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Low Energy Disruption of Lipid Containing Biomass

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LOW ENERGY DISRUPTION of LIPID CONTAINING BIOMASS

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By

Joaquin Rodriguez

2012

For my mother.

LOW ENERGY DISRUPTION of LIPID CONTAINING BIOMASS

by

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THESIS

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Abstract

Lipid containing biomass from microalgae has received recent research interest as a renewable and sustainable feedstock for biofuel production. Algae is known to offer as much as 30 times the energy density than corn based ethanol, the current world standard for biomass based liquid transportation fuels. The development of large scale systems and methods of extracting lipids from microalgae is still in the early stages of development and significant increases in efficiency and reduction in costs are necessary to substantiate algae as a feasible alternative source of fuel. Traditional conversion of algae feedstock into a refineable feedstock for biofuel can be divided into three primary steps: growth and cultivation, biomass concentration, and lipid extraction and separation. This study focuses on the extraction and separation stage where cells must be lysed and lipids separated from other cell matter before further conversion can take place. Current methods of lipid extraction include mechanical methods, solvent methods, and sonication methods. The focus of this work is the development of a low energy method of disrupting lipid containing biomass to facilitate the extraction of intracellular matter from microalgae biomass in its *wet* form, before any concentration or other processing has occurred. Cavitation offers a potential mechanism to be an effective means of releasing intracellular matter; however traditional ultrasound cavitation reaction systems suffer from the scale up problems. A novel reaction system was developed consisting of a flat plate reactor and a Terfenol-D transducer operating within the audible range. The ability of the reaction system to produce cavitation condition was verified through iodine dosimetry and the Weissler reaction. Sonication of 200 ml samples of KI solution was performed at various frequencies and power levels, samples were examined spectrophotometrically at 355nm. Further, samples of algae were irradiated under similar conditions. The samples are examined visually under magnification to validate the efficacy of the system in disrupting the cell wall and releasing intracellular matter. A summary of the efficiency and efficacy of the reaction system is reported as well as future directives for continued research in low energy methods of lipid extraction from microalgae biomass.

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Chapter 1

Introduction

The use and consumption, worldwide, of petroleum based transportation fuels is widely regarded as unsustainable. Consequently, research in renewable and sustainable alternatives has become a priority throughout the scientific community. Of particular interest are the lipids contained within the many species of microalgae. The extraction and thermochemical conversion of these lipids into direct replacement liquid transportation fuels have been designated as a research priority within the U.S. in an effort to curb the nation's growing dependence on foreign energy sources, reduce greenhouse gas emissions and achieve a more stable global climate (*Ferrell et al. 2010*). Methods to carry out these extractions and conversions have shown promise in establishing algae based biofuels as a viable alternative, however, of all the potential solutions no large scale, commercially viable process has come to light. The focus of the research presented herein is the development of a low energy, high volume process to facilitate the extraction of lipids from microalgae.

Energy sources offered in liquid form offer the highest available energy densities of commonly available fuels. To understand the scale at which any viable alternative must be producible it is essential to understand how the nation utilizes energy sources. The first section presented in this document offers an overview of domestic energy sources and use by sector. The overview serves as justification for the research and development of alternatives. A case is then made for algae as a preferred option when compared to other biomass derived fuels. The case is made on the basis of required land mass, theoretical and potential energy yields, and resource consumption and competition in terms of inputs.

Section two begins a technical review of the current state of lipid extraction from algae. Starting in the pond or photobioreactor the required inputs to common algae cultivation systems are outlined. Some extraction methods require preprocessing of the algae stream before any mechanical or solvent based extractions can occur. Current methods of separating lipids from microalgae can be divided into three basic groups, mechanical extraction, solvent extraction and sonication extraction.

The third section of this manuscript focuses on research taken place at the Materials Research and Technology Institute focused on developing a novel method for facilitating the extraction of lipids from microalgal biomass in an efficient, highly scalable process utilizing cavitation reaction engineering. The use of cavitation to facilitate chemical reactions or as a useable form of mechanical energy is a relatively new field. Through the use of a giant magnetostrictive transducer cavitation was incited through the generation of a strong, audible range acoustic field.

When a liquid is subjected to an acoustic field of sufficient amplitude and frequency a pressure wave is induced that is capable of exceeding the shear strength of the liquid resulting in the rapid formation and subsequent collapse of a microbubble. This bubble formation and collapse is the phenomenon known as cavitation. Cavitation creates a unique environment in which high temperature and high pressure reactions can take place in a relatively cool liquid. The cavitation environment also has the potential to bring about substantial physical changes within the reaction system, both to the reaction equipment itself and to any suspended solids within the working liquid of the system.

This thesis explores the possible use of cavitation reactions to disrupt lipid containing biomass in a manner which facilitates the extraction of lipids and other intracellular matter. In doing so it also explores the ability to incite cavitation within an audible range acoustic field.

1.1. U.S. energy overview

The consumption and production of petroleum based fuels are a driving factor for the research presented in this document. Petroleum markets are characterized by volatility and unpredictability; coupled with the nations long standing dependence on petroleum as a major energy source, petroleum plays a major role in the economics of energy markets.

When compared to natural gas, coal and other sources, historically, petroleum has remained the major source of energy. Since 1950 petroleum has consistently accounted for approximately 40% of total energy consumption in the US. Further, the transportation sector is almost entirely dependent on petroleum, consuming 70% of total petroleum use in 2009, up from 52% in 1950 (*ELA 2011*). This rise is indicative of an increasing dependence on liquid transportation fuels. More importantly, this steady increase in consumption of petroleum is accompanied by a steady decrease in production. Production levels in 2010 are approximately equal to levels in 1950 of almost 5.5 million barrels per day, levels have steadily decreased since a peak of 9.6 million barrels per day in 1970 (*ELA 2011*).

Algae derived fuels could potentially serve as a supplement or replacement for diesel and aviation fuels, sectors which have also seen marked increases in consumption in the past 50 years. By the year 2000 the aviation sector consumed jet fuels at a rate of 99% as opposed to being nearly entirely powered by gasoline in 1950. Since 1965 aviation fuel has accounted for 12% of petroleum transportation fuels. In recent years diesel fuel has accounted for 20% of petroleum consumed for transportation, up from only 11% in 1975 (*ELA 2011*).

The volatility of pricing of petroleum follows suit with most commodities, however this volatility is incredibly sensitive to a number of influencing social, political and economic factors. As seen most recently the increase in price of crude oil beginning in 2004 showed that prices could be

greatly affected without any interruption in supply. Pressure from increased demand from China, the U.S., and Europe drove prices upward, adding to uncertainty of the ability of oil producing nations to meet the increased demand. Social and political unrest in the Middle East combined with the threat of natural disasters drove prices further upward. By 2008 crude oil prices had far exceeded the cost of production (*Bebrens et al. 2011*). As prices rose seemingly uncontrollably it became clear that consumer demand for gasoline held constant and was not significantly affected by increase in fuel prices. The financial crisis of 2008 signaled the onset of decreasing fuel demand and prices finally began to stabilize. Economic recovery and further unrest in the Middle East, namely conflict in Libya, have caused price to surge again since 2010. Figure 1.1 shows crude oil prices from 2000 to 2011.

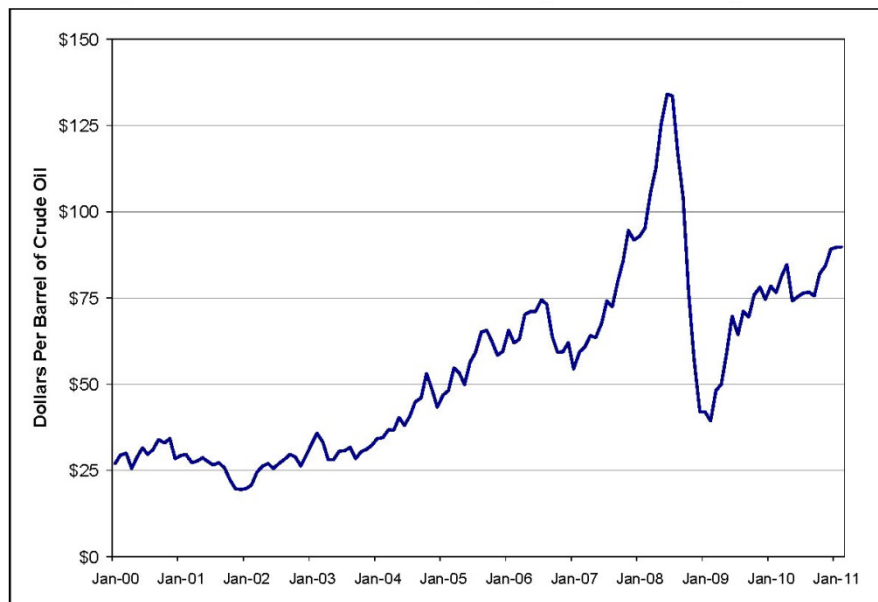


Figure 1.1 Futures price of crude oil from January 2000 to January 2011 from Congressional Research Services, 2011.

As demand for petroleum based fuels rises and domestic production declines or at least fails to increase proportionately it is clear that a sustainable domestic source of liquid transportation fuel

would serve to stabilize domestic costs by creating a market less vulnerable to so many outside influences.

Efforts to develop renewable fuels in the US have focused on ethanol derived from biomass. These first generation biomass derived fuels set the framework for renewable and sustainable petroleum alternatives.

1.2. A brief history of biomass as fuel

Production of biomass derived fuels began in 1981 with the introduction of the nation's primary biofuel, bio ethanol, to the US liquid transportation fuel market. Since then the industry has grown to produce 13.5 billion gallons annually for domestic consumption. Liquid fuels derived from biomass in the US primarily fall under one of two categories, bioethanol or biodiesel; the majority of which are bioethanol which comprise 98% of the nation's current production of biofuels (*ELA 2011*).

Bioethanol has played a primary role in the nation's development of biomass derived fuels. Bioethanol is essentially an ethyl alcohol produced through a process of fermentation, distillation and dehydration of starch based feedstock, whereas biodiesel production consists of oils, fats or greases converted to diesel fuel through a process known as transesterification. The processes and methods by which these fuels are developed is not the focus of this thesis therefore the details of them are not outlined here. Of more importance to this work are the limitations of the various feedstocks available for conversion.

