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REMOVAL OF CYCLOHEXANE FROM A CONTAMINATED AIR STREAM USING A DENSE PHASE MEMBRANE BIOREACTOR

THESIS

Michael G. Roberts, Captain, USAF

AFIT/GES/ENV/05M-03

DEPARTMENT OF THE AIR FORCE
AIR UNIVERSITY

AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

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AFIT/GES/ENV/05M-03

REMOVAL OF CYCLOHEXANE FROM A CONTAMINATED AIR STREAM USING A DENSE PHASE MEMBRANE BIOREACTOR

THESIS

Presented to the Faculty

Department of Systems and Engineering Management

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Environmental Engineering and Science

Michael G. Roberts, BS

Captain, USAF

March 2005

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Michael G. Roberts

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Abstract

The purpose of this research was to determine the ability of a dense phase membrane bioreactor to remove cyclohexane, a volatile organic compound in JP-8 jet fuel, from a contaminated air stream using a biologically active film for degradation. The research answered questions regarding applications of membrane bioreactors, the ability of cyclohexane to diffuse through a dense phase membrane, growth of a viable microbial culture, and determination of the performance capabilities of the reactor. To answer these questions, a literature review was conducted and laboratory experiments were performed. Through the design, construction, and testing of the dense phase membrane bioreactor used for this research, it was determined that the reactor removed cyclohexane from a contaminated air stream at an average elimination capacity of 321.4 +/- 76.2 g m^{-3} hr^{-1} with a 95% confidence interval.

The successful removal of cyclohexane with the dense phase membrane bioreactor in this research effort filled a vacant niche in the scientific body of knowledge surrounding membrane bioreactor technology. Current technology applications, laboratory techniques, and data analysis are discussed.

REMOVAL OF CYCLOHEXANE FROM A CONTAMINATED AIR STREAM USING A DENSE PHASE MEMBRANE BIOREACTOR

1.0 Introduction

1.1 Background

The release of volatile organic compounds (VOCs) into the atmosphere is one of many challenging environmental problems facing the world today. When released into the atmosphere, VOCs can combine with other gases to form greenhouse gases potentially contributing to global warming (Godish, 2004). Hydrocarbons commonly found in fuels and fuel additives may be among the worst of all volatile organic compounds due to the adverse health effects associated with these compounds. Aromatic hydrocarbons such as benzene, toluene, ethyl benzene and *m*, *p*, and *o*-xylene (BTEX) have all been classified as potential human carcinogens by the American Council of Government Industrial Hygienists (ACGIH, 2004). Because of the hazardous nature of these volatile organic hydrocarbons, many have been listed as Hazardous Air Pollutants (HAPs) by the Clean Air Act Amendments of 1990, Section 112, Hazardous Air Pollutants. A chemical that has been designated as a HAP is subject to restrictive regulations set by the Environmental Protection Agency (EPA) in the National Emission Standards for Hazardous Air Pollutants (NESHAPs) (EPA, 1990).

Currently in the United States Air Force, jet propulsion fuel 8 (JP-8) is the fuel most commonly used in jet powered aircraft (ATSDR, 1998). JP-8 consists of many volatile hydrocarbons to include the benzene, toluene, ethyl benzene, xylene, cumene, cyclohexane, and naphthalene, all of which are listed as HAPs (Westbrook, *et al*, 2001). A study performed by the American Toxic Substances and Disease Registry (ATSDR)

found that exposure to JP-8 through inhalation can result in nervous system distress including headaches, anorexia, poor coordination, and difficulty concentrating (ATSDR, 1998). The vast amount of JP-8 stored and handled on Air Force installations throughout the world creates the potential for a significant release of hydrocarbons into the atmosphere. For example, volatilization occurs from JP-8 storage tanks. Currently, open venting to the atmosphere through fuel storage tank vent ports releases pressure in the tanks (UFC 3-460-03, 2003). This open venting also releases thousands of pounds of VOCs into the atmosphere each year (AFIOH, 2004).

Another source of JP-8 vapors is the result of purging aircraft fuel cells prior to entry. Fuel cells on aircraft are entered by sheet metal workers for maintenance or for repair of damaged cells. Aircraft fuel cells are considered confined spaces by the Occupational Safety and Health Administration (OSHA) due to the fuel cells being large enough for a person to get their entire body into, having restricted means of entry and exit, and not being designed for continuous employee occupancy (OSHA, 1993). Prior to workers entering a fuel cell, it must be purged, or forcefully flooded with air, for a period of time to remove any potentially harmful vapors or gases and to ensure sufficient oxygen is present. During this purging, JP-8 vapors lingering in the cell are forced out and released into the atmosphere. As regulatory pressure increases and studies continue to show the adverse health effects of JP-8, the Air Force will be forced to control atmospheric releases in an efficient and cost effective manner.

1.2 Bioremediation Techniques

Bioremediation is an environmental restoration technique that involves stimulating the growth of natural organisms, mainly bacteria, which can biodegrade contaminants (Masters, 1998). The bioremediation of environmental releases of hydrocarbons is a widely studied and accepted practice for contaminated soil, groundwater and liquid impoundments. Within the last 15 years, bioremediation of waste vapor streams has become a mature technique (Attaway, *et al*, 2001). Bioremediation of contaminated media has developed as an attractive alternative to physicochemical techniques due to low cost and complete degradation of the compound of interest (Parvatiyar, *et al*, 1996).

Systems most commonly studied and used for remediation of waste gases are conventional bioreactor designs including bioscrubbers, biotrickling filters, and packed beds (Min, *et al*, 2002). Bioscrubbers use microorganisms suspended in liquid and rely on the transfer of the pollutant from the gas phase to a hydrophobic organic phase for bioavailability to the microorganisms (DeVinny, *et al*, 1999). Biotrickling filters usually employ synthetic, inorganic growth media and receive liquid nutrient and buffer through a nozzle system positioned on top of the system (DeVinny, *et al*, 1999). In conventional packed bed bioreactor designs, microorganisms with the ability to degrade the target compound are grown on various media such as peat or compost and kept moist by trickling or spraying of water on the developed biofilm. To remove the compound from the waste gas, the gas stream is passed through the reactor where the target compound diffuses through the water layer around the microorganisms and is then degraded by various metabolic pathways, theoretically ending with the formation of carbon dioxide,

water, and adenosine triphosphate (ATP) which the microorganisms use for growth and energy (Maier, *et al*, 2000). Conventional bioreactor designs are plagued with problems, however, to include plugging, or overcrowding of biomass in filter media, gas channeling, support media acidification, toxic cometabolism, backpressure fluctuations, and difficult moisture control due to evaporation at high flow rates (Attaway, *et al*, 2001).

Though not as widely used as conventional bioreactor designs, membrane bioreactors are also used to treat contaminated air. Membrane bioreactors have a diffusive membrane immersed in a liquid bath that provides support for biological growth and efficient target compound transport to the biofilm for degradation. A membrane allows separation of the liquid and gas phases of the reactor. By separating the phases, the problems of plugging, gas channeling, toxic cometabolism, and difficult moisture control observed in conventional bioreactor designs are eliminated (Reij, *et al*, 1998).

Two types of membrane bioreactors have been studied recently, to include hollow fiber microporous membranes and dense phase membranes. Hollow fiber membranes utilize hydrophobic, microporous membranes as the support/transport structure. These are membranes with pore sizes of approximately 0.5 μm through which waste gases are passed to a biofilm grown on the outside of the membrane which degrades the target compounds (Ergas, *et al*, 1999). While less problematic than conventional designs, hollow fiber membranes are prone to plugging of the pores and often their use is prohibited due to high cost. Dense phase membranes utilize nonporous hydrophobic materials such as silicone rubber that exhibit high permeability to oxygen and hydrophobic organic compounds (Attaway, *et al*, 2001). Dense phase membranes such as silicone are readily available from medical suppliers, significantly less expensive than

hollow fiber membranes, and not prone to plugging of pores. Dense phase membranes have been shown in recent research to provide similar contaminant removal rates in comparison to hollow fiber membranes, and have shown up to 30% better removal of contaminants in comparison to conventional bioreactor designs (Attaway, *et al*, 2001).

1.3 Research

A dense phase membrane bioreactor was assembled and tested for its removal of cyclohexane vapors from a contaminated air stream. Cyclohexane was used as a representative compound of the cyclic alkanes present in JP-8. Aromatic compounds such as benzene and toluene diffuse through silicone resulting in successful bioreactor tests similar to the tests performed in this research (England, 2003; Attaway, *et al*, 2001). Cyclohexane is a light non-aqueous phase liquid, so its hydrophobicity made it a prime candidate for diffusion through a dense phase membrane. The biofilm employed for the degradation of the cyclohexane vapors was derived from a combination of microorganisms in a composted soil sample, microorganisms found by Air Force Research Laboratory (AFRL) researchers in a JP-8 storage tank at the Paramount Refinery in Los Angeles, California, and from activated sludge obtained from the Fairborn, Ohio waste water treatment plant.

1.4 Research Objectives

The overall objective of this research was to determine if a dense phase membrane bioreactor could successfully remove the volatilized components of JP-8 jet

fuel, specifically cyclohexane, from a contaminated air stream. During the course of this research effort, a number of questions were answered:

1. What is a membrane bioreactor and where is this technology being applied?

2. Would cyclohexane diffuse through the dense phase membrane?

3. If diffusion occurred, would the biofilm derived from the Paramount Refinery, composted soil, and activated sludge grow on the dense phase membrane and eventually degrade the cyclohexane?

4. If successful degradation occurred, what was the removal rate the membrane bioreactor could achieve?

1.5 Methodology

The overall research methodology employed to answer the research questions involved several components. The following are the main methodological steps:

1. A comprehensive literature review was conducted with emphasis on bioreactor technology, specifically current uses of dense phase membrane bioreactors in remediation roles.

2. A membrane bioreactor was set up to test the removal of cyclohexane vapors from a contaminated air stream.

3. The diffusion of cyclohexane through the membrane in the reactor, prior to biofilm establishment, was tested by analysis of influent and effluent water and air streams using gas chromatography and flame ionization detection.

4. A viable batch of cyclohexane-degrading microorganisms was grown and used to inoculate the bioreactor for growth of the biofilm on the membrane's outer surface.

5. The removal of cyclohexane from the contaminated air stream was measured as the biofilm grew to visible thickness and thereafter for an extended period of time. Reactor robustness and response was tested by varying air flow rates and influent load.

1.6 Study Scope and Limitations

1. This study focused on dense phase membrane technology, not hollow fiber membranes or conventional bioreactors. In particular, a dual tube dense phase reactor was used.

2. Cyclohexane was used a compound representative of the cyclic alkanes contained in JP-8. Other compounds, such as aromatics and straight chain alkanes, have been shown in recent research to transfer across dense phase membranes (Attaway, et al, 2001, England, 2003, and Cole, 2001). By proving the ability of the dense phase membrane to transfer cyclic alkanes, a step toward establishing the ability of all compounds in JP-8 to transfer and be degraded was made. However, demonstrating the ability of the membrane to transfer isolated compounds does not prove its effectiveness when loaded with multiple compounds at the same time. Further research should be performed to demonstrate the ability to transfer all compounds together prior to fielding a similar design.

3. Due to the small scale of the test bioreactor, actual results may not scale up directly to a full size operational unit. Further experimentation should be performed on a full scale unit prior to field deployment.

