior to re-use (Hammer, 2008: 262). Ion exchange produces high purity water which is suitable for use in industry or scientific research (Eldridge, 1995: 61).

1.3.2.3 Ozonation

Ozonation is used in the USA and Europe including the UK to replace chlorine disinfection treatment to avoid any harmful by-products such as trihalomethanes, haloacetic acids. Ozone is a powerful oxidizing agent which effectively destroys most organic compounds and biological contaminants (Pilkington, 1995: 77). The main disadvantages of ozonation are outlined below (Stevenson, 1997:22):

- Ozone decays rapidly
- Ozone is potentially hazardous by inhalation
- Probably most expensive disinfectant/oxidizing agent
- It requires on-site generation as it is cannot be stored.

1.3.2.4 Ultraviolet light

When water passes through a tube with ultraviolet lamps operating at a wavelength of 254nm it inactivates bacteria by distorting the DNA structure using treatment times as short as a few seconds (Pilkington, 1995: 77). When UV treatment is used alone, it requires no additional chemicals and no by-products are formed during treatment. As with ozone, UV treatment provides no residual disinfection so the distribution system must be carefully monitored (Stevenson, 1997:35).

1.3.2.5 Ultrasound

Ultrasound refers to sound of high frequency (greater than 20 kHz), which is inaudible to humans. When ultrasound passes through a liquid it generates cavitation bubbles which on collapse can generate high temperatures (5000 Kelvin) and pressures (several thousand atmospheres). Cavitation can also produce radicals

(H●, HO● and HOO●) during bubble collapse, providing energy for chemical and biological reactions. Ultrasound has shown great potential for drinking water and sewage treatment to remove chemical and microbial pollution. Ultrasound can be used for biological decontamination; removing microorganisms (bacteria, algae and fungi) through cavitational effects on cell structure and functional organs of microorganisms. Organic pollutants such as dyes can be degraded using ultrasound through the production of radicals produced during cavitation (H●, HO● and HOO●). Radicals generated by cavitation can enter the bulk solution and react with pollutants (Mason, 2003 and Mason and Lorimer, 2002:131−143).

1.4 Cyanobacterial blooms and eutrophication

Cyanobacteria are photosynthetic bacteria, also known as blue-green algae. Eutrophication is defined as harmful biological effects that occur in water bodies due to high levels of plant nutrients (nitrogen and phosphorus). Generally this results in enhanced plant growth and visible cyanobacterial or algal blooms (Chorus, 1999: 13). Further sections in this thesis deal with the implications and management of algal blooms**.**

1.4.1 Factors causing cyanobacterial blooms

Natural sunlight intensity is the most important factor for algal blooms. Cyanobacteria and algae contain chlorophyll A for photosynthesis. At high light intensities green algae grow rapidly increasing turbidity and decreasing available light. Cyanobacteria require less light energy than other organisms and can lead to their dominance in water bodies. It is also established that cyanobacterial blooms are caused by high concentrations of phosphorus (P) and nitrogen (N) supporting growth of other phytoplankton. Thus, water with high turbidity i.e. low light availability with high P and N can also result in cyanobacterial blooms. Cyanobacteria have a maximum growth rate at temperatures above 25°C. In the UK, most cyanobacteria blooms occur in summer, but in hot countries the problems can be all year round (WHO, 1998:1−2).

1.4.2 Effects of cyanobacterial blooms (biological, engineering, economic and social effects) (Anderson, 2009; Fay, 1983: 77−78; Palmer, 1980: 36−39)

1.4.2.1 Biological effects

Cyanobacterial blooms result in oxygen sensitive species such as plants, fish, and microorganisms reducing in numbers (macrophyte decline). This is due to blooms on the surface resulting in low light intensities at the bottom and damaged ecosystems (Anderson, 2009). Aquatic plants act as a home and food for fish and shellfish but these rapidly deplete without adequate sun light. Fish will die in nutrient rich lakes due to low oxygen levels, shortage of food and changes in pH or temperature.

1.4.2.2 Engineering effects

Current filtration systems are unable to cope with cyanobacterial blooms in water supplies, resulting in blockage of filtration systems. In 2007, the city of Wuxi (China) suffered from severe water shortages due to extensive cyanobacterial blooms (Xinhua, 2007). Cyanobacterial blooms can be removed with chemical coagulants. Cyanobacteria can grow on filters blocking and reducing efficiency. Furthermore, odours, algal metabolites, toxins and other complex organic contaminates caused by cyanobacterial activity are difficult to remove in routine water treatment processes. Increasing chlorine concentrations can kill cyanobacteria but can also result in harmful disinfection by-products (Anderson, 2009).

1.4.2.3 Economic effects

Biological and engineering processes which are required to combat eutrophication are expensive (Fay, 1983: 77−78). Additional engineering is required to deal with cyanobacterial blooms resulting from eutrophication and this further increases the operating cost of water treatment plants (additional manpower, equipment damage and higher chemical dosing). Agriculture and fishing revenue will also be reduced during a bloom, with potential dramatic knock on effects in the tourism industry.

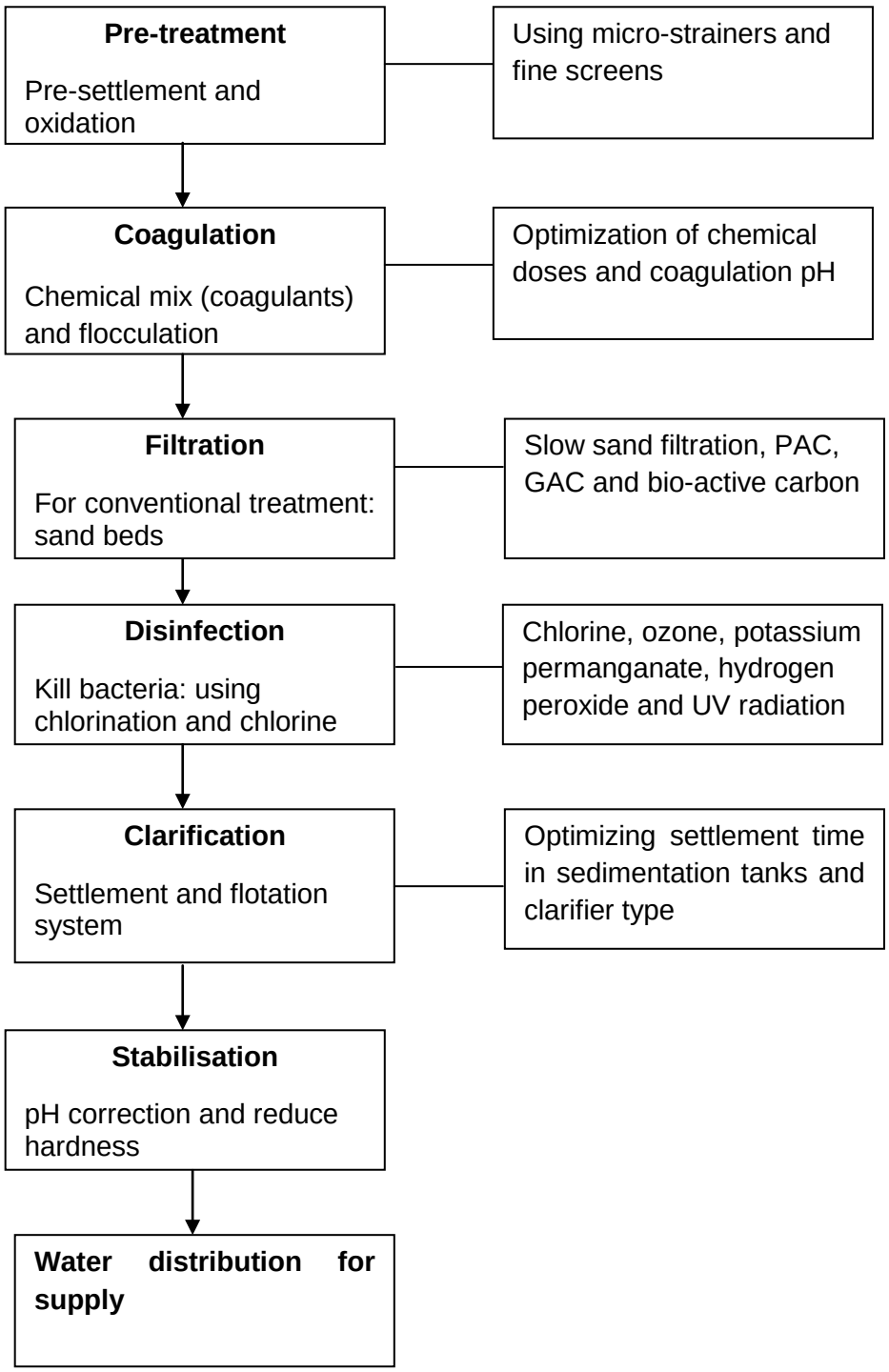
1.4.2.4 Social effects

Global cyanobacterial blooms have recently generated a growing amount of global publicity and public concern due to their associated undersirable odours/colours and human toxicity. Cyanobacterial toxins cause liver damage, neural toxicity and tumour promotion. The impacts of toxic cyanobacteria on health are further discussed in chapter 3.

1.5 Algal removal in drinking water

Algae removal is a major concern for drinking water companies. Treatment processes for algae blooms are outlined in “Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management”. The flow chart below indicates the techniques employed in the removal of algae (Chorus, 1999: 267−301).

Figure 1.2 Algae and algal toxin removal in drinking water treatment process (Chorus, 1999: 267−301)



1.5.1 Pre-treatment

Coarse screens are employed to remove large debris in water resources but do not deal with cyanobacteria or cyanobacterial toxins. Micro−strainers and fine screens may be applied, but some species such as *Microcystis aeruginosa* can pass though such filters. Recently, activated carbon has shown high efficiencies (Upadhyayula, 2009).

1.5.2 Chemical coagulation

Coagulation converts soluble substances into insoluble particles by employing coagulants such as aluminium and iron sulphates or chlorides and removing certain micro-organisms including cyanobacteria, but chemical doses and coagulation pH must be carefully controlled for efficient treatment. Unfortunately, chemical coagulation does not remove soluble algae toxins.

1.5.3 Clarification

Sludge requires long periods of up to 4 to 6 hours to settle in sedimentation tanks to removal cyanobacterial toxins. Clarifiers have longer flocculation times and are more effective for algal removal. Dissolved air flotation (DAF) is more effective than sedimentation in algae rich waters. DAF is a water treatment process which forces air under pressure into water (Figure 1.3). Once the particles (algae) reach the surface, they can be collected by a skimmer. Neither conventional sedimentation, nor dissolved air flotation, is effective in removing algal toxins (Anderson, 2009). High turbidity may cause problems during dissolved air flotation treatment.

Figure 1.3 Diagram of a dissolved air flotation system (The Open University, 1995:32)

1.5.4 Filtration

Filtration removes suspended particles. Different granular media (direct rapid filtration, slow sand filtration, powdered activated carbon (PAC), granular activated carbon (GAC) and biologically active carbon) may increase efficiency of removal rates. Slow sand filtration is effective in algal cell reduction (Mouchet, 1998). However, high concentrations of algae may result in filter blockage.

1.5.5 Disinfection

Oxidation processes combined with disinfection can control water quality within the distribution system reducing biological hazards. Dissolved algae toxins can also be removed at this stage. Table 1.3 provides an overview of current oxidation techniques with expected removal rates for microcystins. Microcystins is the most common algal toxin, and is regularly used as an indicator of algal toxins. The results are only based on extracellular toxin concentration (toxin outside algal cells).

Table 1.3 Summary of oxidation technique performance on microcystin toxins (Anderson, 2009 and Palmer, 1980)

Oxidation technique	**Expected removal (extracellular)**	**Comments**
Ozonation (post clarification)	>98%	Rapid and efficient, but expensive
Free chlorine (post filtration)	>80%	pH and chlorine dose must be controlled
Chloramine	Negligible	Ineffective
Chlorine dioxide	Negligible	Ineffective
Potassium permanganate	95%	Effective only with soluble toxins
Hydrogen peroxide	Negligible	Ineffective
UV radiation	Negligible	Only work at very high doses

1.6 Advanced technologies for algae control

A number of approaches are currently available to control algal blooms in water. These include physical, biological, chemical and natural methods.

1.6.1 Physical control of algal blooms

Physical methods refer to those without chemical or biological reactions. Activated carbon can be employed as filter medium. Granular activated carbon (GAC) filtration can be employed prior to other treatment processes. Studies indicated that GAC effectively reduces algae toxins. Wang used a combination of pre-ozonation and powdered activated carbon to adsorb cyanobacteria and their associated toxins (Wang, 2007). Maatouk demonstrated pre-chlorination with powdered activated carbon removed algae toxins (Maatouk, 2002). Combinations of oxidation and activated carbon filters may be a better approach contributing to effective to control of living algae cells and algae toxins in drinking water treatment.

Light-shading can be used to control *Microcystis aeruginosa* in water. *Microcystis aeruginosa* is one the most common toxic blooming algae species. Long periods of

Light-shading can stop photosynthesis in the algae, removing food sources resulting in death. Studies which involved Light-shading lasting for 6–9 days in conjunction with aeration reduced algal biomass (chlorophyll A) by 81% (Chen, 2009).

UV irradiation at 254nm has been reported in control *Microcystis aeruginosa* growth. Tao suggests UV irradiation may have an operational value in controlling cyanobacteria due to cell membrane damage, which results in loss of division capability for reproduction (Tao, 2010).

1.6.2 Biological control

Biological techniques are sophisticated and involve the removal or addition of particular biological species or groups of species to a water system. Some species, ranging from bacteria to fish exhibit strong inhibition activity against some algae species, which control blooms on site. However, these organisms or species must not interfere with dynamics of other organisms. Careful environmental impact assessment of these techniques is vital for widespread applications including assessing potential harm on local ecosystems.

Kim (2007) demonstrated *Pseudomonas fluorescens* HAK−13 has a potential use in bio-controlling harmful algal blooms. Planktivorous fish reduced cyanobacterial biomass in a reservoir in Russia. Plantivorous fish refer to fish which consume plankton, such as *Carassius auratus* (Prokopkin, 2006). It was observed that *Streptomyces neyagawaensis*, an aquatic bacterium isolated from the sediment of a eutrophic lake (Lake Juam, Korea) inhibited the growth of *Microcystis aeruginosa* at lab scale of (25 mL *Microcystis aeruginosa*) (Choi, 2005).

1.6.3 Chemical control

Chemical controls such as the use of algaecides will result in an additional form of water pollution. Once algae are killed/controlled, the introduced chemical pollutant must then be remediated before water is considered safe for consumption. Some herbicides can be used to inhibit growth of plants. Mohr (2007) found chloroacetamide metazachlor had negative effects on the macrophyte biomass of *Potamogeton natans*, *Myriophyllum verticillatum* and filamentous green algae (*Cladophora glomerata*). However, it has been argued by Anderson that single

exposure of aquatic macrophytes to metazachlor at nominal concentrations of >5 μgL^{-1} is likely to have pronounced long-term effects on certain algae, aquatic biota and ecosystem function.

Copper sulphate treatment for algal control led to a reduction in Chlorophyll A concentrations, but it also affected other parameters such as dissolved oxygen content, thus requiring two months for the copper concentrations to return to normal background levels following copper sulphate application (Hullebusch, 2002). This renders these treatments expensive and impractical since they can result in additional pollution which kills plants, fish and other aquatic life.

Lab-scale electrochemical treatment was investigated to assess the effects on *Microcystis aeruginosa*. In Xu's study, $Ti-RuO_2$ and graphite were used as anode and cathode. Electrolysis was effective at inhibiting *Microcystis aeruginosa*, with an inhibition ratio of 91.51% obtained at a current density of 12 mA cm^{-2} (Xu, 2007); but current research are still at small lab-scale.

1.6.4 Other methods

A traditional and more holistic method for controlling algae blooms in water employs the use of barley straw bales. The toxicity of the straw to phytoplankton can be explained by the leaching of phenols and oxidised phenolics from the bales as they decompose (Everall, 1997). Some evidence suggests this is not the most practical/effective solution for large areas of water but it is suitable for smaller lakes (Ferrier, 2005).

Ultrasonic treatments have been reported to effectively inhibit cyanobacterial growth by acoustic cavitation, which can collapse gas vacuoles (Lee, 2000). The extent of algal growth is influenced by ultrasonic parameters like frequency, intensity and treatment time (Joyce, 2010). Power ultrasound combined with an electromagnetic treatment can control algal growth in cooling towers (Mason, 2003). Ultrasound can improve coagulation treatments of algae. Liang used a flow system at flow rate of 20 min/mL and treatment time is 15 seconds (Heng, 2009). In this research, ultrasound is applied as a control measure for algal (blue-greens) blooms.

Various options are available to control algal blooms and these can be divided into two categories:

- Reduction of algal populations in water by the addition of algaecides
- Manipulating the whole environment against algal dominance (biomanipulation techniques)

Nutrient deprivation is the best long-term sustainable solution for cyanobacterial blooms (Heisler, 2008). However, it is not practical in most cases as farmers are reluctant to reduce the amount of fertilizer (thus reducing crop yields) or increase set-back distances (which will reduce the available growing area) (National Rivers Authority, 1990). Thus researchers are still looking for effective and practical methods to control algal blooms that occur in nature.

2.0 Introduction to ultrasound

2.1 Theory

Ultrasound is classified as sound at a frequency beyond human hearing (20 kHz − 100 MHz), it can be sub-divided into three regions (Figure 2.1):

- Power ultrasound (20 − 100 kHz) is used for plastic welding, cleaning, cutting and to influence chemical reactions
- Ultrasound at intermediate frequencies (100 kHz − 2 MHz) that can be employed for sonochemistry
- High frequency low power ultrasound (5 − 10 MHz) which is used for medical scanning (Mason, 1999: 4)

Figure 2.1 Frequency ranges of sound (Mason 1999:4)

2.1.1 Ultrasonic applications

It has been recognized for many years that power ultrasound has great potential applications in a wide variety of processes in research and applied industries.

Table 2.1 Some applications of ultrasound (Mason and Lorimer, 2002: 5, Shoh, 1988: 97−120)

Field	Application
Biology, Biochemistry	Homogenisation and cell disruption
Engineering	Drilling, grinding and cutting
Dentistry	Cleaning and drilling of teeth
Geography, Geology	Locating mineral and oil deposits and measuring depths of water bodies and oceans
Industrial	Cleaning engineering parts, dispersing solids in paint, inks and resins
Medicine	Ultrasonic imaging (2−10 MHz) is employed to diagnose illness of heart, breast, liver, etc. Low frequencies (20−50 kHz) are used in the treatment of muscle strain and dissolution of blood clots

2.2 Cavitation

Direct sound effects are not in a frequency range that could directly affect chemical bonding since the power is too low even for the excitation of rotational motion. It is reported that the energy density of a sound field is only 10^{-2} eV per μm^3, which cannot directly affect chemical reactions (Mason and Peters, 2002: 5).

The phenomenon of cavitation is responsible for producing chemical and biological effects using power ultrasound (Atchley and Crum, 1988: 1−62). Ultrasound is transmitted through liquids via a wave which compress and stretch the molecular structure of the medium (Figure 2.2). The stretching results in the liquid breaking

down and the formation of voids or microbubbles. Once produced, these micro-bubbles also known as cavitation bubbles grow in successive compression and rarefaction cycles reaching an equilibrium size (Mason and Lorimer, 1988: 35−42). However the acoustic field is often unstable so the bubbles collapse liberating large amounts of energy (Mason, 1999: 8−10).

Figure 2.2 Development and collapse of cavitation bubbles (Mason, 1999: 10)

During cavitation bubble collapse, temperatures may reach 5000 Kelvin (K) and pressures of up to several thousand atmospheres (Gogate, 2002 and Mason, 1999: 10).

There are two types of cavitation: transient and stable. Transient cavitation bubbles collapse violently after a few cycles whereas stable cavitation bubbles oscillate, often non-linearly and can exist for many cycles. It is generally thought that transient cavitation bubbles are mainly responsible for sonochemical effects (Mason, 1991:21).

2.3 Factors affecting cavitation (Brennan, 2006: 223−224 and Mason, 1999: 10−15)

Based on literature reviews, parameters affecting cavitation can be divided into acoustic factors (frequency, intensity), external (temperature, pressure, gas type and content) and other factors (sonication time, viscosity and surface tension of the medium).

2.3.1 Acoustic factors

Sound is a form of energy and the equation for intensity of ultrasound may be expressed as:

$$I = P_A^2 / 2\rho c$$

This means larger intensities (I) result in greater acoustic pressures (P_A). 'ρ' refers to the density of medium through which ultrasound is transmitted and 'c' indicates the velocity of sound. Increased intensity is associated with an increase in cavitational effect. However, there are some limits to the power input of a system (Mason, 1999: 14):

1. A minimum intensity of sonication is required to reach the cavitation threshold and this is dependent on the frequency.
2. Excessive micro-bubble formation may be produced by high ultrasonic powers but this will reduce the transfer of acoustic energy as the bubbles act as a barrier absorbing ultrasonic energy.
3. Ultrasound will lose some power when ultrasonic energy is transferred from the generator to medium.
4. The transducer will be eventually degraded as cavitation bubbles erode the surface of a transducer.

With increasing ultrasonic frequencies, the rarefaction phase shortens and more power is required at higher frequencies to achieve the same cavitational effects at lower frequencies. For example, ten times more power is required to make water cavitate at 400 kHz than at 10 kHz. When the ultrasonic frequency is increased into the MHz region, cavitiation becomes more difficult to produce in liquids, since increasing frequency results in less time for the formation of cavitation bubbles (Neis

et al., 2001). Transducers operating at high frequencies are not capable of generating very high ultrasonic powers (Mason and Lorimer, 2002: 56).

2.3.2 External factors

Increasing the temperature of the reaction medium raises the vapour pressure of the medium, producing cavitation at lower acoustic intensities but with a less violent collapse. If a liquid is sonicated at its boiling point, there will not be any great sonochemical effects, since large numbers of cavitation bubbles will form, fill with vapour and act as a barrier to sound transmission (Mason, 1999: 13).

Increasing external pressure makes the bubbles harder to form but leads to a more rapid and violent bubble collapse. In this way, more energy will be produced for sonochemical reactions (Mason and Lorimer, 1988:49).

Dissolved gas in liquids can act as nuclei for cavitation but the collapse intensity is lower due to a greater sound dampening effect in the micro-bubble (Mason and Lorimer, 1988:47−49).

2.3.3 Other parameters

Extending the time of sonication generally increases the sonochemical effect.

For liquids that have high viscosity or surface tensions a higher intensity of ultrasound must be used to achieve similar effects to those obtained in lower viscosity and surface tension (Mason, 1991: 22) .

2.4 Physical and chemical effects of acoustic cavitation (Mason and Peters, 2002: 12−21)

The main theory of cavitation is the so-called “Hot-Spot” theory (Fitzgerald *et al.*, 1956). When cavitation bubbles collapse, they can generate extremely high temperatures (2000−5000K) and pressures (1000−4000 atm), shown in Table 2.2.

Table 2.2 Cavitation effects at 3 MHz (Mason and Peters, 2002:12)

Table 2.2 has been removed due to third party copyright. The unabridged version of the thesis can be viewed at the Lanchester Library, Coventry University

The heated gas inside the bubbles is surrounded by a 200nm liquid shell at a temperature of about 1500−2000K. For this reason cavitation bubbles are considered to be localised micro-reactors for physical and chemical reactions.

There are two other theories for cavitation: plasma and electrical theory. The plasma theory assumes that the energy of cavitation results from highly charged microplasma formed inside collapsing bubbles. The electrical theory suggests that the asymmetric collapse in bubbles will produce strong electrical fields, resulting in electrical discharge (Mason and Peters, 2002: 12). In this thesis, we will focus on the 'hot-spot' theory as it is the most widely accepted theory for cavitation.

2.4.1 Physical effects

Physical effects occur after bubble collapse and these include radiation forces, standing waves and acoustic streaming which results in the conversion of ultrasonic energy to heat. Cavitation bubble collapse results in strong pressure waves. Oscillation of stable cavitation bubbles and the collapse of transient bubbles induces molecular and particle movement and is termed 'microstreaming'. The release of shock waves and the effects of microstreaming can result in intensive shear stress in a liquid (Mason and Peters, 2002:13−15).

2.4.2 Chemical effects (Mason and Peters, 2002:15−16)

High temperature and pressure inside collapsing cavitation bubbles can produce radicals which may affect synthetic reactions or can be employed as a biocide since free radicals can react with cell membranes leading to breakage of cell walls.

Cavitation bubbles contain vapour and when this vapour is subjected to high pressure and temperature, the homolytic bonds of the solvent breaks down producing radicals such as H● and HO●. The generation of such radicals in water is shown below:

$$H_2O \rightarrow HO\bullet + H\bullet$$
$$H\bullet + O_2 \rightarrow HO_2\bullet$$
$$2HO\bullet \rightarrow H_2O_2$$
$$2HO_2\bullet \rightarrow H_2O_2 + O_2$$

Radicals produced inside the bubble will first react with chemicals in the bulk media at the interface.

On collapse the liquid will rapidly rush into space occupied by cavitation bubbles producing intense shock waves and shear forces capable of breaking polymer chains in the liquid (Mason, 1991: 25−26).

Figure 2.3 Cavitation effects in a homogeneous liquid (Mason, 1991: 26)

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The result of sonochemical reactions in a liquid phase depends on both the frequency and intensity. For low frequency ultrasound (20 kHz − 100 kHz), the main effects are the result of the physical (mechanical) forces of cavitation with some radicals produced. However for high frequency ultrasound (100 kHz −2 MHz) mechanical effects are reduced and more free radicals are produced (Mason, 1991: 25−26). In either case increasing the intensity will increase the number of cavitation bubbles. In general in order to achieve effective treatment, the ultrasonic parameter settings (frequency and intensity) must be optimised.

2.5 Ultrasonic equipment

Ultrasound is generated by transducers, which convert either mechanical or electrical energy to ultrasonic waves. There are three main types: gas driven, liquid driven and electromechanical. Gas driven transducers are operated by passing a jet of gas through an orifice into a resonating cavity. This type of system is generally used for atomisation. For liquid driven transducers, liquid is forced by a powerful pump through an orifice to emerge in a chamber as a jet which vibrates a thin steel blade producing cavitation bubbles. There are two types of electromechanical transducers: piezoelectric and magnetostrictive (Table 2.3).

Table 2.3 Piezoelectric and magnetostrictive transducers (Mason and Peters, 2002: 23−26)

Transducer Type / Properties	Piezoelectric	Magnetostrictive
Working mechanism	Reversing charges cause fluctuations in dimensions of crystal sections, which transmit ultrasonic vibration to a medium	Metal will vibrate when it is affected by a magnetic field. The magnetic field can be produced by a series of short pulses
Current common material	Lead zirconate titanate, barium titanate and lead metaniobate	Cobalt/iron combinations and aluminium/iron with chromium
Application	Sonochemistry laboratory applications	Industrial applications requiring heavy-duty continuous work
Advantage and disadvantage	Highly efficient, frequency range from 20 kHz to many MHz but the ceramic material will degrade under high temperature so a cooling system is required	Robust, durable construction, effective force but frequency range is limited below 100 kHz. Energy losses are in the format of heat

In this work we have employed three main types of ultrasonic equipment on a small Lab-scale. These include 40 kHz bath (Langford Sonomatic), 20 kHz probe (Vibra-cell, Sonics & Materials), and a multi-frequency bath 580, 864 and 1146 kHz (Meinhart).

2.5.1 Ultrasonic bath

An ultrasonic bath generally consists of a container which holds the reaction medium with one or several transducers clamped to the base. It is the most accessible and cheapest ultrasonic equipment and usually the frequency is set at 40 kHz (Figure 2.4). Energy is transmitted vertically as sound waves from the base of the bath into the reaction medium. Santos (2007) concluded different variables must be taken into account when sonication is generated within an ultrasonic bath:

- Water temperature inside the bath
- Frequency of ultrasonic energy
- Position in which the sample container is situated within the bath
- Use of detergent in water to lower surface tension thus enhancing acoustic cavitation in liquids achieving better results due to lower surface tensions

The advantages of ultrasonic cleaning baths are that they are widely available and has a reasonably even distribution of energy through the reaction vessel. However the power is lower than a probe system and temperature control is difficult is achieve (Mason and Lorimer, 1988: 215).

Some specialised high frequency baths have been designed for sonochemical research studies, (Figure 2.5). The transducers are piezoelectric and offer a wide frequency range. A cooling system is added to control the experimental temperature. Compared with probes, the advantage is that there will be no erosion of the transducer face directly into the reaction and so no fragments of metal enter the reaction. There is however a limitation of volume for the reaction cells (Mason and Lorimer, 1988: 215).

Figure 2.4 Ultrasonic cleaning bath (Mason, 1999: 42)

Figure 2.5 Improved ultrasonic bath for sonochemistry (Mason and Lorimer,2002: 279)

2.5.2 Ultrasonic probes

Ultrasonic probes can induce high ultrasonic energy in a reaction system because it directly transmits energy into the system. Transducers are piezoelectric and the overall structure is illustrated in Figure 2.6. A generator provides the source of alternating electrical frequency to the transducer. The horn acts as a means of

transfer of energy from the transducer to the reaction. There are four different shapes of horns: uniform cylinder, linear taper (or cone), exponential taper and stepped. We only used a uniform cylinder which functions as an energy extender from the transducer but does not amplify the vibration. The other forms of horn magnify the vibrational amplitude.

The advantage of a probe is that the power is high and the power settings can be adjusted. However, tip erosion may occur resulting in contamination of reaction mixtures (Mason and Lorimer, 1988: 215).

Figure 2.6 Ultrasonic probe for sonochemistry (Mason, 1991: 74)

2.6 Scale-up

Scale-up is vital for ultrasonic applications where ultrasound has been proven effective at a Lab-scale. To achieve the best cavitational effects, optimized conditions must be taken into account. Three main factors must be considered: reaction medium characteristics (viscosity, vapour pressure and concentration of dissolved gas), reaction conditions (temperature and pressure) and type of ultrasonic system employed (Mason, 1991: 92−93).

Bath systems can be upscale simply by enlarging the bath and either adding more transducers or using a submersible transducer which is commonly used in cleaning

applications. There are two ways in which probe systems can be scaled up: batch reactors and flow systems. Batch reactors can produce high power ultrasound localised at the tip of the horn but it is difficult to use a single probe to sonicate a large reaction volume and provide sufficient energy density throughout. Flow systems employ a flow loop outside the main reactor providing an ultrasonic system which can deal with large volumes (Mason and Peters, 2002:286−292).

In our experiments, we used the following ultrasonic equipment and the volume ranged from 1 − 5 litres, which achieved Scale-up from small (200 and 400 mL) to medium Lab-scale:

- 20 kHz ultrasonic probe (designed and constructed at Southeast University)
- Sonolator (Sonic Corporation)
- 16 kHz and 20 kHz Dual Frequency Reactor (DFR, Advanced Sonics Processing Systems)
- 20 kHz Vibrating Tray (Advanced Sonics Processing Systems)

2.7 Sonochemistry in environmental treatment and biological decontamination

Ultrasound has shown great potential for applications in the field of environmental remediation. Ultrasonic irradiation can remove surface contamination and biofilms, wash soils by efficiently removing organic and inorganic contamination, control airborne pollution and treat sewage sludge (Mason, 2002: 131).

In this study we have focused on biological decontamination using ultrasound with particular reference to algae. Main biological contaminants of interest considered include microorganisms (especially bacteria), their colonies and spores (Mason and Lorimer, 2002: 132). The use of biocides for conventional biological contamination may cause some problems due to microbes developing resistance to disinfection, the production of secondary environmental pollution (disinfection by-products) and high treatment costs. Ultrasound can be thought of as a friendly (“green”) biocide to the environment providing a method for the removal of biological contaminants using reduced levels of biocide. Ultrasound renders biocides more effective because it is able to inactivate bacterial cells and increase the porosity of cell membranes. However, this is dependent on ultrasonic frequency, intensity and sonication time. A range of experiments using different species of bacteria have been investigated

under different ultrasonic parameters. Ultrasonic treatments have demonstrated a clear inactivation effect on bacteria and algal cells (Mason, 2007).

2.7.1 Mechanism of ultrasonic effects on cellular material

The mechanism of the effect of ultrasound on cellular material is based on cavitation and associated shear stress. Transient cavitation generates high shear forces which can rupture cells and produce free radicals which are toxic to biological cells (Mason, 2002:131). In addition stable cavitation can produce a steady flow surrounding the bubble, known as a micro-streaming that can also generate shear forces (Firzzell, 1988: 287−290). Figure 2.7 illustrates ultrasonic damage to cells. Figure 2.7A shows untreated *E. coli* cells and Figure 2.7B the cells following 2 minutes treatment using a 20 kHz probe at 750 W revealing that the cell boundaries are damaged In Figure 2.7C, untreated *S. cerevisiae* illustrate a uniform cell wall, but after 2 minutes treatment, cell wall and cell organelles were damaged (Figure 2.7D, Cameron, 2008).

Figure 2.7 Transmission electron micrographs of cells (Cameron, 2008)

The nature of microorganisms determines their sensitivity to ultrasound. Factors which greatly influence sensitivity are cell size, shape of microorganisms, cell wall composition, and physiological state. Larger cells are more readily damaged because they have a greater surface area in contact with cavitation. Round cells are difficult to break ultrasonically as sound energy can be reflected. Ultrasound is transferred through thin cell walls more easily than thick cell walls. Active and vegetative cells

are more easily destroyed than their inactive spores as the size of spores are smaller and cell walls are thicker (Tiehm, 2001: 32).

2.7.2 Ultrasonic destruction of biological contaminants in water (Phull, 2001: 16 −21)

Ultrasound can inactive a wide range of microorganism such as bacteria (*E. coli, S. aureus, B. subtilis, and P. aeruginosa*) and fungi. A combination of 70 kHz ultrasound and the antibiotic (gentamicin sulfate) resulted in a decrease of up to 97% in the *E. coli* counts in a biofilm following 2 hours treatment (Piyasena, 2003). The removal rate of sewage fungi using 42 kHz (0.14 Wcm^{-3}) ultrasound was reported to be 99.92% although the species of fungi was not mentioned (Dehghani, 2007).

Zooplankton often block the purification system of water treatment plants. Power ultrasound inactivates zooplankton cells and cleans filters. Ultrasound is also employed for algae control and can be combined with an electromagnetic anti-scaling treatment to clean water in cooling towers (Mason, 2007). *Bacillus subtilis* are difficult to remove using conventional treatment, but can be inactivated using a 20 kHz probe (0.24 Wcm^{-3}) and 15 minutes treatment (Joyce, 2003). The protozoa, *Cryptosporidium parvum*, can be reduced using ultrasound as an aid to conventional disinfection (chlorination). Using a 1000 kHz (2.3 Wcm^{-3}) bath, the number of viable oocysts was reduced to 99.99 % following a 10 min treatment time (Olivera, 2008).

Current trends focus on forming less disinfection by-products. Low power ultrasound is capable of enhancing the effects of chemical biocides using lower working concentrations. Additionally, ultrasound can be combined with ultraviolet light, TiO_2 and ozone. The potential application of ultrasound to treat biological contaminants in water continues to expand either using it alone or in combination with other technologies thus providing a bright future for environmental protection.

3.0 Introduction to algae (blue-green)

3.1 Cyanobacteria

Cyanobacteria are photosynthetic bacteria with some similar characteristics of eukaryotic algae including physical characteristics and photosynthetic functions (Whitton and Potts, 2000: 1−13). In the past, it was difficult to distinguish cyanobacteria from algae due to similarities in size. During the twentieth century, researchers established cyanobacteria are prokaryotic (no nucleus) and other algae are eukaryotic (complex structure with a nucleus) (Chorus and Bartram, 1999).

Cyanobacteria are also known as blue-green algae due to their colour. They consist of blue-green, blue-green algae, myxophyceaens, cyanophyceans, cyanophytes, cyanobacteria, etc. (Whitton and Potts, 2000: 1−13). In this research, algae (blue-greens) are referred to as Cyanobacteria.

Cyanobacteria have existed on earth for billions of years and are the origin of all plants. A long evolutionary history has resulted in algae tolerance to conditions such as low oxygen, high temperature, high pH and low light. Cyanobacteria can fix nitrogen and can also live in waters containing low nutrient levels. Although cyanobacteria can grow inland, in freshwater and coastal waters, in this study we will focus on freshwater blue-greens (Mur, 1999).

Cyanobacteria have two forms: unicellular and filamentous, and they occur singly or in colonies. Sticky biopolymer frameworks (exopolysaccharides) may help maintain colonies. Some planktonic species have intracellular gas vacuoles for buoyancy control. The taxonomy of cyanobacteria is complicated, as no single system exists to cover all ecological features. However, two main groups exist (Bergey, 1994):

- Non-filamentous unicellular with order of Chroococcales, Pleurocapsales
- Filamentous with order of Oscillatoriales, Nostocales and Stigonematales

There has been a great deal of interest in cyanobacteria blooms because of increasing environmental concerns relating to the pollution of drinking water resources. In most cases, these algae blooms are toxic resulting in human health

problems. Therefore a number of studies of cyanobacteria bloom formation and control have been undertaken. Effective management of blooms and control measures are essential for safe drinking water. To help with methods of controlling blooms, it is necessary to have some understanding of cyanobacteria cell structure, morphological diversity, growth rates and occurrence.

3.2 Cell structure of cyanobacteria

Although cyanobacteria are also known as blue-green algae, there are differences between a cyanobacteria and eukaryotic algae cells. Cells are the fundamental organizational unit of all living systems. Cytoplasmic membrane surrounds the cellular contents providing a safe external environment. There are two types of cells: prokaryotic or eukaryotic and both have similar overall functions; growth and reproduction. However, they differ with regard to cell structure (Figure 3.1). A prokaryotic cell is simple in that it does not have membrane-bound compartments or a nucleus. Prokaryotic cells only contain nuclear material such as DNA (deoxyribonucleic acid), DNA−binding proteins and RNA (ribonucleic acid). The presence of a nucleus determines if a cell is prokaryote or eukaryote (Atlas, 1995:4).

Figure 3.1 Comparison of structure between prokaryotes and eukaryotes (Pearson Education, 2010)

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In the evolutionary tree, there are three principal lines for cellular evolution: eukarya, achaea and bacteria (Atlas, 1995:5). Figure 3.2 illustrates this three kingdom classification system indicating algae and cyanobacteria are not in the same kingdom. Microbiologically, bacteria and archae belong to prokaryotic cells, whereas fungi, algae and protozoa belong to eukaryotic cells.

Figure 3.2 Phylogenetic tree showing evolutionary relationships (Taylor, 2005)

Most cyanobacteria cells range from 2 − 5μm in diameter and the cell structure is only visible using an electron microscope. The general structure of cyanobacteria is similar to a typical bacterial cell but relatively large compared with a prokaryote cell. The cell is protected by a multi-layered cell wall and a sheath which may extend from the cell wall surrounding the entire cell. A cell wall encloses the cellular contents including: thylakoids, chromosomes and various granules required for growth and reproduction (Fay, 1983: 4−18).

Figure 3.3 Typical cyanobacteria cells (Koning, 1994)

3.2.1 Wall and sheath

Individual cells of cyanobacteria are typically composed of thick, gelatinous cell walls with two principal layers:

- An inner murein or peptidoglycan layer adjacent to the cell membrane
- An outer lipoprotein layer

The inner layer supports and strengthens the wall, while the outer lipoprotein layer controls transport of solutes similar to bacteria cells secreting materials into the sheath. The sheath is usually located outside the cell wall, providing protection against exposure to high levels of sunlight (Sze, 1998:22).

3.2.2 Nuclear apparatus

DNA (deoxyribonucleic acid), DNA−binding proteins and RNA (ribonucleic acid) are contained in the central nucleoplasmic region. These are commonly referred to as chromosomes. Ribosomes are the site of protein synthesis. Ribosomes are composed of RNA (called ribosomal RNA or rRNA) and protein (Fogg, 1973:59−61).

3.2.3 Cell membrane and thylakoids

Cells of cyanobacteria are maintained by an elective semi-permeable thin membrane that maintains protoplasts in an osmotical balance. The structure of the membrane resembles a flattened sac with thylakoids situated in the outer region of the cytoplasm.

Thylakoids are the site of photosynthesis. Thylakoids are reproduced in the cell membrane and may disintegrate in aging/dying cells. Thylakoids contain several photosynthetic pigments of cyanobacteria with major absorption bands illustrated in the table below (Fay, 1983:10):

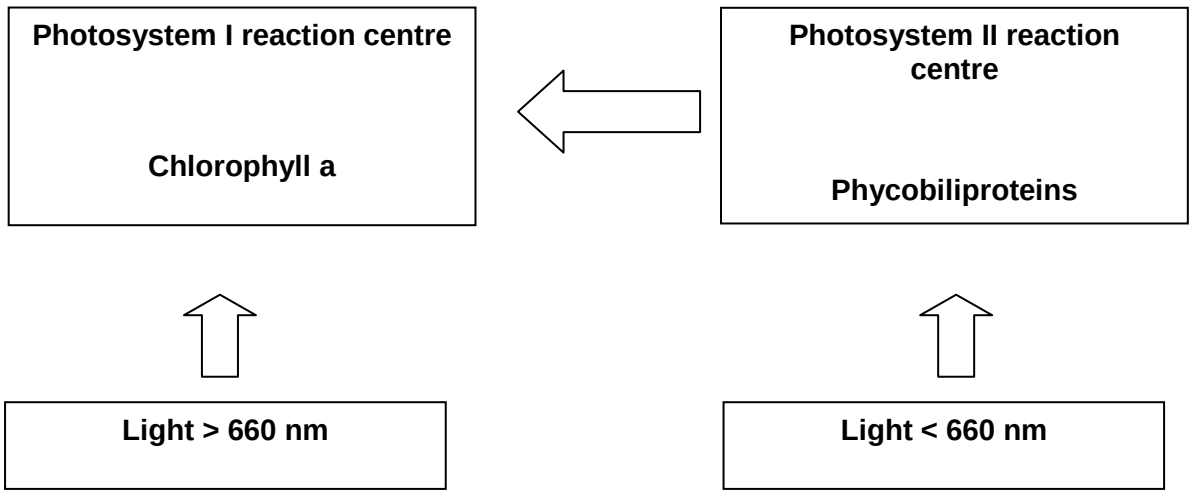
Table 3. 1 The major photosynthetic pigments of cyanobacteria (Fay, 1983:10)

There are three groups of photosynthetic pigments in cyanobacterial cells: chlorophylls, carotenoids and phycobiliproteins. Light energy is trapped by phycobiliproteins and transferred to chlorophyll with high efficiency (Fay, 1983:10-11). Figure 3.4 illustrates the absorption spectra for the three groups of photosynthetic pigments: chlorophyll, carotenoids and phycobiliproteins along with the cell absorption spectra *in vivo* (*Anacystis nidulans*).

Figure 3.4 Absorption spectra of *Anacystis nidulans* cells (Fay, 1983: 11**)**

Photosynthesis is the process by which photosynthetic organisms transfer sunlight energy to release oxygen from water and fix carbon dioxide to sugar. For cyanobacteria, there are two photo systems, which are illustrated in Figure 3.5. Photosystem I has a reaction centre with chlorophyll A. Photosystem II transfers energy to photosystem I through a series of membrane–bound carriers (cytochrome *b*, plastoquinone, cytochrome *c* and plastocyanin). Photosynthetic organisms are sensitive to environmental influences, especially light (Fay, 1983: 13).

Figure 3.5 Light reactions in photosynthesis of cyanobacteria



3.2.4 Cytoplasmic inclusions

Cytoplasm is heterogenous and contains some granular material required for growth and reproduction: carboxysomes, cyanophycin, starch and polyphosphate granules. The function of carboxysomes is to catalyze the fixation of CO_2. Cyanophycin is mainly located at the cell periphery and serves as nitrogen storage. Carbohydrate is stored in starch granules. Polyphosphate granules are highly electron-dense serving as phosphate stores (Shively, 1988: 195–203, Shively, 1988: 204–206, and Allen, 1988:207–213).

3.2.5 Gas vacuoles

Gas vacuoles are buoyancy-regulating organelles, which are located in the cytoplasm. The content of gas vacuoles is determined by the surrounding cytoplasm and external medium. Gas vacuoles will collapse when a cell is subjected to pressures of several atmospheres (Hayes, 1988:213–222).

3.2.6 Reproduction

Generally, cyanobacteria reproduce asexually using binary fission, which results in the division of an original cyanobacteria cell into two cells. During this process, the original (mother) cell extends to double its original size and splits into two (daughter) cells. For filamentous cyanobacteria in adverse conditions, there are certain modes for reproduction such as akinetes, hormogonia, and hormocysts. Some cyanobacteria can produce endogenous or exogenous spores for asexual reproduction (Fay, 1983: 24－27). To date there is no evidence of true sexual reproduction in cyanobacteria (Aloisie, 2008).

Table 3.2 Types of cyanobacteria reproduction

Types	***Features***
Binary fission	Most common method
Akinete formation	Mode during adverse conditions
Hormogonia	Forming short pieces of filaments
Hormocysts	Germinating from one or both ends
Fragmentation	Mother cell divides into two or more fragments
Spores	Reproductive cells are produced inside the cell walls (rapid reproduction)

3.3 Occurrence in nature

Cyanobacteria existed on earth from very early times, with some fossils dating from 3000 million years ago. Cyanobacteria are organic matter producers and oxygen providers thus, playing an important role in the evolution process of earth.

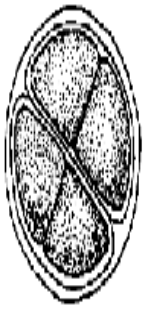
Natural algae grow poorly in flowing water but they are regularly found in still water bodies. Light limits algae growth near the water surface since strong light harms algae growth since high light intensity will increase temperatures, increasing the volume of gas inside vacuoles leading to breakage of gas vacuoles structure. The regeneration of gas vacuoles requires energy from photosynthesis. The growth rate of cyanobacteria is dependent on nutritional factors and specific inorganic nutrients (carbon, nitrogen, phosphorus and silicon) determine the levels of algal growth. Algae blooms occur in nutrient rich waters but cyanobacteria can also survive in many different environmental conditions (pH, temperature, oxygen and light intensity) (Bartram, 1999: 12−24).

Some specific species of cyanobacteria can colonize bare areas of rock and soil. They can also live in poor nutrition areas (volcanic ash, desert and rocks) where no other microalgae can exist. Another remarkable feature of cyanobacteria is their ability to survive at extremely high and low temperatures. Thus cyanobacteria are found as common inhabitants of hot springs but also in Arctic and Antarctic lakes (WHO, 2003: 136−137).

3.4 Morphological diversity of cyanobacteria

In this chapter, the classification of cyanobacteria (Table 3.3) is mainly based on systematic bacteriology (Bergey, 1994: 377−414), Fay's principal groups for blue−greens (Fay, 1983: 5) and WHO's basic morphology of cyanobacteria (Mur, 1999: 27 −30). There are five sections in this classification and each is illustrated using basic morphology, reproduction mode, order name, cells picture and representative genera, which are the main features for taxonomic classification.

Table 3.3 Classification of cyanobacteria (Bergey, 1994, Fay, 1983and Mur, 1999)

Section	Morphology	Reproduction	Order	Figure	Representative genera
I	Unicellular Colonial	Binary fission	Chroococcales		Chamaesipho Gloeobacter Gloeocapsa Gloeothece Microcystis Prochlorococcus Synechocystis
II	Unicellular Colonial	Budding Multiple fission	Pleurocapsales		Cyanocystis Myxosarcina Pleurocapsa-group
III	Filamentous Non-differentiated	Trichome Fragmentation Hormogonia	Oscillatorriales		*Oscillatoria* *Microcoleus* *spirulina* *Pseudanabaena*
IV	Filamentous Heterocystous	Trichome Fragmentation Hormogonia Akinetes	Nostocales		*Anabaena* *Nostoc* *Gloeotrix* *Rivularia* *Scytonema*
V	Branched Filamentous Heterocystous	Trichome Fragmentation Hormogonia Akinetes	Stigonematales		Chlorogloeopsis Fischerella Geitleria Iyengariella Nostochopsis Stigonema

Based on Bergey's manual of determinative bacteriology (ninth edtion), there are two basic categories for cyanobacteria (Bergey, 1994: 379):

1. Unicellular or nonfilamentous cells held together by outer walls: unicellular or colonies (Section I and Section II).
2. Filamentous, trichome of cells: branched or unbranched: (Section Ⅲ, Section Ⅳ and Section Ⅴ).

3.4.1 Unicellular and colonial forms (Hoek, 1995: 33)

Unicellular cells are spherical, ovoid or cylindrical, illustrated in Table 3.3 (Order Chroococcales and Pleurocapsales) and are held together using exopolysaccharides. The main reproduction mode is binary fission converting a single mother cell to two small daughter cells. Budding is a supplemental reproduction mode. For some species in the order Pleurocapsales, exospores are budded off from the upper ends of cells (Mur, 1999: 30).

For Order Chroococales, the most common cell shape is spherical and cells are surrounded by a thin mucilage. However, cells of *Chamaesiphon* are oval or pear−shaped. Reproductive mode is via exospores and most species of Order Chroococales live in water or on rocks. There is a genus known as *Microcystis*, the subject of study in this thesis, which is infamous because it poses a serious risk to public health due to the formation of toxic blooms in water.

For Order Pleurocapsales, algae are unicellular and may form colonies. Reproduction is via cell division and the formation of endospores. The cells of Genus *Cyanocysits* are spherical or pear−shaped.

3.4.1.1 Genus *Microcystis*

Microcystis is the main genus found in cyanobacteria blooms. The cells are oval to spherical in shape and 3−8 µm in diameter. They usually form colonies and contain gas vacuoles. Young colonies are usually spherical, but older colonies can be irregular in shape. *Microcystis* can form toxic blooms in water bodies. Some specific features of Genus *Microcystis* are that they (Bergey, 1994: 384):

- possess gas vacuoles
- have a tendency to form large 3−dimensional algal blooms in wide area
- are toxic
- contain ß-cyclocitral, causing odour issues in drinking water

Microcystis can grow rapidly in nature. The growth of most cyanobacteria is limited by high intensity light. However, *Microcystis* utilise gas vacuoles to regulate their position in water, thus maintaining optimal light intensity for growth (Graham and Wilcox, 2000:124−125).

Microcystis colonies are prevalent during early summer in lakes and ponds**.** *Microcystis* blooms have been reported globally in Europe, Australia, United States and China (Mur, 1999:45−46).

Problems caused by *Microcystis* blooms include contamination of water, fish kill, poisoning of animals and human health risks (liver cancer). Therefore, effective control measures are required to maintain safe drinking water and an ecological balance.

Figure 3.5 Micrograph of *Microcystis aeruginosa* and surface bloom (Chorus and Bartram, 1999:45−46)

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Microcystis aeruginosa is a species of genus *Microcystis*, which is one of the most adaptable species in algae blooms. Single cells of *Microcystis aeruginosa* can join together in groups to form colonies and float on the surface of water bodies. Single *Microcystis aeruginosa* cells are typically spherical, ovoid or capsule−shaped with dimensions of 4−9 µm. The cell structure is illustrated below:

Figure 3.6 Ultrastrucuture of *Microcystis aeruginosa* cell (Reynolds, 1981)

Figure 3.6 illustrates a cell surrounded by cell wall (c.w.) and plasmalemma (p.). The nucleoplasm (n.) contains ribosomes, and there are several storage granules: polyphosphate bodies (p.b.), structured granules (s.g.), glycogen granules (g.), poly− ß − hydroxybutyrate (P.H.B.) and carboxysomes (c.). Gas vacuoles (g.v.) are contained within the cell. *Microcystis aeruginosa* cells are similar to typical blue− green algae cells. Cells inclusions include lipid droplets, granules and gas vacuoles. Phycobilisomes are located on the outer face of the thylakoids, which are the photosynthetic site, producing energy for algae growth. Gas vacuoles are shown in greater detail in Figure 3.7.

Figure 3.7 Gas vacuoles of *Microcystis aeruginosa* (Reynolds, 1981)

Although high light intensity harms growth, *Microcystis aeruginosa* grow rapidly at the water surface and can combat too much light intensity by regulating their position in water using gas vacuoles. Nutrients are an important factor for growth. Dissolved phosphorus (P) and nitrogen (N) help renew cell growth in old colonies, warm temperatures (20 – 40 ^{o}C) stimulate algae growth and reduced oxygen concentrations can also increase the growth of *Microcystis* colonies.

During early spring *Microcystis* cells begin annual cycles of regrowth. Cells float to the water surface via regenerated gas vacuoles. They accelerate division and growth in summer to form colonies and algal concentration levels reach their peak at this time. Some colonies are large enough to be seen by the naked eye. When the temperature drops, *Microcystis* sink to the bottom of the water body into mud or sediment and become dormant surviving on stored food. Structured granules, polyphosphate and carboxysomes decline in autumn and winter because they provide energy for algae to survive at low temperatures. Anoxic environments are good for dormancy and in the next spring, residual food storage also provides the

energy for cell division forming new colonies and the growth cycle starts again (Reynolds, 1981).

3.4.2 Filamentous cyanobacteria (Hoek, 1995: 33–34)

Filamentous cyanobacteria are formed by daughter cells resulting from repeated cell divisions and are usually trichome structure, chains of cells may be straight or coiled in a regular spiral. Cell size and shape vary with filamentous forms. There are some other reproduction methods e.g. fragmentation, hormogonia and akinetes (Fay, 1983: 5–7).

For Order Oscillatotiales, algae cells are filamentous and their reproduction mode is hormogonia. In the genus *Oscillatoria*, the trichome is cylindrical with no mucilage. Single cells are disc–shaped with species able to live in sea, freshwater and sewage. Each cell of Genus *Lyngbya* is enclosed by a sheath and occurs in sea and freshwater. The cells of Genus *Microcoleus* occur in salt water and are held together in colonies by a sheath.

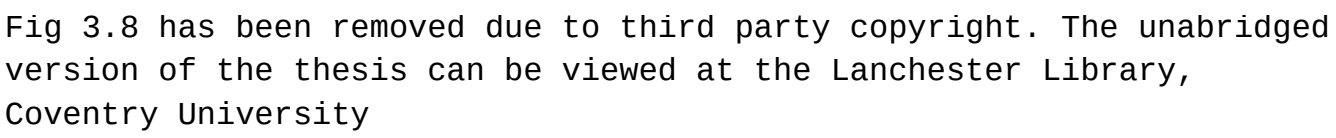
For Order Nostocales, cells are filamentous and reproduce via hormogonia. In genus *Nostoc*, cells are unbranched and covered by a sheath. Colonies may be round like small black grapes and can be found in freshwater or on damp soil. Cells of genus *Anabaena* are not contained by a sheath and they can form blooms in fresh water. The cells of genus *Aphanizomenon* lie side by side in bundles. One of the species *Aphanizomenon flos–aquae* can form algae blooms. There are about 60 species in genus *Scytonema* with cylindrical trichomes.

For order Stigonematales, filaments are multi-striate and the mode of reproduction is hormogonia. The habitats of genus *Stigonema* are on rock or in freshwater.

3.4.1.1 Genus *Spirulina*

Genus *Spirulina* belongs to the order Nostocales. *Spirulina* are free-floating filamentous cyanobacteria characterized by cylindrical, multi-cellular trichomes in an open left-hand helix. *Spirulina* occurs naturally in tropical and subtropical lakes. *Spirulina* is cultivated around the world and used as a food supplement (Fay, 1983: 81).

Figure 3.8 Spirulina (Culture Collection of Autotrophic Organisms, CCAO)



1.3.2.2 Genus *Anabaena*

Genus *Anabaena* belongs to the order Nostocales. They are filamentous cells, which can form into large colonies in water. *Anabaena* resembles a string of beads with spherical cells scattered along the filament. *Anabaena* can be found in bloom−causing algae.

Figure 3.9 Anabaena (Mur, 1999:45)

3.5 Cyanobacterial toxins

The most notable feature of these algae in terms of public health impact is that a range of species can produce toxins. Each toxin has specific properties with distinct health impacts including liver damage, neuron toxicity and tumour promotion.

3.5.1 Toxic cyanobacteria

The occurrence of toxic cyanobacteria is a worldwide environmental issue. There are at least 46 toxic species, including *Microcystis spp., Anabaena spp., Nostoc spp., etc*. *Microcystis* is the most toxic genus. According to a WHO's report, approximately 60% of cyanobacterial samples contain toxins and environmental factors (light intensity and temperature) can influence growth (WHO, 2003: 137). Illness resulting from toxic cyanobacteria has been reported and guidelines for human health safety have been set by WHO (Chorus and Cavalieri, 2000: 208). Table 3.4 shows three levels of risk and when cell numbers reach 10^5 per mL in drinking water, which may cause long-term illness.

Table 3.4 Guidelines for safe drinking water at three different levels of risk
(Chorus and Cavalieri, 2000: 208)

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3.5.2 Cyanotoxins

There are three main categories of cyanotoxins based on chemical structure (NRA, 1990: 27):

1. Neurotoxins (some of which are alkaloids)
2. Hepatotoxins (cyclic peptides)
3. Lipopolysaccharides (LPS) (compounds of fats and sugars)

Cyanobacterial toxins and their toxicity are outlined in Table 3.5.

Table 3.5 Cyanobacterial toxins and their toxicity (WHO, 2003: 140 and Sivonen and Jones, 1999: 57)

Cyanotoxins	Cyanobacterial genera	Target organ
Hepatotoxins Microcystins in general (Microcystin-LR, Microcystin-YR, Microcystin-RR, Nodularin)	*Microcystis* *Anabaena* *Planktothrix (Oscillatoria)* *Nostoc* *Hapalosiphon*	Liver
Neurotoxins Anatoxin-a (alkaloid) Anatoxin-a (S) Saxitoxins	Anabaena Aphanizomenon Lyngbya Cylindrospermopsis	Nerve synapse Nerve axons
Lipopolysaccharides (LPS)	All	Potential irritant exposed tissue

3.5.2.1 Hepatotoxins

Hepatotoxins are the most common cyanobacterial toxin resulting in liver damage by altering cells in the cytoskeleton inducing cancer. Signs of poisoning in animals are weakness, vomiting, cold extremities, piloerection, diarrhea, heavy breathing and death. Hepatotoxins are featured as they are composed of cyclic peptides. Microcystin-LR is the best studied of all the algal toxins because of its prevalence in nature (NRA, 1990: 28).

3.4.2.1.1 Microcystin (WHO, 2004:197)

The most common types of cyanotoxins are Microcystin (MCs) (NRA, 1990: 28). MCs are strongly hepatotoxic and known to initiate tumor-promoting activity. The WHO published a guideline value for MC−LR of 0.001 (mg/litre) (WHO, 2004: 407).

Microcystins are low molecular weight monocyclic peptides, which consist of seven amino acids, three D-amino acids and two common but variable L-amino acids. For example, Microcystin-LR contains leucine (L) and arginine (R) whereas Microcystin-LA contains leucine (L) and alanine (A). As illustrated in Figure 3.10, R_1 and R_2 can be substituted by any amino acid.

Figure 3.10 Molecular structure of Microcystins (NRA, 1996:4)

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There are more than 70 Microcystin variants. Microcystin-LR is more toxic than the other 70 structure variants of Microcystins. Microcystin-LR is soluble, stable and does not change structure in 300ºC water. Microcystins can be oxidised by ozone or ultra-violet light.

3.5.2.2 Neurotoxins

Neurotoxins usually affect nerve cells. For cyanobacteria, there are three main neurotoxins: anatoxin-a, anatoxin-a(s) and saxitoxin. Most neurotoxins have the chemical structure of alkaloids. Neurotoxins can result in paralysis, respiratory arrest, muscular tremor, salivation, staggering and convulsions (NRA, 1990: 27). Neurotoxins are mainly reported to cause animal poisoning (WHO, 2003: 142).

3.5.2.3 Lipopolysaccharides

Lipopolysaccharides (LPS) vary in chemical composition and consist of combinations of fats and sugars; some also contain phosphate. Research suggests that LPS are responsible for skin irritation following contact with polluted water bodies (NRA, 1990: 29).

3.5.2.4 Fate of cyanotoxins in environment (Chorus and Bartram, 1999: 95−99)

Cyanotoxins are contained in living cyanobacterial cells and are released to the surrounding water during cell aging, death and lysis. In high intensity sunlight, microcystins can slowly break down, while other cyanotoxins such as Anatoxins degrade more rapidly in sunlight. Cylindrospermopsins break down slowly at a temperature of 50ºC. Cyanotoxins can be absorbed in the sediments of lakes and ponds. Some aquatic bacteria (*Pseudomonas fluorescens* and *Streptomyces neyagawaensis*) can biodegrade microcystins (Kim, 2007 and Choi, 2005). Bioaccumulation in fish, mussels and zooplankton has also been reported and these fish and mussels then should not be consumed. Cyanotoxins, especially Microcystins are very stable.

4.0 Review of the literature on ultrasonic effects on algae (blue-greens)

Cyanobacteria are photosynthetic bacteria with some characteristics of algae. Cyanobacteria are also known as blue-green algae due to the presence of phycobiliproteins such as phycocyanin (blue) and Chlorophyll A (green). Cyanobacterial or algal blooms occur frequently and are considered a worldwide environmental issue. Algae blooms have generated a great deal of publicity and media coverage and public concern due to odour problems and the presence of algal toxins (e.g. Microcystin) which result in liver damage, neuron toxicity and promotes tumour growth. They have also been shown to be hazardous to domestic, wild animals and humans (WHO, 2003). A dominant blue-green algae species found in algae blooms is *Microcystis aeruginosa*, which produces microcystin toxins.

A number of approaches are currently available to control algal blooms in water bodies. These include minimising nutrient loading, addition of algaecides, aeration or artificial mixing, use of filters embedded with activated carbon and a more traditional holistic method employing barley straw bales (National Rivers Authority, 1990). Although all methods work to some degree all have limitations. Minimising nutrient loading is not generally practiced as it is very difficult to get support from the local agricultural communities. Excessive use of algaecides can itself lead to additional problems of pollution in water bodies. Although aeration or artificial mixing, filters embedded with activated carbon and barley straw do control algae blooms they cannot be easily applied on a large-scale to reservoirs (Harper, 1992).

A more recent approach to algae control is using ultrasound. This technology has been used in environmental protection for many years on treatment of air, soil and sewage (Mason, 2001). Ultrasound provides energy for chemical and biological reactions via cavitation, which generates extreme conditions such as high temperature (5000 Kelvin) and high pressure (several thousand atmospheres) producing radicals such as HO● and H●. Free radicals attack the cell surface breaking cell walls resulting in leakage of cell contents (Koda, 2009). Sonochemical control of algae is only in the early stages of development but initial reports indicated inhibition of cyanobacterial growth was due to the rupturing of gas vacuoles within cells resulting in cell lysis (Lee, 2001). In 2009, Graham-Rowe reported ultrasound may offer a new technology to control algal blooms using high intensity ultrasound where algal cells burst, sink and die without access to adequate sunlight (Graham-

Rowe, 2009). To date there has been few reports outlining ultrasonic parameters such as frequency and power consumption for algae removal. These parameters play a vital role in sonochemical treatment (Mason, 2002). Further research investigating the mechanism of ultrasonic effects on algal cells is required to help understand the treatment process at a cell ultra-structure level and thus optimizing ultrasonic parameter settings. Ultrasound may provide a solution for algae toxins, as it has been reported to degrade algae toxins (microcystin). Researchers have also applied ultrasound treatment for algal bloom control on a large-scale field application.

4.1 Ultrasonic parameter settings for algae removal

Tang *et al.* employed 1.7 MHz ultrasound (intensity 0.6 Wcm^{-3}) for algae removal using *Spirulina plantensis*. Although the actual ultrasonic equipment and sample volume was not documented in this paper, samples were taken following 0, 1, 3, 5, 7 and 9 minutes ultrasonic treatment. Treated, samples were taken then re-cultured for six days. Following 1 minute treatment there was no decrease in algae numbers. However, after 3 minutes sonication algae growth was prevented for 2 − 3 days; with longer treatments achieving better results. Although, algae kept growing after 5, 7 and 9 minutes treatment, the growth rates were inhibited. The optical density (560nm) of algae in control groups (without sonication) increased from 0.2 − 0.8 after 6 days culture. But the optical density of algae after 9 minute treatment only increased from 0.2 − 0.3 after 6 days culture. Additionally, algae samples were also sonicated 1 minute daily, 2 minutes every other day, 4 minutes every three days, 6 minutes every five days and 12 minutes every 11 days. After 14 days test, the optical density of algae in the control group increased from 0.1 to more than 1.5. The optical density of algae sonicated 1 minute every day and 2 minutes every other day increased from 0.1 − 0.3, but the other treated algae still showed high growth rates. Results indicated that ultrasonic energy input is an important parameter for algae removal. Shorter but more frequent ultrasonic treatments were more effective for inhibiting algae growth than longer but less frequent ultrasonic treatments (Tang, 2003).

Hao *et al.* applied high and low frequencies of 1.7 MHz (intensity 0.07 Wcm^{-3}) and 20 kHz (intensity 0.014 Wcm^{-3}) to treat *Spirulina plantensis*. For 20 kHz, 800 mL algae was sonicated for 5 minutes achieving 43.8% reduction however at 1.7 MHz, the reduction percentage was 62.5%. Results indicated 1.7 MHz (0.014 Wcm^{-3}) is more effective than 20 kHz (0.070 Wcm^{-3}). Researchers believed this was due to high

frequency ultrasound at 1.7 MHz (0.014 Wcm^{-3}) is closer to the resonance frequency of gas vacuoles in algae (Hao, 2004).

Zhang *et al.* investigated a range of ultrasonic frequencies: 20, 80, 150, 410, 690 and 1320 kHz using 1000 mL *Microcystis aeruginosa* for 10 minutes treatment. The intensity for all frequencies using ultrasonic cells was set to 0.080 Wcm^{-3}. Algae reduction results decreased in the following order: 20 < 80 < 150 < 410 < 690 < 1320 kHz, with higher frequencies achieving higher reduction results. Ultrasonic frequency was also studied using different intensities: 80 kHz at 0.032, 0.048, 0.064 and 0.080 Wcm^{-3}. Reduction results decreased in the following order: 0.032 < 0.048 < 0.064 < 0.08 Wcm^{-3}. High intensity is more efficient than low intensity for algae cell removal, but the authors also reported higher powers would increase microcystin concentration following sonication. Zhang's research suggested that low ultrasonic powers can achieve algal control and low algal toxin concentrations in water. Three frequencies (20, 80 and 150 kHz) were tested against intensities of 0.032, 0.048 and 0.08 Wcm^{-3}. An intensity under 0.048 Wcm^{-3} did not increase the extracellular algal toxin concentration and treatment at the same intensity, showed little difference at different frequencies. Results indicate higher frequencies and intensities worked more efficiently, but higher intensities increased the toxin concentrations. High ultrasonic frequencies with low intensities should be studied for algae removal (Zhang, 2006).

Mahvi *et al.* applied a 42 kHz cleaning bath (intensity 0.07 Wcm^{-3}) to sonicate 400, 700, and 1000 mL of blue green algae for 30, 60, 90, 120 and 150 seconds. Ultrasonic irradiation resulted in a decrease in algae cell numbers with the reduction percentage being 8.55, 35.22, 67.22, 90.67 and 100% respectively. Mahvi *et al.* concluded that 42 kHz ultrasonic irradiation was effective for algae removal (Mahvi, 2005), however, the power input for the cleaning bath was high. Joyce *et al.* reported tests with the 40 kHz cleaning bath (intensity 0.02 Wcm^{-3}) resulted in no reduction but an increase of 4.20% in cell numbers indicating a declumping effect (Joyce *et al.*, 2010). This demonstrates that both the intensity of ultrasound and applied frequency are important parameters and algae removal is dependent on both.

Giordano and Leuzzi applied an ultrasonic generator with a frequency of 1 MHz and a maximum acoustic intensity of 20 W (intensity 3 Wcm^{-3}) to treat 100 mL of unicellular algae *Scenedesmus quadricauda*. Giordano found that only high acoustic intensities resulted in a disruptive effect and concluded that the intense energy of

shock waves produced by collapsing cavitation bubbles inactivated algae cells. Giordano also indicated that increasing the irradiation time increased the number of cells destroyed, but this did not follow a simple exponential law between degradation of algae and sonication time. Results indicated that high intensity ultrasound can break unicellular algae cells (Giordano and Leuzzi, 1976). Simon (1974) employed an ultrasonic cleaning bath at 0.1 Wcm^{-3} without mentioning the applied frequency to sonicate *Anabaena cylindrica* (blue-green algae). Simon reported, since algae cell walls are thick; it is difficult to achieve efficient cell disruption with small numbers of cells (< 100 µl) in suspension. Results from this study suggested that for some algal species, ultrasound is unable to break algae cells, even at high ultrasonic intensities (Simon, 1974).

From the literature it is known that ultrasonic algae removal at a Lab-scale is determined by a number of parameters: intensity, frequency, sonication time and volume. These parameters are the main focus of my research. The effect of ultrasound in the removal of harmful algae is dependent on all of these parameters rather than only one.

Although some researchers reported little difference in algae removal rate constants at lower frequency ranges, our results demonstrate at low intensities a declumping effect occurs at 40 kHz while at 20 kHz very low levels of inactivation are observed. This may be explained by differences in the way that the ultrasonic power is delivered to each system. The bath provides a relatively even power distribution from bath water through the flask containing algae samples whereas the 20 kHz horn delivers concentrated energy directly from the horn tip immersed within the algae sample. Although the overall power entering the algae suspension is similar to that provided by the bath in the case of the horn the energy is more concentrated resulting in bulk mixing.

Generally, high ultrasonic frequencies and intensities achieve high reduction rates for algae due to subsequent effect on gas vacuoles (Hao, 2004). The main disadvantage is that high frequencies and intensities require high energy input, increasing water treatment costs. Some researchers e.g. Ma (2005) suggest high ultrasonic intensities will break algae cell walls releasing toxins into water resulting in a major hazard for public health. Therefore it is vital to determine ultrasonic parameter settings to optimizing this technology based on algae reduction, energy costs and control of toxins. Different ultrasonic frequencies and intensities must be investigated to

determine optimized conditions. It is important that effective parameter settings on a laboratory scale will also work at a pilot scale because this is vital for practical applications in algae control in drinking water sources.

4.2 Ultrasonic mechanisms in cyanobacterial control

The possible mechanisms of ultrasonic algae control have been reported by very few scientists. Mechanistic studies help us determine how ultrasound works during treatment and more importantly to select effective and energy saving parameters settings for treatment.

Hao *et al.* suggested inhibition mechanisms for algae removal is attributed to ultrasonic cavitation. In his research, ultrasonic cavitation was evaluated using cavitation noise spectrum and KI dosimetry to prove cavitation occurred during treatment. Bloom-forming algae cells (*Spirulina platensis*) contain gas vacuoles which consist of stacks of cylindrical vacuoles. Cavitation disrupts gas vacuoles resulting in cell collapse due to pressures which exceed the cell wall strength. In figure 4.1, differential interference microscopy of ultrasonically treated cells clearly illustrates damage to gas vacuoles and cells induced by ultrasound (Hao, 2004). Lee (Figure 4.2) applied transmission electron microscopy to indicate that gas vacuoles of a single *Microcystis aeruginosa cell* collapsed following treatment with ultrasound of 28 kHz, 0.12 Wcm^{-3} and 30 seconds treatment (Lee, 2001).

Figure 4.1 Differential interference microscopy of cells (*Spirulina platensis*) showing details of the cell surface (Hao, 2004)

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Figure 4.2 Transmission electron microscopy of cells (*Microcystis aeruginosa*) showing details of the cell surface (Lee, 2000)

Another method which is used to evaluate gas vacuoles in sonicated cyanobacteria was reported by Lee *et al.* Flow cytometry is a rapid method for measuring fluorescence and light scattering of individual cells in large populations providing information relating to size, shape and intracellular structure. Side scatter light (SSC) intensity is used to distinguish granulated from non-granulated cells. Lee suggested sonicated cyanobacteria had lower SSC intensity than untreated cyanobacteria. Lee used disappearance of intact clusters of algae to indicate the complete collapse of gas vacuoles following sonication. Forward light scatter versus side light scatter intensity was used to identify live versus dead sub-populations in microbial viability studies. Results obtained by Lee can also be explained as the algae have been killed or injured using ultrasound. Lee also reported regeneration of algae using analysis by flow cytometry demonstrating ultrasonic treatments (28 kHz, 0.07 Wcm^{-3} for 30 seconds treatment) did not kill algae cells but rather resulted in cell injury. Three points can be taken from Lee's research (Lee, 2000):

- Flow cytometry can be used for rapid analysis of algae viability
- The theory of ultrasonic irradiation breaking algae gas vacuoles must be proved by more direct evidence
- Algae can regenerate after ultrasonic treatment using 28 kHz, 0.07 Wcm^{-3} for 30 seconds treatment

Tang *et al.* compared gas-vacuolated cyanobacteria *Microcystis aeruginosa* with non gas-vacuole cyanobacteria *Synechococcus* by sonicating at 1.7 MHz ultrasound for 5

minutes (intensity 0.6 Wcm^{-3}). Different results were observed with an inhibition of growth rate for *Microcystis aeruginosa* whereas *Synechococcus* grew rapidly after ultrasonic treatments. Results indicate that the only algae containing gas vacuoles were sensitive to ultrasonic irradiation. Tang also suggested the mechanism of ultrasonic treatment was disruption of gas vacuoles via cavitation. Cavitation was detected by determining electric conductivity during sonication in algae suspensions as cavitation provides sufficient energy to dissolve N_2 and O_2 in water to form NO, which is further oxidized to form NO_2 which will combine with H_2O to form HNO_3 (nitric acid) and HNO_2 (nitrous acid), so that the electric conductivity change of water can be used to detect the cavitational yield (Feng, 2002). It was concluded that greater cavitation yields were produced in algae suspensions with gas vacuoles (Tang, 2004).

Another inhibition mechanism is possibly due to the fact that ultrasound may damage cyanobacterial photosynthesis. Photosynthesis is the process that photosynthetic organisms transfer sunlight energy to release oxygen from water and fix carbon dioxide into sugar. Cyanobacteria have two photo systems; Photosystem I has a reaction centre with chlorophyll A and Photosystem II transfers energy to photosystem I through a series of photosynthetic pigments. Zhang *et al.* sonicated *Microcystis aeruginosa* with 25 kHz ultrasound (intensity of 0.32 Wcm^{-3}) for 5 minutes. Results indicate chlorophyll A concentration and phycocyanin (PC) (photosynthetic pigment) absorbance reduced immediately after sonication. This research concluded that ultrasound damaged the photosynthetic function of organs in algae inhibiting photosynthesis reducing algal growth (Zhang, 2006). Tang (2003) reported 5 minutes ultrasonic treatment (1.7 MHz, 0.5−0.6 Wcm^{-3}) reduced absorption peaks of phycocyanin at 625nm *in vivo*.

There are two main mechanisms that are universally accepted for ultrasonic algae removal. Firstly, ultrasound breaks gas vacuoles in algae via cavitation. Gas vacuoles are the buoyancy-regulating organelles of cyanobacteria by which they regulate their position in water maintaining optimal light intensity for growth. When gas vacuoles collapse, cyanobacteria sink to the bottom of water bodies where there is less light for photosynthesis. The second mechanism is that ultrasound damages algae resulting in cell death, affecting photosynthetic functions such as those required by chlorophyll A. Once the photosystem is damaged, algal cells die due to food shortages.

4.3 Ultrasonically degradation of microcystin toxin

The most notable feature for cyanobacteria in terms of public health is that a range of species can produce toxins which pose a direct health impacts including liver damage, neuron toxicity and tumour promotion. Toxins are also hazardous to domestic or wild animals and humans. The most common types of toxins are microcystins (MCs), which are strongly hepatotoxic and known to initiate tumor-promoting activity (Figure 4.3). The WHO published guideline value for MC−LR is 0.001 (mg/litre) (WHO, 2003).

Figure 4.3 Common molecular structure of microcystins (NRA, 1996:4)

In Ma *et al.*'s (2005) research, a dilute solution of microcystin (extracted from *Microcystis* suspension) was sonicated using 20 kHz, 150 kHz, 410 kHz and 1.7 MHz ultrasound at an intensity of 0.03 Wcm^{-3} for all frequencies. Following 20 minutes treatment, toxin concentrations were reduced by 54.7, 70.6, 65.2 and 53.9%, respectively. Results indicate intermediate frequencies had an improved effect on microcystin degradation. The effect of ultrasonic intensity was also studied by sonicating dilute solutions of microcystin using 20 kHz at 0, 0.03, 0.06 and 0.09 Wcm^{-3}. After 5 minutes treatment, the reduction percentage was 0, 18.1, 50.2, 63.6% respectively. Ma *et al.* found that increasing ultrasonic power resulted in an increase in microcystin degradation. The results of ultrasonic power on microcystin degradation are very similar to that obtained for the degradation of pentachlorophenol, acephatement and CCl_4 due to extreme conditions (high temperature and pressure) resulting from cavitation, which breaks C−C, C=C, C−N, C−O bonds (Chowdhury, 2009). According to Ma *et al.*'s research, ultrasonic

irradiation offers an efficient method for degradation of microcystin when dissolved in water with 150 kHz being the optimum frequency (Ma *et al.*, 2005).

Song *et al.* demonstrated ultrasonic irradiation at 640 kHz lead to the rapid degradation of microcystin-LR (MC−LR) (extracted from *Microcystis* suspension) using 640 kHz within 2.5 minutes treatment. It was reported that commercial MC−LR standard degrades more rapidly than toxin extracted from algae suspension using ultrasonic treatments, resulting in inhibition of cyanobacterial exudates in extracts (Song *et al.*, 2005).

2−methylisoborneol (MIB) and geosmin (GSM) are microbial by-products associated with blue-green algae resulting in taste and odour problems. Song *et al.* used a 640 kHz ultrasonic bath to sonicate MIB and GSM for 40 minutes achieving over 90% removal of compounds. As the yield of OH● radicals increased through acoustic cavitation, degradation rates increase along with increasing temperature. Ultrasound can also reduce taste and odour problems along with algae blooms and toxins. This research was based on commercial chemicals, which may not indicate effective field applications using natural samples. Further research is required to determine ultrasonic effects on MIB and GSM in algae suspensions (Song *et al.*, 2007).

Hudder *et al.* evaluated ultrasonic detoxification of MC−LR in water supplies. The ultrasonic transducer was operated at 640 kHz (intensity 0.5 Wcm^{-3}) and irradiation time of 90 minutes resulted in 99% of MC−LR degraded. Comparison experiments were undertaken between toxin and treated toxin reaction products (TTRP) to determine if ultrasonic degradation of toxins occurred at histopathology and genetic level. Lactate dehydrogenase (LDH) is a good indicator of hepatotoxicity and assays showed even the lowest dose of toxin can result in significant LDH leakage above background levels. The TTRP had no LDH activity, proving sonication of MC−LR reduces toxicity. Gene expression of liver cells indicated algae toxins are harmful to animal health. However, no genes responded to microcystin exposure using TTRP, demonstrating ultrasonic treatment reduces the hazards of algae toxins. Results of quantitative Polymerase Chain Reaction (PCR) also supported the results obtained by DNA analysis. Hudder's study indicated that microcystin was toxic to animals and the effects were expressed at a genetic level, but ultrasound can reduce algae toxicity. In this study, power ultrasound was applied for a relatively long irradiation time. High ultrasonic powers and long treatment times may be required to reduce toxin problems (Hudder *et al.*, 2007).

Toxin and odour issues are one of the main problems associated with algae blooms and cannot be ignored during treatment. Recent studies on ultrasonic algae removal mainly focus on algae cell control or inactivation. Although toxin and odour issues will reduce with decreasing numbers of algae cells, it is important to study the mechanism of toxin degradation following sonication to prove ultrasonic treatments are a safe method to control algae blooms. A typical algal toxin, microcystins-LR is a cyclic peptide which is composed of C−C and C=C bonds. Sonochemists have already demonstrated that ultrasound can break C−C, C=C, C−N, C−O bonds via cavitation (Chowdhury *et al.*, 2009). Thus it should be possible for ultrasound to break chemicals bonds in the algae toxin molecule. This is an added advantage for ultrasonic treatments on algae blooms as most other control measures such as algaecides, light-shading, biological controls do not solve the toxin and odour issues during treatments.

4.4 Large-scale applications

Lee *et al.* (2002) applied an ultrasonic irradiation system (USIS) to treat a lake contaminated by algae blooms. Liquid volume of Lake Senba (Japan) was 365,000 m^3, with a mean depth of 1 meter. The USIS consisted of a water jet circulator, drive liquid suction pump and ultrasonic irradiation module. This commercial ultrasonic irradiation equipment (Honda Electronics Co. Ltd, Toyohashi, Japan) operated at 200 kHz frequency (intensity 0.1 Wcm^{-3}). 10 units of USIS equipment were installed in the lake and the exact location was optimized using a computer simulation to completely treat the lake water. Liquid flow rates of the water jet circulator were 5.61 m^3/min and the retention time of treated water in the ultrasonic chamber was 4.7 seconds (optimized ultrasonic irradiation time for algae removal). Ultrasonic field treatments were undertaken in the lake for one year. The effect of the USIS was evaluated by monitoring the water quality of the lake two years before and following treatment. Lake water and sediment samples were collected monthly and analysed for chlorophyll A, suspended solids, chemical oxygen demand (COD), total nitrogen and total phosphorus. Results indicated that the USIS decreased chlorophyll A concentrations, suspended solids, COD, total nitrogen and total phosphorus even during the summer season. However, the concentration of chlorophyll A and suspended solids increased in the following year when no ultrasonic treatment was employed, although they were lower than prior to treatment. Lee's work is very important for large-scale ultrasonic applications for algae control and indicates this is

an effective treatment when applied in natural eutrophic water bodies. However, it should be noted the energy consumption was not reported and power of ultrasound was relatively high, which may have resulted in high energy costs. Additionally, once the ultrasonic treatment was stopped the algae blooms re-occurred the following year suggesting ultrasonic treatment must be applied each year at certain periods to prevent algae blooms occurring (Lee *et al.*, 2002).