Production of biofuel has increased dramatically since the phasing out of *methyl tertiary butyl ether* began in 2005. Ethanol has been designated and mandated as a replacement oxygenate for consumer fuel. Present levels of production are triple what they were in 2005; this sharp upturn in

production has led to increasing concerns about the realities of large scale biofuel production. The United Nations Food and Agriculture Organization has substantiated claims that low energy conversion efficiency of biofuel has had detrimental effects on food security by promoting large-scale land conversion (FAO 2008). Studies have also shown that fuel crops that double as food crops can result in increased prices of food products derived from these crops when competition for arable land exists (Koh & Ghazoul 2008). Worldwide the concerns worsen, widespread deforestation is occurring in an effort to create more available land for biofuel crops. The destruction of forest land results in devastating and irreversible damage to local ecosystems including a decrease in biodiversity. Authors have also questioned claims that biofuel production has a net positive effect on greenhouse gas emission (Fargione et al. 2008; Smeets et al. 2009)

1.3. The algae argument

As biofuel production increases, concerns over the true nature of its sustainability will likely follow suit. Research has suggested that biofuels derived from microalgae have potential to address these concerns and serve as a suitable feedstock for biofuel production (Patil et al. 2008; Chisti 2007; Hu et al. 2008; Dismukes et al. 2008). Microalgae are very efficient producers of biomass, they divide rapidly and grow year-round. In addition algae are very simple organisms compared to land-based crops which consume energy through the growth of supporting plant structures, microalgae have no leaves, roots or stems; structures which offer little usable biomass for biofuel production. Certain species of algae which have been designated as potential biofuel feedstocks have very high oil content up to 50% of dry weight under normal growing conditions. When combined with the fact that oil energy density is approximately double the energy density of carbohydrates (Spolaore et al. 2006) the potential energy content of microalgae as a biofuel feedstock is much more attractive than conventional terrestrial biofuel crops.

Microalgae also have advantages from a research standpoint; microbial organisms are well-suited to cultivation and experimentation within a controlled laboratory environment. Their rapid multiplication allows algae colonies to adapt rapidly to changing biological conditions. This establishes microalgae as a good candidate for bioengineering. Further, as microbial organism, microalgae do not require energy intensive preprocessing and pretreatment that is characteristic of land-based crops.

Select species of microalgae also have the possibility of being cultivated in wastewater streams or even brackish water. Terrestrial crops more often than not require the use of large amounts of fresh water for growing, some of which is lost to evaporation. If microalgae are cultivated in a closed system water losses are minimized, as are nutrient losses characteristic of irrigation cultivation of land-based fuel crops and agricultural runoff. These factors address many of the concerns of ecosystem damage from large-scale production of biofuels from land-based crops.

Traditional biofuel sources from staple food crops such as corn, soybeans, and sugarcane create competition between growing these staples for food and growing them for biofuel production. This competition leads to immense pressure on land suitable for agriculture; the necessary growing environment for microalgae does not rely on arable land that could otherwise be used for raising food crops. In terms of land usage microalgae not only offer the ability to be cultivated on non-arable land but in terms of energy yield per unit land even modest estimates show that microalgae has significant advantages over traditional biofuel crops. As much as 13 times higher than corn ethanol, 40 times higher than soybean based biodiesel and 3 times as much as palm oil; with annual yields reported as high as 60 tons dry weight and 20 tons oil per hectare (*Mohemani & Borowitzka, 2006*).

Large scale production of algae-based biofuels is relatively untested and unreported. Although large-scale cultivation of algae is not necessarily a new industry, achieving profitability in the biofuels market has proven to be a significant challenge. The development of highly efficient and cost effective methods of taking algae from the pond pump is necessary before algae can truly be considered a suitable option for large-scale biofuel production. But most important is the establishment of biofuel production from microalgae as a net energy producer. Until conclusive results show a production process that confirms the net energy balance ratio (the amount of energy produced divided by the amount of energy spent in production) of algae-based biofuel production is at least greater than one, it is necessary to pursue advancements in production technologies. The purpose of this thesis is the development of a novel and efficient means of disrupting lipid containing microalgae biomass in an effort to facilitate the extraction of lipids. And in doing so, increase the energy efficiency of obtaining a biological crude oil replacement from microalgae. Steps taken to refine algal oil into bio diesel or other biofuels are outside the scope of this thesis; although, research in those areas is also of great importance in the furtherance of microalgae-based biofuel production. This thesis aims to focus mainly on the extraction stage of the biofuel production process from microalgae.

Chapter 2

Microalgae Derived Biofuel

Cultivation of microalgae as a food source has existed for several hundred years, earliest reports of microalgae cultivation as a potential fuel or energy source were published in 1957 (*Golueke et al. 1957*). Following the fuel crisis of the 1970's the National Renewable Energy Lab launched a major effort into examining the possibility of algae-based fuels through the Aquatic Species Program (*Sheenan et al. 1998*).

Production of biofuel from microalgae consists of a series of steps in a process that can be broken down into two main stages; the growth and cultivation of algae biomass and harvest and extraction of lipids contained within that biomass. Figure 2.1 shows the major steps in the algae biofuel production process. An understanding of the current state-of-the-art in lipid extraction from algae-based biomass is necessary if the goal is to increase the efficiency of current processes or to develop a new process as is the aim of the work presented here.

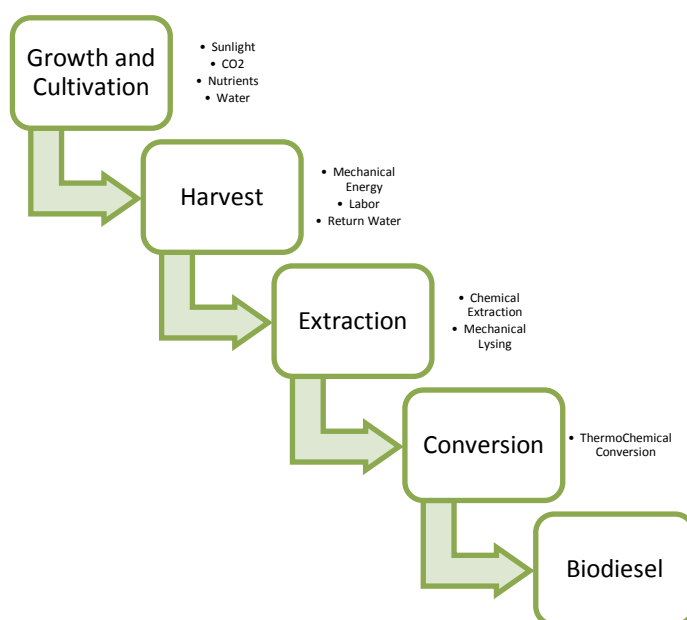


Figure 2.1 Basic block diagram of algae biodiesel production.

2.1. Algae

Algae consist of a vast amount of aquatic, photosynthetic species. Macro algae are more plant like in structure whereas microalgae more closely resemble bacteria in structure and form. It is estimated that over 300,000 species of algae exist and is generally accepted that many more have yet to be discovered. Of the known species, only a small percentage have been examined for their suitability as biofuel feedstock. Research has focused on a number of fast growing algae capable of accumulating large amounts of lipids. *Chlamydomonas reinhardtii*, *Dunaliella salina*, and various *Chlorella* species, as well as *Botryococcus braunii* are amongst some of the green algae species suitable for biofuel production; *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*, and other heterokonts including *Nannochloropsis* and *Isochrysis spp.* are classified as diatoms and have also been studied (Scott *et al.* 2010).

2.2. Growth and cultivation

The growth of microalgae for use as a biofuel source centers on balancing inputs in an effort to maximize intracellular lipid production, duplication rate and other factors. The growth and cultivation of algae biomass is not the focus of this thesis, nonetheless a short summary on the basic principles and influencing factors of algae cultivation are provided below.

2.2.1. Inputs

In general the growth and cultivation of microalgae requires inputs of light, nutrients, carbon and water. Table 2.1 outlines some of the major influencing factors on algae growth. The availability of these nutrients have significant influence on the design of the growth system, perhaps most significant of which is light availability.

Table 2.2. Major factors influence algae growth and common solutions. (Richmond 2004)

Limiting Factor	Solution
Light Availability	Lower Cell Density Or Light Path/Mixing
Photo Inhibition	Dark Light Alteration, Distribution Of Light Intensity
Carbon Availability	Aeration/CO ₂ Injection
Oxygen Accumulation	Aeration/Degassing
High Or Low Temperature	Cooling/Heating
Competition	High Inoculation Volume/Isolation/Extremofiles
Shear Stress	Reduced Flow Speed
Nutrient Availability	Constant Supply/Nutrient Monitoring
Physiological Limitations	Genetic Manipulation/Selective Breeding

. Controlling the available light is necessary in order for algae to benefit from efficient photosynthetic activity while avoiding a light condition that promotes photo inhibition. Photo inhibition is a protective molecular mechanism that protects the algae from excess light intensity by dissipating the light as heat and fluorescence. If an overly intense light situation occurs photo-bleaching may occur which results in damage to the photosynthetic reaction abilities of the algae. These effects greatly decrease algae growth rates to almost half the maximum productivity. It is generally accepted that under natural light conditions peak irradiance from daylight can cause photo inhibition and photo damage (*Chisti, 2007*).

Nutrients play a major role in the cultivation of algae. Like any other organism algae require a number of micro and macro nutrients. These nutrients and their availability are often a limiting factor of growth rate under natural conditions. In cultivation systems such as ponds or photo bioreactors it becomes essential to control the availability of these nutrients. The most essential nutrients to most algae species are nitrogen, phosphorus and sulfur; components which comprise a considerable amount of the total dry mass of the cell. Sodium, potassium and chloride are also essential to the intracellular environment. Certain diatoms also require silica as a major structural

component of the cell wall. In addition to these nutrients metals are required to facilitate cell metabolism, most commonly iron, magnesium, copper and zinc. A number of other non-essential compounds and vitamins have been shown to increase growth rates but high concentrations of certain elements can be toxic to algae and greatly decreased growth rates (*Croft et al. 2006*). Careful and continuous monitoring of nutrient availability and concentration in the growth medium is essential to maximizing growth rates.

Carbon can be considered a nutrient but in terms of algae cultivation is more accurately referred to as a structural component. In highly dense cultures of algae growing under natural conditions, where carbon availability is limited by diffusion from atmospheric CO₂, carbon may become a limiting factor. The optimization of carbon dioxide injection is important to creating optimal growth environments for highly dense cultures and has been shown to increase growth rates (*Morais & Costa 2007*). Addition of carbon in its mineral form is also a possibility for certain strains of algae that are able to absorb carbonates directly (*Wang et al. 2008*).

A notable dichotomy has developed in recent years between algae growth in CO₂ emissions from flue gasses. The direct use of flue gasses has been shown to positively affect algae growth rates and lipid yield (*Doucha & Livansky, 2006*). The positive effects swing both ways, flue gases also contain considerable amounts of nitrogen oxides, compounds which algae are known to absorb. Experiments on large scales have investigated the ability of algae to reduce nitrogen oxide emissions from commercial power generation equipment and have shown that removal rates as high as 90% are possible (*Pulz, 2007*).

Water is an essential input to the algae growth system, not only does it provide a suitable medium for the cultivation of aquatic species but it is necessary for carbon fixation during photosynthesis. Water also serves as a solvent for the required nutrients. Major issues regarding

water use during algae cultivation are the availability of freshwater and evaporation. Even saltwater systems that suffer from evaporation require the use of freshwater for replenishing to avoid an increase in salinity. Evaporation is not an issue for closed photo bioreactor systems but plays a major role in the maintenance of open pond systems.

Systems have been developed that show that algae can be cultivated in wastewater streams and even be used in wastewater treatment (*Mulbry et al. 2008*). The high resilience of algae and the available nutrients and vitamins available in effluent streams validates the option. Evaluation of the streams and the nutrient content as well as determination of the existence of any toxic compounds is necessary in order to disqualify any unsuitable streams. Seawater presents another alternative for algae cultivation, especially when enriched with nutrients from an outside source, such as a wastewater stream (*Goldman & Stanley, 1974; Takaji et al. 2006*). Although seawater would limit the available number of species it does contain the majority of the nutrients necessary for algae cultivation. Most importantly seawater would mitigate the need for freshwater and algae cultivation system.