1.7 Significance

This research expanded the body of knowledge currently existing for membrane bioreactor technologies. The successful demonstration of this system was a step forward in the quest for more sustainable control technologies that could some day be used by the Air Force, the Department of Defense, and the civilian sector. Membrane bioreactors excel in sustainability, ease of design, construction, and field application. This technology requires very little external input of energy or fuel, allows for complete conversion of wastes, and can be adapted to a wide range of pollutants. Membrane bioreactors could be the wave of the future for environmental control technology.

1.8 Summary

Jet propellant-8, the jet fuel of choice for the United States Air Force, contains many compounds considered hazardous air pollutants due to their volatility and potential adverse health effects. Current practices in use by the Air Force allow for significant release of these compounds. To control these releases, a sustainable, low cost control strategy should be developed and employed. Membrane bioreactor technology could be a viable technology to fulfill this role. This research examined the applicability of a membrane bioreactor to this problem and demonstrated the removal efficiency of a laboratory scale membrane bioreactor for a representative compound. Through continued research on membrane systems similar to the one tested in this research, the successful development and deployment of such systems throughout the Air Force could soon prove a viable option for air pollution control in the future.

2.0 Literature Review

2.1 Introduction

The goal of this research was to examine the impacts of volatile organic compounds from JP-8 jet fuel on the environment and human health and to design an air pollution control system capable of removing those volatile components from a contaminated air stream. To accomplish these goals, a multidisciplinary research approach was pursued. This research included a study of JP-8 jet fuel including its uses, storage, and environmental fate and transport, a study of applicable regulations governing potential emissions from JP-8 use and storage, current air pollution control technologies capable of controlling volatile organic emissions, and sections devoted to the design of the membrane bioreactor used in this project.

2.2 Study of Jet Fuel

In this section, JP-8 is examined from its development and use throughout the United States Air Force (USAF) and North Atlantic Treaty Organization (NATO) operations, the environmental fate and transport of JP-8 in various systems, human health effects associated with exposure to jet fuel, and the determination to use cyclohexane as a representative compound for JP-8 in laboratory tests.

2.2.1 Development, Usage, and Storage of JP-8

Jet propulsion fuel 8 was developed by the USAF following the Vietnam conflict in the 1970's. During the Vietnam conflict, jet propulsion fuel 4 (JP-4) was used to

power the United States' primary fighter and jet powered bomber aircraft. JP-4 was essentially a mix of aviation gasoline and high flash point kerosene (BP Fuels, 2004). While JP-4 was an effective propellant, it was also highly explosive, as observed during the Vietnam conflict. JP-8 was developed as a lower flash point blend similar to Jet A-1 fuel used in commercial aircraft. JP-8 is essentially Jet A-1 with added fuel system icing, corrosion, and static dissipation inhibitors (Army Fuel Guide, 2000). The lower flash point of JP-8 was desirable due to the increased fire safety of JP-8 in comparison to JP-4 (BP Fuels, 2004).

The USAF began its implementation of JP-8 to all jet powered aircraft in 1979, and completed this transition in 1995. JP-8 is covered by the specification MIL-DTL-83133. JP-8 is currently the fuel specified for all U.S Air Force and U.S. Army turbine powered aircraft, as well as all NATO turbine powered aircraft. The NATO designation for JP-8 is F-34 jet fuel (Army Fuel Guide, 2000). JP-8 is very similar to kerosene, and kerosene data is often used as surrogate data for JP-8 in many environmental remediation and health effect roles (ATSDR, 1998).

2.2.2 Environmental and Health Effects of JP-8

JP-8 and the Navy's equivalent fuel, JP-5, were detected at 22 of the 1,445 sites listed on the National Priorities Listing (NPL) sites throughout the United States (ATSDR, 1998). NPL sites are those sites designated by the Environmental Protection Agency as the country's most polluted sites under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly known as Superfund, which was enacted on December 11, 1980 (CERCLA Overview, 2004). The detection of

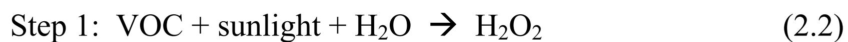
jet fuel spurred the need for a comprehensive health effects study. This study was performed and completed by the Agency for Toxic Substances and Disease Registry (ATSDR) in August of 1998.

The ATSDR study examined JP-8's environmental fate and found that environmental effects vary widely due to the diverse nature of environmental behavior among the chemicals in the fuel (ATSDR, 1998). JP-8 consists of some highly water soluble chemicals that are likely to be transported by groundwater flow following releases from underground storage tanks or leaking distribution systems. Other constituents are less soluble, but readily partition and sorb to soil particles due to their affinity for organic substrates, calculated for hydrogeological transport systems as K_{oc}, the partition coefficient of a compound between organic carbon and water (Domenico and Schwartz, 1998).

This research focused on constituents that are readily volatile and easily released from JP-8 storage systems such as benzene, toluene, ethyl benzene, xylene isomers, and the four to ten carbon alkanes and low carbon number cyclic alkanes. These easily volatilized chemicals, when released into the atmosphere and combined with nitric oxide compounds, undergo a photo oxidation reaction in which the organics are readily converted into ozone, the precursor to photochemical smog as shown in Equation 2.1 (Masters, 1998).

$$NO_x + VOC + Sunlight \rightarrow O_3 \qquad (2.1)$$

Another potential fate of volatile organic release is the formation of acid rain through a two step chemical reaction. The first step combines the VOCs with water and photo oxidizing sunlight to form hydrogen peroxide, and the second step consists of the hydrogen peroxide combining with sulfur oxides and ozone to form acidic compounds which precipitate out as acid precipitation, as seen in Equations 2.2 and 2.3 (Acid Rain, EPA, 2004).



$$\text{Step 1: } VOC + \text{sunlight} + H_2O \rightarrow H_2O_2 \quad (2.2)$$

$$\text{Step 2: } SO_2 + H_2O_2 + O_3 \rightarrow H_2SO_4 \quad (2.3)$$

A third potential fate for atmospheric release of volatile organic compounds is a surface water load created by the volatile compounds solubilizing in airborne precipitation and falling back down to the earth, entering into the water cycle (ATSDR, 1998). Though the solubility of most volatile organics is low, on a grand scale this could lead to a significant surface water load.

Human exposures to constituents of JP-8 released into the environment could result from intake of contaminated drinking water, breathing of contaminated air, or through dermal contact with contaminated soils. Though the exact metabolic pathways of many jet fuel constituents are not completely understood, research has been conducted on kerosene exposure. Extensive kerosene exposure causes vomiting, diarrhea, swelling of the stomach, stomach cramps, drowsiness, restlessness, irritability, and loss of consciousness (ATSDR, 1998). Coughing, pneumonia, and difficult or painful breathing after drinking kerosene suggest that kerosene has entered the lungs. In addition, drinking

large amounts of kerosene has resulted in patients entering comas, experiencing convulsions, and may even cause death. Dermal kerosene exposure has shown to make skin itchy, red, and sore, and has even shown to result in blistering and peeling of the skin in sensitive individuals (ATSDR, 1998). Case studies of JP-5 exposure have shown nervous system effects including headaches, lightheadedness, anorexia (loss of appetite), poor coordination, and difficulty concentrating. Laboratory tests have also shown that continual dermal exposure to JP-8 and JP-5 cause cancer in mice, but there have been no tests showing development of cancer in humans (ATSDR, 1998).

The Air Force's Occupational Exposure Limit (OEL) for exposure to JP-8 through inhalation is set at 350 mg m^{-3} averaged over an 8 hour work day, and the Short Term Exposure Limit (STEL) is 1800 mg m^{-3} averaged over a 15 minute exposure period (Smith, 1999). The ATSDR has derived an intermediate-duration inhalation Minimal Risk Level (MRL) of 300 mg m^{-3} for JP-5 and JP-8. An MRL is an estimate of daily human exposure to a noncarcinogenic substance over a specific period that is likely to be without an appreciable risk of adverse effects (ATSDR, 1998). The occupational exposure levels set by the Occupational Safety and Health Administration (OSHA) for the allowable airborne concentration of petroleum products in a workroom during an 8 hour day, 40 hour work week is 400 mg m^{-3} (ATSDR, 1998). This exposure limit is often applied to occupational JP-8 exposure.

2.2.3 Cyclohexane Use and Properties

For the purpose of this research, cyclohexane, a cyclic alkane present in JP-8, was used as a compound representative of the most volatile constituents of JP-8.

Cyclohexane is primarily used in the production of adipic acid, a nylon intermediate. It is also used in many solvent applications, as fuel for camp stoves, and as an ingredient in fungicidal applications (Cyclohexane, EPA, 1994). There are only four producers of cyclohexane in the United States, including Champlin, Chevron, Phillips, and Texaco. All production plants are located in southeast Texas. Environmental presence of cyclohexane can result from releases of crude oil, volcanoes, tobacco smoke, and exhaust gases from automobiles (Cyclohexane, EPA, 1994). Cyclohexane is considered volatile, with a vapor pressure of 77 mm of mercury. The Henry's constant for cyclohexane is 0.195 atm m^3 mol^{-1} at 25 ºC, which indicates that cyclohexane readily partitions from the aqueous phase to the gas phase at equilibrium conditions (Cyclohexane, EPA, 1994). The estimated K_{OC} for cyclohexane is 482, which indicates a moderate potential for solid adsorption (Cyclohexane, EPA, 1994). Cyclohexane is slightly soluble in water, with a solubility of 55 mg L^{-1} (Cyclohexane, EPA, 1994).

Health effects of cyclohexane include microscopic liver and kidney damage from dermal exposure in rabbits, and human case studies have shown that approximately 23% of inhaled concentrations of cyclohexane are absorbed through the lungs and metabolized (Cyclohexane, EPA, 1994). Distribution of cyclohexane in rabbits was found mostly in fatty tissue and some brain distribution, with concentrations 50 to 80 times higher in fatty tissue (Cyclohexane, EPA, 1994). Cyclohexane is metabolized through the hepatic, vascular, and renal systems, with microsomal hydroxylases oxidizing cyclohexane to cyclohexanol in the presence of nicotinamide adenine dinucleotide phosphate with hydrogen (NADPH) and oxygen (Cyclohexane, EPA, 1994; Fox, 2004). Other metabolites of cyclohexane in mammals include trans-cyclohexane-1,2-diol,

cyclohexanone, and adipic acid (Cyclohexane, EPA, 1994). Once metabolized, cyclohexane is eliminated either as unchanged cyclohexane or as one of its metabolites in the urine. Cyclohexane has shown to be a nervous system depressant in humans at high concentrations causing dizziness and unconsciousness and is listed as a hazardous air pollutant (HAP) under the Clean Air Act Amendments of 1990. OSHA has set a permissible exposure limit for cyclohexane at 300 ppm over an 8 hour time weighted average work day (Cyclohexane, EPA, 1994). The American Conference of Governmental Industrial Hygienists (ACGIH) has also set a Threshold Limit Value (TLV) of 300 ppm over and 8 hour time weighted average (OSHA, 2004). The properties of cyclohexane are summarized in Table 2.1.