An enclosure study was undertaken by Ahn *et al.* to identify if field applications for ultrasonic algae removal were feasible. Cylindrical plastic enclosures that were 0.6 m in diameter, 0.7m deep and contained 200 L pond water were constructed in a small eutrophic pond. Ultrasonic radiation was applied in an upward direction from a depth of 40 cm using custom-made ultrasonic equipment (USP-s, Morko Co., Daegu, Korea). The intensity and frequency was 0.63 Wcm^{-3} and 22 kHz respectively and the period of ultrasonic operation and operation/pulse were 40 and 120 seconds over 10 days. Surface water samples were collected every 3 days to determine algae cell concentrations, chlorophyll A, nitrogen, phosphorus, pH values and dissolved oxygen (DO). The pH value, dissolved oxygen, total nitrogen and total phosphorus decreased following 3 days treatment, indicating ultrasonic irradiation inhibited photosynthesis in algae. No floating scum of cyanobacteria was observed in the sonication enclosure. Chlorophyll A concentrations decreased to one-fourth of the initial level and no release of microcystin toxin was reported. In the control field, the total nitrogen and total phosphorus level also decreased. The cyanobacterial concentration in the sonicated enclosures dropped from 66% to 0.3% within 3 days, whereas the cyanobacterial concentration in control enclosures increased from 66% to 91%. Ahn *et al.* concluded that their ultrasonic treatments were effective (Ahn *et al.*, 2003).

In another later study, two neighbouring algae (Korean) blooming lakes were studied by Ahn *et al*, which were 200 m apart but of similar size (7000 and 9000 m^3). Both ponds were shallow with a mean depth of 2 meters and highly eutrophic. One ultrasonic device (USP-s, Morko Co., Daegu, Korea) was used to treat a whole pond of 9000 m^3, ultrasonic frequency of 22 kHz (intensity 0.63 Wcm^{-3}). Cyclic ultrasonication treatments were programmed to work for 85 seconds of every 115 seconds for 25 days. The ultrasonic device was submerged in one corner of the pond and two water pumps fed water to be sonicated. Since cyanobacteria mainly float on the water surface, this region was sampled for temperature, conductivity, dissolved oxygen chlorophyll A, total nitrogen, total dissolved nitrogen, total particulate

nitrogen, total phosphorus, total dissolved phosphorus and total particulate phosphorus. Ultrasonic treatments reduced dissolved oxygen, indicating photosynthesis was repressed in the treated pond. Total nitrogen and total phosphorus concentrations were also reduced. Average levels of chlorophyll A and total algae numbers in the treated pond reduced by 61 and 53% respectively. Results from Ahn *et al.* indicate ultrasonic treatment can provide a control solution to algae blooms. However this work did not take into account algal toxins in relation to water quality. Furthermore, algae concentrations in solid lake sediment should also be analysed, as algae may sink to the bottom of lake following sonication. It is important to identify the condition and metabolic activity of algae after sonication. This is vital to ensure algal growth is inhibited after ultrasonic irradiation rather than enhancing reproduction, which was reported by some researchers (Ahn *et al.*, 2007).

Some researchers e.g. Mason (2004) have suggested that there are limits to the efficiency of the processes employing ultrasonic probes since cavitation is only achieved in a localised area near the surface of the probe. Cavitation can also be achieved using a liquid jet (hydrodynamic cavitation) which can be very efficient with high pump efficiencies (Kalumuck, 2003). Jet cavitation was employed to identify if it had a negative effect on cyanobacterial growth. In Xu *et al.*'s study, jet cavitation equipment consisted of a 6 L tank, high pressure self-priming pump with a uni-directional piston, motor with an electric power (1.1 kW with speed of 2850 rpm), control valves, cavitation tube and main and by-pass lines connected to the discharge side of the pump through pipes. Algae volume was 5 L and experiments were carried out at 0.55 or 0.4 Mpa pump pressures. Results indicated removal effects by jet cavitation strongly depends on the hydraulic characteristics of cavitation tubes, inlet pressure, cavitation number (dimensionless number used to characterise the conditions of cavitation in jet devices), light and different algae initial concentration. High inlet pressures (0.55 Mpa) and longer treatment times (up to 114 hours) achieved higher algae reductions. In addition to the direct mechanical effects of cavitation hydrogen peroxide is produced and this had an oxidizing effect on algae cells (Xu *et al.*, 2006). One problem with such jets is the possibility that the cavitation tubes could become blocked by algae during long term use.

In summary, large-scale applications must be able to deal with complex natural conditions such as temperature, pH value, nutrient concentration, depth of water, etc. Since few effective methods exist to deal with large-scale algae blooms, ultrasonic treatment may provide a suitable solution. The effectiveness of this technology

requires long term monitoring of water quality before, during and after treatment in addition to identification of ecosystem changes. The production of algae toxins is an issue of great importance and concern for drinking water safety. Ultrasonic parameters such as frequency, intensity, sonication time must be determined in an effective and energy saving way. In this project, our lab-scale studies were designed to determine and optimise parameter combinations for large-scale field applications to control harmful algal growth.

5.0 Experimental details

Table 5.1 Ultrasonic treatment units used for small lab-scale experiments

Equipment	Additional information
Experimental volume	200 mL, 400 mL
Frequency (kHz)	40 kHz bath (Langford Sonomatic, see section 5.1.2) 20kHz probe (Vibra-cell, Sonics & Materials) 580, 864 and 1146 kHz Multi-frequency bath (Meinhart)

Table 5.2 Ultrasonic treatment units used for middle-scale experiments (≤ 5 L water)

Equipment	Additional information
Experimental volume	1,1.5, 3, 5L
Frequency (kHz)	20 kHz ultrasonic probe (designed and constructed by Prof. W. Wu at Public Health Department of Southeast University, China, see section 5.1.7) Sonolator (Sonic Corporation) 16 kHz and 20 kHz Dual Frequency reactor (DFR, Advanced Sonics Processing Systems) 20 kHz Vibrating Tray (Advanced Sonics Processing Systems)

Table 5.3 Analysis equipment

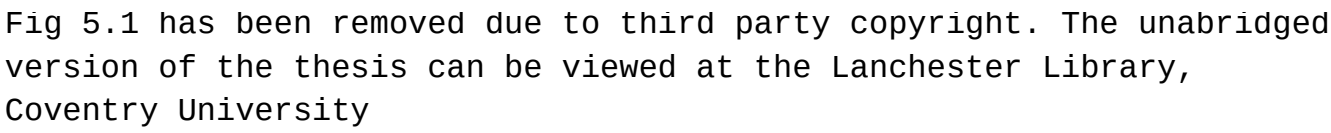
Cell counts	Haemocytometer (Weber, BS748)
Cell counts/biomass (Chlorophyll A)	Colorimeter (Corning)
Microcystis toxin detection	HPLC (SHIMADZU, LC−20AD, SPD−20A, CTO−20AC)
Cell health (Chlorophyll A)	UV-visible spectrometer (optical density) (SHIMADZU, 2450PC), fluorospectrometer (SHIMAZU RF5301), flow cytometer (BD FACSCalibur)

5.1 Sonication equipment

5.1.1 Cleaning bath

The bath is the most accessible and cheapest type of ultrasonic equipment. The construction consists of a stainless steel tank with transducers clamped to its base. The power and frequency is controlled by the type and number of transducers. The low intensity bath system uses a power density of 5 Wcm^{-2} at the transducer radiating surface area. The picture below illustrates ultrasonic energy is produced by transducers which must then pass through the bath medium to the reaction vessel to achieve chemical or biochemical reactions. In sonochemical experiments, a flat-bottom flask is preferred as the reaction vessel in an ultrasonic bath as it has better energy transmission than a round bottomed flask. To achieve uniform transmission of the sound waves a surfactant Decon 90 (Decon® Laboratories Limited, UK) is added to the bath water to lower the surface tension. For each experiment, the same reaction vessel is placed at the same position in the bath to ensure reproducibility of experiments. A Langford Sonomatic 40 kHz bath was used in this work.

Figure 5.1 Ultrasonic cleaning bath (Mason, 1999: 42)



5.1.2 Probe systems

Ultrasonic probe systems are the most efficient method of transmitting ultrasonic energy directly into a reaction vessel and the energy input can be up to 100 times greater than an ultrasonic cleaning bath (Mason and Lorimer, 1988: 215). Modern probe systems are based on piezoelectric transducers and the overall construction is shown in figure 5.2.

Figure 5.2 Ultrasonic probe (Mason, 1999: 58)

A detachable horn transmits vibrations from the upper fixed horn through a length of metal. Different length and shapes of detachable horns effect efficiency. The minimum length of a titanium alloy horn is half a wavelength, which will give an exact mirror of the vibrational amplitude supplied at one end to the other. Tip erosion is a significant problem since this reduces overall length of the horn due to resulting in loss of efficiency. Tips are usually replaceable, which is more cost effective than replacing the whole titanium horn which would be expensive. A probe tip costs under one hundred pounds but a whole horn device may cost several thousand pounds (£6,000-10,000). There are four common shapes of horns used with commercial probes: uniform cylinder, linear taper or cone, exponential and stepped (Mason and Lorimer, 2002:281). Most probes are set at 20 kHz frequency with variable amplitude for different ultrasonic intensity. For this work, a Sonics and Materials 20 kHz VC 600 probe system was used.

5.1.3 High multi-frequency bath

The multi-frequency bath consists of a power amplifier, transducer and reaction vessel. Two modes can be applied for sonochemical reactions; standard and pulse. The multi-frequency bath is illustrated in the figure below. Frequencies used in tests were: 580, 864 and 1146 kHz and the intensity could be set from 0% to a maximum power setting for energy input. The advantage of multi-frequency bath is that different frequencies can be employed in the same reaction vessel giving accurate and reproducible results.

Figure 5.3 Multi-frequency bath (Meinhardt)

5.1.4 Sonolator

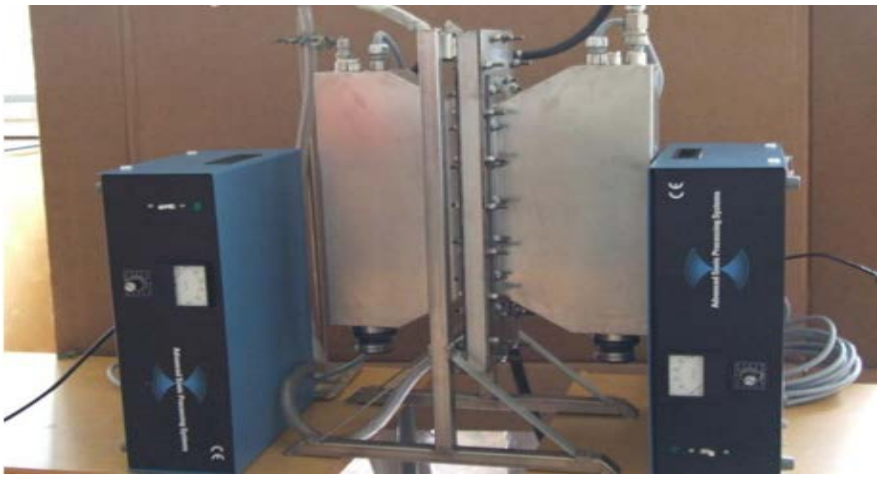
The Sonolator produces cavitational energy by forcing a liquid through a small orifice which then strikes a knife-like blade set in its path. The rapid flow of fluid across the blade produces hydrodynamic cavitation and allows very effective mixing, homogenizing, emulsifying and dispersing. The Sonolator is equipped with a pump to circulate liquid in the system. 5L algal suspension was sonicated using the Sonolator.

Figure 5.4 Sonolator (Sonic Corporation)

5.1.5 Dual frequency reactor

Dual frequency reactor (DFR) is equipped with two sonicating metal plates. The plates enclose a flow system and are driven at different frequencies, 16 and 20 kHz. The two plates are similar to two bases of a ultrasonic baths facing toward each other and separated by 5 cm. A pump is employed to feed the liquid/slurry to the bottom of the reactor in a vertical position for circulating runs. This configuration avoids air gaps within the reaction column. The dimensions of the DFR are as follows: ~12 x 50 cm with space of 0.25 cm, surface area = 0.06m^2 and volume = 1.2 litre.

Figure 5.5 Dual Frequency Reactor (Advanced Sonics Processing Systems)



5.1.6 Vibrating tray

The vibrating tray has a surface area of 21 x 58 cm = 0.12 m^2 but only part of the entire surface is active. Equipment consists of a suspended tray with a 20 kHz transducer attached to the base where the base of the tray vibrates (resonates) and samples are placed directly in the tray during treatment. This type of equipment has been used for large-scale continuous processing of coal and metal ores at rates up to 20 tons per hour. In our experiments, the vibrating tray was used to assess the effect of inactivation on algae removal.

Figure 5.6 Vibrating Tray (Advanced Sonics Processing Systems)

5.1.7 20 kHz ultrasonic probe designed and constructed in Southeast University (China)

A proto-type purpose-made ultrasonic probe was designed and constructed at Southeast University, China. The ultrasonic transducer was set at frequency of 20 kHz with a nominal power output of 200 W. The ultrasonic device consisted of three parts: acoustic components (ultrasonic transducer), ultrasonic generator and mechanical components with ancillary equipment. The ultrasonic transducer is constructed using piezoelectric ceramic material (Lead Zirconate Titanate) with ferroelectric ceramics. The advantage of the purpose-made ultrasonic probe is that the transducer is waterproof, so it can be used under water.

Figure 5.7 20 kHz ultrasonic probe (Southeast University, China)

5.2 Culture of cyanobacteria

Cyanobacteria used in this project were one strain of *Microcystis aeruginosa* which was purchased from the Culture Collection of Algae and Protozoa (CCAP − strain number 1450/15). Toxin producing *Microcystis aeruginosa* were also purchased from the French culture collection (PCC 7806). All algae were cultured using blue-green medium (BG11 − CCAP) which involved preparing nine stock solutions, as outlined in the Table 5.4 below.

5.2.1 Culture media

1. 829 mL of distilled water was placed in a 1 litre glass bottle and sterilized by autoclaving at 121ºC for 15 minutes.
2. Stock solutions were prepared by weighing out the chemicals as specified in Table 5.4 and sterilized by autoclaving at 121ºC for 15 minutes.
3. **Stock 1** was prepared by adding 15.0g $NaNO_3$ to 1000 mL of distilled water.
4. **Stock 2−8** solutions were each prepared separately by adding 2.0g K_2HPO_4, 3.75g $MgSO_4.7H_2O$, 1.80g $CaCl_2.2H_2O$, 0.30g Citric acid, 0.30g Ammonium ferric citrate green, 0.05g $EDTANa_2$, and 1.00g Na_2CO_3 to 500 mL of distilled water.
5. **Stock 9** solution was prepared by adding 2.86g H_3BO_3, 1.81g $MnCl_2.4H_2O$, 0.22g $ZnSO_4.7H_2O$, 0.39g $Na_2MoO_4.2H_2O$, 0.08g $CuSO_4.5H_20$, and 0.05g $Co(NO_3)_2.6H_2O$ to 1 liter of distilled water.
6. Once the stock solutions had cooled, 100 mL of Stock 1 (sterile graduate cylinder), 10 mL (each) of Stock 2−8 (10 mL sterile pipette) and 1 mL of Stock 9 (1 mL sterile pipette) was added to 829 mL of sterile distilled water in a laminar flow cabinet. This 1 litre solution was thoroughly mixed and placed in the fridge until required for use.

N.B. The use of careful aseptic techniques is vital in media preparation to minimize the risk of algae cultures becoming contaminated with bacteria from the environment.

Table 5.4 BG11 stock solution components

Stocks 1	Per litre
(1) $NaNO_3$	15.0g
Stock 2−8	Per 500 mL
(2) K_2HPO_4	2.0g
(3) $MgSO_4.7H_2O$	3.75g
(4) $CaCl_2.2H_2O$	1.80g
(5) Citric acid	0.30g
(6) Ammonium ferric citrate green	0.30g
(7) $EDTANa_2$	0.05g
(8) Na_2CO_3	1.00g
Stock 9: Trace metal solution:	Per litre
H_3BO_3	2.86g
$MnCl_2.4H_2O$	1.81g
$ZnSO_4.7H_2O$	0.22g
$Na_2MoO_4.2H_2O$	0.39g
$CuSO_4.5H_20$	0.08g
$Co(NO_3)_2.6H_2O$	0.05g

Source: CCAP (Culture Collection of Algae and Protozoa)

5.2.2 Algae culture

1. A range of glass conical flasks (100, 250, 500 and 1000 mL) with a magnetic fleas, foam bungs, tin foil lids and autoclave tape were sterilized by autoclaving at

121 º C for 15 minutes.

2. A small volume of algae was added to fresh sterile BG11 every three days to ensure sufficient nutrients for a healthy culture.
3. When starting new algae cultures: 5 mL algae was added to 50 mL BG11 media (25 mL algae to 250 mL BG11 media, 100 mL algae to 1000 mL of BG11 media).
4. A sterile magnetic flea was added to each conical flask to ensure sufficient mixing during culture.
5. Algal suspensions were placed in the plant growth room and incubated at 25ºC along with being exposed to 12−hour incandescent lights and 12−hours darkness to reproduce natural day and night cycles.

5.3 Algae analysis

5.3.1 Spectrophotometer (optical density)

Optical density measures the amount of light absorbed by an algal suspension. Higher absorbance indicates the presence of more algal cells. The experimental method is outlined below:

1. The spectrophotometer (Corning) was zeroed by placing distilled water in a 1 mL cuvette and the optical density was read at 680nm.
2. A sample of algae suspension was removed from the growth room and placed in a 1 mL cuvette and the absorbance reading was measured at 660nm to determine the Chlorophyll A concentration. This was done in triplicate to ensure statistical accuracy.
3. Data was recorded
4. Equipment was cleaned

5.3.2 Haemocytometer

A haemocytometer consists of a thick glass microscope slide which contains a chamber on the slide surface. The chamber is engraved with a grid of perpendicular lines of standard length and depth. Therefore it is possible to enumerate the number of algal cells in a specific volume of fluid calculating cell concentration. The experimental method is outlined below:

1. A haemocytometer (Weber, BS748) was cleaned using 70% IMS (industrial

methylated spirits) and tissue paper. IMS was allowed to completed evaporate to ensure it did not have adverse effects on the algae cells.

2. The haemocytometer cover slip was placed on the slide and 1 mL of algae suspension was loaded on to the haemocytometer using a pipette. Any excess liquid was removed from the haemocytometer slide using tissue.
3. The haemocytometer grid was located under the 10X objective and the microscope was fine focused until the algal cells were visible.
4. All algal cells located within the haemocytometer grid were counted using the 40X objective. This was done in triplicate to ensure statistical accuracy.
5. Data was recorded.
6. The equipment was cleaned

5.3.3 HPLC C18 column – toxin detection

Filtered algae suspensions or the prepared microcystin-LR analytical standard (MC−LR) (Sigma, 10 µg/mL in methanol) were analyzed using HPLC (Shimadzu) with an Ultrasphere 5 nm ODS (Beckman 4.6 mm* 25 cm) column and a chromatograms at UV 238 nm. The mobile phase was water: acetonitrile gradient from 75:25 to 25:75. The analysis time was 30 minutes with a flow rate of 1.5 mL/min and an injection volume of 25 µL.

5.3.4 Measurement of intact algal cells and metabolic activity

The spectrum of intact algal cells was measured using a UV-Vis spectrometer (optical density) (Shimadzu, 2450 PC) at a wavelength scale of 360 − 800nm and a fluorometer (Shimazu, RF5301) at a fixed exctiation wavelength 465nm with an emission spectrum from 475-850 nm. UV-Vis spectrometer analysis of untreated intact *Microcystis aeruginosa* cells indicated an absorption spectra in vivo consisting of three groups of photosynthetic pigments: chlorophyll (420 and 680 nm), carotenoids (430nm) and phycobiliproteins (620nm) (Tang, 2003 and Fay, 1983). UV-Vis analysis was used to determine ultrasonic effects on the photosynthetic pigments. Analysis with the fluorometer indicated the phycobiliproteins peak (665nm) can be used to assess ultrasonic effects on algal photosynthetic functions as other pigments are relatively unstable under different temperature and culture light conditions (Hao, 2004).

1. Equipment was ‘zeroed’ by placing distilled water in a 1 mL cuvette.
2. A sample of algae suspension was placed in a 1 mL cuvette and the absorbance reading was measured to determine the chlorophyll A concentration. This was done in triplicate to ensure statistical accuracy.
3. Data was recorded.
4. Equipment was cleaned.

5.3.5 Flow cytometer – cell integrity and viability

Microcystis species were stained with 1.0µL SYTO−9 and 1.0µL Propidium Iodide (PI) from a LIVE/DEAD Baclight bacterial viability kit (Invitrogen, L10316) for 1 mL algae samples. Standard settings employed in the experiment were: FSC = E00, SSC = 242, green fluorescence (FL1) = 510, orange fluorescence (FL2) = 550 and red fluorescence (FL3) = 610. All parameters were set on a logarithmic amplification by default four dot plots: FSC V’s Counts, FSC V’s SSC, FL1 V’s Counts and FL1−FL3.

- Stained algae samples were placed in a tube
- All samples were vortexed prior to analysis
- Equipment was switched to standby
- Machine was set to ‘Run’ and samples were analysed
- Results were acquired and digital signals are processed and presented using BD CellQuest Pro Software (BD Biosciences, USA)

5.3.6 Data analysis

All data was processed using SPSS (Statistical Product and Service Solutions) software (12.0, SPSS Inc., Chicago Ill, USA). All results are an average of the three independent trials SD (Standard Deviation). Data was subjected to analysis of variance (ANOVA). P-value less than 0.05 were considered statistically significant.

5.4 Experimental methods

5.4.1 Experimental Quality Control

- All experiments were performed in triplicate.
- All the analysis equipment was allowed to warm up for 30 minutes prior to use.
- Optical density ranges from 1.5 to 2.5 at 680nm using the colorimeter.
- Syringe filters with a pore size 0.7μm were used to filter samples prior to HPLC analysis.
- UV-Vis spectrometer was calibrated using distilled water at 680nm prior to analysis for Chlorophyll A peak using *Microcystis aeruginosa.* Refer to section 5.3.4.
- A cooling system was employed for all experiments to maintain a temperature below 25ºC. For high powers, temperature was controlled under 30ºC.
- Controls were performed in parallel to each experiment to compare ultrasonic effects on algae and analyzed by haemocytometer and optical density.

5.4.2 Determination of the acoustic power of different ultrasonic equipment

Prior to undertaking any ultrasonic treatments, calibration was completed to determine the actual power in each system. Temperature of a varied volume (200 and 400 mL) of water (H_2O) was recorded every ten seconds over a set period time (180 seconds) of continuous sonication at the following frequencies: 20, 40, 850, 864 and 1146 kHz. This was completed in triplicate and the average of each was recorded to calculate the power of ultrasound. Power is calculated using the equations in section 6.1.

5.4.3 Sonication of algae (OD 0.2, 200 and 400 mL) using the 20 kHz ultrasonic probe (Vibra-cell, Sonics & Materials)

200 or 400 mL standard suspensions of *Microcystis aeruginosa* at an OD at 680 nm of 0.2 (6.00×10^6 cells per mL) was placed in a 250 mL flask and sonicated for 30 minutes using a 20 kHz probe (intensity 0.0015 Wcm^{-3} (200 mL), 0.0023 Wcm^{-3} (400 mL) and a ice bath to maintain the temperature below 25ºC. Samples were taken at 0, 5, 10, 20 and 30 minutes and the cell number was calculated using a

haemocytometer. The temperature was recorded and chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at excitation wavelength 465nm. A control algae sample (unsonicated) was sampled at 0, 5, 10, 20 and 30 minutes.

5.4.4 Sonication of algae (OD at 680 nm 0.2, 200 and 400 mL) using the 40 kHz ultrasonic bath (Langford Sonomatic)

200 or 400 mL standard suspensions of *Microcystis aeruginosa* at an OD at 680 nm of 0.2 (6.00×10^6 cells per mL) was placed in a 250 mL flask and sonicated for 30 minutes using a 40kHz bath (intensity 0.0200 Wcm^{-3},200 mL), 0.0047 Wcm^{-3} (400 mL) and a ice bath was employed to maintain the temperature below 25−30ºC. Samples were taken at 0, 5, 10, 20 and 30 minutes and the cell number was calculated using a haemocytometer. Temperature was recorded and the chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at exctiation wavelength 465nm with an emission spectrum from 475-850 nm. A control algae sample (unsonicated) was sampled at 0, 5, 10, 20 and 30 minutes.

5.4.5 Sonication of algae (OD at 680 nm 0.2, 200 and 400 mL) using the Meinhart multi-frequency generator (580, 864 and 1146 kHz)

200 or 400 mL standard suspensions of *Microcystis aeruginosa* at an OD at 680 nm of 0.2 (6.00×10^6 cells per mL) was placed sonicated for 30 minutes using a multi-frequency bath with a cooling system to control the temperature below 25ºC. Samples were taken at 0, 5, 10, 20 and 30 minutes and the cell number was calculated using a haemocytometer. Temperature was recorded and the chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at emission wavelength 465nm. A control algae sample (unsonicated) was sampled at 0, 5, 10, 20 and 30 minutes.

5.4.6 Sonication of algae (OD at 680 nm 0.2, 5L) using the Sonolator for 5 hours

5L standard suspension of *Microcystis aeruginosa* at an OD at 680 nm of 0.2 at 680 nm (6.00 $\times 10^6$ cells per mL) was sonicated for 5 hours using a pumping system with a cooling system to control the temperature below 25ºC. Samples were taken after 0, 1, 2, 3, 4 and 5 hours ultrasonic treatment. The exposure time in reactor was 1.09 minutes. Intensity was controlled to maximum by adjusting the pressure control before treatment. The manufacturer claimed at maximum pumping speed, the frequency generated was 30 kHz. For single pass test, 1 L standard suspensions of algae were circulated in the ultrasonic system for 5 minutes. Following each treatment time samples were removed and the cell number was calculated using a haemocytometer. The temperature was recorded and the chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at emission wavelength 465nm. A control algae sample (unsonicated) was sampled at 0, 1, 2, 3, 4 and 5 hours.

5.4.7 Sonication of algae (OD at 680 nm 0.2, 5L) using the 20 kHz ultrasonic probe (Southeast University, China) for 30 minutes

5L raw water (natural field samples) of *Microcystis aeruginosa* at an OD at 680 nm of 0.2 (6.00 $\times 10^6$ cells per mL) was placed in an 8L tank and sonicated for 30 minutes using a 20 kHz probe (intensity 0.0015 W/cm^3). The temperature was recorded and samples were taken at 0 (surface), 21.5 (middle) and 43cm (bottom) from surface following 0, 5, 10, 20 and 30 minutes ultrasonic treatment. Chlorophyll A concentration (cells counts) was measured using a spectrometer (optical density). A control algae sample (unsonicated) was sampled at 0, 5, 10, 20 and 30 minutes.

5.4.8 Sonication (circulating) of algae (OD at 680 nm 0.15, 3.5L) using 16 kHz and 20 kHz Dual frequency reactor (DFR) at 40% intensity for 60 minutes

3.5L standard suspension of *Microcystis aeruginosa* with an OD at 680 nm of 0.15 at 680 nm (4.5×10^6 cells per mL) was sonicated for 1 hour using 16 kHz and 20 kHz reactor with cooling. Samples were taken after 0, 1, 2, 5, 10, 15, 20, 30 and 60 minutes ultrasonic treatment. Following 60 minutes treatment, exposure time in the

reactor was 17 minutes temperature was controlled under 25 ºC. Two plates of DFR reactor worked at 16 kHz and 20 kHz. Following each treatment time samples were removed and the cell number was calculated using a haemocytometer. Chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at emission wavelength 465nm. Algal cell viability was analysed using a flow cytometer. Cell viability enumerates live or dead cells in a sample, which can be reported using cell number and staining to identify live and dead sub-populations (Maria, 2004). A control algae sample (unsonicated) of 3.5L was sampled at 0, 1, 2, 5, 10, 15, 20, 30 and 60 minutes.

5.4.9 Sonication (static) of algae (OD at 680 nm 0.25, 1L) using 16 kHz and 20 kHz Dual frequency reactor (DFR) at 40% intensity for 10 minutes

1L standard suspension of *Microcystis aeruginosa* at an OD at 680 nm of 0.25 at 680 nm (9.7 $\times 10^6$ cells per mL) was sonicated for 10 minutes using 16 kHz and 20 kHz reactor (no cooling). Samples were taken after 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 minutes ultrasonic treatment. The total exposure time in the reactor was 10 minutes and the temperature was controlled under 30ºC. Two plates of DFR reactor worked at 16 kHz and 20 kHz. Following each treatment time samples were removed and the cell number was calculated using a haemocytometer. Chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 – 800nm and fluorometer at emission wavelength 465nm. Algal cell viability was analysed using a flow cytometer. A control algae sample (unsonicated) was sampled at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 minutes.

5.4.10 Sonication (circulating) of algae (OD at 680 nm 0.15, 3.5 L) using 16 kHz and 20 kHz Dual frequency reactor (DFR) at 60% intensity for 20 minutes

3.5L standard suspension of *Microcystis aeruginosa* at an OD at 680 nm of 0.15 at 680 nm (5.30 $\times 10^6$ cells per mL) was sonicated for 20 minutes using 16 kHz and 20 kHz reactor with cooling. Samples were taken after 0, 1, 2, 5, 10, 15 and 20 minutes ultrasonic treatment. The total exposure time in the reactor was 7 minutes and the temperature was controlled under 30 ºC. Two plates of DFR reactor worked at 16

kHz and 20 kHz. Following each treatment time samples were removed and the cell number was calculated using a haemocytometer. Chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at emission wavelength 465nm. Algal cell viability was analysed using a flow cytometer. A control algae sample (unsonicated) was sampled at 0, 1, 2, 5, 10, 15 and 20 minutes.

5.4.11 Sonication (static) of algae (OD at 680 nm 0.25, 1L) using 16 kHz and 20 kHz Dual frequency reactor (DFR) reactor at 60% intensity for 10 minutes

1L standard suspension of *Microcystis aeruginosa* at an OD at 680 nm of 0.25 at 680 nm (5.30 $\times 10^6$ cells per mL) was sonicated for 10 minutes using 16 kHz and 20 kHz reactor. Samples were taken after 0, 1, 2, 3, 5 and 10 minutes ultrasonic treatment. The total exposure time in the reactor was 10 minutes and the temperature was controlled under 30ºC. Two plates of DFR reactor worked at 16 kHz and 20 kHz. Following each treatment time samples were removed and the cell number was calculated using a haemocytometer. Chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at emission wavelength 465nm. Algal cell viability was analysed using a flow cytometer. A control algae sample (unsonicated) was sampled at 0, 1, 2, 3, 5 and 10 minutes.

5.4.12 Sonication of algae (OD at 680 nm 0.18, 1.5L) using 20 kHz vibrating tray reactor for 5 minutes

1.5 L standard suspension of *Microcystis aeruginosa* at an OD at 680 nm of 0.18 at 680 nm (5.30 $\times 10^6$ cells per mL) was sonicated for 5 minutes using a 20 kHz tray. Samples were taken after 0, 0.5, 1, 2, 3 and 5 minutes ultrasonic treatment. Following each treatment time samples were removed and the cell number was calculated using a haemocytometer. Chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at emission wavelength 465nm. Algal cell viability was analysed using a flow cytometer. A control algae sample (unsonicated) was sampled at 0, 0.5, 1, 2, 3 and 5 minutes.

5.4.13 Cell integrity and viability test using the 20 kHz probe, 580 kHz and 1146 kHz high multi-frequency bath at 40% intensity and flow cytometer analysis

200 mL standard suspension of *Microcystis aeruginosa* with an OD at 680 nm of 0.2 at 680 nm (6.00×10^6 cells per mL) was sonicated for 30 minutes using 580 kHz high multi-frequency bath at 40% intensity. Samples were taken after 0, 5, 10, 20 and 30 minutes ultrasonic treatment. Following each treatment time samples were removed and cell number was calculated using a haemocytometer. Chlorophyll A concentration was measured using a spectrometer (optical density) and UV-Vis spectrometer at wavelength scale 360 − 800nm and fluorometer at emission wavelength 465nm. Algal cell viability was analysed using a flow cytometer. The operation process was described in section 5.3.5. Standard settings for flow cytometry analysis were: FSC = E00, SSC = 242, green fluorescence (FL1) = 550, orange fluorescence (FL2) = 550 and red fluorescence (FL3) = 610. All parameters were set on a logarithmic amplification by default four dot plots: FSC vs. Counts, FSC vs. SSC, FL1 vs. Counts and FL1−FL3. Further information on flow cytometry can be found in Section 6.4.1.

5.5 Algae toxin determination using HPLC

5.5.1 Calibration of MC−LR

MC−LR standard (LGC Standards, MCLR−A) were purchased from Sigma. The following MC−LR standards (0, 0.2, 0.4, 1 and 1.5µg/mL) were prepared to make a calibration curve. Samples were analyzed by HPLC (Shimadzu) with an Ultrasphere 5 nm ODS (Beckman 4.6 mm* 25 cm) column and chromatograms at UV 238 nm. The mobile phase was water: acetonitrile gradient ranging from 75:25 to 25:75 over 30 minutes. The flow rate was 1.5 mL/min and injection volume was 25 µL.

5.5.2 Determination of MC−LR concentration in *Microcystis aeruginosa* suspension

Microcystis aeruginosa suspension was filtered to determine the toxin concentration outside algae cells. *Microcystis aeruginosa* cells were then lysed using cell breaker

and filtered to determine the total toxin concentration of algae cells. *Microcystis aeruginosa* suspension was sonicated for 30 minutes and filtered to determine the toxin concentration of algae cells after ultrasonic treatment.

5.6 Lower limit tests of optical density

5.6.1 Optical density test of algae pellet and supernatant

50 mL standard suspensions of *Microcystis aeruginosa* with an OD at 680 nm of 2.0 at 680 nm (6.00×10^6 cells per mL) was placed in a 50 mL centrifuge tube and centrifuged at 5000 RPM for 5 minutes. Following centrifugation the algae pellet and supernatant were retained and both were analysed using a haemocytometer, optical density, UV-visible spectrometer (optical density) and fluorometer (optical density). Controls were run for all experiments.

5.6.2 Optical density baseline test

10 mL standard suspensions of *Microcystis aeruginosa* with an OD at 680 nm of 2.0 at 680 nm (6.00×10^6 cells per mL) was placed in a 10 mL cell breaker tube and inserted to the cell high speed mixing disrupter (Braun, Model no: 8531625) and centrifuged at the maximum setting for 10 minutes. Following 10 minutes of treatment the disrupted algal suspension was retained and analysed using a haemocytometer, optical density, UV-visible spectrometer (optical density) and fluorometer (optical density). Controls were run for all experiments.

5.7 Resistance test

100 mL standard suspension of *Microcystis aeruginosa* was inactivated/killed by boiling for 10 minutes and 100 mL *Microcystis aeruginosa* was sonicated using a 20 kHz probe (0.0179 Wcm^{-3}) for 30 minutes. 100 mL inactivated *Microcystis aeruginosa* suspension was added to 200 mL live algae suspension (LIVE+DEAD) and cultured under normal conditions. 100 mL sonicated *Microcystis aeruginosa* suspension was

added to 200 mL live algae suspension (LIVE+SONICATED) and cultured under normal conditions. Mixed samples were given nutrient media every two days and growth/condition of the culture was assessed using haemocytometer, optical density, UV-visible spectrometer (optical density) and fluorometer (optical density). Controls were run for all experiments.

6.0 Results and discussion

The following chapter is divided in three parts: determination of acoustic power for different types of ultrasonic equipment; sonication of algae; at a Lab-scale (200 and 400 mL), middle scale (1−5 litres) using commercial ultrasonic equipment and a mechanistic study of the ultrasonic processes on algal cells using flow cytometry.

6.1 Determination of the acoustic power of different ultrasonic equipment

Ultrasonic equipment converts electrical power to mechanical energy. This energy is transmitted to a medium via sound waves. Some energy loss is through attenuation and heating and the rest produces cavitation (Mason, 1999). Not all cavitational energy is involved in chemical or physical reactions as some energy is consumed in sound re-emission (harmonics and sub−harmonics). The actual energy input of ultrasonic equipment into the reaction is vital for the study of sonochemical reactions. Recording energy input ensures that results from any subsequent work can be directly compared (Suslick, 1994).

There are two main methods to measure the amount of ultrasonic power entering a reaction. The most common measurement is calorimetry which depends on temperature increases caused by cavitation during sonication. The other method is chemical dosimetry, which employs sonochemical generations of chemical species which indicates the acoustic power in a reaction; Iodine dosimetry is the most conventional method (Mason and Peters, 2002).

In this work, the acoustic power entering each ultrasonic system was determined by calorimetry. The temperatures of different volumes of water (H_2O) (200 and 400 mL) was recorded every ten seconds over a set period of time (180 seconds) starting at ambient and using continuous sonication at frequencies of 20, 40, 580, 864 and 1146 kHz. This was carried out in triplicate, with the average recorded and used to calculate ultrasonic power. Power is calculated using the equations below. T

indicates temperature and t is time (seconds). Cp relates to the heat capacity of water at 25 ºC (J Kg^{-1} K^{-1}) and M is mass of H_2O (kg).

Power = (dT/dt) Cp (H_2O) M

Intensity = Power/ Area

Energy Density = Power/ Volume

Dosage = power × ultrasonic time

Table 6.1 and 6.2 illustrate calorimetry calculations at 200 and 400 mL respectively. Table 6.3 illustrates calorimetry results for the 20 kHz probe, 40 kHz bath and multi-frequency bath (580, 864 and 1146 kHz) using two different volumes (200 and 400 mL). Three different power settings were employed with the multi-frequency bath: 40%, 80% and max power setting. dT/dt value relates to the change in temperature from time zero. Power in watt (W) and power density (W mL^{-1} and Wcm^{-3}) are calculated using the equations above. W mL^{-1} describes power density in density in millilters and Wcm^{-3} indicates cubic centimetre power. A visual comparison of ultrasonic power is presented in Figure 6.1. All calorimetry calculations are presented in detail in Appendix 1.

Table 6.1 Calculation of ultrasound power using different ultrasonic equipment and 200 mL water

Frequency (kHz)	Polynomial equation	Power = $dT/dt \times C_p \times m$ (Watt)
20	$y = -1E-08x^3 - 4E-06x^2 + 0.0213x + 22.412$ $R^2 = 0.9996$	0.0213 ˚C/Sec x 4.19 J /(g x ˚C) x 200g = **17.85W**
40	$y = -6E-08x^3 + 3E-05x^2 + 0.0255x + 23.353$ $R^2 = 0.9998$	0.0255˚C/Sec x 4.19 J / (g x ˚C) x 200g = **21.369W**
580 (40%)	$y = -3E-08x^3 + 8E-06x^2 + 0.0021x + 21.512$ $R^2 = 0.9883$	0.0021 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **1.7598W**
580 (80%)	$y = 2E-09x^3 - 2E-05x^2 + 0.0256x + 23.171$ $R^2 = 0.9992$	0.0256 ˚C /Sec x 4.19 J/(g x ˚C) x 200g = **21.4528W**
580 (max)	$y = -1E-07x^3 - 1E-05x^2 + 0.0588x + 29.939$ $R^2 = 0.9993$	0.0588 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **49.2744W**
864 (40%)	$y = 1E-08x^3 - 3E-06x^2 + 0.005x + 23.848$ $R^2 = 0.995$	0.005 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **4.19W**
864 (80%)	$y = -2E-07x^3 + 6E-05x^2 + 0.0198x + 21.856$ $R^2 = 0.9999$	0.0198 ˚C /Sec x 4.19 J/(g x ˚C) x 200g = **16.5924W**
864 (max)	$y = 2E-07x^3 - 1E-04x^2 + 0.0687x + 22.054$ $R^2 = 0.9982$	0.0687 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **57.5706W**
1146 (40%)	$y = -4E-08x^3 + 6E-06x^2 + 0.0031x + 24.549$ $R^2 = 0.9914$	0.0031 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **2.5978W**
1146 (80%)	$y = -2E-07x^3 + 8E-05x^2 + 0.0148x + 21.346$ $R^2 = 0.9997$	0.0148 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **12.4024W**
1146 (max)	$y = -7E-07x3 + 0.0002x2 + 0.0296x + 20.18$ $R^2 = 0.9996$	0.0296 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **24.8048W**

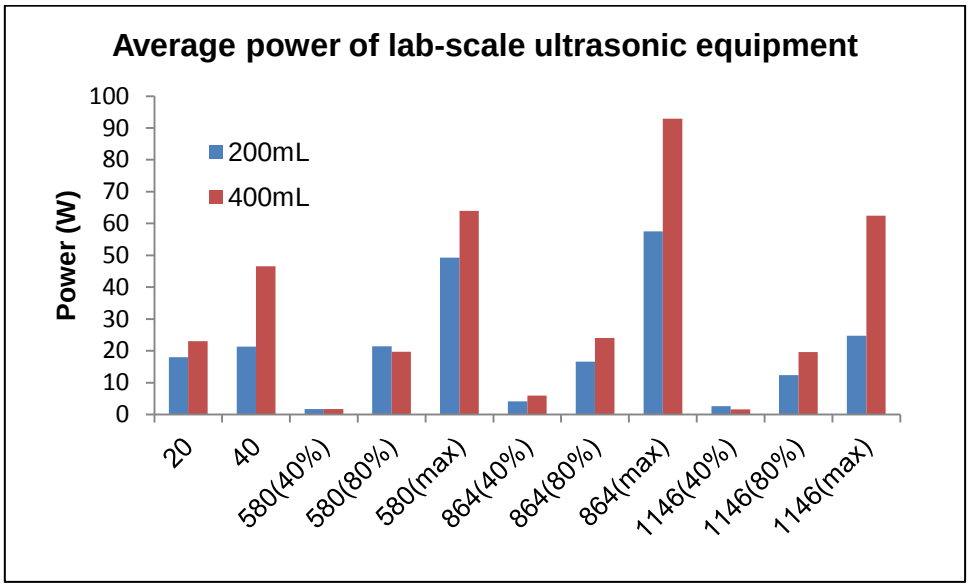
Table 6.2 Calculation of ultrasound power using different ultrasonic equipment and 400 mL water

Frequency (kHz)	Polynomial equation	Power = $dT/dt \times C_p \times m$ (Watt)
20	$y = 2E{-}07x^3 - 8E{-}05x^2 + 0.0135x + 21.335$ $R^2 = 0.9806$	0.0135 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **22.6260W**
40	$y = 3E{-}07x^3 - 9E{-}05x^2 + 0.0278x + 22.482$ $R^2 = 0.9982$	0.0278 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **46.5928W**
580 (40%)	$y = -8E{-}08x^3 + 2E{-}05x^2 + 0.001x + 21.989$ $R^2 = 0.9811$	0.001 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **1.6760 W**
580 (80%)	$y = -5E{-}09x^3 + 2E{-}05x^2 + 0.0118x + 22.223$ $R^2 = 0.9995$	0.0118 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **19.7768W**
580 (max)	$y = 7E{-}08x^3 - 4E{-}05x^2 + 0.0382x + 22.321$ $R^2 = 0.9997$	0.0382 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **64.0232W**
864 (40%)	$y = -2E{-}08x^3 + 2E{-}06x^2 + 0.0036x + 21.619$ $R^2 = 0.9859$	0.0036 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **6.0336W**
864 (80%)	$y = 2E{-}07x^3 - 5E{-}05x^2 + 0.0145x + 21.464$ $R^2 = 0.9988$	0.0145 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **24.3020W**
864 (max)	$y = -7E{-}08x^3 - 1E{-}05x^2 + 0.0554x + 21.368$ $R^2 = 0.9991$	0.0554 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **92.8504W**
1146 (40%)	$y = -6E{-}08x^3 + 2E{-}05x^2 - 0.001x + 22.394$ $R^2 = 0.9677$	0.001 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **1.6400W**
1146 (80%)	$y = -1E{-}07x^3 + 2E{-}05x^2 + 0.0117x + 23.518$ $R^2 = 0.9964$	0.0117 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **19.6092W**
1146 (max)	$y = -8E{-}08x^3 + 9E{-}06x^2 + 0.0373x + 22.605$ $R^2 = 0.9998$	0.0373˚C/Sec x 4.19 J /(g x ˚C) x 400g = **62.5148W**

Table 6.3 Calorimetry of ultrasonic equipment

Equipment	Frequency (kHz)	Power	Volume (mL)	dT/dt	Power (W)	Power density (WmL^{-1})	Power density (Wcm^{-3})
20 kHz probe	20		200	0.0213	17.85	0.0892	0.0179
			400	0.0135	22.63	0.0566	0.0226
40 kHz bath	40		200	0.0255	21.37	0.1059	0.0214
			400	0.0278	46.59	0.1165	0.0466
Multi-frequency bath	580	40%	200	0.0021	1.76	0.0088	0.0018
			400	0.0010	1.68	0.0042	0.0017
		80%	200	0.0256	21.45	0.1073	0.0216
			400	0.0118	19.78	0.0495	0.0198
		Max	200	0.0588	49.27	0.2456	0.0493
			400	0.0382	64.02	0.1600	0.0640
	864	40%	200	0.0050	4.19	0.0210	0.0042
			400	0.0036	6.03	0.0151	0.0060
		80%	200	0.0198	16.59	0.0830	0.0166
			400	0.0145	24.30	0.0608	0.0243
		Max	200	0.0687	57.57	0.2879	0.0576
			400	0.0554	92.85	0.2321	0.0929
	1146	40%	200	0.0031	2.60	0.0130	0.0026
			400	0.0010	1.64	0.0041	0.0016
		80%	200	0.0148	12.40	0.0620	0.0124
			400	0.0117	19.61	0.0490	0.0196
		Max	200	0.0296	24.80	0.1240	0.0248
			400	0.0373	62.51	0.1563	0.0625

Figure 6.1 Power of lab-scale ultrasonic equipment



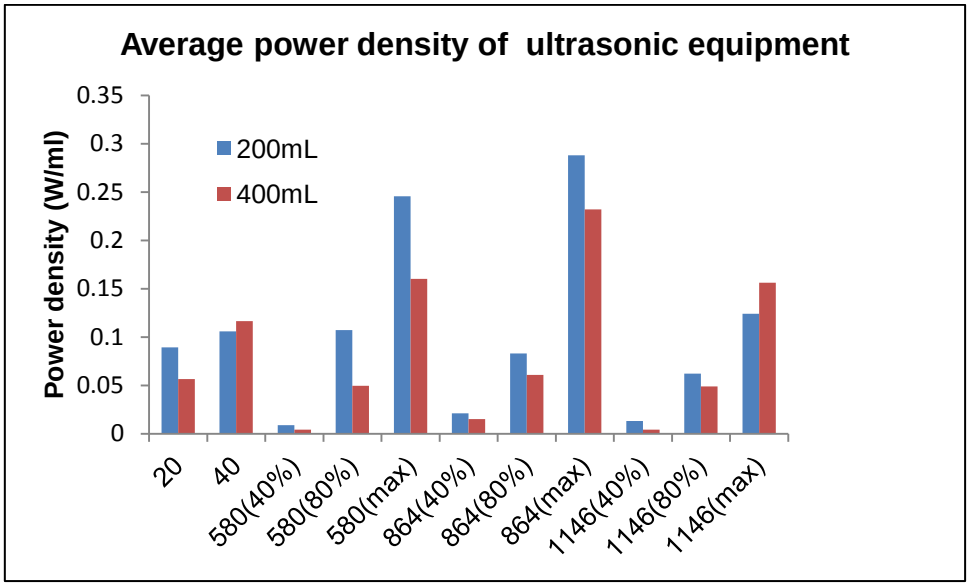
Using 200 mL volume of water, the highest input power was obtained using 864 kHz (max power setting) and the lowest input power was obtained at 580 kHz (40% intensity). At low frequencies, the power of a 40 kHz bath is higher than that of a 20 kHz probe. At high frequencies, the power increases in the expected order:

40% < 80% < max power setting

Using 400 mL volume, the highest power is using 864 kHz (max power setting) and the lowest power is obtained at 580 kHz (40% max power setting). Under the conditions used the power of a 40 kHz bath was higher than a 20 kHz probe.

A comparison of power density for ultrasonic equipment is presented in Figure 6.2.

Figure 6.2 Power density of lab-scale ultrasonic equipment



Power density is calculated as the power per unit volume, indicating the actual power input per unit volume. Using 200 mL volume, the highest power density is using 864 kHz (max power setting) and the lowest is obtained at 1146 kHz (40% power setting). At low frequencies, the power density of a 40 kHz bath is higher than that of 20 kHz probe. At high frequencies, the power density increases in the expected order:

40% < 80% < max power setting

Using 400 mL volume, the highest power density is using 864 kHz (max power setting) and the lowest power density is obtained at 1146 kHz (40% power setting). The power density of a 40 kHz bath is higher than that of a 20 kHz probe. Power density increases with increasing intensities. Generally, power densities of 400 mL are lower than 200 mL at each frequency.

Comparison of Figure 6.1 and Figure 6.2 indicates that low ultrasonic power may not result in lowest power densities. For example, at 400 mL the lowest power is obtained with 580 kHz (40% power setting) but the lowest power density was

calculated to be 1146 kHz (40% power setting). The explanation for this is that the power density is dependant on both power input and volume.

In this thesis, calorimetry was used as a measure upon which to base ultrasonic effects in a liquid using different ultrasonic equipment and power settings. A summary of the relationships between ultrasonic powers (W) and power densities (Wcm^{-3}) is:

- For low frequencies (bath and probe), power density of a 20 kHz probe is lower than a 40 kHz bath at both 200 and 400 mL
- For high frequencies; increasing power settings increased the power densities
- The order of power densities for the systems studied is:
- High frequencies at low power (40%) < low frequency < high frequencies at high power (80% and max power setting)

6.2 Sonication of Microcystis aeruginosa at small lab-scale

The effects of ultrasound on cyanobacteria were monitored at a range of different frequencies, intensities and volumes (200 and 400 mL). The frequencies employed were 20 (one intensity), 40 (one intensity), 580 (40%, 80%, and maximum intensity), 864 (40%, 80%, and maximum intensity) and 1146 kHz (40%, 80%, and maximum intensity) over 30 minute treatment.

6.2.1 Sonication of algae (optical density 0.2 at 680 nm, 200 and 400 mL) using the 20 kHz ultrasonic probe (Vibra-cell, Sonics & Materials)

Figure 6.3 Inactivation of 200 mL *Microcystis aeruginosa* using the 20 kHz probe (haemocytometer and spectrophotometer)

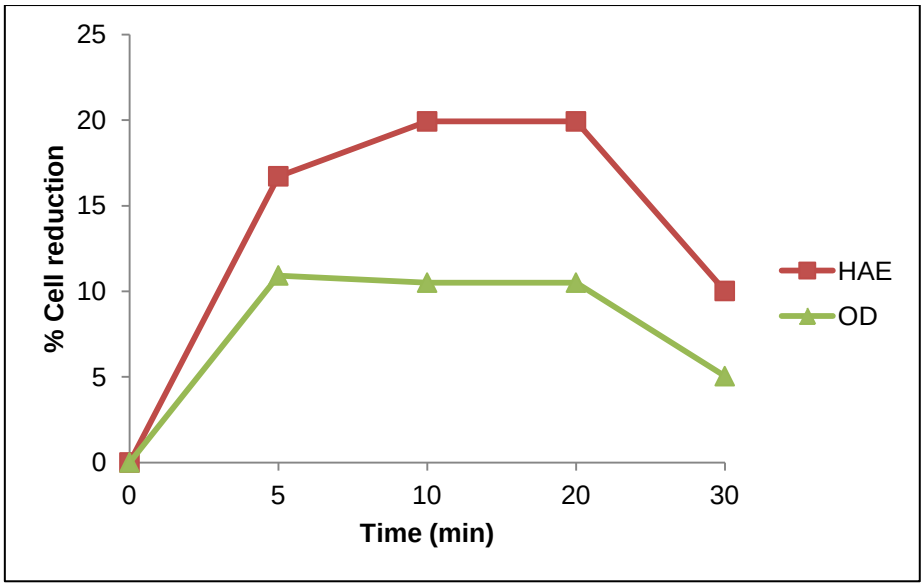
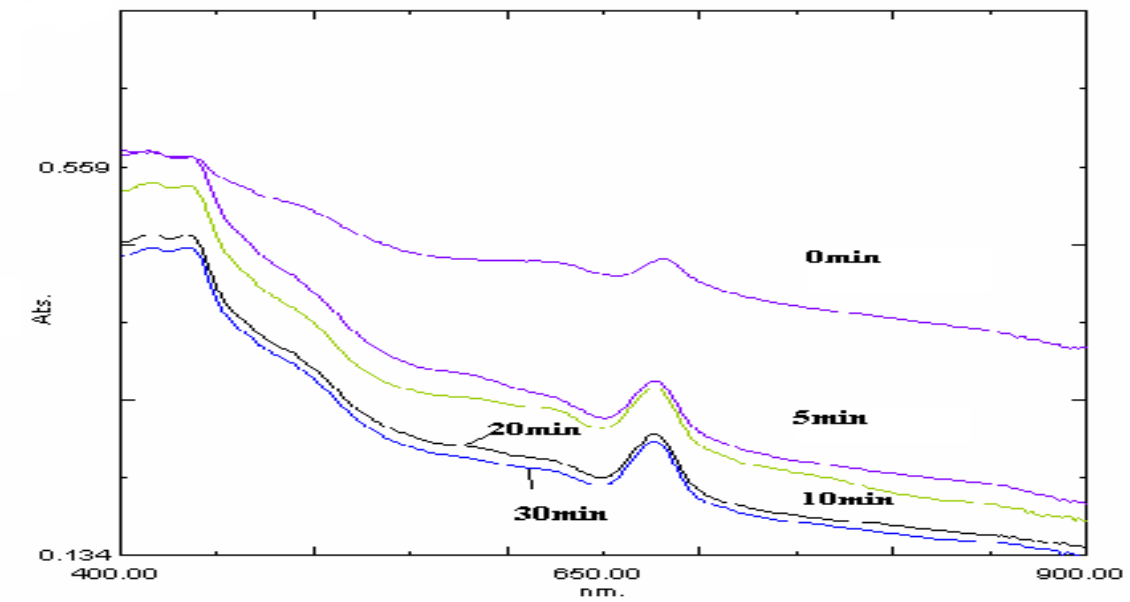


Figure 6.4 Inactivation of 200 mL *Microcystis aeruginosa* using the 20 kHz probe (UV–visible spectrophotometer)



The sonication of 200 mL algae suspension with 20 kHz probe (0.0179 Wcm^{-3}) resulted in a fluctuation of algal cell numbers (Figure 6.3). The algae concentration decreased during the first 10 minutes with a reduction of 19.92% (HAE) and 10.50% (OD at 680nm). However, after 20 minutes treatment the algae concentrations began to increase, probably due to the declumping effect of sonication on algal cells and resulted in low removal rates following 30 minutes treatment. The absorption spectrum of intact algal cells was measured from 400 - 800 nm using a UV-Vis spectrophotometer to observe the ultrasonic effects on photosynthetic systems. The algal absorption spectrum at 0 minutes (live) in Figure 6.4 indicates untreated intact *Microcystis aeruginosa* which consists of three groups of photosynthetic pigments: chlorophyll (420 and 680nm), carotenoid (430nm) and phycobiliprotein (620nm) (Tang, 2003 and Fay, 1983). Complete inactivation following boiling for 30 minutes was demonstrated by UV-Vis spectrophotometer analysis in Appendix 3, Figure 35, (Day 1) with the disappearance of the phycobiliproteins (620nm) peak. Following sonication a reduction in phycobiliproteins (620nm) was observed, indicating ultrasound damaged photosynthetic pigments, which may inhibit photosynthesis thus reducing algal growth. The results in Figure 6.4 suggest sonication with a 20 kHz probe (0.0179 Wcm^{-3}) has a small inactivation effect on the algae although some declumping was indicated in Figure 6.3.

Figure 6.5 Inactivation of 400 mL *Microcystis aeruginosa* using the 20 kHz probe (haemocytometer and spectrophotometer)

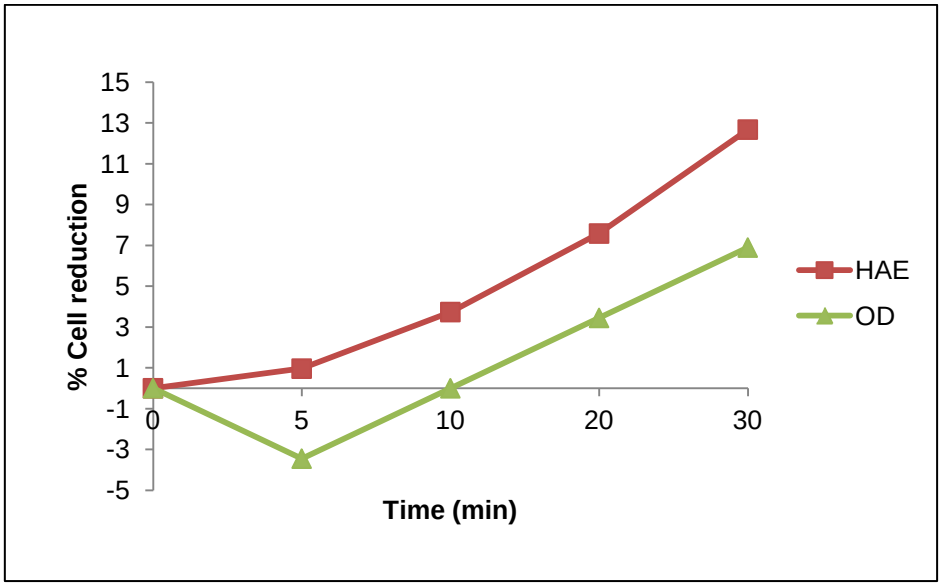
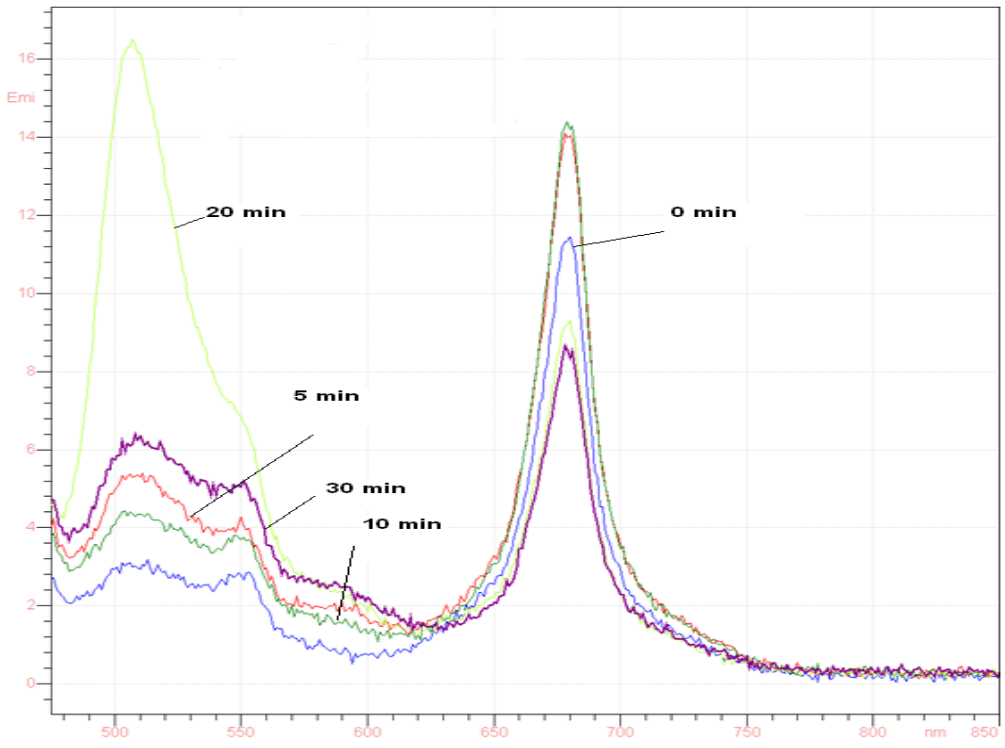


Figure 6.6 Inactivation of 400 mL *Microcystis aeruginosa* using the 20 kHz probe (Fluorometer)

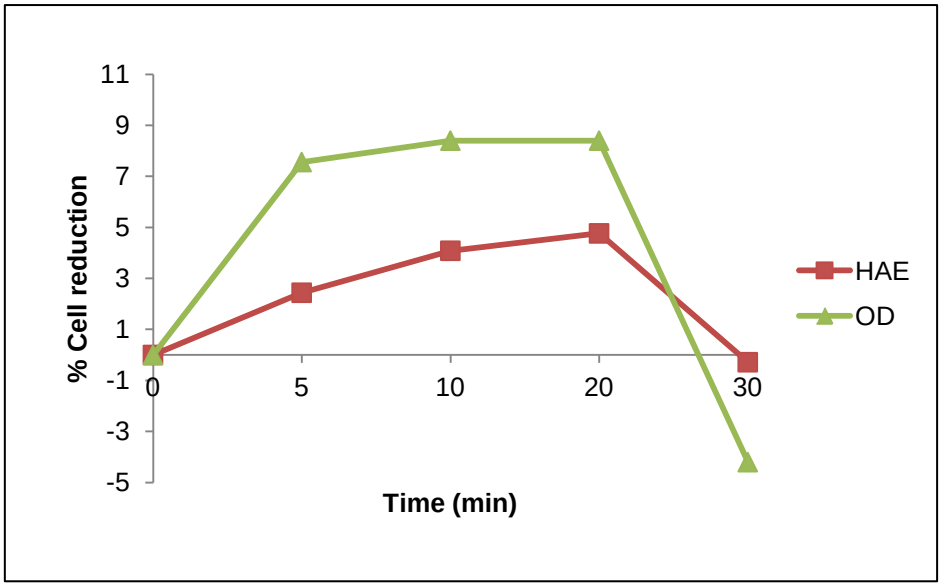


The sonication of 400 mL algae suspension with a 20 kHz probe (0.0226 Wcm^{-3}) resulted in a reduction of algae concentration over 20 minutes treatment time. Sonication demonstrated a small, but continuous decease in cell numbers by haemocytometer over 30 minutes treatment although there was a small increase of optical density at 680 nm, indicating an increase in chlorophyll A concentration. UV-Vis spectrophotometer results (Appendix 3, Figure 2) showed peaks around 620 nm decreased slightly during treatment. The fluorescence spectrum of intact algal cells without sonication is indicated in Figure 6.6 (0 minute). Completed inactivation following boiling for 30 minutes was observed by fluorometry in Appendix 3, Figure 35 (Day 1), with the disappearance of phycobiliproteins (620nm) peak. Phycobiliproteins (665nm) are sensitive to sonication indicated by decreases during ultrasonic treatment; hence photosynthetic pigments were damaged by ultrasound (Hao, 2004).

From the above, when 200 and 400 mL of *Microcystis aeruginosa* suspensions were exposed to a 20 kHz probe the inactivation was not significant. For the 20 kHz using 200 mL (0.0178 Wcm^{-3}), a small decrease in the number of algal cells was observed using both haemocytometer and optical density at 680 nm, indicating inactivation. However, the removal rate was lower than 15% and there were fluctuations in haemocytometer and optical density at 680 nm results during the treatment. For the 20 kHz probe using 400 mL (0.0226 Wcm^{-3}), there was also a small decrease in cell numbers indicated using haemocytometry. Although the removal rate of algal cells in volume of 400 mL is low, it continued rather than fluctuated as demonstrated with the with 200 mL volume. This may be due to a higher intensity of sonication in a 400 mL volume since higher intensity may lead to higher reduction of cell numbers (Joyce, 2003).

6.2.2 Sonication of algae (optical density 0.2 at 680 nm, 200 and 400 mL) using a 40 kHz ultrasonic bath (Langford Sonomatic)

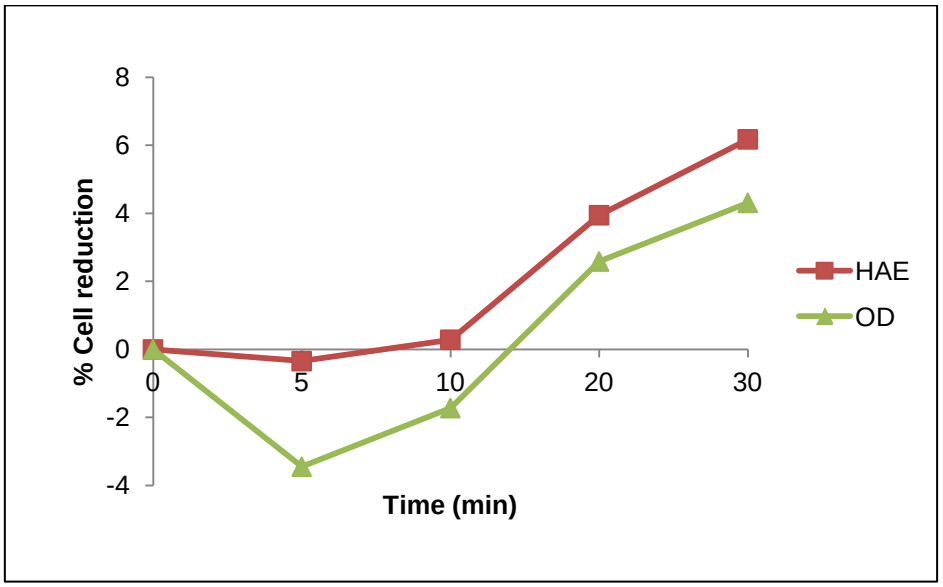
Figure 6.7 Inactivation of 200 mL Microcystis aeruginosa using a 40 kHz bath (haemocytometer and spectrophotometer)



The sonication of 200 mL algae suspension with 40 kHz bath (0.0214 Wcm^{-3}) resulted in an apparent fluctuation of algae concentration. Algae concentrations estimated by both haemocytometer and optical density at 680 nm decreased during the first 20 minutes, but after this time detected cell numbers increased. Algal cells appeared very small but still green when viewed under a microscope following 30 minutes treatment, which may be due to changes in the algae cell membrane induced by ultrasonic oxidation. After 30 minutes treatment, the algae concentration was higher than the initial concentration by haemocytometer and the optical density at 680 nm was equal to the initial value. A possible explanation is two effects of ultrasound were present during sonication (a) inactivation of algae cells that leads to a loss in concentration and (b) breaking apart clumps of algae and thus producing more individual cells. Sonication with low frequency ultrasound (20 and 40 kHz) show little effect on algae removal after 30 minutes. For the UV–visible spectrophotometer results (Appendix 3, Figure 3), the peak before treatment was smaller than peaks following sonication at 680nm. From these results we can conclude that 40 kHz

ultrasound affects the algal cells and provided a declumping effect, which increased apparent cell numbers following treatment.

Figure 6.8 Inactivation of 400 mL *Microcystis aeruginosa* using the 40 kHz bath (haemocytometer, spectrophotometer)



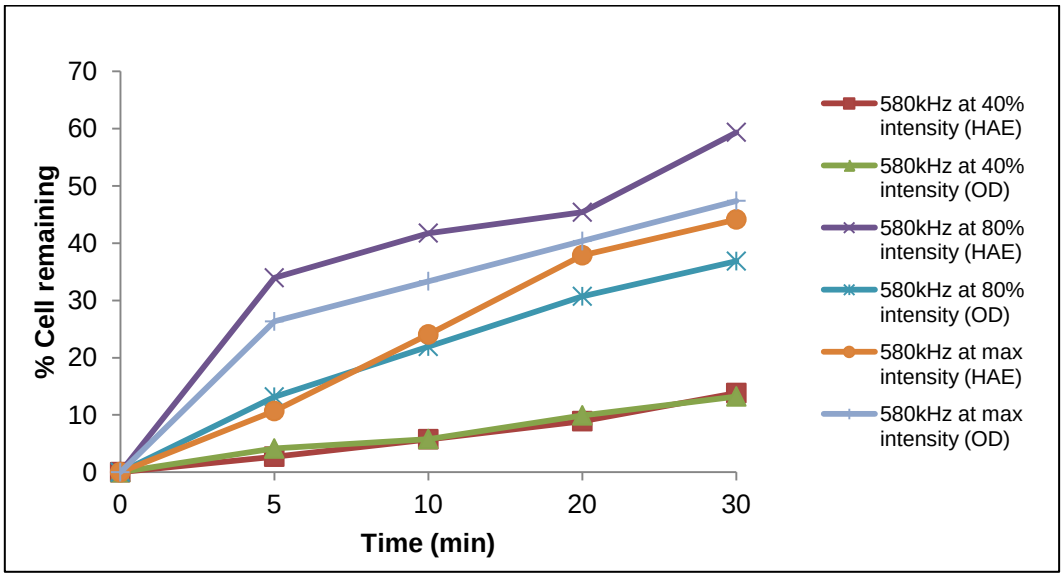
The sonication of 400 mL algae suspension with 40 kHz bath (0.0466 Wcm^{-3}) resulted in a reduction of algae concentration. Optical density at 680 nm results illustrated a increase in algal cells following 10 minutes treatment of -1.72%. Following 30 minutes treatment, small reductions by both haemocytometer and optical density at 680 nm were obtained. UV-Vis spectrophotometer results showed that the peaks around 600 nm decreased slightly during the treatment. Fluorometer results indicated peaks around 665 nm decreased, but peaks around 500 nm increased. The increasing peaks around 500 nm may be the result of damage to the algal photosynthetic pigments as it corresponds to the fluorometric wavelength used to observe dead algal cells (Lee, 2000). Both UV-Vis spectrophotometer and fluorometer results (Appendix 3, Figure 4) showed ultrasonic damage on algal photosynthetic pigments reducing growth.

The results demonstrated that at 40 kHz using 200 mL (0.0214 Wcm^{-3}), an increase in algal cell numbers was recorded using both haemocytometer and optical density at 680 nm, suggesting declumping of algae into individual cells. This may be a result of

low cavitation effect under these conditions (Joyce, 2003). For the 40 kHz bath using 400 mL (0.0466 Wcm^{-3}) a slight decrease in algae cell numbers was observed indicating a small inactivation effect. This differs from the 200 mL results and this may be due to slight increases in ultrasonic intensity entering different volumes of suspension contained in the same vessel.

6.2.3 Sonication of algae (optical density 0.2 at 680 nm, 200 and 400 mL) using the Meinhart multi-frequency generator (580, 864 and 1146 kHz)

Figure 6.9 Inactivation of 200 mL *Microcystis aeruginosa* using the 580 kHz bath at 40%, 80% and maximum power setting (haemocytometer and spectrophotometer)



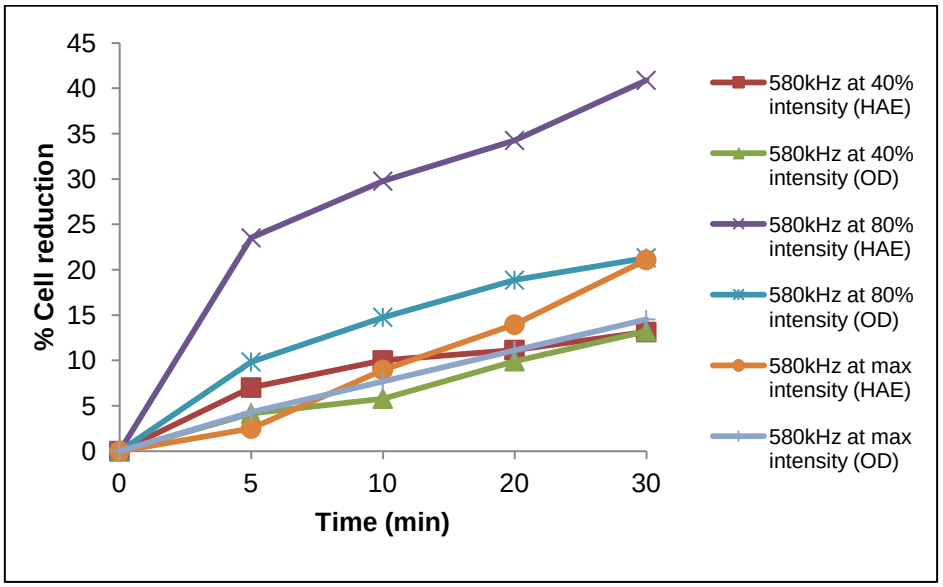
Sonication of 200 mL algae suspension with a 580 kHz bath (40% power setting, 0.0018 Wcm^{-3}) resulted in a reduction of algae concentration. There is a clear correlation between results obtained by haemocytometer and optical density at 680 nm. UV-Vis spectrophotometer results (Appendix 3, Figure 5) showed phycobiliproteins peaks around 620 nm decreased during treatment, indicating damage to the algal photosynthetic system.

The sonication of 200 mL algae suspension with a 580 kHz bath (80% power setting, 0.0216 Wcm^{-3}) resulted in an inactivation of algal cells. The algal concentration indicated by both haemocytometer and optical density at 680 nm measurements reduced over 30 minutes treatment. The percentage algal cells reduction by optical density at 680 nm was slightly lower than that obtained using haemocytometry. With increasing intensity the percentage reduction was higher than 580 kHz (40% power setting). Following treatment the UV-Vis spectrophotometer results (Appendix 3,

Figure 7) demonstrate 580 kHz (80% power setting) can effectively control algae as the peaks around 620 nm decreased.

The sonication of 200 mL algae suspension with 580 kHz bath (at the maximum power setting, 0.0493 Wcm^{-3}) resulted in a reduction of algae cell numbers. Haemocytometer and optical density at 680 nm results corresponded well, illustrating a reduction in algal cells following treatment. However, with highest power setting at 580 kHz, the removal rate as shown by haemocytometer was lower than 580 kHz (80%), which may indicate that the intensity was beyond the optimum intensity for this frequency. UV-Vis spectrophotometer results (Appendix 3, Figure 9) showed 580 kHz (maximum power setting) can effectively control algae, as phycobiliproteins peaks around 620 nm decreased during treatment, which may resulted from reduction of algal growth.

Figure 6.10 Inactivation of 400 mL *Microcystis aeruginosa* using the 580 kHz bath at 40%, 80% and maximum power setting (haemocytometer and spectrophotometer)

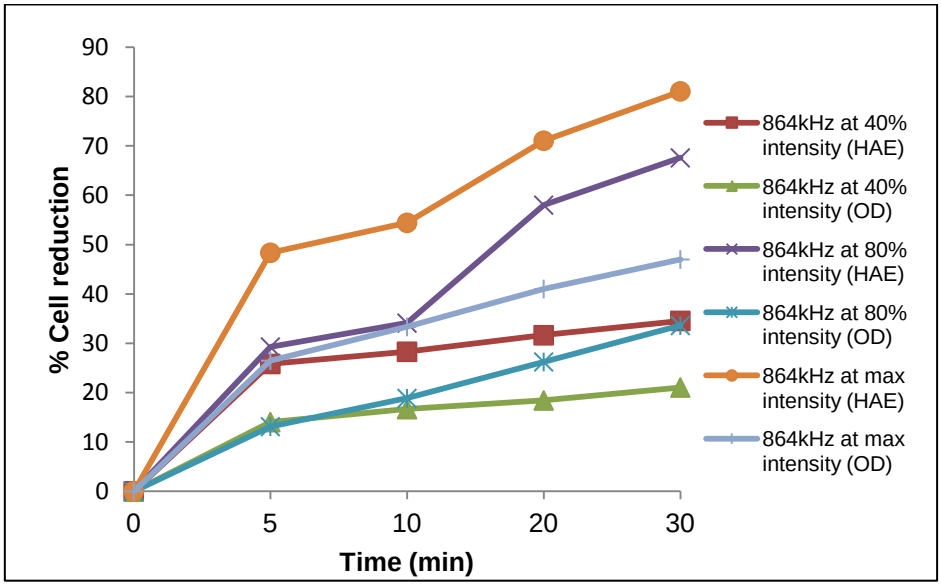


The sonication of 400 mL algae suspension with 580 kHz bath (40% power setting, 0.0017 Wcm^{-3}) resulted in a reduction of algae cell numbers (Figure 6.10). Over 30 minutes treatment a small but detectable reduction was obtained both by haemocytometer and optical density at 680 nm. UV-Vis spectrophotometer results (Appendix 3, Figure 6) showed that absorbance peaks around 600 nm decreased slightly during treatment, while fluorometer results (Appendix 3, Figure 6) for photosynthetic pigments peaks around 500 (chlorophyll A) and 665 nm (phycobiliproteins) also decreased.

The sonication of 400 mL algae suspension with 580 kHz bath (80% power setting, 0.0198 Wcm^{-3}) resulted in an inactivation. Over 30 minutes ultrasonic treatment, the algae cell concentrations decreased over time. A rapid decrease in algae cell numbers was observed during the first 10 minutes treatment. Haemocytometer and optical density at 680 nm results correlated well, illustrating an inactivation effect. UV-Vis spectrophotometer results (Appendix 3, Figure 8) showed phycobiliproteins peaks around 620 nm hardly decreased. However fluorometry results (Appendix 3, Figure 8) showed phycobiliproteins absorbance peaks round 665 nm decreased whilst those around 500 nm increased during treatment, which may indicate an increase in damaged cells.

The sonication of 400 mL algae suspension using a 580 kHz bath (maximum power setting, 0.0640 Wcm^{-3}) resulted in inactivation. Haemocytometer and optical density at 680 nm results correspond well, illustrating a reduction in algal cells following treatment with ultrasound. However at the highest power setting for 400 mL at this frequency, the removal rate by haemocytometer is lower than that at 80% power for 200 mL volume. This observation may due to the ultrasonic system going beyond the upper limitation of intensity for this frequency. UV-Vis spectrophotometer results (Appendix 3, Figure 10) showed that peaks around 620 nm and fluorometry emission peaks (Appendix 3, Figure 10) around 665 nm decreased during treatment which may indicate damage on algal photosynthetic system reducing algal growth.

Figure 6.11 Inactivation of 200 mL *Microcystis aeruginosa* using the 864 kHz bath at 40%, 80% and maximum power setting (haemocytometer and spectrophotometer)



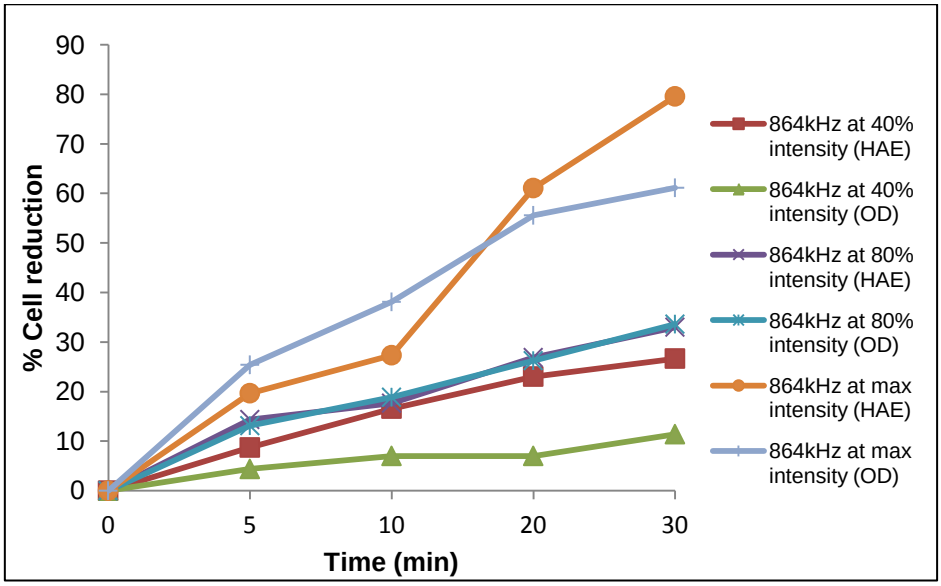
The sonication of 200 mL algae suspension with the 864 kHz bath (40% power setting, 0.0042 Wcm^{-3}) resulted in a reduction of algal cell numbers. A rapid decrease in algae cell numbers was observed during the first 5 minutes treatment. Over 30 minutes treatment, the reduction indicated by haemocytometer and optical density at 680 nm readings was slow but continuous. Haemocytometer and optical density at 680 nm results correspond well, illustrating a reduction in algal cells following treatment with ultrasound. UV-Vis spectrophotometer results (Appendix 3, Figure 11) showed peaks around 620 nm decreased during treatment. Fluorometer results (Appendix 3, Figure 11) indicated that peaks around 665 nm decreased during treatment which may indicate damage on algal photosynthetic system reducing algal growth.

The sonication of 200 mL algae suspension with an 864 kHz bath (80% power setting, 0.0166 Wcm^{-3}) resulted in a significant inactivation of 67.60% by haemocytometer and 33.61% by optical density at 680 nm. 30 minute ultrasonic treatment led to a reduction of algal concentration by haemocytometer and optical density at 680 nm and the reduction rate was higher than at the 40% power setting

because of the higher intensity. UV-Vis spectrophotometer peaks (Appendix 3, Figure 13) around 620 nm decreased with time and fluorometry results (Appendix 3, Figure 13) indicated that peaks around 665 nm decreased during treatment which may indicate damage on algal photosynthetic system reducing algal growth.

Sonication of 200 mL algae suspension with an 864 kHz bath (maximum power setting, 0.0576 Wcm^{-3}) resulted in a reduction of algae cell numbers. Results using a haemocytometer and spectrophotometer showed a reduction in algal cells following treatment with ultrasound. 864 kHz bath (maximum power setting, 0.0576 Wcm^{-3}) achieved the highest removal rate by haemocytometer in the 864 kHz frequency range. UV-Vis spectrophotometer results (Appendix 3, Figure 15) showed that 864 kHz sonication at the maximum power setting can inactivate algal cells effectively. UV-Vis spectrophotometer peaks around 620 nm decreased during treatment and fluorometry results (Appendix 3, Figure 15) indicated that phycobiliproteins peaks around 665 nm decreased during treatment which may indicate damage on algal photosynthetic system.