2.2.2. Open ponds and photobioreactors

An understanding of the necessary inputs for algae growth is important to create a distinction between the available controllability of operating parameters in open ponds or photo bioreactors. The biggest distinction between these two systems is their exposure to external environmental conditions. Artificial ponds serve to contain the algae growth medium but do not generally include means for isolating the system from the environment, whereas closed photo bioreactor systems create closed loop growing system in which algae cultivation is physically separated from the external environment. Figure 2.2 shows a few of the available systems for cultivating algae.

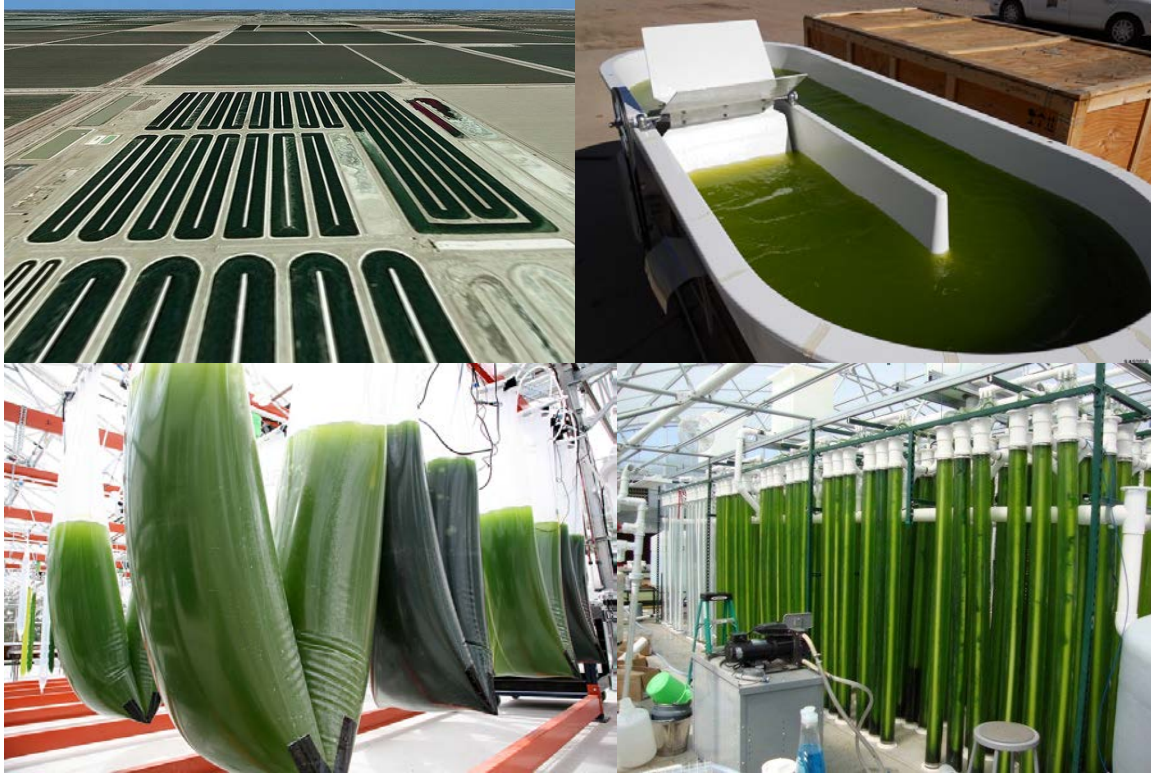


Figure 2.2. Clockwise from top left: Open raceways at Pacific Northwest National Labs, Small raceway at New Mexico State University – Fabian Garcia Science center, Tubular reactors at Pittsburg State University, Bag type reactor at Synthetic Genomics – La Jolla Ca.

Raceway ponds are the most common open algae cultivation system, essentially a shallow oval-shaped pond where a paddle or pump circulates the medium. Open systems are limited to very hardy species; their openness to the environment makes controlling cultivation conditions extremely difficult. The introduction of invasive species can also greatly affects the integrity of the cultivation system (*Sheenan et al. 1998*).

Closed photo bioreactor systems greatly facilitate the ability to cultivate a monoculture, a culture consisting of a single species. Photo bioreactors can be divided into three general groups vertical column photo bioreactors, tubular photo bioreactors and flat plate photo bioreactors (*Ugwu et al. 2008*). Vertical column photo bioreactors are essentially a large transparent column in which air and carbon dioxide are sparged from the bottom of the column and are allowed to diffuse freely

upwards. This bubbling limits oxygen accumulation and provides excess CO₂ for photosynthesis. This system provides exceptional control over light availability, temperature, alkalinity and nutrients. This controllability however comes at a high equipment cost and in general is not suited for large-scale application.

Tubular systems are more common in large-scale experiments and commercial ventures. Tubular systems suffer from carbon limitation, decreased nutrient availability and overtime oxygen accumulation. To address these issues the long plastic tubes characteristic of tubular systems must be pumped. This pumping is known to cause significant cell damage during the growth phase and also results in added costs on large scale systems.

Flat plate systems focus on maximizing light availability, they consist of two large flat plastic panels in which the growth medium flows through internal channels. Typically these systems are no more than 10 cm thick. Flat plate systems benefit from increased positionability, however they can also be very costly.

Generally, open systems and ponds are significantly less expensive to construct when compared with closed photo bioreactor systems. Photo bioreactors are more expensive but offer far more control over the cultivation parameters. The various systems available for algae cultivation all have their own advantages and disadvantages and choosing the correct system will rely heavily on the goals of the researcher or commercial concern. For the purposes of this work a simple closed photo bioreactor and concentrated nutrient solution were used to maintain a viable monoculture of algae.

2.3. Harvest and Concentration

After cultivation a crucial step in the extraction of lipids from microalgae is the harvesting stage. Microalgae grow in a relatively low concentration in terms of biomass weight per volume of water. This harvesting stage is essential for facilitating further extraction or processing of the algae biomass, no large scale process currently exists that allows for lipid extraction from biomass without first concentrating the biomass and physically removing it from its growing medium. The process and reaction system developed and evaluated in this thesis addresses the issues of increased cost and energy input required during the harvesting stage by allowing for the processing of a fully wet non-concentrated algae stream. To understand the implications of such a process it is important to understand the current state-of-the-art of the harvesting, concentration and extraction process.

Separating the biomass from its growth medium is traditionally done by one of three basic methods, sedimentation and flocculation, filtration or centrifugation (*Molina-Grima et al. 2003*). Of those three, filtration and centrifugation are both high-energy and high maintenance processes. Sedimentation occurs naturally at slower rates but can be accelerated through the use of flocculants or surfactants, both of which add significant cost and may not be suitable for large-scale application. These processes are still capable of lowering the water content of algae from 99% to 90%, in most cases more drying of the algae must occur before proceeding to the extraction phase. A filter press, drum dryer or high-speed centrifuge can be used to decrease the water content to between 20 and 40%. Solar drying or steam based drying such a spray drying or drying on a fluidized bed can be used to bring the water content down to as little as 10%, which is required for some extraction processes.

These drying processes add significant energy requirements and costs to the algae biofuel process stream. Solar drying seems attractive, however the amount of water lost to evaporation and

further space requirements make it less suitable for large-scale applications. It is clear that a process which limits the amount of drying or concentration necessary before extraction is much needed.

2.4. Mechanical extraction

Mechanical extraction methods include pressing, bead milling, and homogenization. The nature of these methods minimizes contamination while maintaining the integrity of intracellular lipids and fatty acids on a chemical level (*Greenwell et al. 2010*). Pressing is a simple application of high pressure to rupture the cell wall. Homogenization forces concentrated biomass through an orifice and a prompt pressure drop and high sheering action cause the cell wall to rupture and release lipids. Bead milling is a more commonly used mechanical extraction method. Grinding media, glass or other hard small spherical beads are vigorously agitated in a vessel causing significant disruption of the algae and releasing lipids. The application of mechanical extraction processes on *Chlorella protothecoides* was shown to recover 14.9% for French press homogenization and 18.8% for bead milling (*Doucha 2008*). Mechanical methods require high energy inputs but are typically more suited to large scale applications when combined with methods that weaken the cell wall prior to disruption such as acid or alkali and enzymatic pretreatments. Mechanical methods can also be effective when used in conjunction with solvent methods to increase the percentage of oil released and decrease the amount of solvent required.

2.5. Solvent extraction

Solvent extraction relies heavily on the fact that oil has a high solubility in organic solvents and the fact that organic solvents have the ability to easily degrade the cell wall. Organic solvents suited for oil recovery include benzene, cyclohexane, hexane acetone and chloroform (*Mercer & Armenta 2011*).

Organic solvents should be chosen that meet the following criteria: they should be insoluble in water; they should only solvate the desired compound; have a low boiling point and a significantly different density of water. It is also important that the solvent be inexpensive, reusable and easily sourced (*Banerjee et al. 2002*). The polarity of the solvent is also an important factor in solvent selection, the polarity of the solvent should match the target compound, for example nonpolar lipids extracted by nonpolar hexane.

Extractions that combine mechanical methods and solvent methods show significant increases in the percentage of extracted oil (*Ying Shen et al. 2009; Cooney et al. 2009*). The addition of mechanical disruption to solvent extraction increases the ability of the solvent to make physical contact with the lipid material. Hexane extraction combined with wet milling showed an increase in lipid recovery from 6.3% to 25.3%. When using a bead beater in combination with hexane extraction lipid recovery increased from 5.6% to 18.8% (*Ying Shen et al. 2009*). The combination of these two methods increases cost and energy input requirement thus decreasing the efficiency and net energy balance ratio of the process.

Another option reported by *Cooney et al. 2009* is accelerated solvent extraction which carries out traditional organic solvent extraction at elevated temperatures and pressures. Accelerated solvent extraction that exceeds the boiling point of the solvent greatly modifies the solvent power available; water can be used as a solvent at high temperatures and pressures in a process known as low polarity water extraction.

The Bligh and Dyer technique uses a combination of chloroform, methanol and water to extract lipids from a biological matrix (*Bligh & Dyer, 1959*). This classical method was originally intended to extract lipids from the tissue of fish and is considered a benchmark for comparison of other extraction methods.

There are advantages and disadvantages to the use of organic solvents for lipid extraction from microalgae. The solvents used are generally inexpensive and readily available. Results obtained from solvent extractions in terms of total lipids recovered are easily reproducible. On larger scales organic solvents suffer from several limitations. Organic solvents are often highly flammable and extremely toxic. Complete recovery of the solvent after extraction is an expensive and energy intensive process and often solvent residues remain in the final product. There are also significant safety concerns with handling such large volumes of organic solvents.