Table 2.1: Cyclohexane Properties (Cyclohexane, EPA, 1994)

Characteristic/Property	**Data**
CAS No.	110-82-7
Synonyms	hexahydrobenzene, hexamethylene, hexanapthene
Molecular Formula	C_6H_{12}
Physical State	Liquid
Molecular Weight	84.16
Melting Point	6.47 ºC
Boiling Point	80.7 ºC @ 1 atm
Water Solubility	55 mg L^{-1} at 25 ºC
Density	0.7781
Vapor Density (Air = 1)	2.9
K_{oc}	482
Log K_{ow}	3.44
Vapor Pressure	77 mm Hg at 20 ºC
Reactivity	flammable, reacts with oxidizing materials
Flash Point	18 ºC
Henry's Law Constant	0.194 atm m^3 mol^{-1} @ 25 ºC
Fish Bioconcentration Factor	240
Odor Threshold	300 ppm in air

2.3 Applicable Rules and Regulations

This section presents the rules and regulations governing the release of volatile organic compounds. The overarching federal regulation that governs releases of volatiles is the Clean Air Act. Through various amendments to the Act since its inception in 1970, regulations have become increasingly more restrictive as research advances the body of knowledge available concerning the pollutants' impacts on human health and the environment. Many of the most volatile compounds in JP-8 are considered hazardous air

pollutants (HAPs) due to their toxicity either to humans or environmental systems. This section includes an analysis of the Clean Air Act, the amendments made in 1990, the determination of hazardous air pollutants and regulations pertaining to their control, and the application of membrane bioreactors as a viable control technology for JP-8 emissions.

2.3.1 Air Pollution Regulation

The environmental regulatory structure in the United States is best described as centralized command with decentralized control. The Federal government, through the Environmental Protection Agency, promulgates Federal regulations that serve as minimum standards for state implementation. The states are then responsible for enforcement of Federal laws, as well as establishment of any extra stipulations required specifically for those states due to local environmental stresses. Extra stipulations to Federal regulations are enacted by state legislatures and promulgated in state regulations. The structure of the environmental regulatory system is set this way due to the nature of environmental concerns. In general, environmental concerns are local issues that require local knowledge of polluting systems in relation to sensitive ecosystems or potentially exposed populations for effective regulation (Clean Air Act, EPA, 1993).

Air pollution regulation in the United States began in 1955 when the Clean Air Legislation authorized the Public Health Service, then in the Department of Health, Education, and Welfare, to conduct air pollution research and training for state programs (Godish, 2004). In 1963, Congress passed the original Clean Air Act in response to declining air quality in the postwar United States. This original act mainly provided for

increased and better funded research as well as increased regulatory authority from the federal government (Godish, 2004). In 1967, the Air Quality Act was passed. This act required the National Air Pollution Control Administration (NAPCA) to establish air quality criteria and issue control technique information. Since air pollution control was still a relatively new science at that time, it took years for NAPCA to develop this guidance, and the Air Quality Act of 1967 was largely ineffective at the time (Godish, 2004).

The first set of amendments to the Clean Air Act came in 1970, when public concern for environmental problems was at the forefront of American life and politics. The amendments of 1970 transferred the responsibilities of the NAPCA to the newly formed EPA, required the establishment of National Ambient Air Quality Standards (NAAQS), and required that states develop State Implementation Plans (SIPs) for control of atmospheric releases (Godish 2004). Another set of amendments to the Clean Air Act was passed in 1977. Of the provisions outlined in these amendments, the most notable was the authority given to the EPA to regulate stratospheric ozone destroying chemicals. The regulation and banning of these chemicals is widely known as an environmental regulatory success story (Godish 2004). The most significant amendments to the Clean Air Act came in 1990. Under the 1990 amendments, increased emphasis was applied to regulations of motor vehicle emissions, regulation of hazardous and toxic air pollutants, acidic deposition control, stratospheric ozone protection, permitting requirements, and enforcement (Godish 2004).

2.3.2 Permitting Challenges

The permitting system set in place by the Clean Air Act Amendments of 1990 requires owners of atmospheric pollution sources to acquire and maintain permits restricting the amount of pollution they release over given time periods. This system allows the atmosphere in localized areas to be analyzed and regulated based on total pollutant load. The Clean Water Act controls pollution in much the same way, requiring National Pollutant Discharge Elimination System (NPDES) permits prior to release of the chemicals of concern for that area (Clean Air Act, EPA, 1993). Permits are particularly stringent in those areas that are out of compliance for release and atmospheric concentration of pollutants, known as Non-Attainment Areas (NAAs). Many military bases are located in or near NAAs, and permitting on these bases can present many challenges to base operations.

2.3.3 Hazardous Air Pollutants

The most significant provision of the 1990 amendments was the identification and specific regulation of 189 hazardous air pollutants. Prior to the 1990 amendments, only 7 HAPs had been identified. Chemicals were identified as hazardous or toxic air pollutants due to their potential for hazard to health (carcinogenicity) or the environment. By using hazard to the environment as a criteria for identification of a chemical as a hazardous air pollutant, Congress and the EPA's intent to protect wildlife, aquatic life, and natural resources was illustrated (Godish, 2004). For control of HAPs, regulations require the use of Maximum Available Control Technology (MACT). MACT is to be achieved through process changes, enclosure of polluting operations, collection and treatment of

released pollutants, or design, equipment, or work practice modifications (Godish, 2004). The use of MACT for the control of HAPs is directly applicable to this research effort. Of the volatile emissions identified in JP-8, those listed as hazardous air pollutants include benzene, 1,3-butadiene, cumene, ethyl benzene, hexane, naphthalene, toluene, 2,2,4-trimethylpentane, xylene isomers, and cyclohexane (Westbrook, *et al*, 2001 and Hazardous Air Pollutants, EPA, 2004).

For sources of hazardous air pollutants, emissions are regulated based on the total atmospheric load of the pollutant of concern on a yearly basis. As of now, none of the JP-8 storage tank farms are listed as major sources. Typical emissions from a 40,000 gallon storage tank result in atmospheric loads of less than 10 pounds of volatile organic load to the atmosphere per year (AFIOH, 2004). This research effort is specifically focused on the development of membrane bioreactor control technology's application to controlling emissions from the hundreds of storage tanks throughout the world. The current practice of open venting of JP-8 storage tanks to release pressure could be considered a significant release of volatile organic compounds when viewed on the grand scale of loading from all of the storage tanks all over the world (UFC, 2003). The use of a membrane bioreactor to break down these volatile emissions as they are released from the storage tanks would serve as a great leap in establishment of an effective, low maintenance Maximum Available Control Technology to ease regulatory pressure on bases throughout the country and potentially decrease permitting requirements in non-attainment areas, saving installations money and manpower.

2.4 Existing Air Pollution Control Technologies

In this section, existing air pollution control strategies that could be applied to the volatile JP-8 tank release are examined. Currently there are a number of processes being used to remove volatile organic compounds from contaminated effluent gas streams. However, of the existing remediation technologies being used today, many require disposal of contaminated media, input of additional fuel to operate, or present safety hazards that would exclude them as applicable control measures. In this overview of conventional systems, the theory of operation, advantages, and disadvantages of carbon adsorption, incineration, conventional bioreactors, and membrane bioreactors will be presented.

2.4.1 Carbon Adsorption Systems

The process of adsorption involves molecular diffusion of gas phase contaminants from the gas stream to the surface of a sorbent medium where they are bound by van der Waals forces (Godish, 2004). Adsorption systems utilize a wide variety of sorbent media to include activated carbon, silica gel, activated alumina, and zeolites. Activated carbon is most widely used in adsorption systems due to its high affinity for nonpolar compounds with molecular weights of 45 or greater, its low cost, and its relative insensitivity to the presence of water vapor in gas streams (Godish, 2004 and Zerbonia, *et al*, 1995). Carbon is "activated" through high temperature oxidation of wood or coal in the absence of oxygen (Godish, 2004).

Carbon adsorption systems have many advantages. These systems have proven to be very effective, with 99% removal of VOCs attainable (Zerbonia, *et al*, 1995). Carbon

adsorption systems are predictable, with constant effluent concentrations achievable even with widely varying influent loads. Lastly, carbon adsorption systems are easily tailored to the process they are being used to control, with predictable break through times based on pollutant load (Zerbonia, *et al*, 1995). These advantages have made carbon adsorption systems popular for gaseous pollutant control in the past.

While carbon adsorption systems do a great job of removing the VOC from the gas phase, the owner of the process is still responsible for disposal or treatment of the contaminated media, which results in increased cost and maintenance. Carbon adsorption systems have also shown variable results in low VOC concentration applications. For virtually every adsorption application, removal efficiency is enhanced by lower operating temperatures and higher organic concentration loads (Zerbonia, *et al*, 1995). Due to the dependence of adsorption systems on particle diffusion as the main transport process involved in removal, fewer contaminant particles diffusing (low concentration) results in decreased likelihood of interaction with the adsorbing surface (Zerbonia, *et al*, 1995). Due to the difficulty of removal of low concentration VOCs and increased complication of remediation from disposal of contaminated media, carbon adsorption systems may not be optimal for use in low VOC emission systems, such as JP-8 storage tanks.

2.4.2 Incineration

Thermal oxidation, or incineration, is a process that converts hydrocarbon or oxygenated waste compounds to carbon dioxide and water (Godish, 2004). Waste gases are preheated and injected into a reaction chamber where they are exposed to extremely high temperatures which oxidizes the waste. There are two main types of incineration

systems, thermal oxidation and catalytic oxidation. Thermal oxidation consists of superheating the waste stream to temperatures of 750 to 1000 ºC. Catalytic oxidation consists of passing the waste stream over a catalytic bed which lowers the activation energy required to destroy the molecule, then heating the stream to 350 to 500 ºC (Zerbonia, *et al*, 1995). For low VOC concentration systems, incineration systems require extra fuel to sustain combustion of the waste.

Incineration systems provide complete destruction of waste compounds, so secondary treatment of contaminated media is not required, as it is with carbon adsorption systems (Zerbonia, *et al*, 1995). However, incineration systems are plagued with complications. The fuel required to sustain combustion results in increased operating costs and handling of another potentially hazardous product. Incineration systems can also be adversely affected by temperature fluctuations of influent waste streams, fluctuations in influent waste concentrations, fouling from particulate matter or polymers, as well as deactivation of catalyst materials in catalytic incinerators (Zerbonia, *et al*, 1995). Another obvious disadvantage of using an incineration system at a fuel tank farm is the hazard of explosion. A localized, tank top system could never be safely utilized at the operating temperatures required for thermal oxidation. Due to the explosion hazard, high operating costs, and variability of volatile organic compound release from JP-8 storage tanks, incineration is not an applicable control technology.

2.4.3 Conventional Bioreactors

Bioreactors are systems which take advantage of the ability of microorganisms to destroy most organic compounds and mineralize these compounds into carbon dioxide,

water, microbial growth, and inorganic salts. Bioreactors used for remediation of contaminated gas streams utilize either attached boils or aqueous suspended microorganisms as the growth medium and require only moisture, a carbon source (waste), and macro and micronutrients to support growth. Boilers rely on the establishment of a concentration gradient between the waste stream and the biofilm or aqueous biological solution which creates the driving force behind diffusion and subsequent degradation. Three types of conventional bioreactors will be discussed in this section: boilers, bioscrubbers, and biotrickling filters.

2.4.3.1 Boilers

Boilers are systems in which a humid polluted gas stream is passed through a packed media bed. The packed bed typically consists of organic material such as peat or wood, covered by a degrading biofilm. The biofilm is a microcolony of bacterial cells attached to a surface and encased in adhesive polysaccharides excreted by the cells. Biofilms trap nutrients for growth, and the polysaccharide casing prevents damage to the colony that could be the result of hydraulic forces created in flowing environments (Madigan, *et al*, 2003). The bacterial cells within biofilms communicate via biofilm specific genes which encode proteins that serve as communication packets among the cells. Through this intercellular communication, cells within the biofilm begin fulfilling their specific roles, from production of the polysaccharide casing to producing chemotactic agents whose purpose is to recruit nearby compatible cells (Madigan, *et al*, 2003).