Figure 6.12 Inactivation of 400 mL *Microcystis aeruginosa* using the 864 kHz bath at 40%, 80% and maximum power setting (haemocytometer and spectrophotometer)

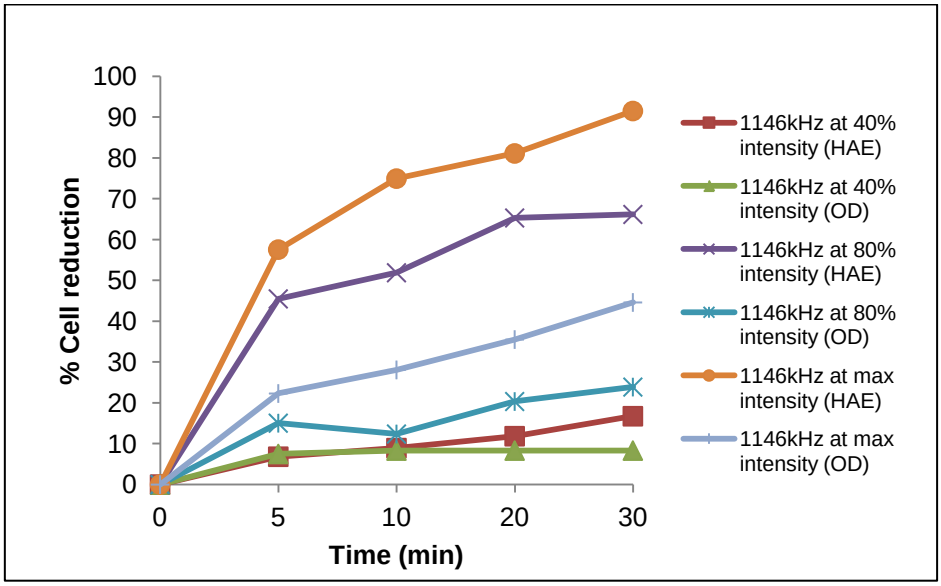


The sonication of 400 mL algae suspension with 864 kHz bath (40% power setting, 0.0060 Wcm^{-3}) resulted in a reduction of algal cell numbers. The linear relationship of haemocytometer and optical density at 680 nm results showed a good degree of agreement of the trend, illustrating a reduction in algal cells. UV-Vis spectrophotometer results (Appendix 3, Figure 12) showed that peaks around 620 nm decreased over 30 minutes treatment. Fluorometer results (Appendix 3, Figure 12) phycobiliproteins peaks around 665 nm decreased during treatment which may indicate damage on algal photosynthetic system.

The sonication of 400 mL algae suspension with 864 kHz bath (80% power setting, 0.0243 Wcm^{-3}) resulted in an inactivation. Haemocytometer and optical density at 680 nm results correlated well, illustrating a reduction in algal cells following treatment with ultrasound. UV-Vis spectrophotometer results (Appendix 3, Figure 14) showed peaks around 620 nm decreased during treatment. Fluorometer results (Appendix 3, Figure 14) indicated peaks at 665 nm decreased.

The sonication of 400 mL algae suspension with 864 kHz bath (maximum power setting, 0.0929 Wcm^{-3}) resulted in a decrease of algal cell numbers. Following 30 minutes treatment, the concentration of algae decreased rapidly. 864 kHz bath (maximum power setting, 0.0929 Wcm^{-3}) achieved the highest removal rate by haemocytometer and optical density at 680 nm in the 864 kHz frequency range at 400 mL volume. UV-Vis spectrophotometer results (Appendix 3, Figure 16) showed peaks around 620 nm decreased significantly during treatment. Fluorometry results (Appendix 3, Figure 16) indicated peaks at 680 nm decreased over time.

Figure 6.13 Inactivation of 200 mL *Microcystis aeruginosa* using the 1146 kHz bath at 40%, 80% and maximum power setting (haemocytometer and spectrophotometer)

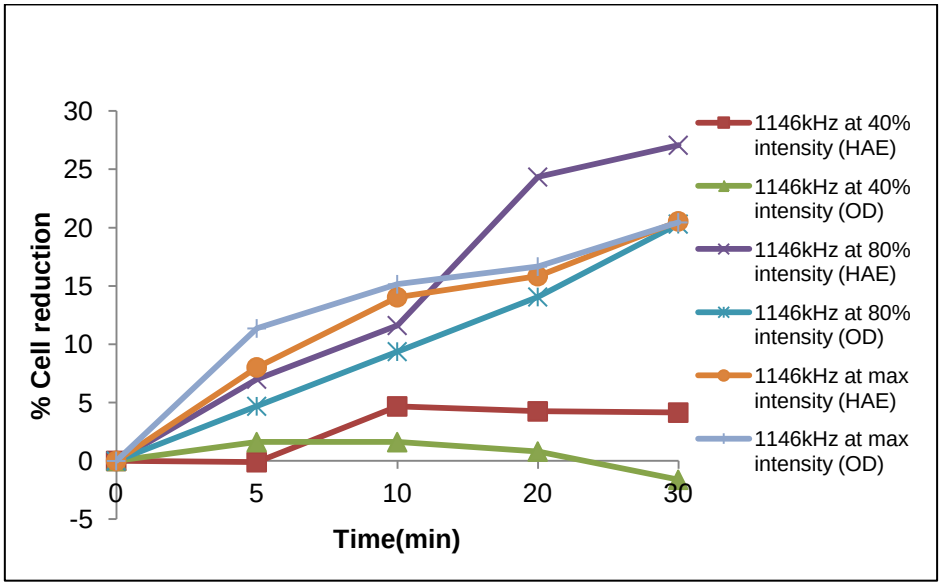


The sonication of 200 mL algae suspension using an 1146 kHz bath (40% power setting, 0.0026 Wcm^{-3}) resulted in a reduction of algae cell numbers. The reduction by both haemocytometer and optical density at 680 nm is continuous but low. No significant reduction at 620nm was observed by UV-Vis spectrophotometry (Appendix 3, Figure 17). Fluorometer results (Appendix 3, Figure 17) showed that peaks around 665 nm decreased during treatment.

The sonication of 200 mL algae suspension with 1146 kHz bath (80% power setting, 0.0124 Wcm^{-3}) resulted in an inactivation. The concentration of the algae decreased over 30 minutes treatment. A rapid decrease in algae cell numbers was observed during the first 5 minutes. A fluctuation in optical density at 680 nm was observed between 5 and 20 minutes treatment and this differed from the haemocytometer results. UV-Vis spectrophotometer results (Appendix 3, Figure 19) showed 1146 kHz (80% power setting) is suitable for algae control, as peaks around 620 nm decreased during treatment. Fluorometry results (Appendix 3, Figure 19) indicated peaks at 680 decreased over time.

The sonication of 200 mL algae suspension with 1146 kHz bath (maximum power setting, 0.0248 Wcm^{-3}) resulted in a significant inactivation. Results from both haemocytometer and optical density at 680 nm correlated well, illustrating a reduction in algal cells over a 30 minute treatment. The highest removal rate by haemocytometer at 1146 kHz was with a 200 mL volume. UV-Vis spectrophotometer results (Appendix 3, Figure 21) confirmed that using this frequency at a maximum power setting is effective for controlling algae since peaks around 620 nm decreased during treatment. Fluorometry results (Appendix 3, Figure 21) indicated peaks at 680 decreased during the treatment.

Figure 6.14 Inactivation of 400 mL *Microcystis aeruginosa* using the 1146 kHz bath at 40%, 80% and maximum power setting (haemocytometer and spectrophotometer)



The sonication of 400 mL algae suspension using a 1146 kHz bath at 40% power setting (0.0016 Wcm^{-3}) resulted in a fluctuation in algal cell numbers. Following 30 minutes treatment the concentration of the algae by haemocytometer decreased slightly over time. However, optical density at 680 nm results showed an increase in chlorophyll A concentration during treatment. This suggested that a declumping effect was occurring (Joyce, 2003). UV-Vis spectrophotometer results (Appendix 3,

Figure 18) showed peaks around 620 nm and fluorometer peaks (Appendix 3, Figure 18) at 680 nm both decreased over time.

The sonication of 400 mL of algae suspension at 1146 kHz using 80% power setting (0.0196 Wcm^{-3}) also resulted in inactivation. Over the 30 minutes treatment time haemocytometer and optical density at 680 nm results corresponded well, showing a reduction in algal cells following treatment with ultrasound. These results represented the highest removal rate by haemocytometer. UV-Vis spectrophotometer results (Appendix 3, Figure 20) showed peaks around 600 nm decreased slightly during treatment. Fluorometry results (Appendix 3, Figure 20) indicated that the peaks at 680 nm decreased over time.

The sonication of 400 mL algae suspension with 1146 kHz bath (maximum power setting, 0.0625 Wcm^{-3}) also resulted in a reduction in algae cell numbers. Haemocytometer and optical density at 680 nm results agreed well although the results obtained at the maximum power setting did not show higher inactivation levels (20.55% by haemocytometer and 20.45% by optical density at 680 nm) compared with at 80% intensity (27.07% by haemocytometer and 20.31% by optical density at 680 nm). UV-Vis spectrophotometer results (Appendix 3, Figure 22) showed peaks around 620 nm decreased slightly during treatment. Fluorometer results (Appendix 3, Figure 22) indicated peaks at 680 nm decreased over time. Both UV-Vis spectrophotometer and fluorometer results indicated phycobiliproteins are sensitive to sonication as they decreased during ultrasonic treatment (Hao, 2004).

Summary

From the above it can be seen that ultrasound can induce two different effects on algal cells. In broad terms inactivation occurs at high power (≥ 0.0022 Wcm^{-3}) and de-agglomeration at low power (≤ 0.0042 Wcm^{-3}). All experiments were carried out over 30 minutes at different frequencies, different powers and in triplicate. The results are summarised as percentage cell reduction in 200 mL suspension (Table 6.4) and in 400 mL suspension (Table 6.5) and the % cell reduction was calculated as follows:

$$\% \text{ cell reduction} = (1 - C_{30}/C_0) \times 100\%$$

Table 6.4 Effect of ultrasound on 200 mL *Microcystis aeruginosa* algal suspensions

Freq. (kHz)	Power Setting	Intensity (Wcm^{-3})	% Reduction (HAE)	% Reduction (OD at 680 nm)
580	40%	0.0018	13.81	13.22
580	80%	0.0216	59.33	36.84
580	maximum	0.0493	44.12	47.37
864	40%	0.0042	34.55	21.05
864	80%	0.0166	67.60	33.61
864	maximum	0.0576	81.09	47.01
1146	40%	0.0026	16.75	8.33
1146	80%	0.0124	66.19	23.89
1146	maximum	0.0248	91.54	44.63

Using 580 kHz bath

Using 580 kHz bath, the highest removal rate indicated by haemocytometer readings was achieved using 580 kHz (80% power setting, 0.216 Wcm^{-3}) and the lowest was using 580 kHz (40% power setting, 0.0018 Wcm^{-3}). The highest removal rate by optical density at 680 nm was obtained using 580 kHz (maximum power setting, 0.0493 Wcm^{-3}) and lowest was using 580 kHz (40% power setting, 0.0018 Wcm^{-3}). The removal rate by optical density at 680 nm increased with increasing intensity. High ultrasonic intensity can damage photosynthetic pigments (Zhang, 2006). There was no large difference between the removal rate by haemocytometer at 80% and

maximum power setting, indicating there may be an upper limitation on intensity at this frequency.

Using 864 kHz bath

Using 864 kHz bath, the highest removal rate by haemocytometer and optical density at 680 nm is achieved using 864 kHz (maximum power setting, 0.0576 Wcm^{-3}). The lowest removal rate by haemocytometer and optical density at 680 nm was obtained using 864 kHz (40% power setting, 0.0042 Wcm^{-3}). The removal rate increased with increasing intensity at this frequency. The results at 864 kHz are higher than 580 kHz, possibly because at a higher frequency more free radicals are produced to inactivate algal cells (Joyce, 2003).

Using 1146 kHz bath

Using 1146 kHz bath, the highest removal rate by haemocytometer is achieved using 1146 kHz (maximum power setting, 0.0248 Wcm^{-3}) and the lowest is using 1146 kHz (40% power setting, 0.0018 Wcm^{-3}). The highest removal rate by optical density at 680 nm was obtained using 1146 kHz (maximum power setting, 0.0493 Wcm^{-3}) and lowest was using 1146 kHz (40% power setting, 0.0026 Wcm^{-3}). The removal rate increased with increasing intensity. Although the intensity of 1146 kHz (maximum power setting, 0.0248 Wcm^{-3}) was lower than 864 kHz (maximum power setting, 0.0576 Wcm^{-3}), the removal rate indicated by the haemocytometer data was much higher, which may due to more free radicals being produced to inactivate algal cells.

Table 6.5 Effect of ultrasound on 400 mL *Microcystis aeruginosa* algal suspensions

Freq. (kHz)	Power Setting	Intensity (W/cm^3)	% reduction (HAE)	% reduction (OD at 680 nm)
580	40%	0.0017	13.13	13.22
580	80%	0.0198	40.89	21.31
580	maximum	0.0640	21.08	14.53
864	40%	0.0060	26.66	11.40
864	80%	0.0243	32.99	33.61
864	maximum	0.0929	79.56	61.11
1146	40%	0.0016	4.14	−1.61
1146	80%	0.0196	27.07	20.31
1146	maximum	0.0625	20.55	20.45

Using 580 kHz bath

Using 580 kHz bath, the highest removal rate by haemocytometer and optical density at 680 nm was achieved using 580 kHz (80% power setting, 0.0198 Wcm^{-3}) and the lowest was using 580 kHz (40% power setting, 0.0017 Wcm^{-3}). The removal rates ranged from 13 − 22% (except in the case of the result by haemocytometer at 80% power setting, 0.0198 Wcm^{-3}), which are relatively low at this frequency. This may be due to the fact that at 580 kHz; higher intensities are required to produce cavitation effects (Joyce, 2003).

Using 864 kHz bath

Using 864 kHz bath, the highest removal rate by haemocytometer and optical density at 680 nm was achieved using 864 kHz (maximum power setting, 0.0929 Wcm^{-3}). The lowest removal rate by haemocytometer and optical density at 680 nm was obtained using 864 kHz (40% power setting, 0.0060 Wcm^{-3}). The removal rate increased with increasing intensity at this frequency. The results at 864 kHz were higher than 580 kHz, similar to the effects recorded at 200 mL, indicating with higher frequency; more free radicals were produced to inactivate algal cells.

Using 1146 kHz bath

Using 1146 kHz bath, the highest removal rate by haemocytometer was achieved using 1146 kHz (80% power setting, 0.0196 Wcm^{-3}) and the lowest was using 1146 kHz (40% power setting, 0.0016 Wcm^{-3}). The highest removal rate by optical density at 680 nm was obtained using 1146 kHz (maximum power setting, 0.0625 Wcm^{-3}) and lowest was using 1146 kHz (40% power setting, 0.0016 Wcm^{-3}). The sonication of 1146 kHz (40% power setting 0.0016 Wcm^{-3}) resulted in a declumping effect, which resulted in an increase in the optical density at 680 nm over 30 minutes treatment. The observation of declumping rather than kill is almost certainly the result of the low intensity used as low intensity may break up bacterial clumps into a greater number of individual bacteria in a suspension (Joyce, 2003).

General comparison 200 and 400 mL against HAE vs. OD

At 200 mL, the highest removal rate by haemocytometer was achieved using 1146 kHz (maximum power setting, 0.0248 Wcm^{-3}) and the highest removal rate by optical density at 680 nm was using 580 kHz (maximum power setting, 0.0493 Wcm^{-3}). The result obtained at 1146 kHz (maximum power setting, 0.0248 Wcm^{-3}) by optical density at 680 nm were only slightly lower than that at 580 kHz (maximum power setting, 0.0493 Wcm^{-3}). From this we can deduce that 864 kHz (maximum power setting, 0.0576 Wcm^{-3}) was the most effective parameter setting at 200 mL.

At 400 mL, the highest removal rate by haemocytometer and optical density at 680 nm was achieved using 864 kHz (maximum power setting, 0.0929 Wcm^{-3}), demonstrating it was the most effective parameter setting of those tested. A

declumping effect was observed using 1146 kHz (40% power setting 0.0016 Wcm^{-3}), indicating low intensity does not inactivate algae cells.

The removal rates at 200 mL are higher than 400 mL since with increasing volume, power density decreases. Less cavitation effects are produce per unit volume to inactive algal cells.

In section 6.2.3, the trends of haemocytometer and optical density at 680 nm results agree well but the reductions indicated by haemocytometer are usually higher than those indicated by optical density at 680 nm readings. To date this has not been reported in the literature but may be due to the fact that when algal cells are disrupted by ultrasound cell debris is produced, maintaining optical density at 680 nm.

UV-Vis and optical density at 680 nm results did not agree as well as fluorometer and UV-Vis. One possible explanation is that the absorption of chlorophyll B and carotenoids may have interfered with the chlorophyll A results (Zhang, 2006).

In conclusion, it is clear that there was a significant effect of applied ultrasonic frequency and intensity on *Microcystis aeruginosa*. Sonication can cause declumping and/or inactivation depending on the conditions. Generally there is competition between (a) inactivation of algae cells leading to a reduction in cell numbers and (b) deagglomeration of algae clumps to produce more individual cells. Overall ultrasonic effects are summarized below (Joyce, 2010):

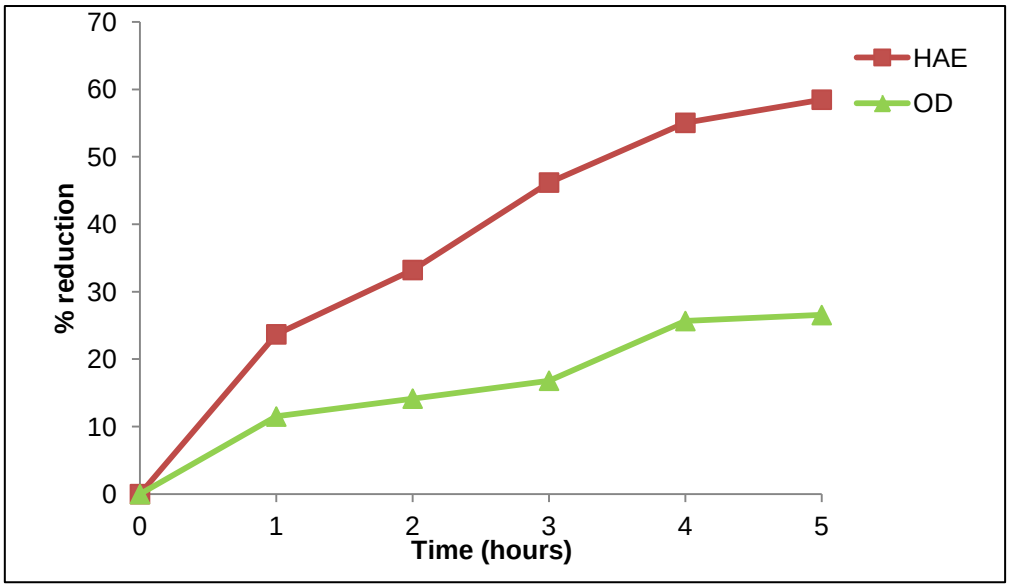
- High power ultrasound results in a reduction of algal cell numbers by rupturing algal cell walls
- Low power ultrasound may lead to an apparent initial rise in algal cell numbers (declumping) by breaking up flocs of algae into single cells, without the power to inactivate
- Higher frequency ultrasound produces more free radicals which can also attack algal cell walls

6.3 Sonication of *Microcystis aeruginosa* at a medium size laboratory scale

As has been demonstrated in section 3.2; sonication of *Microcystis aeruginosa* at small Lab-scale provides a suitable and effective method to reduce cell numbers in a suspension. In this section, we focus on medium-scale (industrial pilot) ultrasonic equipment. Experiments were undertaken to assess ultrasonic control of algae using the following equipment and volumes: Sonolator (Sonic Corporation, 5L), and 16 kHz and 20 kHz Dual Frequency Reactor (DFR, Advanced Sonics LLC, 1L (static) and 3.5 L (circulating).

6.3.1 Sonication of algae (optical density 0.2 at 680 nm, 5L) using the Sonolator for 5 hours

Figure 6.15 Inactivation of 5 litre *Microcystis aeruginosa* using the Sonolator (haemocytometer and spectrophotometer)



The Sonolator is equipped with a pump to circulate liquid in the system. The exposure time is 1.09 minutes as the flow rate of the system is 4600cm^3/min. Following ultrasonic treatments with the Sonolator, the concentration of algae steadily decreased. UV-Vis spectrophotometer results (Appendix 3, Figure 23) showed that this equipment was able to damage algal photosynthetic system in algae

cells because the peaks around 620nm decreased during treatment. Fluorometer and spectrophotometer results agree well (Appendix 3, Figure 23), both showing a reduction in phycobiliproteins following treatment.

For tests involving the Sonolator, cavitation is produced by forcing liquid through a small orifice which then strikes a knife-like blade set in its path (hydrodynamic cavitation) (Mason and Lorimer, 2002:269). Our tests demonstrated that the Sonolator can reduce algal cell numbers although the treatment time was long (5 hours). It is interesting to note that the reduction indicated by haemocytometer counts appeared much lower than indicated by optical density at 680 nm readings. UV-visible spectrophotometer and fluorometer results confirmed that ultrasound decreased chlorophyll A levels in algae suspensions, indicating injury to the algae cells.

From this study it is clear sonication by hydrodynamic cavitation was only effective following long exposure times. However, it does indicate that there may be a potential for the use of this ultrasonic flow systems to reduce algae cell numbers. Hydrodynamic cavitation generates direct mechanical effects on algal cells along with the production of hydrogen peroxide which has an oxidizing effect. Xu reported 114 hours treatment can effectively inhibit algal growth and inactivation rates were greatly affected by parameter setting such as hydraulic characteristics of cavitation tubes, inlet pressure, orifice shape and size (Xu, 2006). Further optimization of hydrodynamic cavitation parameter settings is required to enhance the efficiency of this system.

6.3.2 Sonication (circulating) of algae (optical density 0.15 at 680 nm, 3.5L) using 16 kHz and 20 kHz DFR reactor at 40% power setting for 60 minutes

Figure 6.16 Inactivation of 3.5L *Microcystis aeruginosa* using DFR (circulating) at 40% power setting for 60 minutes (haemocytometer and spectrophotometer)

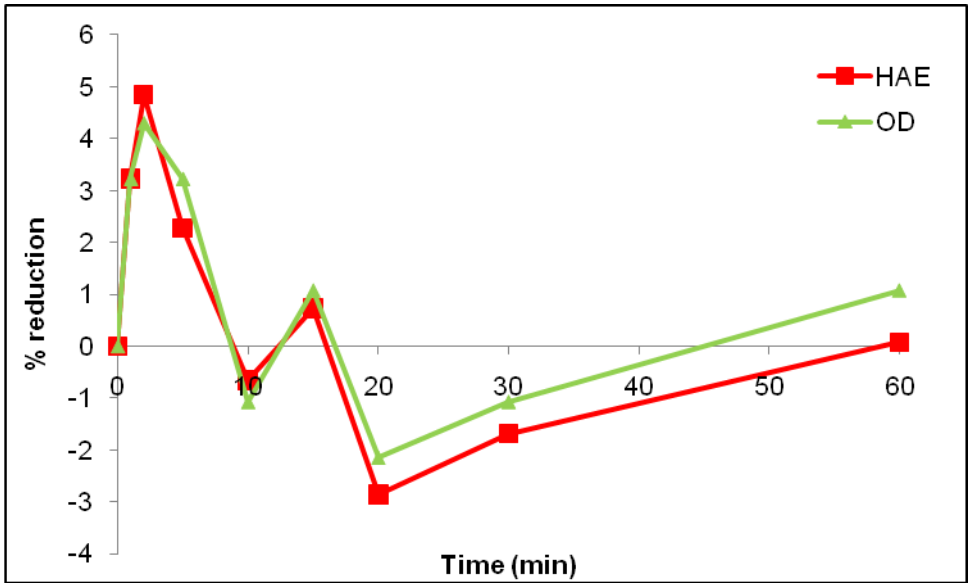


Figure 6.17 Inactivation of 3.5L *Microcystis aeruginosa* DFR (circulating) (Flow cytometer) at 40% power setting

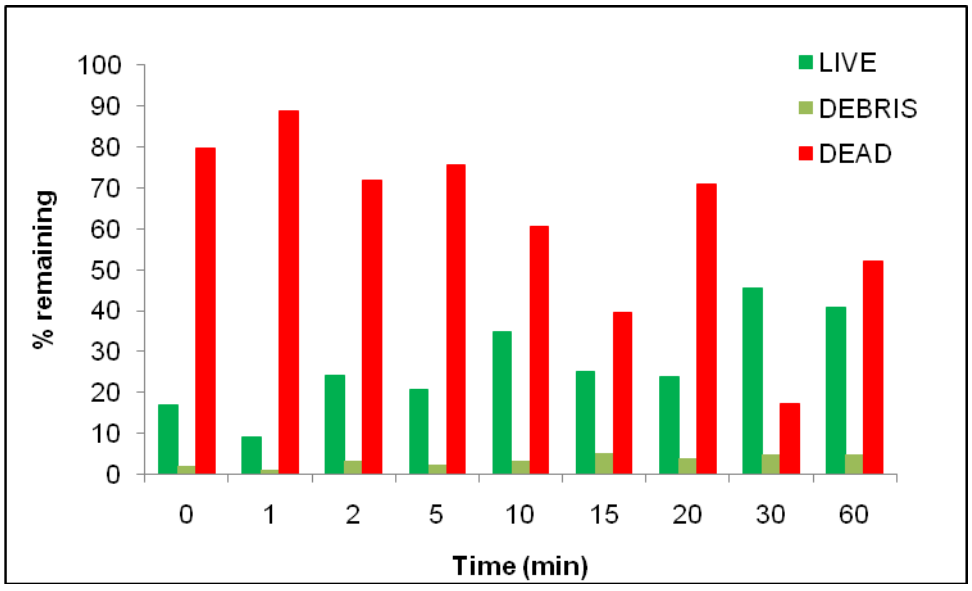
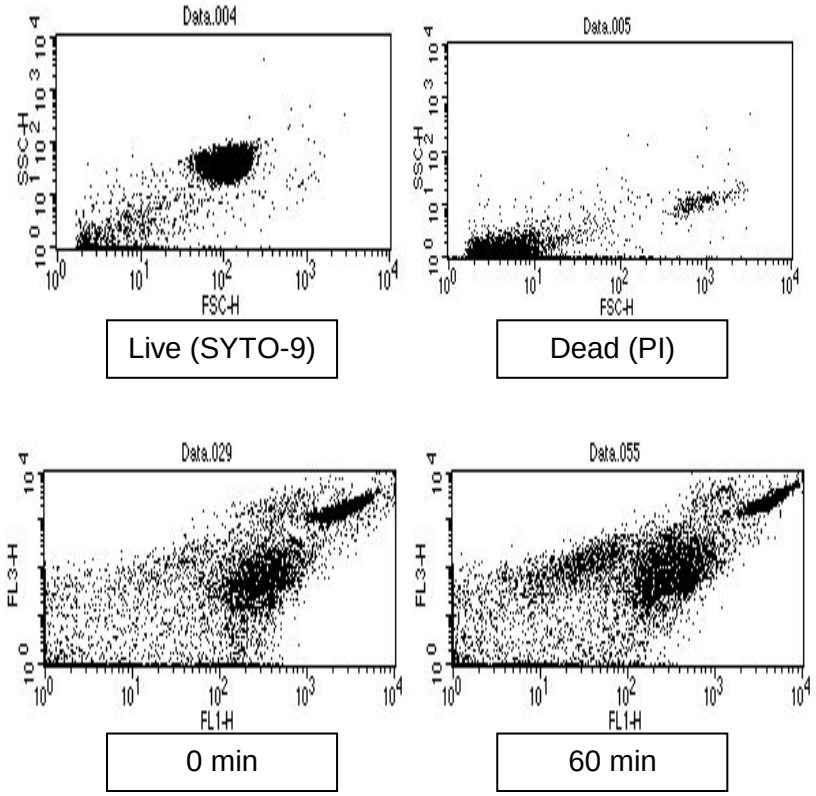


Figure 6.18 Inactivation of 3.5L *Microcystis aeruginosa* DFR (circulating) (Flow cytometry) at 40% power setting (graphs of live, dead, before and after treatment)



The above FCM dot plots indicate the quadrant position for live and dead sub-populations using live (7 day culture) and dead (boiled for 30 minutes) controls, which were stained with SYTO-9 and PI. At 0 minutes the majority of cells appear in the live position and following DFR (40%) for 60 minutes there appears to be a third subpopulation present which may be between a live and dead state (viable but with reduced metabolic functions).

The effect of 16 kHz and 20 kHz Dual Frequency Reactor (DFR) with circulating mode at 40% power setting resulted in a fluctuation by both haemocytometer and optical density at 680 nm, illustrating a declumping effect. The reduction assessed by haemocytometer counts and optical density at 680 nm readings corresponded well following 60 minutes treatment, indicating a low removal rate. UV-Vis spectrophotometer results (Appendix 3, Figure 24) showed peaks around 620 nm that did not decrease significantly with ultrasonic treatment, indicating phycobiliproteins of algae cells was not damaged. Fluorometer results (Appendix 3,

Figure 24) illustrated a slightly reduction of peaks at 665 nm following 60 minutes treatment with ultrasound.

UV-Vis spectrophotometer results and fluorometer results showed that the algae cells were healthy prior to treatment, but flow cytometry indicated two sub-populations, live and dead cells, suggesting that flow cytometry may be the more sensitive method of analysis compared to haemocytometry counts. Flow cytometry (FCM) is a tool for automated algal cell counting providing information on cell size, biomass and condition of cells (live, damaged and particulate matter or cell debris). Further research was taken and discussed in section 6.4.1 mechanism study using flow cytometer.

Staining with SYTO−9 and PI revealed three sub-populations: live, dead and cell debris or particulate matter (Figure 6.18). With increasing treatment times the live subpopulation reduced and dead sub-population increased over 10 minutes. Over 50 minutes treatment the results of live and dead sub-populations fluctuated, indicating a declumping effect. In Figure 6.17, percentage remaining of live cell increased and dead cell decreased during 60 minutes treatment. There was no obvious shift from live to dead regions which indicated ultrasound did not have a significant inactivation in Figure 6.18 effect on algal cells using DFR at 40% intensity in circulating mode.

Overall the results demonstrated a declumping effect using the DFR (circulating) at 40% power setting indicating at these conditions the DFR system at this intensity is not suitable for algae bloom control.

6.3.3 Sonication (static) of algae (optical density 0.25 at 680 nm, 1L) using 16 kHz and 20 kHz DFR reactor at 40% power setting for 10 minutes

Figure 6.19 Inactivation of 1L *Microcystis aeruginosa* using DFR (static) at 40% power setting for 10 minutes (haemocytometer and spectrophotometer)

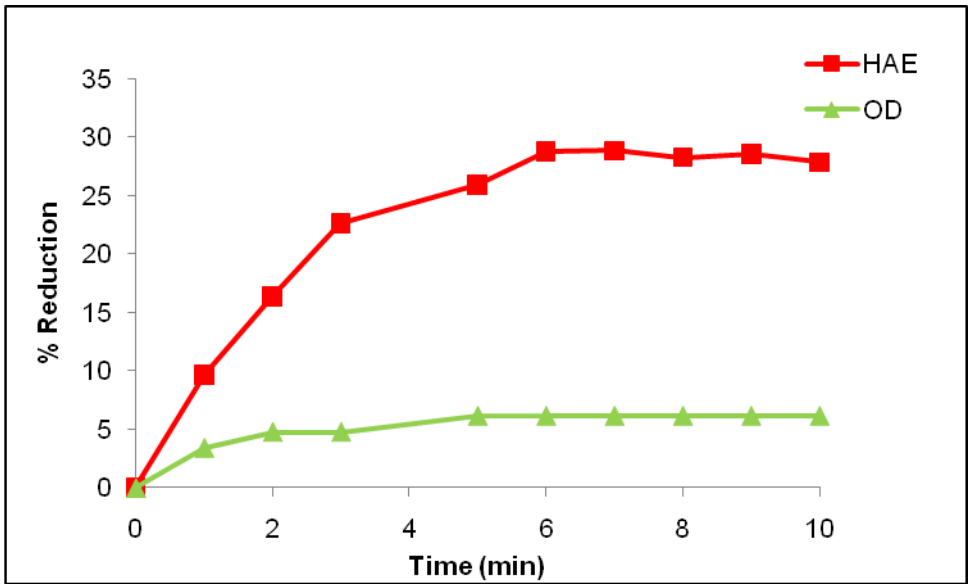


Figure 6.20 Inactivation of 1L *Microcystis aeruginosa* DFR (static) (Flow cytometer) at 40% power setting (figure)

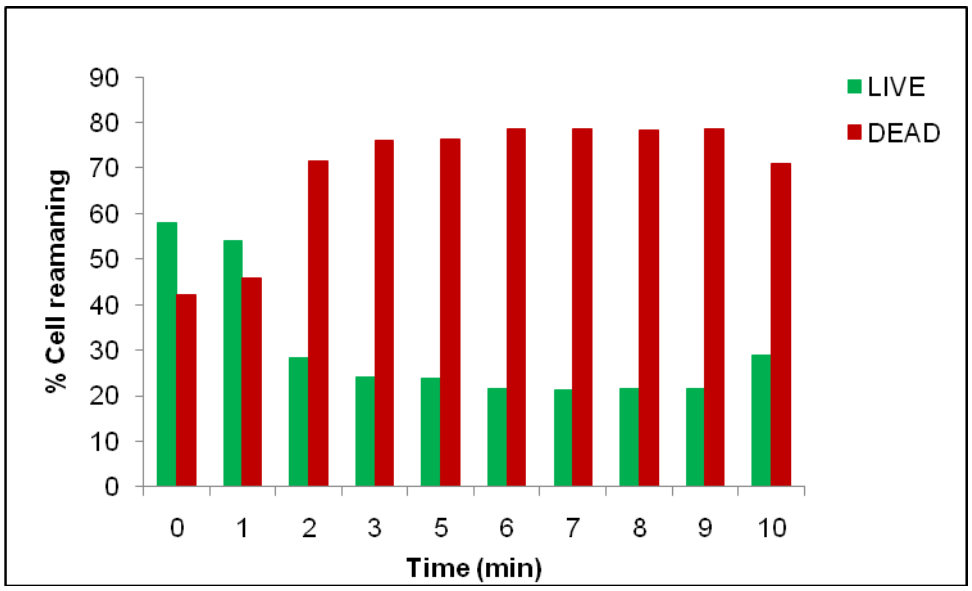
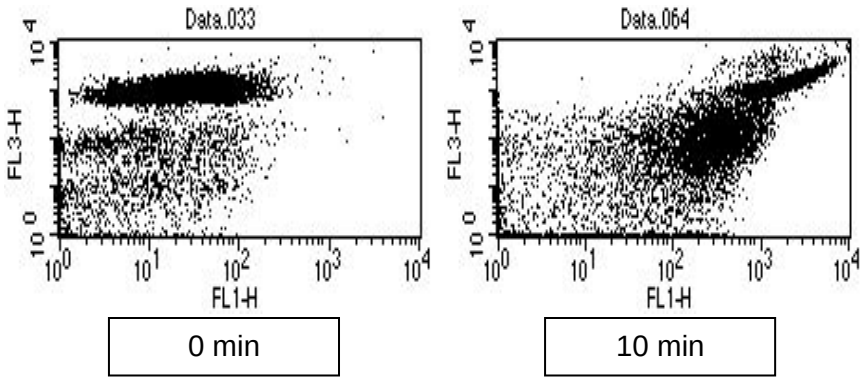


Figure 6.21 Inactivation of 1L *Microcystis aeruginosa* DFR (static) (Flow cytometry) at 40% power setting (graphs before and after treatment)



Using this equipment in the static (non-circulating) mode allows the algae suspensions to be in continuous contact with the vibrating plates of the DFR. The effect of 16 kHz and 20 kHz DFR reactor (static) at 40% power setting, for 10 minutes treatment on 1 litre algae suspensions resulted in a slight inactivation. Although there is a continous small reductions; UV-Vis spectrophotometer results (Appendix 3, Figure 25) illustrated the peaks around 620 nm decreased slightly during treatment, indicating phycobiliproteins were not badly damaged. Fluorometer results (Appendix 3, Figure 25) also illustrated peaks at 665 nm decreased very slightly during treatment time, indicating the algal photosynthetic system was slightly affected by sonication.

Staining with SYTO−9 and PI revealed two sub-populations: live and dead. With increasing treatment times, live sub populations reduced and the dead sub-population increased (Figure 6.20). In Figure 6.21, there was an obvious shift from live to dead regions which indicated that algae cells are injured following sonication. Please refer to Section 6.4 for further discussions.

In conclusion, the effects of the DFR at 40% power setting in static mode are more effective than circulating mode since the exposure time is longer. This confirms increasing ultrasonic exposure results in greater algae cell reductions.

6.3.4 Sonication of algae (optical density 0.15 at 680 nm, 3.5 litre) using 16 kHz and 20 kHz DFR reactor (circulating) at 60% power setting for 20 minutes

Figure 6.22 Inactivation of 3.5L *Microcystis aeruginosa* using DFR (circulating) at 60% power setting for 20 minutes (haemocytometer and spectrophotometer)

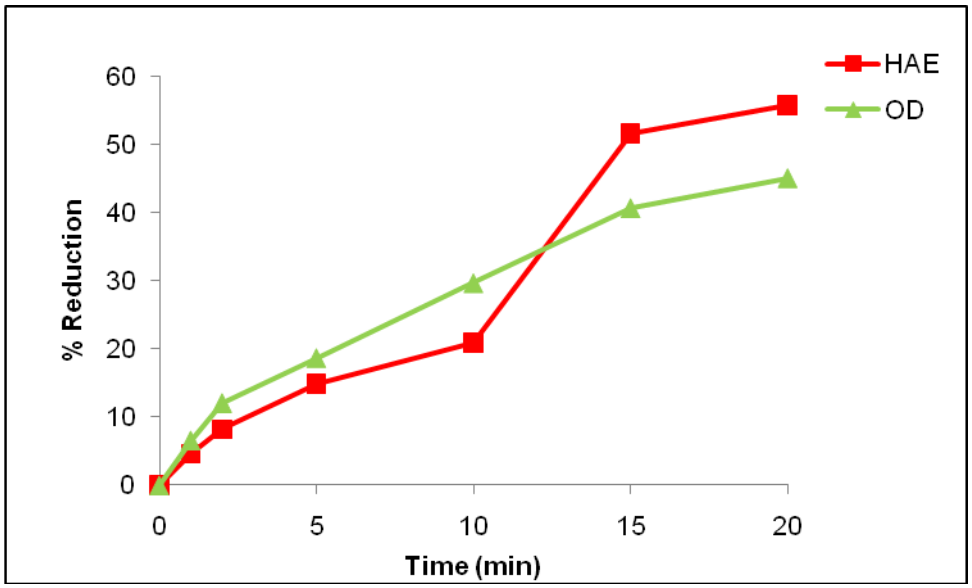


Figure 6.23 Inactivation of 3.5L *Microcystis aeruginosa* DFR (circulating) (Flow cytometer) at 60% power setting

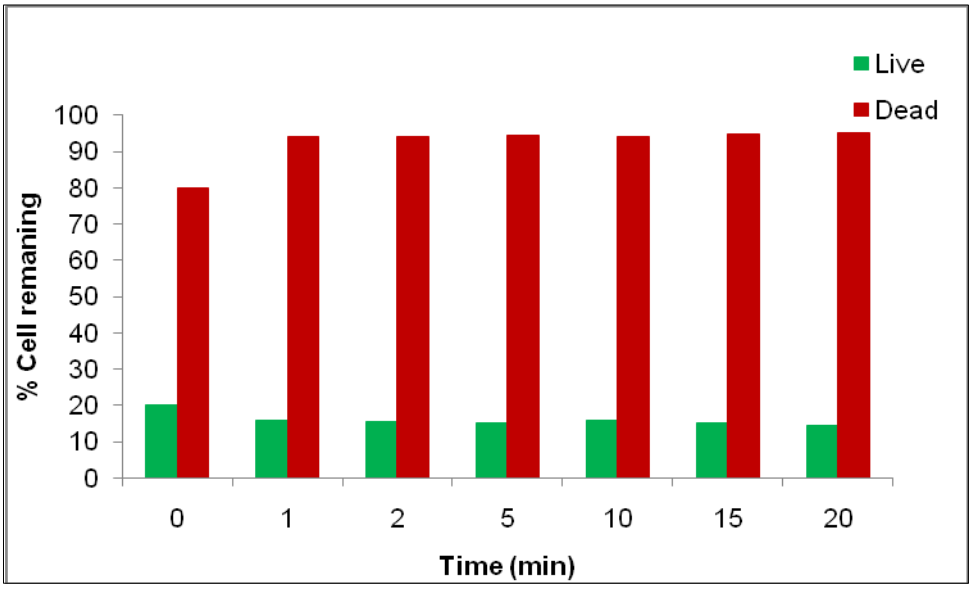
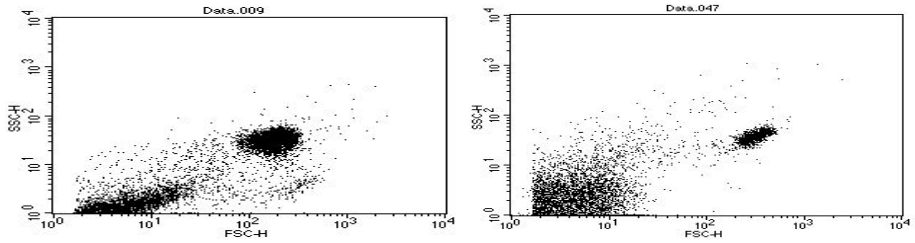


Figure 6.24 Inactivation of 3.5L *Microcystis aeruginosa* DFR (circulating) (Flow cytometry) at 60% power setting (graphs before and after treatment)



The effect of ultrasonic treatments with the 16 kHz and 20 kHz DFR reactor (circulating) at 60% power setting over 20 minutes treatment resulted in a reduction in the algae cell numbers by haemocytometer and optical density at 680 nm. The percentage algal cells reduction indicated by optical density at 680 nm readings was slightly lower than that obtained using haemocytometer readings over 20 minutes treatment. UV-Vis spectrophotometer results (Appendix 3, Figure 27) illustrated the peaks around 620 nm decreased slightly during treatment, indicating phycobiliproteins was not badly damaged. Fluorometer results (Appendix 3, Figure 27) also illustrated peaks at 665 nm decreased very slightly during treatment time, indicating little damage to cells.

Flow cytometry illustrates a slight increase in algal cell numbers in the dead sub-population, while fluorometry results showed fluctuations in algal cell activity. This indicates flow cytometry and may reveal more details relating to cell viability since the fluorometric measurements are more sensitive than haemocytometer or optical density at 680 nm (Lee, 2000). Joyce suggested that there was competition between inactivation and declumping effects during sonication (Joyce, 2003). Results demonstrate algal cells may remain in a condition or state between live and dead during sonication (Joyce, 2009), which could cause the fluctuations in flow cytometry results.

6.3.5 Sonication of algae (optical density 0.25 at 680 nm, 1 litre) using 16 kHz and 20 kHz DFR (static) reactor at 60% power setting for 10 minutes

Figure 6.25 Inactivation of 1L *Microcystis aeruginosa* using DFR (static) at 60% power setting for 10 minutes (haemocytometer and spectrophotometer)

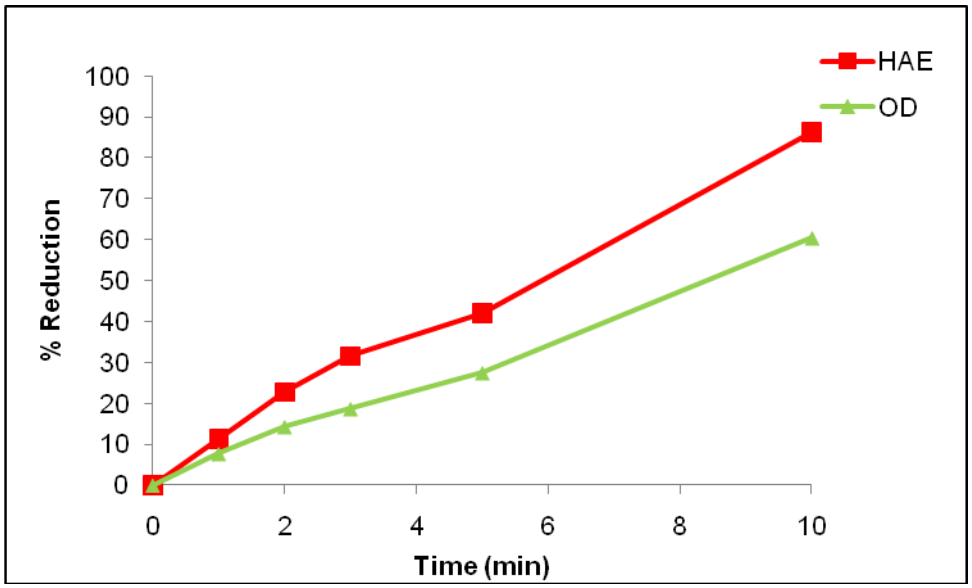


Figure 6.26 Inactivation of 1L *Microcystis aeruginosa* DFR (static) (Flow cytometer) at 60% power setting

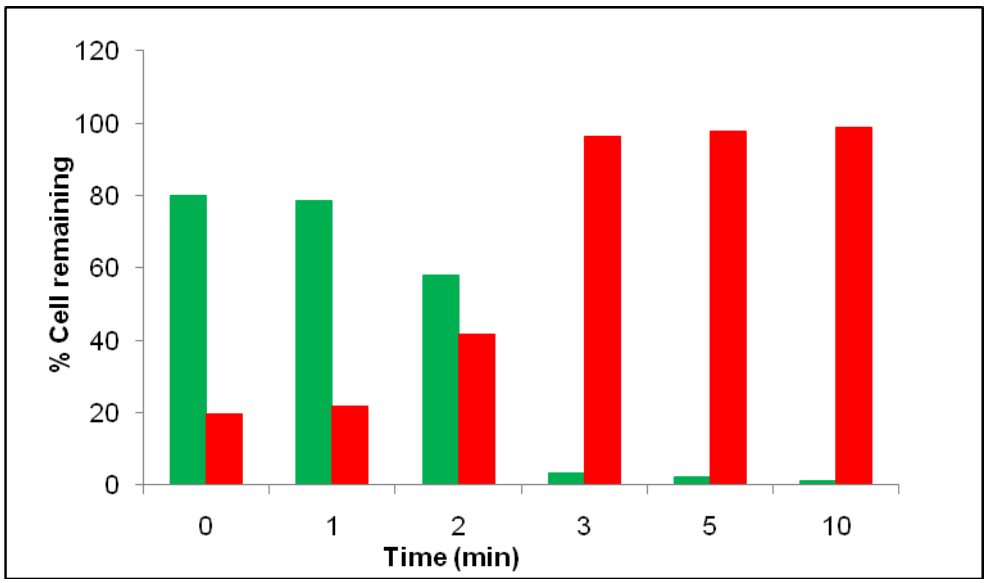
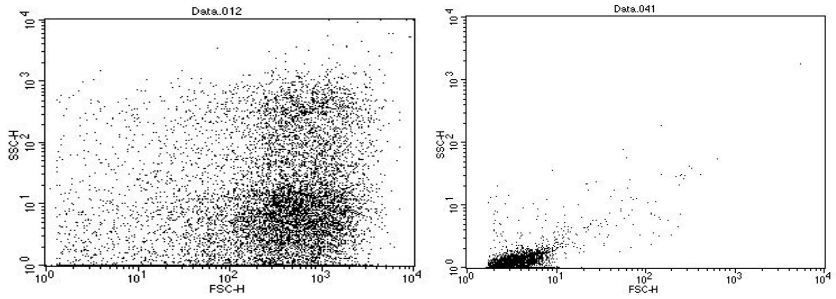


Figure 6.27 Inactivation of 1L *Microcystis aeruginosa* DFR (static) (Flow cytometry) at 60% power setting (graphs before and after treatment)



The effect of the 16 kHz and 20 kHz, DFR reactor (static) at 60% power setting resulted in a decease in algae concentration by haemocytometer and optical density at 680 nm over 10 minutes treatment. The power used was high power at 60% intensity and the removal rate by haemocytometer and optical density at 680 nm was also relatively high following 10 minutes treatment. A significant decrease in phycobiliproteins peaks was observed by both UV-Vis spectrophotometer and fluorometer (Appendix 3, Figure 26), indicating algae cells were injured following ultrasonic treatments (Zhang, 2006). Staining with SYTO−9 and PI stain revealed two sub-populations: live and dead. With increasing treatment times, live sub-populations reduced and dead sub-populations increased. An inactivation effect occurred during 10 minutes treatment. Results for the 16 and 20 kHz DFR at 60% intensity (% HAE reduction of 86.16% and % OD at 680 nm reduction of 60.44%) indicate low frequency but high intensity ultrasound is effective for algae control than low intensities of 40% intensity using DFR (% HAE reduction of 27.85% and % OD at 680 nm reduction of 6.12%).

Table 6.6 Sonication of algae using DFR reactor (Circulating)

Power setting (Circulating)	Volume (L)	Intensity (Wcm^{-3})	Resident time (min)	% HAE	% OD (680nm)	% FCM (live)	UV−Vis peaks decrease over time	Fluoro peaks decrease over time	Temperature (°C)
40%	3.5	−	17	0.07	1.08	40.92	No, but slightly decrease (Appendix 3, Figure 24)	No, but slightly decrease (Appendix 3, Figure 24)	16.8 − 17.2
60%	3.5	−	7	55.65	45.05	14.60	No, but slightly decrease (Appendix 3, Figure 27)	No, but slightly decrease (Appendix 3, Figure 27)	12.7 − 29.6

Table 6.7 Sonication of algae using DFR reactor (Static)

Power setting (Static)	Volume (L)	Intensity (Wcm^{-3})	Resident time (min)	% HAE	% OD (680nm)	% FCM (live)	UV−Vis peaks decrease over time	Fluoro peaks decrease over time	Temperature (°C)
40%	1	0.0256	10	27.85	6.12	28.96	No, but slightly decrease (Appendix 3, Figure 25)	Yes (Appendix 3, Figure 25)	16.9 − 21.17
60%	1	0.0177	10	86.16	60.44	1.10	No, but slightly decrease (Appendix 3, Figure 26)	Yes (Appendix 3, Figure 26)	16.4 − 26.03

Algal growth is greatly affect by temperature with negative effects on viability when cultured above 30 ^{o}C (Bartram, 1999: 12−24). In this experiment, temperature increases ranged between 16 − 27^{o}C during these tests but this appeared to have had no deleterious effect on algae cells numbers.

During ultrasonic treatments with the 16 and 20 kHz DFR (circulating) at 40% power setting the concentration of algae fluctuated during 60 minutes treatment ending with a slight reduction. Following ultrasonic treatments with the 16 and 20 kHz DFR (circulating) at 60% power setting, the removal rate increased due to increase of intensity, indicating ultrasonic intensity plays an important role in algae control. A possible explanation for the fluctuation following treatment is that two ultrasonic effects are presented during treatment (a) inactivation of algae cells resulting in loss in concentration and (b) disruption of algae producing more individual cells in suspension. Sonication using low frequency ultrasound but at a low intensity provided little effect on algae removal.

Following ultrasonic treatments with the 16 and 20 kHz DFR (static) at 40% intensity, the concentration of algae decreased slightly over a 10 minute treatment. However ultrasonic treatment with the DFR (static) at 60% intensity reduced the concentration of algae cells significantly after 10 minutes treatment, indicating inactivation. The static mode resulted in higher removal rate because the suspension was continuously exposed to ultrasound in the reactor itself whereas during circulation exposure to ultrasound only occurred while the suspension was passing through the reactor.

The DFR is designed to introduce high power ultrasound into a flow system (Mason, 1994). The DFR is equipped with two vibrating metal plates which face each other and are separated by 25 cm. Many transducers are employed in the system, providing high ultrasonic powers to each plate. Employing a combination of two different frequencies (16 and 20 kHz) results in a 'beat' frequency, which provides high energy and continuously oscillates in the two metal plates (Tatake, 2002). In this way, the DFR provides greater intensities than expected from a simple doubling of two single plates since ultrasound can reverberate and improve effects.

DFR experiments indicate sonication has two effects on algae. Firstly, declumping which breaks algae clumps into individual algae cells and secondly inactivation which can damages chlorophyll A in algae cells. These two effects were confirmed by UV-Visible spectrophotometer, fluorometer and flow cytometry (Lee, 2000, Hao, 2004, and Zhang, 2006).

6.3.6 Sonication of algae (optical density 0.18 at 680 nm, 1.5 litre) using a vibrating tray reactor for 5 minutes

Figure 6.28 Inactivation of 1.5L *Microcystis aeruginosa* using a vibrating tray for 5 minutes (haemocytometer and spectrophotometer)

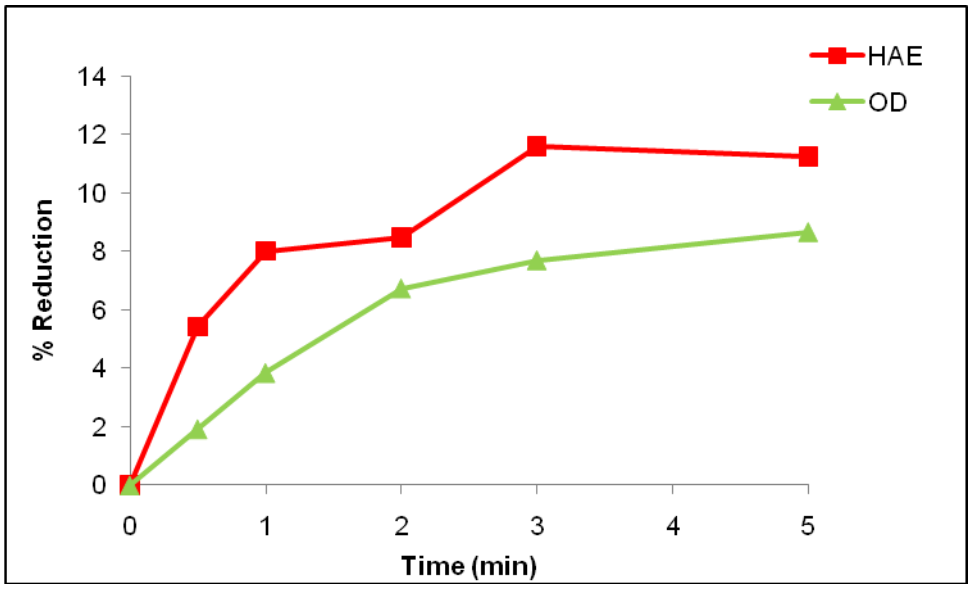


Figure 6.29 Inactivation of 1.5L *Microcystis aeruginosa* using a vibrating tray (Flow cytometer) for 5 minutes

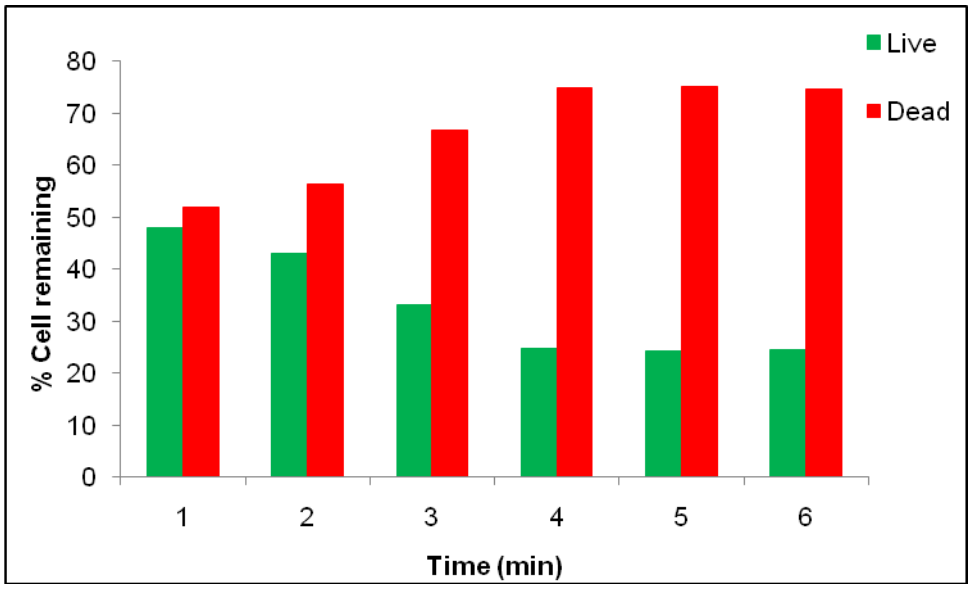
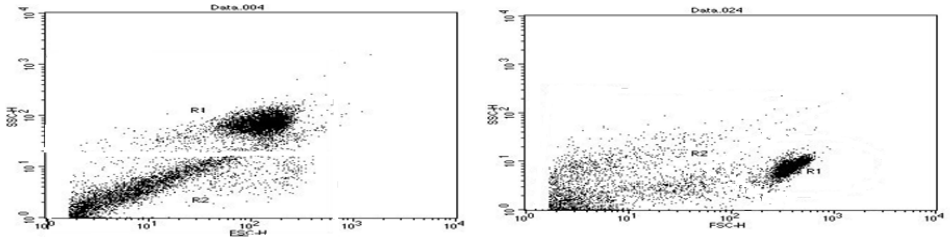


Figure 6.30 Inactivation of 1.5L *Microcystis aeruginosa* using a vibrating tray (Flow cytometry) (graphs of before and after treatment)



0 min | 6 min

The vibrating tray (20 kHz) is designed for the continuous processing of coal and metal ores. Vibrations are transmitted to the base of the tray and then into the reaction medium in the tray; in the flow mode this provides a short residence time (seconds). The vibrating tray is also employed in heavy duty chemical processing of solid and liquid reactions for extraction (Mason, 1991:101−102).

The effect of ultrasonic treatments with the vibrating tray (20 kHz) resulted in slight inactivation as shown by haemocytometer and optical density at 680 nm measurements over 6 minutes treatment. UV-Vis spectrophotometer results showed the peaks around 620 nm decreased during the treatment, indicating chlorophyll A of algae cells was damaged. Fluorometer results showed the chlorophyll A peak around 680 nm decreasing with increasing treatment times. There is small decrease in the size of phycobiliproteins peaks when analysed by both UV-Vis spectrophotometer and fluorometry (Appendix 3, Figure 28) (Zhang, 2006).

Flow cytometry revealed two main sub-populations in the sample: live and dead. With increasing treatment times, live sub-populations reduced rapidly over 3 minutes treatment. Following 3 minutes treatment, the entire population shifted to the left quadrant of the graph, indicating cell size decrease. Following 5 minutes treatment, the main sub-population was injured or dead cells.

Conclusions

The results of sonication of *Microcystis aeruginosa* at a medium-laboratory scale can be summarised as follows:

- Sonication can have two effects on algae: declumping or inactivation depending upon the power used
- The Sonolator can reduce algae cell concentration in a recirculating flow system but only over an extended time period.
- For the 16 and 20 kHz DFR, since the applied frequencies are low, intensity plays an important role during treatment
- For the vibrating tray, inactivation effects are low, indicating this equipment is not suitable for algae control
- Haemocytometer and flow cytometer results agree well, confirming that ultrasound damages algae cells during treatment
- Small/medium pilot-scale ultrasonic treatments show potential for the inactivation of algae. Ultrasonic irradiation may thus provide a suitable method for algal bloom control in large-scale applications

Most large-scale ultrasonic systems are designed at low frequencies (20 − 40 kHz) and high power inputs. We have tested low frequency systems at a medium lab-scale (ultrasonic probe, Dual Frequency Reactor (DFR) and Vibrating tray), which can be developed into large-scale applications. Results indicate that low frequency but high intensity ultrasonic equipment can reduce algae cell numbers. By employing high power settings, it is possible to achieve effective inactivation using the Dual Frequency Reactor (DFR). Thus, ultrasonic flow systems may be able to inhibit algae blooms on a large-scale providing that there is suitable optimisation of the ultrasonic parameters involved e.g. frequencies, energy and dosage. However, the balance of energy cost and removal rates must be carefully considered.

6.4 Mechamisms study of ultrasonic effects on algae

6.4.1 Mechanisms study using flow cytometer

Flow cytometry (FCM) is a useful tool for automated algal cell counting, reducing analysis time and minimizing human error. Direct analysis provides information on cell size, biomass and condition of cells (live, damaged, dead and particulate matter or cell debris). Highly sensitive stains and fluorescent molecular probes are required to undertake microalgae research using flow cytometry (Marie, 2004).

FCM measures algal cells in a liquid suspension by aligning suspensions in a very narrow stream (10 to 20 μm wide) with focused light sources. Each time a particle (cell) passes through the beam, light is scattered. The angular intensity depends on refractive index, size and shape of particles. Algal cells contain chlorophyll, which emit fluorescence at an excitation wavelength of 488 nm (Lee, 2000). Light pulses are detected by photodiodes and these are converted into digital signals processed by a computer.

Forward scatter (FSC) and side scatter (SSC) detectors respond when a particle passes through an excitation beam. Both parameters are dependent on cell size, but SSC is affected by cell surface and internal cellular structure and so can detect dead or damaged cells.

Fluorescent dyes can identify specific molecules within cells resulting in a much more sensitive analysis. Lee (2000) stained *Microcystis* aeruginosa with 1.5μl SYTO−9 and 1.5μl Propidium Iodide (PI) from a LIVE/DEAD Baclight bacterial viability kit. Fluorescence of SYTO−9 and PI was detected at 510 nm (FL1, green) and 610 nm (FL3, red) band pass filters, respectively (Lee, 2000). Brussaard (2001) reported the use of SYTO−9 and Calcein-Am to stain live and dead algal cells (Brussaard, 2001).

Algal samples contain many small particles and cell debris, which increase background noise levels. Marie (2004) recommended increasing discriminator threshold to reduce the number of events observed by flow cytometry. Furthermore, gas vacuoles may interfere with light scattering results (SSC). Regel (2000) collapsed gas vacuoles using a Scholander bomb (30 second at 1200 kPa) however gas vacuoles represent the regeneration and viability of *Microcystis aeruginosa*, which is vital in our research.

In our tests, 1 mL *Microcystis* species was stained with 1.0 µl SYTO−9 and 1.0 µl Propidium Iodide (PI) from a LIVE/DEAD Baclight bacterial viability kit (Invitrogen, L10316). Standard settings employed in the experiments were: FSC = E00, SSC = 242, green fluorescence (FL1) = 510, orange fluorescence (FL2) = 550 and red fluorescence (FL3) = 610.

6.4.1.1 Cell integrity and viability test using the 20 kHz probe, 580 kHz and 1146 kHz high multi-frequency bath at 40% intensity and flow cytometer analysis

Figure 6.31 Inactivation of 200 mL *Microcystis aeruginosa* using 20 kHz probe for 30 minutes (haemocytometer and spectrophotometer)

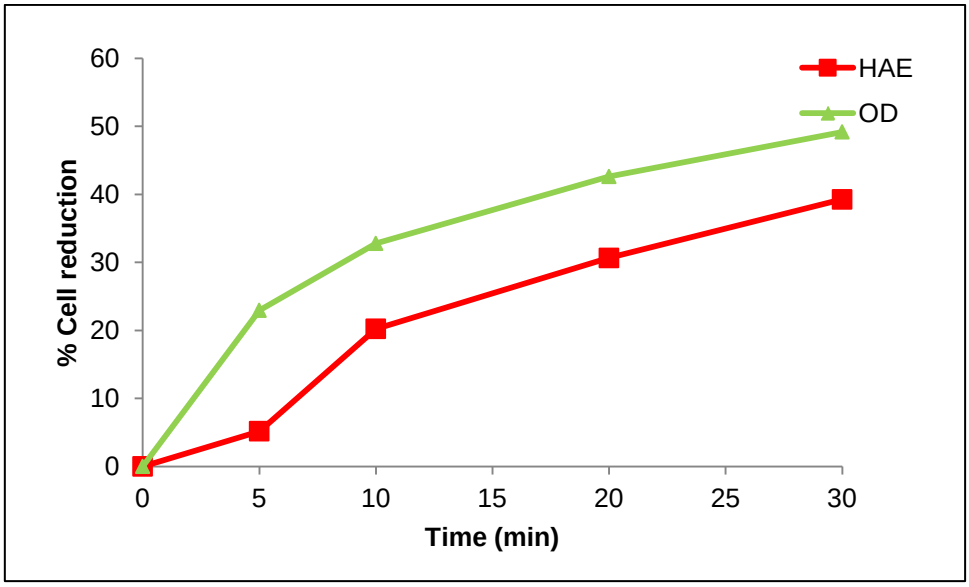


Figure 6.32 Inactivation of 200 mL *Microcystis aeruginosa* using 20 kHz probe for 30 minutes (flow cytometry)

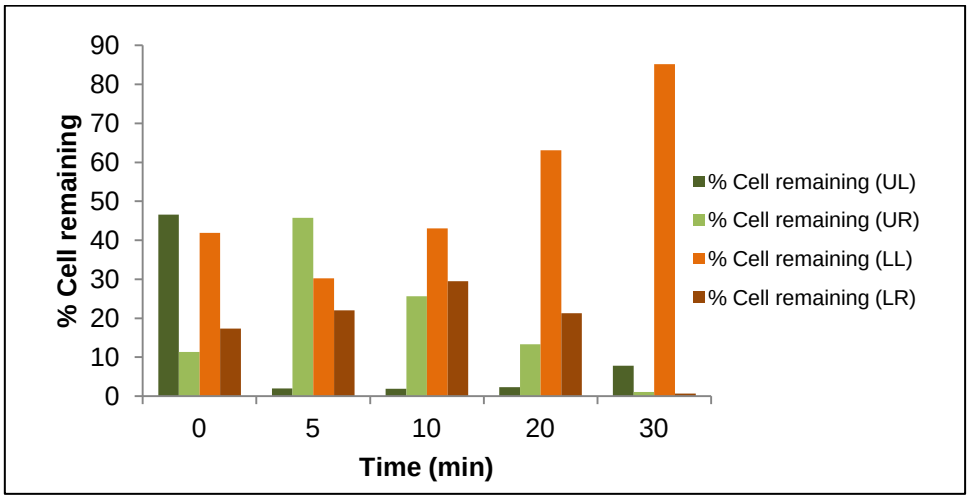
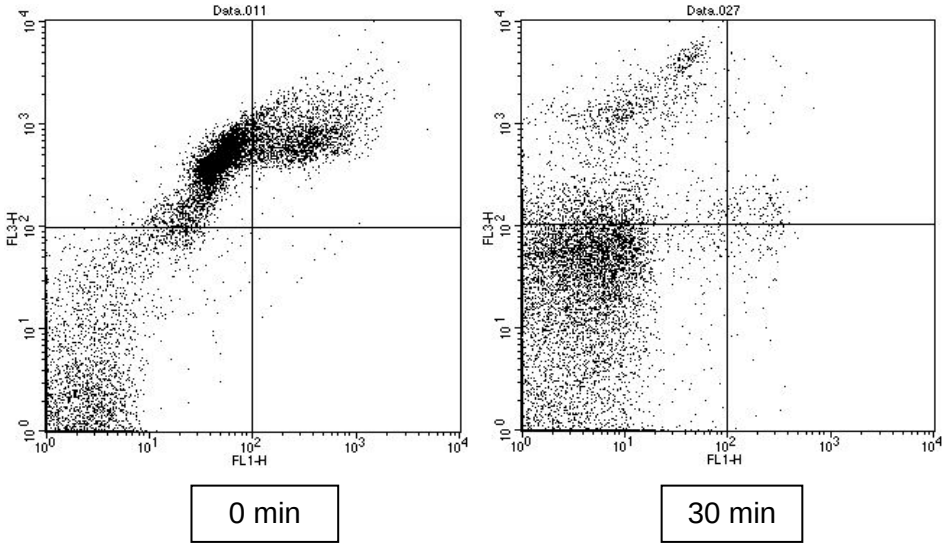


Figure 6.33 Inactivation of 200 mL *Microcystis aeruginosa* using 20 kHz probe for 30 minutes (flow cytometry)



No significant effect of temperature was observed on algal cells in the range 18 − 25ºC. After ultrasonic treatment with the 20 kHz probe (0.0403 Wcm^{-3}), haemocytometer and optical density at 680 nm results were found to both show an inactivation effect (Figure 6.31). UV-Vis spectrophotometer peaks (Appendix 3, Figure 29) around 620 nm decreased with ultrasonic treatment, indicating phycobiliproteins of algae cells was damaged. Fluorometer results (Appendix 3,

Figure 29) showed peaks around 665 nm disappeared with ultrasonic treatment times, which may indicate damage to phycobiliproteins by sonication.

UV-Vis spectrophotometer results and fluorometer results showed that the algae cells were healthy prior to treatment and, as expected, flow cytometry indicated two sub-populations live and dead cells. After sonication flow cytometry using SYTO−9 and PI probes revealed three sub-populations live, dead but also cell debris/particulate matter. With increasing treatment times, live sub-populations reduced and dead sub-populations increased.

6.4.1.2 Inactivation of 200 mL *Microcystis aeruginosa* using 580 kHz bath for 30 minutes

Figure 6.34 Inactivation of 200 mL *Microcystis aeruginosa* using 580 kHz bath for 30 minutes (haemocytometer and spectrophotometer)

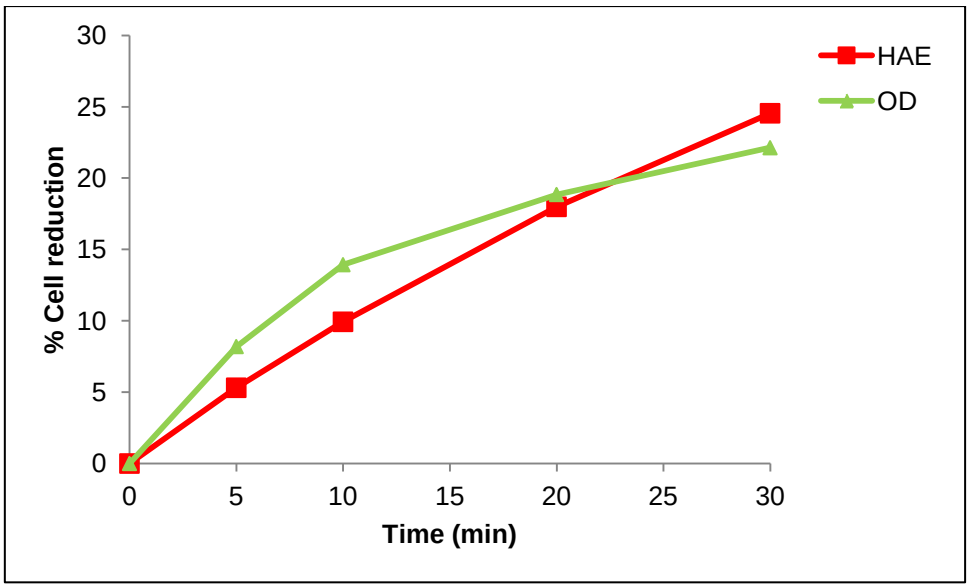


Figure 6.35 Inactivation of 200 mL *Microcystis aeruginosa* using 580 kHz bath for 30 minutes (flow cytometry)

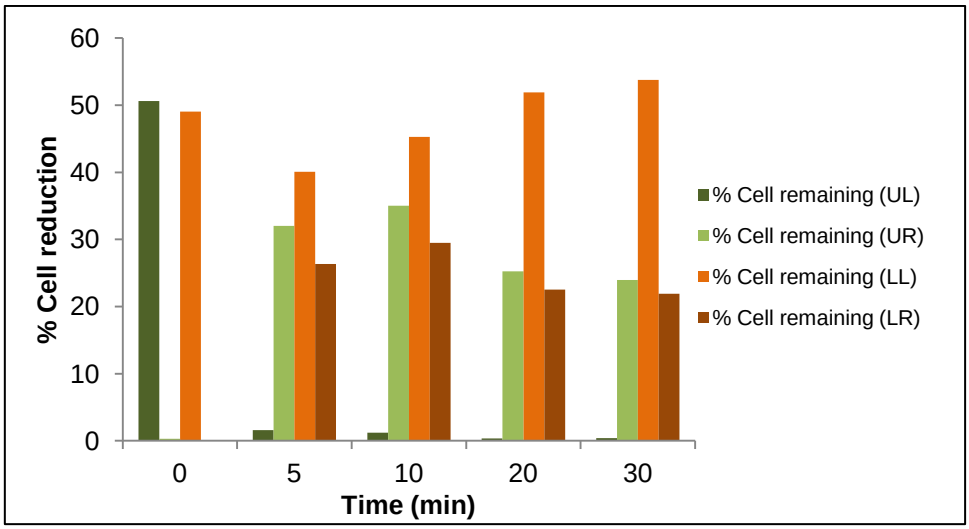
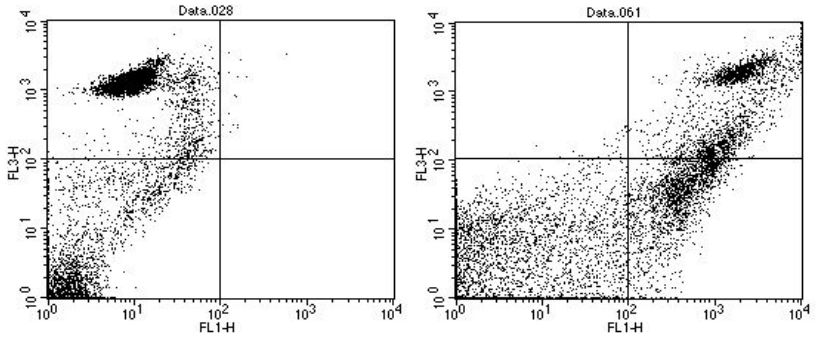


Figure 6.36 Inactivation of 200 mL *Microcystis aeruginosa* using 580 kHz bath for 30 minutes (flow cytometry)



No significant effect of temperature was observed on algal cells in the range 12 − 25ºC. Following ultrasonic treatments with the 580 kHz bath (40% power setting, 0.0042 Wcm^{-3}) both the haemocytometer and optical density at 680 nm results decreased slightly over 30 minutes indicating inactivation. UV-Vis spectrophotometer peaks (Appendix 3, Figure 30) around 620 nm only decreased slightly during treatment, indicating that the phycobiliproteins of algae cells was not badly damaged. Fluorometer results (Appendix 3, Figure 30) also illustrated phycobiliproteins peaks decreased very slightly during treatment. Using SYTO−9 and PI probes for flow cytometry, dead sub-populations increased showing a decrease in algal cell viability.

6.4.1.3 Inactivation of 200 mL *Microcystis aeruginosa* using 1146 kHz bath for 30 minutes

Figure 6.37 Inactivation of 200 mL *Microcystis aeruginosa* using 1146 kHz bath for 30 minutes (haemocytometer and spectrophotometer)

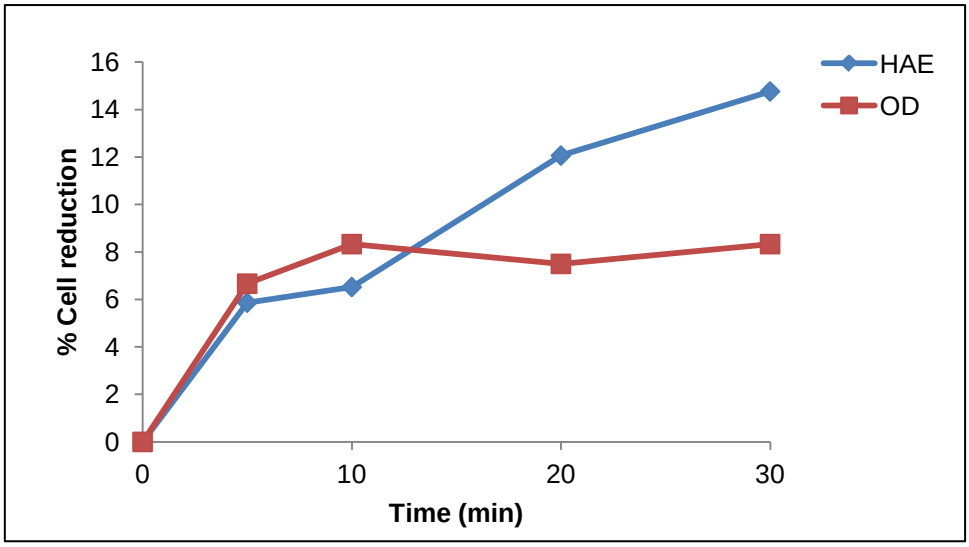


Figure 6.38 Inactivation of 200 mL *Microcystis aeruginosa* using 1146 kHz bath for 30 minutes (flow cytometry)

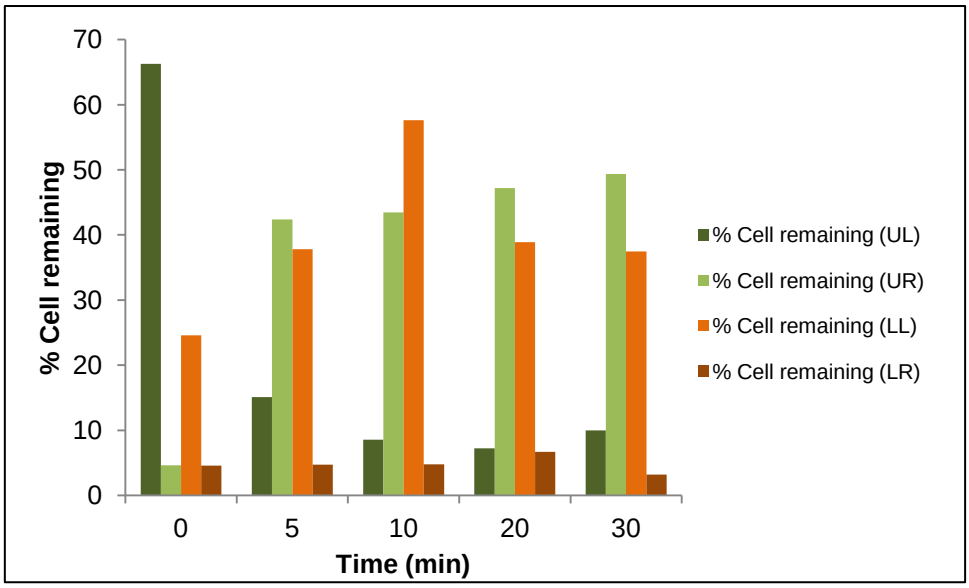
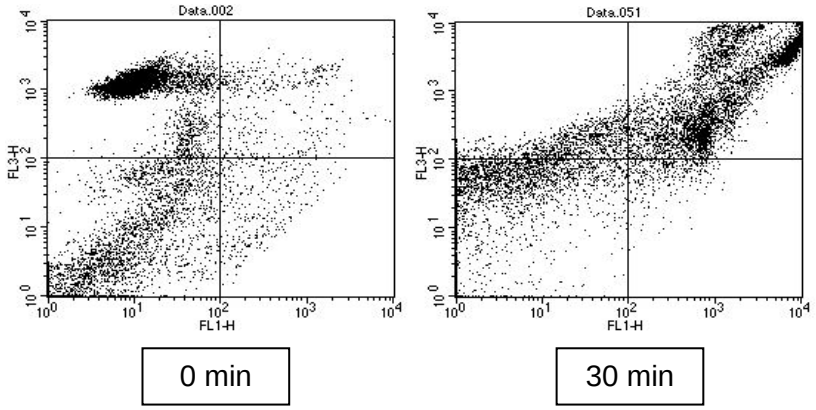


Figure 6.39 Inactivation of 200 mL *Microcystis aeruginosa* using 1146 kHz bath for 30 minutes (flow cytometry)



No significant effect of temperature was observed on algal cells between 12 − 22ºC. The effect of ultrasonic treatments with 1146 kHz bath (40% power setting, 0.0018 Wcm^{-3}) resulted in an optical density at 680 nm decrease over 30 minutes. The haemocytometer results showed a cell reduction over the first 10 minutes treatment but thereafter little change occurred. UV-Vis spectrophotometer peaks (Appendix 3, Figure 31) around 620 nm decreased slightly during treatment, indicating phycobiliproteins in the algae cells was not badly damaged. Fluorometer results (Appendix 3, Figure 31) also showed phycobiliproteins peaks decreasing very slightly during treatment time. Flow cytometry showed live sub-populations reduced and dead sub-populations increased over a 10 minutes treatment time. During the subsequent 20 minutes treatment, live sub-populations increased and dead sub-populations decreased. Over the total 30 minutes treatment, results from flow cytometry (Figure 6.38) indicate declumping had occurred.

Table 6.8 Summary of the effects of ultrasound on 200 mL *Microcystis aeruginosa* algal suspensions

Frequency (kHz)	**20**	**580**	**1146**
Power (Wcm^{-3})	0.0403	0.0041	0.0018
Volume (mL)	200	200	200
Sonication time (minutes)	30	30	30
% HAE	39.25	24.55	14.77
% OD at 680 nm	49.18	22.13	8.33
UV-Vis (620nm, 680nm) Peaks	Decreased	Decreased	Increased
Fluoro (670nm) Peaks	Decreased	Decreased	Increased
FCM live sub-population	Decreased	Decreased	Increased

In general inactivation effects, where algal numbers are reduced, predominate following treatment at a high intensity of 20 kHz (0.0403 Wcm^{-3}). A declumping effect was observed using the 1146 kHz bath and 200 mL (40% intensity, 0.0018 Wcm^{-3}). Haemocytometer counts and optical density at 680 nm results agree well for most experiments, illustrating a reduction in algal cells following treatment with ultrasound.