2.6. Sonication extraction

The use of ultrasound as a primary extraction method and as a supplement to solvent methods has been reported to damage cell walls and release intracellular matter (*Cravotto et al. 2008; Wei et al 2008*). The mechanism by which ultrasound can rupture the cell wall is a phenomenon known as cavitation which is a primary focus of this work and described in detail in the following chapter. Ultrasound extraction methods can greatly reduce the extraction time and quantity of solvent necessary for extraction. The efficient mixing provided by ultrasound increases surface contact and facilitates the release of cell contents into the growing medium.

Ultrasound shows promise as a method of cell disruption however it suffers from limitations in scale up ability (*Asakura et al. 2008*), there are also significant energy cost and equipment complexity in commercial ultrasound equipment. This work explores and evaluates the efficacy of cavitation based extractions initiated not by ultrasound, but by audible range acoustic fields.

Chapter 3

The low energy question

The wavelengths of acoustic fields in the ultrasonic range are generally considered too large to directly affect biological organisms, the method by which the organisms are disrupted is known as cavitation. Cavitation has been reported as an effective means of disrupting biological organisms (*Cooney et al. 2009; Cravatto et al. 2008, Gogate & Kabadi 2009; Savant et al. 2008*). This research however has focused mainly on the use of high-frequency ultrasound equipment to incite cavitation. As discussed previously ultrasonic methods of cell disruption have limiting factors in terms of scale up. When compared to mechanical processes ultrasonic disruption is far more energy efficient than bead milling or homogenization. This increase in efficiency shows no practical significance when coupled with the inability to create large-scale ultrasonic systems.

This work focuses on technology that can potentially increase the efficiency of the acoustic cavitation based reactions in a manner that is suitable for large-scale lipid extraction from biomass. A thorough understanding of cavitation and its effects is necessary if an efficient system is to be designed.

3.1. Cavitation

Cavitation can be defined as the inception, growth and the rapid collapse of a microscopic bubble within a fluid system. There exist three main modes by which cavitation can be incited: acoustic cavitation, hydrodynamic cavitation and cavitation incited by laser (Shah et al. 1999)

Cavitation relies on impurities to act as nucleation sites within a liquid; in pure liquids void of any dissolved gases or suspended particles pressures on the order of 1000 atm are required before any cavitation can occur. This is due to the high tensile strength characteristic of such liquids, liquids

of this nature however are rare and in most conditions liquids will contain impurities. When an acoustic field is applied to a liquid medium the nature of sound waves exposes medium to alternating cycles of compression and rarefaction. If pressure at an impurity during the rarefaction stage falls below the vapor pressure of the liquid cavitation may occur. Figure 3.1 shows the stages of cavitation incited by a high-intensity acoustic field. The presence of impurities or nucleation sites is essential to facilitating cavitation at negative pressures of less than 1 atm. These nucleation sites can consist of gas microbubbles within the liquid as well as gas cavities present on any dissolved solids and particles within the liquid. It is reasonable to consider cavitation a surface phenomenon. The behavior of cavitation bubbles under an oscillating field is often referred to as bubble dynamics (Ghandi & Kumar, 1994).

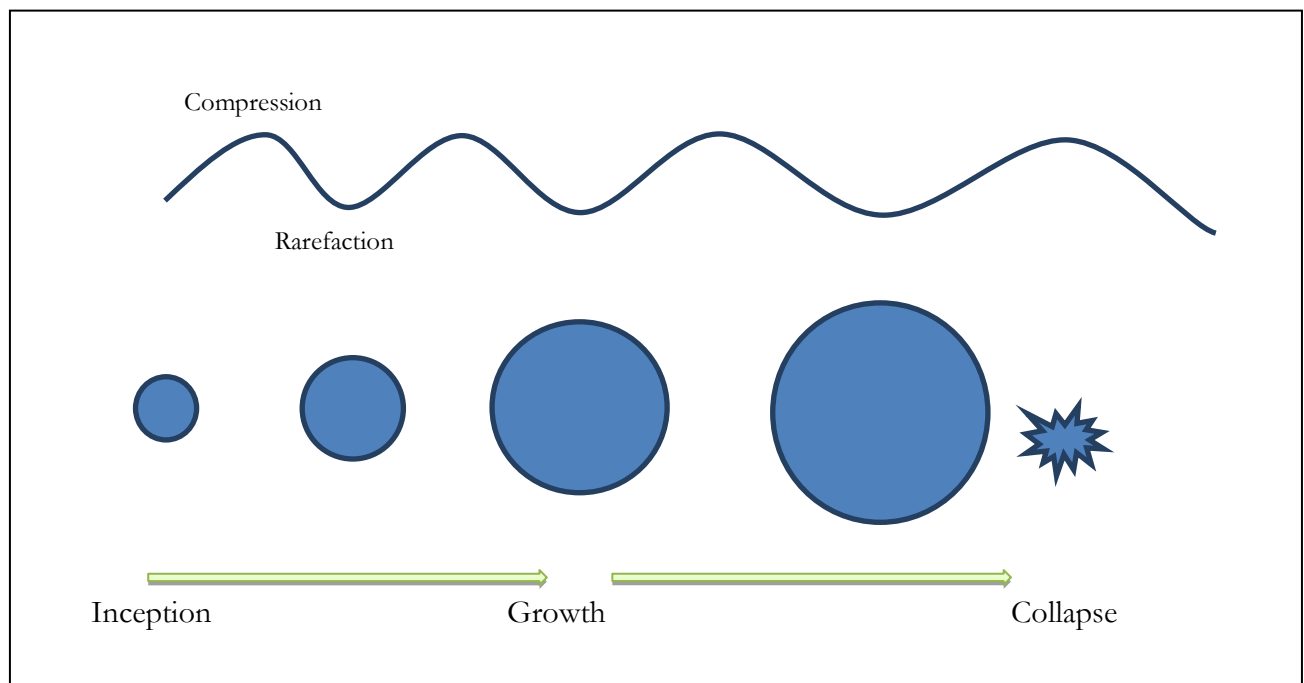


Figure 3.1 Stages of Cavitation including the inception of the cavitation bubble, the growth until a maximum bubble size and the collapse during the compression cycle of the pressure wave.

3.1.1. Bubble Dynamics

The existence of cavitation bubbles is short-lived, the growth and subsequent collapse occurred within a timespan on the order of only a few microseconds. The growth phase occurs during the rarefaction cycle of the oscillating pressure field. When compared to the collapse phase the growth phase is considered very slow. During growth enough heat exchange between the contents within the bubble and the surrounding liquid is assumed to take place to ensure isothermal conditions. The inertia of the liquid allows for bubble growth and expansion to carry over into the compression cycle before collapse. As the cycle continues the compressive pressure field coupled with the inward rushing liquid begins to reduce the radius of the bubble. Because this compression and eventual collapse of the bubble happen so quickly the amount of heat transfer between the bubble and the surrounding liquid is negligible and the collapse can be treated as adiabatic. It is during this adiabatic compression that extreme temperatures and pressures can be achieved. *Suslick 1989* reported temperatures as high as 5500°C and pressures as high as 500 atmospheres.

When the cavitation bubble collapses near a solid wall or another bubble the collapse occurs asymmetrically nearest the solid boundary. This asymmetric collapse gives rise to short-lived micro-jets approximately 100 μ in diameter with the velocity between 100 and 500 m/s (*Ghandi & Khumar, 1994*). There is also a surface phenomenon which occurs when the bubble wall velocity exceeds the velocity of sound giving rise to shockwaves. Both of these effects tremendously increase mass transfer rates often resulting in erosion or fracture of nearby surfaces. This damage is well known and reported throughout fluid mechanics.

3.1.2. Physical and Chemical Effects of Cavitation

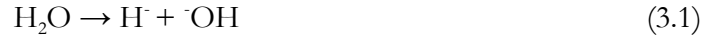
Chemistry induced by cavitation and ultrasound reactions is commonly referred to as sonochemistry. The collapse of cavitation bubbles and intense environment created within and

around the bubble are universally accepted as the source of homogeneous sonochemistry. The upper limits of temperature and pressure achieved during bubble collapse are difficult to measure, as are heating and cooling rates. *Suslick, 1999* reports heating and cooling rates of at least 10^{10} K/s. The existence of intense temperature and pressure, in such a short amount of time allows cavitation to create severe physical damage and greatly accelerated or altered chemical reactions in an essentially cold liquid and provides a unique mechanism for generating high-energy chemistry.

Cavitation is proven to accelerate a number of heterogeneous reactions including hydrogenation of alkenes and even dehydrogenation reactions. The effect of cavitation on two phase reactions may be attributed to the influence of the micro-jets created during bubble collapse, such as convection and degassing. Cavitation also negates the need for purification of solvents and reagents in a number of reactions, most notably in the preparation of the Grignard reagent. Avoiding costly purification is especially important in an industrial setting which translates directly to economic benefit. Cavitation has also been shown to replace the role of these transfer catalysts in two-phase organic reactions where the reactants are insoluble in each other.

The most common applications of the chemical effects of cavitation deal with the acceleration of reaction rates. Cavitation also brings rise to unpredicted chemical reactions where they would normally not occur. For instance applying ultrasound to water leads to the development of highly active yet short-lived compounds including hydrogen peroxide and hydroxyl radicals. This is the basis for a reaction involving the liberation of iodide from potassium iodine discussed in detail in section 3.4.1 in this manuscript. Specific reactions that benefit from cavitation are extensive; an understanding of the basic chemical processes that can be affected by cavitation and understanding the probable mechanism by which cavitation effects these processes is more suited to this thesis.

When aqueous solutions that would normally be unreactive are subject to an acoustic field of sufficient intensity to incite cavitation the following major reactions occur (*Merouani et al. 2010*):



The adiabatic collapse of the cavitation bubble creates a severe enough environment for the thermal disassociation of H_2O vapor within the bubble to occur releasing highly active hydroxyl radical $\cdot\text{OH}$ and the hydroperoxyl radical OOH^\cdot . These radicals then reform to create hydrogen peroxide.



These highly reactive species can then recombine, react with other gaseous species, or diffuse into the bulk medium and react with molecules in the solute. This reaction pathway is generally accepted as the mode by which cavitation effects homogenous reactions.

Cavitation and the application of ultrasound also influence a number of physical processes. As cavitation takes advantage of the existence of dissolved gases within a liquid it can quickly degas a solvent by consuming all of these nucleation sites with cavitation events.

The use of cavitation to facilitate emulsification is employed industrially to easily emulsify a number of immiscible liquids. The extreme tensile stress exerted on the liquid during cavitation as well as the shockwaves created during bubble collapse contribute to the fragmentation of droplets and efficient mixing.

The physical effects of cavitation are more important to the novel acoustic cavitation process outlined in this thesis. As described earlier, cavitation near a solid boundary results in the asymmetric collapse of the cavitation bubble and the formation of intense micro-jets. Figure 3.2 illustrates this phenomenon. Microalgae suspended in a liquid medium offer a number of free surfaces for gas microbubbles to attach to and act as nucleation sites for cavitation. When cavitation occurs near the cell wall of microalgae the intense micro-jets can have damaging effects eventually leading to the rupture and degradation of the cell wall.