The microorganisms in the biofilm obtain macro and micronutrients from the organic growth media, obtain carbon from the pollutant, and obtain the moisture needed from the humidity in the waste stream. As the waste stream passes through the bed, contaminants are absorbed into the biofilm and to the support media, where they are degraded and a favorable concentration gradient is established. Biofilters utilize absorption, adsorption, and degradation processes to remediate contaminated effluents (DeVinny, *et al*., 1999). Biofilters exhibit rapidly adaptable removal when exposed to varying influent concentrations as one would expect from actual processes. In a matter of a few hours, biofilters are capable of "ramping up" effectively to increased pulse or step loads (Deshusses, *et al*, 1996).

Difficulties associated with conventional biofilter design are the control of moisture, prevention of channeling in the system, and controlling increasing pressure drop in the reactor. Constant moisture content must be maintained in the waste stream to prevent drying of the bed and ultimately killing of the microorganisms. Also, the biological growth has been observed to build up in areas, essentially increasing resistance to flow along some pathways and channeling the flow through the reactor. Channeling leads to increased waste stream velocity through the bed resulting in decreased residence time, short-circuiting the removal process. As the biological growth becomes denser, increased pressure drop across the bed is commonly observed (Thalasso *et al*, 2001). Table 2.2, adapted from the DeVinny text, lists advantages and disadvantages of conventional biofilters.

Table 2.2: Conventional Biofilter Summary (Devinny, *et al*, 1999)

Advantages	***Disadvantages***
Simple, flexible design with low operating and capital costs	Large surface are required
Low pressure drop	Particulate matter may clog medium
No further waste streams produced	Moisture and pH difficult to control
Suitable for low pollutant concentrations in waste air	Less suitable for high concentrations
	Dissolution of gas into liquid is the rate limiting step, long residence times required

2.4.3.2 Bioscrubbers

Bioscrubbers utilize suspended aqueous biological growth to degrade contaminants. Bioscrubbing can be performed in stirred tank, spray tower, or bubble column configurations (DeVinny, et al, 1999). As with biofilters, water solubility of the target contaminant often becomes the rate limiting process. Reliability of bioscrubbers is lower than biofilters due to washout of the process. Washout occurs when the influent mass flow rate of the carbon source does not allow for the growth rate of the microorganisms to keep up with the effluent mass flow rate of the liquid medium (Yeom and Daugulis, 2001). Table 2.3 lists advantages and disadvantages associated with bioscrubbing devices.

Table 2.3: Bioscrubber Summary (Devinny, *et al*, 1999)

Advantages	***Disadvantages***
No medium disposal required	High operating costs
Ability to handle variable loads	Need for complex chemical growth media
Moderate capital cost	Reliant on good gas dissolution, thus, it removes only highly soluble contaminants efficiently
Can handle high flow rates	Mechanical maintenance often required

2.4.3.3 Biotrickling Filters

Biotrickling filters use both microorganisms fixed in a biofilm and suspended microorganisms to degrade contaminants. Biotrickling filters are generally constructed in packed tower arrangements, with a biofilm growing on the packing material and suspended microorganisms in water that is constantly recirculated (trickled) over the packed media. Waste gas flows either co-current or counter current to the water flow. Contaminants that absorb into the liquid phase from the gas phase are metabolized by the suspended organisms, and contaminants that adsorb to the biofilm are degraded by the film taking advantage of processes utilized by both biofilters and bioscrubbers (Devinny, *et al*, 1999). In a study of ethyl acetate removal during polyurethane manufacturing, it was observed that biotrickling filters allow for better control of pressure drop, pH, and nutrient feed in comparison to other biofiltration techniques, and the use of synthetic media provided increased longevity over natural media used in other configurations

(Chang, *et al*, 2001). While biotrickling filters have advantages over other biofiltration devices, excessive biomass growth leading to clogging of the filters remains a problem. Clogging was controlled by both decreased nutrient addition (which ultimately led to decreased removal) as well as bed washing with sodium hydroxide (Weber and Hartmans, 1996). Table 2.4 lists advantages and disadvantages of biotrickling filters.

Table 2.4: Biotrickling Filter Summary (Devinny, *et al*, 1999)

Advantages	***Disadvantages***
Moderate operating and capital costs	Dissolution of gas into liquid is the rate limiting step, so long residence times are required
Effective removal of pollutants	Further waste streams produced
Effective removal of acid producing pollutants	Clogging by biomass
Low pressure drop	Increased structure maintenance

2.4.4 Membrane Bioreactors

Membrane bioreactors utilize a permeable membrane to separate the liquid and gas phases of the reactor vessel. The basic premise of using a membrane is to create a controllable, predictable delivery method to transport gaseous nutrients and/or substrate to an attached biofilm. The hydrophobic membrane acts as the contaminant or nutrient transport medium, allowing gaseous contaminants to diffuse through the membrane while preventing water from passing through (Devinny, *et al*, 1999). Membrane technology has been applied in many environmental remediation roles including pollutant extraction from wastewater, wastewater aeration systems, and recently gas phase contaminant

removal (Reij, *et al*, 1998). Membrane bioreactors allow for complete separation of the aqueous and gaseous phases in the system, allowing for more reliable control of operating parameters in the system. The moisture control, pH control, and pressure drop increase that plague conventional bioreactor configurations are virtually eliminated with membrane systems due to the separation of gas and water phases. Membrane systems also have an advantage over conventional reactors because they do not rely on diffusion of the contaminant into the water phase prior to degradation. As the biofilm is established on the outer surface of the membrane, the hydrophobic contaminants are able to pass from the membrane directly into the biofilm without complete solubilization required, greatly increasing mass transfer ability in the reactor (Reij, *et al*, 1998). Disadvantages of membrane systems include high construction costs (particularly due to the use of hollow fiber microporous membranes), and lack of data on long term reliability of the systems (Reij, *et al*, 1998). Table 2.5 lists advantages and disadvantages of membrane bioreactor systems (Rishell, 2002).

Table 2.5: Membrane Bioreactor Summary (Rishell, 2002).

Advantages	***Disadvantages***
Moisture and pH easily controlled	High construction costs of HFMB's
Pressure drop stays constant	Long term reliability undetermined
Increased mass transfer ability	
Degradation in biofilm and in suspended culture	
Gas and water flow rates easily variable for exact retention times	

2.5 Membrane Technology

Two functional classes of membrane bioreactors are currently in use in environmental remediation roles: extractive membrane bioreactors (EMB's) and membrane aerated systems (Attaway, *et al*, 2001). Extractive membrane bioreactors rely on contaminant transport across the membrane from a contaminated gaseous phase to a microorganism rich water phase where the contaminants are degraded. A membrane aerated system supplies oxygen or essential gaseous nutrients to a liquid solution in which a biofilm is being used to degrade a liquid contaminant. In this section an overview of membrane usage in wastewater and groundwater remediation applications will be presented as well as an in depth analysis of their applications in gas phase pollutant remediation.

2.5.1 Membrane Usage in Wastewater Treatment

In a 2003 study performed by Semmens, *et al*. at the University of Minnesota, wastewater contaminated with ammonium acetate was remediated. The most unique aspect of the bioreactor used in this research was its ability to remove both chemical oxygen demand (COD) and complete mineralization of ammonium acetate in one reactor. This is unique because the complete mineralization of ammonium acetate requires both nitrification (aerobic process) and denitrification (anaerobic process). Nitrification is the conversion of ammonium to nitrate and nitrite using oxygen as the electron acceptor. Denitrification is the conversion of the nitrate and nitrite into nitric oxide, nitrous oxide, and nitrogen gas using nitrate as the electron acceptor (Maeir, *et al*, 2000). Both are accomplished in membrane aerated bioreactors because the biofilm that grows on the membrane becomes stratified. The biofilm closest to the membrane is rich in oxygen making nitrification possible. The nitrification taking place close to the membrane uses up most of the oxygen, so the biofilm farthest from the membrane is oxygen poor making denitrification possible (Semmens, *et al*, 2003).

For Semmens' research effort, a 7.0 L reactor was constructed using two vertical 63 mm i.d. polyvinyl chloride (PVC) tubes connected together in parallel. The membrane module inside each PVC tube consisted of four individually potted bundles of polyethylene hollow fiber microporous membranes from Mitsubishi Rayon Corporation. The membrane fibers had an outside diameter of 280 µm, and each fiber bundle consisted of 400 fibers (Semmens *et al*, 2003). The bioreactor was seeded with a sample of activated sludge from the municipal wastewater treatment plant in St. Paul, MN. The influent consisted of a solution of ammonium acetate with a nutrient solution of basal

mineral salts at varying flow rates from 31.4 to 62.8 mL min^{-1}. Air was supplied to the membrane at a rate of 4.2 L min^{-1} (Semmens, *et al*, 2003). Analysis was performed using standard methods for pH, alkalinity, total suspended solids, ammonia, nitrate, and COD over 190 days of run time. Results showed that removal of COD and nitrogen rose to over 90% for each after 40 days of operation (Semmens, *et al*, 2003). Three separate stages were run over the course of the 190 day effort, with each stage adding higher concentrations of ammonium to the influent. Removal rates continued to stay high until approximately 140 days of run time. At this time, the researchers observed no sludge waste coming from the process, increased biosolids content, and a shift in biofilm color from brown to black. Along with these observations, removal efficiency suffered. It was hypothesized that as the biofilm grew to a thickness of approximately 600µm, the gas flow became increasingly channelized reducing the positive effects of biofilm stratification (Semmens, *et al*, 2003). The researchers ultimately stated that the design of the membrane modules used in this bioreactor was clearly inappropriate for this type of application and that the reactor failed because it was choked with biomass (Semmens, *et al*, 2003). Though the bioreactor did ultimately fail under extremely high loading rates, final removal rates for nitrogen were as high as 2 g m^{-2} day^{-1} and corresponding COD removal rates reached 10 g m^{-2} day^{-1} (Semmens *et al*, 2003). The final results of this research showed that membrane aerated bioreactors are adaptable to unique situations in which aerobic and anaerobic processes must occur for complete degradation of a waste, and final designs must account for significant biofilm growth.

Ahn *et al.* (2001) directly compared a membrane bioreactor wastewater treatment system to a membrane filtration system. The membrane filtration system tested

in this research was similar in operation to a reverse osmosis filter, in that high vacuum pressure was applied to one side of the membrane to create a driving force for the wastewater across the membrane. Particles larger than the pore size of the membrane are physically filtered out of the wastewater. Removal of biochemical oxygen demand (BOD) and COD were used as indicators of performance. During this research, it was observed that filtration resistance in the membrane bioreactor was over one order of magnitude less than the resistance to filtration in the direct membrane separation system. Results of the research showed that due to the steady concentration of dissolved carbon in the membrane bioreactor system from active biodegradation, the effluent from the membrane bioreactor was consistently of higher quality than the effluent from the direct filtration unit (Ahn, *et al*, 2001).

2.5.2 Membrane Usage in Groundwater Remediation

Gas transfer through membranes is also gaining popularity in groundwater remediation roles due to the ability to reliably transfer exact dissolved gas concentrations required for given remediation scenarios over a wide range of depths in the soil and to minimize gas losses to the vadose zone in soil systems (Roggy, *et al*, 2002). However, studies focusing on oxygen transfer for remediation of benzene, toluene, ethylbenzene, and xylene as well as injecting oxygen and methane for remediation of trichloroethene have shown gas transfer losses due to buildup of biofilms and inorganic precipitates on membranes (Chiang, *et al*, 1999; Hartley, *et al*, 1999; Benner, *et al*, 2000; and Newell, *et al*, 2000).