Figure 6.40 Live and dead sub-populations using flow cytometer

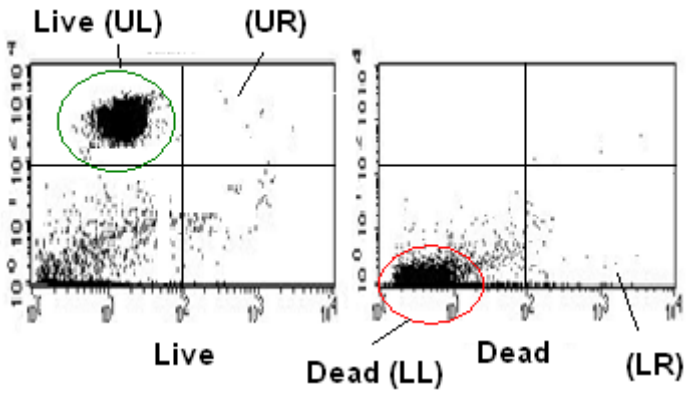
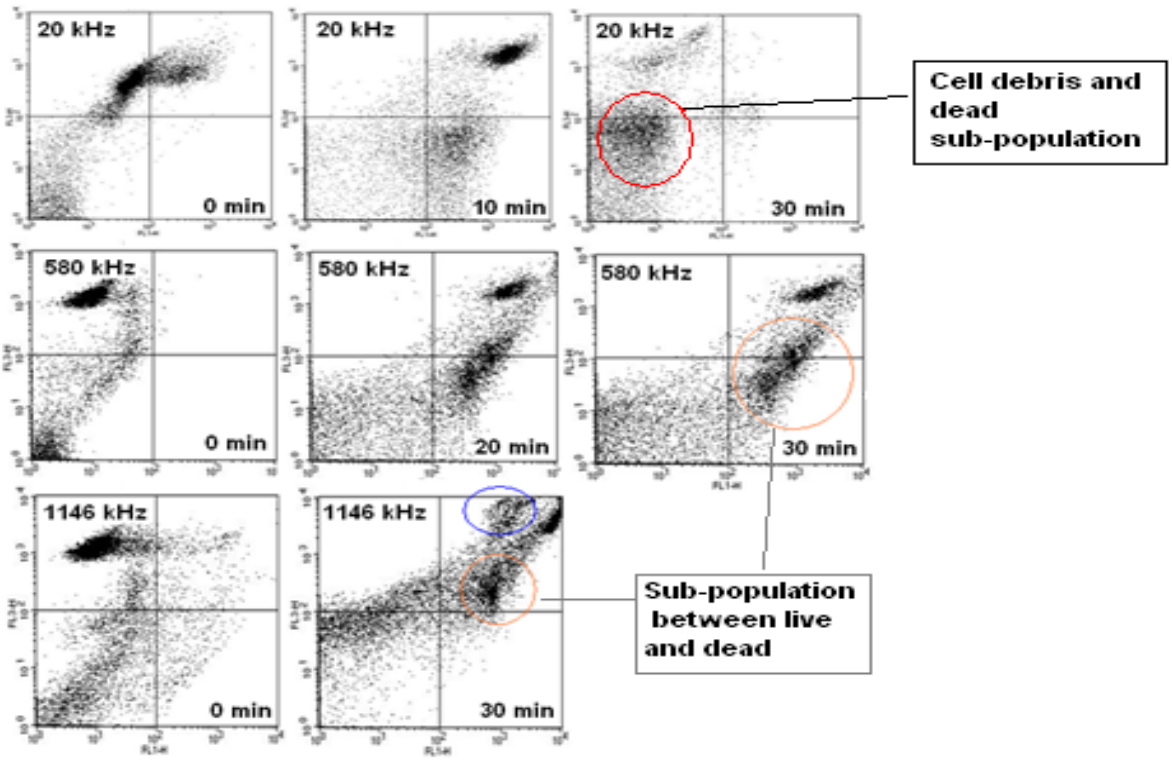


Figure 6.40 indicated that live sub-populations remained in UL (upper left) quadrant and dead sub-populations positioned in the LL (lower left) quadrant.

Figure 6.41 Flow cytometer results for mechanism study using 20, 580 and 1146 kHz



For flow cytometry results, using 20 kHz probe (0.0403 Wcm^{-3}), live sub-populations in UL (upper left, live position) quadrant shifted to LL (lower left, dead position) quadrant, indicating algae cells were inactivated. For the 580 kHz bath (40% intensity, 0.0041 Wcm^{-3}) at 20 minutes treatment, there was a large sub-populations at LR (lower right) quadrant, which was between live and dead sub-populations, indicating the effect of sonication was not a full inactivation. Some algae were between live and dead states (live but not fully metabolically active). This could be due to the low intensity, which can not generate enough mechanical power to disrupt algal cells. For 1146 kHz bath (40% intensity, 0.0018 Wcm^{-3}), most sub-populations remained in UL (upper left) and UR quadrant (upper right), indicating sonication did not inactivate algal cells.

A further comparison was made as sonication results by haemocytometer and optical density at 680 nm are similar but using different parameter settings (Figure 6.41). The flow cytometer results demonstrated differences:

Using 20 kHz probe (0.0403 Wcm^{-3}) at 10 minutes treatment, live sub-populations shifted to UL (upper left) quadrant due to high power.

For the 580 kHz bath (40% intensity, 0.0041 Wcm^{-3}), a large third sub-population appeared at LR (lower right) quadrant following 20 minutes treatment. This is a significantly different result from that obtained using the 20 kHz probe. In both cases the reduction in live cells as monitored by haemocytometer was similar, but flow cytometer indicates that the "dead" algae cells are not completely dead after treatment at 580 kHz. They are live, but probably not in the sense of fully metabolically active. We believe that this may due to the difference in the cavitation effects 20 vs. 580 kHz; in the former case the effects are mainly mechanical and almost instantaneous whereas at 580 kHz a lower mechanical effect is accompanied by the production of a greater amount of free radicals. Ultrasonically generated free radicals (H●, HO● and HOO●) will not chemically disrupt cell walls but will react with membrane components (lipids) eventually resulting in cell lysis but this may require long reaction times (greater than 30 minutes sonication) (Firzzell, 1988: 287−290).

For 1146 kHz bath (40% intensity, 0.0018 Wcm^{-3}), no large sub-population remained in LL (lower left) quadrant, indicating sonication did not inactivate algal cells.

Table 6.9 The effects of ultrasound on 200 mL *Microcystis aeruginosa* algal suspensions with similar results

Frequency (kHz)	**20**	**580**	**1146**
Power (Wcm^{-3})	0.0403	0.0041	0.0018
Sonication time (minutes)	10	20	30
% HAE	20.23	17.99	14.77
% OD at 680nm	32.79	18.85	8.33

Flow cytometer results allow us to draw the following conclusions about the possible mechanisms involved in the effects of sonication on algae:

- At low frequencies but high powers, acoustic cavitation leads mainly to mechanical effect i.e. the high shear forces generated can lead to the direct rupture of cells. At these low ultrasonic frequencies there are less radicals generated than at the higher ranges and so chemical damage to cells is small (Koda, 2009).

- At high frequencies with medium powers, the cavitation collapse energy is smaller than at low frequencies leading to less direct mechanical damage through sheer forces (Joyce, 2010). On the other hand more radicals are produced leading to inactivation but not necessarily cell rupture i.e. the cells are not necessarily metabolically dead.

- At high frequencies but low powers the energy input is sufficient to break algae cell clusters into individual cells, leading to a declumping effect (Joyce, 2003).

6.4.2 Sonication of algae (optical density 0.2 at 680 nm, 5L) using a 20 kHz ultrasonic probe (Southeast University, China) for 30 minutes

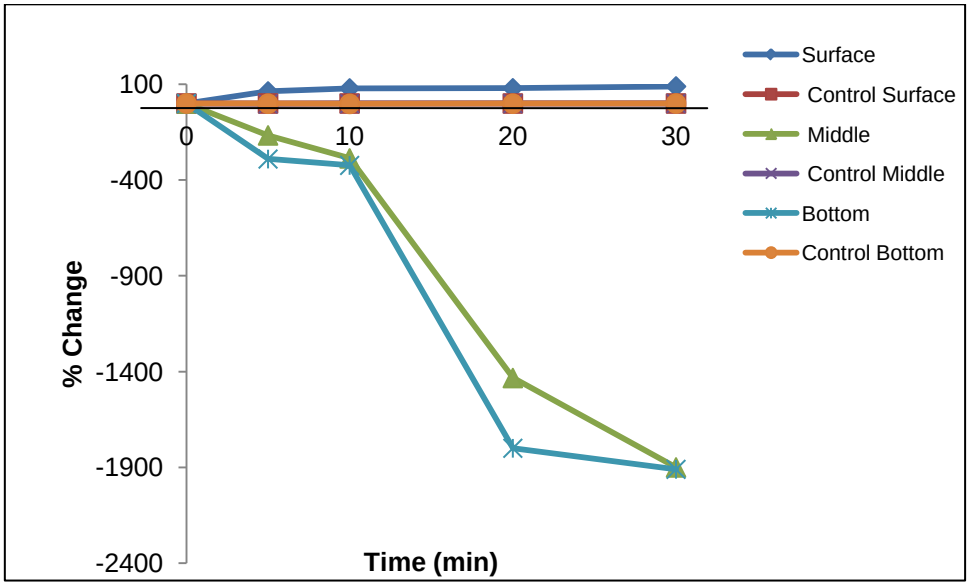
This set of experiments was carried out in China with a group of researchers from South East University (Nanjing) with whom the Sonochemistry Centre is collaborating. The aim was to determine whether ultrasound, applied to a natural floating algae bloom in Lake Taihu would cause blooming algal cells to sink and thus offer a possible treatment for algae infected lakes.

Cells of microalgae were taken from Taihu Lake, the third largest freshwater lake in China (Ding, 2009). The main species of cyanobacteria found in this bloom was *Microcystis.* Experiments and analysis were undertaken within 3 hours of sampling due to the distance between sampling points and the laboratory. The colour and consistency of natural algae water was green and viscous. When observed under a microscope, algae were arranged in groups and it was difficult to observe individual cells.

Figure 6.42 Location of Taihu Lake in the map and ultrasonic probe

The algae was placed in a reactor consisting of a large tank through the bottom of which an ultrasonic probe was positioned (Figure 6.42). Samples were taken from this tank at three different distances from the surface: at 0 (surface), 21.5 (middle) and 43 cm (bottom of tank). A control group was placed in a similar reactor to determine whether algal cells would sink to the bottom without sonication.

Figure 6.43 Inactivation of 4L *Microcystis aeruginosa* using the 20 kHz probe (spectrophotometer)



Surface samples (0 cm from surface) were tested during treatment and showed that the concentration of algae decreased from an optical density at 680 nm of 0.5 to 0.1 at 680nm over 30 minutes. It was noticeable that the algae concentration reduced rapidly after the first 5 minutes of ultrasonic treatment.

Samples taken in the middle of the ultrasonic reactor (21.5 cm from surface), showed an increase in the concentration of algae from an optical density at 680 nm of 0.01 to 0.2 at 680nm over 30 minutes.

Samples taken from the bottom of the ultrasonic reactor (43cm from surface) showed an increase in algae concentration from an optical density at 680 nm (680nm) of 0.01 – 0.22 over 30 minutes treatment.

Control group (no sonication) showed algal cells remained at the water surface. Ultrasonic treatment had an inactivation effect on algal cells with cells sinking down to the bottom of reactor.

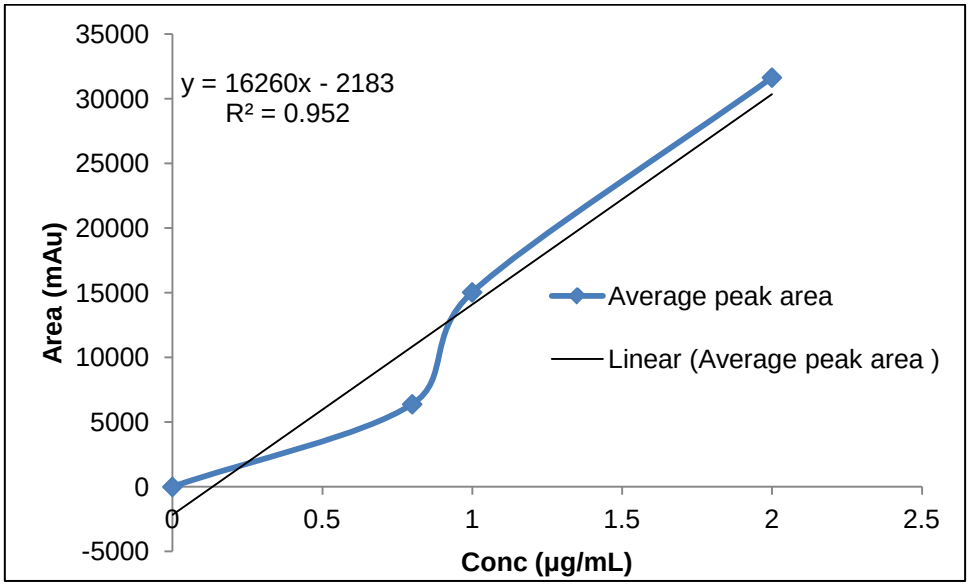
In summary natural algae bloom samples were treated with a 20 kHz probe over 30 minutes. During this time they were found to sink, suggesting that sonication was disrupting gas vacuoles within the cells (Hao, 2004). This indicates that ultrasonic treatment at this frequency and power (20 kHz) may be used to treat natural algae blooms in the field or in natural eutrophic water. These results support our mechanism theories that at low ultrasonic frequencies but high power, ultrasound can control algae blooms by disruption of gas vacuoles through mechanical effects.

6.5 Algae toxin determination using HPLC

Microcystins are named after *Microcystis aeruginosa* which produces these specific toxins. The general structures of microcystins are complex. The signature group is amino acid Adda (2s, 3s, 8s, 9s)-3-amino-9methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (WHO, 2004). In our study, microcystins are determined using high-performance liquid chromatography (HPLC), which is a form of column chromatography which separates, identifies and quantifies compounds. HPLC employs a column to hold the chromatographic packing material, a pump to run mobile phases through the column, and a detector to show the retention times, indicating quantities of the compounds present. The HPLC equipment used in our laboratory is HPLC−PDA/UV (SHIMADZU), which is equipped with pump (LC−20AD), PDA /UV detector (SPD−M20A) and C18 5 µm 2.5×4mm (5 mm) column (SUPELCO).

A HPLC calibration plot of Microcystin-LR (Enzo Life Sciences, ALX−350−012−C050) was produced using concentrations of 0.8, 1.0 and 2.0 µg/mL (Figure 6.44).

Figure 6.44 Calibration of Microcystin-LR using Shimadzu HPLC system



Total microcystin concentration was measured, which includes toxin present inside and outside algal cells. The total Microcystin−LR concentration was 1.00 μg/mL. The HPLC limits of detection in our lab was 0.05μg/mL.

No microcystin was detectable in any of the algal suspensions treated for 30 minutes using a 20 kHz probe (0.040.3 Wcm^{-3}), 580 kHz bath (40% power setting, 0.0041 Wcm^{-3}) or 1146 kHz bath (40% power setting, 0.0018 Wcm^{-3}) suggesting that the toxin concentration released after sonication is lower than 0.05μg/mL.

Results suggested that sonication did not disrupt all algal cells. The detection limit of our HPLC analyses did not reach the WHO guidelines (WHO, 2004) (0.001 μg/mL). Tests showed following 30 minutes sonication, extracellular toxin concentrations did not increase to the levels of total toxin concentration, indicating ultrasonic treatment may not release algal toxins or it may decompose the toxin in water (Ma, 2005).

6.6 Lower limit tests of optical density at 680 nm

6.6.1 Analysis of algae pellet and supernatant using optical density at 680 nm

A 50 mL standard suspension of *Microcystis aeruginosa* with an OD at 680 nm of 0.2 at 680 nm (6.00×10^6 cells per mL) was placed in a 50 mL tube and centrifuged at 5000 RPM for 5 minutes. Following centrifugation the algae pellet and supernatant were retained. The cells number of supernatant was by haemocytometer was 3.0×10^5 per mL and 0.05 by optical density at 680 nm. Average pellet concentration was 0.65 by optical density at 680 nm. Since large numbers of algal cells were in clusters, haemocytometer results were unable to provide an accurate number. Results indicate that algal cell numbers are much higher in the pellet than the supernatant as all cells sink to contribute to the pellet during centrifugation. UV-Vis spectrophotometer and fluorometer results (Appendix 3, Figure 32) also contribute to the algal cell numbers are much higher in supernatant than pellet.

6.6.2 Optical density at 680 nm baseline test

10 mL standard suspension of *Microcystis aeruginosa* was placed in a 10 mL cell breaker and centrifuged at the maximum setting (2000 RPM) for 10 minutes. Following 10 minutes treatment, the disrupted algal suspension showed an increase in optical density at 680 nm from 0.2 to 0.3 since intracellular photosynthetic pigments (chlorophylls, carotenoids, and phycobiliproteins) were released into water after disruption. Following cell disruption, no intact algal cells could be observed in the haemocytometer chamber using microscopy. UV-Vis spectrophotometer results and fluorometer results (Appendix 3, Figure 33) illustrated the shape of phycobiliproteins peaks were not affected.

Results demonstrate mechanical disruption of algal cell release photosynthetic pigments since no intact algal cells could be observed in the haemocytometer chamber. However the peaks of Phycobiliproteins did not reduced when analyzed using UV-Vis spectrophotometer and fluorometer. Results indicate mechanical disruption did not damage photosynthetic pigments. Ultrasonic effects on algal photosynthetic pigments appear to be due to cavitation, which produces high temperatures, high pressures and free radicals which damage photosynthetic algal pigments (Mason, 1994).

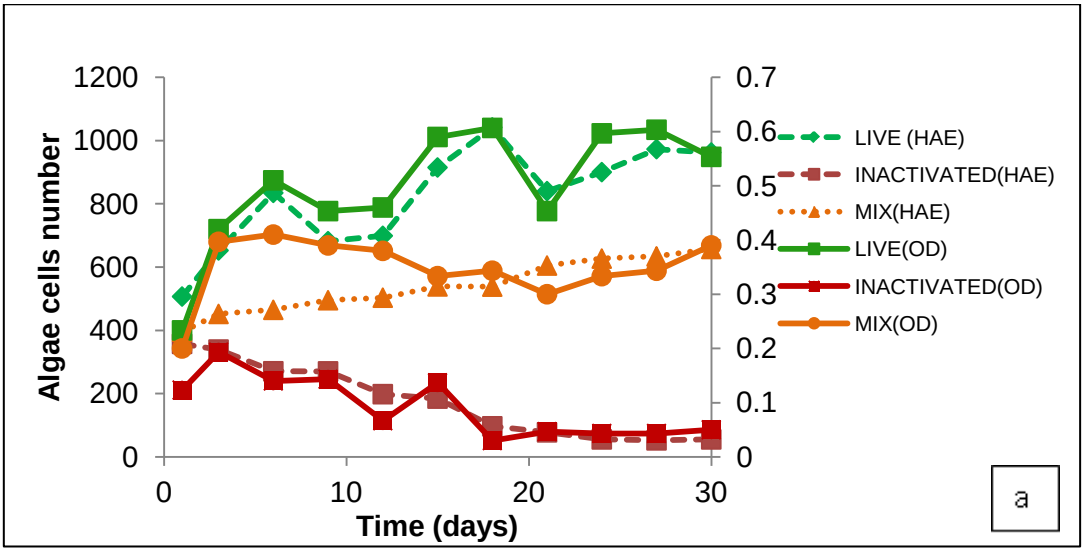
6.7 Resistance test

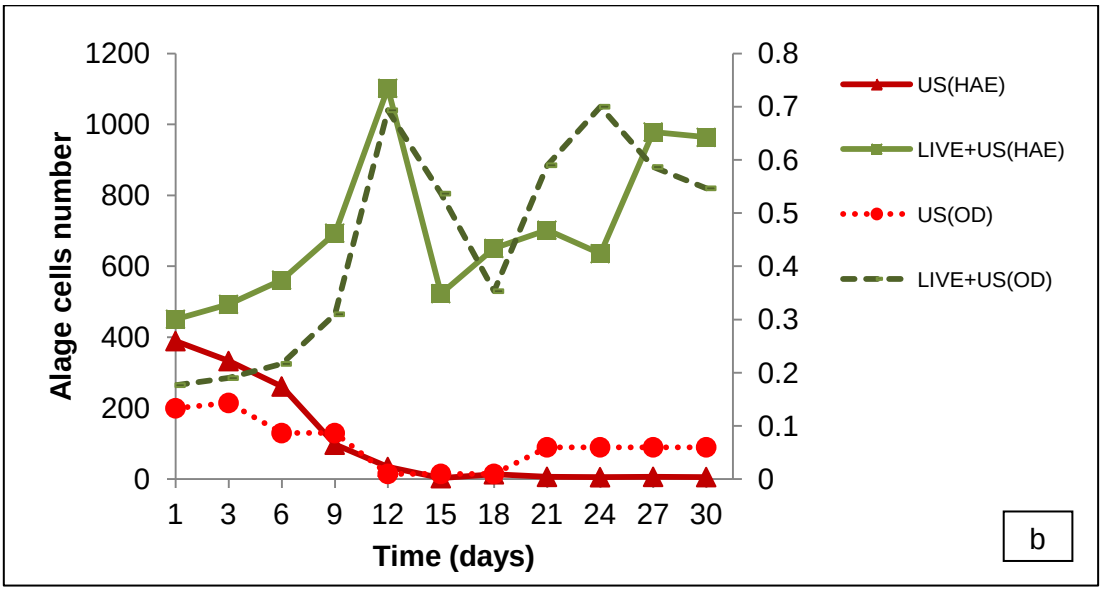
100 mL standard suspension of *Microcystis aeruginosa* was inactivated or killed by boiling for 10 minutes (INACTIVETION). 100 mL inactivated *Microcystis aeruginosa* suspension was added to 200 mL live algae suspension (LIVE+INACTIVATION) and cultured under normal conditions. Standard algal suspensions was also cultured (LIVE). 100 mL sonicated *Microcystis aeruginosa* suspension (SONICATED) was added to 200 mL live algae suspension (LIVE+SONICATED) and cultured under normal conditions. Culture medium of BG11 was added every two days after sampling.

Results indicated that LIVE algae cells grew rapidly over 30 days in culture. The concentration of inactivated algae fluctuated but decreased continuously, except for some small fluctuations on day 3 and 15. Mixed populations of LIVE+DEAD algal cells kept growing but at a slower rate than the live group. Cell numbers of the sonicated algae continued to decrease over 30 days culture. Mixed population of LIVE+SONICATED algae showed an increase in the number of algal cells but the concentration was lower than LIVE populations. Haemocytometer and optical density at 680 nm results correlated well. UV-Vis spectrophotometer and fluorometer results also confirmed similar trends.

These resistance tests demonstrate fresh algae cells kept growing under our culture conditions, which was observed using haemocytometry and optical density at 680 nm. Dead algae cells cannot regrow after inactivation. When live algal cells were mixed with dead cells, they will continue to grow, but at a lower rate than live algal cells. When algae were sonicated with the 20 kHz probe (0.0179 Wcm^{-3}), no re-growth was observed. When live algae were mixed with sonicated algae, cells continue to grow but the concentration was lower than untreated algae. By comparison when live algae were mixed with dead algae as well as sonicated algae growth can be inhibited. For practical applications, sonicated or treated algal cells may offer a solution to inhibit growth of live algae cells in ponds or lakes.

Figure 6.45 Resistance test on *Microcystis aeruginosa* for 30 days (haemocytometer and spectrophotometer)





7.0 Conclusions

Power ultrasound was studied to determine its effects on *Microcystis aeruginosa* at small and medium laboratory-scales. Flow cytometry was employed in order to study the mechanism of the effects of ultrasound on algae.

7.1 Sonication of *Microcystis aeruginosa* at 200 and 400 mL

Microcystis aeruginosa was sonicated using 200 and 400 mL suspensions with an optical density at 680 nm of 0.2 at 680nm to assess the effect of different parameters (volume, intensity, frequency and sonication time) on algae removal or inactivation. This was carried out to determine the settings required for optimum algae removal with ultrasound in terms of energy costs.

The frequencies employed were 20, 40, 580 (40%, 80%, and maximum intensity), 864 (40%, 80% and maximum) and 1146 kHz (40%, 80% and maximum) over a 30 minute treatment. The calorimetry results are showed in Table 7.1.

Table 7.1 Calorimetry results

Equipment	Frequency (kHz)	Power	Volume (mL)	Power density (Wcm^{-3})
20 kHz probe	20		200	0.0179
			400	0.0226
40 kHz bath	40		200	0.0214
			400	0.0466
Multi-frequency bath	580	40%	200	0.0018
			400	0.0017
		80%	200	0.0216
			400	0.0198
		Max	200	0.0493
			400	0.0640
	864	40%	200	0.0042
			400	0.0060
		80%	200	0.0166
			400	0.0243
		Max	200	0.0576
			400	0.0929
	1146	40%	200	0.0026
			400	0.0016
		80%	200	0.0124
			400	0.0196
		Max	200	0.0248
			400	0.0625

The results were also analysed in terms of process efficiency (Mason 1994) where ultrasonic power is taken into account (Mason 1994). This approach uses a concept of ultrasonic 'dosage' where power and exposure time are considered. In the case of treatment at different powers but with the same treatment time of 30 minutes, the efficiency is calculated using the formula below:

Efficiency = % reduction / intensity

A part of this work, involving a study of efficiency, has already been published and is

summarised in Table 7.2 (Joyce, 2010). The order of effectiveness in terms of frequency was found to be 40 < 20 < 1146 < 580 < 864 kHz. However, in terms of efficiency the order of reduction is not quite the same and increases in the order 20 < 1146 < 864 < 580 kHz. It is interesting to note that the order of reduction in term of efficiency is different from the order of reduction in term of frequency. This emphasises the need to include efficiency when analysing results (Joyce, 2010).

The results obtained at 40 kHz (0.0213 Wcm^{-3}) treatment resulted in a declumping effect and for this reason it is excluded.

Table 7.2 Ultrasonic treatment using 200 ml algae (Joyce, 2010)

Table 7.2 has been removed due to third party copyright. The unabridged version of the thesis can be viewed at the Lanchester Library, Coventry University

A more complete list of the results are displayed using % algal cell reduction and efficiency in Table 7.3 and 7.4

Table 7.3 Effect of ultrasound on 200 mL *Microcystis aeruginosa* algal suspensions

Freq. (kHz)	Power Setting	Intensity (Wcm^{-3})	% Reduction (HAE)	Efficiency (HAE)	% Reduction (OD at 680 nm)	Efficiency (OD at 680 nm)
20		0.0178	10.02	562.92	5.04	283.15
40		0.0214	−0.28	N/A	−4.20	N/A
580	40%	0.0018	13.81	7672.22	13.22	7344.44
580	80%	0.0216	59.33	2746.76	36.84	1705.56
580	maximum	0.0493	44.11	894.93	47.37	960.85
864	40%	0.0042	34.55	8226.19	21.05	5011.90
864	80%	0.0166	67.60	4072.29	33.61	2024.70
864	maximum	0.0576	81.09	1407.81	47.01	816.15
1146	40%	0.0026	16.75	6442.31	8.33	3203.85
1146	80%	0.0124	66.19	5337.90	23.89	1926.61
1146	maximum	0.0248	91.54	3691.13	44.63	1799.60

Ultrasound can induce two different effects on algal cells; inactivation at high power and de-agglomeration at low power (Joyce, 2010). Using 200 mL volume, the highest reduction was obtained using 1146 kHz (maximum power setting, 0.0248 Wcm^{-3}) and the lowest was obtained at 40 kHz. The highest reduction as measured by optical density at 680 nm was observed at 580 kHz (maximum power setting, 0.0493 Wcm^{-3}) and the lowest is using 40 kHz bath (0.0214 Wcm^{-3}). At low frequencies, 40 kHz bath (0.0214 Wcm^{-3}) results demonstrated a de-agglomeration effect. At high frequencies, for each frequency, remaining algal concentration decreased in the following order:

40% < 80% < maximum power setting

Frequency studies revealed that high frequencies were able to produce high algal cell reductions when measured using haemocytometer and optical density at 680 nm. It is interesting to note that the reduction results at 580 kHz (40% power setting, 0.0018 Wcm^{-3}) and 1146 kHz (40% power setting, 0.0026 Wcm^{-3} were similar to those obtained using a 20 kHz probe (0.0178 Wcm^{-3}), which may due to the similar intensities.

Three frequencies were found to be operating at similar intensities: 20 kHz (0.0178 Wcm^{-3}), 864 kHz (80% power setting, 0.0168 Wcm^{-3}) and 1146 kHz (80% power setting, 0.0124 Wcm^{-3}). Despite this similarity, the higher frequencies gave significantly higher removal rates by haemocytometer and optical density at 680 nm.

As the frequency of sonication is increased the rarefaction time during the acoustic cycle reduces. This means that the production of cavitation bubbles becomes more difficult to achieve in the available time and so greater sound intensities (power) are required to achieve cavitation (Mason, 2002). Raising the frequency of sonication also increases the free radical production through the decomposition of water induced by cavitation bubble collapse (Petrier, 1992). Free radicals oxidize cell walls inactivating algal cells. Sonication at higher frequencies inactivates algae by this route in addition to the mechanical forces of cavitational collapse.

Based upon power measurements these studies can be sub−divided into three ranges:

1. Low intensity (0.0018 − 0.0042 Wcm^{-3}): 580 kHz (40% power setting, 0.0018 Wcm^{-3}), 864 kHz (40% power setting, 0.0042 Wcm^{-3}) and 1146 kHz (40% power setting, 0.0026 Wcm^{-3})

2. Medium intensity (0.0124 − 0.0248 Wcm^{-3}): 20 kHz (0.0178 Wcm^{-3}), 40 kHz (0.0214 Wcm^{-3}), 580 kHz (80% power setting, 0.0216 Wcm^{-3}), 864 kHz (80% power setting, 0.0166 Wcm^{-3}), 1146 kHz (80% power setting, 0.0124 Wcm^{-3}) and 1146 kHz (maximum power setting, 0.0248 Wcm^{-3})

3. High intensity (0.0493 − 0.0576 Wcm^{-3}): 580 kHz (maximum power setting, 0.0493 Wcm^{-3}) and 864 kHz (maximum power setting, 0.0576 Wcm^{-3})

In the low intensity range, although only small reductions are obtained at 580 kHz (40% power setting, 0.0018 Wcm^{-3}), 864 (40% power setting, 0.0042 Wcm^{-3}) and 1146 (40% power setting, 0.0026 Wcm^{-3}). However at these frequencies the efficiencies are relatively high.

In the medium intensity range, reduction was measured using a haemocytometer and increases in the following order:

40 < 20 < 580 (80%) < 1146 (80%) < 864 (80%) < 1146 (maximum)

A slight difference in order occurs when the remaining algal concentration is measured using optical density at 680 nm:

40 < 20 < 1146 (80%) < 864 (80%) < 580 (80%) < 1146 (maximum)

Although the optical density at 680 nm results are slightly different from haemocytometer results, it is clear that 1146 kHz (maximum power setting, 0.0248 Wcm^{-3}) produces the best reduction.

When the efficiency of the process is estimated using a haemocytometer it increased in the following order (note that the 40 kHz results were omitted from these calculations due to the fact that it gave rise to declumping):

20 < 580 (80%) < 1146 (maximum) < 864 (80%) < 1146 (80%)

The corresponding order of efficiency by optical density at 680 nm differed slightly:

20 < 580 (80%) < 1146 (maximum) < 1146 (80%) < 864 (80%)

It is interesting to note that 1146 kHz (maximum power setting, 0.0248 Wcm^{-3}) using the multi-frequency bath gave the highest removal rate by haemocytometer and optical density at 680 nm, but the efficiency was only the third highest. This reinforces the need to include efficiency when analysing results of this type. Efficiency determines the optimal ultrasonic parameter settings in terms of cost in energy consumption as well as effectiveness. Under these conditions 864 kHz (80% power setting, 0.0166 Wcm^{-3}) and 1146 (80% power setting, 0.0248 Wcm^{-3}) appear to be the best control parameters.

In the high intensities range, both 580 kHz (maximum power setting, 0.0493 Wcm^{-3}) and 864 kHz (maximum power setting, 0.0576 Wcm^{-3}) achieved high inactivation rates, but the efficiency was relatively low when compared with results at 40% and 80% power settings.

Table 7.4 Effect of ultrasound on 400 mL *Microcystis aeruginosa* algal suspensions

Freq. (kHz)	Power Setting	Intensity (Wcm^{-3})	% Reduction (HAE)	Efficiency (HAE)	% Reduction (OD at 680 nm)	Efficiency (OD at 680 nm)
20		0.0226	12.68	561.06	6.90	305.31
40		0.0466	6.18	132.62	4.31	92.49
580	40%	0.0017	13.13	7723.53	13.22	7776.47
580	80%	0.0198	40.89	2065.15	21.31	1076.26
580	maximum	0.0640	21.08	329.38	14.53	227.03
864	40%	0.0060	26.66	4443.33	11.40	1900.00
864	80%	0.0243	32.99	1358.02	33.61	1383.13
864	maximum	0.0929	79.56	856.40	61.11	657.80
1146	40%	0.0016	4.14	2756.25	−1.61	N/A
1146	80%	0.0196	27.07	1381.12	20.31	1036.22
1146	maximum	0.0625	20.55	328.8	20.45	327.2

Using 400 mL volume, the highest reduction by haemocytometer and optical density at 680 nm was achieved using 864 kHz (maximum power setting, 0.0929 Wcm^{-3}) and the lowest was obtained at 1146 kHz (40% power setting, 0.0016 W/cm^{-3}).

In general, these results show that high frequencies tended to achieve high reductions. At low frequencies, the reductions indicated by the haemocytometer and optical density at 680 nm were low.

For each frequency the removal rates increased in the order of increasing power setting. As with the 200 mL results the studies can be sub−divided into three ranges:

1. Low intensity (0.0016 − 0.0060 Wcm^{-3}): 580 kHz (40% power setting, 0.0017 Wcm^{-3}), 864 kHz (40% power setting, 0.0060 Wcm^{-3}) and 1146 kHz (40% power setting, 0.0016 Wcm^{-3})

2. Medium intensity (0.0196 − 0.0466 Wcm^{-3}): 20 kHz (0.0226 Wcm^{-3}), 40 kHz (0.0466 Wcm^{-3}), 580 kHz (80% power setting, 0.0198 Wcm^{-3}) and 864 kHz (80% power setting, 0.0243 Wcm^{-3})

3. High intensity (0.0640 − 0.0929 Wcm^{-3}): 580 kHz (maximum power setting, 0.0640 Wcm^{-3}), 864 kHz (maximum power setting, 0.0929 Wcm^{-3}) and 1146 kHz (maximum power setting, 0.0625 Wcm^{-3})

At the low intensity ranges, for 580 kHz (40% power setting, 0.0017 Wcm^{-3}) and 864 kHz bath (40% power setting, 0.0060 Wcm^{-3}), removal rates by haemocytometer and optical density at 680 nm were low. In the case of 1146 kHz (40% power setting, 0.0016 Wcm^{-3}), the reduction by optical density at 680 nm was −1.61%, indicating a declumping effect.

In the medium intensity ranges, reduction using haemocytometer increases in the following order:

40 < 20 < 1146 (80%) < 864 (80%) < 580 (80%)

However there was a slight difference when measuring reduction using optical density at 680 nm which increased in the following order:

40 < 20 < 1146 (80%) < 580 (80%) < 864 (80%)

Although slightly different in terms of order both sets of results show that the most effective settings are 580 kHz (80% power setting, 0.0198 Wcm^{-3}) and 864 kHz (80% power setting, 0.0243 Wcm^{-3}). The efficiency was calculated to help determine optimum parameter settings. Efficiency, as indicated by haemocytometer readings increased in the following order:

40 < 20 < 864 (80%) < 1146 (80%) < 580 (80%)

Efficiency by optical density at 680 nm increased in the same order:

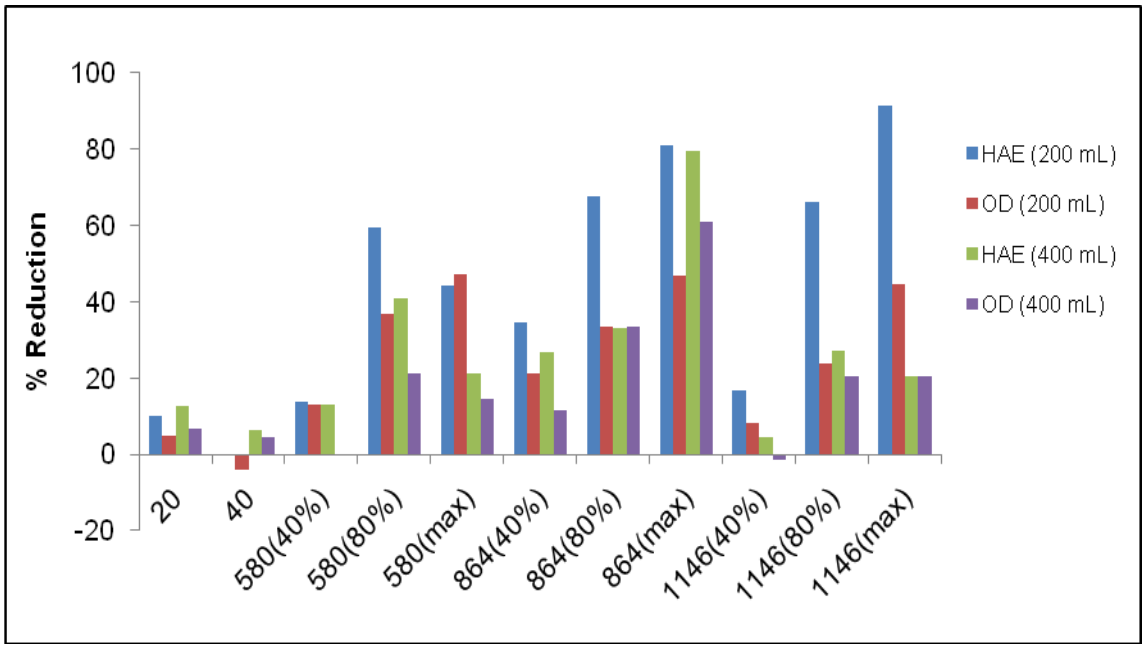
40 < 20 < 864 (80%) < 1146 (80%) < 580 (80%)

Efficiency by haemocytometer and optical density at 680 nm correlated well, indicating that 580 kHz (80% power setting, 0.0198 Wcm^{-3}) is the most effective for algae control.

From results at the high intensity range, 864 kHz (maximum power setting, 0.0060

Wcm^{-3}) achieved the highest reduction by haemocytometer and optical density at 680 nm with 400 mL, but the efficiency was relatively low. For 580 kHz (maximum power setting, 0.0640 Wcm^{-3}) and 1146 kHz (maximum power setting, 0.0625 Wcm^{-3}), removal rates and efficiency by haemocytometer and optical density at 680 nm were also low.

Figure 7.1 Percentage cells reduction of *Microcystis aeruginosa* at 200 and 400 mL



Using both the low frequency probe and 40 kHz bath the ultrasonic inactivation is low but the former is slightly more efficient possibly because the energy is introduced directly into suspension and the effects were better at higher powers. With the 40 kHz bath and 200 mL volume declumping effects are observed.

The directly measured ultrasonic effects on algae using high frequencies were variable although the effects were similar when the intensities used were comparable. The most pronounced inactivation result was obtained with 1146 kHz (maximum intensity) and 200 mL suggesting that the reduction in algal cells improves with increasing frequency. At higher volumes, the ultrasonic inactivation effect was reduced. Generally, high ultrasonic frequencies and intensities achieved high reduction rates for algae which can be attributed to free radical production and cavitation effects on the gas vacuoles. High frequencies at low intensities resulted in a relatively low inactivation effect. However when the efficiency of sonication was

taken into account the results indicated that low powers at high frequencies were more efficient than high powers.

These results provided a guide to the ultrasonic parameter settings which are important to optimise algae reduction with ultrasound based on energy costs.

7.2 Sonication of *Microcystis aeruginosa* at a medium laboratory−scale

Studies were undertaken on small or medium pilot-scale (industrial) ultrasonic equipment all of which were low frequency. Experiments were undertaken to assess the control of algae using the following volumes and equipment: Sonolator (Sonic Corporation, 5L), Dual Frequency Reactor (DFR, 16 kHz and 20 kHz, Advanced Sonics LLC, 1L and 3.5 L) and a Vibrating Tray (20 kHz Advanced Sonics LLC, 1.5L). Some experiments were also carried out using an ultrasonic probe desiged and constructed at Southeast University, China (20 kHz, 4L).

Tests with the Sonolator employed 5L standard suspensions of *Microcystis aeruginosa* (OD at 680 nm of 0.2) sonicated for 5 hours with a cooling system to keep the temperature below 30ºC. Haemocytometer and spectrophotometer results showed that over a period of 30 minutes the concentration of algae reduced by 58.48% by haemocytometer and 26.55% by optical density at 680 nm during treatment.

Tests involving the 16 and 20 kHz DFR were run using two modes: circulating and static at two power settings: 40% and 60%. In the circulating mode at 40% power setting, the concentration of algae fluctuated over 60 minutes treatment time but did show a slight overall reduction. For circulating mode at 60% power setting, the sonication effect gave a slightly better inactivation. The static system at 40% intensity gave a more effective result than circulation with a continuous but slight decrease in concentration over 10 minutes treatment. For static modes at 60% intensity, the effect was much greater leading to a significant decrease in the concentration of algae cells after 10 minutes treatment.

Following ultrasonic treatments with the 20 kHz vibrating tray, the sonication effect resulted in a small but continuous reduction over 6 minutes treatment.

Table 7.5 Effect of ultrasound on *Microcystis aeruginosa* algal suspensions at small or medium lab-scale

	Volume (Litre)	Resident time (minutes)	% Reduction (HAE)	% Reduction (OD at 680 nm)	% FCM (live remaining)
Sonolator	5	1.09	85.6	22.67	–
DFR (Circulating, 40% power setting)	3.5	17	0.07	1.03	40.92
DFR (Circulating, 60% power setting)	3.5	7	33.48	45.05	14.60
DFR (Static, 40% power setting)	1	10	27.84	6.14	28.96
DFR (Static, 60% power setting)	1	10	79.25	60.44	1.10
Vibrating Tray	1.5	6	11.25	8.65	24.61

Although the Sonolator gave the highest reduction in algal cells as indicated using haemocytometer, the optical density at 680 nm result was much lower. Sonolator results indicated that hydrodynamic cavitation can control algae in water. However, based on our experiments this requires a treatment time longer than 5 hours.

Using 16 and 20 kHz DFR, the most effective inactivation effect was obtained in the static mode (60% power setting) by haemocytometer and optical density at 680 nm and the lowest was obtained using the circulating mode (40% power setting). The reason for the greater inactivation is that in the static test the whole sample is in the ultrasonic zone of the reactor for the entire time whereas in the circulating mode a larger volume is used and only part of the suspension is in the active zone of the reactor at any one time.

The laboratory-scale vibrating tray resulted in a low reduction as assessed by both haemocytometer and optical density at 680 nm. This system may have the potential

for large-scale treatment since it has been used industrially for washing processes e.g. coal beneficiation (Mason and Lorimer, 2002).

As might be expected, the overall deactivation results using larger-scale low frequency systems ≤ 20 kHz appeared to be less than those employed to treat 200 and 400 mL volumes at higher frequencies. Medium-scale systems showed the same two effects on algae; declumping of algae groups into individual cells and inactivation. Damage to the chlorophyll A in algae was confirmed using UV-Visible spectrophotometer, fluorometer and flow cytometer.

This study is the first to explore the use of large commercially available ultrasonic systems for the control of algae but not large enough for use in the water industry. Results show that with further Scale-up and optimisation similar systems could be developed and made available for industrial scale algae treatment.

One significant issue that has not yet been addressed is that once ultrasonic treatments as a control measure are turned off, algal blooms regrow in the following year (Lee, 2002). Algae bloom management involves the combination of a number of approaches and requires maintenance and control of nutrient levels in water, balancing ecosystems using biomanipulation techniques and applying advanced effective control or removal methods.

7.3 A study of the mechanisms of ultrasonic effects on algae

The mechanism of ultrasonic algae control has also been studied in this work. Mechanistic studies help to determine how ultrasound works during treatment and more importantly allows for the selection of the most effective and energy saving parameters to be employed for treatment. Our mechanistic study was based on the analysis results of reduction in algal cell numbers, indicated by UV-Visible spectrometry, fluorometry and flow cytometry. UV-Visible spectrophotometer was used to determine the chlorophyll A levels in algae. Chlorophyll A acts as a photosynthetic organ producing food for algae growth, so reductions in chlorophyll A can result in inhibition of natural algae growth. If algal cells are injured or stressed, the chlorophyll A peak at 680nm decreases. A fluorometer (RF−5301PC − SHIMAZU) was also employed to measure the chlorophyll A fluorescence of algae, an additional measure of algae condition.

Flow cytometry (FCM) is a useful tool for automated algal cell counting, which reduces analysis time and minimizes human error. Direct analysis provides information on the cell size; biomass and condition of cells (live, injured, dead and residual cell debris or particulate matter). Specific stains and fluorescent molecular probes are required to undertake microalgae research using flow cytometry. Forward scatter (FSC) and side scatter (SSC) detectors reflect or refract light when a particle passes through the excitation beam. These parameters are dependent on cell size, but SSC is also affected by cell surface and internal cellular structure therefore detecting dead or injured cells (Maria, 2004).

Table 7.6 Effect of ultrasound on 200 mL *Microcystis aeruginosa* algal suspensions for mechanism study

Frequency (kHz)	**20**	**580**	**1146**
Power (Wcm^{-3})	0.0410	0.0042	0.0016
Volume (mL)	200	200	200
Sonication time (min)	30	30	30
% reduction (HAE)	39.25	24.55	14.77
% reduction (OD at 680 nm)	49.18	22.13	8.33
UV-Vis (620nm, 680nm) peaks	Decreased	Decreased	Increased
Fluoro (660nm) peaks	Decreased	Decreased	Increased
FCM live sub-population	Decreased	Decreased slightly	Increased

The results of these studies at three different frequencies are shown in Table 7.6. Using the 20 kHz probe (0.0410 Wcm^{-3}), UV-Visible spectrophotometer results showed chlorophyll A peaks reduced over 30 minutes treatment. Fluorometry measurements also indicated a reduction in chlorophyll A concentration and flow cytometry revealed a reduction in live sub-populations during treatment, indicating an overall inactivation effect. With 580 kHz (40% power setting, 0.0042 Wcm^{-3}) similar but smaller inactivation effects were observed. In the case of 1146 kHz (40% power setting, 0.0016 Wcm^{-3}) very small reductions were observed using a haemocytometer and by optical density at 680 nm. Chlorophyll A peaks using UV-Visible spectrophotometer and fluorometer both showed a slight increase over this treatment time indicating possible declumping. This was backed up by flow cytometry where a slight increase in live sub-populations was observed during treatment. For all above tests, haemocytometer and flow cytometer results corresponded; indicating flow cytometry is a reliable, rapid and accurate method of analysis.

From the evidence, there are several possible contributions to the overall mechanism through which ultrasound can influence algae removal as identified below, but it is likely that more than one of these is in operation at any one time depending on the conditions used:

1. Acoustic cavitation via mechanical effects can induce sufficient shear forces to directly rupture algae cells.
2. Acoustic cavitation can directly rupture gas vacuoles within the cells again via mechanical effects. This will result in a loss in buoyancy (sinking). Once algae cells have sunk below the surface they are unable to undergo photosynthetic processes. This will result in food shortage within the cell resulting in death.
3. At higher ultrasonic frequencies the mechanical energy of cavitation is less but a larger proportion of free radicals is produced from the ultrasonic degradation of water (Mason, 1999: 10-15). The free radicals can chemically attack and weaken the algae cell walls.
4. At higher frequencies the free radicals can also damage Chlorophyll A leading to a loss in photosynthetic viability.
5. At low powers ultrasonic energy can cause the declumping of algae.

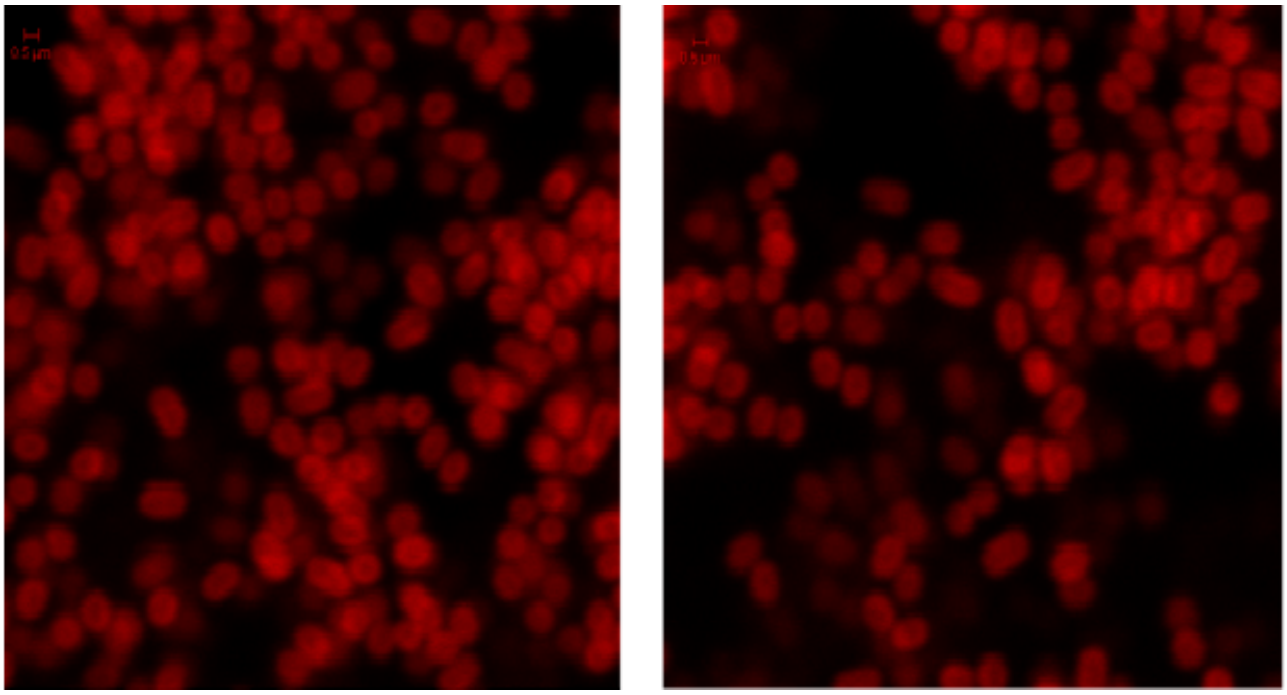
Confocal laser scanning microscopy was employed to observe the effects of ultrasound on the health of the overall algae population. This method proved ideal in terms of assessing the live / dead cells present but unfortunately the highest magnification objective lens available (40X) which was not powerful enough to observe the gas vacuoles in the algal cells so it was not possible to determine the level of damage to them following sonication (Figure 7.2). However we do have evidence from the field tests in China that following sonication at 20 kHz the algae cells sank, suggesting vacuole disruption.

Flow cytometry was able to demonstrate the declumping effect that occurred at 1146 kHz (40% power setting, 0.0016 Wcm^{-3}) and to differentiate the cell reduction at different frequencies in terms of differing proportions of mechanical vs. chemical effects. Unlike 20 kHz ultrasound, the higher frequencies e.g. 580 kHz produced a sub-population which was between live and dead cells (live but not fully metabolically active). This can be attributed to free radical attack on the cells with less direct mechanical damage.

Based on our literature review (Section 4.0) few researchers have investigated small-scale studies (200−1000 mL). Hao *et al.* applied high and low frequencies of 1.7 MHz (intensity 0.07 Wcm^{-3}) and 20 kHz (intensity 0.014 Wcm^{-3}) to treat 800 mL *Spirulina plantensis* (Hao, 2004). Zhang *et al.* investigated a range of ultrasonic frequencies to control *Microcystis aeruginosa* using 1000 mL (Zhang, 2006). Joyce *et al.* investigated the effects of different ultrasonic frequencies and intensities on

Microcystis aeruginosa using 200 mL (Joyce, 2010). Large-scale ultrasonic applications on natural ponds and lakes have only been reported by Lee (2002), Ahn (2003) and Ahn (2007), but to date no work has been undertaken using medium-scale commercial ultrasonic equipment. This work reports on medium-scale equipment for the first time.

Figure 7.2 Confocal laser scanning microscopy results with *Microcystis aeruginosa* using 1146 kHz (maximum power setting) and 200 mL (before and after treatment, autofluorescence)



Our research examined three different commercially available ultrasonic systems that are capable of scaling-up for large-scale treatment. These systems all operated at low frequencies but could be used in a flow configuration. The small-scale laboratory equipment used in the first part of the investigation revealed that algal treatment at low frequency required high powers to inactivate algae cells. This proved to be the case in this medium-scale treatment indeed intensity is a very important factor when considering Scale-up. If the intensity is too low, declumping effects may occur. The importance of radical production through cavitation at high frequencies could not be

tested on a larger-scale due to the use of lower frequencies, however damage to chlorophyll A was observed by UV-Vis and fluorometry under some conditions.

Further research into large-scale applications is required and may focus on an improvement to the acoustic efficiency of transducers and also on the combination of low frequency mechanical effects combined with some high frequency sonication to generate radicals.

8.0 Further work

The work reported in this thesis confirms that ultrasonic treatment may offer a successful means of control for algae blooms in drinking water sources. The following experiments would provide useful information for the continued progression of these studies:

1. If larger-scale equipment was available then experiments using high frequency sonication would help to determine whether the promising results at a laboratory scale could also be transferred to pilot and industrial scale.

2. Studies of the mechanisms of the effects of ultrasound on algae have been restricted by the absence of a high objective lens for the confocal microscope. Higher power magnification would enable a study of the ultrasonic effects on algae at cell sub-cellular structure level. In this work, a third sub-population between live and dead sub-populations was observed using flow cytometry. It would be interesting to employ a high objective lens to assess cell damage in this third sub-population and also to determine if ultrasonically damaged algae cells can re-grow under normal culture conditions.

3. *Microcystis aeruginosa* was chosen for our research as it is the main, and most toxic species in algal blooms. Further work should be carried out to determine if ultrasound is effective on control of other harmful toxin producing species such as Anabaena or Spirulina (WHO, 2003c).

4. An interesting unanswered question is whether ultrasound can sonochemically destroy any liberated toxin. Additional analysis techniques (Enzyme-Linked Immuno Sorbent Assay, ELISA) could be employed to detect algae toxin concentrations before and after sonication and thus help answer this question. Microcystin-LR is the most common algae toxin and very stable in natural environments. Current techniques used in drinking water treatment have proved to be ineffective for the degradation of such toxins (WHO, 2004). Any free radicals produced during cavitation may react with algae toxins altering their chemical structure and reducing risks to human health in drinking water sources, but high level free radicals may also affect human health.

5. Further work in required to determine the benefits of sonication in conjunction with other current treatments (e.g. algaecides, biomanipulation techniques, filtration, UV and ozone)

Most of the work in this thesis was laboratory based using cultured algae, but clearly it is important to study ultrasound on large-scale natural settings. Such applications for algae bloom control must be effective and able to deal with variations in already complicated natural conditions such as temperature, pH, nutrient content, depth of water, etc. Few effective methods exist to deal with large-scale algae blooms. Current filtration systems are unable to cope with cyanobacterial blooms in water supplies and regularly result in blockage of filters. Chemical controls such as the use of algaecides will result in an additional form of water pollution. Thus, ultrasonic treatment may provide a suitable solution.

Prior to investigating the possibility of large applications, long term monitoring of water quality before, during and after ultrasonic treatment is required. The concentration and species of algae, bacteria, diatoms and protozoa must be assessed in terms of ultrasound treatment to ensure ultrasound has no harmful effects on the environment.

One of the main issues relating to the application of ultrasound on a large-scale is energy efficiency. Our work has shown that high ultrasonic frequencies and low intensities resulted in high reduction rates for algae but this is at a laboratory-scale. For flow systems at lower frequencies high power is required. The actual power requirement for large-scale treatment is not yet determined but could increase water treatment costs. However power ultrasound may achieve this goal by optimization of parameter settings and employing a circulating system, pulse mode, or in combination with algaecides, UV and ozone (Joyce, 2009). These latter combinations will certainly reduce the power requirement but will need to be investigated (Mason, 2007).

9.0 References

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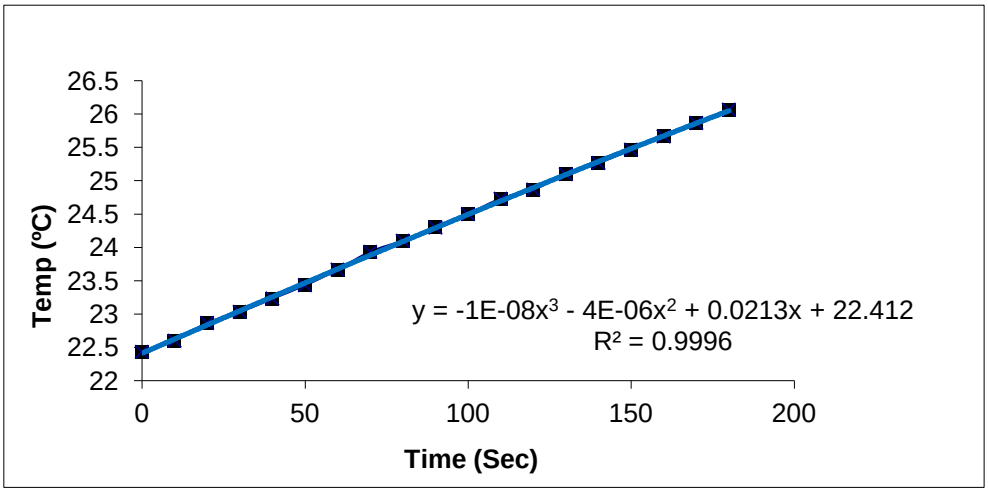
Appendix 1 CALORIMETRY FOR ULTRASONIC EQUIPMENT

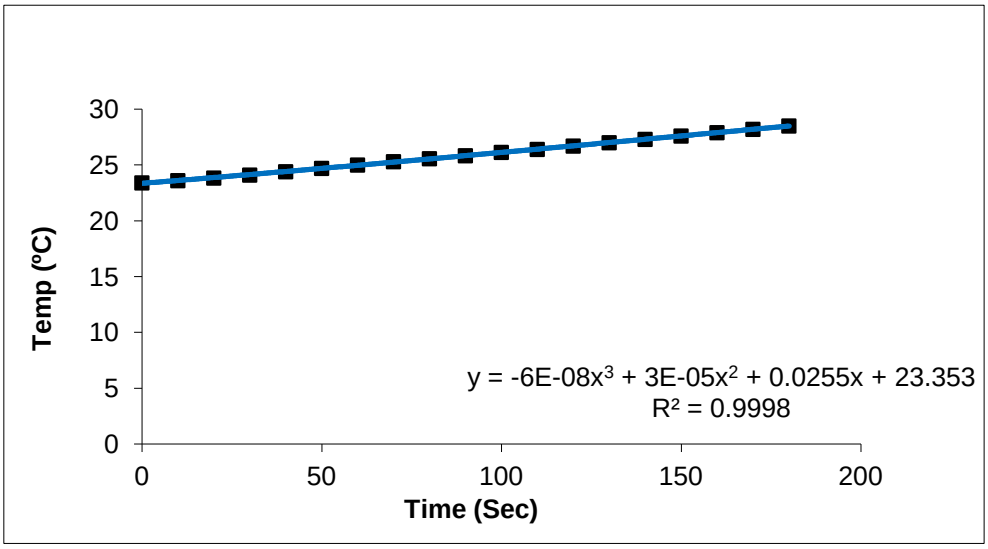
20 kHz ultrasonic probe (Sonics & Materials), 200 ml of water, in a 250 mL beaker

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	22.1	23.2	22	22.43333	0.665833	2.968051
10	22.3	23.4	22.1	22.6	0.7	3.097345
20	22.6	23.7	22.3	22.86667	0.737111	3.22352
30	22.7	23.9	22.5	23.03333	0.757188	3.287356
40	22.9	24.1	22.7	23.23333	0.757188	3.259058
50	23.1	24.3	22.9	23.43333	0.757188	3.231242
60	23.3	24.6	23.1	23.66667	0.814453	3.44135
70	23.6	24.8	23.4	23.93333	0.757188	3.163737
80	23.8	25	23.5	24.1	0.793725	3.293466
90	24	25.2	23.7	24.3	0.793725	3.26636
100	24.2	25.4	23.9	24.5	0.793725	3.239695
110	24.4	25.69	24.1	24.73	0.844808	3.416125
120	24.5	25.8	24.3	24.86667	0.814453	3.275279
130	24.8	26	24.5	25.1	0.793725	3.162253
140	24.9	26.2	24.7	25.26667	0.814453	3.223428
150	25.1	26.4	24.9	25.46667	0.814453	3.198113
160	25.3	26.6	25.1	25.66667	0.814453	3.173193
170	25.4	26.9	25.3	25.86667	0.896289	3.465033
180	25.7	27	25.5	26.06667	0.814453	3.124499



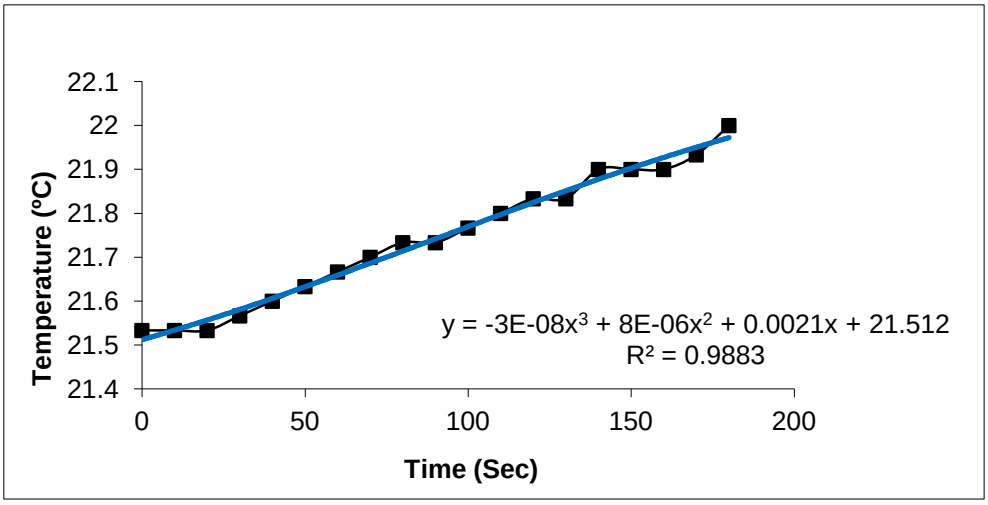
40 kHz bath (Langford Sonomatic), 200 mL of water, in a 250 mL beaker

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	22	23.2	25	23.4	1.509967	6.45285
10	22.1	23.4	25.3	23.6	1.609348	6.81927
20	22.3	23.6	25.6	23.83333	1.662328	6.974801
30	22.6	23.8	25.9	24.1	1.670329	6.930827
40	22.9	24.1	26.2	24.4	1.670329	6.845612
50	23.2	24.4	26.5	24.7	1.670329	6.762467
60	23.5	24.7	26.8	25	1.670329	6.681317
70	23.8	25	27.1	25.3	1.670329	6.602092
80	24.1	25.3	27.3	25.56667	1.616581	6.323002
90	24.4	25.6	27.5	25.83333	1.563117	6.050774
100	24.7	25.9	27.8	26.13333	1.563117	5.981313
110	25	26.1	28.1	26.4	1.571623	5.953119
120	25.3	26.4	28.4	26.7	1.571623	5.88623
130	25.6	26.7	28.7	27	1.571623	5.820827
140	25.9	27	29	27.3	1.571623	5.756862
150	26.2	27.3	29.3	27.6	1.571623	5.694288
160	26.5	27.6	29.6	27.9	1.571623	5.633059
170	26.8	27.9	29.9	28.2	1.571623	5.573132
180	27.1	28.2	30.2	28.5	1.571623	5.514468



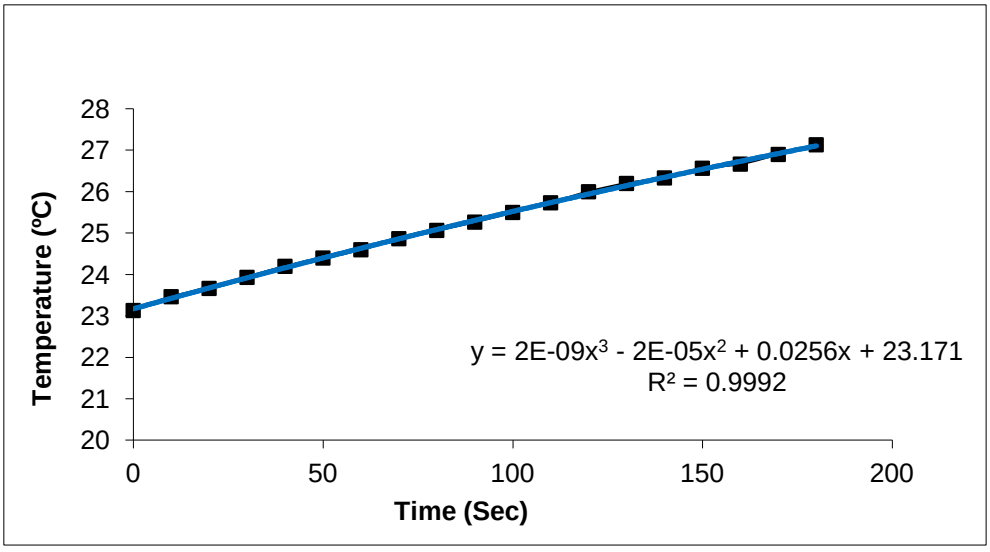
580 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting 40%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	21.4	21.9	21.3	21.53333	0.321455	1.492825
10	21.4	21.9	21.3	21.53333	0.321455	1.492825
20	21.4	21.9	21.3	21.53333	0.321455	1.492825
30	21.4	21.9	21.4	21.56667	0.288675	1.338525
40	21.4	22	21.4	21.6	0.34641	1.603751
50	21.4	22.1	21.4	21.63333	0.404145	1.86816
60	21.5	22.1	21.4	21.66667	0.378594	1.747356
70	21.5	22.1	21.5	21.7	0.34641	1.59636
80	21.5	22.2	21.5	21.73333	0.404145	1.859564
90	21.5	22.2	21.5	21.73333	0.404145	1.859564
100	21.6	22.2	21.5	21.76667	0.378594	1.739329
110	21.6	22.2	21.6	21.8	0.34641	1.589037
120	21.6	22.3	21.6	21.83333	0.404145	1.851047
130	21.6	22.3	21.6	21.83333	0.404145	1.851047
140	21.7	22.3	21.7	21.9	0.34641	1.581782
150	21.7	22.3	21.7	21.9	0.34641	1.581782
160	21.7	22.3	21.7	21.9	0.34641	1.581782
170	21.8	22.3	21.7	21.93333	0.321455	1.4656
180	21.8	22.4	21.8	22	0.34641	1.574592



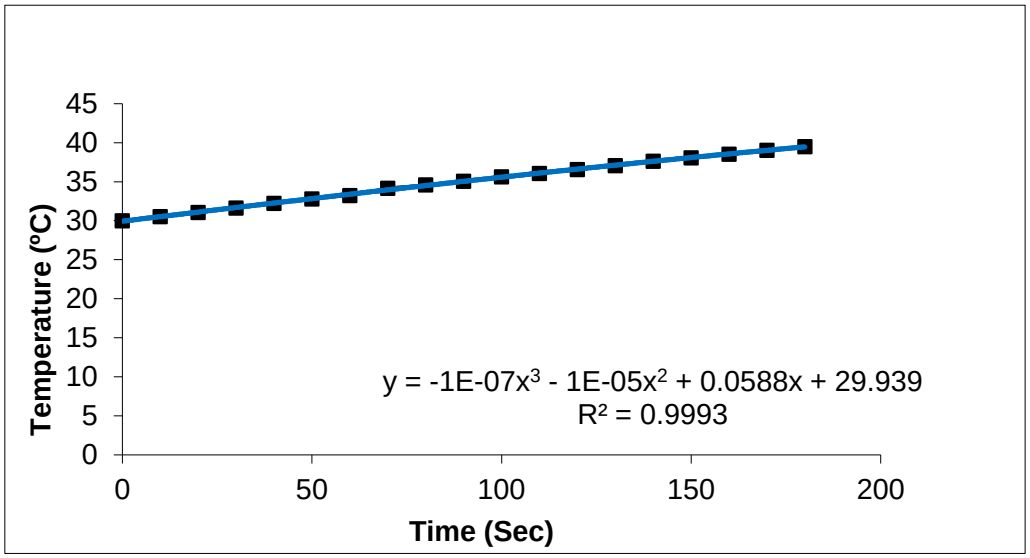
580 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting 80%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	22.3	26.7	20.4	23.13333	3.231615	13.96952
10	22.8	26.9	20.7	23.46667	3.153305	13.43738
20	22.9	27.1	21	23.66667	3.121431	13.18915
30	23.2	27.3	21.3	23.93333	3.066486	12.81261
40	23.6	27.4	21.6	24.2	2.946184	12.17431
50	23.7	27.6	21.9	24.4	2.91376	11.94164
60	23.8	27.8	22.2	24.6	2.884441	11.72537
70	24.1	28.1	22.4	24.86667	2.926317	11.76803
80	24.3	28.2	22.7	25.06667	2.829016	11.28597
90	24.5	28.4	22.9	25.26667	2.829016	11.19663
100	24.7	28.6	23.2	25.5	2.787472	10.93126
110	24.9	28.8	23.5	25.73333	2.746513	10.67298
120	25.2	29.1	23.7	26	2.787472	10.72105
130	25.5	29.2	23.9	26.2	2.718455	10.37578
140	25.7	29.3	24	26.33333	2.706166	10.27658
150	25.9	29.6	24.2	26.56667	2.761038	10.39287
160	25.9	29.8	24.3	26.66667	2.829016	10.60881
170	26.3	29.9	24.5	26.9	2.749545	10.22136
180	26.5	30.2	24.7	27.13333	2.804164	10.33476



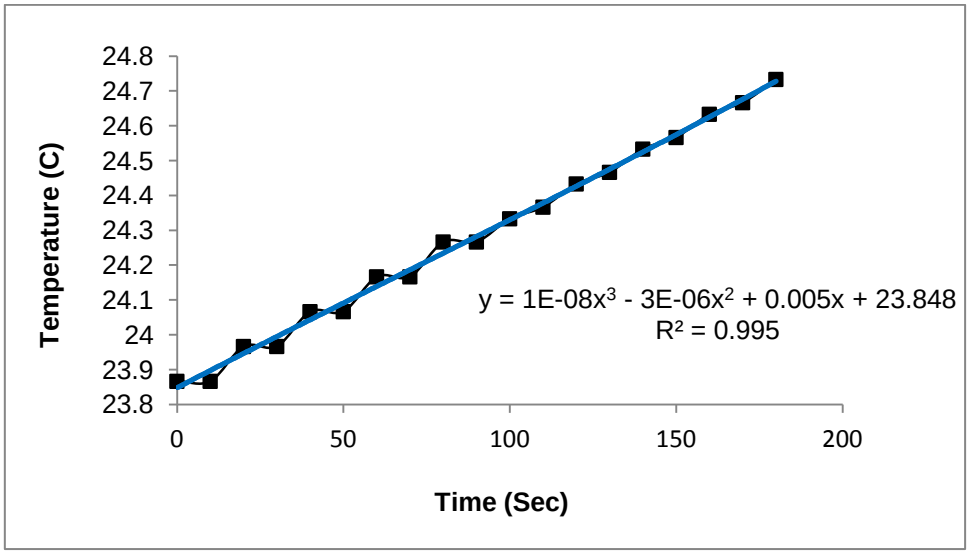
580 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting max

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	31	29	30	30	1	3.333333
10	31.5	29.6	30.5	30.53333	0.950438	3.11279
20	32	30.2	31	31.06667	0.90185	2.90295
30	32.6	30.8	31.5	31.63333	0.907377	2.868421
40	33.3	31.4	32	32.23333	0.971253	3.013196
50	33.9	32	32.5	32.8	0.984886	3.002701
60	34.4	32.3	33	33.23333	1.069268	3.217455
70	35.3	33.6	33.6	34.16667	0.981495	2.87267
80	35.9	33.9	34	34.6	1.126943	3.25706
90	36.5	34.2	34.5	35.06667	1.250333	3.565589
100	37.2	34.6	35.1	35.63333	1.379613	3.871694
110	37.7	35	35.5	36.06667	1.436431	3.98271
120	38.3	35.4	36	36.56667	1.530795	4.186313
130	38.9	35.8	36.5	37.06667	1.625833	4.38624
140	39.7	36.2	37	37.63333	1.833939	4.873178
150	40.1	36.6	37.5	38.06667	1.817507	4.774538
160	40.6	37	38	38.53333	1.858315	4.822616
170	41.2	37.4	38.5	39.03333	1.955335	5.009397
180	41.8	37.7	39	39.5	2.095233	5.304387



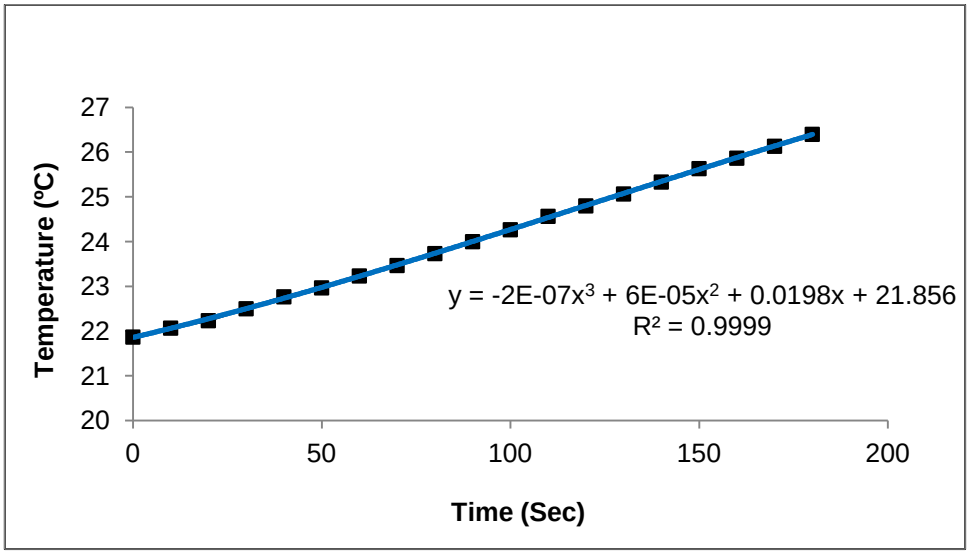
864 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting 40%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	25.3	22.3	24	23.86667	1.504438	6.303511
10	25.3	22.3	24	23.86667	1.504438	6.303511
20	25.4	22.4	24.1	23.96667	1.504438	6.27721
30	25.4	22.4	24.1	23.96667	1.504438	6.27721
40	25.5	22.5	24.2	24.06667	1.504438	6.251127
50	25.5	22.5	24.2	24.06667	1.504438	6.251127
60	25.6	22.6	24.3	24.16667	1.504438	6.22526
70	25.6	22.6	24.3	24.16667	1.504438	6.22526
80	25.7	22.7	24.4	24.26667	1.504438	6.199607
90	25.7	22.7	24.4	24.26667	1.504438	6.199607
100	25.7	22.8	24.5	24.33333	1.457166	5.988354
110	25.8	22.8	24.5	24.36667	1.504438	6.174164
120	25.8	22.9	24.6	24.43333	1.457166	5.963845
130	25.9	22.9	24.6	24.46667	1.504438	6.148929
140	25.9	23	24.7	24.53333	1.457166	5.939536
150	26	23	24.7	24.56667	1.504438	6.123899
160	26	23.1	24.8	24.63333	1.457166	5.915424
170	26.1	23.1	24.8	24.66667	1.504438	6.099072
180	26.1	23.2	24.9	24.73333	1.457166	5.891508



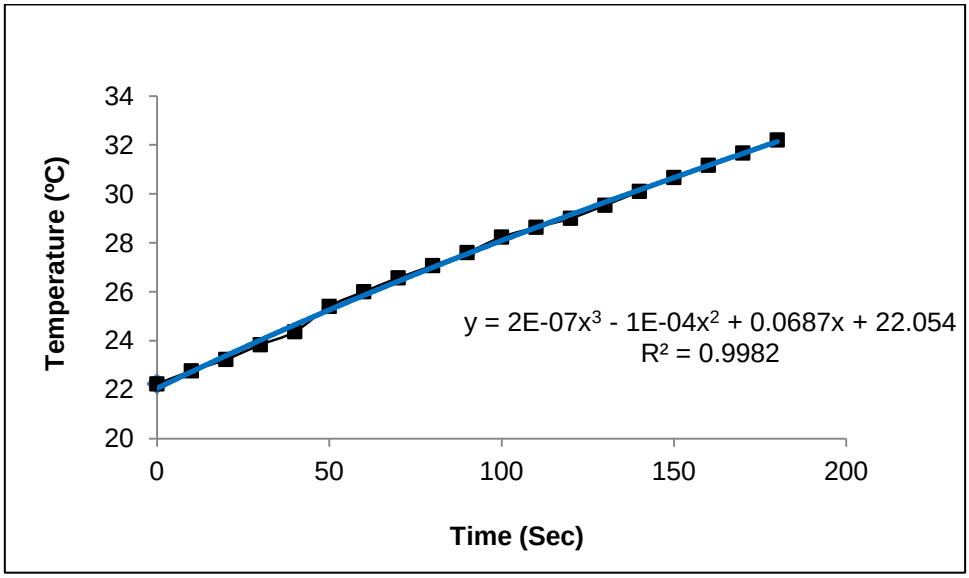
864 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting 80%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	23	21.1	21.5	21.86667	1.001665	0.578312
10	23.1	21.5	21.6	22.06667	0.896289	0.517472
20	23.3	21.8	21.6	22.23333	0.929157	0.536449
30	23.6	22	21.9	22.5	0.953939	0.550757
40	23.9	22.3	22.1	22.76667	0.986577	0.5696
50	24.2	22.5	22.2	22.96667	1.078579	0.622718
60	24.5	22.7	22.5	23.23333	1.101514	0.635959
70	24.8	22.9	22.7	23.46667	1.159023	0.669162
80	25.1	23.2	22.9	23.73333	1.193035	0.688799
90	25.4	23.4	23.2	24	1.216553	0.702377
100	25.7	23.7	23.4	24.26667	1.250333	0.72188
110	26	24	23.7	24.56667	1.250333	0.72188
120	26.2	24.3	23.9	24.8	1.228821	0.70946
130	26.5	24.5	24.2	25.06667	1.250333	0.72188
140	26.8	24.8	24.4	25.33333	1.28582	0.742369
150	27.1	25.1	24.7	25.63333	1.28582	0.742369
160	27.4	25.3	24.9	25.86667	1.342882	0.775314
170	27.6	25.6	25.2	26.13333	1.28582	0.742369
180	27.9	25.9	25.4	26.4	1.322876	0.763763



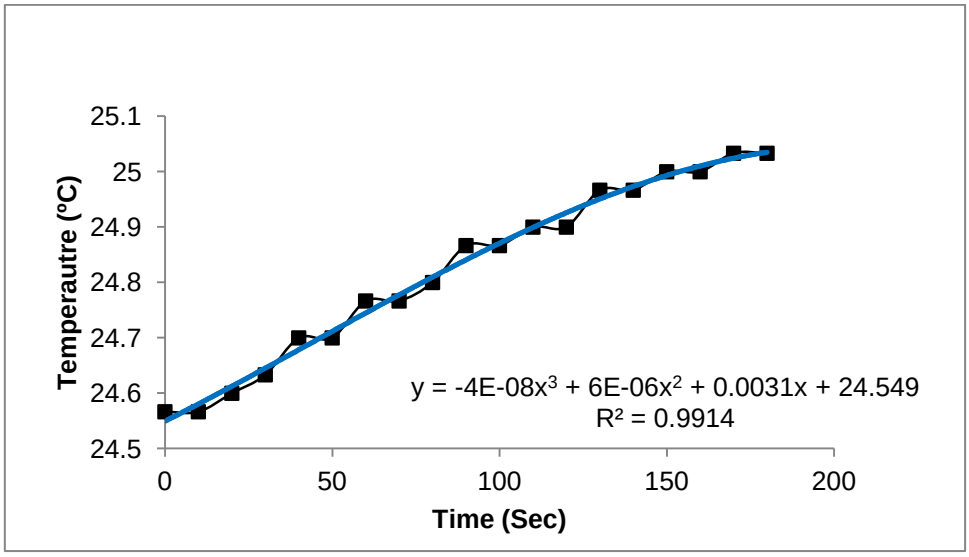
864 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting max

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	24	23.1	19.6	22.23333	2.324507	1.342055
10	24.3	23.4	20.6	22.76667	1.929594	1.114052
20	24.7	23.7	21.3	23.23333	1.747379	1.00885
30	25.1	24.1	22.3	23.83333	1.41892	0.819214
40	25.6	24.5	23	24.36667	1.305118	0.75351
50	26.6	25.5	24.1	25.4	1.252996	0.723418
60	27.1	26.1	24.8	26	1.153256	0.665833
70	27.6	26.6	25.5	26.56667	1.050397	0.606447
80	28.1	27.1	26	27.06667	1.050397	0.606447
90	28.5	27.6	26.7	27.6	0.9	0.519615
100	29.1	28.1	27.5	28.23333	0.80829	0.466667
110	29.4	28.5	28	28.63333	0.70946	0.409607
120	29.6	29	28.4	29	0.6	0.34641
130	30	29.6	29	29.53333	0.503322	0.290593
140	30.5	30.1	29.7	30.1	0.4	0.23094
150	31	30.6	30.4	30.66667	0.305505	0.176383
160	31.5	31.1	30.9	31.16667	0.305505	0.176383
170	31.9	31.6	31.5	31.66667	0.208167	0.120185
180	32.4	32.1	32.1	32.2	0.173205	0.1