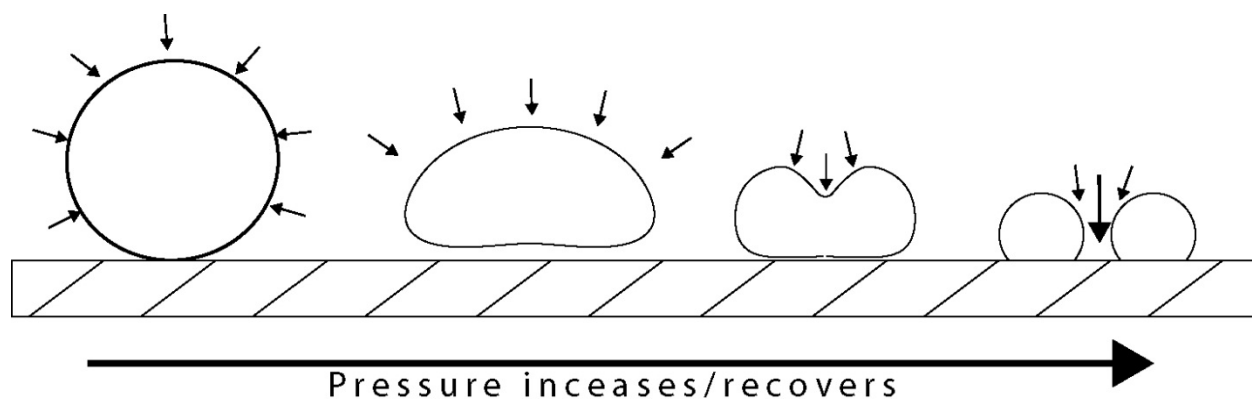


Figure 4. Cavitation near a solid boundary resulting in the formation of a microjet.

3.2. Some effects of audible sound

Most efforts in examining the effects of cavitation have centered on the use of power ultrasound generated by piezoelectric transducers. Reports of audible sound creating sonochemical effects are limited; *Gaines, 1932* and *Flosdorf et al. 1936* are the most complete accounts of the physical and chemical effects of audible range sound utilizing a magnetostrictive oscillator to impart

an acoustic field to a liquid through direct contact. *Gaines* reported number of intensely destructive physical effects of his magnetostrictive oscillator including charring of the interiors of corks, a 6 cm high fountain on the surface of the water directly above the oscillating element. He also reports erosion of metals and glass which he attributes directly to the formation of cavitation. *Flosdorf et al.* reports conclusively that oxidations previously attributed only to ultrasonic cavitation can be achieved through sonication in the audible range.

The most recent mention of low frequency vibrations for ultrasonic effects is the use of a large mechanical oscillator for soil decontamination (*Mason, 2004*). The author suggests that the effects of high amplitude low-frequency vibrations did not incite cavitation acoustically but rather result in hydrodynamic cavitation due to rapid pressure drop. The ability of low frequency vibrations created by magnetostrictive oscillators to create sonochemical effects and incite cavitation is a primary motivation for this work.

3.3. Terfenol-D

The reactor designed in this thesis utilizes a low frequency magnetically biased transducer consisting of a Terfenol-D magnetostrictive element. Magnetostriction is a phenomenon in which a ferromagnetic material undergoes a dimensional change or induced strain due to changes in its magnetization. Early magnetostrictive materials consisted of iron, nickel and cobalt and exhibited magnetostrictive strains of -9, -40 and -60 ppm respectively (*Restorf, 1994*). The discovery of giant magnetostrictive elements terbium and dysprosium in the mid-1960s exhibited strains greater than 1500 ppm, this discovery was limited to cryogenic temperatures (*Meeks & Timme 1976*). Efforts at the Naval Surface Warfare Center developed the first giant magnetostrictive alloy consisting of terbium, dysprosium and iron; an alloy capable of strains up to 2000 ppm at room temperature (*Clarke, 1980*).

3.3.1. Giant magnetostrictive alloy

Two regimes of the magnetostrictive process are observed. Spontaneous magnetostriction consists of the strain that occurs when a ferromagnetic material is cooled below its Curie temperature allowing for magnetic moments to transition from an unordered paramagnetic state to order domains.

The second regime of magnetostriction is the strain induced by changes in magnetization of a ferromagnetic material below the current temperature. This magnetostriction process is attributed to the coupling of the crystal lattice of the material to the magnetic domains. If a material exhibits positive magnetostriction the crystal lattice is elongated in the direction of the magnetization vector, resulting in asymmetrical lattice spacing and induced strain dependent on the orientation of the magnetic domains. The limit of magnetostriction occurs once all magnetic moments are outlined with the applied magnetic field; this limit is termed the saturation magnetostriction.

Terfenol-D consists of a cubic crystal structure and features large magnetostrictive anisotropy and a positive magnetostrictive strain coefficient. The Terfenol D element within the transducer employed in this work is manufactured as a monolithic crystal through a free stand zone melt process. Figure 3.2 illustrates the crystallographic structural changes within a Terfenol D rod during magnetization.

The growth structure of Terfenol D crystals is dendritic sheets in the $[11\bar{2}]$ direction within 19.5° of the nearest $[111]$ axis. The $[111]$ axes are also consider the magnetically easy axes. Greatest magnetostriction will result in the magnetization vector M rotating from $[111]$ to $[11\bar{1}]$.

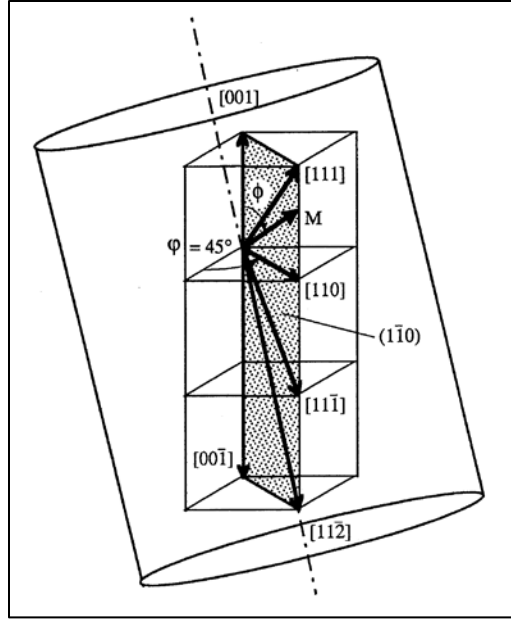


Figure 3.3. Crystallographic structure of TerfenolD monolithic rod under magnetization vector M (Cedell, 1995 as reported in Calkins 1997).

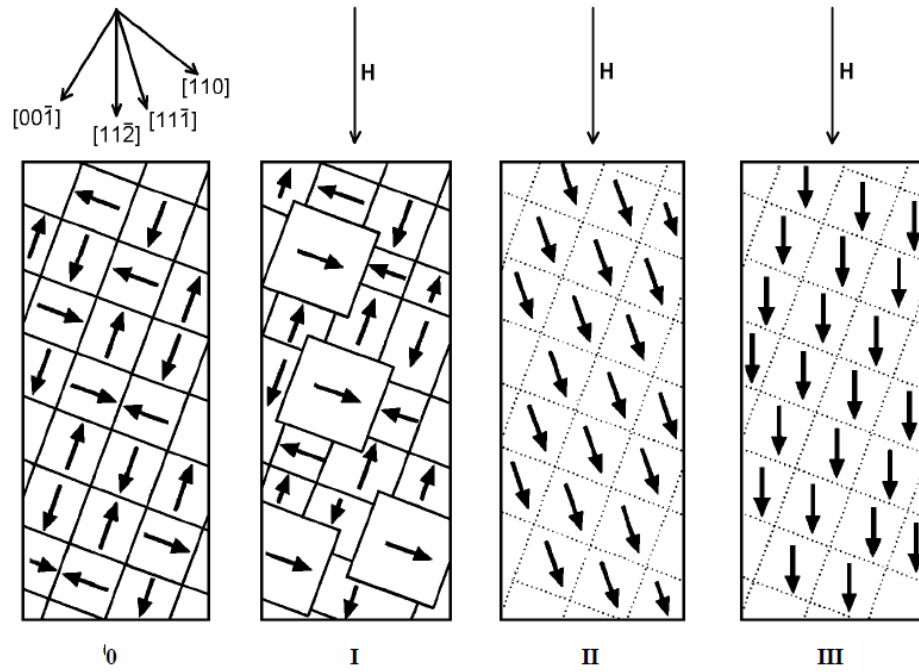


Figure 3.4. Stages of magnetostriction (Dapino, 1999).

Figure 3.4 shows the development of magnetostrictive strain as the magnetization vector moves as described previously. At state zero the magnetic domain vectors within the material have no specific orientation and result in a net zero magnetization. During state I the more closely aligned vectors take precedence and their magnetostrictive contribution is amplified. States II and III reflect further application of the applied field and the eventual theoretical saturation and complete alignment of the magnetic fields.

Traditional power ultrasound equipment employs piezo-ceramic transducers which convert electrical energy directly into mechanical energy and vibration. When compared to piezo-ceramic transducers, magnetostrictive transducers benefit from high dynamic strains which makes them well-suited to operation at resonance. Dynamic strains as high as 4000 ppm are observed in Terfenol transducers compared to 1600 for piezo-ceramic transducers operating at resonance (*Claeysen et al. 2002*). The low Young's modulus of Terfenol D means it can produce low frequency oscillators of compact size. *Moffet et al. 1991* reported higher output strains for Terfenol D when compared to PZT4 piezo ceramic at all power factors. When considered as an option for a lab sized, low frequency transducer capable of ultrasound results Terfenol D is well suited.

3.4. Experimental Methods

In order to establish cavitation as a suitable low-energy method for disrupting algae biomass, it became necessary to verify the ability of an audible range acoustic field to initiate cavitation. Additionally, examining the cavitation intensity and cavitation yield of the reaction system provided the groundwork for comparing the efficiency of audible range cavitation reactions against ultrasound cavitation reactions and hydrodynamic cavitation reactions.

The commonly accepted method for calibration and comparison of cavitation efficiency in a wide range of reactors is a reaction consisting of the decomposition of potassium iodide resulting in

the liberation of free iodine in the form I_3^- (Gogate *et.al.* 2005, Koda *et.al.* 2003, Suslick *et.al.* 1997). This reaction is known as the Weissler Reaction (Weissler *et.al.* 1950).

3.4.1. Weissler Reaction

As discussed previously the cavitation environment creates a number of short-lived, yet highly active species; notably the OH^\cdot radical. The Weissler Reaction relies on the fact that the formation of these hydroxyl radicals is solely dependent on the existence of the cavitation condition. When sufficient cavitation occurs in the potassium iodide solution these free radicals react with the potassium iodine to free iodine, this free iodine then reacts with the remaining potassium iodide resulting in the formation of the tri-iodide ion, I_3^- . The reaction in its simplest form is as follows:



This triiodide ion can easily be quantified spectrophotometrically, when starch solution is added to the original solution after reaction the solution turns blue.

Reagent grade potassium iodine was dissolved in distilled, deionized water to create a .1 mol/L solution. This potassium iodine solution was prepared 1 L at a time and stored in an airtight nonreactive container until it was used. Samples of 200 mL were irradiated in the acoustic reactor at a frequency of approximately 1430 hertz, the approximate resonating frequency of the vibrating plate in the reactor. It should be noted that outside of this narrow resonant frequency much less evidence of cavitation was observed.