In a 2002 Strategic Environmental Research Development Program (SERDP) funded study, performed by Roggy, *et al*. at the University of Minnesota, membranes were used to transfer hydrogen to a laboratory-scale soil system contaminated with tetrachloroethene for reductive dehalogenation. The focus of the Roggy study was to quantify the effects of biofouling and iron sulfide buildup on the membrane surfaces with respect to mass transfer of the injected gas to a groundwater system. This study showed that biofouling and precipitate buildup increase resistance to mass transfer by creating tortuous paths of transfer to the contaminated groundwater. While resistance was increased, causing nearly an 80% decrease in mass transfer in stagnant systems, in systems flowing at velocities typical of groundwater systems, the increased resistance resulted in negligible changes to mass transfer of the gas into the groundwater (Roggy, *et al*, 2002). The Roggy research also concluded that in groundwater systems using membranes to transfer gases, the systems should be installed in a manner that would make them easily removable for acid washing to periodically remove biological buildup to prevent channeling.

In a study by Bruce and Schroeder (2002), groundwater contaminated with nitrate was remediated using a hollow fiber membrane bioreactor. The system was an *ex-situ* treatment process, which passed the nitrate contaminated groundwater along a membrane separating the contaminated groundwater from a denitrifying culture. The nitrate diffused through the membrane and was eliminated by hydrogenotrophic denitrification. The hydrogenotrophic denitrification culture was used to circumvent the addition of an organic substrate to the culture (Bruce and Schroeder, 2002). Removal ranged from 92% to 96% with influent concentrations of 20 to 40 mg L^{-1} nitrate as nitrogen. Removal

capacities achieved ranged from 2.7 to 5.2 g NO_3^- -N m^{-2} d^{-1} (Bruce and Schroeder, 2002).

2.5.2 Membrane Usage in Gas Phase Pollutant Remediation

Throughout the research conducted on membrane extraction of gaseous pollutants for biodegradation, two types of membranes are typically used. The first is the hollow fiber microporous membrane (HFMB), and the second is the dense phase membrane. A hollow fiber membrane bioreactor (HFMB) utilizes a hydrophobic microporous membrane bundle immersed in water as both a support structure for the growth of the biofilm and as a method to transport the substrate and electron acceptor to the microorganisms in the biofilm (Ergas, *et al*, 1999). The microscopic pores are too small for bacteria and water to enter into the membrane, but large enough to allow gaseous substances to pass through to the attached biofilm. A dense phase membrane differs from the hollow fiber microporous membrane in that it does not have micropores in the membrane. Instead, it depends on the dissolution and consequent diffusion of the gaseous contaminant into and through the membrane structure to reach the biofilm attached to the outer wall. For successful membrane applications, it is essential that the contaminant of concern is 100 – 10,000 times more permeable through the membrane than air (Yeow, *et al*, 2002).

2.5.2.1 Hollow Fiber Microporous Membrane Applications

In a 1999 study performed by Ergas, *et al.*, a polypropylene HFMB was tested. The HFMB consisted of 2,400 membrane fiber bundles with an inner diameter of 200

μm, an outer diameter of 250 μm, and length of 19.5 cm. The overall surface area of the HFMB was 0.37 m^2 (Ergas, *et al*, 1999). This membrane was immersed in water in a glass cylinder with aluminum end caps. The system was configured in a counter current flow pattern, with water and air flows traveling in opposite directions along the length of the vessel. The reactor was inoculated with microorganisms obtained from the activated sludge unit of the Amherst, MA wastewater treatment facility. A basal mineral salts solution was also added to the liquid phase of the reactor to support biofilm growth. Samples of influent and effluent water and gas were analyzed using a Varian 3500 gas chromatograph (GC) and a flame ionization detector. During the course of the research, toluene was passed through the reactor at an influent concentration of 200 ppm_v at a flow rate set to achieve a gas residence time of 1.8 seconds inside the reactor.

Samples were taken every two days during the course of the test. Sample results showed toluene removal as high as 62% in the first three days, with a sharp decline to only 28% removal on day four. Following the sharp decrease in removal seen on day four, a gradual increase in removal efficiency was observed and reached a peak removal of 72%. The sharp decrease and gradual increase observed in removal efficiency were attributed to starvation conditions in the liquid phase followed by slow growth of the biofilm on the hollow fiber membrane leading to more efficient removal (Ergas, *et al*, 1999). The maximum elimination capacity reported from this study was 42 g m^{-3} min^{-1}.

In a 1996 study performed by Parvatiyar, *et al*., a bioreactor similar to the one used in the previous study was employed using a polysulfone hollow fiber membrane cartridge to biodegrade a toluene contaminated air stream (Parvatiyar, *et al*, 1996). The Parvatiyar study varied influent toluene concentrations from 200 ppm_v to 600 ppm_v at air

flow rates from 40 to 80 milliliters per minute. The maximum toluene removal achieved was 84%, observed at an influent flow rate of 80 milliliters per minute at 600 ppm_v (Parvatiyar, *et al*., 1996).

Another application of a HFMB system used to remove contaminants from a contaminated effluent was the 2002 study performed by Min, *et al*. in which a HFMB was used to nitrify (aerobically convert) nitric oxide to nitrate (Min et al., 2002). A synthetic effluent stream designed to mimic that resulting from coal burning applications was created in the laboratory. The influent gas consisted of 15% CO_2, 5% O_2, 77% N_2 and 100 ppm NO (Min, *et al*., 2002). This influent was directed through a reactor containing a bundled fibrous membrane with inner diameter of 200µm, outer diameter of 284 µm, and porosity of 42%. The influent gas was transferred through the membrane to nitrifying bacteria which quickly oxidized the NO to nitrate (NO_3^-). Maximum removal was achieved with an elevated influent gas temperature of 55 ºC, and ranged from 69% to 73% removal.

2.5.2.2 Dense Phase Membrane Applications

Hollow fiber membranes have proven to be effective, but researchers have experienced problems such as biomass plugging at high organic load rates, requirement of constant back flushing, and significant material expense (Attaway, *et al*, 2001). Dense phase membranes eliminate the problem of biofilm plugging due to their lack of pores and are constructed from common rubber materials such as silicone and latex, keeping their costs low. Dense phase membranes, specifically silicone rubber or polydimethylsiloxane (PDMS), have been primarily utilized for aeration purposes due

their high oxygen permeability (Reij, *et al*, 1998). Dense phase membranes can also be tailored more effectively for the transport of specific compounds. Dense phase membranes rely on a compound's solubility into the dense phase material itself for transport, so selected contaminants can be extracted from influent mixtures through preferential transfer in the membrane. Preferential removal can be a major advantage of using dense phase membranes over hollow fiber membranes, which rely on bulk transport of contaminants through the micropores in the HFMB (Reij, *et al*, 1996).

A 2001 study examined the effectiveness of using a dense phase membrane to degrade BTEX compounds (benzene, toluene, ethylbenzene, *m*-xylene, *o*-xylene, and *p*-xylene) using microorganisms obtained from an industrial portage site in Charleston, South Carolina (Attaway, *et al*, 2001). For this research two distinct BTEX degrading isolates were defined by plating them on basal salts agar exposed to BTEX vapors (Attaway, *et al*, 2001). The reactor was inoculated with only the two *Pseudomonas putida* isolates, which were fed a basal mineral salts solution and liquid BTEX, directly into the liquid phase of the reactor to establish the biofilm. Oxygen was used as the electron acceptor and was supplied by diffusion through the membrane. The dense phase membrane bioreactor was constructed from two 15.24 meter lengths of Dow-Corning Silastic tubing with inner diameter of 1.02 mm and outer diameter of 2.15 mm. The membrane tubing was arranged in a spiral configuration inside a cylindrical polypropylene shell with dimensions of 6.5 cm x 18.5 cm (Attaway, *et al*, 2001). This reactor was set up with counter current water/air flow.

Analysis of influent and effluent gas and liquid was performed with a Hewlett-Packard 5890-A gas chromatograph and a flame ionization detector. Influent

concentration of BTEX vapors was set at 600 ppm_v at a flow rate of 250 ml min^{-1}. This flow rate equates to a residence time of 6 seconds inside the reactor. Required residence times are typically longer for dense phase systems than HFMB systems due to the lower mass transfer due to the decreased mass flow rate across the membrane material (Attaway, *et al*, 2001). Influent concentration was varied up to 2600 ppm_v, and additional biofilm growth was observed during increased influent concentration (Attaway, *et al*, 2001). Overall removal of the BTEX substrate was found to be as high as 98% removal at influent concentration of 700 ppm_v, or 30 μg cm^{-2} hr^{-1}. To obtain consistent performance greater than 98% for BTEX removal, a loading rate of approximately 23 μg cm^{-2} hr^{-1} or less was required (Attaway, *et al*, 2001). At the beginning of testing of the reactor, Attaway *et al* observed high removal efficiency followed by a sharp decrease, then a gradual increase in removal efficiency much like that observed by Ergas, *et al* (1999). The observance of this phenomenon further emphasizes the theory that suspended organisms are in a starvation state prior to the introduction of the substrate and rapidly remove all of the substrate until mass transfer through the liquid phase becomes limiting. Once liquid phase mass transfer is limiting, removal increases as the biofilm is established on the outer wall of the membrane (Attaway, *et al*, 2001).

In a 1995 study performed by Frietas dos Santos, *et al.*, a dense phase membrane reactor was used to aerobically degrade 1,2-dichloroethane (DCE). A spirally wound silicone membrane with overall surface area of 2.5 m^2 was used to transfer the DCE to the degrading biofilm on the outer surface of the membrane. Influent DCE concentrations of 0.65 mg L^{-1} were optimally reduced to 0.06 mg L^{-1} for a removal

efficiency of 91% (Freitas dos Santos, *et al*, 1995). The main advancement this research made to membrane bioreactor technology was the development of a way to control toxic byproducts created through metabolism of wastes. During the destruction of DCE, the microorganisms produced hydrochloric acid (HCl), which could have created an acidic aqueous environment for the microorganisms, eventually inactivating the degradation process or even killing off the microorganisms completely. However, since the gas and water phases in a membrane bioreactor are kept separate, pH was easily controlled in the liquid medium during recirculation with the addition of a buffer solution (Frietas dos Santos, *et al*, 1995). This would be virtually impossible in a conventional bioreactor configuration, making the advantage of membrane systems obvious. Frietas dos Santos, *et al.* (1995) also compared the membrane bioreactor directly to a bioscrubber in terms of overall mass transfer efficiency, and found that the membrane bioreactor was 289% more efficient at mass transfer than the bioscrubber (Frietas dos Santos, *et al*, 1995).

Later work was performed with a variety of compounds in dense phase membrane systems, including the removal of benzene and toluene using silicone and latex membranes by Cole (2001), removal of methane using a silicone membrane by Rishell (2002), and removal of toluene using a silicone membrane performed by England (2003). Cole (2001) found that with low liquid flow rates (2.5 mL min^{-1}) and exact influent concentrations of benzene and toluene, near 100% removal efficiency could be achieved. Another important finding during the Cole (2001) study was that removal achieved by membrane bioreactors does not directly scale up. In his research, two bioreactors were constructed, one exactly twice the length of the other. Identical influent concentrations and water and air flow rates were set for both reactors, but the larger reactor removed

only slightly more of the VOC's than the smaller reactor, implying that there are limitations to the amount of contaminated gas that bioreactors can remove (Cole, 2001).