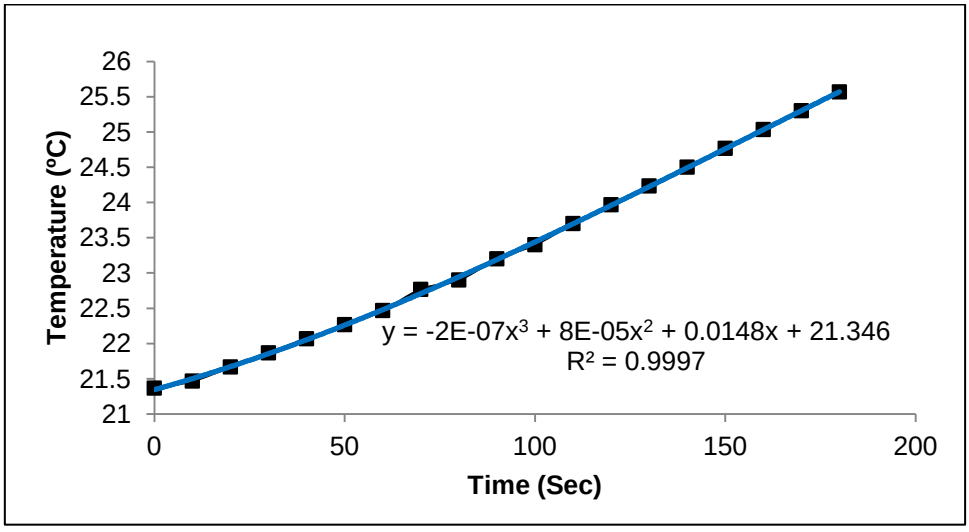
1146 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting 40%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	26.3	27	20.4	24.56667	3.625374	14.75729
10	26.3	27	20.4	24.56667	3.625374	14.75729
20	26.4	27	20.4	24.6	3.649658	14.83601
30	26.4	27.1	20.4	24.63333	3.682843	14.95065
40	26.5	27.1	20.5	24.7	3.649658	14.77594
50	26.5	27.1	20.5	24.7	3.649658	14.77594
60	26.6	27.2	20.5	24.76667	3.7072	14.96851
70	26.6	27.2	20.5	24.76667	3.7072	14.96851
80	26.6	27.2	20.6	24.8	3.649658	14.71636
90	26.7	27.3	20.6	24.86667	3.7072	14.90831
100	26.7	27.3	20.6	24.86667	3.7072	14.90831
110	26.7	27.3	20.7	24.9	3.649658	14.65726
120	26.7	27.3	20.7	24.9	3.649658	14.65726
130	26.8	27.4	20.7	24.96667	3.7072	14.8486
140	26.8	27.4	20.7	24.96667	3.7072	14.8486
150	26.8	27.4	20.8	25	3.649658	14.59863
160	26.8	27.4	20.8	25	3.649658	14.59863
170	26.8	27.5	20.8	25.03333	3.682843	14.71176
180	26.8	27.5	20.8	25.03333	3.682843	14.71176



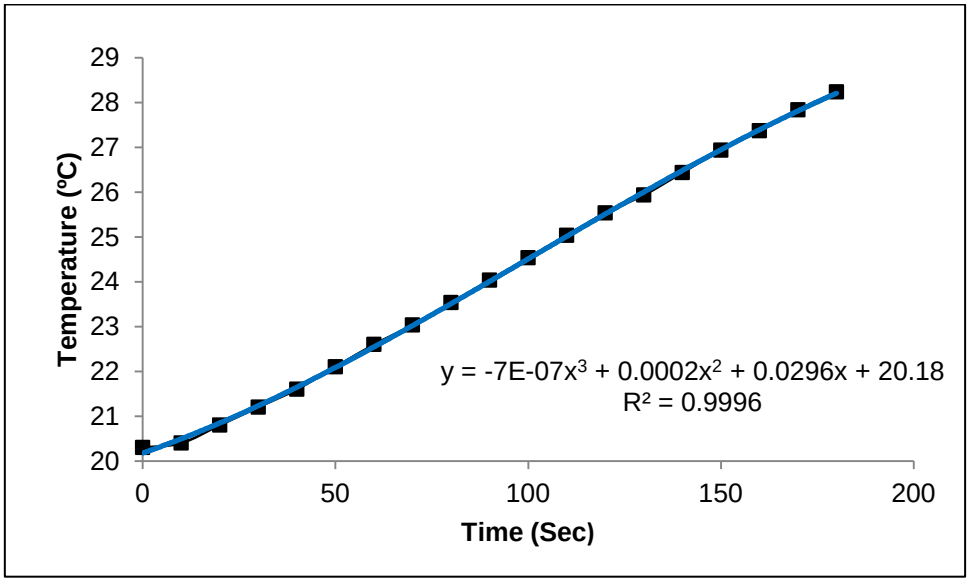
1146 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting 80%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	22	21	21.1	21.36667	0.550757	0.31798
10	22.1	21.1	21.2	21.46667	0.550757	0.31798
20	22.3	21.3	21.4	21.66667	0.550757	0.31798
30	22.5	21.5	21.6	21.86667	0.550757	0.31798
40	22.7	21.7	21.8	22.06667	0.550757	0.31798
50	22.9	21.9	22	22.26667	0.550757	0.31798
60	23.1	22.1	22.2	22.46667	0.550757	0.31798
70	23.4	22.4	22.5	22.76667	0.550757	0.31798
80	23.6	22.5	22.6	22.9	0.608276	0.351188
90	23.9	22.8	22.9	23.2	0.608276	0.351188
100	24.1	23	23.1	23.4	0.608276	0.351188
110	24.4	23.3	23.4	23.7	0.608276	0.351188
120	24.6	23.6	23.7	23.96667	0.550757	0.31798
130	24.8	23.9	24	24.23333	0.493288	0.2848
140	25	24.2	24.3	24.5	0.43589	0.251661
150	25.2	24.5	24.6	24.76667	0.378594	0.218581
160	25.4	24.8	24.9	25.03333	0.321455	0.185592
170	25.6	25.1	25.2	25.3	0.264575	0.152753
180	25.8	25.4	25.5	25.56667	0.208167	0.120185



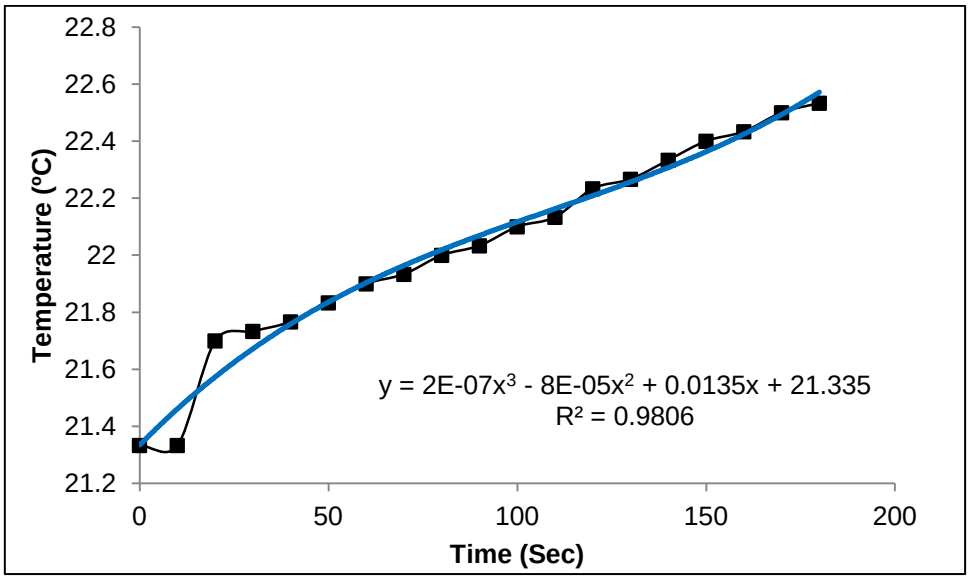
1146 KHz bath (Langford Sonomatic), 200 mL distilled water, in the bath itself, at power setting max

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	20.5	20	20.4	20.3	0.264575	0.152753
10	20.6	20.1	20.5	20.4	0.264575	0.152753
20	21	20.5	20.9	20.8	0.264575	0.152753
30	21.4	20.9	21.3	21.2	0.264575	0.152753
40	21.8	21.3	21.7	21.6	0.264575	0.152753
50	22.3	21.8	22.2	22.1	0.264575	0.152753
60	22.8	22.3	22.7	22.6	0.264575	0.152753
70	23.2	22.8	23.1	23.03333	0.208167	0.120185
80	23.7	23.3	23.6	23.53333	0.208167	0.120185
90	24.2	23.8	24.1	24.03333	0.208167	0.120185
100	24.7	24.3	24.6	24.53333	0.208167	0.120185
110	25.2	24.8	25.1	25.03333	0.208167	0.120185
120	25.7	25.3	25.6	25.53333	0.208167	0.120185
130	26.1	25.7	26	25.93333	0.208167	0.120185
140	26.6	26.2	26.5	26.43333	0.208167	0.120185
150	27.1	26.7	27	26.93333	0.208167	0.120185
160	27.5	27.2	27.4	27.36667	0.152753	0.088192
170	28	27.6	27.9	27.83333	0.208167	0.120185
180	28.4	28	28.3	28.23333	0.208167	0.120185



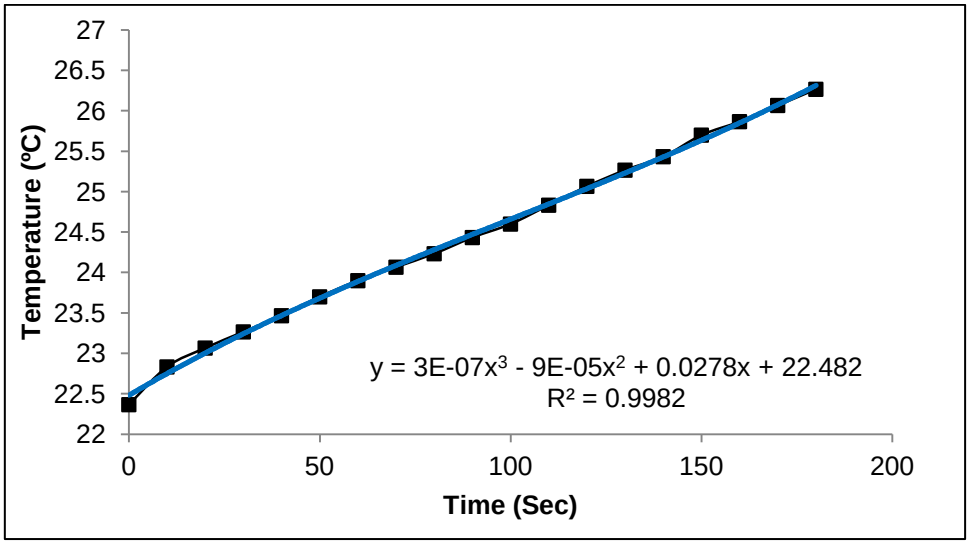
20 kHz ultrasonic probe (Sonics & Materials), 200 ml of water

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	21.5	21.1	21.4	21.33333	0.208167	0.120185
10	21.5	21.1	21.4	21.33333	0.208167	0.120185
20	21.5	22.1	21.5	21.7	0.34641	0.2
30	21.5	22.2	21.5	21.73333	0.404145	0.233333
40	21.5	22.2	21.6	21.76667	0.378594	0.218581
50	21.6	22.3	21.6	21.83333	0.404145	0.233333
60	21.7	22.3	21.7	21.9	0.34641	0.2
70	21.7	22.4	21.7	21.93333	0.404145	0.233333
80	21.8	22.4	21.8	22	0.34641	0.2
90	21.8	22.5	21.8	22.03333	0.404145	0.233333
100	21.9	22.5	21.9	22.1	0.34641	0.2
110	21.9	22.6	21.9	22.13333	0.404145	0.233333
120	22	22.7	22	22.23333	0.404145	0.233333
130	22	22.8	22	22.26667	0.46188	0.266667
140	22.1	22.8	22.1	22.33333	0.404145	0.233333
150	22.1	22.9	22.2	22.4	0.43589	0.251661
160	22.2	22.9	22.2	22.43333	0.404145	0.233333
170	22.2	23	22.3	22.5	0.43589	0.251661
180	22.3	23	22.3	22.53333	0.404145	0.233333



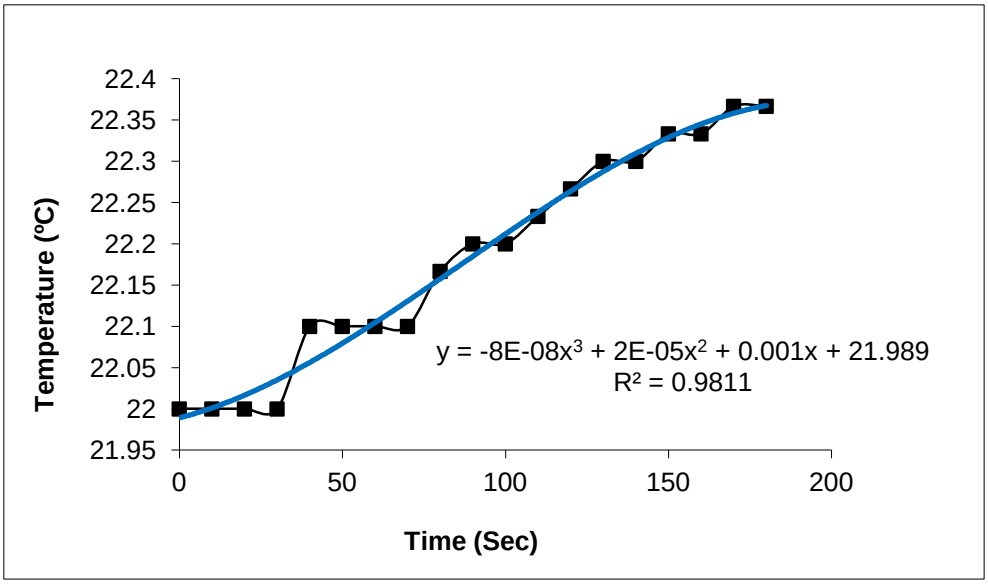
40 kHz bath (Langford Sonomatic), 400 mL of water

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	22.1	23	22	22.36667	0.550757	2.462401
10	22.7	23.5	22.3	22.83333	0.61101	2.675957
20	23	23.6	22.6	23.06667	0.503322	2.182033
30	23.2	23.7	22.9	23.26667	0.404145	1.737014
40	23.4	23.9	23.1	23.46667	0.404145	1.72221
50	23.7	24.1	23.3	23.7	0.4	1.687764
60	23.9	24.3	23.5	23.9	0.4	1.67364
70	24.1	24.5	23.6	24.06667	0.450925	1.873649
80	24.3	24.7	23.7	24.23333	0.503322	2.076983
90	24.5	24.9	23.9	24.43333	0.503322	2.059982
100	24.6	25.1	24.1	24.6	0.5	2.03252
110	24.9	25.3	24.3	24.83333	0.503322	2.026801
120	25.1	25.6	24.5	25.06667	0.550757	2.197169
130	25.3	25.9	24.6	25.26667	0.650641	2.575095
140	25.4	26.1	24.8	25.43333	0.650641	2.55822
150	25.7	26.3	25.1	25.7	0.6	2.33463
160	25.8	26.5	25.3	25.86667	0.602771	2.330302
170	26	26.7	25.5	26.06667	0.602771	2.312422
180	26.2	26.9	25.7	26.26667	0.602771	2.294815



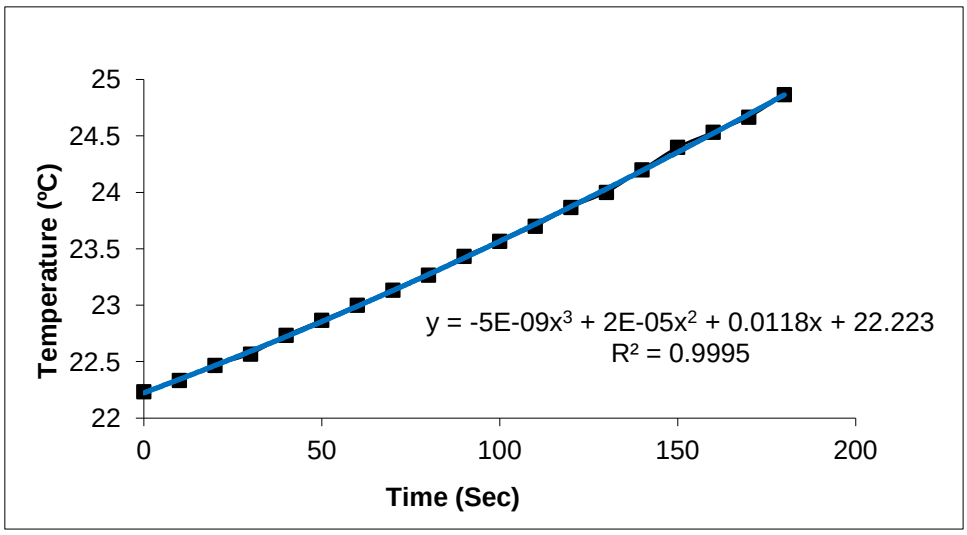
580 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting 40%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	22.4	22.6	21	22	0.87178	3.962635
10	22.4	22.6	21	22	0.87178	3.962635
20	22.4	22.6	21	22	0.87178	3.962635
30	22.4	22.6	21	22	0.87178	3.962635
40	22.5	22.7	21.1	22.1	0.87178	3.944705
50	22.5	22.7	21.1	22.1	0.87178	3.944705
60	22.5	22.7	21.1	22.1	0.87178	3.944705
70	22.5	22.7	21.1	22.1	0.87178	3.944705
80	22.5	22.8	21.2	22.16667	0.85049	3.836797
90	22.6	22.8	21.2	22.2	0.87178	3.926936
100	22.6	22.8	21.2	22.2	0.87178	3.926936
110	22.6	22.8	21.3	22.23333	0.814453	3.663206
120	22.6	22.9	21.3	22.26667	0.85049	3.819566
130	22.7	22.9	21.3	22.3	0.87178	3.909326
140	22.7	22.9	21.3	22.3	0.87178	3.909326
150	22.7	22.9	21.4	22.33333	0.814453	3.646803
160	22.7	22.9	21.4	22.33333	0.814453	3.646803
170	22.7	23	21.4	22.36667	0.85049	3.802489
180	22.7	23	21.4	22.36667	0.85049	3.802489



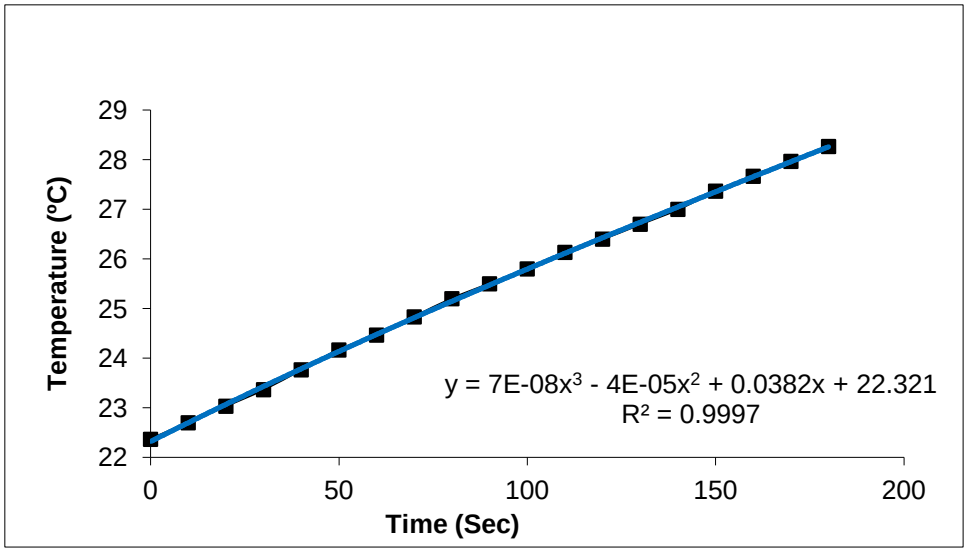
580 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting 80%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	21.8	21.9	23	22.23333	0.665833	2.99475
10	21.9	22	23.1	22.33333	0.665833	2.981341
20	22.1	22.1	23.2	22.46667	0.635085	2.826789
30	22.2	22.2	23.3	22.56667	0.635085	2.814263
40	22.4	22.3	23.5	22.73333	0.665833	2.928883
50	22.5	22.4	23.7	22.86667	0.723418	3.163635
60	22.6	22.5	23.9	23	0.781025	3.395761
70	22.8	22.6	24	23.13333	0.757188	3.273146
80	22.9	22.7	24.2	23.26667	0.814453	3.500513
90	23	22.9	24.4	23.43333	0.83865	3.578875
100	23.1	23	24.6	23.56667	0.896289	3.803205
110	23.2	23.2	24.7	23.7	0.866025	3.654116
120	23.3	23.4	24.9	23.86667	0.896289	3.755399
130	23.4	23.6	25	24	0.87178	3.632416
140	23.5	23.9	25.2	24.2	0.888819	3.672808
150	23.7	24.1	25.4	24.4	0.888819	3.642703
160	23.8	24.2	25.6	24.53333	0.945163	3.852567
170	23.9	24.3	25.8	24.66667	1.001665	4.060805
180	24.1	24.6	25.9	24.86667	0.929157	3.736558



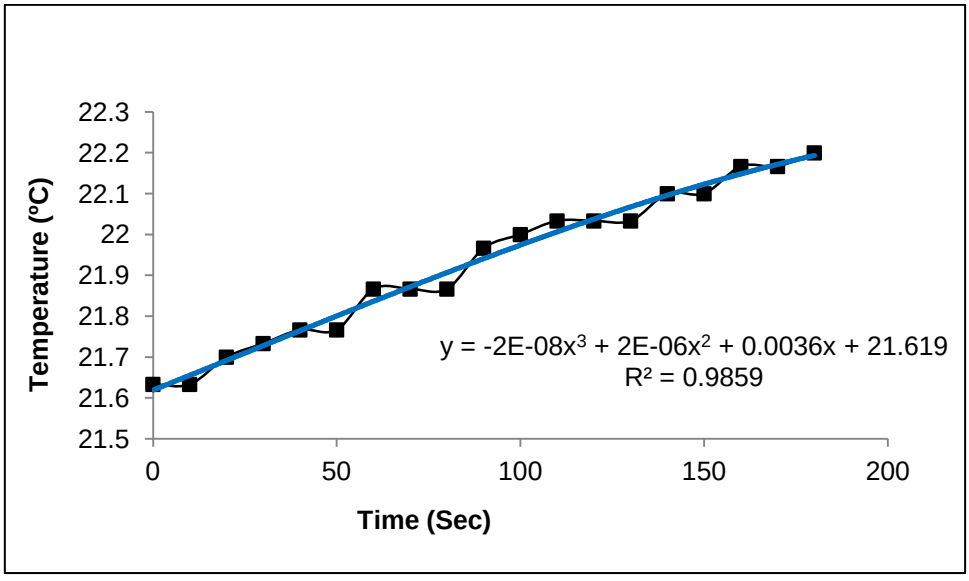
580 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting max

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	22.6	22	22.5	22.36667	0.321455	1.437206
10	22.9	22.4	22.8	22.7	0.264575	1.165529
20	23.2	22.8	23.1	23.03333	0.208167	0.903762
30	23.5	23.2	23.4	23.36667	0.152753	0.65372
40	23.9	23.6	23.8	23.76667	0.152753	0.642717
50	24.3	24	24.2	24.16667	0.152753	0.632079
60	24.6	24.3	24.5	24.46667	0.152753	0.624329
70	25	24.6	24.9	24.83333	0.208167	0.838255
80	25.4	24.9	25.3	25.2	0.264575	1.049901
90	25.7	25.2	25.6	25.5	0.264575	1.03755
100	26	25.5	25.9	25.8	0.264575	1.025485
110	26.4	25.7	26.3	26.13333	0.378594	1.448701
120	26.7	25.9	26.6	26.4	0.43589	1.651098
130	27	26.2	26.9	26.7	0.43589	1.632546
140	27.3	26.5	27.2	27	0.43589	1.614407
150	27.7	26.8	27.6	27.36667	0.493288	1.802515
160	28	27.1	27.9	27.66667	0.493288	1.78297
170	28.3	27.4	28.2	27.96667	0.493288	1.763844
180	28.6	27.7	28.5	28.26667	0.493288	1.745124



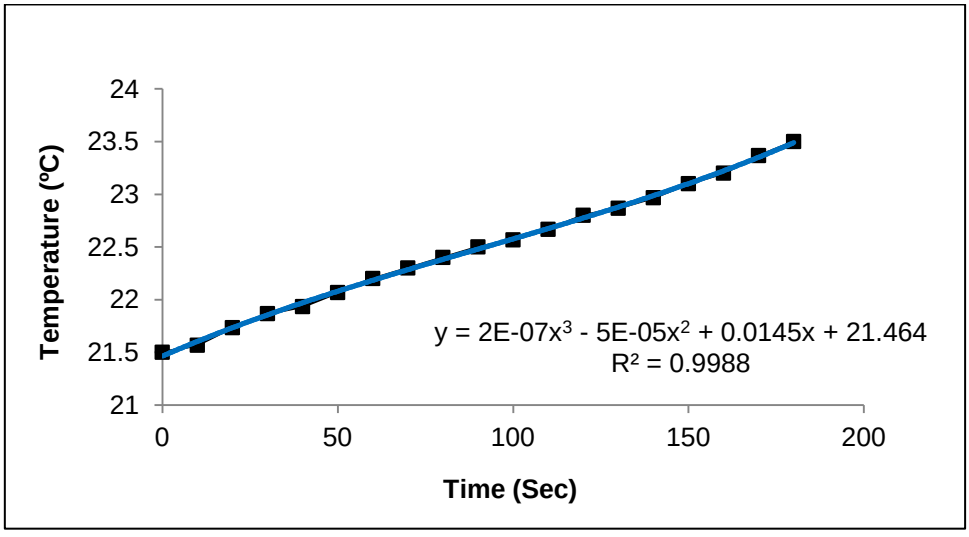
864 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting 40%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	21.7	21.1	22.1	21.63333	0.503322	2.326605
10	21.7	21.1	22.1	21.63333	0.503322	2.326605
20	21.7	21.2	22.2	21.7	0.5	2.304147
30	21.8	21.2	22.2	21.73333	0.503322	2.3159
40	21.8	21.2	22.3	21.76667	0.550757	2.530277
50	21.8	21.2	22.3	21.76667	0.550757	2.530277
60	21.9	21.3	22.4	21.86667	0.550757	2.518706
70	21.9	21.3	22.4	21.86667	0.550757	2.518706
80	21.9	21.3	22.4	21.86667	0.550757	2.518706
90	22	21.4	22.5	21.96667	0.550757	2.50724
100	22	21.5	22.5	22	0.5	2.272727
110	22	21.6	22.5	22.03333	0.450925	2.046558
120	22	21.6	22.5	22.03333	0.450925	2.046558
130	22	21.6	22.5	22.03333	0.450925	2.046558
140	22.1	21.7	22.5	22.1	0.4	1.809955
150	22.1	21.7	22.5	22.1	0.4	1.809955
160	22.1	21.8	22.6	22.16667	0.404145	1.823211
170	22.1	21.8	22.6	22.16667	0.404145	1.823211
180	22.2	21.8	22.6	22.2	0.4	1.801802



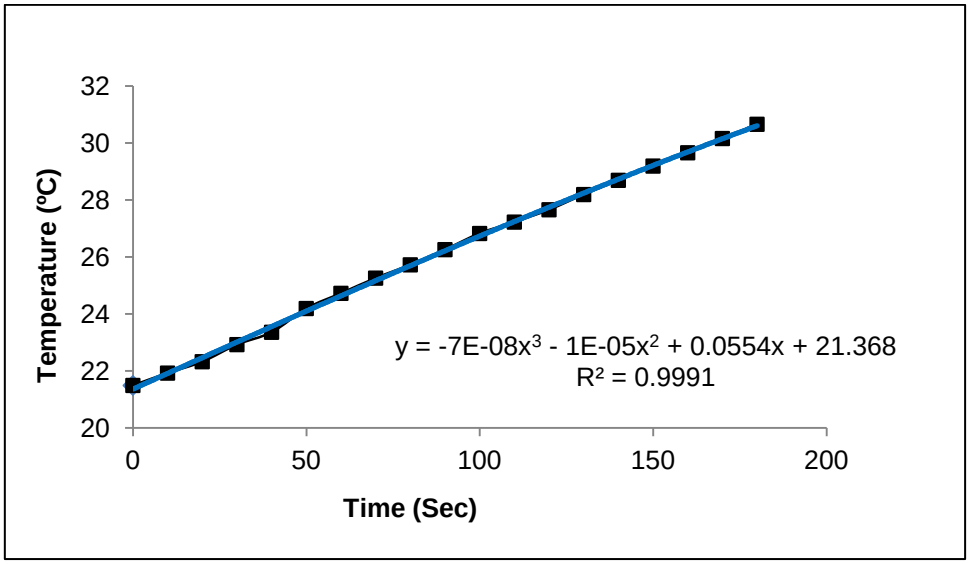
864 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting 80%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	22.1	22.3	20.1	21.5	1.216553	5.658384
10	22.2	22.4	20.1	21.56667	1.274101	5.907733
20	22.4	22.6	20.2	21.73333	1.331666	6.127296
30	22.5	22.8	20.3	21.86667	1.36504	6.24256
40	22.6	22.9	20.3	21.93333	1.422439	6.485285
50	22.7	23.1	20.4	22.06667	1.457166	6.603472
60	22.8	23.3	20.5	22.2	1.493318	6.72666
70	22.9	23.4	20.6	22.3	1.493318	6.696495
80	23	23.5	20.7	22.4	1.493318	6.6666
90	23.2	23.5	20.8	22.5	1.479865	6.577177
100	23.3	23.6	20.8	22.56667	1.537314	6.812321
110	23.4	23.7	20.9	22.66667	1.537314	6.782266
120	23.6	23.8	21	22.8	1.56205	6.851096
130	23.6	23.9	21.1	22.86667	1.537314	6.722946
140	23.6	24.1	21.2	22.96667	1.550269	6.750082
150	23.8	24.2	21.3	23.1	1.571623	6.803564
160	23.9	24.3	21.4	23.2	1.571623	6.774239
170	24.1	24.5	21.5	23.36667	1.628906	6.971065
180	24.2	24.7	21.6	23.5	1.664332	7.082263



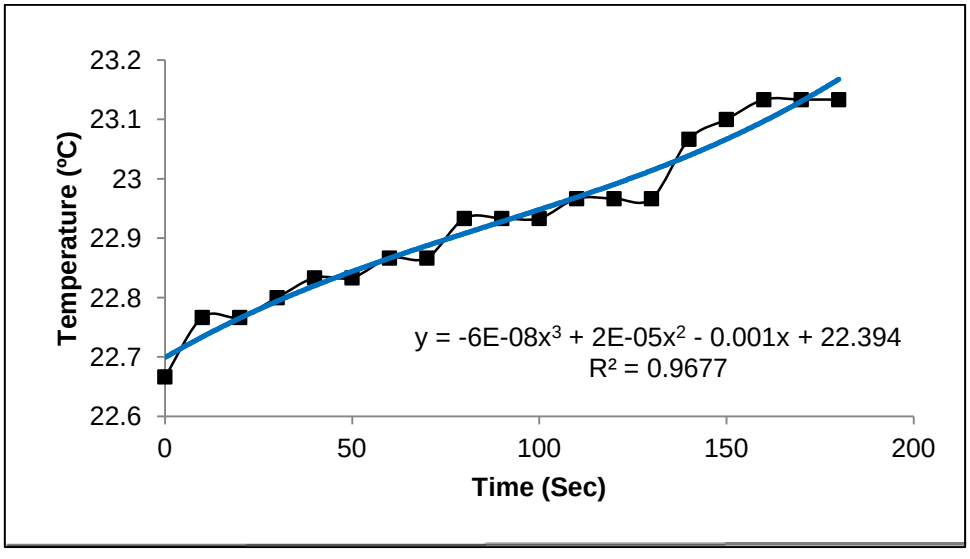
864 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting max

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	21.8	23.1	19.6	21.5	1.769181	8.228747
10	21.8	23.4	20.6	21.93333	1.404754	6.404653
20	22	23.7	21.3	22.33333	1.234234	5.52642
30	22.4	24.1	22.3	22.93333	1.011599	4.411044
40	22.6	24.5	23	23.36667	1.001665	4.286727
50	23	25.5	24.1	24.2	1.252996	5.177671
60	23.3	26.1	24.8	24.73333	1.40119	5.665189
70	23.7	26.6	25.5	25.26667	1.464013	5.794246
80	24.1	27.1	26	25.73333	1.517674	5.897696
90	24.5	27.6	26.7	26.26667	1.594783	6.071509
100	24.9	28.1	27.5	26.83333	1.70098	6.339056
110	25.2	28.5	28	27.23333	1.778576	6.53088
120	25.6	29	28.4	27.66667	1.814754	6.559353
130	26	29.6	29	28.2	1.92873	6.839469
140	26.3	30.1	29.7	28.7	2.088061	7.275475
150	26.6	30.6	30.4	29.2	2.253886	7.718786
160	27	31.1	30.9	29.66667	2.311565	7.791793
170	27.4	31.6	31.5	30.16667	2.396525	7.944283
180	27.8	32.1	32.1	30.66667	2.482606	8.095455



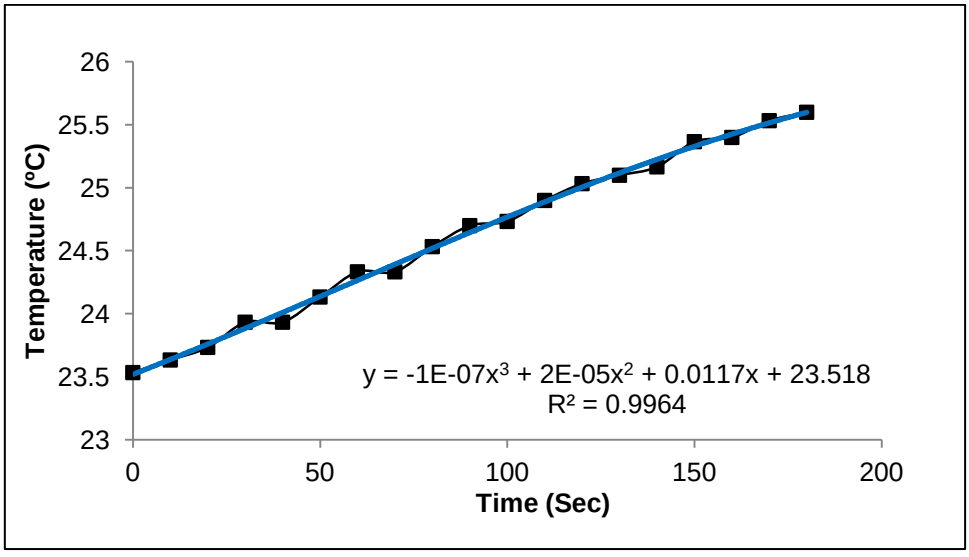
1146 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting 40%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	23.1	22.6	22.3	22.66667	0.404145	1.782993
10	23.2	22.7	22.4	22.76667	0.404145	1.775162
20	23.2	22.7	22.4	22.76667	0.404145	1.775162
30	23.2	22.7	22.5	22.8	0.360555	1.581382
40	23.2	22.8	22.5	22.83333	0.351188	1.538052
50	23.2	22.8	22.5	22.83333	0.351188	1.538052
60	23.3	22.8	22.5	22.86667	0.404145	1.767399
70	23.3	22.8	22.5	22.86667	0.404145	1.767399
80	23.4	22.9	22.5	22.93333	0.450925	1.966243
90	23.4	22.9	22.5	22.93333	0.450925	1.966243
100	23.4	22.9	22.5	22.93333	0.450925	1.966243
110	23.4	22.9	22.6	22.96667	0.404145	1.759703
120	23.4	22.9	22.6	22.96667	0.404145	1.759703
130	23.4	22.9	22.6	22.96667	0.404145	1.759703
140	23.5	23.1	22.6	23.06667	0.450925	1.954877
150	23.5	23.1	22.7	23.1	0.4	1.731602
160	23.6	23.1	22.7	23.13333	0.450925	1.949243
170	23.6	23.1	22.7	23.13333	0.450925	1.949243
180	23.6	23.1	22.7	23.13333	0.450925	1.949243



1146 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting 80%

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	24.7	23.6	22.3	23.53333	1.201388	5.105049
10	24.8	23.7	22.4	23.63333	1.201388	5.083447
20	24.9	23.7	22.6	23.73333	1.150362	4.847032
30	25	24	22.8	23.93333	1.101514	4.602427
40	25	24	22.8	23.93333	1.101514	4.602427
50	25.2	24.2	23	24.13333	1.101514	4.564285
60	25.4	24.4	23.2	24.33333	1.101514	4.52677
70	25.4	24.4	23.2	24.33333	1.101514	4.52677
80	25.6	24.6	23.4	24.53333	1.101514	4.489867
90	25.7	24.8	23.6	24.7	1.053565	4.265447
100	25.8	24.8	23.6	24.73333	1.101514	4.453561
110	25.9	25	23.8	24.9	1.053565	4.231186
120	26	25.2	23.9	25.03333	1.059874	4.233852
130	26.1	25.2	24	25.1	1.053565	4.197472
140	26.2	25.3	24	25.16667	1.106044	4.394877
150	26.4	25.5	24.2	25.36667	1.106044	4.360226
160	26.4	25.6	24.2	25.4	1.113553	4.384066
170	26.6	25.6	24.4	25.53333	1.101514	4.314024
180	26.6	25.8	24.4	25.6	1.113553	4.349816



1146 KHz bath (Langford Sonomatic), 400 mL distilled water, in the bath itself, at power setting max

Time [sec]	Temp [ºC]	Temp [ºC]	Temp [ºC]	AV	SD	SE
0	23.9	20	24	22.63333	2.281082	10.07842
10	24.4	20.1	24.5	23	2.511971	10.92161
20	24.8	20.5	24.7	23.33333	2.454248	10.51821
30	25.1	20.9	25	23.66667	2.396525	10.12616
40	25.5	21.3	25.4	24.06667	2.396525	9.957861
50	25.9	21.8	25.8	24.5	2.338803	9.546135
60	26.2	22.3	26.1	24.86667	2.223361	8.941129
70	26.5	22.8	26.4	25.23333	2.107922	8.353718
80	26.9	23.3	26.8	25.66667	2.050203	7.987805
90	27.2	23.8	27.1	26.03333	1.93477	7.431893
100	27.4	24.3	27.3	26.33333	1.761628	6.689727
110	27.7	24.8	27.6	26.7	1.646208	6.165572
120	28	25.3	27.9	27.06667	1.530795	5.655647
130	28.3	25.7	28.2	27.4	1.473092	5.376248
140	28.6	26.2	28.5	27.76667	1.357694	4.889655
150	28.9	26.7	28.8	28.13333	1.24231	4.415793
160	29.2	27.2	29.1	28.5	1.126943	3.954185
170	29.5	27.6	29.4	28.83333	1.069268	3.708443
180	29.8	28	29.7	29.16667	1.011599	3.468341

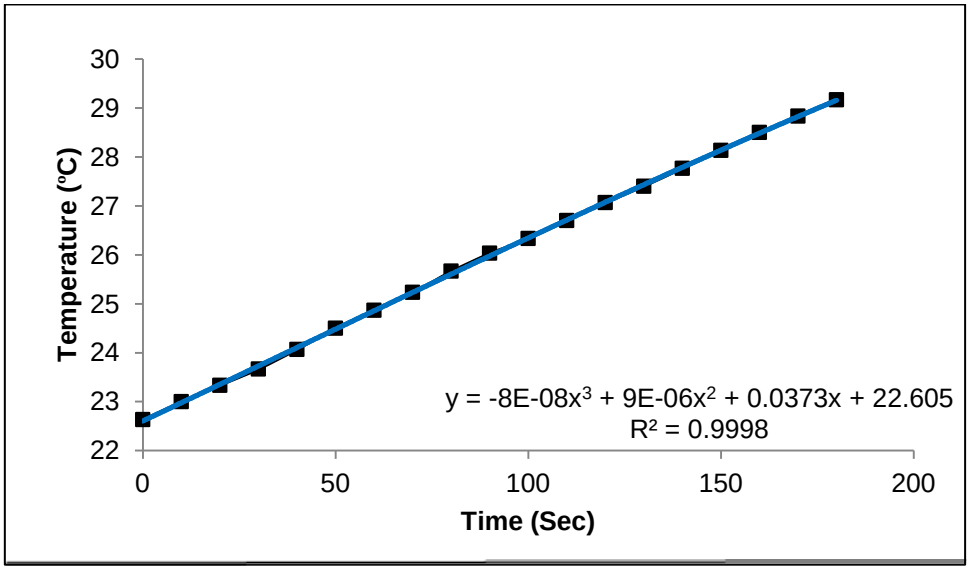


Table 1.1 Calculation of ultrasound power using different ultrasonic equipment and 200 mL water

Frequency (kHz)	**Polynomial equation**	**Power = $dT/dt \times C_p \times m$ (Watt)**
20	$y = -1E-08x^3 - 4E-06x^2 + 0.0213x + 22.412$ $R^2 = 0.9996$	0.0213 ˚C/Sec x 4.19 J /(g x ˚C) x 200g = **17.85W**
40	$y = -6E-08x^3 + 3E-05x^2 + 0.0255x + 23.353$ $R^2 = 0.9998$	0.0255˚C/Sec x 4.19 J / (g x ˚C) x 200g = **21.369W**
580 (40%)	$y = -3E-08x^3 + 8E-06x^2 + 0.0021x + 21.512$ $R^2 = 0.9883$	0.0021 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **1.7598W**
580 (80%)	$y = 2E-09x^3 - 2E-05x^2 + 0.0256x + 23.171$ $R^2 = 0.9992$	0.0256 ˚C /Sec x 4.19 J/(g x ˚C) x 200g = **21.4528W**
580 (max)	$y = -1E-07x^3 - 1E-05x^2 + 0.0588x + 29.939$ $R^2 = 0.9993$	0.0588 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **49.2744W**
864 (40%)	$y = 1E-08x^3 - 3E-06x^2 + 0.005x + 23.848$ $R^2 = 0.995$	0.005 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **4.19W**
864 (80%)	$y = -2E-07x^3 + 6E-05x^2 + 0.0198x + 21.856$ $R^2 = 0.9999$	0.0198 ˚C /Sec x 4.19 J/(g x ˚C) x 200g = **16.5924W**
864 (max)	$y = 2E-07x^3 - 1E-04x^2 + 0.0687x + 22.054$ $R^2 = 0.9982$	0.0687 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **57.5706W**
1146 (40%)	$y = -4E-08x^3 + 6E-06x^2 + 0.0031x + 24.549$ $R^2 = 0.9914$	0.0031 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **2.5978W**
1146 (80%)	$y = -2E-07x^3 + 8E-05x^2 + 0.0148x + 21.346$ $R^2 = 0.9997$	0.0148 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **12.4024W**
1146 (max)	$y = -7E-07x3 + 0.0002x2 + 0.0296x + 20.18$ $R^2 = 0.9996$	0.0296 ˚C/Sec x 4.19 J/(g x ˚C) x 200g = **24.8048W**

Table 1.2 Calculation of ultrasound power using different ultrasonic equipment and 400 mL water

Frequency (kHz)	Polynomial equation	Power = $dT/dt \times C_p \times m$ (Watt)
20	$y = 2E{-}07x^3 - 8E{-}05x^2 + 0.0135x + 21.335$ $R^2 = 0.9806$	0.0135 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **22.6260W**
40	$y = 3E{-}07x^3 - 9E{-}05x^2 + 0.0278x + 22.482$ $R^2 = 0.9982$	0.0278 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **46.5928W**
580 (40%)	$y = -8E{-}08x^3 + 2E{-}05x^2 + 0.001x + 21.989$ $R^2 = 0.9811$	0.001 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **1.6760 W**
580 (80%)	$y = -5E{-}09x^3 + 2E{-}05x^2 + 0.0118x + 22.223$ $R^2 = 0.9995$	0.0118 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **19.7768W**
580 (max)	$y = 7E{-}08x^3 - 4E{-}05x^2 + 0.0382x + 22.321$ $R^2 = 0.9997$	0.0382 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **64.0232W**
864 (40%)	$y = -2E{-}08x^3 + 2E{-}06x^2 + 0.0036x + 21.619$ $R^2 = 0.9859$	0.0036 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **6.0336W**
864 (80%)	$y = 2E{-}07x^3 - 5E{-}05x^2 + 0.0145x + 21.464$ $R^2 = 0.9988$	0.0145 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **24.3020W**
864 (max)	$y = -7E{-}08x^3 - 1E{-}05x^2 + 0.0554x + 21.368$ $R^2 = 0.9991$	0.0554 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **92.8504W**
1146 (40%)	$y = -6E{-}08x^3 + 2E{-}05x^2 - 0.001x + 22.394$ $R^2 = 0.9677$	0.001 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **1.6400W**
1146 (80%)	$y = -1E{-}07x^3 + 2E{-}05x^2 + 0.0117x + 23.518$ $R^2 = 0.9964$	0.0117 ˚C/Sec x 4.19 J /(g x ˚C) x 400g = **19.6092W**
1146 (max)	$y = -8E{-}08x^3 + 9E{-}06x^2 + 0.0373x + 22.605$ $R^2 = 0.9998$	0.0373˚C/Sec x 4.19 J /(g x ˚C) x 400g = **62.5148W**

Appendix 2 RESULTS USING HAEMOCYTOMETER AND OPTICAL DENSITY

Result 1 Inactivation of 200 mL *Microcystis aeruginosa* using the 20 kHz probe (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	%HAE	OD	OD	OD	AV	C/C0	%OD
0	602	589	556	582.3333	1	0	0.2	0.2	0.195	0.198333	1	0
5	561	455	439	485	0.832856	16.71437	0.175	0.175	0.18	0.176667	0.890756	10.92437
10	532	325	542	466.3333	0.800801	19.91986	0.18	0.175	0.175	0.1775	0.894958	10.5042
20	557	419	423	466.3333	0.800801	19.91986	0.175	0.175	0.18	0.1775	0.894958	10.5042
30	532	491	549	524	0.899828	10.01717	0.19	0.185	0.19	0.188333	0.94958	5.042017

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	582.3333	23.71357	13.69104	523.4256	641.2411	556.00	602.00
	5	3	485.0000	66.30234	38.27967	320.2959	649.7041	439.00	561.00
	10	3	466.3333	122.50034	70.72560	162.0256	770.6410	325.00	542.00
	20	3	466.3333	78.54510	45.34804	271.2165	661.4502	419.00	557.00
	30	3	524.0000	29.81610	17.21434	449.9327	598.0673	491.00	549.00
	Total	15	504.8000	77.10864	19.90937	462.0987	547.5013	325.00	602.00
OD	0	3	.1983	.00289	.00167	.1912	.2055	.20	.20
	5	3	.1767	.00289	.00167	.1695	.1838	.18	.18
	10	3	.1767	.00289	.00167	.1695	.1838	.18	.18
	20	3	.1767	.00289	.00167	.1695	.1838	.18	.18
	30	3	.1883	.00289	.00167	.1812	.1955	.19	.19
	Total	15	.1833	.00939	.00242	.1781	.1885	.18	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	11293.600	4	2823.400	10.267	.001
	Within Groups	2750.000	10	275.000		
	Total	14043.600	14			
OD	Between Groups	.001	4	.000	8.667	.003
	Within Groups	.000	10	.000		
	Total	.001	14			

Result 2 Inactivation of 400 mL *Microcystis aeruginosa* using the 20 kHz probe (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	%HAE	OD	OD	OD	AV	C/C0	%OD
0	582	591	594	589	1	0	0.2	0.19	0.19	0.1933333	1	0
5	578	588	584	583.3333	0.990379	0.962083	0.2	0.2	0.2	0.2	1.034483	−3.48828
10	541	571	589	567	0.962649	3.735144	0.195	0.19	0.195	0.1933333	1	0
20	527	564	542	544.3333	0.924165	7.583475	0.18	0.19	0.19	0.1866667	0.965517	3.48267
30	492	524	527	514.3333	0.873231	12.67685	0.175	0.185	0.18	0.18	0.931034	6.896552

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	589.0000	6.24500	3.60555	573.4866	604.5134	582.00	594.00
	5	3	583.3333	5.03322	2.90593	570.8301	595.8366	578.00	588.00
	10	3	567.0000	24.24871	14.00000	506.7629	627.2371	541.00	589.00
	20	3	544.3333	18.61003	10.74451	498.1034	590.5632	527.00	564.00
	30	3	514.3333	19.39931	11.20020	466.1428	562.5239	492.00	527.00
	Total	15	559.6000	31.67198	8.17767	542.0606	577.1394	492.00	594.00
OD	0	3	.1933	.00577	.00333	.1790	.2077	.19	.20
	5	3	.2000	.00000	.00000	.2000	.2000	.20	.20
	10	3	.1933	.00289	.00167	.1862	.2005	.19	.20
	20	3	.1867	.00577	.00333	.1723	.2010	.18	.19
	30	3	.1800	.00500	.00289	.1676	.1924	.18	.19
	Total	15	.1907	.00799	.00206	.1862	.1951	.18	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	11293.600	4	2823.400	10.267	.001
	Within Groups	2750.000	10	275.000		
	Total	14043.600	14			
OD	Between Groups	.001	4	.000	8.667	.003
	Within Groups	.000	10	.000		
	Total	.001	14			

Result 3 Inactivation of 200 mL Microcystis aeruginosa using a 40 kHz bath (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	%HAE	OD	OD	OD	AV	C/C0	%OD
0	592	593	577	587.3333	1	0	0.2	0.2	0.195	0.1983333	1	0
5	561	577	581	573	0.975596	2.440409	0.185	0.185	0.18	0.1833333	0.92437	7.563025
10	532	581	577	563.3333	0.959137	4.086266	0.185	0.18	0.185	0.1816667	0.915966	8.403361
20	557	577	544	559.3333	0.952327	4.76731	0.185	0.185	0.175	0.1816667	0.915966	8.403361
30	544	624	599	589	1.002838	−0.28377	0.21	0.2	0.21	0.2066667	1.042017	−4.20168

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	587.3333	8.96289	5.17472	565.0683	609.5984	577.00	593.00
	5	3	573.0000	10.58301	6.11010	546.7104	599.2896	561.00	581.00
	10	3	563.3333	27.20907	15.70916	495.7423	630.9244	532.00	581.00
	20	3	559.3333	16.62328	9.59745	518.0388	600.6278	544.00	577.00
	30	3	589.0000	40.92676	23.62908	487.3323	690.6677	544.00	624.00
	Total	15	574.4000	23.85013	6.15808	561.1922	587.6078	532.00	624.00
OD	0	3	.1983	.00289	.00167	.1912	.2055	.20	.20
	5	3	.1833	.00289	.00167	.1762	.1905	.18	.19
	10	3	.1833	.00289	.00167	.1762	.1905	.18	.19
	20	3	.1817	.00577	.00333	.1673	.1960	.18	.19
	30	3	.2067	.00577	.00333	.1923	.2210	.20	.21
	Total	15	.1907	.01100	.00284	.1846	.1968	.18	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	2195.600	4	548.900	.952	.474
	Within Groups	5768.000	10	576.800		
	Total	7963.600	14			
OD	Between Groups	.002	4	.000	20.591	.000
	Within Groups	.000	10	.000		
	Total	.002	14			

Result 4 Inactivation of 400 mL Microcystis aeruginosa using the 40 kHz bath (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	%HAE	OD	OD	OD	AV	C/C0	% OD
0	584	579	585	582.6667	1	0	0.2	0.19	0.19	0.1933333	1	0
5	591	584	579	584.6667	1.003432	−0.34325	0.195	0.205	0.2	0.2	1.034483	−3.44828
10	583	578	582	581	0.99714	0.286041	0.19	0.2	0.2	0.1966667	1.017241	−1.72414
20	547	558	574	559.6667	0.960526	3.947368	0.18	0.19	0.195	0.1883333	0.974138	2.586207
30	544	539	557	546.6667	0.938215	6.17849	0.18	0.185	0.19	0.185	0.956897	4.310345

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	582.6667	3.21455	1.85592	574.6813	590.6521	579.00	585.00
	5	3	584.6667	6.02771	3.48010	569.6930	599.6403	579.00	591.00
	10	3	581.0000	2.64575	1.52753	574.4276	587.5724	578.00	583.00
	20	3	559.6667	13.57694	7.83865	525.9397	593.3937	547.00	574.00
	30	3	546.6667	9.29157	5.36449	523.5851	569.7482	539.00	557.00
	Total	15	570.9333	17.06905	4.40721	561.4808	580.3859	539.00	591.00
OD	0	3	.1933	.00577	.00333	.1790	.2077	.19	.20
	5	3	.2000	.00500	.00289	.1876	.2124	.20	.21
	10	3	.1967	.00577	.00333	.1823	.2110	.19	.20
	20	3	.1883	.00764	.00441	.1694	.2073	.18	.20
	30	3	.1850	.00500	.00289	.1726	.1974	.18	.19
	Total	15	.1927	.00753	.00194	.1885	.1968	.18	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	3430.267	4	857.567	13.220	.001
	Within Groups	648.667	10	64.867		
	Total	4078.933	14			
OD	Between Groups	.000	4	.000	3.167	.063
	Within Groups	.000	10	.000		
	Total	.001	14			

Result 5 Inactivation of 200 mL Microcystis aeruginosa using the 580 kHz bath at 40% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	583	598	593	591.3333	1	0	0.2	0.21	0.195	0.201667	1	0
5	566	587	573	575.3333	0.972943	2.70575	0.19	0.2	0.19	0.193333	0.958678	4.132231
10	542	574	556	557.3333	0.942503	5.749718	0.185	0.2	0.185	0.19	0.942149	5.785124
20	521	553	542	538.6667	0.910936	8.906426	0.17	0.19	0.185	0.181667	0.900826	9.917355
30	510	522	497	509.6667	0.861894	13.8106	0.17	0.18	0.175	0.175	0.867769	13.22314

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	591.3333	7.63763	4.40959	572.3604	610.3062	583.00	598.00
	5	3	575.3333	10.69268	6.17342	548.7713	601.8954	566.00	587.00
	10	3	557.3333	16.04161	9.26163	517.4838	597.1829	542.00	574.00
	20	3	538.6667	16.25833	9.38675	498.2787	579.0546	521.00	553.00
	30	3	509.6667	12.50333	7.21880	478.6067	540.7267	497.00	522.00
	Total	15	554.4667	31.48666	8.12982	537.0299	571.9034	497.00	598.00
OD	0	3	.2017	.00764	.00441	.1827	.2206	.20	.21
	5	3	.1933	.00577	.00333	.1790	.2077	.19	.20
	10	3	.1900	.00866	.00500	.1685	.2115	.19	.20
	20	3	.1817	.01041	.00601	.1558	.2075	.17	.19
	30	3	.1750	.00500	.00289	.1626	.1874	.17	.18
	Total	15	.1883	.01160	.00299	.1819	.1948	.17	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	12178.400	4	3044.600	17.895	.000
	Within Groups	1701.333	10	170.133		
	Total	13879.733	14			
OD	Between Groups	.001	4	.000	5.347	.014
	Within Groups	.001	10	.000		
	Total	.002	14			

Result 6 Inactivation of 400 mL Microcystis aeruginosa using the 580 kHz bath at 40% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	%HAE	OD	OD	OD	AV	C/C0	%OD
0	636	648	627	637	1	0	0.2	0.2	0.2	0.2	1	0
5	592	589	596	592.3333	0.92988	7.012036	0.185	0.195	0.195	0.1916667	0.958333	4.166667
10	571	567	582	573.3333	0.900052	9.994767	0.18	0.195	0.195	0.19	0.95	5
20	538	572	588	566	0.88854	11.146	0.175	0.2	0.185	0.1866667	0.933333	6.666667
30	527	556	577	553.3333	0.868655	13.13448	0.19	0.19	0.2	0.1933333	0.966667	3.333333

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	637.0000	10.53565	6.08276	610.8280	663.1720	627.00	648.00
	5	3	592.3333	3.51188	2.02759	583.6093	601.0573	589.00	596.00
	10	3	573.3333	7.76745	4.48454	554.0379	592.6288	567.00	582.00
	20	3	566.0000	25.53429	14.74223	502.5693	629.4307	538.00	588.00
	30	3	553.3333	25.10644	14.49521	490.9655	615.7012	527.00	577.00
	Total	15	584.4000	33.48731	8.64639	565.8553	602.9447	527.00	648.00
OD	0	3	.2000	.00000	.00000	.2000	.2000	.20	.20
	5	3	.1917	.00577	.00333	.1773	.2060	.19	.20
	10	3	.1900	.00866	.00500	.1685	.2115	.18	.20
	20	3	.1867	.01258	.00726	.1554	.2179	.18	.20
	30	3	.1933	.00577	.00333	.1790	.2077	.19	.20
	Total	15	.1923	.00799	.00206	.1879	.1968	.18	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	12767.600	4	3191.900	10.886	.001
	Within Groups	2932.000	10	293.200		
	Total	15699.600	14			
OD	Between Groups	.000	4	.000	1.222	.361
	Within Groups	.001	10	.000		
	Total	.001	14			

Result 7 Inactivation of 200 mL *Microcystis aeruginosa* using the 580 kHz bath at 80% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	877	873	864	871.3333	1	0	0.19	0.19	0.19	0.19	1	0
5	586	575	566	575.6667	0.660673	33.93267	0.165	0.165	0.165	0.165	0.868421	13.15789
10	510	502	511	507.6667	0.582632	41.7368	0.145	0.15	0.15	0.1483333	0.780702	21.92982
20	488	468	472	476	0.546289	45.37108	0.135	0.13	0.13	0.1316667	0.692982	30.70175
30	359	344	360	354.3333	0.406656	59.33435	0.125	0.11	0.125	0.12	0.631579	36.84211

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	871.3333	6.65833	3.84419	854.7931	887.8735	864.00	877.00
	5	3	575.6667	10.01665	5.78312	550.7839	600.5494	566.00	586.00
	10	3	507.6667	4.93288	2.84800	495.4127	519.9206	502.00	511.00
	20	3	476.0000	10.58301	6.11010	449.7104	502.2896	468.00	488.00
	30	3	354.3333	8.96289	5.17472	332.0683	376.5984	344.00	360.00
	Total	15	557.0000	178.96328	46.20812	457.8934	656.1066	344.00	877.00
OD	0	3	.1900	.00000	.00000	.1900	.1900	.19	.19
	5	3	.1650	.00000	.00000	.1650	.1650	.17	.17
	10	3	.1483	.00289	.00167	.1412	.1555	.15	.15
	20	3	.1317	.00289	.00167	.1245	.1388	.13	.14
	30	3	.1200	.00866	.00500	.0985	.1415	.11	.13
	Total	15	.1510	.02586	.00668	.1367	.1653	.11	.19

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	447667.333	4	111916.833	1548.665	.000
	Within Groups	722.667	10	72.267		
	Total	448390.000	14			
OD	Between Groups	.009	4	.002	125.136	.000
	Within Groups	.000	10	.000		
	Total	.009	14			

Result 8 Inactivation of 400 mL *Microcystis aeruginosa* using the 580 kHz bath at 80% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	621	618	632	623.6667	1	0	0.21	0.2	0.2	0.2033333	1	0
5	482	477	472	477	0.764832	23.51684	0.19	0.17	0.19	0.1833333	0.868421	13.15789
10	445	436	433	438	0.702298	29.77018	0.18	0.16	0.18	0.1733333	0.780702	21.92982
20	411	407	412	410	0.657402	34.25975	0.17	0.16	0.165	0.165	0.692982	30.70175
30	365	372	369	368.6667	0.591128	40.88723	0.17	0.15	0.16	0.16	0.631579	36.84211

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	623.6667	7.37111	4.25572	605.3558	641.9775	618.00	632.00
	5	3	477.0000	5.00000	2.88675	464.5793	489.4207	472.00	482.00
	10	3	438.0000	6.24500	3.60555	422.4866	453.5134	433.00	445.00
	20	3	410.0000	2.64575	1.52753	403.4276	416.5724	407.00	412.00
	30	3	368.6667	3.51188	2.02759	359.9427	377.3907	365.00	372.00
	Total	15	463.4667	90.75073	23.43174	413.2106	513.7227	365.00	632.00
OD	0	3	.2033	.00577	.00333	.1890	.2177	.20	.21
	5	3	.1833	.01155	.00667	.1546	.2120	.17	.19
	10	3	.1733	.01155	.00667	.1446	.2020	.16	.18
	20	3	.1650	.00500	.00289	.1526	.1774	.16	.17
	30	3	.1600	.01000	.00577	.1352	.1848	.15	.17
	Total	15	.1770	.01771	.00457	.1672	.1868	.15	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	115024.400	4	28756.100	1044.410	.000
	Within Groups	275.333	10	27.533		
	Total	115299.733	14			
OD	Between Groups	.004	4	.001	10.412	.001
	Within Groups	.001	10	.000		
	Total	.004	14			

Result 9 Inactivation of 200 mL *Microcystis aeruginosa* using the 580 kHz bath at maximum power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	580	572	591	581	1	0	0.19	0.19	0.19	0.19	1	0
5	521	512	524	519	0.893287	10.67126	0.145	0.14	0.135	0.14	0.736842	26.31579
10	478	468	378	441.3333	0.75961	24.03901	0.13	0.13	0.12	0.1266667	0.666667	33.33333
20	362	377	344	361	0.621343	37.86575	0.12	0.12	0.1	0.1133333	0.596491	40.35088
30	342	321	311	324.6667	0.558807	44.11933	0.11	0.1	0.09	0.1	0.526316	47.36842

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	581.0000	9.53939	5.50757	557.3028	604.6972	572.00	591.00
	5	3	519.0000	6.24500	3.60555	503.4866	534.5134	512.00	524.00
	10	3	441.3333	55.07571	31.79797	304.5177	578.1490	378.00	478.00
	20	3	361.0000	16.52271	9.53939	319.9553	402.0447	344.00	377.00
	30	3	324.6667	15.82193	9.13479	285.3628	363.9705	311.00	342.00
	Total	15	445.4000	101.39583	26.18029	389.2489	501.5511	311.00	591.00
OD	0	3	.1900	.00000	.00000	.1900	.1900	.19	.19
	5	3	.1400	.00500	.00289	.1276	.1524	.14	.15
	10	3	.1267	.00577	.00333	.1123	.1410	.12	.13
	20	3	.1133	.01155	.00667	.0846	.1420	.10	.12
	30	3	.1000	.01000	.00577	.0752	.1248	.09	.11
	Total	15	.1340	.03274	.00845	.1159	.1521	.09	.19

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	136562.267	4	34140.567	46.303	.000
	Within Groups	7373.333	10	737.333		
	Total	143935.600	14			
OD	Between Groups	.014	4	.004	61.829	.000
	Within Groups	.001	10	.000		
	Total	.015	14			

Result 10 Inactivation of 400 mL *Microcystis aeruginosa* using the 580 kHz bath at maximum power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	621	591	595	602.3333	1	0	0.2	0.19	0.19	0.1933333	1	0
5	596	582	584	587.3333	0.975097	2.490315	0.19	0.185	0.185	0.1866667	0.965517	3.448276
10	540	561	544	548.3333	0.910349	8.965136	0.18	0.18	0.18	0.18	0.931034	6.896552
20	507	528	520	518.3333	0.860542	13.94577	0.175	0.175	0.17	0.1733333	0.896552	10.34483
30	482	461	483	475.3333	0.789153	21.08467	0.17	0.165	0.165	0.1666667	0.862069	13.7931

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	602.3333	16.28906	9.40449	561.8691	642.7976	591.00	621.00
	5	3	587.3333	7.57188	4.37163	568.5237	606.1429	582.00	596.00
	10	3	548.3333	11.15049	6.43774	520.6340	576.0327	540.00	561.00
	20	3	518.3333	10.59874	6.11919	492.0046	544.6621	507.00	528.00
	30	3	475.3333	12.42310	7.17248	444.4727	506.1940	461.00	483.00
	Total	15	546.3333	48.81842	12.60486	519.2986	573.3681	461.00	621.00
OD	0	3	.1933	.00577	.00333	.1790	.2077	.19	.20
	5	3	.1867	.00289	.00167	.1795	.1938	.19	.19
	10	3	.1800	.00000	.00000	.1800	.1800	.18	.18
	20	3	.1733	.00289	.00167	.1662	.1805	.17	.18
	30	3	.1667	.00289	.00167	.1595	.1738	.17	.17
	Total	15	.1800	.01018	.00263	.1744	.1856	.17	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	31938.000	4	7984.500	55.940	.000
	Within Groups	1427.333	10	142.733		
	Total	33365.333	14			
OD	Between Groups	.001	4	.000	28.571	.000
	Within Groups	.000	10	.000		
	Total	.001	14			

Result 11 Inactivation of 200 mL *Microcystis aeruginosa* using the 864 kHz bath at 40% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	755	748	766	756.3333	1	0	0.19	0.19	0.19	0.19	1	0
5	569	566	548	561	0.741736	25.82636	0.17	0.16	0.16	0.1633333	0.859649	14.03509
10	566	524	537	542.3333	0.717056	28.2944	0.165	0.155	0.155	0.1583333	0.833333	16.66667
20	548	507	496	517	0.683561	31.6439	0.165	0.15	0.15	0.155	0.815789	18.42105
30	532	482	471	495	0.654473	34.55267	0.15	0.15	0.15	0.15	0.789474	21.05263

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	756.3333	9.07377	5.23874	733.7928	778.8738	748.00	766.00
	5	3	561.0000	11.35782	6.55744	532.7856	589.2144	548.00	569.00
	10	3	542.3333	21.50194	12.41415	488.9196	595.7471	524.00	566.00
	20	3	517.0000	27.40438	15.82193	448.9237	585.0763	496.00	548.00
	30	3	495.0000	32.51154	18.77054	414.2369	575.7631	471.00	532.00
	Total	15	574.3333	98.80838	25.51221	519.6151	629.0516	471.00	766.00
OD	0	3	.1900	.00000	.00000	.1900	.1900	.19	.19
	5	3	.1633	.00577	.00333	.1490	.1777	.16	.17
	10	3	.1583	.00577	.00333	.1440	.1727	.16	.17
	20	3	.1550	.00866	.00500	.1335	.1765	.15	.17
	30	3	.1500	.00000	.00000	.1500	.1500	.15	.15
	Total	15	.1633	.01520	.00392	.1549	.1717	.15	.19

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	131720.000	4	32930.000	66.347	.000
	Within Groups	4963.333	10	496.333		
	Total	136683.333	14			
OD	Between Groups	.003	4	.001	26.029	.000
	Within Groups	.000	10	.000		
	Total	.003	14			

Result 12 Inactivation of 400 mL *Microcystis aeruginosa* using the 864 kHz bath at 40% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	574	582	577	577.6667	1	0	0.19	0.19	0.19	0.19	1	0
5	522	527	533	527.3333	0.912868	8.713214	0.18	0.18	0.185	0.1816667	0.95614	4.385965
10	476	482	488	482	0.834391	16.56088	0.17	0.18	0.18	0.1766667	0.929825	7.017544
20	452	449	433	444.6667	0.769763	23.02366	0.17	0.18	0.18	0.1766667	0.929825	7.017544
30	419	430	422	423.6667	0.73341	26.65897	0.165	0.17	0.17	0.1683333	0.885965	11.40351

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	577.6667	4.04145	2.33333	567.6271	587.7062	574.00	582.00
	5	3	527.3333	5.50757	3.17980	513.6518	541.0149	522.00	533.00
	10	3	482.0000	6.00000	3.46410	467.0952	496.9048	476.00	488.00
	20	3	444.6667	10.21437	5.89727	419.2928	470.0406	433.00	452.00
	30	3	423.6667	5.68624	3.28295	409.5413	437.7921	419.00	430.00
	Total	15	491.0667	58.07442	14.99475	458.9061	523.2272	419.00	582.00
OD	0	3	.1900	.00000	.00000	.1900	.1900	.19	.19
	5	3	.1817	.00289	.00167	.1745	.1888	.18	.19
	10	3	.1767	.00577	.00333	.1623	.1910	.17	.18
	20	3	.1767	.00577	.00333	.1623	.1910	.17	.18
	30	3	.1683	.00289	.00167	.1612	.1755	.17	.17
	Total	15	.1787	.00812	.00210	.1742	.1832	.17	.19

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	46778.267	4	11694.567	266.593	.000
	Within Groups	438.667	10	43.867		
	Total	47216.933	14			
OD	Between Groups	.001	4	.000	11.350	.001
	Within Groups	.000	10	.000		
	Total	.001	14			

Result 13 Inactivation of 200 mL *Microcystis aeruginosa* using the 864 kHz bath at 80% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	835	827	804	822	1	0	0.21	0.21	0.19	0.2033333	1	0
5	623	614	507	581.3333	0.707218	29.27818	0.19	0.18	0.16	0.1766667	0.868852	13.11475
10	576	558	491	541.6667	0.658962	34.10381	0.18	0.17	0.15	0.165	0.811475	18.85246
20	366	347	322	345	0.419708	58.0292	0.16	0.15	0.14	0.15	0.737705	26.22951
30	299	254	246	266.3333	0.324006	67.59935	0.145	0.13	0.13	0.135	0.663934	33.60656

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	822.0000	16.09348	9.29157	782.0216	861.9784	804.00	835.00
	5	3	581.3333	64.53165	37.25736	421.0278	741.6388	507.00	623.00
	10	3	541.6667	44.79211	25.86074	430.3969	652.9364	491.00	576.00
	20	3	345.0000	22.06808	12.74101	290.1799	399.8201	322.00	366.00
	30	3	266.3333	28.57155	16.49579	195.3577	337.3090	246.00	299.00
	Total	15	511.2667	204.51038	52.80435	398.0126	624.5207	246.00	835.00
OD	0	3	.2033	.01155	.00667	.1746	.2320	.19	.21
	5	3	.1767	.01528	.00882	.1387	.2146	.16	.19
	10	3	.1667	.01528	.00882	.1287	.2046	.15	.18
	20	3	.1500	.01000	.00577	.1252	.1748	.14	.16
	30	3	.1350	.00866	.00500	.1135	.1565	.13	.15
	Total	15	.1663	.02635	.00680	.1517	.1809	.13	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	570076.933	4	142519.233	92.150	.000
	Within Groups	15466.000	10	1546.600		
	Total	585542.933	14			
OD	Between Groups	.008	4	.002	13.183	.001
	Within Groups	.002	10	.000		
	Total	.010	14			

Result 14 Inactivation of 400 mL *Microcystis aeruginosa* using the 864 kHz bath at 80% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	630	605	611	615.3333	1	0	0.21	0.21	0.19	0.2033333	1	0
5	527	533	521	527	0.856446	14.35536	0.19	0.18	0.16	0.1766667	0.868852	13.11475
10	508	511	501	506.6667	0.823402	17.6598	0.18	0.17	0.15	0.1666667	0.819672	18.03279
20	456	446	448	450	0.731311	26.86891	0.16	0.15	0.14	0.15	0.737705	26.22951
30	412	418	407	412.3333	0.670098	32.99025	0.145	0.13	0.13	0.135	0.663934	33.60656

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	615.3333	13.05118	7.53510	582.9124	647.7543	605.00	630.00
	5	3	527.0000	6.00000	3.46410	512.0952	541.9048	521.00	533.00
	10	3	506.6667	5.13160	2.96273	493.9191	519.4143	501.00	511.00
	20	3	450.0000	5.29150	3.05505	436.8552	463.1448	446.00	456.00
	30	3	412.3333	5.50757	3.17980	398.6518	426.0149	407.00	418.00
	Total	15	502.2667	72.35179	18.68115	462.1996	542.3338	407.00	630.00
OD	0	3	.2033	.01155	.00667	.1746	.2320	.19	.21
	5	3	.1767	.01528	.00882	.1387	.2146	.16	.19
	10	3	.1667	.01528	.00882	.1287	.2046	.15	.18
	20	3	.1500	.01000	.00577	.1252	.1748	.14	.16
	30	3	.1350	.00866	.00500	.1135	.1565	.13	.15
	Total	15	.1663	.02635	.00680	.1517	.1809	.13	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	72704.933	4	18176.233	312.306	.000
	Within Groups	582.000	10	58.200		
	Total	73286.933	14			
OD	Between Groups	.008	4	.002	13.183	.001
	Within Groups	.002	10	.000		
	Total	.010	14			

Result 15 Inactivation of 200 mL *Microcystis aeruginosa* using the 864 kHz bath at maximum power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	844	804	821	823	1	0	0.2	0.19	0.195	0.195	1	0
5	421	416	438	425	0.516403	48.35966	0.14	0.135	0.155	0.1433333	0.735043	26.49573
10	366	372	387	375	0.45565	54.43499	0.13	0.125	0.135	0.13	0.666667	33.33333
20	211	209	294	238	0.289186	71.08141	0.11	0.11	0.125	0.115	0.589744	41.02564
30	142	137	188	155.6667	0.189145	81.08546	0.1	0.1	0.11	0.1033333	0.529915	47.00855

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	823.0000	20.07486	11.59023	773.1313	872.8687	804.00	844.00
	5	3	425.0000	11.53256	6.65833	396.3515	453.6485	416.00	438.00
	10	3	375.0000	10.81665	6.24500	348.1299	401.8701	366.00	387.00
	20	3	238.0000	48.50773	28.00595	117.5001	358.4999	209.00	294.00
	30	3	155.6667	28.11287	16.23097	85.8304	225.5029	137.00	188.00
	Total	15	403.3333	239.90941	61.94434	270.4759	536.1907	137.00	844.00
OD	0	3	.1950	.00500	.00289	.1826	.2074	.19	.20
	5	3	.1433	.01041	.00601	.1175	.1692	.14	.16
	10	3	.1300	.00500	.00289	.1176	.1424	.13	.14
	20	3	.1150	.00866	.00500	.0935	.1365	.11	.13
	30	3	.1033	.00577	.00333	.0890	.1177	.10	.11
	Total	15	.1373	.03353	.00866	.1188	.1559	.10	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	798198.667	4	199549.667	262.819	.000
	Within Groups	7592.667	10	759.267		
	Total	805791.333	14			
OD	Between Groups	.015	4	.004	71.297	.000
	Within Groups	.001	10	.000		
	Total	.016	14			

Result 16 Inactivation of 400 mL *Microcystis aeruginosa* using the 864 kHz bath at maximum power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	582	607	611	600	1	0	0.21	0.21	0.21	0.21	1	0
5	476	482	488	482	0.803333	19.66667	0.15	0.15	0.17	0.1566667	0.746032	25.39683
10	425	431	452	436	0.726667	27.33333	0.13	0.12	0.14	0.13	0.619048	38.09524
20	217	251	233	233.6667	0.389444	61.05556	0.09	0.09	0.1	0.0933333	0.444444	55.55556
30	133	120	115	122.6667	0.204444	79.55556	0.08	0.08	0.085	0.0816667	0.388889	61.11111

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	600.0000	15.71623	9.07377	560.9587	639.0413	582.00	611.00
	5	3	482.0000	6.00000	3.46410	467.0952	496.9048	476.00	488.00
	10	3	436.0000	14.17745	8.18535	400.7813	471.2187	425.00	452.00
	20	3	233.6667	17.00980	9.82061	191.4120	275.9214	217.00	251.00
	30	3	122.6667	9.29157	5.36449	99.5851	145.7482	115.00	133.00
	Total	15	374.8667	179.29340	46.29336	275.5773	474.1560	115.00	611.00
OD	0	3	.2100	.00000	.00000	.2100	.2100	.21	.21
	5	3	.1567	.01155	.00667	.1280	.1854	.15	.17
	10	3	.1300	.01000	.00577	.1052	.1548	.12	.14
	20	3	.0933	.00577	.00333	.0790	.1077	.09	.10
	30	3	.0817	.00289	.00167	.0745	.0888	.08	.09
	Total	15	.1343	.04829	.01247	.1076	.1611	.08	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	448326.400	4	112081.600	651.890	.000
	Within Groups	1719.333	10	171.933		
	Total	450045.733	14			
OD	Between Groups	.032	4	.008	145.879	.000
	Within Groups	.001	10	.000		
	Total	.033	14			

Result 17 Inactivation of 200 mL *Microcystis aeruginosa* using the 1146 kHz bath at 40% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	749	752	726	742.3333	1	0	0.2	0.2	0.2	0.2	1	0
5	684	694	697	691.6667	0.931747	6.825326	0.185	0.185	0.185	0.185	0.925	7.5
10	678	682	668	676	0.910642	8.935788	0.185	0.18	0.185	0.1833333	0.916667	8.333333
20	649	655	659	654.3333	0.881455	11.85451	0.18	0.18	0.19	0.1833333	0.916667	8.333333
30	627	602	625	618	0.83251	16.74899	0.19	0.175	0.185	0.1833333	0.916667	8.333333

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	742.3333	14.22439	8.21246	706.9980	777.6687	726.00	752.00
	5	3	691.6667	6.80686	3.92994	674.7575	708.5758	684.00	697.00
	10	3	676.0000	7.21110	4.16333	658.0866	693.9134	668.00	682.00
	20	3	654.3333	5.03322	2.90593	641.8301	666.8366	649.00	659.00
	30	3	618.0000	13.89244	8.02081	583.4893	652.5107	602.00	627.00
	Total	15	676.4667	43.48377	11.22746	652.3862	700.5472	602.00	752.00
OD	0	3	.2000	.00000	.00000	.2000	.2000	.20	.20
	5	3	.1850	.00000	.00000	.1850	.1850	.19	.19
	10	3	.1833	.00289	.00167	.1762	.1905	.18	.19
	20	3	.1833	.00577	.00333	.1690	.1977	.18	.19
	30	3	.1833	.00764	.00441	.1644	.2023	.18	.19
	Total	15	.1870	.00775	.00200	.1827	.1913	.18	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	25433.733	4	6358.433	61.257	.000
	Within Groups	1038.000	10	103.800		
	Total	26471.733	14			
OD	Between Groups	.001	4	.000	8.000	.004
	Within Groups	.000	10	.000		
	Total	.001	14			

Result 18 Inactivation of 400 mL *Microcystis aeruginosa* using the 1146 kHz bath at 40% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	629	632	621	627.3333	1	0	0.2	0.21	0.21	0.2066667	1	0
5	625	637	622	628	1.001063	−0.10627	0.2	0.21	0.2	0.2033333	0.983871	1.612903
10	589	598	607	598	0.953241	4.675877	0.2	0.21	0.2	0.2033333	0.983871	1.612903
20	592	599	611	600.6667	0.957492	4.250797	0.195	0.21	0.21	0.205	0.991935	0.806452
30	588	602	614	601.3333	0.958555	4.144527	0.21	0.21	0.21	0.21	1.016129	−1.6129

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	627.3333	5.68624	3.28295	613.2079	641.4587	621.00	632.00
	5	3	628.0000	7.93725	4.58258	608.2828	647.7172	622.00	637.00
	10	3	598.0000	9.00000	5.19615	575.6428	620.3572	589.00	607.00
	20	3	600.6667	9.60902	5.54777	576.7965	624.5368	592.00	611.00
	30	3	601.3333	13.01281	7.51295	569.0077	633.6590	588.00	614.00
	Total	15	611.0667	16.14871	4.16958	602.1238	620.0095	588.00	637.00
OD	0	3	.2067	.00577	.00333	.1923	.2210	.20	.21
	5	3	.2033	.00577	.00333	.1890	.2177	.20	.21
	10	3	.2033	.00577	.00333	.1890	.2177	.20	.21
	20	3	.2050	.00866	.00500	.1835	.2265	.20	.21
	30	3	.2100	.00000	.00000	.2100	.2100	.21	.21
	Total	15	.2057	.00563	.00145	.2026	.2088	.20	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	2774.933	4	693.733	7.919	.004
	Within Groups	876.000	10	87.600		
	Total	3650.933	14			
OD	Between Groups	.000	4	.000	.667	.630
	Within Groups	.000	10	.000		
	Total	.000	14			

Result 19 Inactivation of 200 mL *Microcystis aeruginosa* using the 1146 kHz bath at 80% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	657	648	662	655.6667	1	0	0.19	0.19	0.185	0.188333	1	0
5	333	347	392	357.3333	0.544992	45.50076	0.16	0.16	0.16	0.16	0.849558	15.04425
10	307	318	321	315.3333	0.480935	51.90646	0.165	0.165	0.165	0.165	0.876106	12.38938
20	251	227	204	227.3333	0.346721	65.32791	0.14	0.15	0.16	0.15	0.79646	20.35398
30	308	212	145	221.6667	0.338078	66.19217	0.135	0.145	0.15	0.143333	0.761062	23.89381

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	655.6667	7.09460	4.09607	638.0427	673.2906	648.00	662.00
	5	3	357.3333	30.82748	17.79825	280.7536	433.9130	333.00	392.00
	10	3	315.3333	7.37111	4.25572	297.0225	333.6442	307.00	321.00
	20	3	227.3333	23.50177	13.56875	168.9517	285.7150	204.00	251.00
	30	3	221.6667	81.92883	47.30163	18.1442	425.1892	145.00	308.00
	Total	15	355.4667	167.92935	43.35917	262.4705	448.4628	145.00	662.00
OD	0	3	.1883	.00289	.00167	.1812	.1955	.19	.19
	5	3	.1600	.00000	.00000	.1600	.1600	.16	.16
	10	3	.1650	.00000	.00000	.1650	.1650	.17	.17
	20	3	.1500	.01000	.00577	.1252	.1748	.14	.16
	30	3	.1433	.00764	.00441	.1244	.1623	.14	.15
	Total	15	.1613	.01674	.00432	.1521	.1706	.14	.19

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	378164.400	4	94541.100	56.818	.000
	Within Groups	16639.333	10	1663.933		
	Total	394803.733	14			
OD	Between Groups	.004	4	.001	26.925	.000
	Within Groups	.000	10	.000		
	Total	.004	14			