The reaction chamber was immersed in a bath of cold water in an attempt to minimize any rise in bulk temperature of the solution.

Samples of the solution, approximately 5 ml, were extracted at 1 min., 5 min., 10 min. and 15 min. intervals. Samples were quickly saturated with starch solution consisting of 1 g soluble starch in 100 mL of distilled, deionized water. Drops were added until no obvious change in color could be detected. The starch solution was prepared by boiling and stirring 1 g soluble starch in 100 mL of distilled, deionized water until a clear solution was obtained. The starch solution only reacts with the liberated iodine, thus the exact amount added is inconsequential provided that the solution is fully saturated.

The samples were then transferred by pipette to new, disposable, plastic UV cuvettes, (*BrandTech 759150*) and examined spectrophotometrically at 355 nm.

A number of parameters were monitored continuously throughout the experiment, including bulk temperature of the liquid, true power consumption of the cavitation equipment, immersion depth of the acoustic vibrating plate and shape and accuracy of the waveform.

3.4.2. Cavitation Equipment

The design and construction of the audible range acoustic cavitation reactor saw much iteration. The final system consisted of the low-frequency Terfenol-D transducer, described previously (*220-75-50 Etrema Products Inc. Ames, IA*) supported rigidly over a 500 ml borosilicate reaction vessel. A 316L stainless steel plate, 0.250 inch thick and 6.00 inches in diameter was rigidly coupled to the transducer. No seal was used between plate and the reaction vessel. A physical diagram of the reactor can be seen in Figure 3.5.

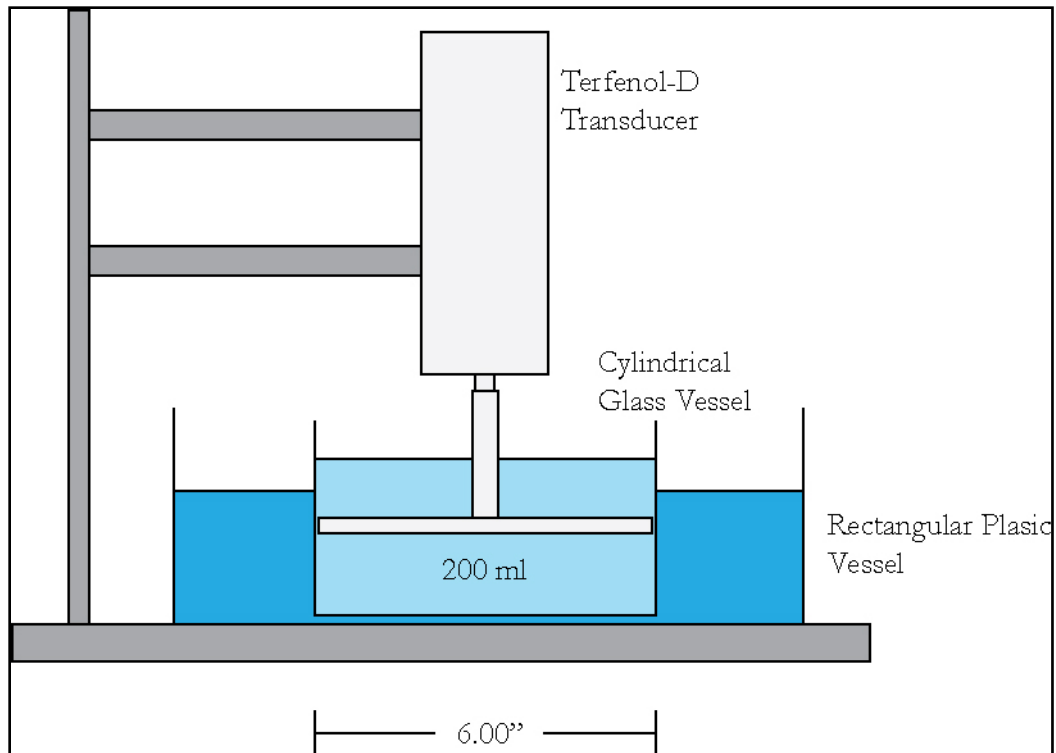


Figure 3.5. Physical diagram of the audible range system.

The design of the reaction plate ensured that the transducer could drive the plate at a resonant frequency while not exceeding its operating limits. To facilitate estimating the resonant frequency of a flat round plate and avoid lengthy solution to the eigenvalue problem a modal analysis was carried out in a finite element solver (*Creo Pro 5.0 – Mechanics, PTC*). Although an analytical solution to the frequency response of a damped flat plate exists (*Martin 2011*) the rigor of such a calculation is outside the scope of this thesis. An FEA approximation proved sufficient enough to fine tune the plate to a resonant frequency using acoustic methods.

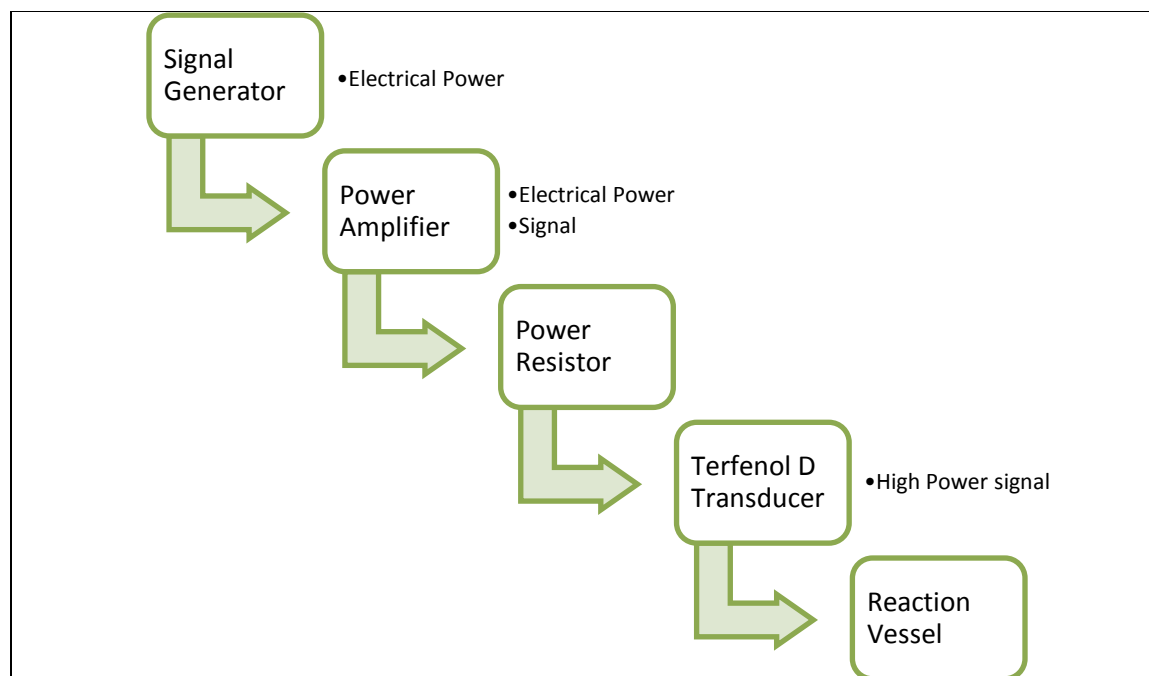


Figure 3.6. Basic operation of the reaction system

A block diagram of the control system can be seen in Figure 3.6. The transducer was driven by a 500 watt power amplifier (*Crown XTi-1000, Crown Audio Inc.*). A hardware signal generator (*BK4017a, Be&K Precision*) was used to drive a square wave at the resonant frequency of the reactor plate. A 4 ohm power resistor was used in line between the power amplifier and the transducer to ensure adequate resistance to prevent activating the short circuit protection cutoff within the amplifier.

3.4.3. Calculations

A calculation methodology was developed that would simplify evaluation of the efficiency of the cavitation reactor while also providing metrics by which the reaction system reported in this thesis could be easily compared to other reaction systems reported in literature. Two main problems were calculated and reported, energy efficiency of the cavitation equipment and the cavitation yield.

Determining these operating parameters would allow for comparison with both ultrasound and hydrodynamic systems as reported by *Merouani et al 2010*, and *Gogate et al 2001*.

Calorimetry was used to determine the energy dissipated from the reactor into the liquid. Temperature rise of a 200 ml sample of DI water was measured over a 5 minute irradiation. If the container is sufficiently insulated the power dissipated into the sample can be evaluated using the following equation:

$$P(W) = mC_p \frac{dT}{dt} \quad (3.9)$$

Where P denotes the power dissipated into the fluid in watts, m denotes the mass of the sample in kg, C_p denotes the heat capacity of the sample in $\frac{J}{kg \cdot K}$ and $\frac{dT}{dt}$ denotes the change in temperature (K) with respect to time.

The energy efficiency of the reactor can then be calculated by a simple efficiency formula:

$$Efficiency \% = \frac{Power\ dissipated\ to\ fluid}{Power\ supplied\ to\ system} \quad (3.10)$$

The ability of the system to effectively create a chemical change can be determined by the cavitation efficiency of the system or:

$$Cavitation\ Yield = \frac{Desired\ chemical\ change}{calorimetric\ power\ density} \quad (3.11)$$

Where the desired chemical change relative to this thesis is the total amount of iodine liberated and the calorimetric power density is the total power dissipated into the solution.

The amount of iodine liberated can be determined through application of the Beer Lambert Law which allows for the determination of the concentration of a solution through spectrophotometric determination of the absorbance over a specified distance.

$$A = \epsilon bc \quad (3.11)$$

Where A is the unitless measured absorbance, ϵ is the molar absorptivity in $\frac{L}{mol \cdot cm}$ a constant for the compound under examination ($26,000 \frac{L}{mol \cdot cm}$, Merouani 2010), b is the path length of the sample as defined by the cuvette and expressed in millimeters. The parameter C is the concentration of the compound in mol per L.

3.4.4. Algal Selection

Nannochloropsis was chosen primarily based on availability and access to continuing cultures if necessary. A starter culture was procured from New Mexico State University - Institute for Energy and the Environment. *Nannochloropsis* exhibits a fast growth rate and nearly 30% of dry weight is oil content consisting mainly of unsaturated fatty acids and a significant percentage of palmitic acid. The combination of acids creates sufficient double bonds to make this algal species well-suited for biodiesel production (Golveia & Oliveira 2009). The starter culture was raised in a simple 3 gallon vertical column photobioreactor in Guillard's f/2 growth medium (Guillard, 1975) under full spectrum LED lights. Samples of algae taken directly from the growth medium were sonicated with no additional solvents or concentration or drying steps.