Rishell (2002) found in the growth of a methanotrophic culture on silicone membrane for removal of methane from a contaminated air stream, transfer of oxygen across the membrane was the rate limiting process, similar to conventional bioreactors. Rishell (2002) concluded that two distinct, independent factors could be altered to increase oxygen transfer to the biofilm: increasing mass transfer through the membrane or increasing mass transfer through the liquid film between the membrane and the biofilm. To increase mass transfer across the membrane, the mass transfer resistance across the membrane must be decreased by using thinner membranes or applying pressure on the gas phase side of the membrane. To increase mass transfer through the liquid film, the liquid film resistance must be decreased, most efficiently by increasing the water velocity through the reactor, increasing the Reynolds number to above 1500 (Rishell, 2002). Cole (2001) and Rishell (2002) show there is a trade-off to be considered between low liquid flow rates to increase contact time with the membrane and the biofilm, resulting in more degradation as observed by Cole (2001), and higher liquid flow rates to decrease the liquid film resistance resulting in increased oxygen transport to the biofilm as observed by Rishell (2002).

England (2003) used different bioreactor designs and operating conditions to perform a number of experiments related to dense phase membrane bioreactors. These experiments examined impacts of recirculated vs. stagnant water flow, determined impacts of nutrient limitation to the biofilm, ability of membrane bioreactors to scale up, effects of increased temperature operation, and the performance of membrane bioreactors

used with transient loading. The same bioreactor used in this study to remove JP-8 components was used by England (2003) to remove toluene from a contaminated air stream. The toluene-removing bioreactor employed 3/8" I.D., 1/2" O.D., 1/16" wall thickness silicone tubing membrane(s). The large, dual tube module was a 77.5 cm in length, 5.72 cm outer diameter clear polyvinyl chloride (PVC) pipe, with two plastic end caps, and a reactor volume of 1990 cm^3. Each silicone tube had an external tube surface area of 263.5 cm^2, for a total surface area of 527 cm^2, with a total lumen volume of 94.11 mL. The reactor had an air flow of 1370 mL min^{-1} and a gas residence time of 4.1 s. Liquid flow rates were maintained at 10 mL min^{-1} throughout the course of experiments run on this reactor. All samples were analyzed using gas chromatography with flame ionization detection of influent and effluent concentrations of toluene.

In many of the experiments, England (2003) showed flexibility of dense phase membrane bioreactor systems. These experiments included the recirculated vs. stagnant water experiment, the nutrient limitation experiment, and the temperature change experiment. In the first, England (2003) tested the effects of water circulation on two different bioreactors, a single silicone tube reactor and the dual silicone tube system described above, used to remove toluene from a contaminated air stream. The first phase of the recirculation experiment was performed by setting up the reactor systems with counter current water recirculation. During this phase of the test, removal rates of 32 mg m^{-2} h^{-1} for the single tube system and 42 mg m^{-2} h^{-1} for the dual tube system were achieved. During the second phase, the water flow was turned off and the water in the reactor was left stagnant. Removal efficiencies of 41 mg $m^{-2}h^{-1}$ for the single tube and 40 mg $m^{-2}h^{-1}$ for the dual tube system were observed. These results suggest that bioreactor

systems do not require flowing water to be effective, decreasing the potential energy requirements of the system and potentially expanding applicability to scenarios in which power supply could be a considerable difficulty (England, 2003).

In the second experiment, nutrient solutions were altered to examine the effect of nutrient starvation on the degrading biofilms. In the first phase of this experiment, a full compliment of nutrients in a solution of nitrogen, phosphorous, sulfur, potassium, magnesium, calcium, sodium and iron were fed to the biofilms. Removal rates were observed at 19 mg m^{-2} h^{-1} for the single tube reactor and 42 mg m^{-2} h^{-1} for the dual tube reactor. In the second phase, the nitrogen in the single tube reactor's nutrient solution was removed. Results showed removal of 13 mg m^{-2} h^{-1}. The phosphorous in the dual tube reactor's nutrient solution was removed, and results of 40 mg m^{-2} h^{-1} removal were observed. Through the study of this system as well as other nutrient starved biofilm scenarios, it was concluded that nutrient cycling occurs within established biofilms, so external nutrient supplementation is not necessary for some systems once viable biofilms have been established.

The third experiment determined the effects of increased temperature on the toluene removal ability of a small single tube membrane bioreactor. During the first phase of this experiment, the reactor was operated at ambient temperature (23º C) for two weeks. During this operation, removal of 17 g m^{-3} h^{-1} toluene was attained. During increased temperature operation, the liquid flow into the reactor was heated to a temperature of 37.5 ºC. The reactor was operated at this temperature for 36 days, during which removal of 20 g m^{-3} h^{-1} toluene was attained. Though the increased temperature reactor removal rates did increase to a small extent, the increase was expected to be more

pronounced than the results showed. The increase was expected, due to the expected increase in biological activity that occurs at higher temperatures, typically on the order of twice the biological activity at a 10 ºC temperature increase (DeVinny, *et al*, 1999). Ultimately, it was deduced that an increase in the Henry's Law Coefficient due to the temperature increase may have resulted in less solubilized toluene, as well as lower diffusion rates into the membrane, preventing removal from increasing as expected (England, 2003).

2.6 Conclusion

Throughout the study of the literature surrounding this research effort, three things have become clear: volatile components of JP-8 can be a significant environmental and health threat if released untreated, the flexibility exhibited by membrane bioreactor systems are the reason they have such exciting possibilities in an unlimited number of environmental remediation roles, and a successful dense phase membrane bioreactor appears to be a viable option for the treatment of the volatile components found in JP-8. Membrane systems, whether microporous or dense phase, have been successfully tailored to remediate air pollution, groundwater, and wastewater. The successful completion of this thesis effort will serve as a step forward toward the goal of establishing a remediation system that is easy to design, inexpensive to construct, requires little maintenance, and allows for complete degradation of the target compounds.

3.0 Methods and Materials

3.1 Overview

This research demonstrated the ability of a membrane bioreactor to transfer and degrade cyclohexane. The bioreactor vessel used consisted of a clear polyvinyl chloride (PVC) outer shell with two inner silicone tube membranes passing through white PVC end caps. Contaminated air was passed through the inside of the tubes, and liquid with nutrient salts solution was passed through the inside of the PVC, outside of the silicone tubes. The bioreactor inoculum was prepared using seed from a microbial consortium known to degrade JP-8 fuel, soil microbes, and activated sludge from the Fairborn, Ohio Wastewater Treatment Plant. Daily measurement of influent and effluent gas concentration was performed. Analysis was accomplished using gas chromatographic separation and flame ionization detection.

3.2 Bioreactor Construction

The dual silicone tube bioreactor used in this project was constructed by laboratory technicians at the University of Missouri-Rolla for use in doctoral research (England, 2003). The dual tube module was a 77.5 cm long, 5.72 cm outer diameter clear PVC pipe with two PVC end caps. The overall reactor volume was 1990 mL. Each silicone tube had an external tube surface area of 263.5 cm^2, for a total surface area of 527 cm^2, with a total lumen volume of 94.11 mL. The silicone tubes employed as the membrane of the reactor were $^3/_8$" I.D., $^1/_2$" O.D., $^1/_{16}$" wall thickness silicone tubing membranes (Cole-Parmer Incorporated, catalog number 06411-12).

The reactor was constructed in a counter current gas/liquid flow configuration. The liquid was fed into the reactor from a 1.0 L Erlenmeyer flask by a Fisher Scientific Variable Flow Mini-Pump (S/N 230215961) peristaltic pump. The flow rate was set at 2.0 L min^{-1} and turned off during initial biofilm growth stages. The liquid flow rate was determined by measuring the amount of time the liquid flow filled a 500 mL graduated cylinder and dividing the volume filled by the time required to fill it. Once the biofilm was established, the liquid flow rate was not altered. In previous studies, it was observed that liquid flow rate did not make a significant difference to the performance of similar bioreactor designs (England, 2003). Once the liquid flowed through the reactor, it was routed back to the Erlenmeyer flask which functioned as a bubble catcher. Figure 3-1 shows the liquid phase schematic.

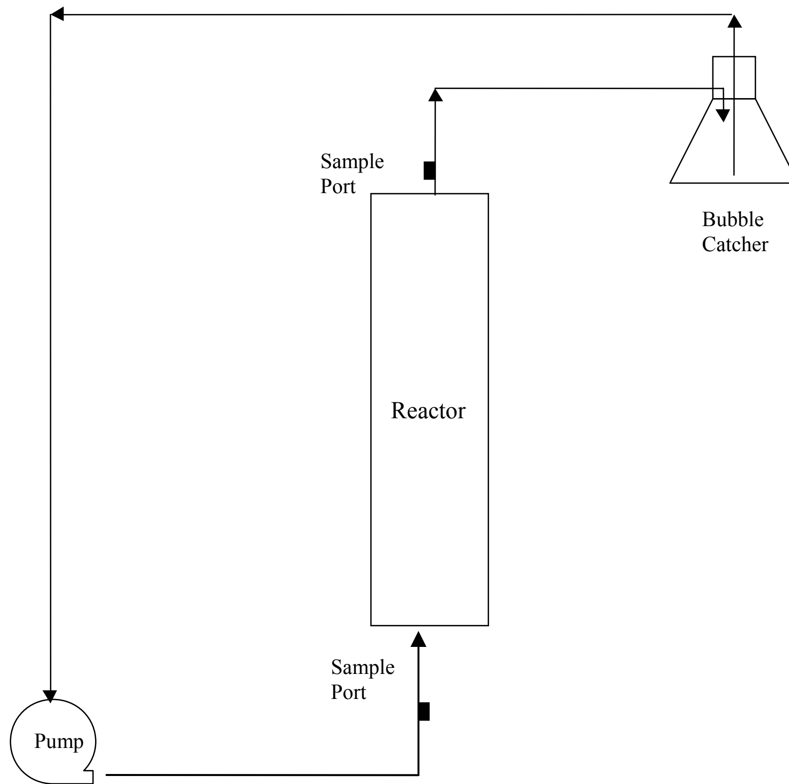


Figure 3-1: Liquid Phase Schematic. Samples ports were installed up and down stream, and a flask was used as a bubble catcher.

The water sample ports were constructed of "T" style Swagelok fittings inserted at upstream and downstream locations in the liquid flow path. Septa for the sample ports were obtained from VOC sample bottles with Teflon® and silicone layers. Valves were also inserted at upstream and downstream locations in the liquid line to seal the reactor when not in use.

Gas flow through the reactor is shown in Figure 3-2. Gas flow was routed from the exhaust port of a Gilian Hi Flow Sampler model HFS-513A air pump (S/N 112-140) through a Swagelok "T" fitting with the flow passing to either a metered valve (as a

bypass) or to a gas tight stopper in a 1.0 liter volumetric flask which contained pure liquid phase cyclohexane. The stopper in this flask had two ports: one for the air coming in from the pump, and the other going out to the second metered valve. This configuration allowed the cyclohexane in the gas phase above the liquid cyclohexane to be directed to the reactor. The flow was then directed to the reactor vessel, and branched off into both silicone tubes by another "T" fitting. All gas flow was routed through Cole Parmer $^{1}/_{8}$" outside diameter stainless steel tubing (Catalog Number 03300-05). Sample ports were installed upstream and downstream of the reactor vessel to allow for sampling of the gas phase contaminants. These sample ports were constructed in like manner to the liquid sample ports, using VOC sampling bottle septa in a "T" fitting. The septa were changed out weekly throughout the course of the project.