Result 20 Inactivation of 400 mL *Microcystis aeruginosa* using the 1146 kHz bath at 80% power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	844	827	826	832.3333	1	0	0.22	0.22	0.2	0.2133333	1	0
5	781	779	762	774	0.929916	7.00841	0.21	0.21	0.19	0.2033333	0.953125	4.6875
10	746	738	723	735.6667	0.883861	11.61394	0.2	0.2	0.18	0.1933333	0.90625	9.375
20	592	589	708	629.6667	0.756508	24.34922	0.185	0.185	0.18	0.1833333	0.859375	14.0625
30	562	577	682	607	0.729275	27.07249	0.17	0.17	0.17	0.17	0.796875	20.3125

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	832.3333	10.11599	5.84047	807.2038	857.4629	826.00	844.00
	5	3	774.0000	10.44031	6.02771	748.0648	799.9352	762.00	781.00
	10	3	735.6667	11.67619	6.74125	706.6614	764.6719	723.00	746.00
	20	3	629.6667	67.85524	39.17624	461.1049	798.2284	589.00	708.00
	30	3	607.0000	65.38348	37.74917	444.5784	769.4216	562.00	682.00
	Total	15	715.7333	95.73361	24.71831	662.7178	768.7488	562.00	844.00
OD	0	3	.2133	.01155	.00667	.1846	.2420	.20	.22
	5	3	.2033	.01155	.00667	.1746	.2320	.19	.21
	10	3	.1933	.01155	.00667	.1646	.2220	.18	.20
	20	3	.1833	.00289	.00167	.1762	.1905	.18	.19
	30	3	.1700	.00000	.00000	.1700	.1700	.17	.17
	Total	15	.1927	.01741	.00450	.1830	.2023	.17	.22

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	109854.933	4	27463.733	14.882	.000
	Within Groups	18454.000	10	1845.400		
	Total	128308.933	14			
OD	Between Groups	.003	4	.001	10.490	.001
	Within Groups	.001	10	.000		
	Total	.004	14			

Result 21 Inactivation of 200 mL *Microcystis aeruginosa* using the 1146 kHz bath at maximum power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	648	639	627	638	1	0	0.205	0.2	0.2	0.201667	1	0
5	265	259	288	270.6667	0.424242	57.57576	0.16	0.155	0.155	0.156667	0.77686	22.31405
10	158	152	169	159.6667	0.250261	74.97388	0.15	0.14	0.145	0.145	0.719008	28.09917
20	113	126	122	120.3333	0.18861	81.13898	0.13	0.13	0.13	0.13	0.644628	35.53719
30	49	52	61	54	0.084639	91.53605	0.12	0.105	0.11	0.111667	0.553719	44.6281

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	638.0000	10.53565	6.08276	611.8280	664.1720	627.00	648.00
	5	3	270.6667	15.30795	8.83805	232.6396	308.6937	259.00	288.00
	10	3	159.6667	8.62168	4.97773	138.2492	181.0841	152.00	169.00
	20	3	120.3333	6.65833	3.84419	103.7931	136.8735	113.00	126.00
	30	3	54.0000	6.24500	3.60555	38.4866	69.5134	49.00	61.00
	Total	15	248.5333	214.48539	55.37989	129.7553	367.3114	49.00	648.00
OD	0	3	.2017	.00289	.00167	.1945	.2088	.20	.21
	5	3	.1567	.00289	.00167	.1495	.1638	.16	.16
	10	3	.1450	.00500	.00289	.1326	.1574	.14	.15
	20	3	.1300	.00000	.00000	.1300	.1300	.13	.13
	30	3	.1117	.00764	.00441	.0927	.1306	.11	.12
	Total	15	.1490	.03163	.00817	.1315	.1665	.11	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	643049.733	4	160762.433	1598.036	.000
	Within Groups	1006.000	10	100.600		
	Total	644055.733	14			
OD	Between Groups	.014	4	.003	172.625	.000
	Within Groups	.000	10	.000		
	Total	.014	14			

Result 22 Inactivation of 400 mL *Microcystis aeruginosa* using the 1146 kHz bath at maximum power setting (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	688	672	684	681.3333	1	0	0.22	0.22	0.22	0.22	1	0
5	624	631	625	626.6667	0.919765	8.023483	0.2	0.19	0.195	0.195	0.886364	11.36364
10	587	592	578	585.6667	0.859589	14.0411	0.19	0.18	0.19	0.1866667	0.848485	15.15152
20	564	574	582	573.3333	0.841487	15.85127	0.18	0.185	0.185	0.1833333	0.833333	16.66667
30	542	538	544	541.3333	0.794521	20.54795	0.175	0.175	0.175	0.175	0.795455	20.45455

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	681.3333	8.32666	4.80740	660.6488	702.0179	672.00	688.00
	5	3	626.6667	3.78594	2.18581	617.2619	636.0715	624.00	631.00
	10	3	585.6667	7.09460	4.09607	568.0427	603.2906	578.00	592.00
	20	3	573.3333	9.01850	5.20683	550.9301	595.7365	564.00	582.00
	30	3	541.3333	3.05505	1.76383	533.7442	548.9225	538.00	544.00
	Total	15	601.6667	50.32845	12.99475	573.7957	629.5376	538.00	688.00
OD	0	3	.2200	.00000	.00000	.2200	.2200	.22	.22
	5	3	.1950	.00500	.00289	.1826	.2074	.19	.20
	10	3	.1867	.00577	.00333	.1723	.2010	.18	.19
	20	3	.1833	.00289	.00167	.1762	.1905	.18	.19
	30	3	.1750	.00000	.00000	.1750	.1750	.18	.18
	Total	15	.1920	.01623	.00419	.1830	.2010	.18	.22

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	35012.000	4	8753.000	194.800	.000
	Within Groups	449.333	10	44.933		
	Total	35461.333	14			
OD	Between Groups	.004	4	.001	66.687	.000
	Within Groups	.000	10	.000		
	Total	.004	14			

Result 23 Inactivation of 5 L *Microcystis aeruginosa* using the Sonolator (haemocytometer and spectrophotometer)

Time [hour]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	402	600	602	534.6667	1	0	0.15	0.21	0.205	0.1883333	1	0
1	128	543	553	408	0.763092	23.69077	0.12	0.19	0.19	0.1666667	0.884956	11.50442
2	117	456	498	357	0.667706	33.22943	0.12	0.18	0.185	0.1616667	0.858407	14.15929
3	86	392	385	287.6667	0.53803	46.19701	0.125	0.17	0.175	0.1566667	0.831858	16.81416
4	79	300	342	240.3333	0.449501	55.04988	0.1	0.15	0.17	0.14	0.743363	25.66372
5	74	292	300	222	0.415212	58.4788	0.1	0.15	0.165	0.1383333	0.734513	26.54867

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	534.6667	114.89706	66.33585	249.2466	820.0868	402.00	602.00
	1	3	408.0000	242.53866	140.02976	-194.4994	1010.4994	128.00	553.00
	2	3	357.0000	208.90428	120.61094	-161.9470	875.9470	117.00	498.00
	3	3	287.6667	174.68352	100.85358	-146.2713	721.6046	86.00	392.00
	4	3	240.3333	141.28812	81.57274	-110.6458	591.3125	79.00	342.00
	5	3	222.0000	128.23416	74.03603	-96.5513	540.5513	74.00	300.00
	Total	18	341.6111	183.64644	43.28588	250.2859	432.9363	74.00	602.00
OD	0	3	.1883	.03329	.01922	.1056	.2710	.15	.21
	1	3	.1667	.04041	.02333	.0663	.2671	.12	.19
	2	3	.1617	.03617	.02088	.0718	.2515	.12	.19
	3	3	.1567	.02754	.01590	.0883	.2251	.13	.18
	4	3	.1400	.03606	.02082	.0504	.2296	.10	.17
	5	3	.1383	.03403	.01965	.0538	.2229	.10	.17
	Total	18	.1586	.03403	.00802	.1417	.1755	.10	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	208166.278	5	41633.256	1.368	.303
	Within Groups	365176.000	12	30431.333		
	Total	573342.278	17			
OD	Between Groups	.005	5	.001	.852	.540
	Within Groups	.015	12	.001		
	Total	.020	17			

Result 24 Inactivation of 3.5L *Microcystis aeruginosa* using DFR (circulating) at 40% power setting for 60 minutes (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	455	501	406	454	1	0	0.155	0.17	0.14	0.155	1	0
1	432	496	390	439.3333	0.967695	3.230543	0.15	0.17	0.13	0.15	0.967742	3.225806
2	446	472	378	432	0.951542	4.845815	0.15	0.165	0.13	0.1483333	0.956989	4.301075
5	437	512	382	443.6667	0.977239	2.276065	0.16	0.16	0.13	0.15	0.967742	3.225806
10	451	521	399	457	1.006608	−0.66079	0.155	0.175	0.14	0.1566667	1.010753	−1.07527
15	453	534	365	450.6667	0.992658	0.734214	0.155	0.175	0.13	0.1533333	0.989247	1.075269
20	462	527	412	467	1.028634	−2.86344	0.16	0.18	0.135	0.1583333	1.021505	−2.15054
30	421	532	432	461.6667	1.016887	−1.68869	0.155	0.175	0.14	0.1566667	1.010753	−1.07527
60	439	511	411	453.6667	0.999266	0.073421	0.155	0.17	0.135	0.1533333	0.989247	1.075269

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	454.0000	47.50789	27.42870	335.9838	572.0162	406.00	501.00
	1	3	439.3333	53.37915	30.81846	306.7322	571.9345	390.00	496.00
	2	3	432.0000	48.53864	28.02380	311.4233	552.5767	378.00	472.00
	5	3	443.6667	65.25591	37.67552	281.5620	605.7713	382.00	512.00
	10	3	457.0000	61.22091	35.34591	304.9188	609.0812	399.00	521.00
	15	3	450.6667	84.52416	48.80005	240.6970	660.6363	365.00	534.00
	20	3	467.0000	57.66281	33.29164	323.7576	610.2424	412.00	527.00
	30	3	461.6667	61.15826	35.30974	309.7411	613.5922	421.00	532.00
	60	3	453.6667	51.58811	29.78441	325.5147	581.8186	411.00	511.00
	Total	27	451.0000	50.98944	9.81292	430.8292	471.1708	365.00	534.00
OD	0	3	.1550	.01500	.00866	.1177	.1923	.14	.17
	1	3	.1500	.02000	.01155	.1003	.1997	.13	.17
	2	3	.1483	.01756	.01014	.1047	.1920	.13	.17
	5	3	.1500	.01732	.01000	.1070	.1930	.13	.16
	10	3	.1567	.01756	.01014	.1130	.2003	.14	.18
	15	3	.1533	.02255	.01302	.0973	.2093	.13	.18
	20	3	.1583	.02255	.01302	.1023	.2143	.14	.18
	30	3	.1567	.01756	.01014	.1130	.2003	.14	.18
	60	3	.1533	.01756	.01014	.1097	.1970	.14	.17
	Total	27	.1535	.01598	.00308	.1472	.1598	.13	.18

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	2918.667	8	364.833	.102	.999
	Within Groups	64679.333	18	3593.296		
	Total	67598.000	26			
OD	Between Groups	.000	8	.000	.103	.999
	Within Groups	.006	18	.000		
	Total	.007	26			

Result 25 Inactivation of 1L *Microcystis aeruginosa* using DFR (static) at 40% power setting for 10 minutes (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	982	1020	988	996.6667	1	0	0.25	0.25	0.25	0.25	1	0
1	902	912	921	911.6667	0.914716	8.528428	0.24	0.24	0.235	0.2383333	0.953333	4.666667
2	832	844	827	834.3333	0.837124	16.28763	0.235	0.23	0.23	0.2316667	0.926667	7.333333
3	769	779	768	772	0.774582	22.54181	0.235	0.235	0.235	0.235	0.94	6
5	721	732	714	722.3333	0.724749	27.52508	0.23	0.23	0.23	0.23	0.92	8
6	671	702	712	695	0.697324	30.26756	0.23	0.23	0.23	0.23	0.92	8
7	682	699	709	696.6667	0.698997	30.10033	0.23	0.23	0.23	0.23	0.92	8
8	711	702	699	704	0.706355	29.36455	0.23	0.23	0.23	0.23	0.92	8
9	697	702	684	694.3333	0.696656	30.33445	0.23	0.23	0.23	0.23	0.92	8
10	695	711	689	698.3333	0.700669	29.93311	0.23	0.23	0.23	0.23	0.92	8

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	996.6667	20.42874	11.79454	945.9189	1047.4145	982.00	1020.00
	1	3	911.6667	9.50438	5.48736	888.0565	935.2769	902.00	921.00
	2	3	834.3333	8.73689	5.04425	812.6297	856.0370	827.00	844.00
	3	3	772.0000	6.08276	3.51188	756.8896	787.1104	768.00	779.00
	5	3	722.3333	9.07377	5.23874	699.7928	744.8738	714.00	732.00
	6	3	695.0000	21.37756	12.34234	641.8952	748.1048	671.00	712.00
	7	3	696.6667	13.65040	7.88106	662.7572	730.5761	682.00	709.00
	8	3	704.0000	6.24500	3.60555	688.4866	719.5134	699.00	711.00
	9	3	694.3333	9.29157	5.36449	671.2518	717.4149	684.00	702.00
	10	3	698.3333	11.37248	6.56591	670.0825	726.5841	689.00	711.00
	Total	30	772.5333	104.26383	19.03588	733.6006	811.4661	671.00	1020.00
OD	0	3	.2500	.00000	.00000	.2500	.2500	.25	.25
	1	3	.2383	.00289	.00167	.2312	.2455	.24	.24
	2	3	.2317	.00289	.00167	.2245	.2388	.23	.24
	3	3	.2350	.00000	.00000	.2350	.2350	.24	.24
	5	3	.2300	.00000	.00000	.2300	.2300	.23	.23
	6	3	.2300	.00000	.00000	.2300	.2300	.23	.23
	7	3	.2300	.00000	.00000	.2300	.2300	.23	.23
	8	3	.2300	.00000	.00000	.2300	.2300	.23	.23
	9	3	.2300	.00000	.00000	.2300	.2300	.23	.23
	10	3	.2300	.00000	.00000	.2300	.2300	.23	.23
	Total	30	.2335	.00632	.00115	.2311	.2359	.23	.25

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	312054.800	9	34672.756	216.524	.000
	Within Groups	3202.667	20	160.133		
	Total	315257.467	29			
OD	Between Groups	.001	9	.000	74.944	.000
	Within Groups	.000	20	.000		
	Total	.001	29			

Result 26 Inactivation of 3.5L *Microcystis aeruginosa* using DFR (circulating) at 60% power setting for 20 minutes (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	572	554	578	568	1	0	0.15	0.16	0.15	0.1533333	1	0
1	544	532	527	534.3333	0.940728	5.92723	0.145	0.15	0.145	0.1466667	0.956522	4.347826
2	522	517	501	513.3333	0.903756	9.624413	0.14	0.14	0.13	0.1366667	0.891304	10.86957
5	494	487	479	486.6667	0.856808	14.31925	0.12	0.13	0.12	0.1233333	0.804348	19.56522
10	432	449	427	436	0.767606	23.23944	0.1	0.12	0.1	0.1066667	0.695652	30.43478
15	398	403	377	392.6667	0.691315	30.86854	0.08	0.1	0.09	0.09	0.586957	41.30435
20	364	369	345	359.3333	0.632629	36.73709	0.075	0.09	0.085	0.0833333	0.543478	45.65217

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	568.0000	12.49000	7.21110	536.9731	599.0269	554.00	578.00
	1	3	534.3333	8.73689	5.04425	512.6297	556.0370	527.00	544.00
	2	3	513.3333	10.96966	6.33333	486.0832	540.5835	501.00	522.00
	5	3	486.6667	7.50555	4.33333	468.0218	505.3115	479.00	494.00
	10	3	436.0000	11.53256	6.65833	407.3515	464.6485	427.00	449.00
	15	3	392.6667	13.79613	7.96520	358.3952	426.9382	377.00	403.00
	20	3	359.3333	12.66228	7.31057	327.8785	390.7882	345.00	369.00
	Total	21	470.0476	73.35903	16.00825	436.6550	503.4403	345.00	578.00
OD	0	3	.1533	.00577	.00333	.1390	.1677	.15	.16
	1	3	.1467	.00289	.00167	.1395	.1538	.15	.15
	2	3	.1367	.00577	.00333	.1223	.1510	.13	.14
	5	3	.1233	.00577	.00333	.1090	.1377	.12	.13
	10	3	.1067	.01155	.00667	.0780	.1354	.10	.12
	15	3	.0900	.01000	.00577	.0652	.1148	.08	.10
	20	3	.0833	.00764	.00441	.0644	.1023	.08	.09
	Total	21	.1200	.02683	.00586	.1078	.1322	.08	.16

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	105845.619	6	17640.937	138.334	.000
	Within Groups	1785.333	14	127.524		
	Total	107630.952	20			
OD	Between Groups	.014	6	.002	39.667	.000
	Within Groups	.001	14	.000		
	Total	.014	20			

Result 27 Inactivation of 1L *Microcystis aeruginosa* using DFR (static) at 60% power setting for 10 minutes (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	562	544	578	561.3333	1	0	0.15	0.16	0.15	0.1533333	1	0
1	492	482	477	483.6667	0.861639	13.8361	0.14	0.15	0.145	0.145	0.945652	5.434783
2	433	411	432	425.3333	0.75772	24.22803	0.13	0.14	0.13	0.1333333	0.869565	13.04348
3	391	387	379	385.6667	0.687055	31.29454	0.12	0.13	0.12	0.1233333	0.804348	19.56522
5	312	300	306	306	0.545131	45.48694	0.1	0.12	0.09	0.1033333	0.673913	32.6087
10	114	105	112	110.3333	0.196556	80.34442	0.07	0.06	0.05	0.06	0.391304	60.86957

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	561.3333	17.00980	9.82061	519.0786	603.5880	544.00	578.00
	1	3	483.6667	7.63763	4.40959	464.6938	502.6396	477.00	492.00
	2	3	425.3333	12.42310	7.17248	394.4727	456.1940	411.00	433.00
	3	3	385.6667	6.11010	3.52767	370.4883	400.8450	379.00	391.00
	5	3	306.0000	6.00000	3.46410	291.0952	320.9048	300.00	312.00
	10	3	110.3333	4.72582	2.72845	98.5938	122.0729	105.00	114.00
	Total	18	378.7222	148.10601	34.90892	305.0708	452.3736	105.00	578.00
OD	0	3	.1533	.00577	.00333	.1390	.1677	.15	.16
	1	3	.1450	.00500	.00289	.1326	.1574	.14	.15
	2	3	.1333	.00577	.00333	.1190	.1477	.13	.14
	3	3	.1233	.00577	.00333	.1090	.1377	.12	.13
	5	3	.1033	.01528	.00882	.0654	.1413	.09	.12
	10	3	.0600	.01000	.00577	.0352	.0848	.05	.07
	Total	18	.1197	.03283	.00774	.1034	.1360	.05	.16

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	371706.278	5	74341.256	746.315	.000
	Within Groups	1195.333	12	99.611		
	Total	372901.611	17			
OD	Between Groups	.017	5	.003	45.575	.000
	Within Groups	.001	12	.000		
	Total	.018	17			

Result 28 Inactivation of 1.5L *Microcystis aeruginosa* using a vibrating tray for 5 minutes (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	502	498	511	503.6667	1	0	0.18	0.18	0.18	0.18	1	0
0.5	478	469	482	476.3333	0.945731	5.42687	0.16	0.175	0.16	0.165	0.916667	8.333333
1	446	475	469	463.3333	0.919921	8.007942	0.17	0.17	0.175	0.1716667	0.953704	4.62963
2	468	452	463	461	0.915288	8.471211	0.16	0.165	0.16	0.1616667	0.898148	10.18519
3	441	439	456	445.3333	0.884183	11.58173	0.16	0.16	0.165	0.1616667	0.898148	10.18519
5	442	447	452	447	0.887492	11.25083	0.155	0.16	0.16	0.1583333	0.87963	12.03704

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	503.6667	6.65833	3.84419	487.1265	520.2069	498.00	511.00
	0.5	3	476.3333	6.65833	3.84419	459.7931	492.8735	469.00	482.00
	1	3	463.3333	15.30795	8.83805	425.3063	501.3604	446.00	475.00
	2	3	461.0000	8.18535	4.72582	440.6665	481.3335	452.00	468.00
	3	3	445.3333	9.29157	5.36449	422.2518	468.4149	439.00	456.00
	5	3	447.0000	5.00000	2.88675	434.5793	459.4207	442.00	452.00
	Total	18	466.1111	21.74691	5.12580	455.2966	476.9256	439.00	511.00
OD	0	3	.1800	.00000	.00000	.1800	.1800	.18	.18
	0.5	3	.1650	.00866	.00500	.1435	.1865	.16	.18
	1	3	.1717	.00289	.00167	.1645	.1788	.17	.18
	2	3	.1617	.00289	.00167	.1545	.1688	.16	.17
	3	3	.1617	.00289	.00167	.1545	.1688	.16	.17
	5	3	.1583	.00289	.00167	.1512	.1655	.16	.16
	Total	18	.1664	.00837	.00197	.1622	.1705	.16	.18

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	7037.111	5	1407.422	16.844	.000
	Within Groups	1002.667	12	83.556		
	Total	8039.778	17			
OD	Between Groups	.001	5	.000	10.785	.000
	Within Groups	.000	12	.000		
	Total	.001	17			

Result 29 Inactivation of 4L *Microcystis aeruginosa* using the 20 kHz probe (Surface, spectrophotometer)

Time	OD	OD	OD	AV	% OD
0	0.5	0.55	0.5	0.516667	0
5	0.156	0.2	0.22	0.192	62.83871
10	0.1	0.12	0.11	0.11	78.70968
20	0.1	0.12	0.1	0.106667	79.35484
30	0.054	0.06	0.056	0.056667	89.03226

Descriptives

OD

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	3	.5167	.02887	.01667	.4450	.5884	.50	.55
5	3	.1920	.03274	.01890	.1107	.2733	.16	.22
10	3	.1100	.01000	.00577	.0852	.1348	.10	.12
20	3	.1067	.01155	.00667	.0780	.1354	.10	.12
30	3	.0567	.00306	.00176	.0491	.0643	.05	.06
Total	15	.1964	.17263	.04457	.1008	.2920	.05	.55

ANOVA

OD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.413	4	.103	240.280	.000
Within Groups	.004	10	.000		
Total	.417	14			

Result 30 Inactivation of 4L *Microcystis aeruginosa* using the 20 kHz probe (Middle, spectrophotometer)

Time	OD	OD	OD	AV	% OD
0	0.0 1	0.0 1	0.01	0.01	0
5	0. 03	0. 025	0. 025	0. 026667	−166.667
10	0. 041	0. 04	0. 035	0. 038667	−286.667
20	0.16	0.15	0.15	0.153333	−1433.33
30	0.2	0.2	0.2	0.2	−1900

Descriptives

OD

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	3	.0033	.00577	.00333	-.0110	.0177	.00	.01
5	3	.0000	.00000	.00000	.0000	.0000	.00	.00
10	3	.0000	.00000	.00000	.0000	.0000	.00	.00
20	3	.1533	.00577	.00333	.1390	.1677	.15	.16
30	3	.2000	.00000	.00000	.2000	.2000	.20	.20
Total	15	.0713	.09039	.02334	.0213	.1214	.00	.20

ANOVA

OD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.114	4	.029	2142.000	.000
Within Groups	.000	10	.000		
Total	.114	14			

Result 31 Inactivation of 4L *Microcystis aeruginosa* using the 20 kHz probe (Bottom, spectrophotometer)

Time	OD	OD	OD	AV	% OD
0	0. 01	0.0 1	0.01	0. 01	0
5	0. 037	0. 04	0.04	0. 039	−290
10	0. 037	0. 04	0.05	0.042333	−323.333
20	0.18	0.2	0.19	0.19	−1800
30	0.183	0.22	0.2	0.201	−1910

Descriptives

OD

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
0	3	.0033	.00577	.00333	-.0110	.0177	.00	.01
5	3	.0133	.02309	.01333	-.0440	.0707	.00	.04
10	3	.0167	.02887	.01667	-.0550	.0884	.00	.05
20	3	.1900	.01000	.00577	.1652	.2148	.18	.20
30	3	.2010	.01852	.01069	.1550	.2470	.18	.22
Total	15	.0849	.09508	.02455	.0322	.1375	.00	.22

ANOVA

OD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.123	4	.031	83.334	.000
Within Groups	.004	10	.000		
Total	.127	14			

Result 32 Inactivation of 200 mL *Microcystis aeruginosa* using 20 kHz probe for flow cytometry (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	562	558	541	553.667	1	0	0.21	0.2	0.2	0.203333	1	0
5	509	552	514	525	0.94822	5.1776	0.15	0.16	0.16	0.156667	0.77049	22.9508
10	452	448	425	441.667	0.79771	20.2288	0.13	0.14	0.14	0.136667	0.67213	32.7869
20	378	398	376	384	0.69356	30.6442	0.11	0.12	0.12	0.116667	0.57377	42.623
30	297	385	327	336.333	0.60747	39.2535	0.105	0.105	0.1	0.103333	0.5082	49.1803

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	553.6667	11.15049	6.43774	525.9673	581.3660	541.00	562.00
	5	3	525.0000	23.51595	13.57694	466.5831	583.4169	509.00	552.00
	10	3	441.6667	14.57166	8.41295	405.4687	477.8647	425.00	452.00
	20	3	384.0000	12.16553	7.02377	353.7792	414.2208	376.00	398.00
	30	3	336.3333	44.73626	25.82849	225.2023	447.4644	297.00	385.00
	Total	15	448.1333	87.49601	22.59137	399.6797	496.5870	297.00	562.00
OD	0	3	.2033	.00577	.00333	.1890	.2177	.20	.21
	5	3	.1567	.00577	.00333	.1423	.1710	.15	.16
	10	3	.1367	.00577	.00333	.1223	.1510	.13	.14
	20	3	.1167	.00577	.00333	.1023	.1310	.11	.12
	30	3	.1033	.00289	.00167	.0962	.1105	.10	.11
	Total	15	.1433	.03653	.00943	.1231	.1636	.10	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	101099.733	4	25274.933	41.584	.000
	Within Groups	6078.000	10	607.800		
	Total	107177.733	14			
OD	Between Groups	.018	4	.005	162.353	.000
	Within Groups	.000	10	.000		
	Total	.019	14			

Result 33 Inactivation of 200 mL *Microcystis aeruginosa* using 580 kHz bath for flow cytometry (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	537	509	555	533.667	1	0	0.2	0.21	0.2	0.203333	1	0
5	512	481	523	505.333	0.94691	5.30918	0.19	0.18	0.19	0.186667	0.91803	8.19672
10	499	465	478	480.667	0.90069	9.93129	0.185	0.17	0.17	0.175	0.86066	13.9344
20	478	423	412	437.667	0.82011	17.9888	0.17	0.165	0.16	0.165	0.81148	18.8525
30	433	398	377	402.667	0.75453	24.5472	0.16	0.16	0.155	0.158333	0.77869	22.1311

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	533.6667	23.18045	13.38324	476.0832	591.2501	509.00	555.00
	5	3	505.3333	21.77919	12.57422	451.2308	559.4359	481.00	523.00
	10	3	480.6667	17.15615	9.90511	438.0484	523.2849	465.00	499.00
	20	3	437.6667	35.36005	20.41514	349.8274	525.5059	412.00	478.00
	30	3	402.6667	28.29016	16.33333	332.3900	472.9433	377.00	433.00
	Total	15	472.0000	53.20983	13.73872	442.5334	501.4666	377.00	555.00
OD	0	3	.2033	.00577	.00333	.1890	.2177	.20	.21
	5	3	.1867	.00577	.00333	.1723	.2010	.18	.19
	10	3	.1750	.00866	.00500	.1535	.1965	.17	.19
	20	3	.1650	.00500	.00289	.1526	.1774	.16	.17
	30	3	.1583	.00289	.00167	.1512	.1655	.16	.16
	Total	15	.1777	.01731	.00447	.1681	.1873	.16	.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	32924.667	4	8231.167	12.261	.001
	Within Groups	6713.333	10	671.333		
	Total	39638.000	14			
OD	Between Groups	.004	4	.001	27.452	.000
	Within Groups	.000	10	.000		
	Total	.004	14			

Result 34 Inactivation of 200 mL *Microcystis aeruginosa* using 1146 kHz bath for flow cytometry (haemocytometer and spectrophotometer)

Time [min]	HAE	HAE	HAE	AV	C/C0	% HAE	OD	OD	OD	AV	C/C0	%OD
0	512	498	507	505.667	1	0	0.2	0.2	0.2	0.2	1	0
5	487	469	472	476	0.94133	5.86684	0.19	0.185	0.185	0.186667	0.93333	6.66667
10	475	474	469	472.667	0.93474	6.52604	0.185	0.18	0.185	0.183333	0.91667	8.33333
20	451	446	437	444.667	0.87937	12.0633	0.18	0.185	0.19	0.185	0.925	7.5
30	443	422	428	431	0.85234	14.766	0.19	0.175	0.185	0.183333	0.91667	8.33333

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	0	3	505.6667	7.09460	4.09607	488.0427	523.2906	498.00	512.00
	5	3	476.0000	9.64365	5.56776	452.0438	499.9562	469.00	487.00
	10	3	472.6667	3.21455	1.85592	464.6813	480.6521	469.00	475.00
	20	3	444.6667	7.09460	4.09607	427.0427	462.2906	437.00	451.00
	30	3	431.0000	10.81665	6.24500	404.1299	457.8701	422.00	443.00
	Total	15	466.0000	27.82086	7.18331	450.5933	481.4067	422.00	512.00
OD	0	3	.2000	.00000	.00000	.2000	.2000	.20	.20
	5	3	.1867	.00289	.00167	.1795	.1938	.19	.19
	10	3	.1833	.00289	.00167	.1762	.1905	.18	.19
	20	3	.1850	.00500	.00289	.1726	.1974	.18	.19
	30	3	.1833	.00764	.00441	.1644	.2023	.18	.19
	Total	15	.1877	.00753	.00194	.1835	.1918	.18	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	10194.000	4	2548.500	39.696	.000
	Within Groups	642.000	10	64.200		
	Total	10836.000	14			
OD	Between Groups	.001	4	.000	7.417	.005
	Within Groups	.000	10	.000		
	Total	.001	14			

Result 35 Resistance test on *Microcystis aeruginosa* for 30 days (Live, haemocytometer and spectrophotometer)

Time [day]	HAE	HAE	HAE	AV	OD	OD	OD	AV
1	472	533	516	507	0.23	0.24	0.23	0.2333333
3	647	638	673	652.6667	0.45	0.4	0.41	0.42
6	812	834	858	834.6667	0.51	0.52	0.5	0.51
9	672	683	691	682	0.45	0.46	0.45	0.4533333
12	695	698	704	699	0.46	0.45	0.47	0.46
15	912	897	936	915	0.6	0.59	0.58	0.59
18	1012	987	1126	1041.667	0.61	0.6	0.61	0.6066667
21	872	814	835	840.3333	0.45	0.46	0.45	0.4533333
24	909	913	879	900.3333	0.6	0.59	0.6	0.5966667
27	1026	991	902	973	0.6	0.61	0.6	0.6033333
30	997	978	912	962.3333	0.55	0.55	0.56	0.5533333

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	1	3	507.0000	31.48015	18.17507	428.7990	585.2010	472.00	533.00
	3	3	652.6667	18.17507	10.49338	607.5173	697.8161	638.00	673.00
	6	3	834.6667	23.00725	13.28324	777.5135	891.8198	812.00	858.00
	9	3	682.0000	9.53939	5.50757	658.3028	705.6972	672.00	691.00
	12	3	699.0000	4.58258	2.64575	687.6163	710.3837	695.00	704.00
	15	3	915.0000	19.67232	11.35782	866.1313	963.8687	897.00	936.00
	18	3	1041.6667	74.09678	42.77980	857.6001	1225.7333	987.00	1126.00
	21	3	840.3333	29.36551	16.95419	767.3854	913.2813	814.00	872.00
	24	3	900.3333	18.58315	10.72898	854.1702	946.4964	879.00	913.00
	27	3	973.0000	63.92965	36.90980	814.1899	1131.8101	902.00	1026.00
	30	3	962.3333	44.61315	25.75742	851.5081	1073.1585	912.00	997.00
	Total	33	818.9091	161.62328	28.13500	761.6000	876.2182	472.00	1126.00
OD	1	3	.2333	.00577	.00333	.2190	.2477	.23	.24
	3	3	.4200	.02646	.01528	.3543	.4857	.40	.45
	6	3	.5100	.01000	.00577	.4852	.5348	.50	.52
	9	3	.4533	.00577	.00333	.4390	.4677	.45	.46
	12	3	.4600	.01000	.00577	.4352	.4848	.45	.47
	15	3	.5900	.01000	.00577	.5652	.6148	.58	.60
	18	3	.6067	.00577	.00333	.5923	.6210	.60	.61
	21	3	.4533	.00577	.00333	.4390	.4677	.45	.46
	24	3	.5967	.00577	.00333	.5823	.6110	.59	.60
	27	3	.6033	.00577	.00333	.5890	.6177	.60	.61
	30	3	.5533	.00577	.00333	.5390	.5677	.55	.56
	Total	33	.4982	.10899	.01897	.4595	.5368	.23	.61

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	805656.727	10	80565.673	58.593	.000
	Within Groups	30250.000	22	1375.000		
	Total	835906.727	32			
OD	Between Groups	.378	10	.038	336.800	.000
	Within Groups	.002	22	.000		
	Total	.380	32			

Result 36 Resistance test on *Microcystis aeruginosa* for 30 days (Dead, haemocytometer and spectrophotometer)

Time [day]	HAE	HAE	HAE	AV	OD	OD	OD	AV
1	361	327	384	357.3333	0.13	0.12	0.12	0.1233333
3	316	358	344	339.3333	0.2	0.18	0.2	0.1933333
6	279	286	247	270.6667	0.14	0.13	0.15	0.14
9	312	261	238	270.3333	0.14	0.15	0.14	0.1433333
12	211	198	186	198.3333	0.06	0.07	0.07	0.0666667
15	128	216	209	184.3333	0.14	0.13	0.14	0.1366667
18	110	98	82	96.66667	0.03	0.03	0.03	0.03
21	32	102	99	77.66667	0.05	0.04	0.05	0.0466667
24	76	39	51	55.33333	0.04	0.05	0.04	0.0433333
27	79	41	37	52.33333	0.05	0.04	0.04	0.0433333
30	82	39	45	55.33333	0.05	0.04	0.06	0.05

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	1	3	357.3333	28.67635	16.55630	286.0973	428.5693	327.00	384.00
	3	3	339.3333	21.38535	12.34684	286.2092	392.4575	316.00	358.00
	6	3	270.6667	20.79263	12.00463	219.0149	322.3184	247.00	286.00
	9	3	270.3333	37.87259	21.86575	176.2526	364.4141	238.00	312.00
	12	3	198.3333	12.50333	7.21880	167.2733	229.3933	186.00	211.00
	15	3	184.3333	48.91148	28.23906	62.8305	305.8362	128.00	216.00
	18	3	96.6667	14.04754	8.11035	61.7706	131.5627	82.00	110.00
	21	3	77.6667	39.57693	22.84975	-20.6479	175.9812	32.00	102.00
	24	3	55.3333	18.87679	10.89852	8.4408	102.2259	39.00	76.00
	27	3	52.3333	23.18045	13.38324	-5.2501	109.9168	37.00	79.00
	30	3	55.3333	23.28805	13.44536	-2.5174	113.1841	39.00	82.00
	Total	33	177.9697	116.29152	20.24376	136.7345	219.2049	32.00	384.00
OD	1	3	.1233	.00577	.00333	.1090	.1377	.12	.13
	3	3	.1933	.01155	.00667	.1646	.2220	.18	.20
	6	3	.1400	.01000	.00577	.1152	.1648	.13	.15
	9	3	.1433	.00577	.00333	.1290	.1577	.14	.15
	12	3	.0667	.00577	.00333	.0523	.0810	.06	.07
	15	3	.1367	.00577	.00333	.1223	.1510	.13	.14
	18	3	.0300	.00000	.00000	.0300	.0300	.03	.03
	21	3	.0467	.00577	.00333	.0323	.0610	.04	.05
	24	3	.0433	.00577	.00333	.0290	.0577	.04	.05
	27	3	.0433	.00577	.00333	.0290	.0577	.04	.05
	30	3	.0500	.01000	.00577	.0252	.0748	.04	.06
	Total	33	.0924	.05443	.00948	.0731	.1117	.03	.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	414969.636	10	41496.964	51.319	.000
	Within Groups	17789.333	22	808.606		
	Total	432758.970	32			
OD	Between Groups	.094	10	.009	181.835	.000
	Within Groups	.001	22	.000		
	Total	.095	32			

Result 37 Resistance test on *Microcystis aeruginosa* for 30 days (Live+Dead, haemocytometer and spectrophotometer)

Time [day]	HAE	HAE	HAE	AV	OD	OD	OD	AV
1	374	402	411	395.6667	0.2	0.2	0.2	0.2
3	416	507	433	452	0.4	0.39	0.4	0.3966667
6	456	467	472	465	0.4	0.41	0.42	0.41
9	512	478	496	495.3333	0.38	0.39	0.4	0.39
12	523	486	501	503.3333	0.37	0.39	0.38	0.38
15	512	598	507	539	0.32	0.34	0.34	0.3333333
18	524	576	515	538.3333	0.33	0.35	0.35	0.3433333
21	579	612	624	605	0.29	0.31	0.3	0.3
24	632	651	598	627	0.33	0.34	0.33	0.3333333
27	638	615	649	634	0.34	0.35	0.34	0.3433333
30	712	633	627	657.3333	0.4	0.39	0.38	0.39

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	1	3	395.6667	19.29594	11.14052	347.7329	443.6004	374.00	411.00
	3	3	452.0000	48.38388	27.93445	331.8078	572.1922	416.00	507.00
	6	3	465.0000	8.18535	4.72582	444.6665	485.3335	456.00	472.00
	9	3	495.3333	17.00980	9.82061	453.0786	537.5880	478.00	512.00
	12	3	503.3333	18.61003	10.74451	457.1034	549.5632	486.00	523.00
	15	3	539.0000	51.15662	29.53529	411.9199	666.0801	507.00	598.00
	18	3	538.3333	32.92922	19.01169	456.5326	620.1340	515.00	576.00
	21	3	605.0000	23.30236	13.45362	547.1137	662.8863	579.00	624.00
	24	3	627.0000	26.85144	15.50269	560.2973	693.7027	598.00	651.00
	27	3	634.0000	17.34935	10.01665	590.9018	677.0982	615.00	649.00
	30	3	657.3333	47.43768	27.38816	539.4916	775.1751	627.00	712.00
	Total	33	537.4545	86.14598	14.99609	506.9085	568.0006	374.00	712.00
OD	1	3	.2000	.00000	.00000	.2000	.2000	.20	.20
	3	3	.3967	.00577	.00333	.3823	.4110	.39	.40
	6	3	.4100	.01000	.00577	.3852	.4348	.40	.42
	9	3	.3900	.01000	.00577	.3652	.4148	.38	.40
	12	3	.3800	.01000	.00577	.3552	.4048	.37	.39
	15	3	.3333	.01155	.00667	.3046	.3620	.32	.34
	18	3	.3433	.01155	.00667	.3146	.3720	.33	.35
	21	3	.3000	.01000	.00577	.2752	.3248	.29	.31
	24	3	.3333	.00577	.00333	.3190	.3477	.33	.34
	27	3	.3433	.00577	.00333	.3290	.3577	.34	.35
	30	3	.3900	.01000	.00577	.3652	.4148	.38	.40
	Total	33	.3473	.05811	.01012	.3267	.3679	.20	.42

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	215610.848	10	21561.085	21.694	.000
	Within Groups	21865.333	22	993.879		
	Total	237476.182	32			
OD	Between Groups	.106	10	.011	134.946	.000
	Within Groups	.002	22	.000		
	Total	.108	32			

Result 38 Resistance test on *Microcystis aeruginosa* for 30 days (Sonication, haemocytometer and spectrophotometer)

Time [day]	HAE	HAE	HAE	AV	OD	OD	OD	AV
1	431	345	392	389.3333	0.15	0.1	0.15	0.133333
3	378	312	309	333	0.15	0.13	0.15	0.143333
6	214	258	312	261.3333	0.09	0.08	0.09	0.086667
9	102	98	92	97.33333	0.08	0.09	0.09	0.086667
12	39	41	23	34.33333	0.01	0.01	0.01	0.01
15	2	4	3	3	0.01	0.01	0.01	0.01
18	16	12	14	14	0.01	0.01	0.01	0.01
21	3	8	9	6.666667	0.06	0.06	0.06	0.06
24	8	5	4	5.666667	0.06	0.06	0.06	0.06
27	7	8	5	6.666667	0.06	0.06	0.06	0.06
30	6	8	4	6	0.06	0.06	0.06	0.06

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	1	3	389.3333	43.06197	24.86184	282.3615	496.3052	345.00	431.00
	3	3	333.0000	39.00000	22.51666	236.1186	429.8814	309.00	378.00
	6	3	261.3333	49.08496	28.33922	139.3995	383.2671	214.00	312.00
	9	3	97.3333	5.03322	2.90593	84.8301	109.8366	92.00	102.00
	12	3	34.3333	9.86577	5.69600	9.8254	58.8413	23.00	41.00
	15	3	3.0000	1.00000	.57735	.5159	5.4841	2.00	4.00
	18	3	14.0000	2.00000	1.15470	9.0317	18.9683	12.00	16.00
	21	3	6.6667	3.21455	1.85592	-1.3187	14.6521	3.00	9.00
	24	3	5.6667	2.08167	1.20185	.4955	10.8378	4.00	8.00
	27	3	6.6667	1.52753	.88192	2.8721	10.4612	5.00	8.00
	30	3	6.0000	2.00000	1.15470	1.0317	10.9683	4.00	8.00
	Total	33	105.2121	144.90380	25.22451	53.8315	156.5928	2.00	431.00
OD	1	3	.1333	.02887	.01667	.0616	.2050	.10	.15
	3	3	.1433	.01155	.00667	.1146	.1720	.13	.15
	6	3	.0867	.00577	.00333	.0723	.1010	.08	.09
	9	3	.0867	.00577	.00333	.0723	.1010	.08	.09
	12	3	.0100	.00000	.00000	.0100	.0100	.01	.01
	15	3	.0100	.00000	.00000	.0100	.0100	.01	.01
	18	3	.0100	.00000	.00000	.0100	.0100	.01	.01
	21	3	.0600	.00000	.00000	.0600	.0600	.06	.06
	24	3	.0600	.00000	.00000	.0600	.0600	.06	.06
	27	3	.0600	.00000	.00000	.0600	.0600	.06	.06
	30	3	.0600	.00000	.00000	.0600	.0600	.06	.06
	Total	33	.0655	.04501	.00783	.0495	.0814	.01	.15

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	660040.848	10	66004.085	122.367	.000
	Within Groups	11866.667	22	539.394		
	Total	671907.515	32			
OD	Between Groups	.063	10	.006	66.800	.000
	Within Groups	.002	22	.000		
	Total	.065	32			

Result 39 Resistance test on *Microcystis aeruginosa* for 30 days (Live+Sonication, haemocytometer and spectrophotometer)

Time [day]	HAE	HAE	HAE	AV	OD	OD	OD	AV
1	421	434	498	451	0.18	0.17	0.18	0.176667
3	492	478	509	493	0.2	0.18	0.19	0.19
6	561	552	571	561.3333	0.21	0.22	0.22	0.216667
9	681	698	702	693.6667	0.32	0.31	0.3	0.31
12	1025	1146	1135	1102	0.7	0.69	0.69	0.693333
15	524	517	528	523	0.56	0.55	0.5	0.536667
18	642	638	672	650.6667	0.36	0.35	0.35	0.353333
21	701	694	711	702	0.6	0.59	0.58	0.59
24	645	651	611	635.6667	0.7	0.7	0.7	0.7
27	1026	998	912	978.6667	0.58	0.59	0.59	0.586667
30	968	978	945	963.6667	0.55	0.55	0.54	0.546667

Descriptives

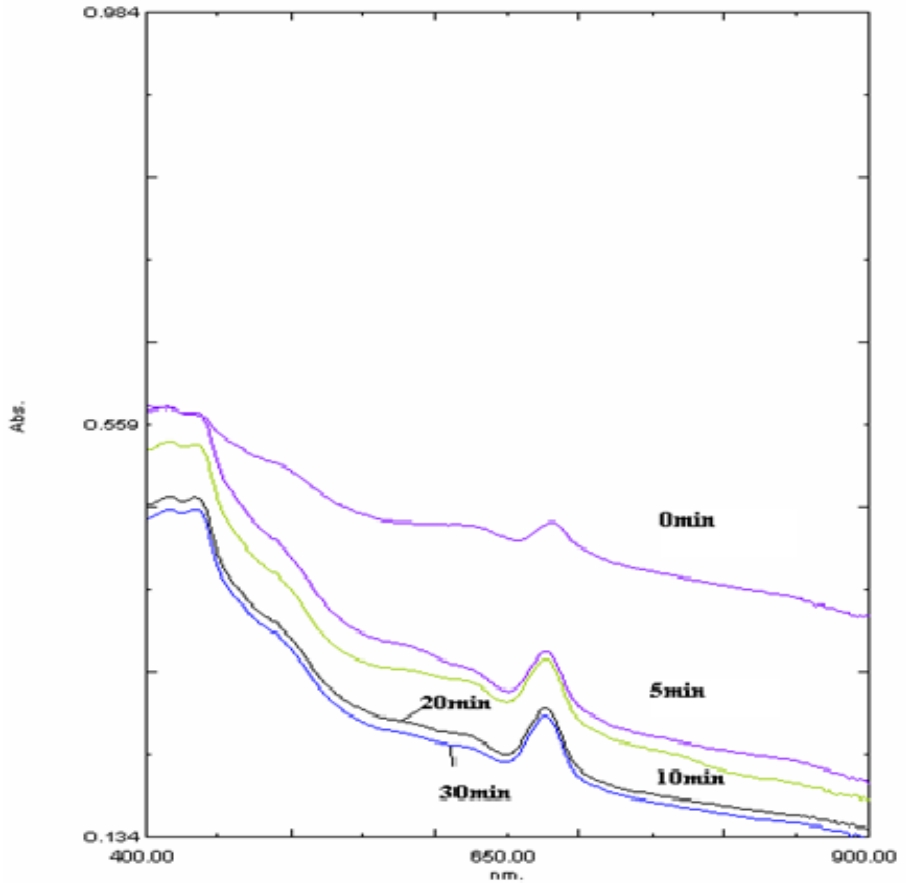
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
HAE	1	3	451.0000	41.21893	23.79776	348.6065	553.3935	421.00	498.00
	3	3	493.0000	15.52417	8.96289	454.4358	531.5642	478.00	509.00
	6	3	561.3333	9.50438	5.48736	537.7231	584.9435	552.00	571.00
	9	3	693.6667	11.15049	6.43774	665.9673	721.3660	681.00	702.00
	12	3	1102.0000	66.91039	38.63073	935.7854	1268.2146	1025.00	1146.00
	15	3	523.0000	5.56776	3.21455	509.1689	536.8311	517.00	528.00
	18	3	650.6667	18.58315	10.72898	604.5036	696.8298	638.00	672.00
	21	3	702.0000	8.54400	4.93288	680.7755	723.2245	694.00	711.00
	24	3	635.6667	21.57159	12.45436	582.0799	689.2535	611.00	651.00
	27	3	978.6667	59.40819	34.29934	831.0885	1126.2448	912.00	1026.00
	30	3	963.6667	16.92139	9.76957	921.6316	1005.7017	945.00	978.00
	Total	33	704.9697	211.71317	36.85453	629.8995	780.0399	421.00	1146.00
OD	1	3	.1767	.00577	.00333	.1623	.1910	.17	.18
	3	3	.1900	.01000	.00577	.1652	.2148	.18	.20
	6	3	.2167	.00577	.00333	.2023	.2310	.21	.22
	9	3	.3100	.01000	.00577	.2852	.3348	.30	.32
	12	3	.6933	.00577	.00333	.6790	.7077	.69	.70
	15	3	.5367	.03215	.01856	.4568	.6165	.50	.56
	18	3	.3533	.00577	.00333	.3390	.3677	.35	.36
	21	3	.5900	.01000	.00577	.5652	.6148	.58	.60
	24	3	.7000	.00000	.00000	.7000	.7000	.70	.70
	27	3	.5867	.00577	.00333	.5723	.6010	.58	.59
	30	3	.5467	.00577	.00333	.5323	.5610	.54	.55
	Total	33	.4455	.19446	.03385	.3765	.5144	.17	.70

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
HAE	Between Groups	1411594.970	10	141159.497	136.662	.000
	Within Groups	22724.000	22	1032.909		
	Total	1434318.970	32			
OD	Between Groups	1.207	10	.121	865.857	.000
	Within Groups	.003	22	.000		
	Total	1.210	32			

Appendix 3 UV-VIS SPECTROPHOTOMETER AND FLUOROMETER RESULTS

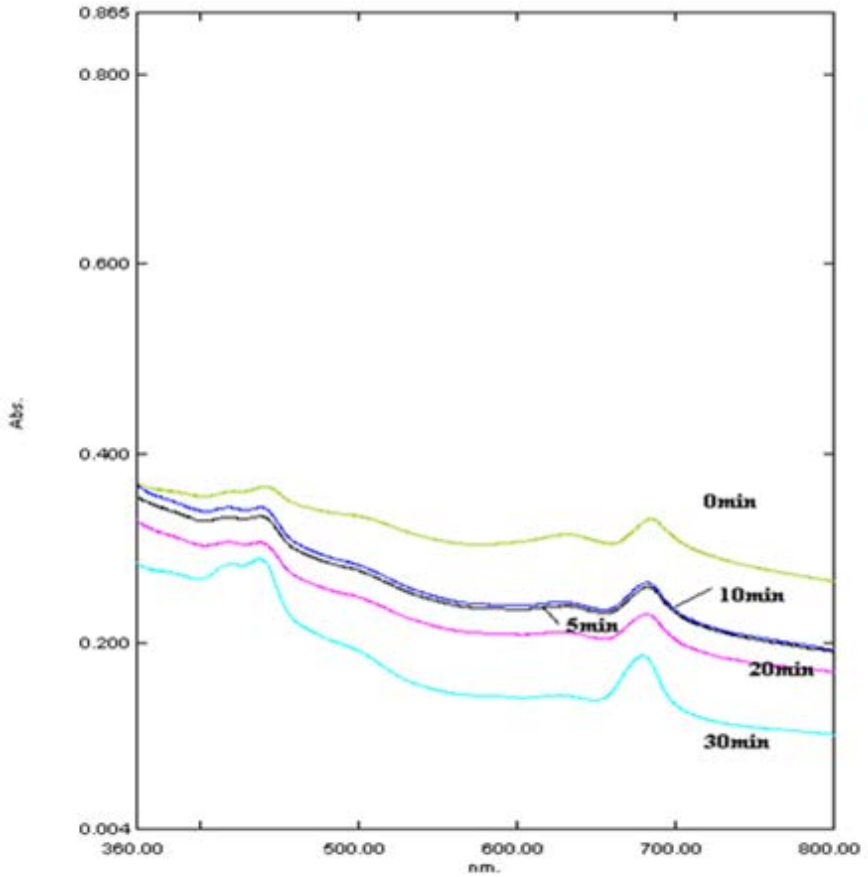
Figure 1 Inactivation of 200 mL *Microcystis aeruginosa* using the 20 kHz probe (UV-Vis spectrophotometer)



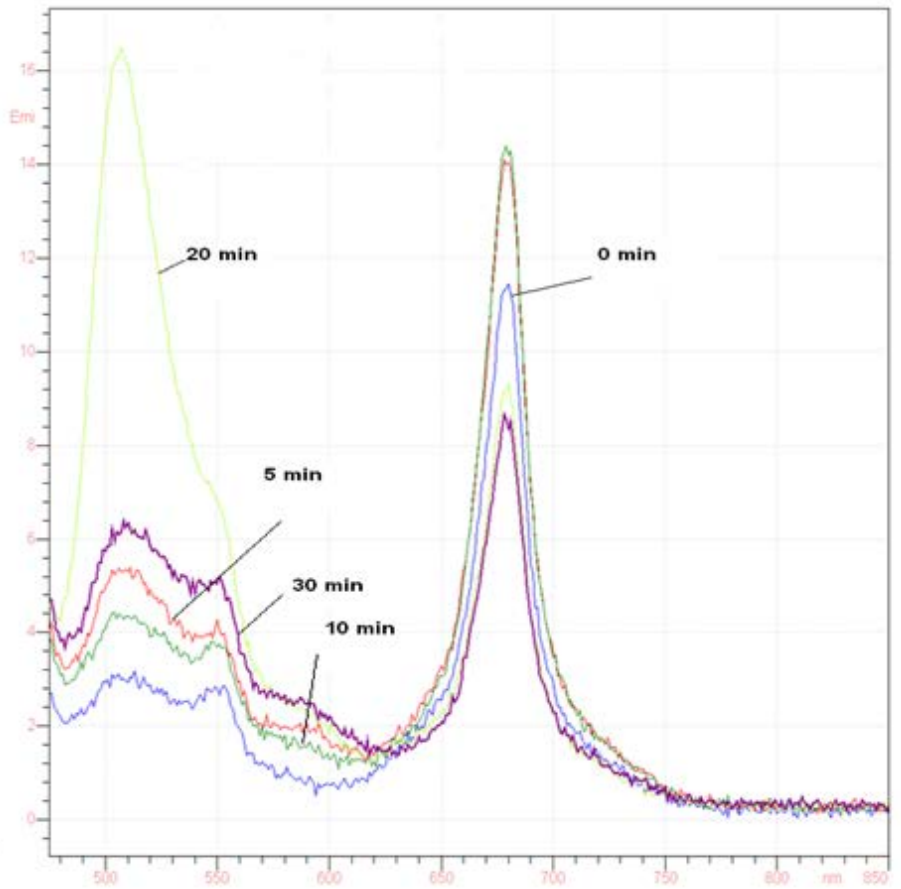
UV-Vis spectrophotometer

Note: No Fluorometer results for 20 kHz, 40 kHz or 580 kHz (40% power setting) and 200 mL, as the fluorometer was not available until 580 kHz (80% power setting.) and 200 mL.

Figure 2 Inactivation of 400 mL *Microcystis aeruginosa* using the 20 kHz probe (UV-Vis spectrophotometer and Fluorometer)

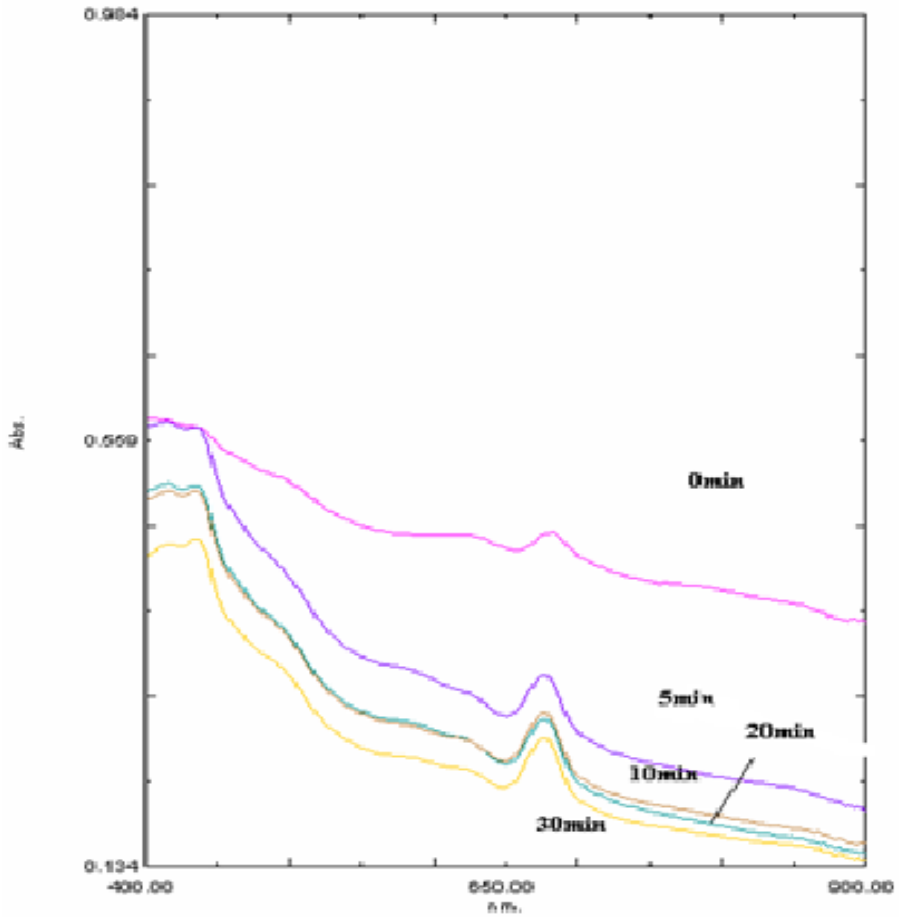


UV-Vis spectrophotometer



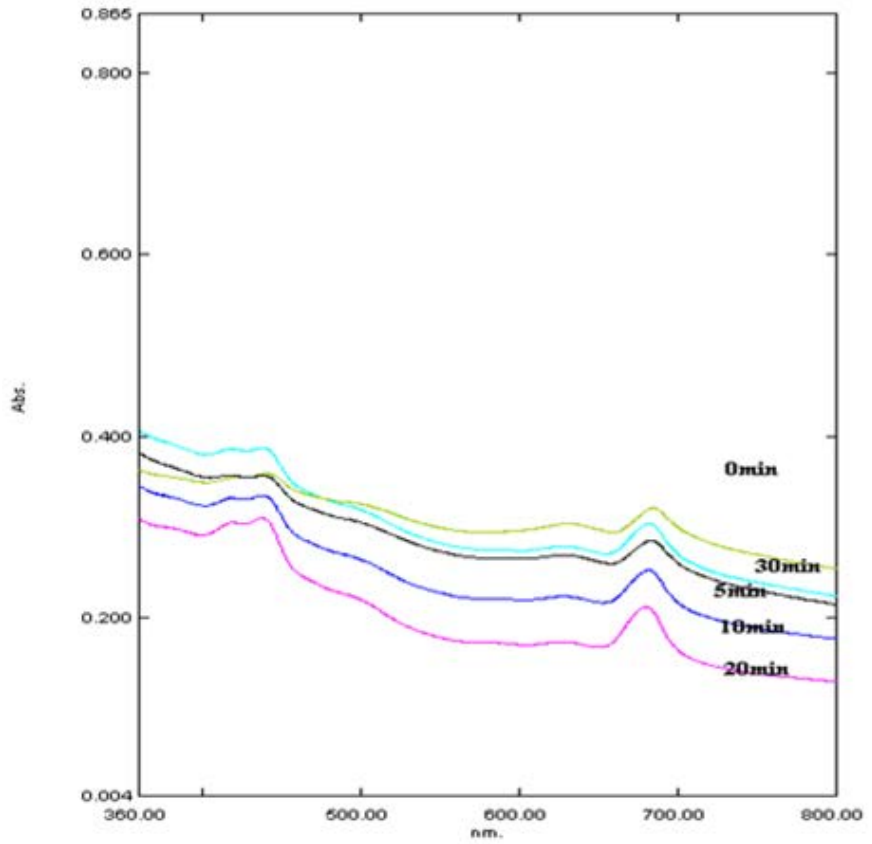
Fluorometer

Figure 3 Inactivation of 200 mL *Microcystis aeruginosa* using the 40 kHz bath (UV-Vis spectrophotometer)

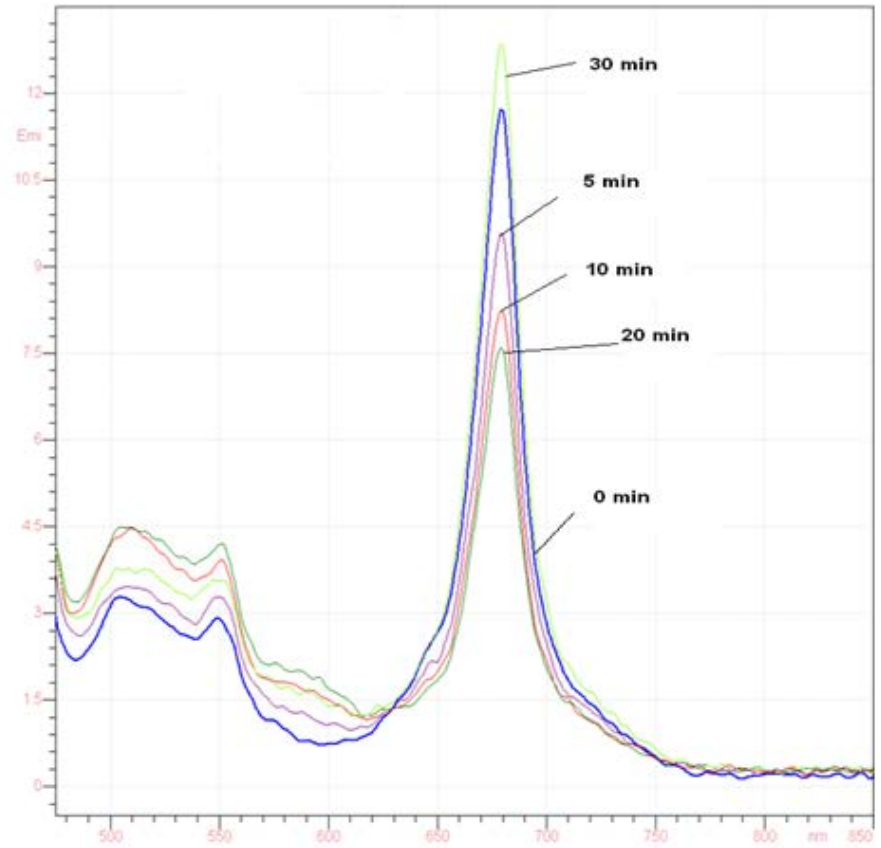


UV-Vis spectrophotometer

Figure 4 Inactivation of 400 mL *Microcystis aeruginosa* using the 40 kHz bath (UV-Vis spectrophotometer and Fluorometer)

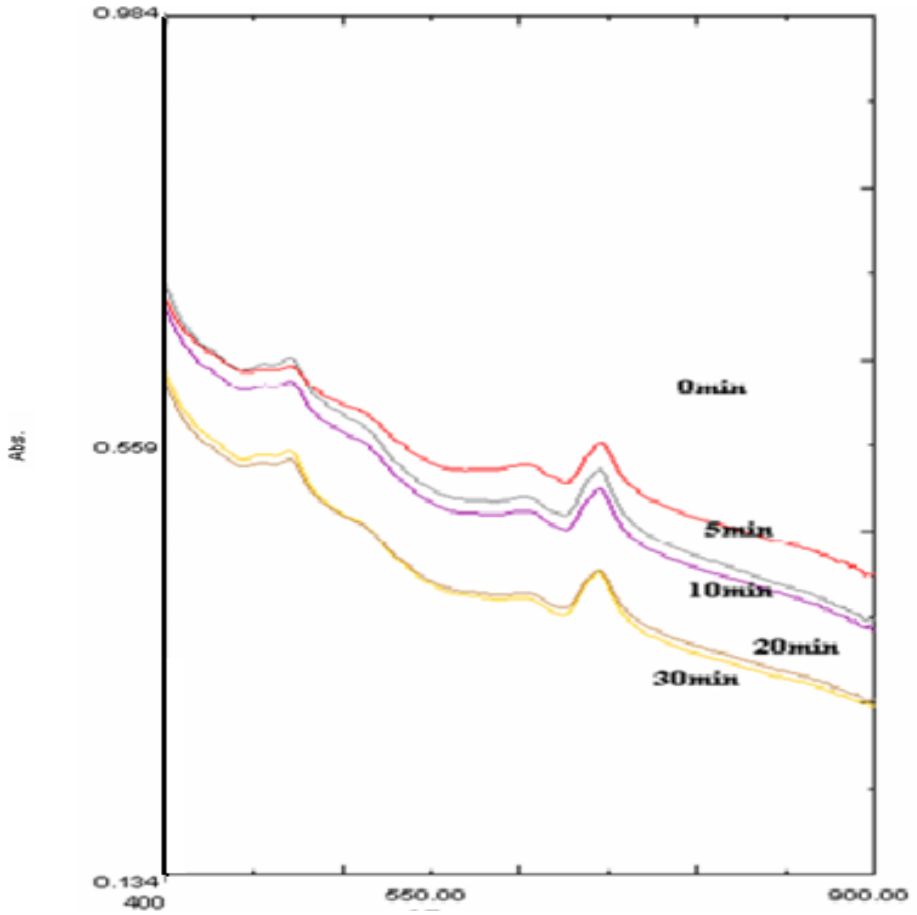


UV-Vis spectrophotometer



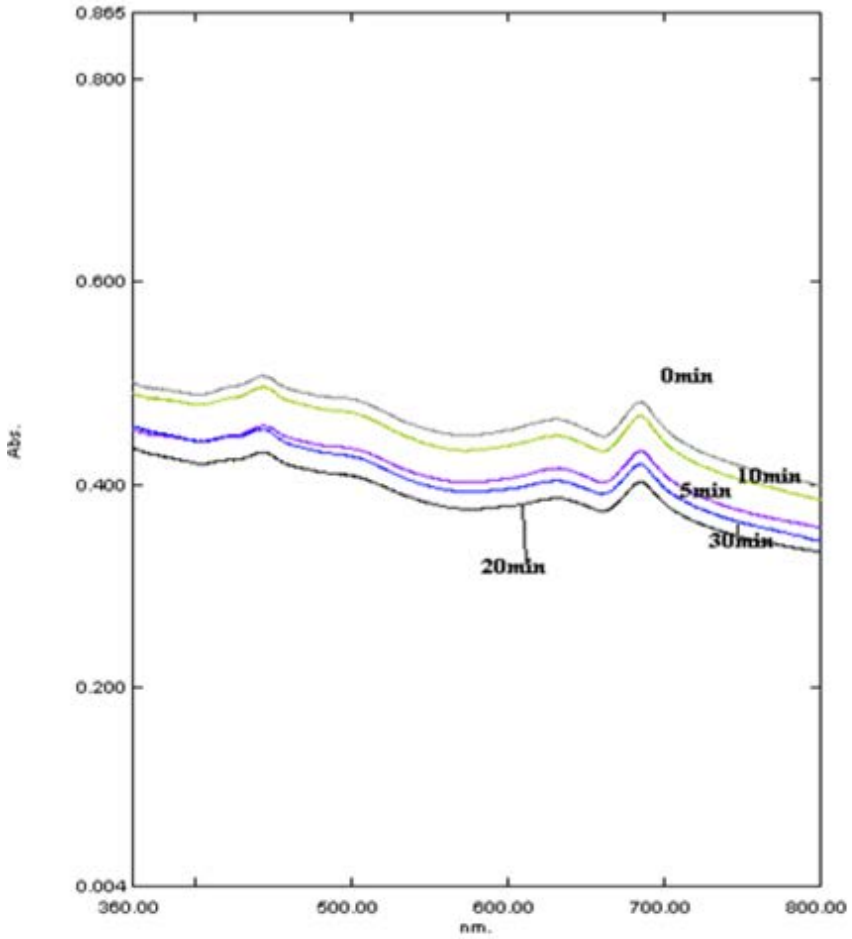
Fluorometer

Figure 5 Inactivation of 200 mL *Microcystis aeruginosa* using the 580 kHz bath (40% power setting) (UV-Vis spectrophotometer)

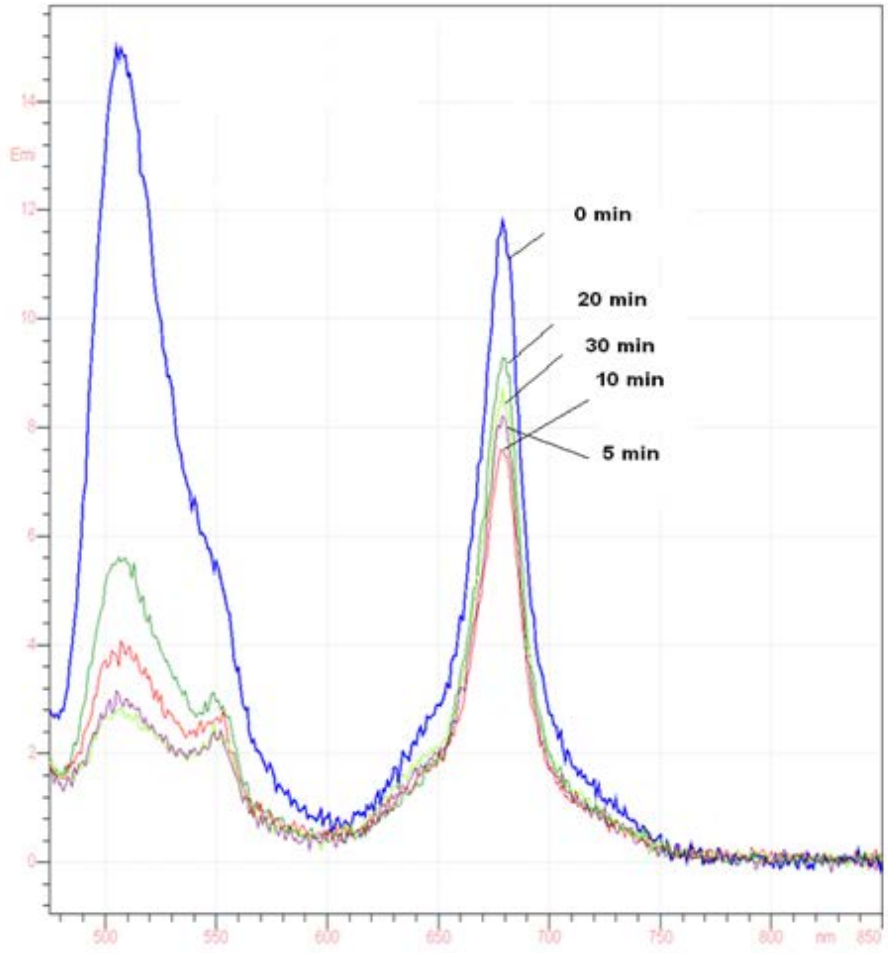


UV-Vis spectrophotometer

Figure 6 Inactivation of 400 mL *Microcystis aeruginosa* using the 580 kHz bath (40% power setting) (UV-Vis spectrophotometer and Fluorometer)

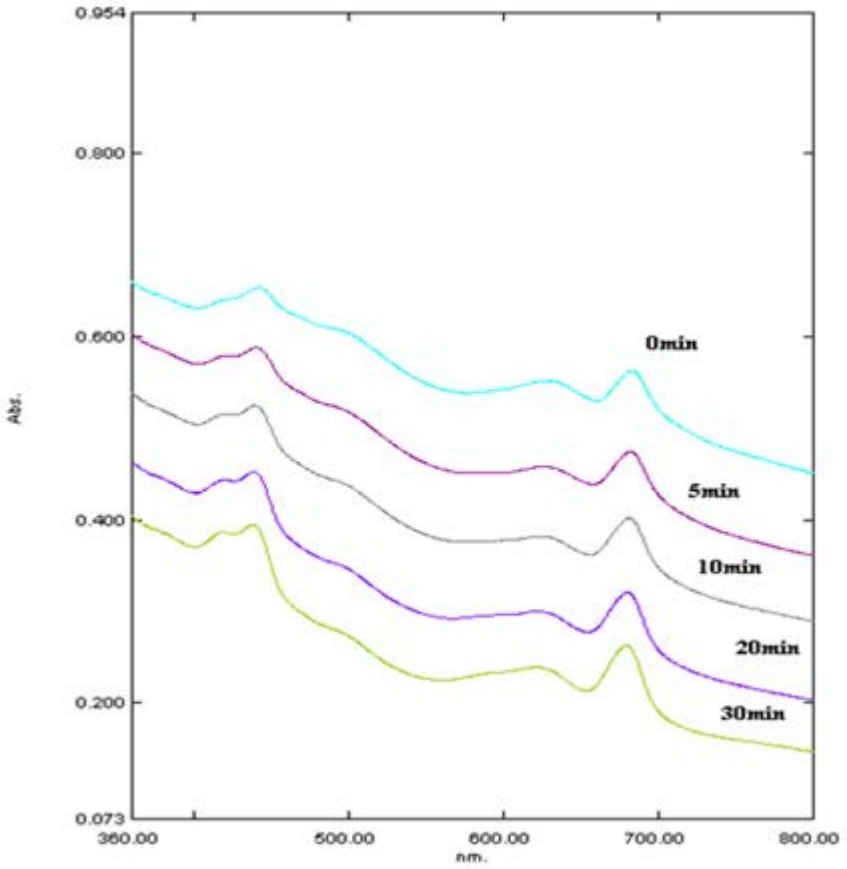


UV-Vis spectrophotometer

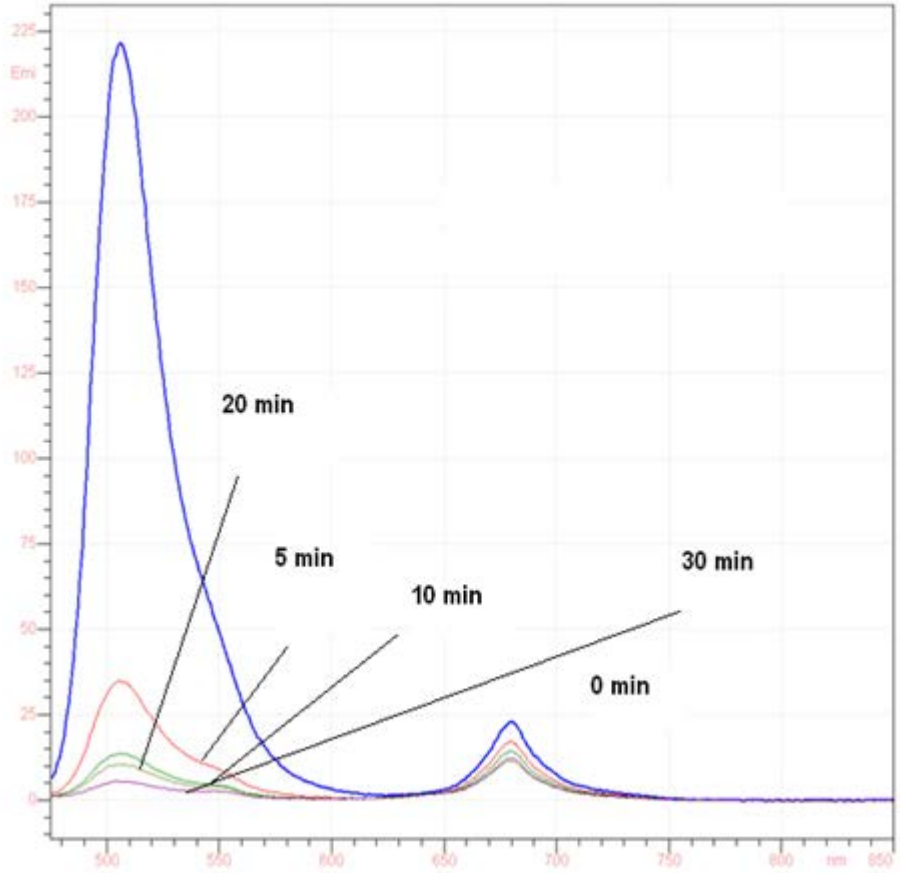


Fluorometer

Figure 7 Inactivation of 200 mL *Microcystis aeruginosa* using the 580 kHz bath (80% power setting) (UV-Vis spectrophotometer and Fluorometer)

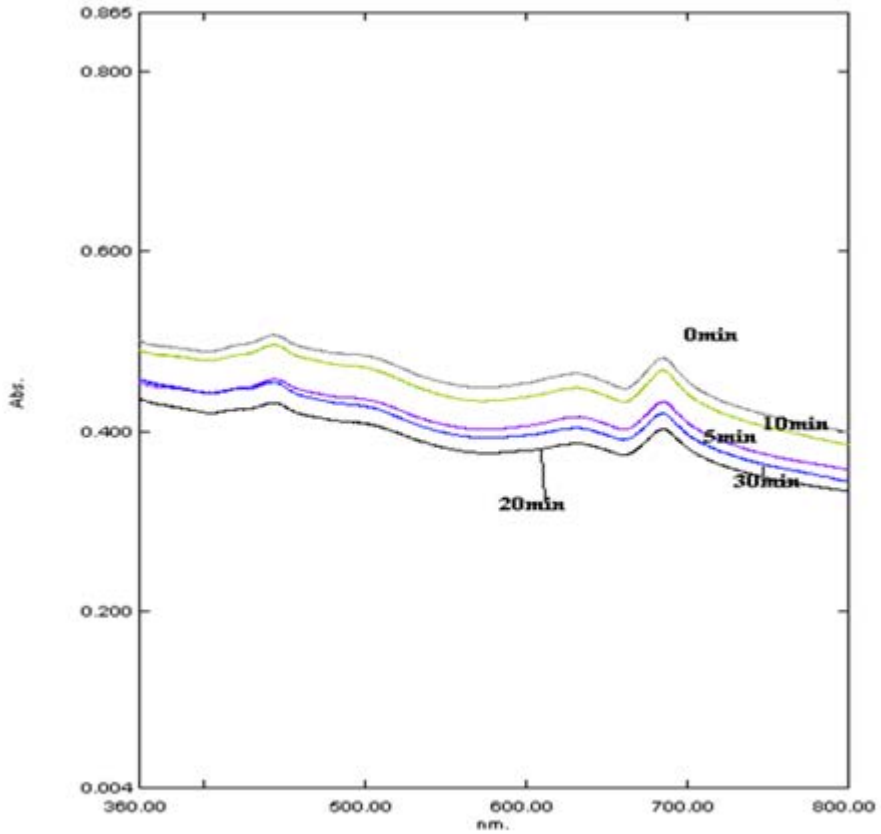


UV-Vis spectrophotometer

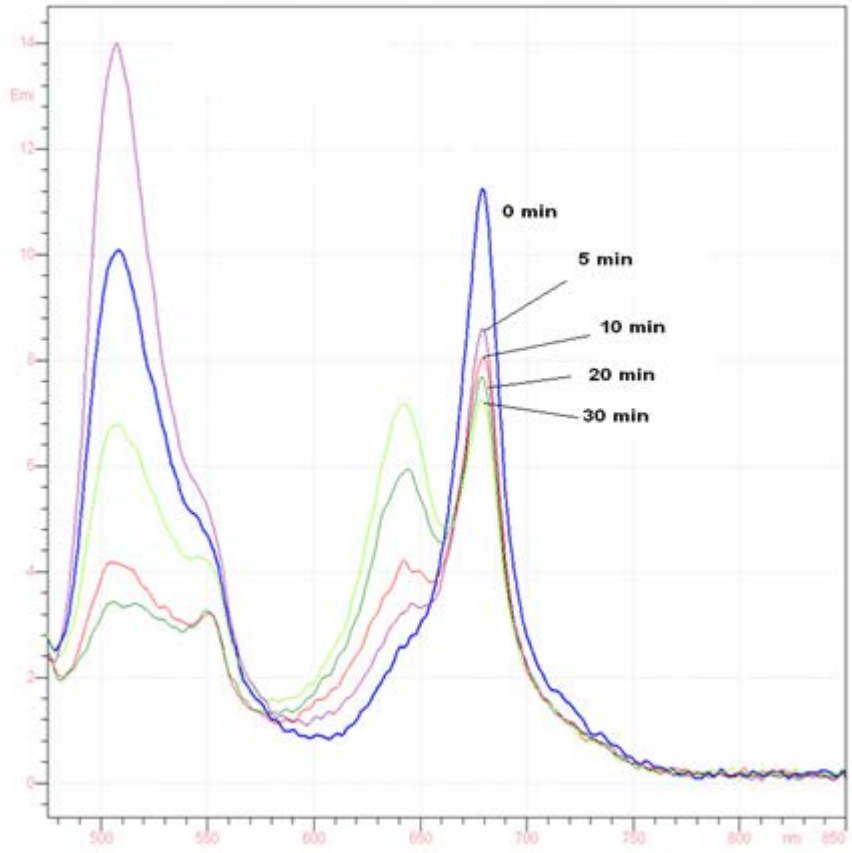


Fluorometer

Figure 8 Inactivation of 400 mL *Microcystis aeruginosa* using the 580 kHz bath (80% power setting) (UV-Vis spectrophotometer and Fluorometer)