Chapter 4

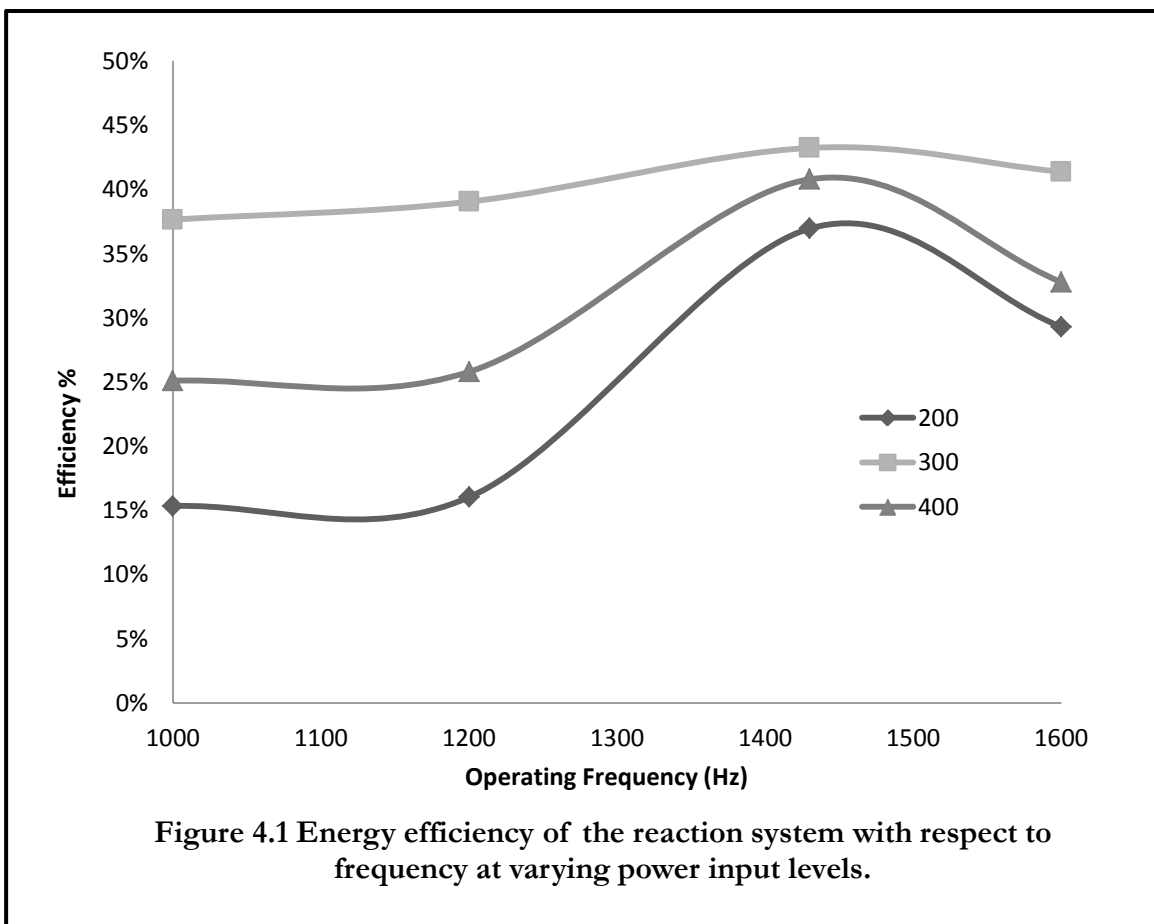
Results & Discussion

The aim of the current work is to create a highly efficient means of disrupting lipid containing biomass and releasing intact intracellular matter, namely intracellular lipids. The ability of the system to incite cavitation is verified through iodine dosimetry and examined spectrophotometrically. The audible range reaction system is compared to other cavitation based reaction systems.

4.1 Energy Efficiency

The cavitation equipment developed and used for comparison in this work, as described above, operate on the same functional principles. Electrical energy is supplied to the system and goes through various changes in form before the inception of cavitation. Generally the system converts the electrical energy to a form of mechanical energy which then creates cavitation. Under the reasonable assumption that the energy used in the chemical reactions is negligible, the remainder of the energy not lost through the series of changes in form must end up as heat generated in the reaction fluid. Through this reasoning the use of calorimetric means to calculate the energy efficiency of the systems is reasonable and allows for comparison to other cavitation equipment that has followed this convention.

The efficiency of the audible range reactor under different operating conditions is shown in Figure 4.1 on the following page. The power amplifier allows for only crude adjustment of the power output, also although the stated output of the amplifier was listed as 500w after 400w severe degradation of signal quality was observed. Peak efficiency of 43.2% was observed at the resonant frequency of the system at the 300w output level. Efficiency at 400w drops significantly, > 10%,

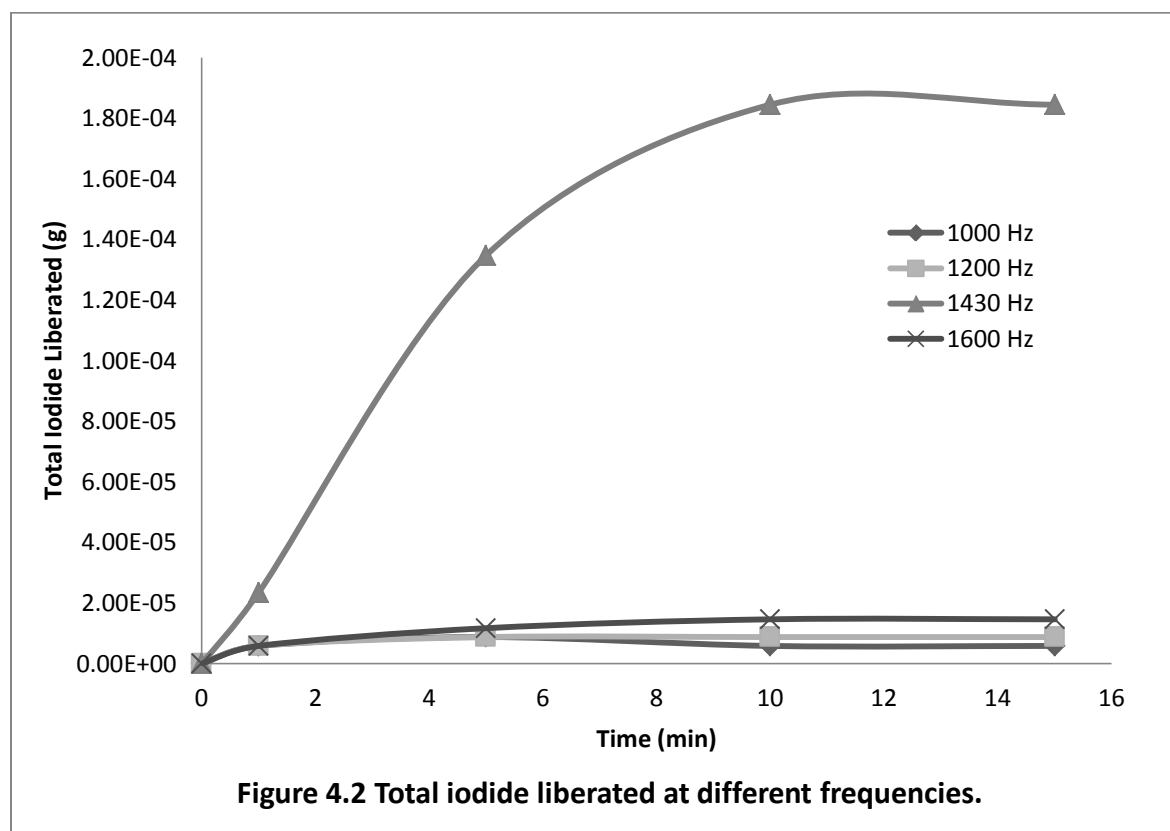


across the majority of frequencies. A common peak in efficiency occurs when the reactor is operating within its resonant range at all power levels. As power output from the amplifier rises from 300w to 400w the decrease in efficiency can be attributed to a decoupling effect within the transducer. At elevated power levels the shape changing properties of the magnetostrictive element decouple from the oscillation of the magnetic field resulting in a decreased power output. Results reported hereafter refer to results obtained at the 300 watt operating condition.

4.2 Iodine Liberation

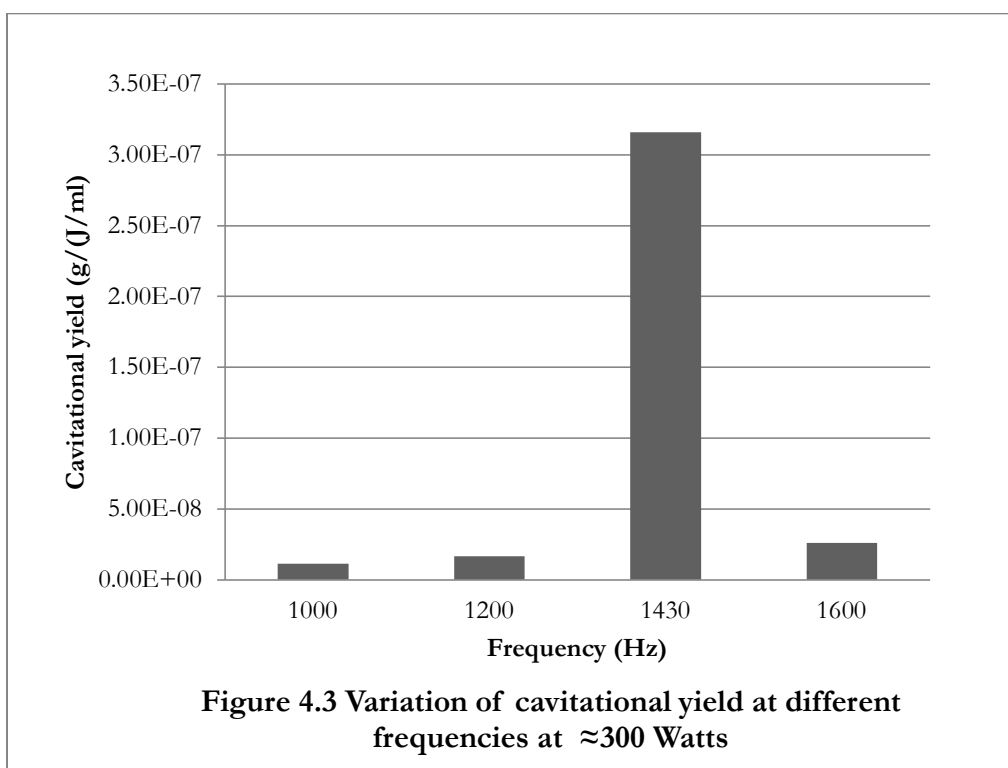
The positive effect of resonance is not limited to an increase in efficiency of the reactor but can be seen more clearly by its effect on the amount of iodine liberated from the KI solution. As seen in figure 4.2 outside the resonant frequency very little iodine liberation occurs. An order of

magnitude separates the total iodine liberated at all frequencies outside the resonant range. At 1000, 1200 and 1600 Hz, what little cavitation effects occur are observed within the first minute of sonication with the amount of liberated iodide stabilizing at maximum levels of 2.93×10^{-5} , 4.39×10^{-5} , 7.32×10^{-5} grams per liter, respectively. Figure 4.2 shows the total iodide liberated during 15 minutes of sonication at four different frequencies. The rate of iodide production decreases after 5 minutes of sonication and further after 10 minutes where a maximum is reached. Peak total iodide liberated stabilizes after 10 minutes at 1.84×10^{-4} . Increased initial rates of reaction can be attributed to dissolved gasses in the solution. Dissolved gasses in the solution act as prime sites for the inception of cavitation. As sonication time increases the solution outgasses and fewer cavitation inception sites are available, thus a corresponding decrease in cavitation reaction rate occurs.