Once the air flow passed through the reactor, it was directed out of the reactor and channeled through silicone tubing to a Gilmont model GF-120 rotameter. The rotameter was placed downstream from the reactor in the system to prevent any obstruction to the feed flow upstream of the reactor, and was used as a visual reference for air flow rate in the reactor. Once the air had passed through the rotameter, it was directed out of the building through a fume hood. The rotameter was calibrated by recording readings during air flow calibration with a Bios Dry Cal and ensuring the reading stayed constant throughout the course of the research.

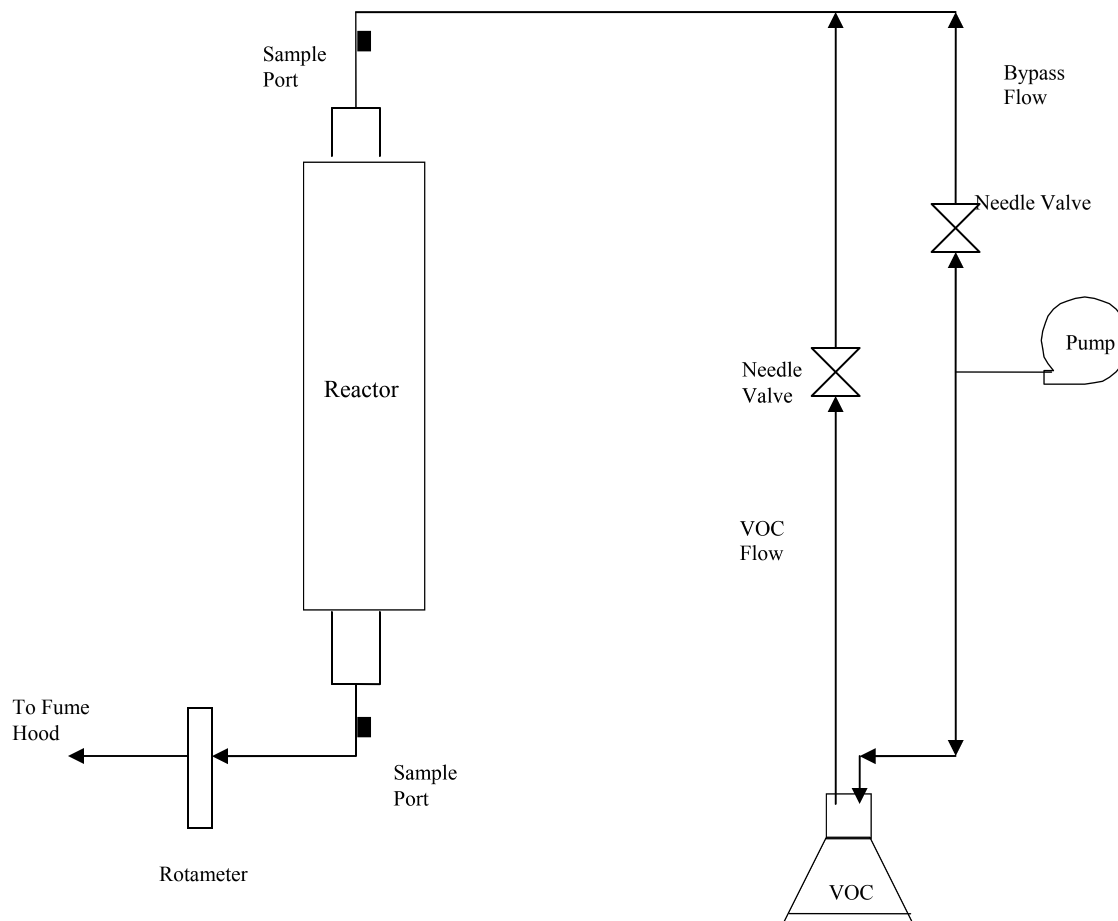


Figure 3-2: Gas Phase Schematic. Needle valves were used to control concentrations entering the reactor, sample ports were installed up and down stream, and a rotameter was used to verify flow rate daily.

Gas flow rates were varied from 1,100 to 1,410 mL per minute, resulting in residence times of 4.0 to 5.1 seconds. This flow rate was calibrated daily with a Bios Dry-Cal primary flow rate standard. Additionally, the rotameter was checked daily as an operational check to ensure the flow rate stayed constant. Figure 3-3 shows the reactor configuration.

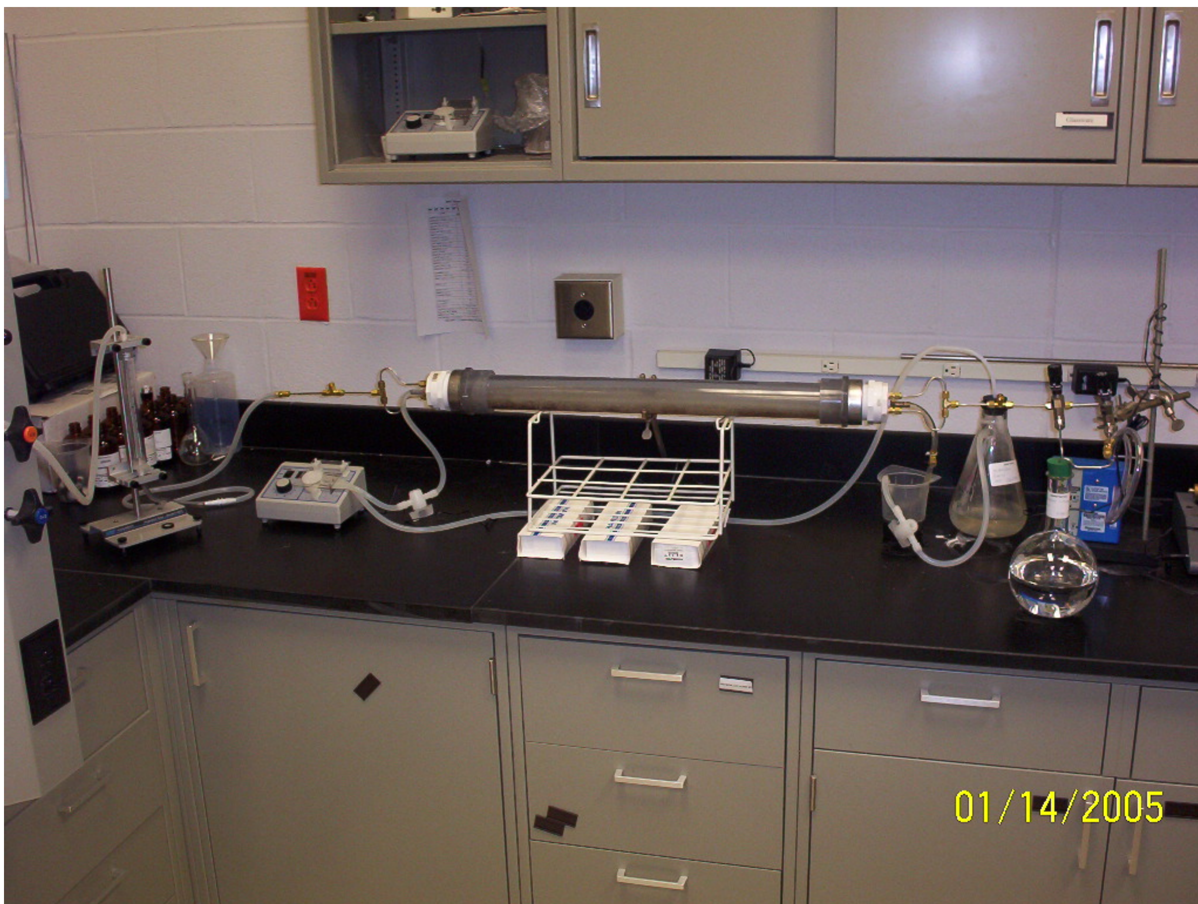


Figure 3-3: Completed Reactor Configuration

3.3. Bacterial Inoculum

The bacteria used to degrade the cyclohexane in this study were obtained from three separate sources and combined: a JP-8 storage tank located at the Paramount Refinery in Los Angeles, California; composted soil from Dr. Charles Bleckmann's residence; and activated sludge from the Fairborn Wastewater Treatment Plant in Fairborn, Ohio. The JP-8 degrading consortium was obtained from personnel working at the Air Force Research Laboratory (AFRL) Fuels Division. The research focus of the Fuels Division personnel was to determine all of the organisms present in the sample through 16s RNA analysis. The Paramount Refinery sample was chosen because, out of

24 samples analyzed by the AFRL personnel, the Paramount sample was the most completely identified. The organisms known to exist in the sample were *Caulobacteraceae* bacterium, *Rhodococcus* species, *Aquabacterium* species, *Bacillus lichenformis, and Alicaligenes* species (AFRL, 2004).

Initially, only the bacterial consortium from the Paramount Refinery sample was used as a seed to degrade cyclohexane. Due to the limited success of this consortium degrading cyclohexane, 50 mL of the composted soil sample was added to the seed culture. The addition of the soil allowed for slightly better degradation of the cyclohexane, but it was decided that the addition of the activated sludge to the seed culture would provide the highest probability of selecting for an organism or organisms capable of aerobically degrading cyclohexane. Ultimately, all three bacterial sources were added to a solution of deionized water and mineral salts to support growth. 100 mL of each bacterial source was added to 600 mL of deionized water in a 1 L container. 100 mL of cyclohexane was added to the solution as a carbon source.

A Hach Biochemical Oxygen Demand (BOD) buffer nutrient solution was also added to support the growth of the culture with essential nutrients. The buffer pillow used was intended for a 3.0 liter BOD buffer solution. The buffer pillow consisted of ammonium chloride, calcium chloride, ferric chloride, magnesium sulfate, monobasic and dibasic potassium phosphate, dibasic sodium phosphate, and deionized water. This culture was placed on a stir plate and stirred at 300 revolutions per minute for 7 days with the cap on loosely to allow oxygen to penetrate the solution. After the initial 7 day mixing period, clouding of the solution was observed, an indication of biological growth.

This solution was constantly stirred for 14 days prior to inoculation of the reactor vessel. Cultures are shown in Figures 3-4 a and b.

a.) Paramount Refinery

b.) Mixed Culture

Figure 3-4: Bacterial Cultures. a.) Biofilm in Paramount Refinery sample. b.) Bacterial inoculum culture during stirred growth.

3.4 Gas Chromatography Methods

For measurement of the cyclohexane in the gas phase, gas chromatographic separation was used in association with flame ionization detection. An Agilent 6890 gas chromatograph (GC) with a flame ionization detector (FID) (S/N US10339021) was used for this work. The column used in the gas chromatograph was an Agilent DB-624 column designed for fuel hydrocarbon analysis. Helium was used as the carrier gas for this application due to its inertness and high resolution qualities, and nitrogen, hydrogen, and compressed air were used as combustion gases for the FID. Gas chromatography procedures are listed in Appendix A. The method used for cyclohexane measurement is shown in Table 3-1.