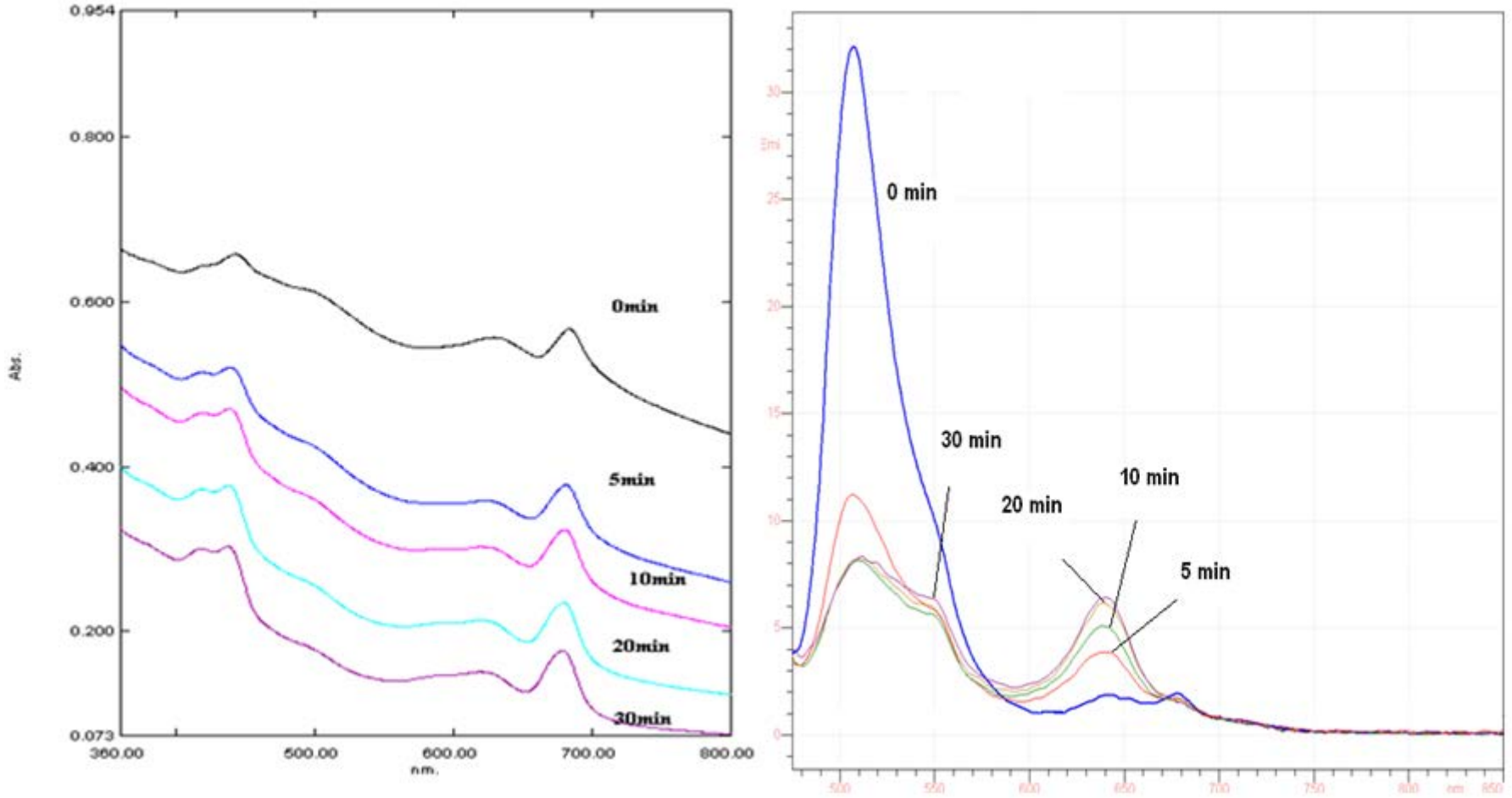


UV-Vis spectrophotometer



Fluorometer

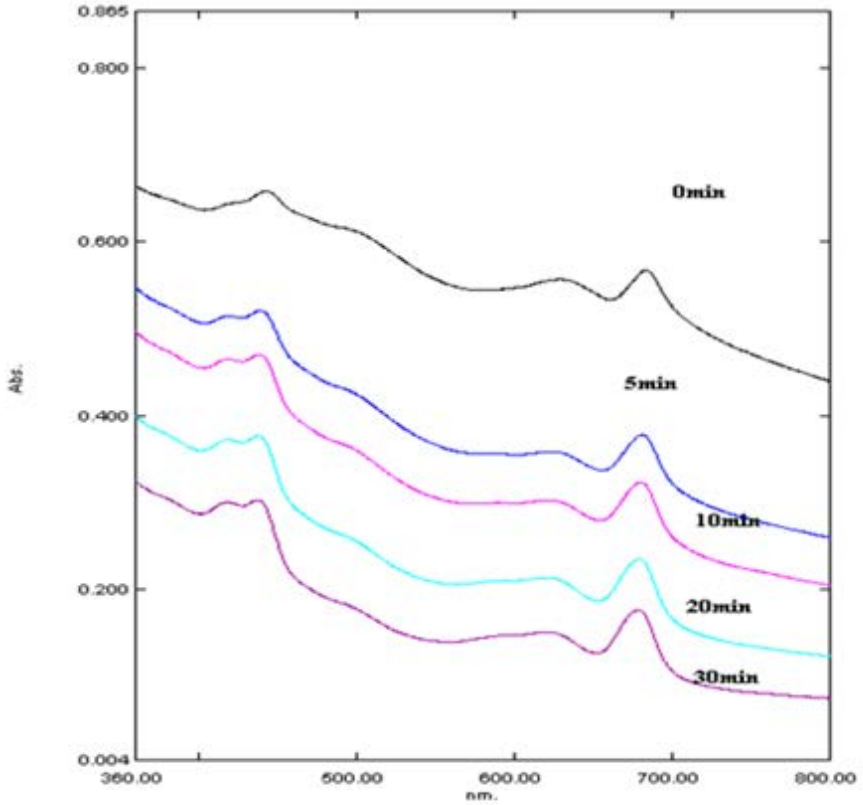
Figure 9 Inactivation of 200 mL *Microcystis aeruginosa* using the 580 kHz bath (maximum power setting) (UV-Vis spectrophotometer and Fluorometer)



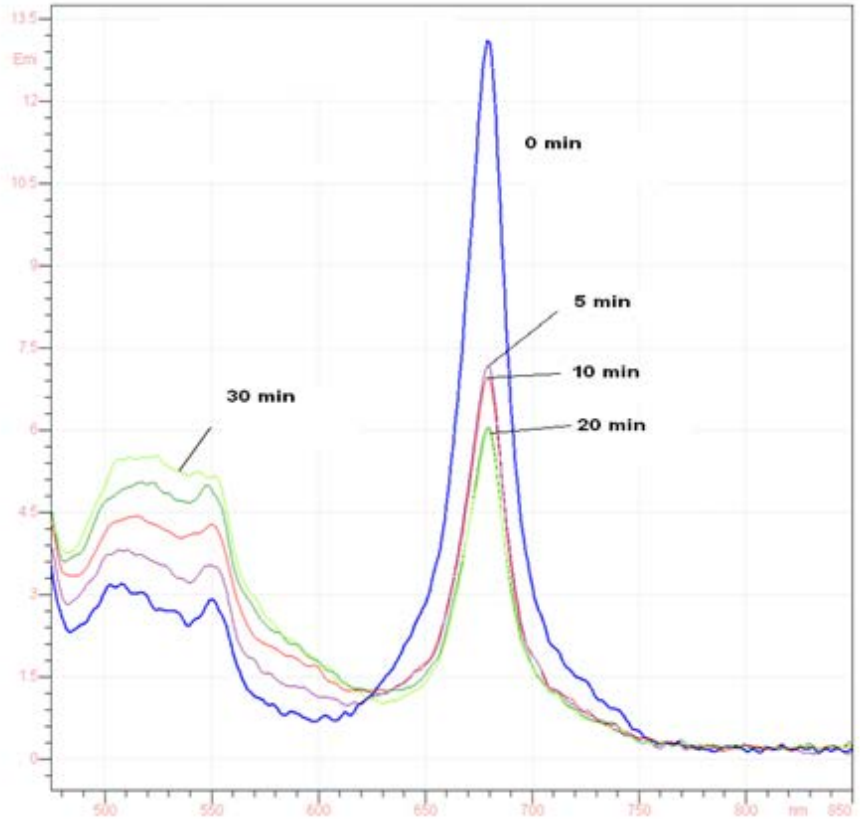
UV-Vis spectrophotometer

Fluorometer

Figure 10 Inactivation of 400 mL *Microcystis aeruginosa* using the 580 kHz bath (maximum power setting) (UV-Vis spectrophotometer and Fluorometer)

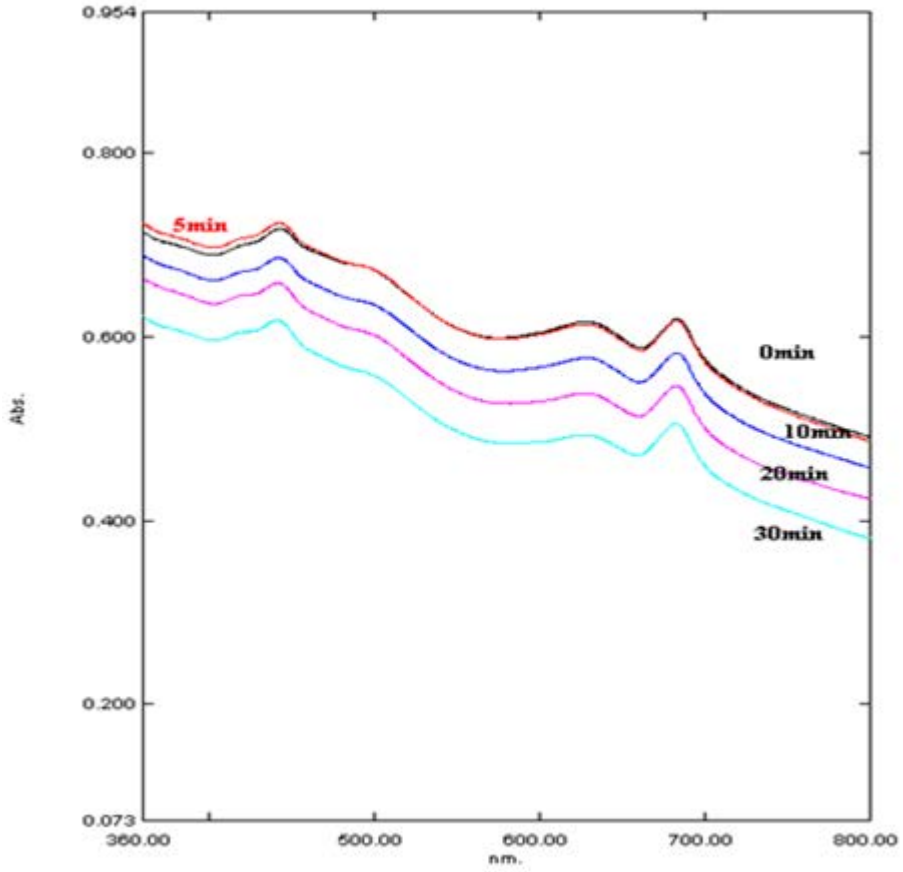


UV-Vis spectrophotometer

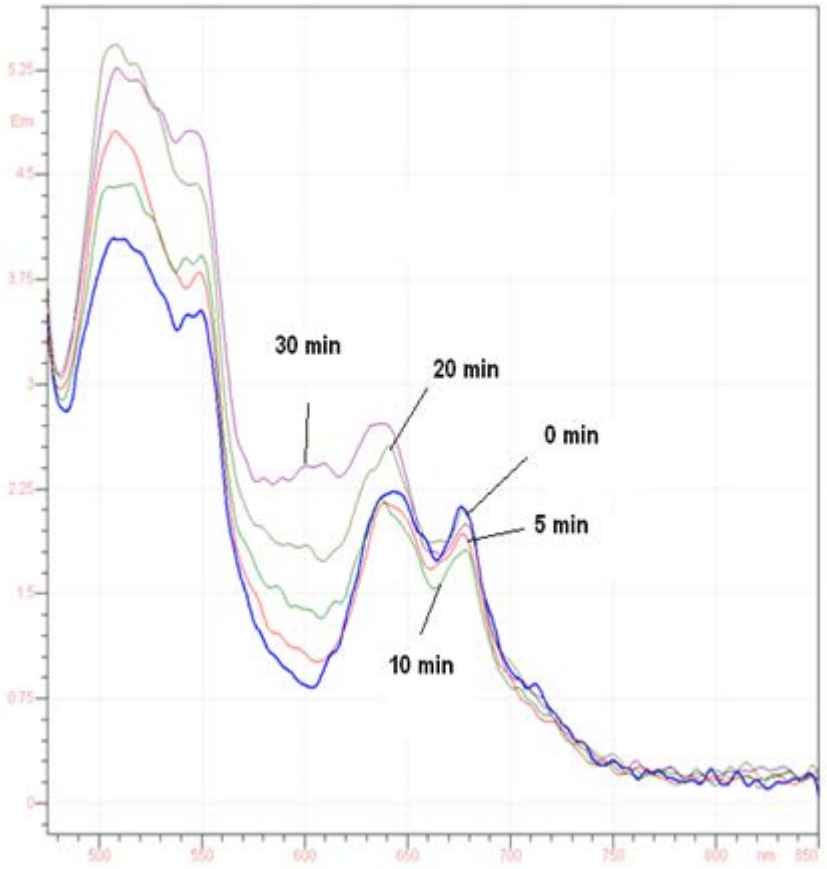


Fluorometer

Figure 11 Inactivation of 200 mL *Microcystis aeruginosa* using the 864 kHz bath (40% power setting) (UV-Vis spectrophotometer and Fluorometer)

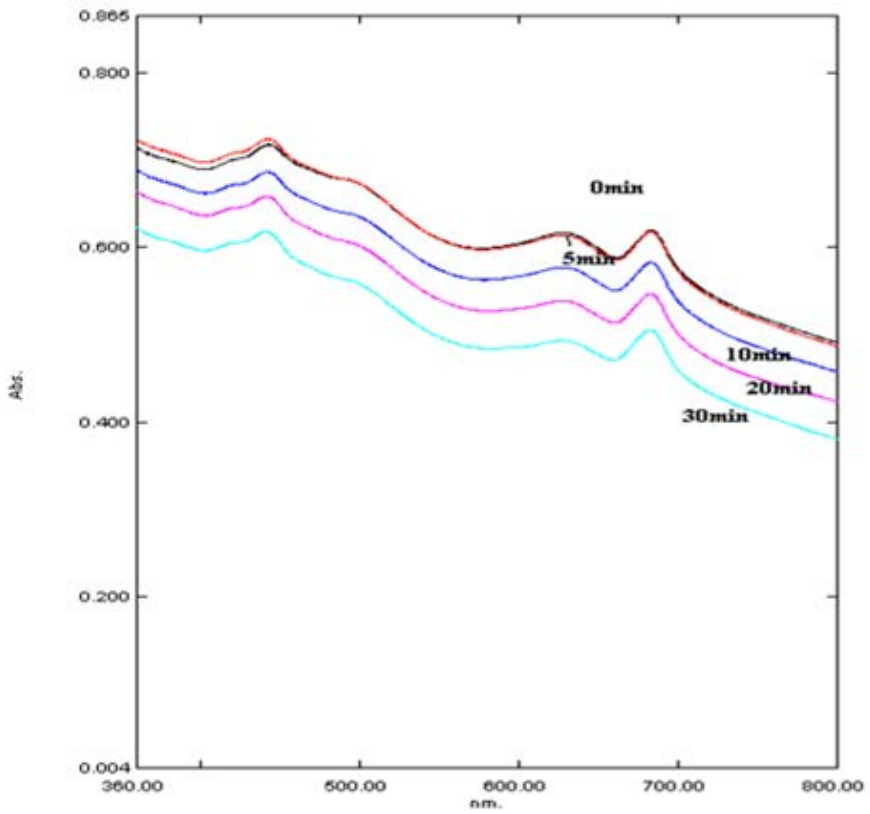


UV-Vis spectrophotometer

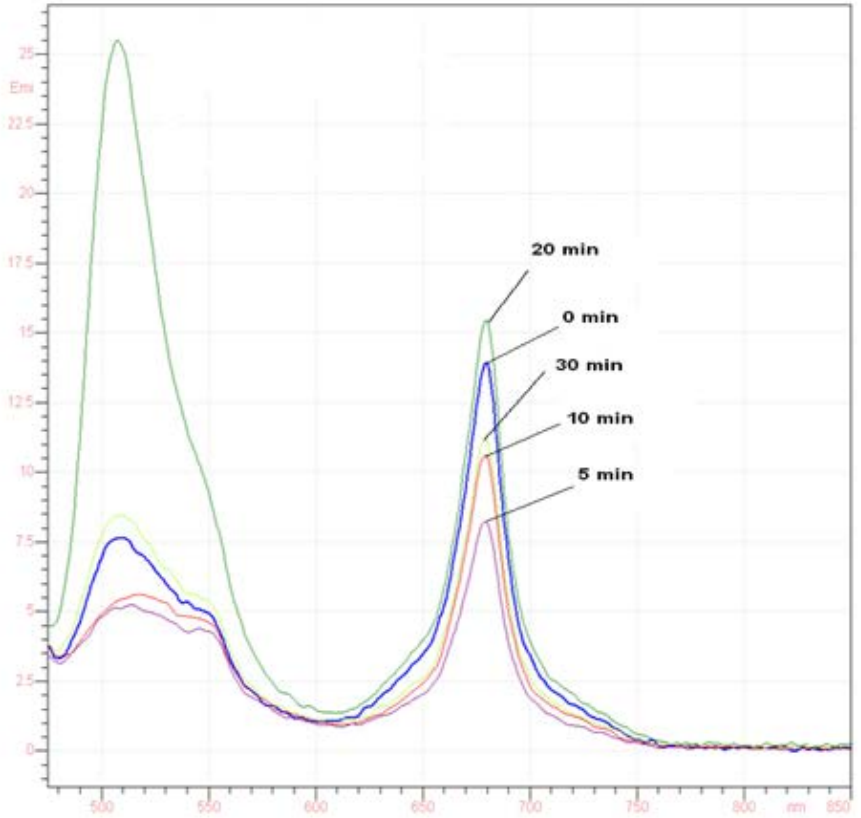


Fluorometer

Figure 12 Inactivation of 400 mL *Microcystis aeruginosa* using the 864 kHz bath (40% power setting) (UV-Vis spectrophotometer and Fluorometer)

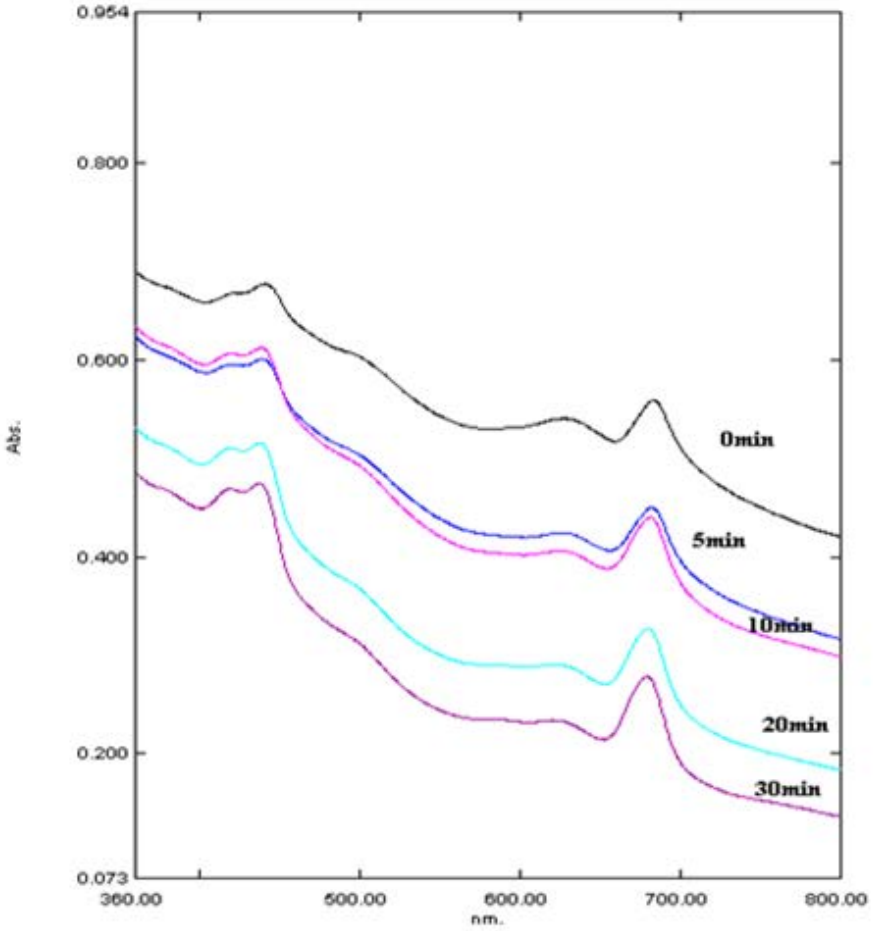


UV-Vis spectrophotometer

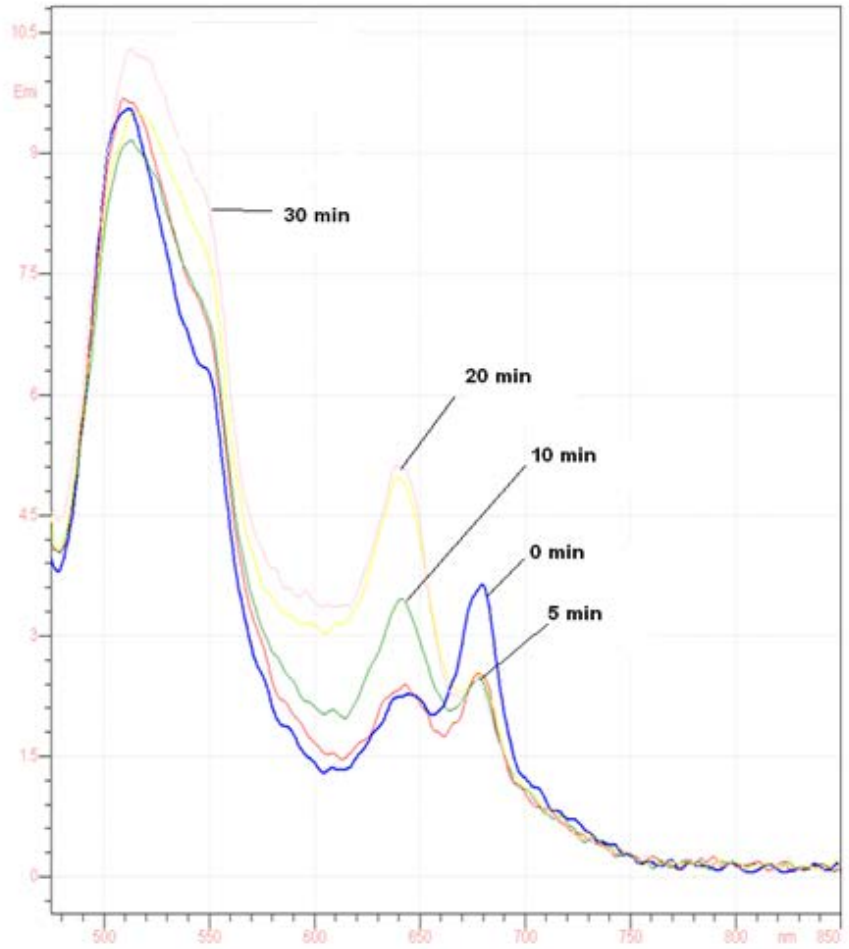


Fluorometer

Figure 13 Inactivation of 200 mL *Microcystis aeruginosa* using the 864 kHz bath (80% power setting) (UV-Vis spectrophotometer and Fluorometer)

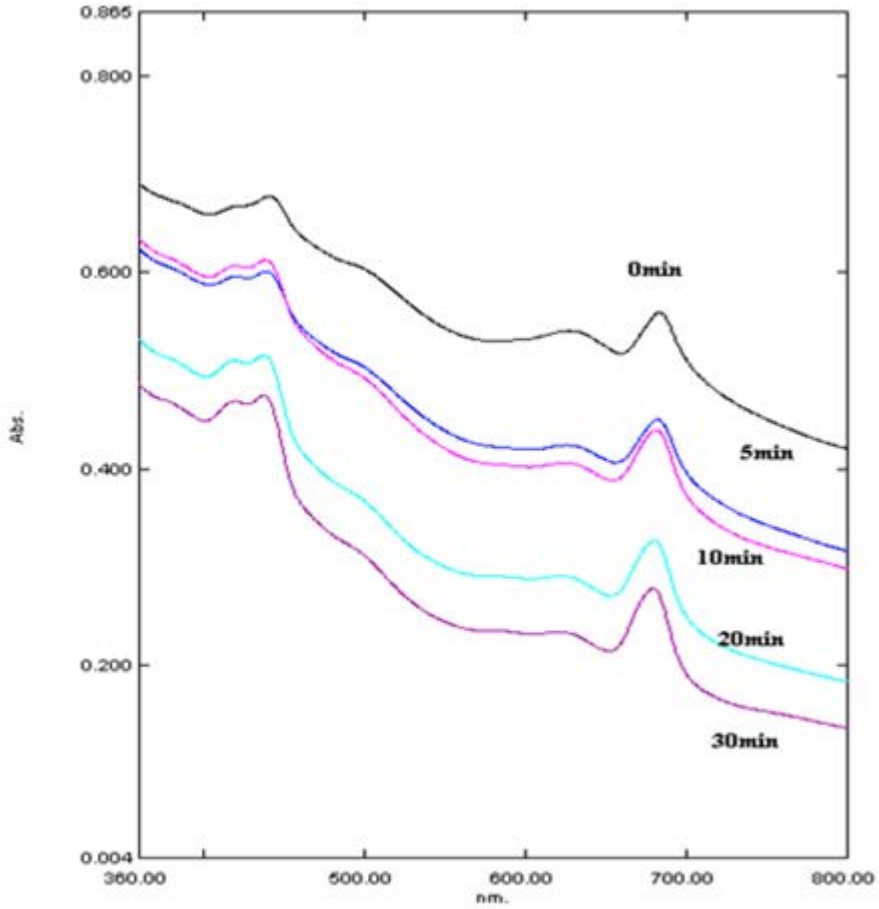


UV-Vis spectrophotometer

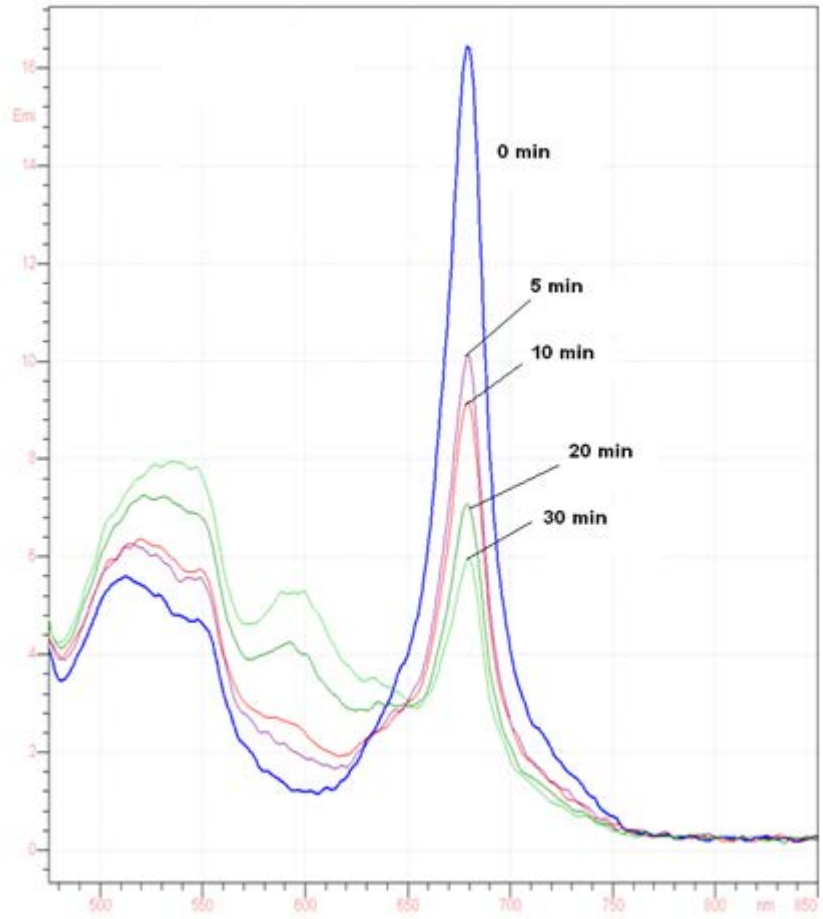


Fluorometer

Figure 14 Inactivation of 400 mL *Microcystis aeruginosa* using the 864 kHz bath (80% power setting) (UV-Vis spectrophotometer and Fluorometer)

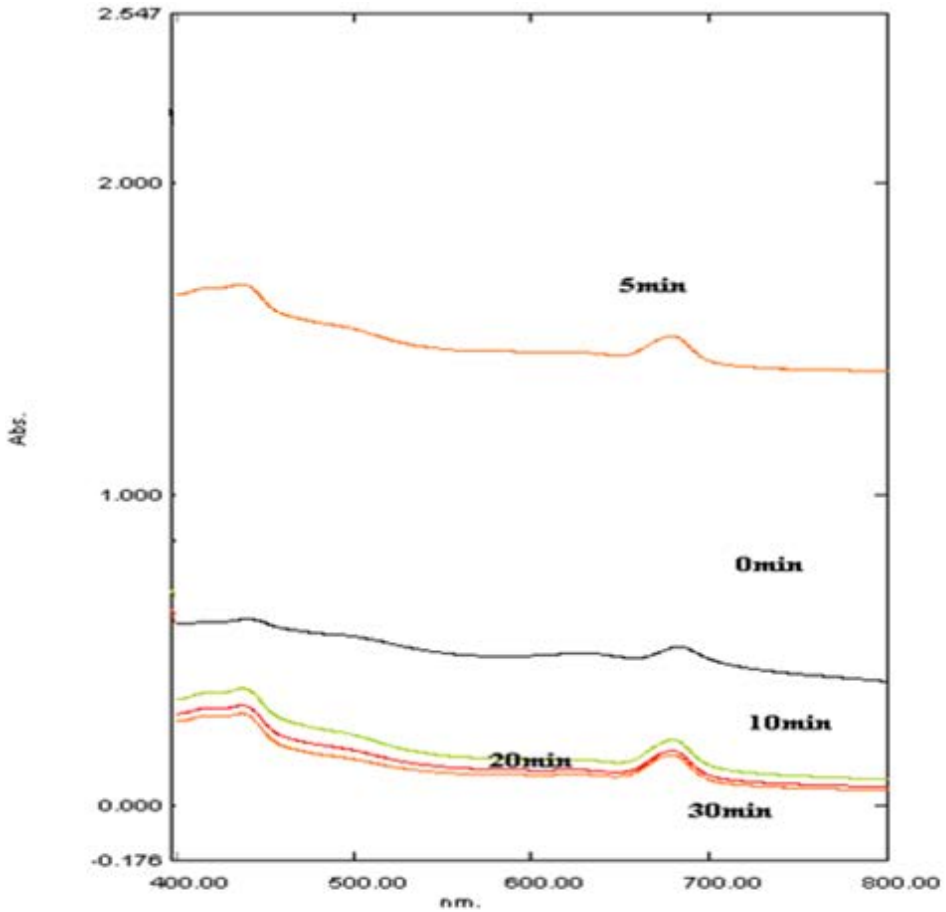


UV-Vis spectrophotometer

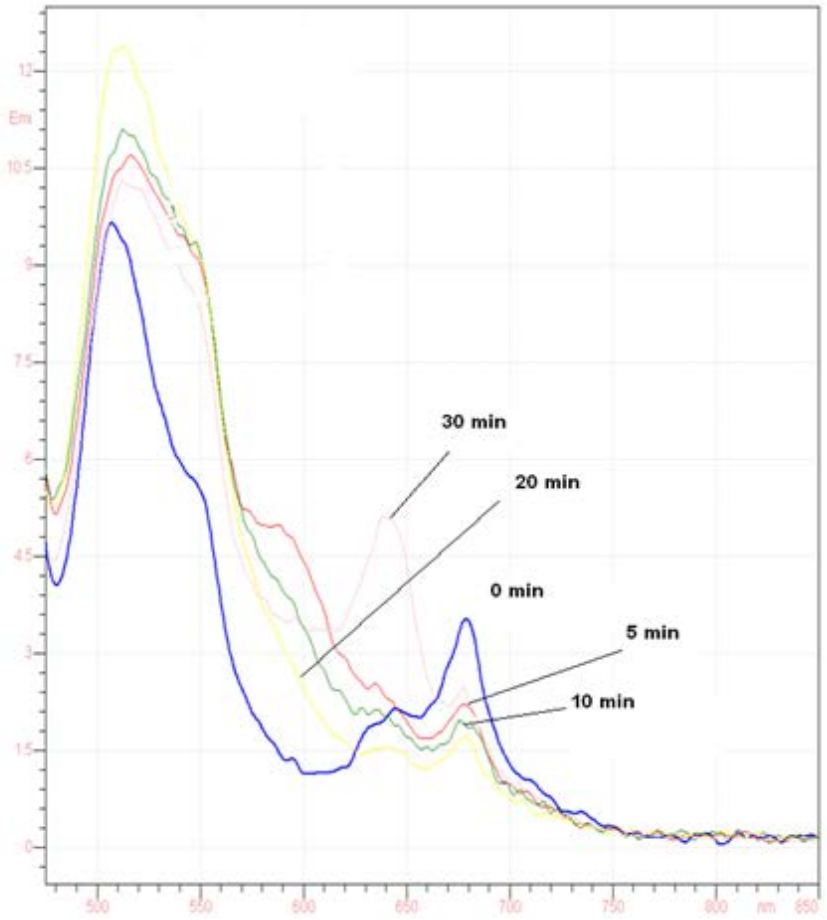


Fluorometer

Figure 15 Inactivation of 200 mL *Microcystis aeruginosa* using the 864 kHz bath (maximum power setting) (UV-Vis spectrophotometer and Fluorometer)

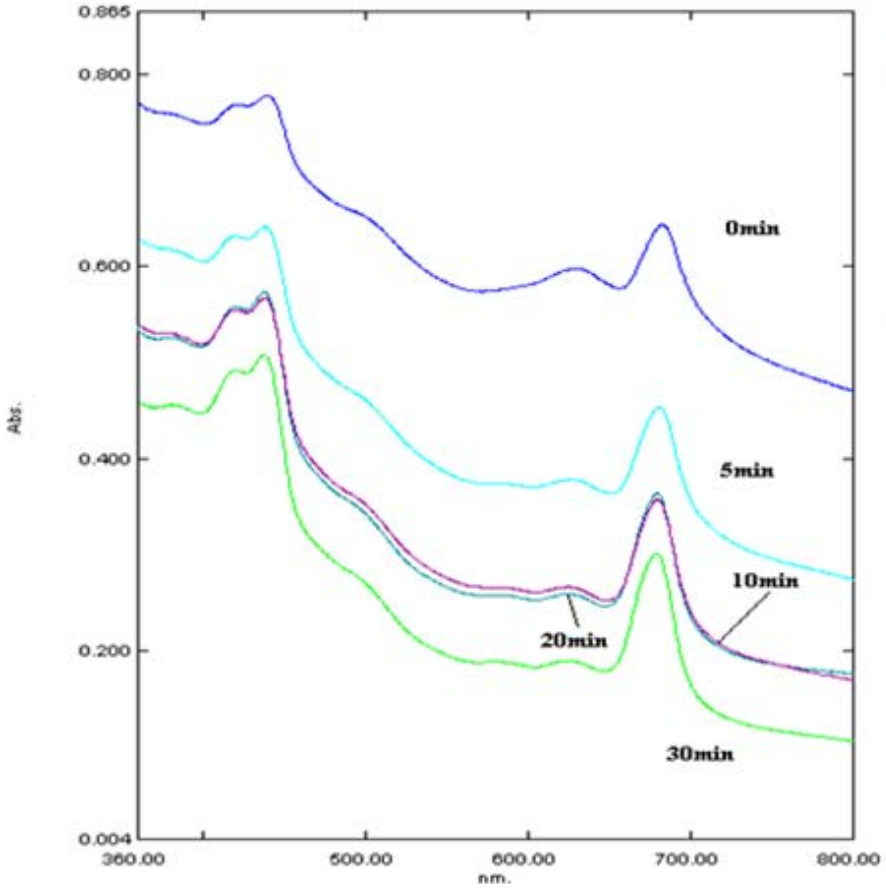


UV-Vis spectrophotometer

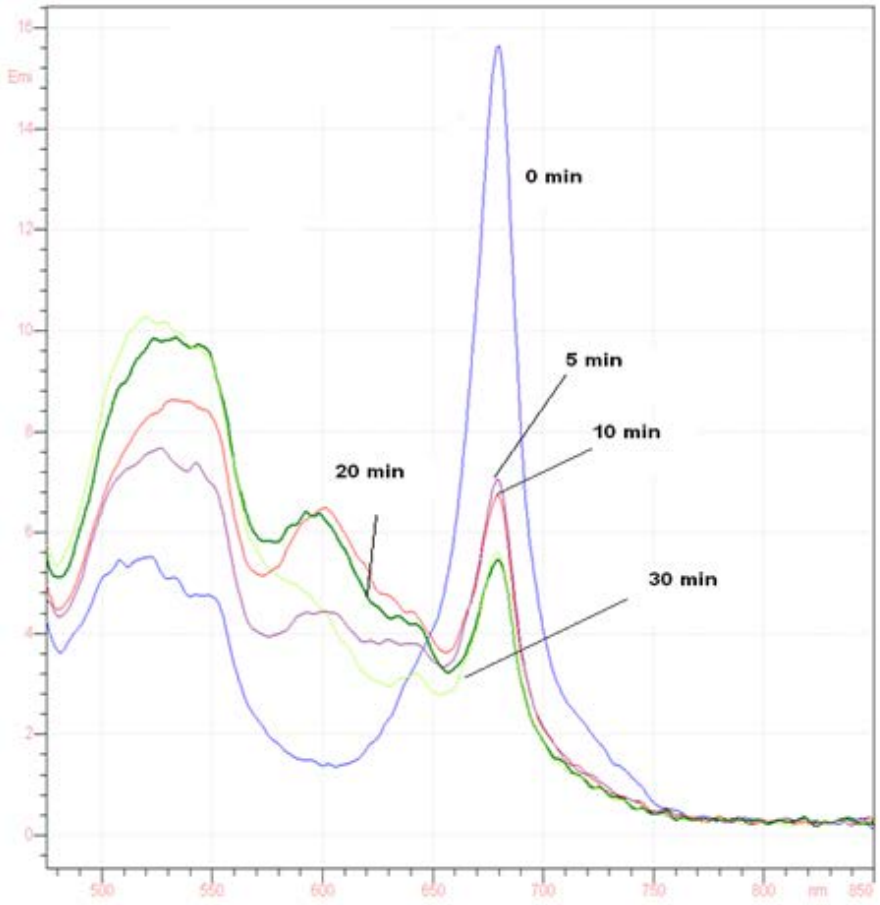


Fluorometer

Figure 16 Inactivation of 400 mL *Microcystis aeruginosa* using the 864 kHz bath (maximum power setting) (UV-Vis spectrophotometer and Fluorometer)

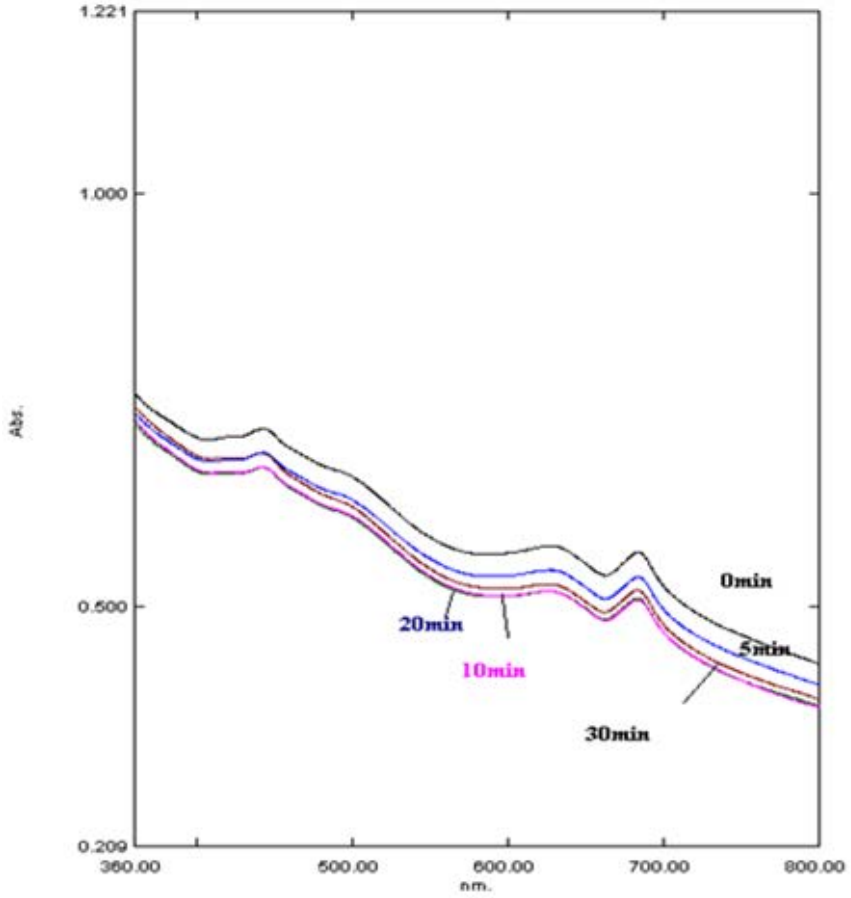


UV-Vis spectrophotometer

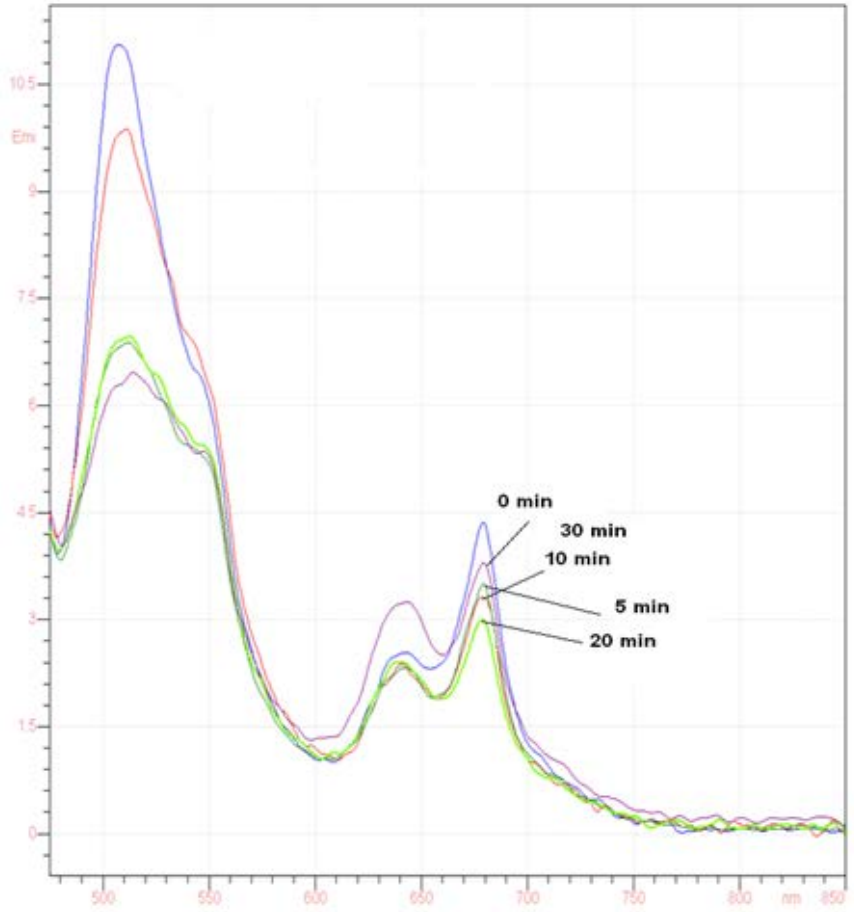


Fluorometer

Figure 17 Inactivation of 200 mL *Microcystis aeruginosa* using the 1146 kHz bath (40% power setting) (UV-Vis spectrophotometer and Fluorometer)

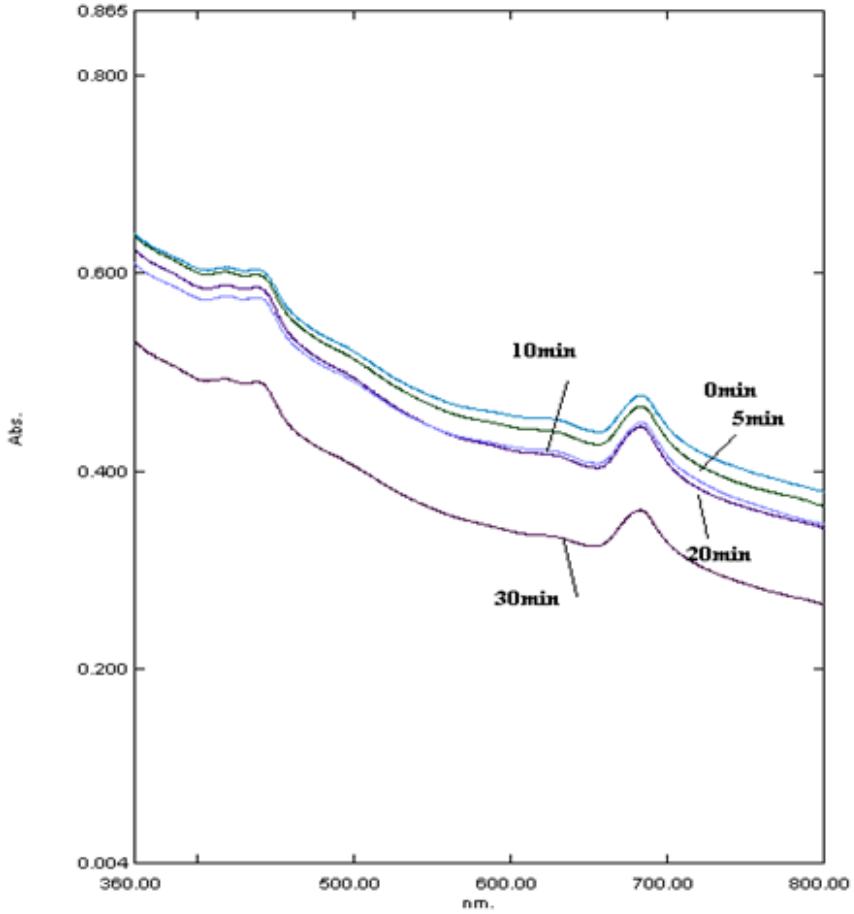


UV-Vis spectrophotometer

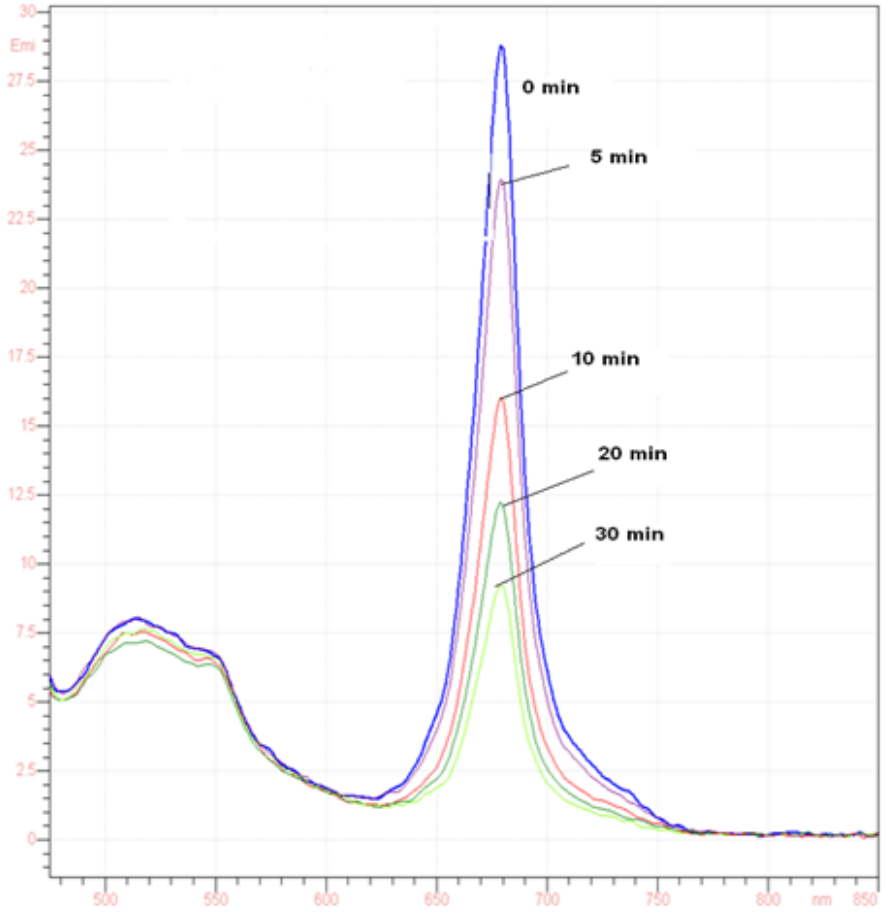


Fluorometer

Figure 18 Inactivation of 400 mL *Microcystis aeruginosa* using the 1146 kHz bath (40% power setting) (UV-Vis spectrophotometer and Fluorometer)

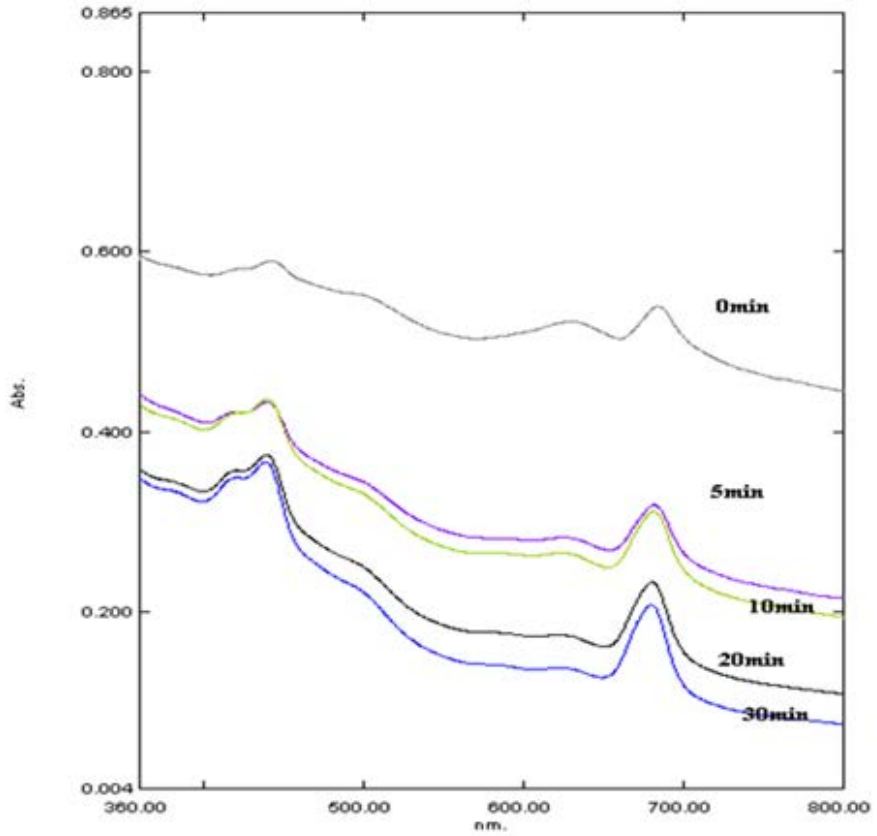


UV-Vis spectrophotometer

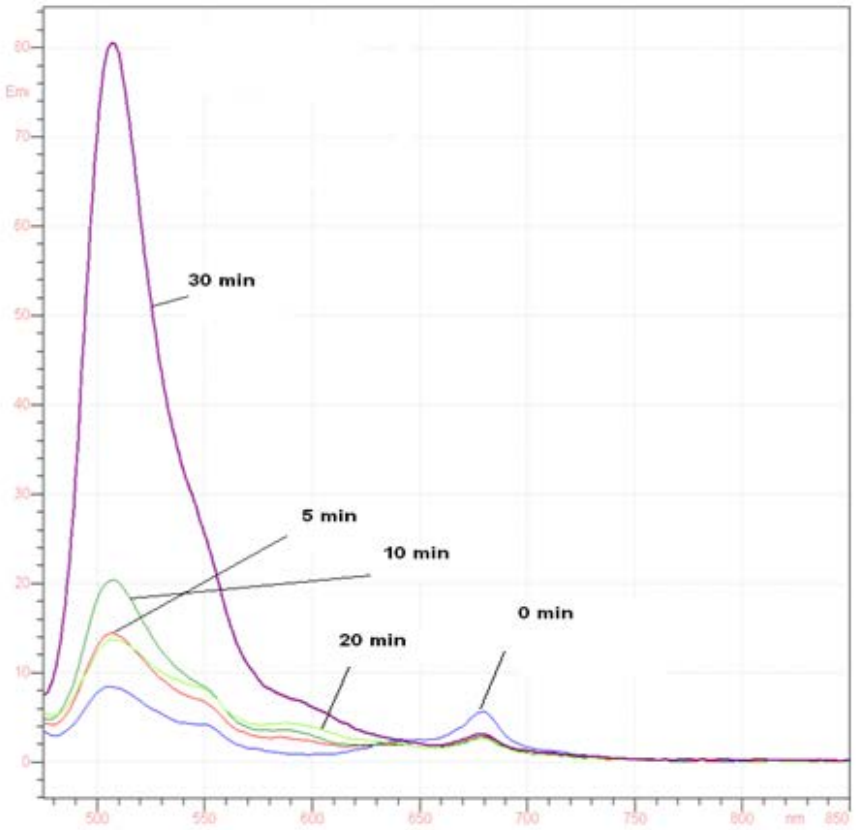


Fluorometer

Figure 19 Inactivation of 200 mL *Microcystis aeruginosa* using the 1146 kHz bath (80% power setting) (UV-Vis spectrophotometer and Fluorometer)

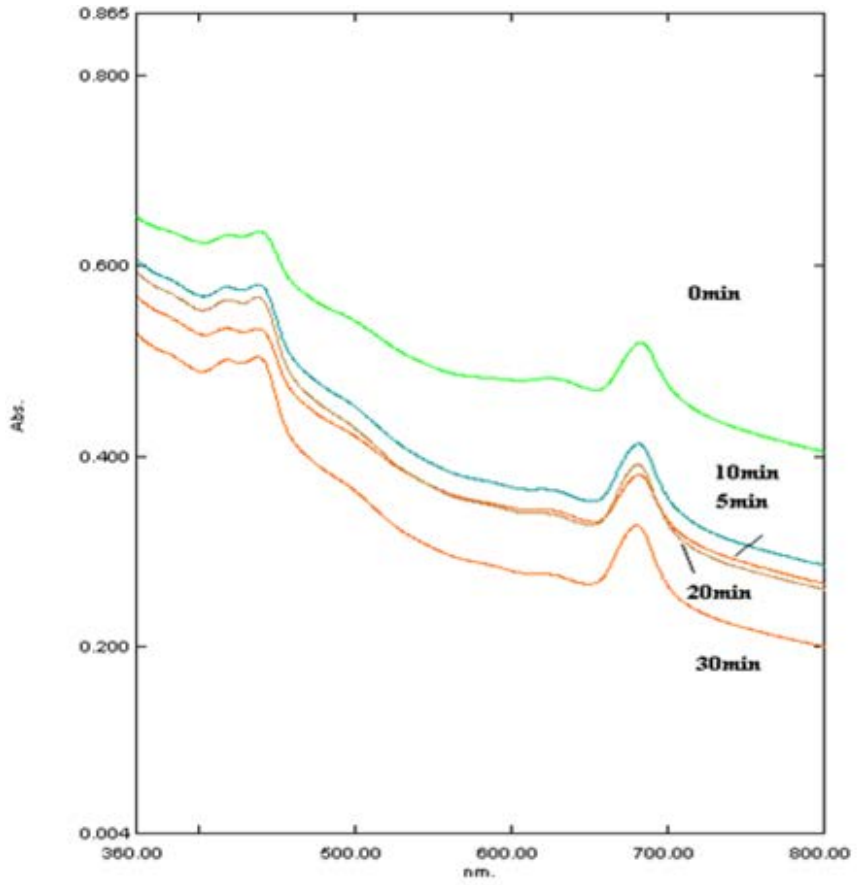


UV-Vis spectrophotometer

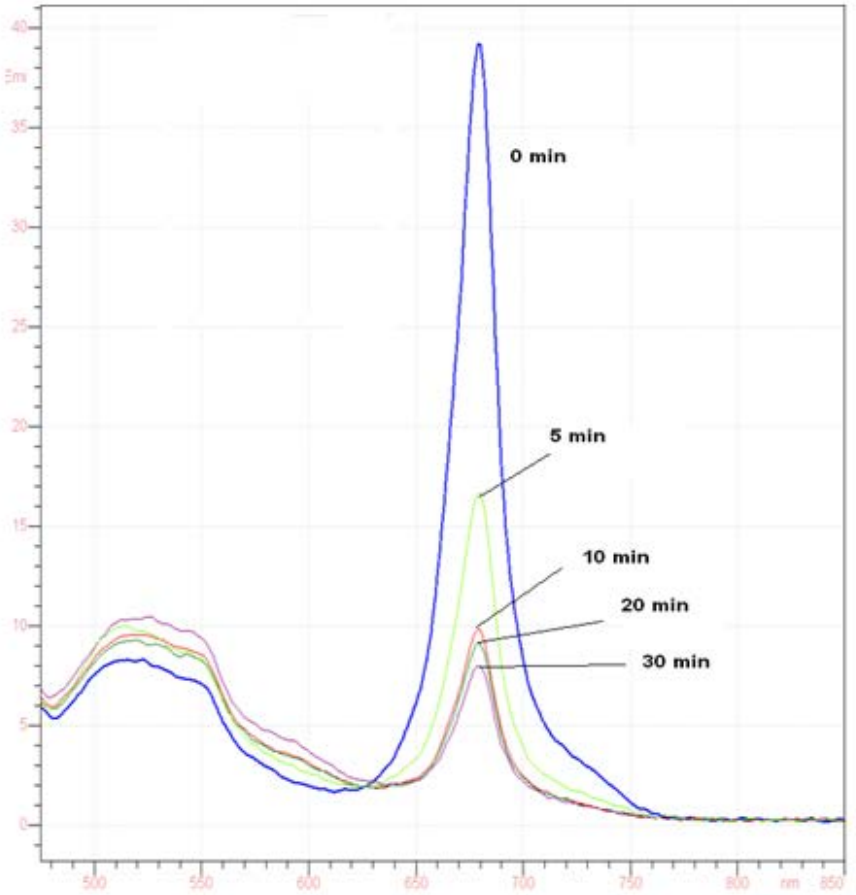


Fluorometer

Figure 20 Inactivation of 400 mL Microcystis aeruginosa using the 1146 kHz bath (80% power setting) (UV-Vis spectrophotometer and Fluorometer)

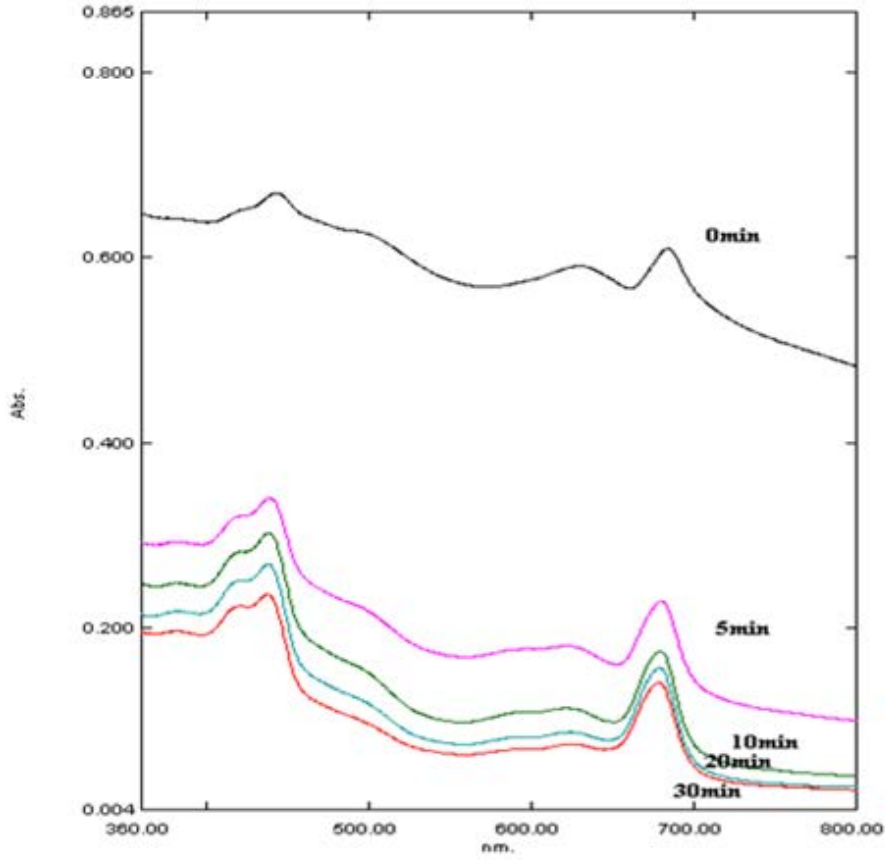


UV-Vis spectrophotometer

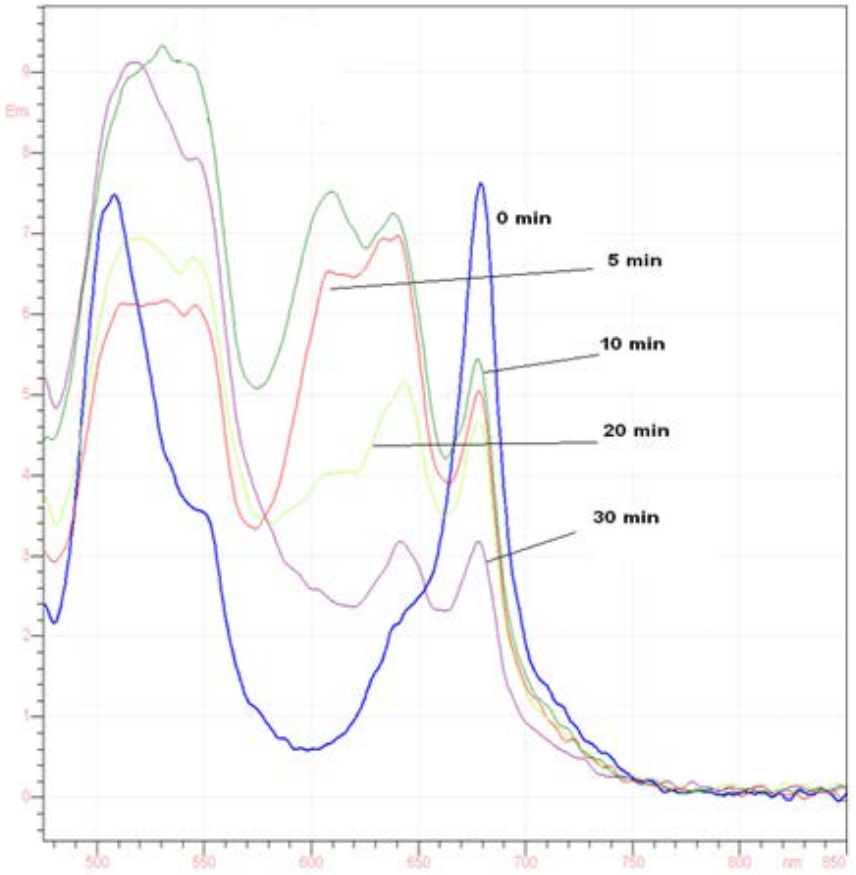


Fluorometer

Figure 21 Inactivation of 200 mL *Microcystis aeruginosa* using the 1146 kHz bath (maximum power setting) (UV-Vis spectrophotometer and Fluorometer)

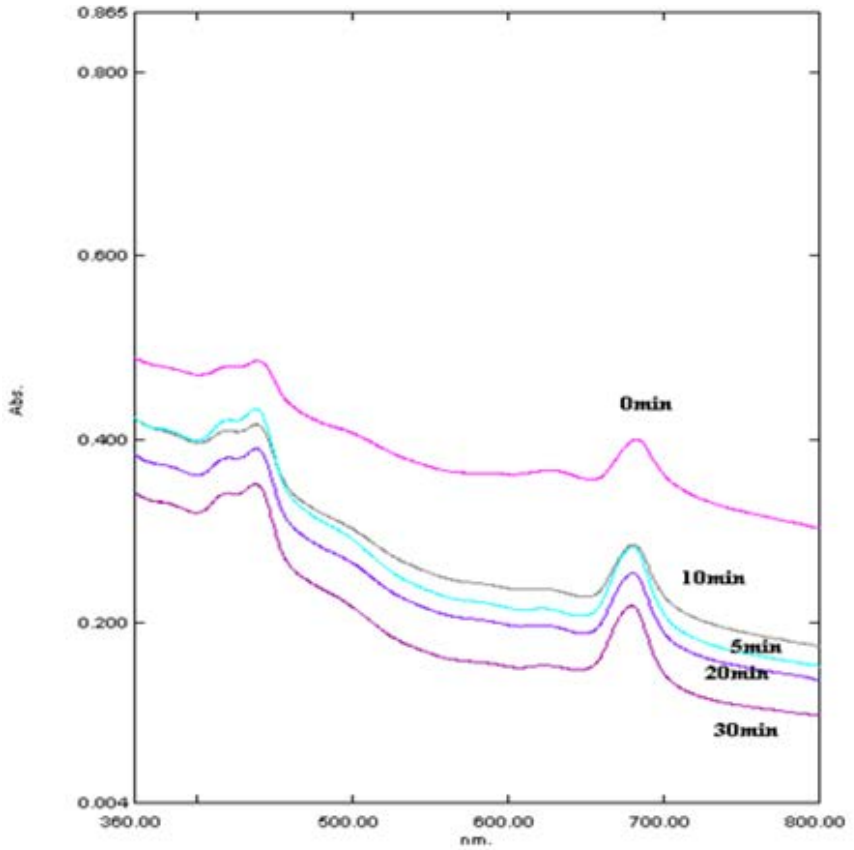


UV-Vis spectrophotometer

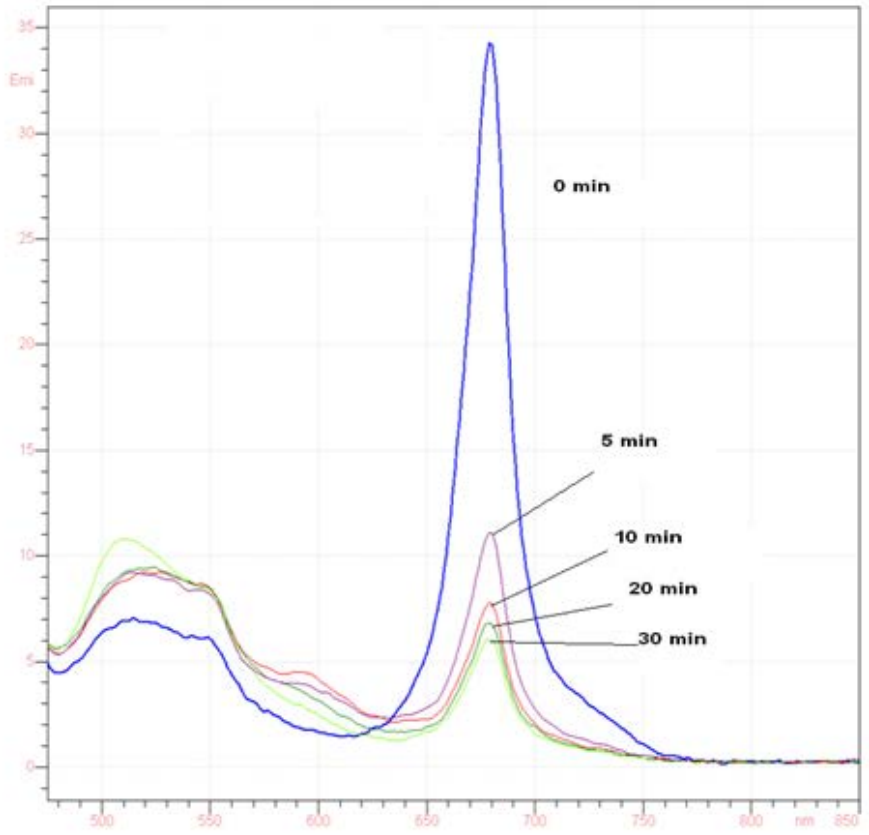


Fluorometer

Figure 22 Inactivation of 400 mL *Microcystis aeruginosa* using the 1146 kHz bath (maximum power setting) (UV-Vis spectrophotometer and Fluorometer)

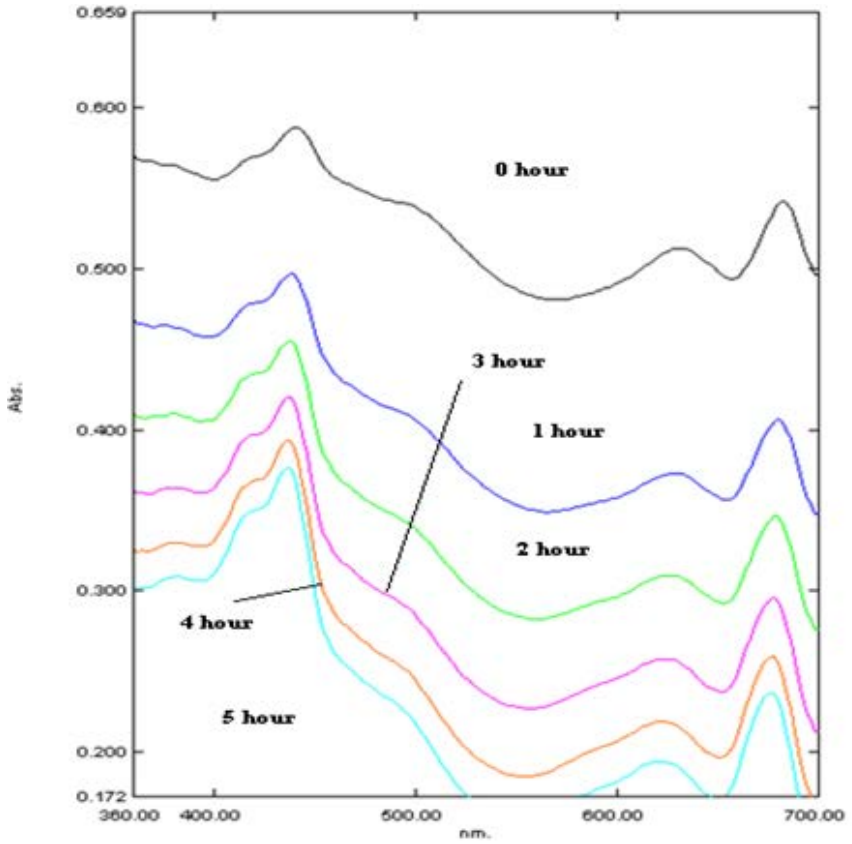


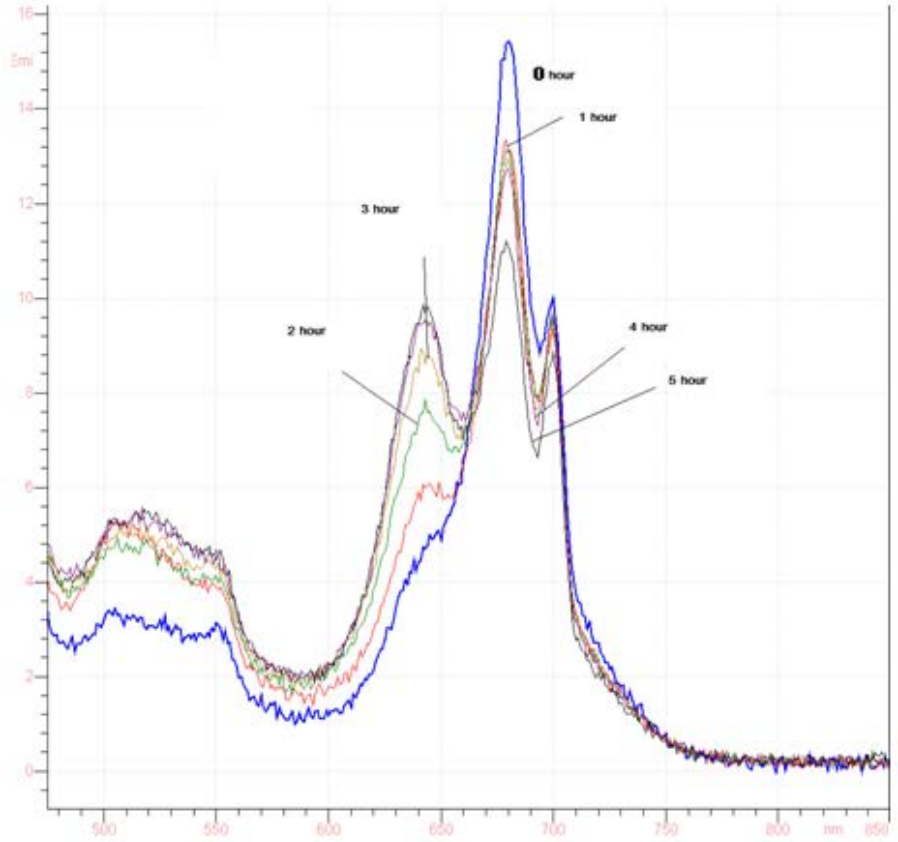
UV-Vis spectrophotometer



Fluorometer

Figure 23 Inactivation of 5 L *Microcystis aeruginosa* using the Sonolator (UV-Vis spectrophotometer and Fluorometer)

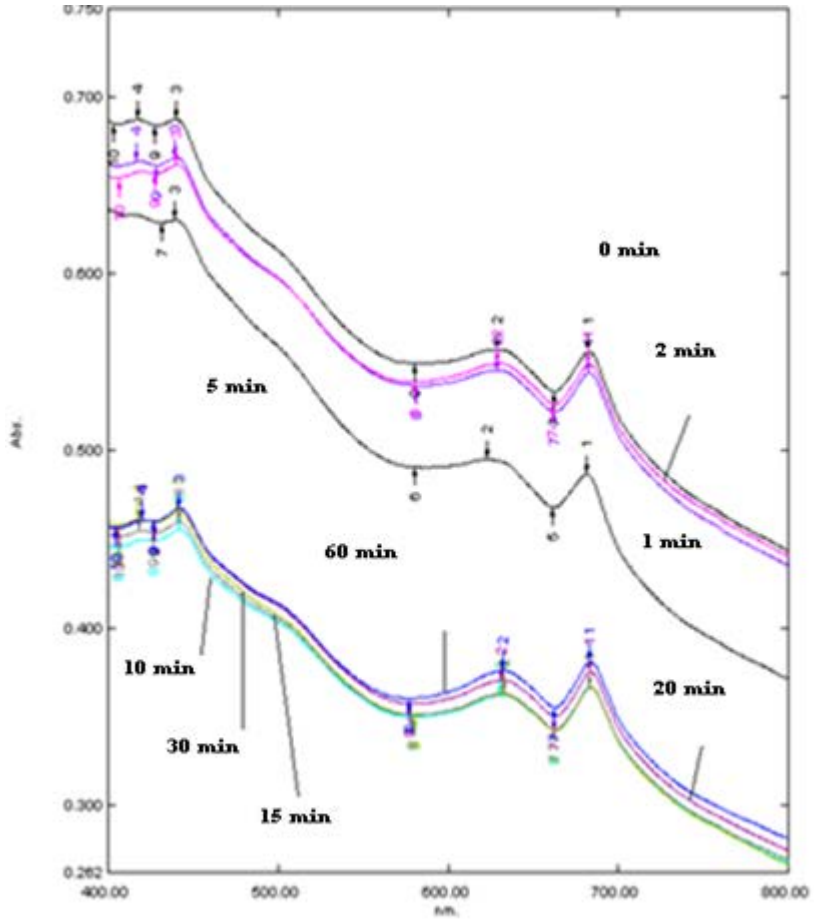




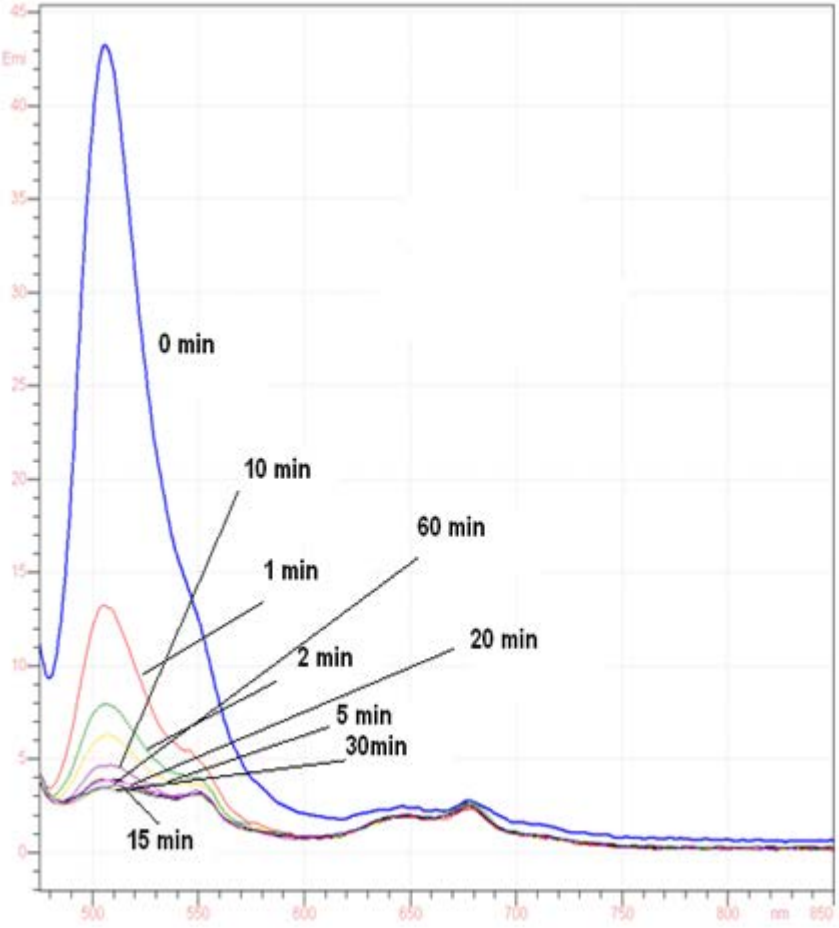
UV-Vis spectrophotometer

Fluorometer

Figure 24 Inactivation of 3.5 L *Microcystis aeruginosa* using DFR (circulating) at 40% power setting (UV-Vis spectrophotometer and Fluorometer)

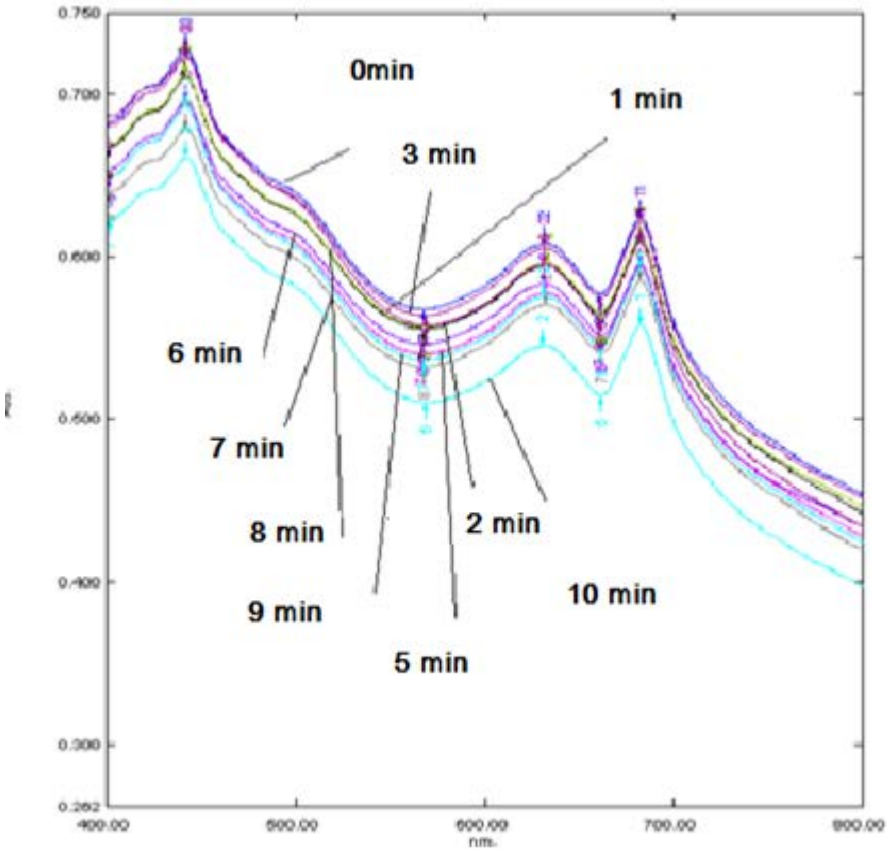


UV-Vis spectrophotometer

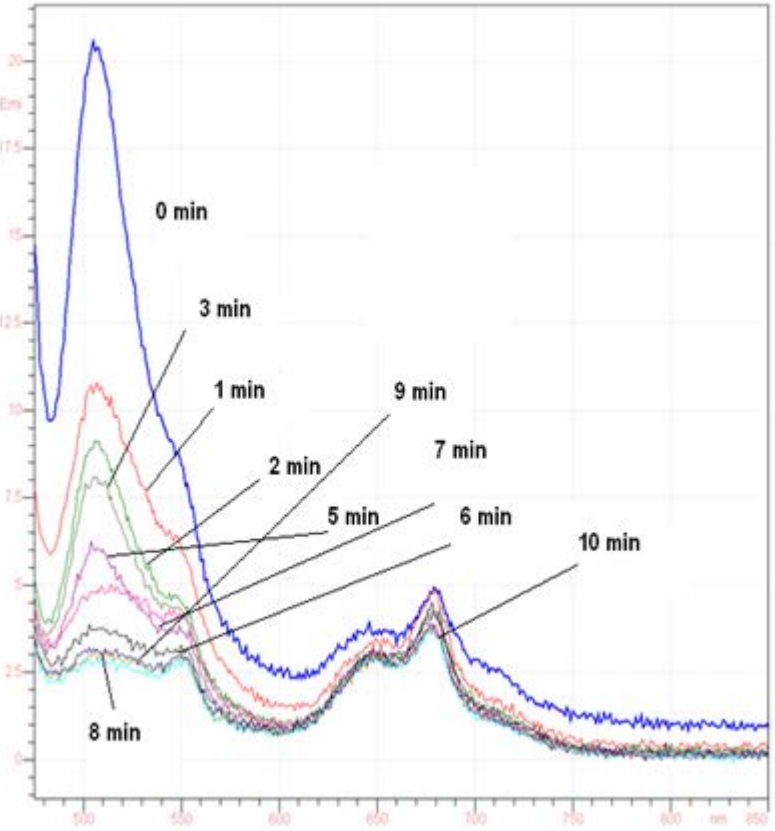


Fluorometer

Figure 25 Inactivation of 1 L *Microcystis aeruginosa* using DFR (static) at 40% intensity (UV-Vis spectrophotometer and Fluorometer)