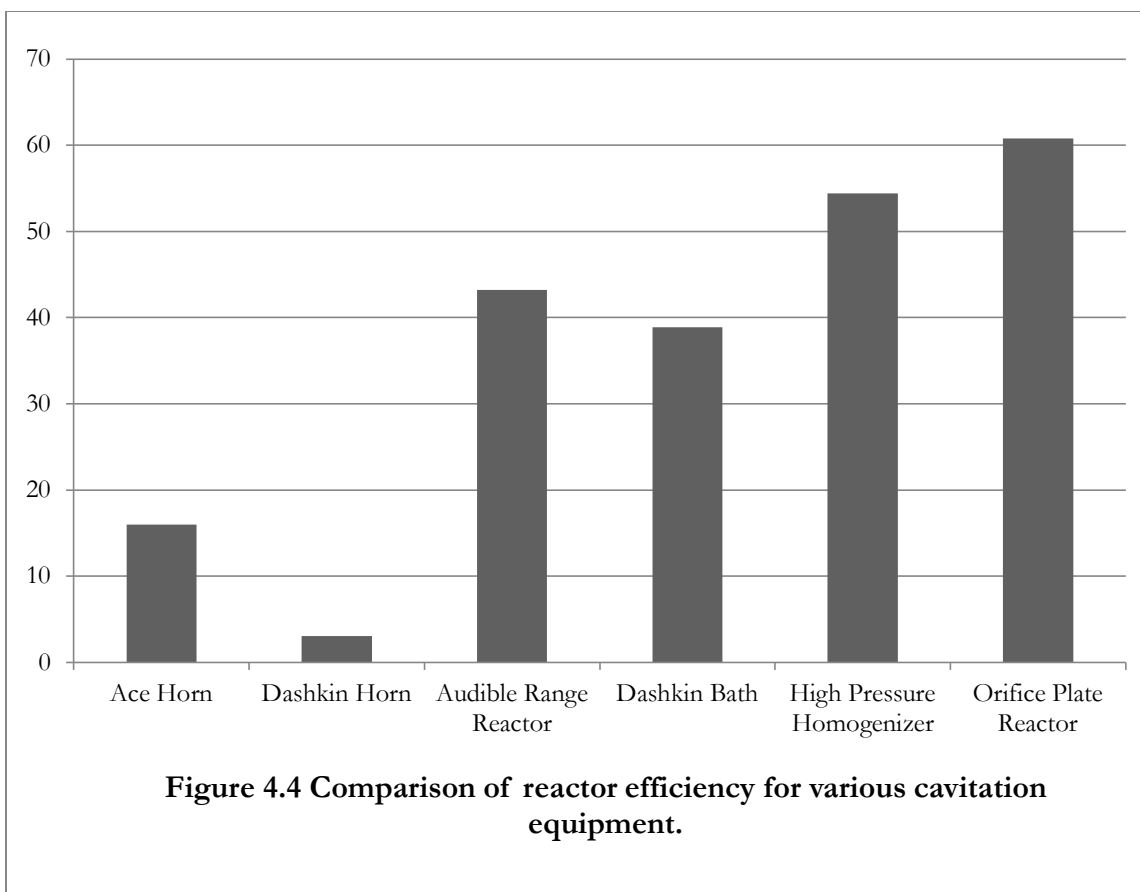
4.3 Cavitation Yield

Combining the energy output by the transducer and the amount of iodide liberated on a volumetric basis gives a measure referred to as cavitation yield. Again, the resonant operating condition results in much higher values for cavitation yield. Figure 4.3 shows the cavitation yield at four different frequencies. The cavitation yield at the resonant operating condition is significantly higher at 3.16×10^{-7} grams per joule per ml than at 1000, 1200 and 1600 Hz.



The experiments with the audible range reactor were designed with the intention of facilitating comparison between this reaction system and other cavitation equipment reported in literature. The most straight forward comparison was possible with the work of *Gogate et al. 2001*; data reported hereafter is adapted from the aforementioned publication.

In terms of energy efficiency the audible range reactor is significantly more efficient than both the Ace horn and Dashkin Horn system, 43.23% versus 16.7% and 3.04% respectively for the Ace horn and Dashkin horn. The horn based systems are only capable of treating small volumes of liquid, the cavitation intensity is also concentrated solely around the horn. The audible range system has similar efficiency to the Dashkin bath (38.86%). Functionally the two systems are similar in that flat vibrational elements attached to transducers distribute a uniform acoustic field into the liquid. A large surface area imparts the field to the liquid as opposed to the horn systems which use a vibrated probe. Two systems reported utilize the hydrodynamic method of inciting cavitation, the high speed homogenizer consists of a rotor and stator which spin at sufficient speed to incite cavitation through direct shear of the fluid over the surfaces of the device, the pilot scale orifice reactor create cavitation through rapid expansion through an orifice plate. These hydrodynamic systems show increased efficiency when compared to all acoustic systems. Figure 4.4 shows a comparison of various cavitation equipment based on the calorimetric efficiency.



A key factor to the performance of a cavitation reaction system is the ability of the system to create intense cavitation as efficiently as possible. Iodide dosimetry and the Weissler reaction allow for the quantification of cavitation intensity by measuring a desired chemical change. Combining the results from the calorimetric energy studies and the iodide liberation studies makes it possible to examine the cavitation yield of the reaction system, essentially making comparisons based on the amount of energy input and cavitation achieved. This gives an accurate portrayal of the system's ability to create cavitation.

Table 4.1 summarizes the measured and calculated operating parameters. The audible range reactor falls in the middle of the field with a cavitation yield that is higher than both acoustic horn

systems but less than the Dashkin bath. Both hydrodynamic systems far exceed the acoustic systems in terms of cavitation yield.

Table 4.3. A summary of results from various equipment used including results from *Gogate et al. 2001*

Equipment	Energy Supplied (W)	Vol (ml)	Time (min)	Calorimetric Energy (W)	Energy Eff. (%)	Iodine Liberated (g)	Cavitation Yield ($\frac{g \cdot ml}{J}$)
Ace Horn (30%)	120	50	10	15.9	16.77	$5.90 \cdot 10^{-6}$	$5.48 \cdot 10^{-9}$
Dashkin Horn	240	50	10	7.31	3.04	$1.02 \cdot 10^{-5}$	$3.53 \cdot 10^{-9}$
Audible Range Reactor	300	200	15	129.7	43.23	$1.84 \cdot 10^{-4}$	$3.16 \cdot 10^{-7}$
Dashkin Bath	120	500	10	46.63	38.86	$8.4 \cdot 10^{-5}$	$5.83 \cdot 10^{-7}$
High Pressure Homogenizer	2090	2,000	30	1,137	54.4	$6.637 \cdot 10^{-5}$	$7.38 \cdot 10^{-5}$
Orifice Plate Reactor	5500	50,000	60	3,344	60.8	$7.50 \cdot 10^{-2}$	$1.90 \cdot 10^{-4}$

The results show that hydrodynamic equipment is capable of producing higher rates of iodide per amount of energy input. Also seen, and most important to the purpose of this research is the performance of the audible range reactor in producing similar results to a bath style ultrasonic cavitation reaction system. This data however is specific to the studied reaction and may not necessarily apply to other desired chemical reactions or desired physical effects. It is however, integral to establishing that vibrations in the audible range are capable of producing efficient cavitation.

4.4 Algal Effects

The primary desired physical component of the cavitation environment created by the audible range system is the ability to disrupt the cell walls of micro algae sufficiently enough to release intra cellular matter, specifically the naturally occurring triacylglycerol.



Figure 6. Left, un-sonicated *Nannochloropsis*, centrifuged. Right, *Nannochloropsis* after sonication, centrifuging. Both samples 24 hours post centrifuge.

Figure 4.5 shows the most obvious physical effect of audible range cavitation on the selected strain of algae. Immediately after processing no changes were observed, even after centrifuging in an effort to facilitate lipid separation no release of lipids was observed. After 24 hours an unexpected result was observed; although initially the algae showed no signs of cell lysis over time the sonicated algae turned white in color. This can be attributed directly to the physical effects of cavitation degrading the cell wall and releasing some intracellular content, namely contents of the cell nearest the cell wall or within it. Clearly, sonication was able to release or at least degrade chlorophyll within the algae that gives it its green color.

Chapter 5

Concluding remarks and future directives

Although most research efforts in cavitation reactions have focused on the use of ultrasound and hydrodynamic cavitation this thesis and the work herein show, conclusively, that cavitation can be incited by an audible range acoustic field. Work also shows, however, that the cavitational yield of an audible range reactor does not far exceed that of an ultrasonic system. Furthermore, research into hydrodynamic cavitation reactions shows that large scale systems are feasible and far surpass the energy efficiency and cavitational yield of both the audible range system reported here and of a variety of ultrasound systems (*Koda et.al. 2003*).

If research into audible range systems is to continue, efforts should be made in creating larger than lab scale systems to validate the claim that audible range acoustic fields have much higher sound densities than ultrasound systems (*Gaines 1932*) and explore whether higher sound density translates to higher efficiency and higher cavitational yield. Work should also focus on further increasing the efficiency of the audible range reactor by creating more resonant structures within the reactor and focusing the energy of those resonant structures into the working liquid. It should be noted that creating resonant structures at low frequencies proves difficult at small scales; the smaller a structure becomes the higher its natural frequencies of vibration become. This makes audible range reactions not necessarily suited to research on a lab scale.

Although this work showed that cavitation can have damaging physical effects on microalgae it is not conclusive whether these physical effects were substantial enough to facilitate the separation of lipids from the biomass. The change in color of the algae after processing can definitely be attributed to some kind of physical disruption, possibly, the release of intracellular matter or other disruption of the cell wall; however a conclusive correlation cannot yet be made linking cavitation to

the facilitation of lipid separation from biomass. Future work should focus on correlating cavitation type, i.e. hydrodynamic versus sonic or ultrasound, and intensity with separation of lipids from biomass and the lysing of cells. Efforts should also be made in exploring different species of algae that may be more suited to lysis by cavitation.

The use of cavitation as a mechanism to disrupt lipid containing biomass and facilitate the extraction of lipids from the biomass is promising. The ability of cavitation reactions to eliminate the need for costly preprocessing of biomass streams could potentially be a game changer in establishing algae-based fuels as a potential alternative to petroleum based liquid transportation fuels. The greater sound density offered by audible range cavitation reaction systems shows promise as a potential large scale system for high volume processing of fully wet algae streams.

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Curriculum Vita

Joaquin Rodriguez, a native El Pasoan entered The University of Texas at El Paso in fall of 2004 after graduating from The Maxine L. Silva Magnet High School for Health Care Professions in the top ten percent of his class. Throughout his undergraduate years in the mechanical engineering program he served on the executive committee of Lambda Chi Alpha Fraternity and was a member of the Tau Beta Pi Honors Society. After receiving his bachelor's degree in spring of 2009 he began working as a research associate at the Materials Research and Technology Institute under Dr. Russell Chianelli.

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