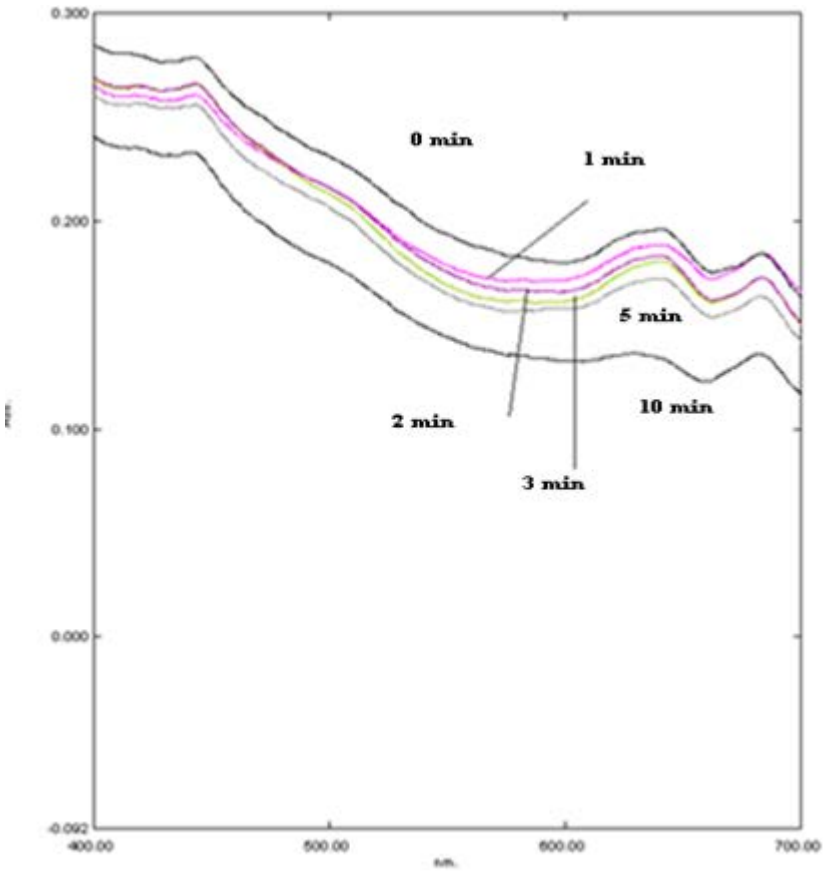


UV-Vis spectrophotometer

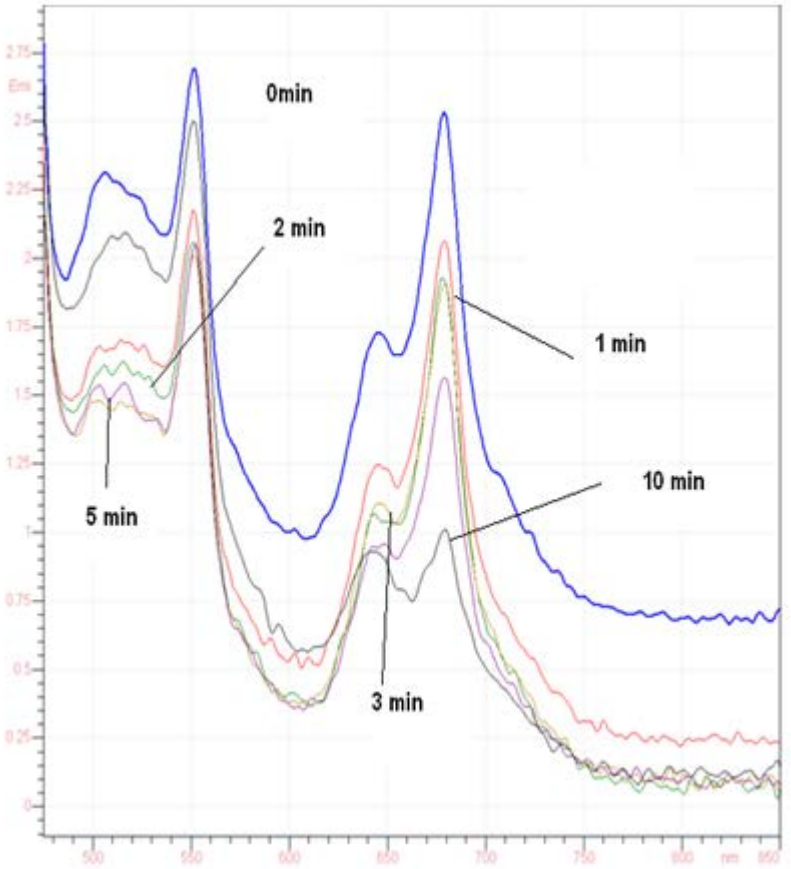


Fluorometer

Figure 26 Inactivation of 1 L *Microcystis aeruginosa* using DFR (static) at 40% power setting (UV-Vis spectrophotometer and Fluorometer)

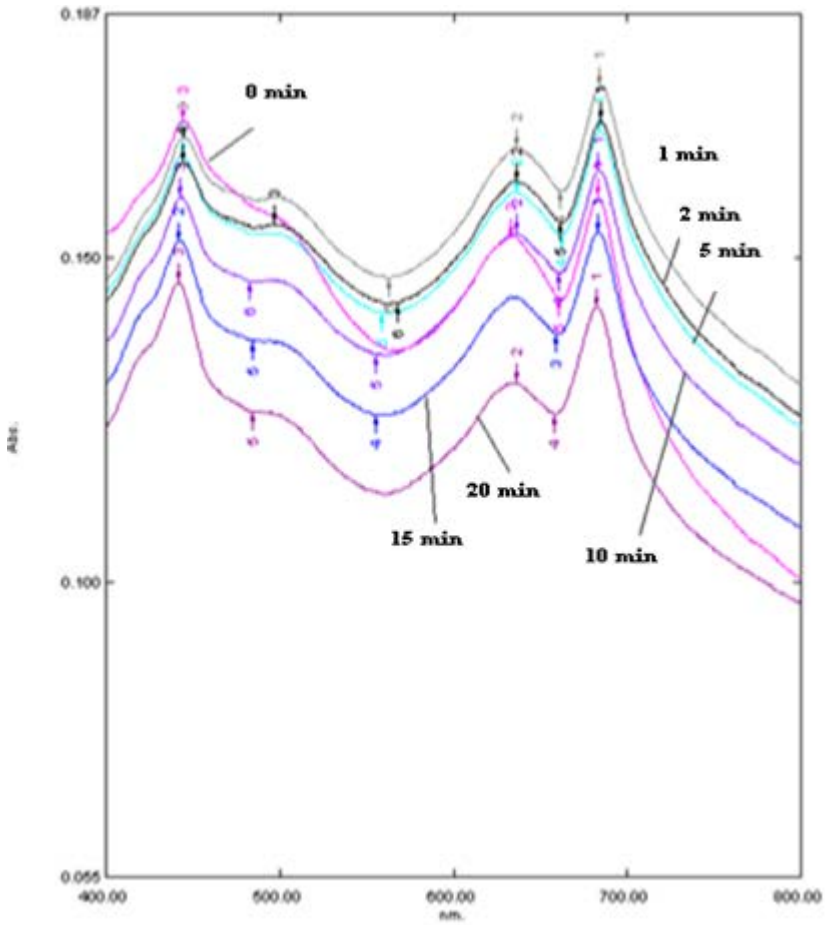


UV-Vis spectrophotometer

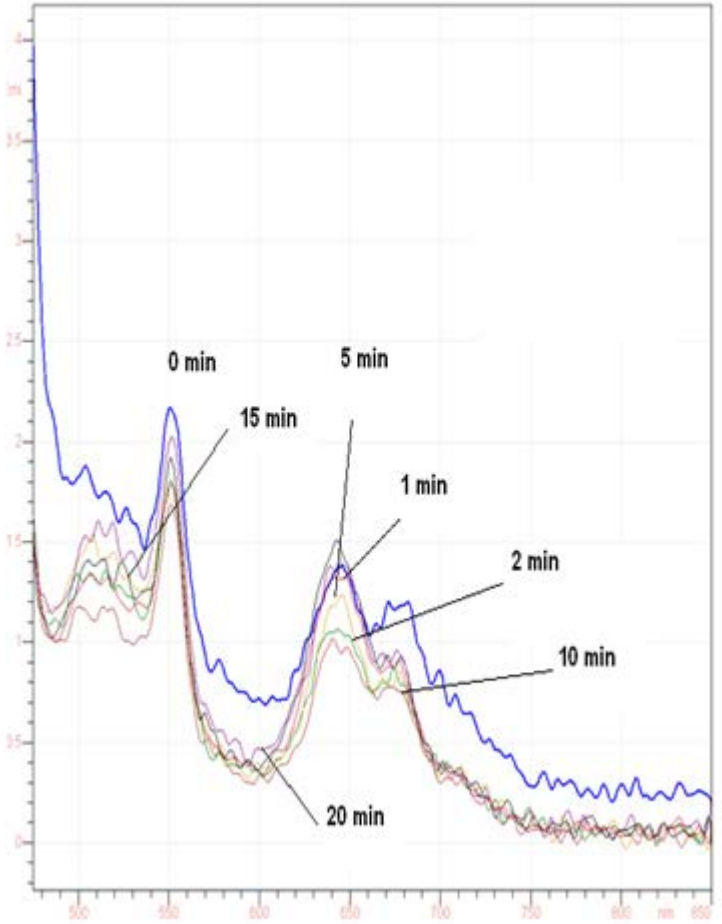


Fluorometer

Figure 27 Inactivation of 3.5 L *Microcystis aeruginosa* using DFR (circulating) at 60% power setting (UV-Vis spectrophotometer and Fluorometer)

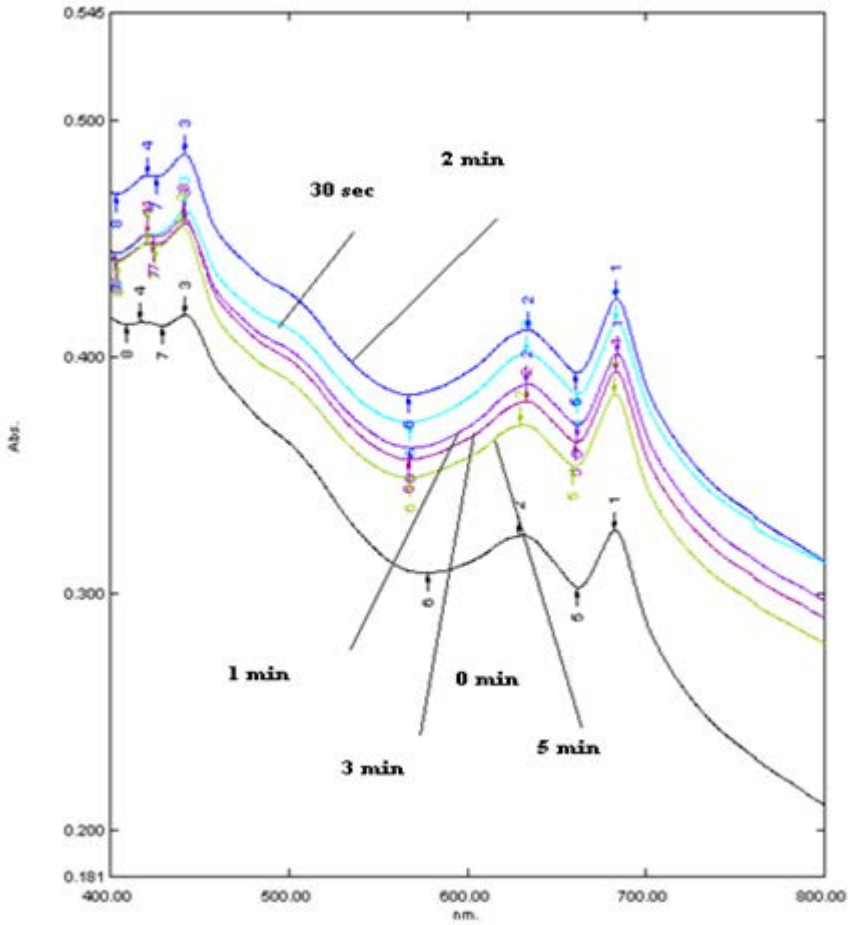


UV-Vis spectrophotometer

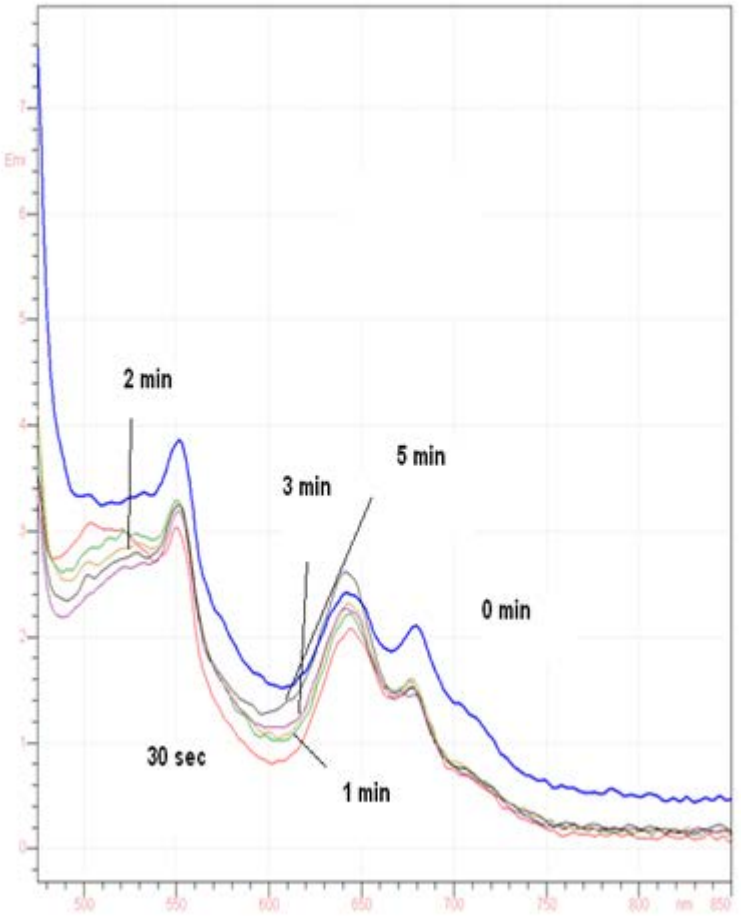


Fluorometer

Figure 28 Inactivation of 1.5 L *Microcystis aeruginosa* using vibrating tray (UV-Vis spectrophotometer and Fluorometer)

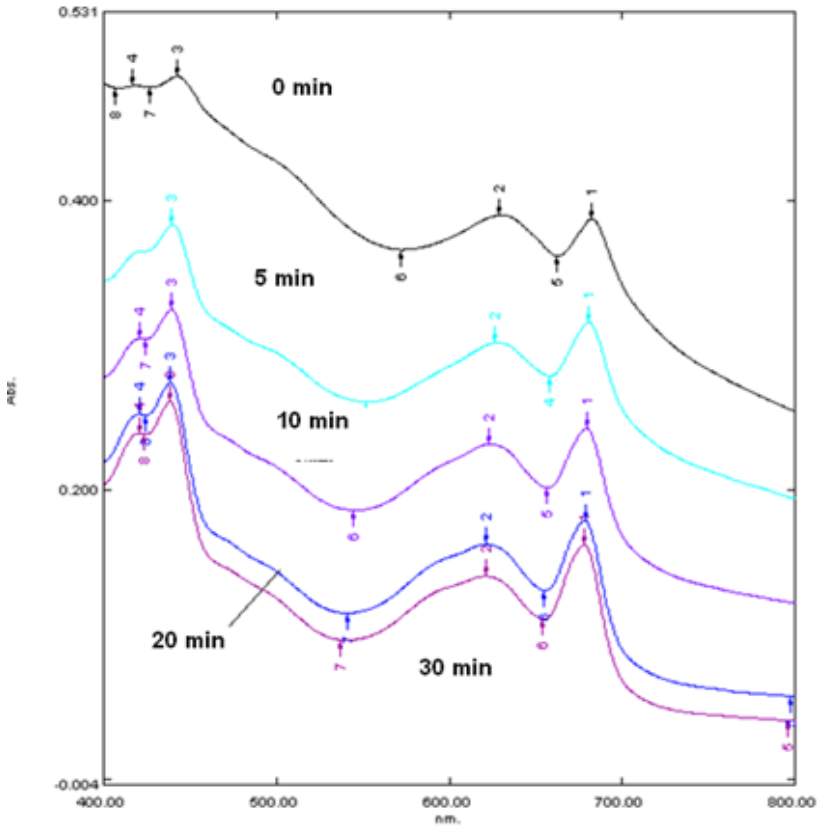


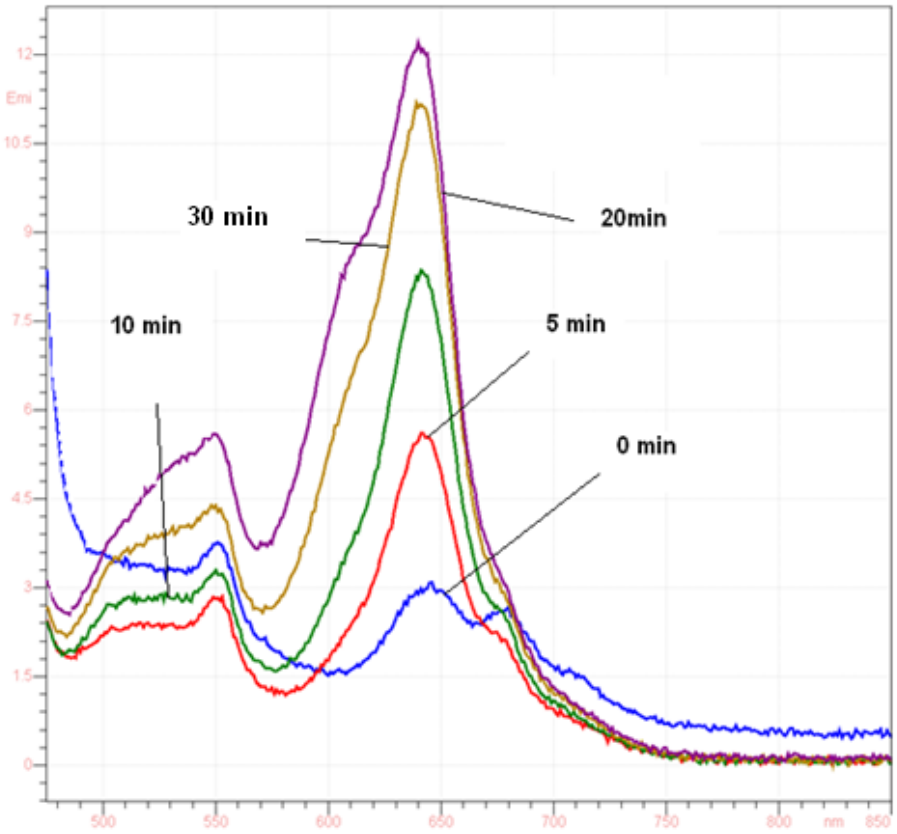
UV-Vis spectrophotometer



Fluorometer

Figure 29 Inactivation of 200 mL *Microcystis aeruginosa* using 20 kHz probe for flow cytometry (UV-Vis spectrophotometer and Fluorometer)

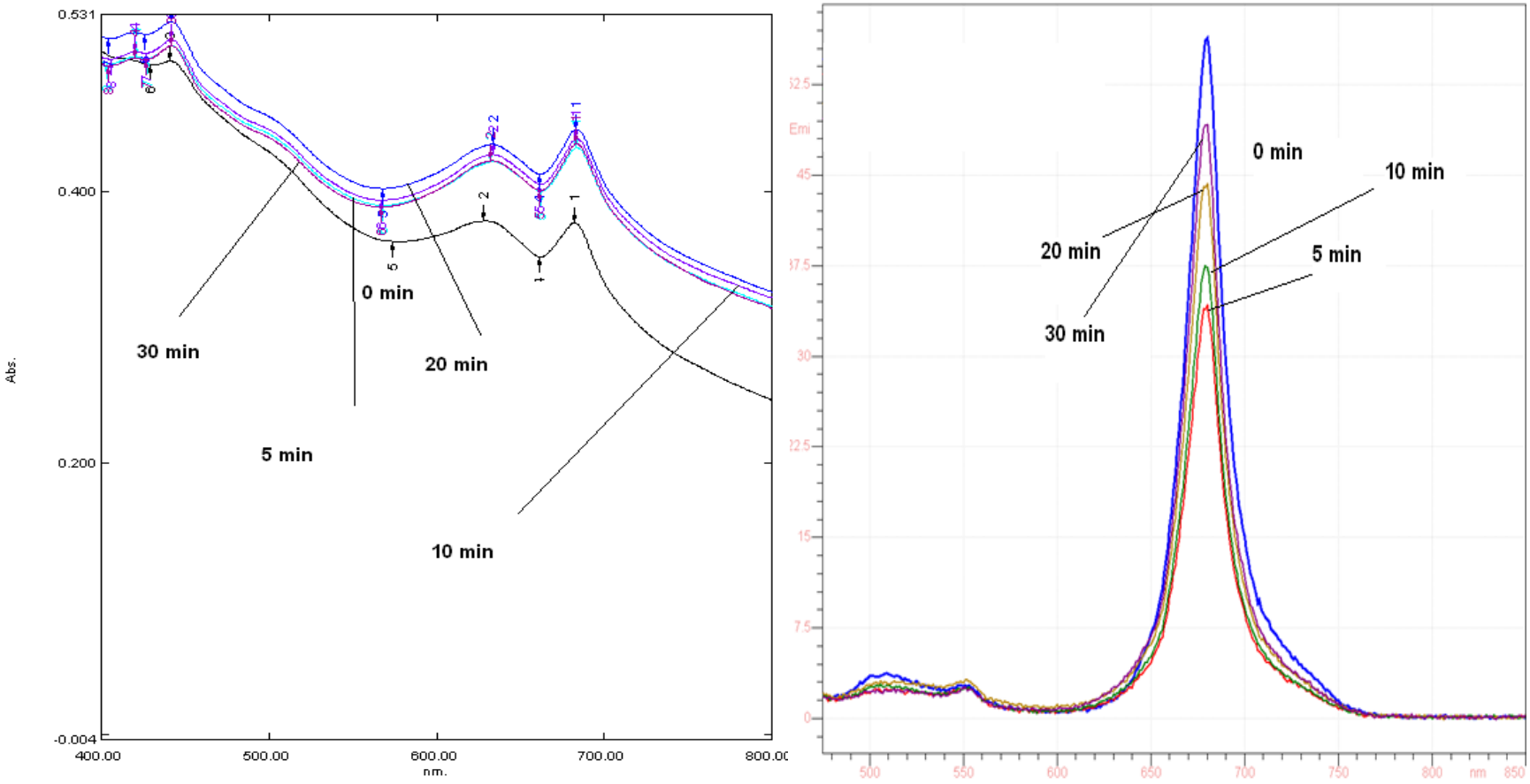




UV-Vis spectrophotometer

Fluorometer

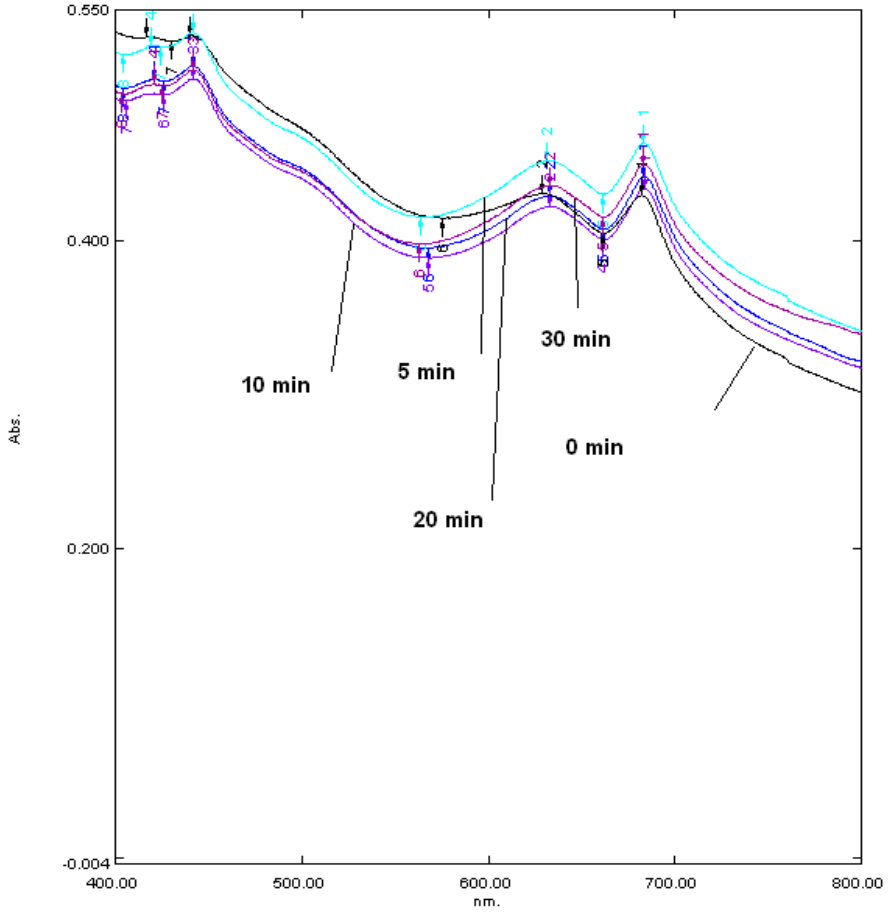
Figure 30 Inactivation of 200 mL *Microcystis aeruginosa* using 580 kHz (40% power setting) for flow cytometry (UV-Vis spectrophotometer and Fluorometer)



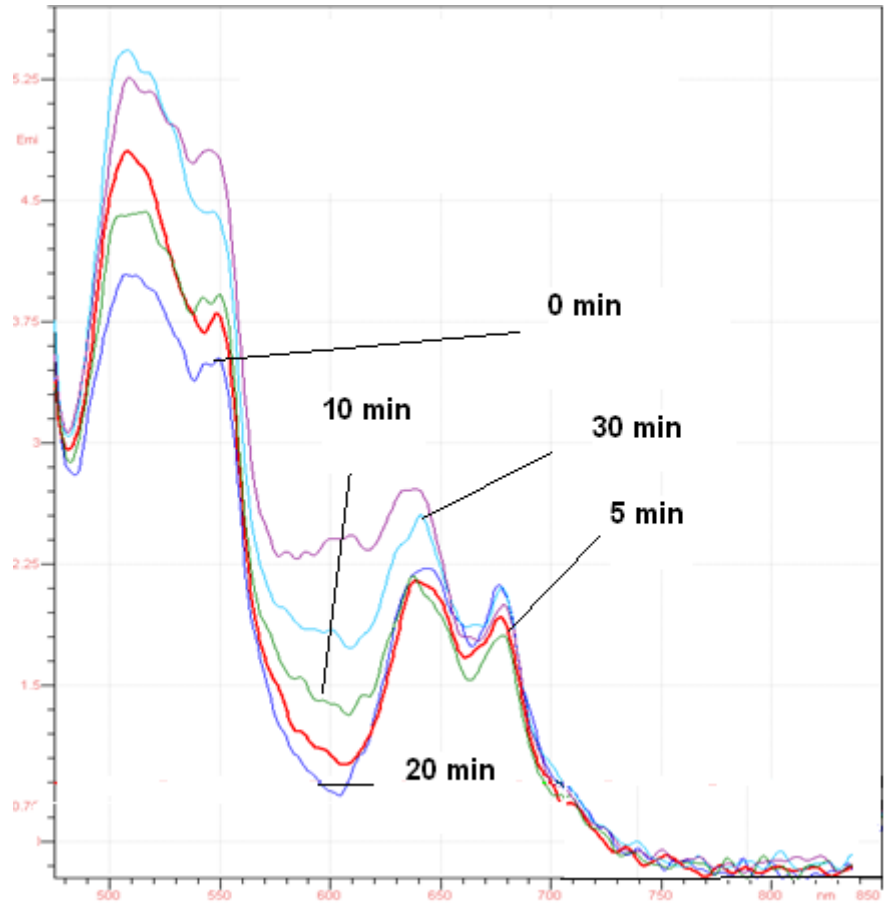
UV-Vis spectrophotometer

Fluorometer

Figure 31 Inactivation of 200 mL *Microcystis aeruginosa* using 1146 kHz (40% power setting) for flow cytometry (UV-Vis spectrophotometer and Fluorometer)

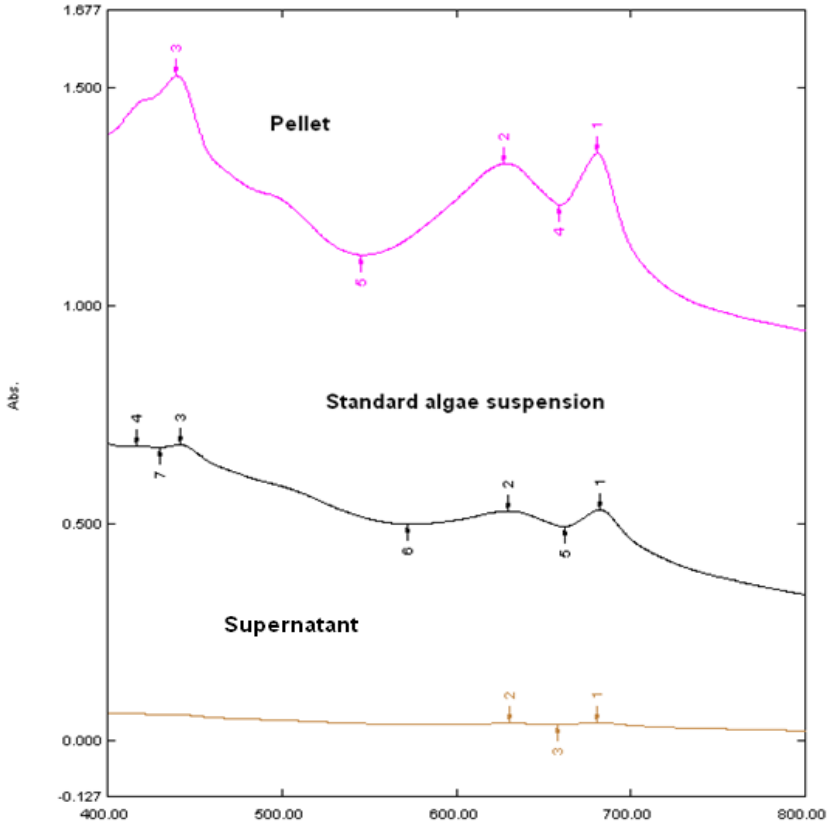


UV-Vis spectrophotometer

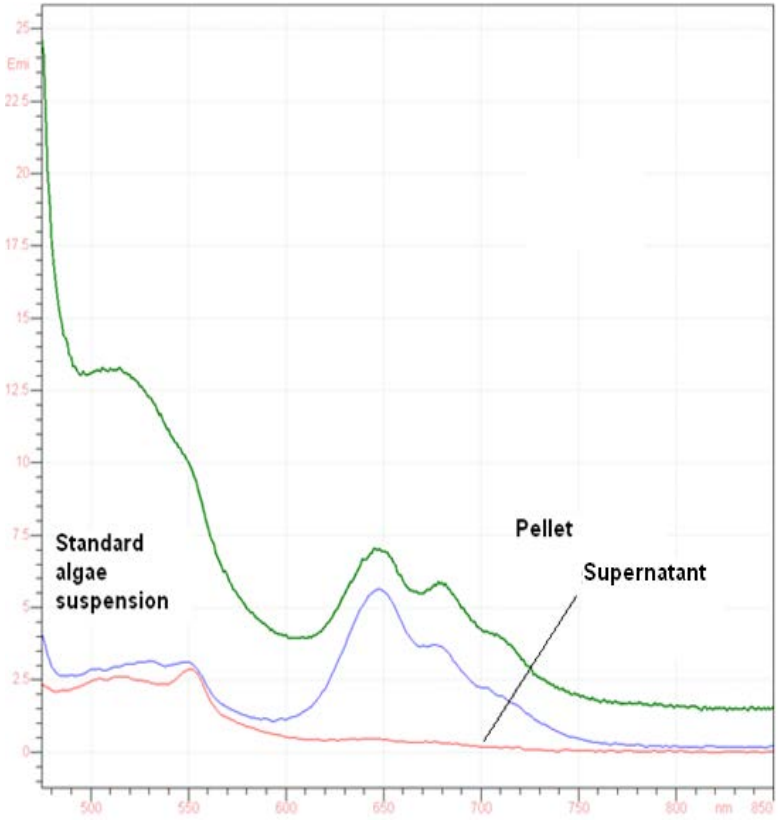


Fluorometer

Figure 32 Optical density test of algae pellet and supernatant (UV-Vis spectrophotometer and Fluorometer)

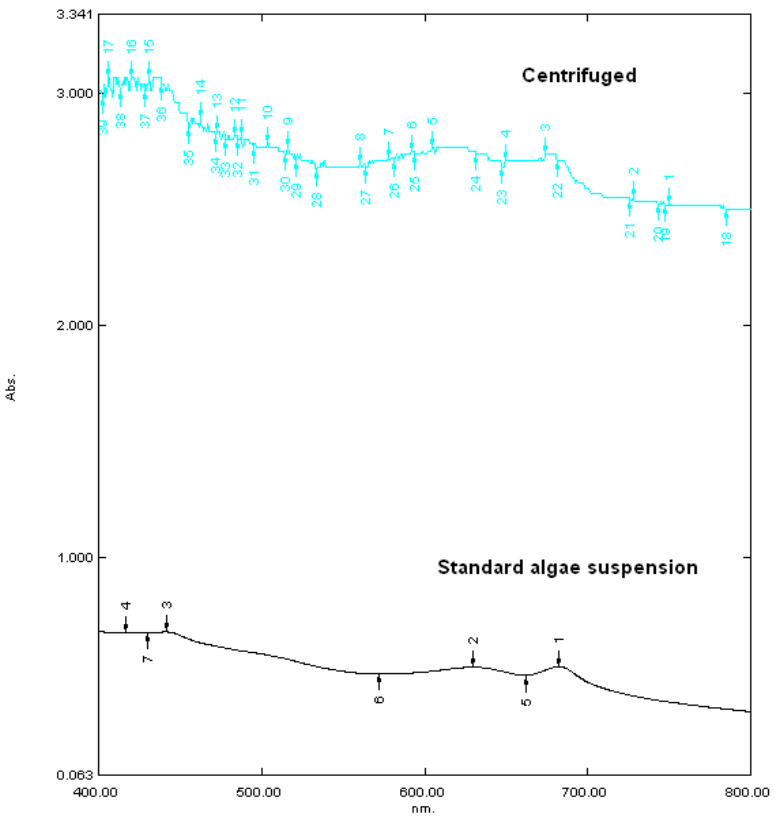


UV-Vis spectrophotometer

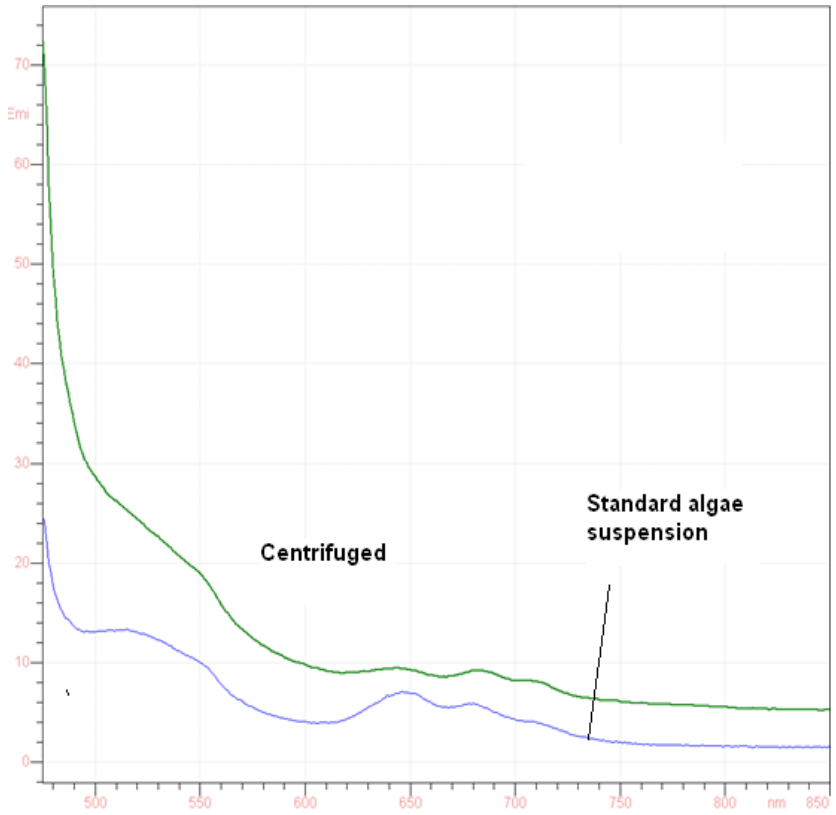


Fluorometer

Figure 33 Optical density baseline test (UV-Vis spectrophotometer and Fluorometer)

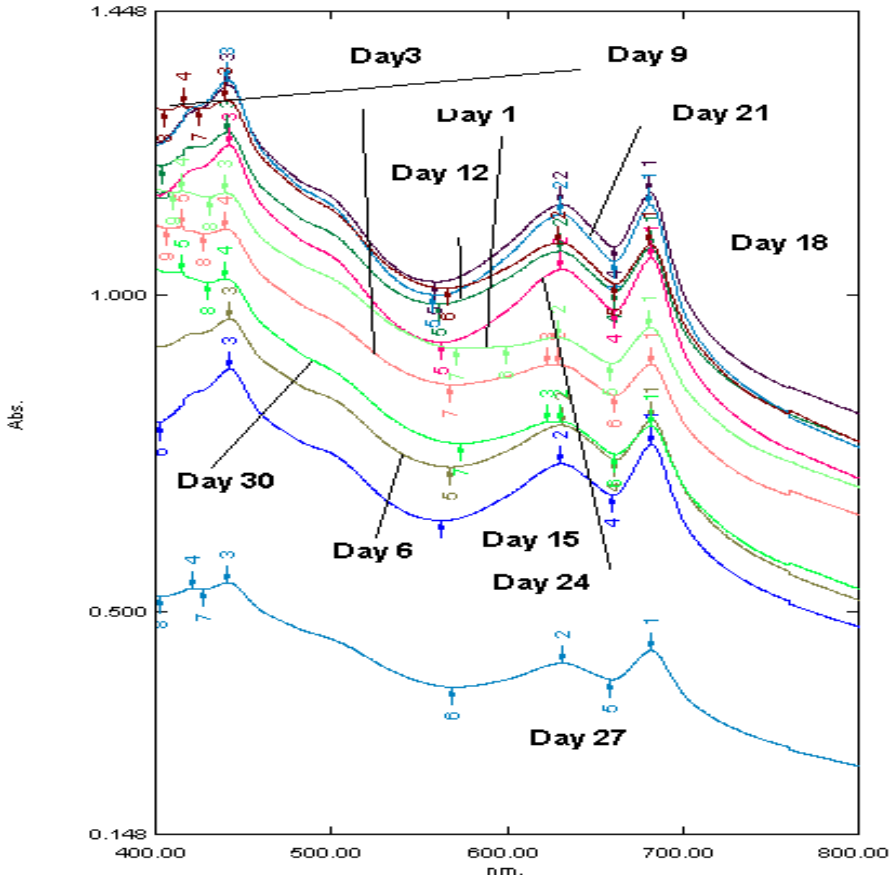


UV-Vis spectrophotometer

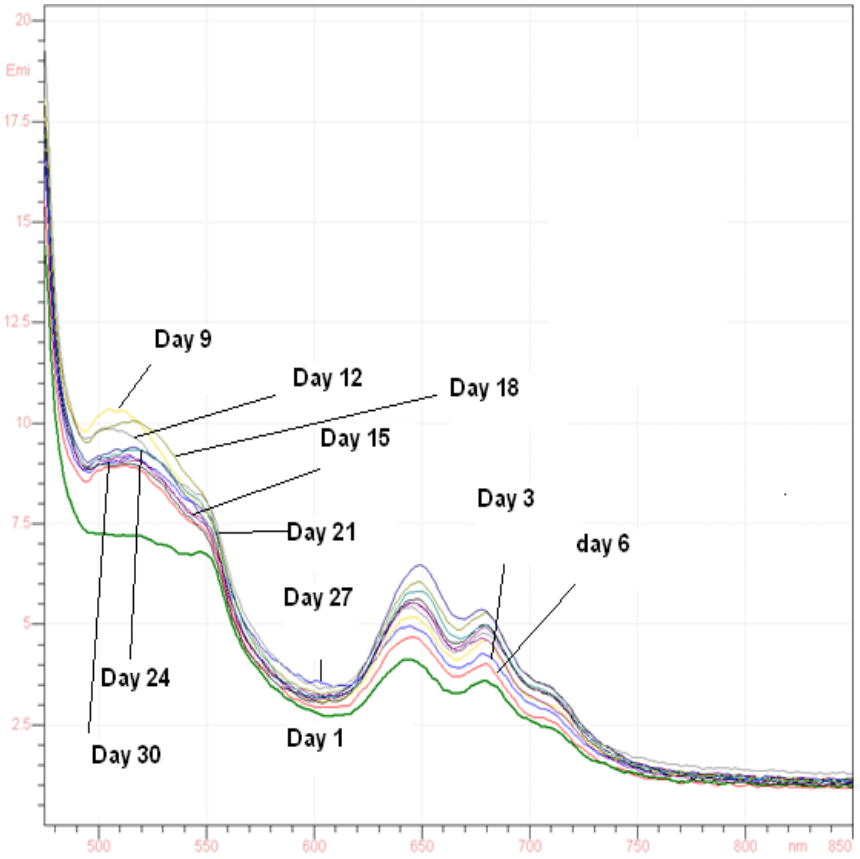


Fluorometer

Figure 34 Resistance test (Live, UV-Vis spectrophotometer and Fluorometer)

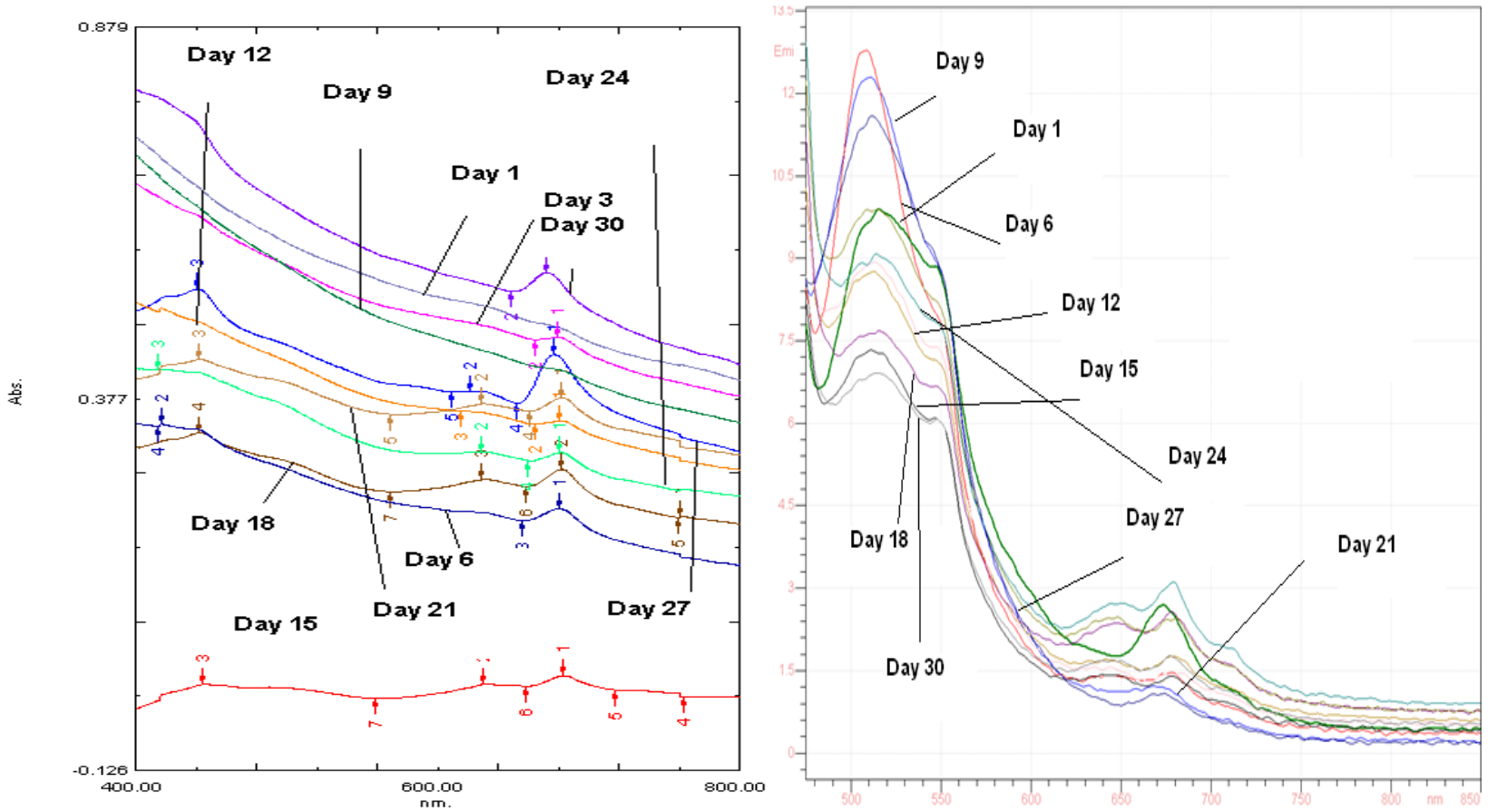


UV-Vis spectrophotometer



Fluorometer

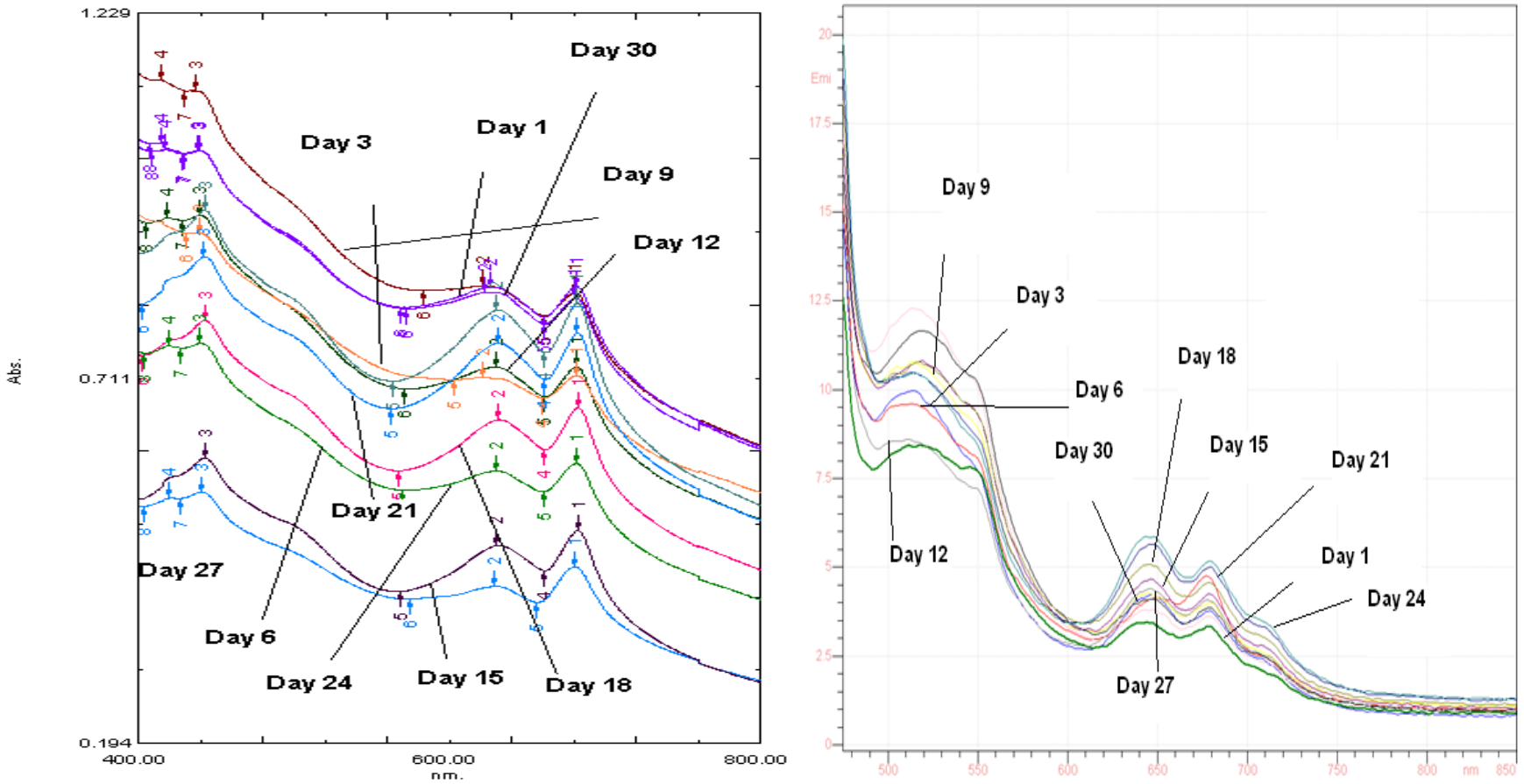
Figure 35 Resistance test (Dead, UV-Vis spectrophotometer and Fluorometer)



UV-Vis spectrophotometer

Fluorometer

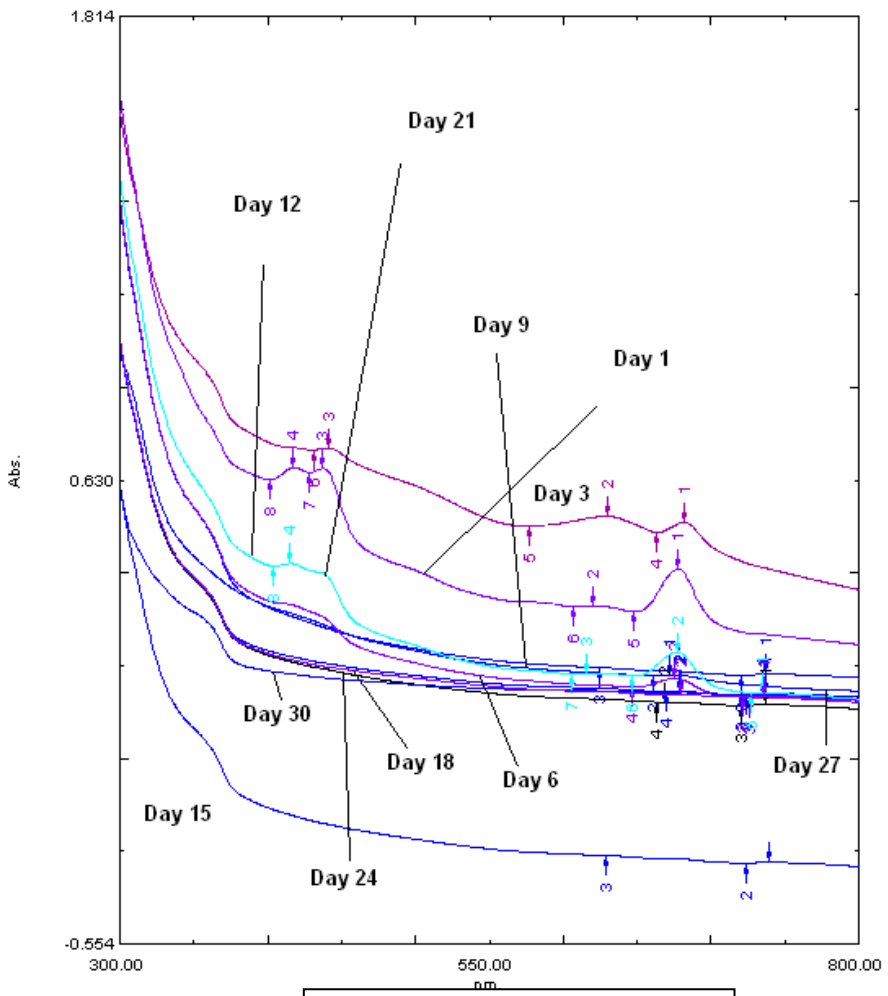
Figure 36 Resistance test (LIVE+DEAD, UV-Vis spectrophotometer and Fluorometer)



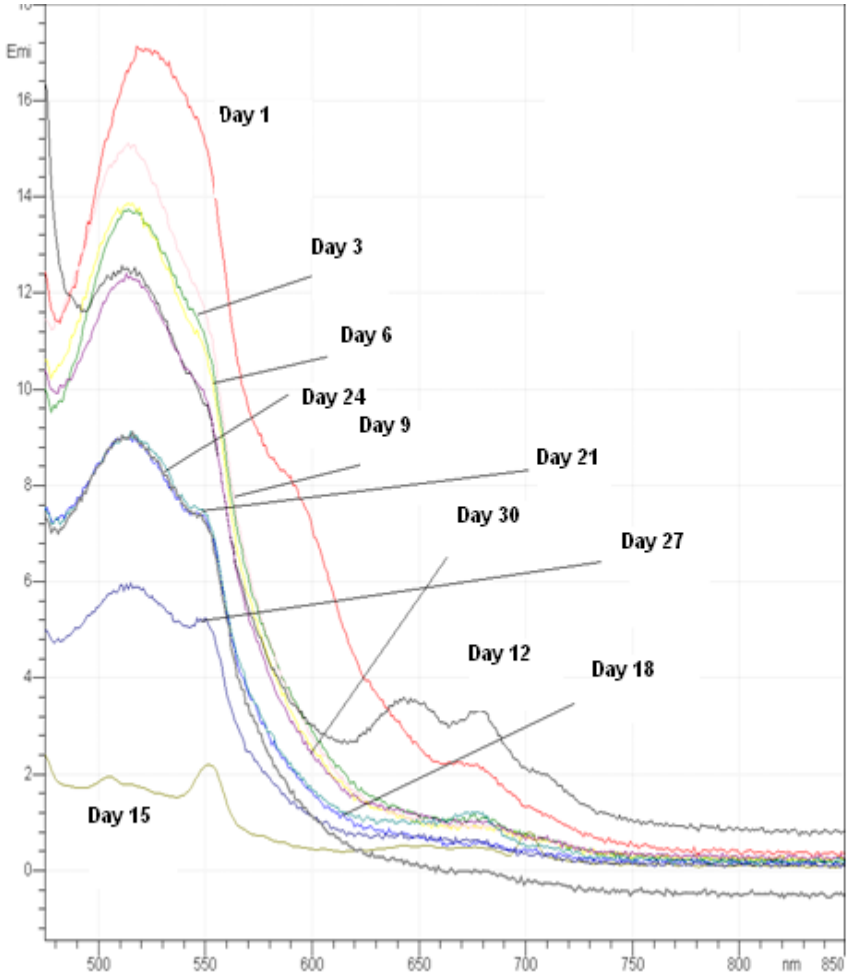
UV-Vis spectrophotometer

Fluorometer

Figure 37 Resistance test (Sonicated, UV-Vis spectrophotometer and Fluorometer)

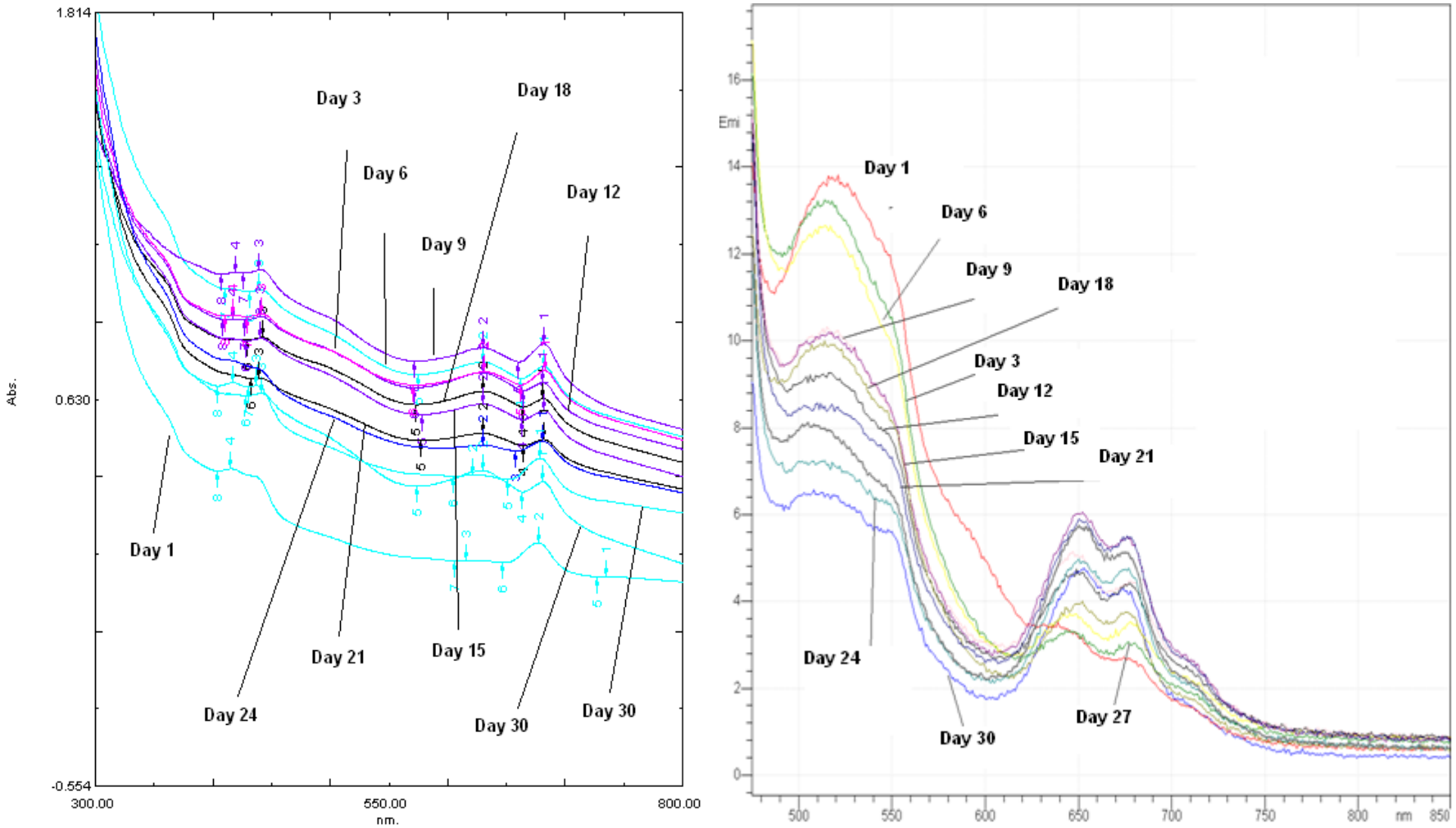


UV-Vis spectrophotometer



Fluorometer

Figure 38 Resistance test (Live+Sonicated, UV-Vis spectrophotometer and Fluorometer)



UV-Vis spectrophotometer

Fluorometer

Appendix 4 results using flow cytometry

Results 1 Inactivation of 3.5L *Microcystis aeruginosa* DFR (circulating) (Flow cytometer) at 40% power setting

Time (min)	Live1	Live2	Live3	LIVE (AV)
0	17.01	16.2	18.6	17.27
1	9.02	9.3	8.9	9.073333
2	24.11	25.12	23.96	24.39667
5	20.64	21.12	20.56	20.77333
10	34.85	35.6	34.7	35.05
15	25.08	26	24.68	25.25333
20	23.73	24.1	23.12	23.65
30	45.49	45.69	45.21	45.46333
60	40.92	40.32	39.73	40.32333
Time (min)	**Dead1**	**Dead2**	**Dead3**	**DEAD(AV)**
0	79.57	80	78.91	79.49333
1	88.76	89.54	72.36	83.55333
2	71.72	69.13	70.96	70.60333
5	75.77	76.45	74.62	75.61333
10	60.67	56.17	62.15	59.66333
15	39.53	41.36	38.39	39.76
20	71.05	72.06	73.62	72.24333
30	17.4	17.68	16.96	17.34667
60	51.97	54.23	56.33	54.17667
Time (min)	**Debris1**	**Debris2**	**Debris3**	**DEBRIS(AV)**
0	2.03	3.8	3.236667	3.022222
1	0.96	1.16	7.373333	3.164444
2	3.41	5.75	5	4.72
5	2.45	2.43	3.613333	2.831111
10	3.25	8.23	5.286667	5.588889
15	5.27	32.64	34.98667	24.29889
20	3.93	3.84	4.106667	3.958889
30	4.83	36.63	37.19	26.21667
60	4.84	5.45	5.5	5.263333

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Live	0	3	17.33	1.528	.882	13.54	21.13
	1	3	9.00	.000	.000	9.00	9.00
	2	3	24.33	.577	.333	22.90	25.77
	5	3	21.00	.000	.000	21.00	21.00
	10	3	35.33	.577	.333	33.90	36.77
	15	3	25.33	.577	.333	23.90	26.77
	20	3	23.67	.577	.333	22.23	25.10
	30	3	45.33	.577	.333	43.90	46.77
	60	3	40.33	.577	.333	38.90	41.77
	Total	27	26.85	11.062	2.129	22.48	31.23
Dead	0	3	79.67	.577	.333	78.23	81.10
	1	3	83.67	10.116	5.840	58.54	108.80
	2	3	70.67	1.528	.882	66.87	74.46
	5	3	75.67	.577	.333	74.23	77.10
	10	3	59.67	3.215	1.856	51.68	67.65
	15	3	39.67	1.528	.882	35.87	43.46
	20	3	72.33	1.528	.882	68.54	76.13
	30	3	17.33	.577	.333	15.90	18.77
	60	3	54.00	2.000	1.155	49.03	58.97
	Total	27	61.41	20.922	4.026	53.13	69.68
Debris	0	3	3.01	.921	.532	.72	5.30
	1	3	3.18	3.634	2.098	-5.85	12.21
	2	3	4.58	1.422	.821	1.05	8.11
	5	3	2.68	.835	.482	.61	4.76
	10	3	5.51	2.622	1.514	-1.01	12.02
	15	3	24.21	16.677	9.628	-17.22	65.64
	20	3	3.98	.134	.077	3.65	4.32
	30	3	26.27	18.425	10.638	-19.50	72.04
	60	3	5.32	.275	.159	4.63	6.00
	Total	27	8.75	11.453	2.204	4.22	13.28

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Live	Between Groups	3172.741	8	396.593	823.692	.000
	Within Groups	8.667	18	.481		
	Total	3181.407	26			
Dead	Between Groups	11131.185	8	1391.398	100.449	.000
	Within Groups	249.333	18	13.852		
	Total	11380.519	26			
Debris	Between Groups	2127.762	8	265.970	3.732	.010
	Within Groups	1282.699	18	71.261		
	Total	3410.461	26			

Results 2 Inactivation of 1L *Microcystis aeruginosa* DFR (static) (Flow cytometer) at 40% power setting (figure)

Time (min)	Live1	Live2	Live3	LIVE (AV)
0	57.86	56.36	59.41	57.87667
1	54.18	55.12	53.36	54.22
2	28.45	28.69	26.3	27.81333
3	24.02	24.3	23.96	24.09333
5	23.73	23.63	24.01	23.79
6	21.48	21.23	21.56	21.42333
7	21.25	21.13	21.45	21.27667
8	21.66	21.62	21.42	21.56667
9	21.48	21.22	21.31	21.33667
10	28.96	28.36	28.22	28.51333
Time (min)	**Dead1**	**Dead2**	**Dead3**	**DEAD(AV)**
0	42.14	43.64	40.59	42.12333
1	45.82	44.88	46.64	45.78
2	71.55	71.31	73.7	72.18667
3	75.98	75.7	76.04	75.90667
5	76.27	76.37	75.99	76.21
6	78.52	78.77	78.44	78.57667
7	78.75	78.87	78.55	78.72333
8	78.34	78.38	78.58	78.43333
9	78.52	78.78	78.69	78.66333
10	71.04	71.64	71.78	71.48667

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Live	0	3	57.88	1.525	.880	54.09	61.67
	1	3	54.22	.881	.508	52.03	56.41
	2	3	27.81	1.316	.760	24.54	31.08
	3	3	24.09	.181	.105	23.64	24.54
	5	3	23.79	.197	.114	23.30	24.28
	6	3	21.42	.172	.099	21.00	21.85
	7	3	21.28	.162	.093	20.88	21.68
	8	3	21.57	.129	.074	21.25	21.89
	9	3	21.34	.132	.076	21.01	21.66
	10	3	28.51	.393	.227	27.54	29.49
	Total	30	30.19	13.428	2.452	25.18	35.21
Dead	0	3	42.12	1.525	.880	38.33	45.91
	1	3	45.78	.881	.508	43.59	47.97
	2	3	72.19	1.316	.760	68.92	75.46
	3	3	75.91	.181	.105	75.46	76.36
	5	3	76.21	.197	.114	75.72	76.70
	6	3	78.58	.172	.099	78.15	79.00
	7	3	78.72	.162	.093	78.32	79.12
	8	3	78.43	.129	.074	78.11	78.75
	9	3	78.66	.132	.076	78.34	78.99
	10	3	71.49	.393	.227	70.51	72.46
	Total	30	69.81	13.428	2.452	64.79	74.82

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Live	Between Groups	5218.881	9	579.876	1126.089	.000
	Within Groups	10.299	20	.515		
	Total	5229.179	29			
Dead	Between Groups	5218.881	9	579.876	1126.089	.000
	Within Groups	10.299	20	.515		
	Total	5229.179	29			

Results 3 Inactivation of 3.5L *Microcystis aeruginosa* DFR (circulating) (Flow cytometer) at 60% power setting

Time	Live1	Live2	Live3	LIVE (AV)
0	20	19.3	21	20.1
1	16	17.2	14	15.73333
2	15.4	15.2	15	15.2
5	15.2	15.1	16.2	15.5
10	15.9	15.6	15.3	15.6
15	15	14.9	15.1	15
20	14.6	14.7	13.9	14.4
Time	**Dead1**	**Dead2**	**Dead3**	**DEAD(AV)**
0	80	80.7	79	79.9
1	84	82.8	86	84.26667
2	84.6	84.8	85	84.8
5	84.8	84.9	83.8	84.5
10	84.1	84.4	84.7	84.4
15	85	85.1	84.9	85
20	85.4	85.3	86.1	85.6

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Live	0	3	20.10	.854	.493	17.98	22.22
	1	3	15.73	1.617	.933	11.72	19.75
	2	3	15.20	.200	.115	14.70	15.70
	5	3	15.50	.608	.351	13.99	17.01
	10	3	15.60	.300	.173	14.85	16.35
	15	3	15.00	.100	.058	14.75	15.25
	20	3	14.40	.436	.252	13.32	15.48
	Total	21	15.93	1.904	.415	15.07	16.80
Dead	0	3	79.90	.854	.493	77.78	82.02
	1	3	84.27	1.617	.933	80.25	88.28
	2	3	84.80	.200	.115	84.30	85.30
	5	3	84.50	.608	.351	82.99	86.01
	10	3	84.40	.300	.173	83.65	85.15
	15	3	85.00	.100	.058	84.75	85.25
	20	3	85.60	.436	.252	84.52	86.68
	Total	21	84.07	1.904	.415	83.20	84.93

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Live	Between Groups	64.380	6	10.730	18.576	.000
	Within Groups	8.087	14	.578		
	Total	72.467	20			
Dead	Between Groups	64.380	6	10.730	18.576	.000
	Within Groups	8.087	14	.578		
	Total	72.467	20			

Results 4 Inactivation of 1L *Microcystis aeruginosa* DFR (static) (Flow cytometer) at 60% power setting

Time	Live1	Live2	Live3	LIVE (AV)
0	79.87	80.01	81.02	80.3
1	78.66	79.26	77.96	78.62667
2	58.04	59.23	57.23	58.16667
3	3.21	4.22	3.45	3.626667
5	2.24	2.61	2.17	2.34
10	1.1	1	0.9	1
Time	**Dead1**	**Dead2**	**Dead3**	**DEAD(AV)**
0	20.13	19.99	18.98	19.7
1	21.34	20.74	22.04	21.37333
2	41.96	40.77	42.77	41.83333
3	96.79	95.78	96.55	96.37333
5	97.76	97.39	97.83	97.66
10	98.9	99	99.1	99

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Live	0	3	80.30	.627	.362	78.74	81.86
	1	3	78.63	.651	.376	77.01	80.24
	2	3	58.17	1.006	.581	55.67	60.67
	3	3	3.63	.528	.305	2.32	4.94
	5	3	2.34	.236	.137	1.75	2.93
	10	3	1.00	.100	.058	.75	1.25
	Total	18	37.34	36.784	8.670	19.05	55.64
Dead	0	3	19.70	.627	.362	18.14	21.26
	1	3	21.37	.651	.376	19.76	22.99
	2	3	41.83	1.006	.581	39.33	44.33
	3	3	96.37	.528	.305	95.06	97.68
	5	3	97.66	.236	.137	97.07	98.25
	10	3	99.00	.100	.058	98.75	99.25
	Total	18	62.66	36.784	8.670	44.36	80.95

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Live	Between Groups	22998.255	5	4599.651	12698.033	.000
	Within Groups	4.347	12	.362		
	Total	23002.601	17			
Dead	Between Groups	22998.255	5	4599.651	12698.033	.000
	Within Groups	4.347	12	.362		
	Total	23002.601	17			

Results 5 Inactivation of 1.5L *Microcystis aeruginosa* using a vibrating tray (Flow cytometer)

Time	Live1	Live2	Live3	LIVE (AV)
0	47.92	48.21	49.22	48.45
0.5	42.96	43.63	42.12	42.90333
1	33.08	33.61	35.12	33.93667
2	24.65	26.36	25.12	25.37667
3	24.3	24.36	24.65	24.43667
5	24.61	24.23	24.17	24.33667
Time	**Dead1**	**Dead2**	**Dead3**	**DEAD(AV)**
0	51.89	51.79	50.78	51.48667
0.5	56.48	56.37	57.88	56.91
1	66.75	66.39	64.88	66.00667
2	75	73.64	74.88	74.50667
3	75.21	75.64	75.35	75.4
5	74.57	75.77	75.83	75.39

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
Live	0	3	48.45	.682	.394	46.75	50.15
	0	3	42.90	.757	.437	41.02	44.78
	1	3	33.94	1.059	.611	31.31	36.57
	2	3	25.38	.883	.510	23.18	27.57
	3	3	24.44	.187	.108	23.97	24.90
	5	3	24.34	.239	.138	23.74	24.93
	Total	18	33.24	9.814	2.313	28.36	38.12
Dead	0	3	51.49	.614	.355	49.96	53.01
	0	3	56.91	.842	.486	54.82	59.00
	1	3	66.01	.992	.573	63.54	68.47
	2	3	74.51	.753	.435	72.64	76.38
	3	3	75.40	.219	.127	74.86	75.94
	5	3	75.39	.711	.410	73.62	77.16
	Total	18	66.62	9.780	2.305	61.75	71.48

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Live	Between Groups	1631.428	5	326.286	645.904	.000
	Within Groups	6.062	12	.505		
	Total	1637.490	17			
Dead	Between Groups	1619.636	5	323.927	609.191	.000
	Within Groups	6.381	12	.532		
	Total	1626.017	17			

Results 6 Inactivation of 200mL *Microcystis aeruginosa* using 20 kHz probe for 30 minutes (flow cytometry)

Time [min]	UL	UL	UL	AV (%UL)	UR	UR	UR	AV (%UR)
0	46.56	45.32	47.12	46.3333	11.34	11.36	12.12	11.6067
5	2	2.1	2.2	2.1	45.77	46.21	44.63	45.5367
10	1.87	1.67	1.36	1.63333	25.59	25.25	25.31	25.3833
20	2.32	3.31	1.23	2.28667	13.33	13	12.99	13.1067
30	7.77	6.78	3.62	6.05667	1.09	1.21	1.03	1.11
Time [min]	**LL**	**LL**	**LL**	**AV (%LL)**	**LR**	**LR**	**LR**	**AV (%LL)**
0	41.93	42.12	40.36	41.47	17.33	1.2	0.4	6.31
5	30.25	30.26	29.12	29.8767	21.98	21.43	24.05	22.4867
10	43.07	42.33	42.01	42.47	29.47	30.75	31.32	30.5133
20	63.1	62.31	60.1	61.8367	21.24	21.38	25.68	22.7667
30	85.14	82.1	83.2	83.48	0.66	9.91	12.15	7.57333

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
UL	0	3	46.33	.921	.532	44.05	48.62
	5	3	2.10	.100	.058	1.85	2.35
	10	3	1.63	.257	.148	.99	2.27
	20	3	2.29	1.040	.601	-.30	4.87
	30	3	6.06	2.167	1.251	.67	11.44
	Total	15	11.68	18.035	4.657	1.69	21.67
UR	0	3	11.61	.445	.257	10.50	12.71
	5	3	45.54	.815	.471	43.51	47.56
	10	3	25.38	.181	.105	24.93	25.83
	20	3	13.11	.193	.112	12.63	13.59
	30	3	1.11	.092	.053	.88	1.34
	Total	15	19.35	15.728	4.061	10.64	28.06
LL	0	3	41.4700	.96597	.55770	39.0704	43.8696
	5	3	29.8767	.65531	.37834	28.2488	31.5046
	10	3	42.4700	.54369	.31390	41.1194	43.8206
	20	3	61.8367	1.55500	.89778	57.9738	65.6995
	30	3	83.4800	1.53922	.88867	79.6564	87.3036
	Total	15	51.8267	19.54687	5.04698	41.0020	62.6514
LR	0	3	6.3100	9.55198	5.51484	-17.4184	30.0384
	5	3	22.4867	1.38153	.79763	19.0547	25.9186
	10	3	30.5133	.94744	.54700	28.1598	32.8669
	20	3	22.7667	2.52399	1.45723	16.4967	29.0366
	30	3	7.5733	6.09098	3.51663	-7.5575	22.7042
	Total	15	17.9300	10.72174	2.76834	11.9925	23.8675

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
UL	Between Groups	4540.266	4	1135.066	846.429	.000
	Within Groups	13.410	10	1.341		
	Total	4553.676	14			
UR	Between Groups	3461.336	4	865.334	4595.832	.000
	Within Groups	1.883	10	.188		
	Total	3463.219	14			
LL	Between Groups	5336.232	4	1334.058	1034.897	.000
	Within Groups	12.891	10	1.289		
	Total	5349.122	14			
LR	Between Groups	1334.345	4	333.586	12.129	.001
	Within Groups	275.034	10	27.503		
	Total	1609.380	14			

Results 7 Inactivation of 200mL *Microcystis aeruginosa* using 580 kHz bath for 30 minutes (flow cytometry)

Time [min]	UL	UL	UL	AV (%UL)	UR	UR	UR	AV (%UR)
0	50.62	50.12	50.56	50.4333	0.31	0.36	0.27	0.31333
5	1.6	1.6	1.4	1.53333	32	32	34.16	32.72
10	1.22	1	1.2	1.14	35.01	37	36.1	36.0367
20	0.37	0.31	0.32	0.33333	25.23	26.3	25.78	25.77
30	0.41	0.21	0.39	0.33667	23.94	24.13	21.36	23.1433
Time [min]	**LL**	**LL**	**LL**	**AV (%LL)**	**LR**	**LR**	**LR**	**AV (%LL)**
0	49.05	47.61	40.11	45.59	0.01	1.91	9.06	3.66
5	40.07	40.12	41	40.3967	26.33	26.28	23.44	25.35
10	45.26	44.13	45.21	44.8667	29.47	17.87	17.49	21.61
20	51.89	50.36	50.36	50.87	22.51	23.03	23.54	23.0267
30	53.75	55.33	53.26	54.1133	21.89	20.33	24.99	22.4033

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
UL	0	3	50.43	.273	.158	49.76	51.11
	5	3	1.53	.115	.067	1.25	1.82
	10	3	1.14	.122	.070	.84	1.44
	20	3	.33	.032	.019	.25	.41
	30	3	.34	.110	.064	.06	.61
	Total	15	10.76	20.541	5.304	-.62	22.13
UR	0	3	.31	.045	.026	.20	.43
	5	3	32.72	1.247	.720	29.62	35.82
	10	3	36.04	.997	.575	33.56	38.51
	20	3	25.77	.535	.309	24.44	27.10
	30	3	23.14	1.547	.893	19.30	26.99
	Total	15	23.60	12.999	3.356	16.40	30.80
LL	0	3	45.5900	4.80012	2.77135	33.6658	57.5142
	5	3	40.3967	.52310	.30201	39.0972	41.6961
	10	3	44.8667	.63846	.36862	43.2806	46.4527
	20	3	50.8700	.88335	.51000	48.6756	53.0644
	30	3	54.1133	1.08177	.62456	51.4261	56.8006
	Total	15	47.1673	5.33241	1.37682	44.2143	50.1203
LR	0	3	3.6600	4.77205	2.75515	-8.1944	15.5144
	5	3	25.3500	1.65430	.95511	21.2405	29.4595
	10	3	21.6100	6.80961	3.93153	4.6940	38.5260
	20	3	23.0267	.51501	.29734	21.7473	24.3060
	30	3	22.4033	2.37203	1.36949	16.5109	28.2958
	Total	15	19.2100	8.80593	2.27368	14.3334	24.0866

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
UL	Between Groups	5907.031	4	1476.758	63744.938	.000
	Within Groups	.232	10	.023		
	Total	5907.263	14			
UR	Between Groups	2355.094	4	588.773	562.795	.000
	Within Groups	10.462	10	1.046		
	Total	2365.556	14			
LL	Between Groups	346.739	4	86.685	16.882	.000
	Within Groups	51.346	10	5.135		
	Total	398.085	14			
LR	Between Groups	930.079	4	232.520	14.949	.000
	Within Groups	155.544	10	15.554		
	Total	1085.623	14			

Results 8 Inactivation of 200mL *Microcystis aeruginosa* using 1146 kHz bath for 30 minutes (flow cytometry)

Time [min]	UL	UL	UL	AV (%UL)	UR	UR	UR	AV (%UR)
0	66.26	65.12	66.23	65.87	4.61	4.23	4.78	4.54
5	15.1	16.1	14.95	15.3833	42.4	42.6	42.1	42.3667
10	8.54	6.78	9.21	8.17667	43.44	43.56	43.25	43.4167
20	7.21	7.22	6.99	7.14	47.19	47.23	47.56	47.3267
30	10	9.66	11.2	10.2867	49.35	49.5	49.16	49.3367
Time [min]	**LL**	**LL**	**LL**	**AV (%LL)**	**LR**	**LR**	**LR**	**AV (%LL)**
0	24.56	24.55	24.63	24.58	4.57	6.1	4.36	5.01
5	37.8	37.56	37.12	37.4933	4.7	3.74	5.83	4.75667
10	57.64	49	46.23	50.9567	4.75	0.66	1.31	2.24
20	38.89	38.96	38.26	38.7033	6.71	6.59	7.19	6.83
30	37.47	37.21	37.56	37.4133	3.18	3.63	2.08	2.96333

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
						Lower Bound	Upper Bound
UL	0	3	65.87	.650	.375	64.26	67.48
	5	3	15.38	.625	.361	13.83	16.94
	10	3	8.18	1.255	.725	5.06	11.29
	20	3	7.14	.130	.075	6.82	7.46
	30	3	10.29	.809	.467	8.28	12.30
	Total	15	21.37	23.226	5.997	8.51	34.23
UR	0	3	4.54	.282	.163	3.84	5.24
	5	3	42.37	.252	.145	41.74	42.99
	10	3	43.42	.156	.090	43.03	43.80
	20	3	47.33	.203	.117	46.82	47.83
	30	3	49.34	.170	.098	48.91	49.76
	Total	15	37.40	17.208	4.443	27.87	46.93
LL	0	3	24.5800	.04359	.02517	24.4717	24.6883
	5	3	37.4933	.34487	.19911	36.6366	38.3500
	10	3	50.9567	5.95134	3.43601	36.1727	65.7406
	20	3	38.7033	.38553	.22259	37.7456	39.6610
	30	3	37.4133	.18175	.10493	36.9618	37.8648
	Total	15	37.8293	8.93711	2.30755	32.8801	42.7785
LR	0	3	5.0100	.94979	.54836	2.6506	7.3694
	5	3	4.7567	1.04615	.60400	2.1579	7.3555
	10	3	2.2400	2.19789	1.26895	-3.2198	7.6998
	20	3	6.8300	.31749	.18330	6.0413	7.6187
	30	3	2.9633	.79739	.46037	.9825	4.9442
	Total	15	4.3600	1.97313	.50946	3.2673	5.4527

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
UL	Between Groups	7546.462	4	1886.616	3083.108	.000
	Within Groups	6.119	10	.612		
	Total	7552.581	14			
UR	Between Groups	4145.011	4	1036.253	21831.168	.000
	Within Groups	.475	10	.047		
	Total	4145.486	14			
LL	Between Groups	1046.765	4	261.691	36.630	.000
	Within Groups	71.442	10	7.144		
	Total	1118.206	14			
LR	Between Groups	39.377	4	9.844	6.507	.008
	Within Groups	15.128	10	1.513		
	Total	54.505	14			