Table 3-1: Gas Chromatography Method

Inlet			
	Temperature (ºC):	235	
	Pressure (psi):	14.1	
	Flow Rate (mL min^{-1}):	44.2	
	Split Ratio	25 to 1	
	Split Flow (mL min^{-1}):	40	
Column			
	Description:	Agilent # 123-1334, DB-624	
		260 ºC Max	
		0.32 mm x 30 m x 1.8 µm	
	Capillary	30.0 m x 320 µm x 1.80 µm nominal	
	Mode:	Constant Pressure	
	Inlet:	Front	
	Detector:	FID	
	Outlet Pressure:	Ambient	
	He Flow:		
		Pressure (psi):	15.1
		Flow (mL min^{-1}):	1.6
		Velocity (cm sec^{-1}):	37
Oven			
	Setpoint (ºC):	240	
	Hold (min):	2.5	
Detector			
	Heater (ºC):	260	
	H_2 Flow (mL min^{-1}):	40.0	
	Air Flow (mL min^{-1}):	450.0	
	N_2 Flow (mL min^{-1}):	25.0	

3.4.1 Standards and Calibration Procedures

Calibration of the GC/FID was accomplished by analyzing the headspace of varying concentrations of cyclohexane in water. The gas phase concentration of the cyclohexane in the headspace of the sample vials was calculated using the universal Henry's constant for cyclohexane shown in Equation 3.1 (Benjamin, 2002).

$$HC_{liquid} = C_{air} \tag{3.1}$$

H = Universal Henry's Constant (unitless)
C_{liquid} = Aqueous Concentration of Cyclohexane (mg L^{-1})
C_{air} = Gas Phase Concentration of Cyclohexane (mg L^{-1})

A Henry's constant of 0.195 atm m^3 mol^{-1} was converted to a dimensionless Henry's constant of 7.9 (Cyclohexane, EPA, 1994). To calibrate the instrument, four varying aqueous concentrations of cyclohexane were analyzed. To prepare these standards, varying amounts of High Pressure Liquid Chromatography (HPLC) grade cyclohexane were added to 200 mL of deionized water in a 250 mL amber glass bottle, capped with Mini-nert® valves, and allowed to equilibrate for 30 minutes. To prepare the standards, aqueous concentrations were determined by converting the volume of cyclohexane injected to mass injected using the specific gravity of cyclohexane (779 µg μL^{-1}), and dividing the injected mass by the volume of the solution to determine the aqueous concentration in milligrams of cyclohexane per liter of solution. Each calibration standard was analyzed six times, averaged, then plotted in Microsoft Excel™ to determine the slope of the calibration curve using linear least squares regression analysis

(Skoog et al, 1998). Two point calibration checks were run daily throughout the course of the project.

3.5 Sampling Plan

To effectively observe the diffusion of the cyclohexane across the silicone membrane and destruction by the microorganisms, a two phased sampling plan was employed. Phase I focused on the ability of the cyclohexane to diffuse across the membrane by calculating mass closure in the reactor, and the focus of Phase II was to observe the behavior of the reactor with the active biofilm in place.

3.5.1 Phase I: Membrane Diffusion

A cyclohexane contaminated airstream was passed through the system prior to inoculation with the microorganisms to determine the integrity of the reactor as well as the ability of the cyclohexane to diffuse across the silicone membrane. This portion of the sampling plan consisted of operating the reactor system at set flow rates and analyzing influent and effluent gas phase concentrations as well as effluent water phase concentrations to ensure mass closure within the reactor system. Determination that all of the mass of contaminants entering the reactor in the vapor phase was accounted for in either the effluent vapor or water phase indicates there were no leaks in the reactor or diffusion through the PVC shell or end caps. During the mass closure experiment, the water phase was not recirculated. The effluent water was disposed of and fresh deionized water was fed into the reactor. This change eliminated the possibility of biasing the water

phase effluent samples through continued mass transport into but not out of the water phase.

For the mass closure experiment, the reactor was set up to run at the set flow rates (1410 mL min^{-1} air flow, 2.0 L min^{-1} water flow), and allowed to operate this way for 4 hours to achieve steady state mass transfer conditions. Three vapor phase influent, vapor phase effluent, and water phase effluent samples were then collected. All vapor phase samples taken during the course of this research effort were obtained using VICI Precision Sampling 250 µL Pressure-Lok® Precision Analytical Syringes, with a sample size of 100 µL per sample. Liquid phase samples were obtained using 5.0 mL syringes. 50 mL of liquid was collected in a 100 mL VOC tight sampling vial and head space analysis was performed. Mass closure was calculated based on percentage of influent mass flow rate compared to effluent mass flow rate, seen in equation 3.2 (England, 2003).

$$\%\ \text{Mass Closure} = \frac{\text{Mass Flow Out}}{\text{Mass Flow In}} x 100\% \qquad (3.2)$$

3.5.2 Phase II: Active Biofilm Sampling

For the remainder of the research effort, sampling was performed throughout the period of growth and establishment of the biofilm on the silicone membrane. Samples were collected each day for 38 days. The sampling plan instituted for Phase II included daily analysis of six influent and six effluent gas samples. Occasional effluent water samples were analyzed, however, water sample results were deemphasized since the exact metabolic pathways of the microorganisms are not fully understood. Results were reported on basis of mass removal rate per membrane surface area (Equation 3.3), mass

removal rate per reactor volume or elimination capacity (E.C., Equation 3.4), and percent removal (Equation 3.5) (DeVinny, *et al*, 1999).

$$\text{Mass Removal per Area} = \frac{\text{Air Flow Rate} * \text{Amount Removed}}{\text{Membrane Area}} \qquad (3.3)$$

$$\text{Elim. Capacity} = \frac{\text{Air Flow Rate} * \text{Amount Removed}}{\text{Module Volume}} \qquad (3.4)$$

$$\% \text{ Removal} = \frac{\text{Effluent Concentration}}{\text{Influent Concentration}} * 100\% \qquad (3.5)$$

3.5.3 pH Monitoring

During the active biofilm sampling phase, the pH of the liquid in the reactor was also monitored daily using an Oakton Instruments pH Testr 3 + pH meter (S/N 35624-86). Three point calibration checks were run daily in accordance with the manufacturer's instructions using Yellow Springs Instruments standard pH 4, 7, and 10 buffer solutions.

3.6 Statistical Analysis

For each set of samples run during Phase I and Phase II of the project, statistical analysis was performed to establish confidence in the sampling results. The method limit of detection for the GC/FID method was calculated using Equation 3.6 (Christian, 2003):

$$LOD = \frac{3.3 * \sigma}{m} \quad (3.6)$$

LOD = Limit of Detection
σ = Standard Deviation of Blanks
m = Slope of Calibration Curve

Also useful to this research was the method limit of quantitation (LOQ), using the y-intercept of the calibration curve as a value representative of the LOQ. The y-intercept was used because any response below that value resulted in a non-real negative concentration using the equation of the line for the calibration curve, presented in equation 3.7.

$$y = mx + b \quad (3.7)$$

y = response from GC
m = slope of calibration curve
x = corresponding gas phase concentration
b = y-intercept

Other statistical analysis determined 95% confidence intervals around the mean of influent and effluent samples taken. By calculating confidence intervals, more complete analysis of system performance was obtained due to the inclusion of standard deviation of the samples taken (Gilbert, 1987). Throughout the statistical analysis used in this research, it was assumed that the distribution of samples was approximately normally distributed. With that assumption and relatively small numbers of samples, the use of the t-statistic was deemed most appropriate for the calculation of the confidence intervals (McClave, 2001). Equation 3.8 was used to calculate the confidence intervals (McClave, 2001).

$$\bar{X} \pm t * \left(\frac{s}{\sqrt{n}} \right) \quad (3.8)$$

$\bar{X}$ = Sample Mean
t = t statistic value for 95% confidence level for given degrees of freedom
s = sample set standard deviation
n = number of samples collected

Error was tracked throughout the course of the project using the summed squares method, in which error for values subtracted are accounted for by calculating the square root of the those errors squared, using Equation 3.9 (Christian, 2003).

$$Error_{Overall} = \sqrt{Error_{Influent}^2 + Error_{Effluent}^2} \quad (3.9)$$

4.0 Results and Discussion

4.1 Overview

This section discusses the results of gas chromatography and mass closure experiments performed with JP-8 and n-pentane, and the challenges faced in the remediation of these chemicals in contaminated air. The reasons for using cyclohexane as the compound representing JP-8 are presented. Then, cyclohexane gas chromatography results, mass closure, and removal capabilities of the membrane bioreactor are displayed and discussed. The order of this chapter is as follows:

4.2: JP-8 Analysis, Transfer, and Challenges

4.3: n-Pentane Analysis, Transfer, and Challenges

4.4: Cyclohexane Gas Chromatography Results

4.5: Cyclohexane Mass Closure Results

4.6: Cyclohexane Removal with Active Biofilm

4.7: Cyclohexane Liquid Phase Results

4.2 JP-8 Analysis, Transfer, and Challenges

The original intent of this research was to determine the ability of the dual silicone membrane bioreactor to remove the volatile compounds from a JP-8 contaminated airstream. Gas chromatographic analysis of these volatiles was attempted by mixing varying concentrations of JP-8 in deionized water and analyzing the headspace of these aqueous solutions. The primary difficulty encountered in this analysis was quantifying the large number of different compounds in the fuel. Each head space

analysis resulted in approximately 100 different peaks in the chromatogram. Using the Autointegration function of the gas chromatograph's software, the area under all of the peaks was summed to create a value representing the concentrations analyzed. The values of the summed areas varied widely between duplicate analyses. Accuracy was no better than 30% between any two given runs at equal concentrations, and often confidence intervals for different concentrations overlapped. The difficulty in reproducible analyses was likely due to several factors, including human error from manual injections, variable volatilities of the different chemicals in JP-8, and depletion of the most volatile compounds from the head space through the repeated analyses.

During the mass closure analysis of JP-8, it became apparent that the peak summation method would not result in an accurate representation of the reactor's removal capability. Each compound in JP-8 has unique permeability properties in the silicone material. Due to these unique properties, some of the compounds exhibited excellent transfer, while others exhibited virtually no transfer across the membrane. For the peak summation method to have given an accurate representation of the removal in the reactor, transfer properties for all of the compounds would have had to be identical. Chromatograms of the gas phase effluent were very different from gas phase influent chromatograms, and did not provide accurate comparisons between influent and effluent concentrations.

Another challenge to the reactor's successful removal of JP-8 was that the microbial culture that had been grown to mineralize the JP-8 had been grown on an aqueous solution of JP-8. In the solution, all of the soluble compounds present in JP-8 were available to the organisms. In the reactor system, the organisms only had access to

those compounds capable of successful transfer through the membrane. Since the exact metabolic pathways of the microbial culture were unknown, it was unclear if the culture would have been able to survive in the reactor. However, since most microbial communities are adaptable to a wide range of different systems, the likelihood of the culture's complete demise was low (Maier, *et al*, 2000).

Because of the difficulties in analysis and varied transfer properties of the JP-8 across the membrane, the focus of the research was changed to examine only one of the compounds found in JP-8. N-pentane was originally chosen as a representative of the volatile organic compounds present in JP-8. N-pentane is a five carbon straight chain alkane, and was believed to represent a large portion of the most volatile straight chain compounds present in JP-8, from C_4 to C_{16}. JP-8 consists of 28% C_4 to C_{16} by weight (Pleil, *et al*, 2000). Also present in JP-8 are polynuclear aromatic hydrocarbons (PAH) and alkyl PAHs like naphthalene and alkyl naphthalene, cyclic ringed molecules such as cyclohexane, hydrazine, mercaptans, chloroform , trichloroethene, tetrachloroethene, and multiple benzene, ethylbenzene, xylene, and toluene variations (Pleil, *et al*, 2000).

4.3 n-Pentane Analysis, Transfer, and Challenges

Analysis of n-pentane was much more straightforward than JP-8. Instead of a large number of peaks to analyze, the chromatograms consisted of only one well defined peak. Retention time in the column was 2.0 minutes. Replicate analyses of the standard concentrations used to create a calibration curve resulted in a maximum of only 3.4% variablity among 6 replicates. The calibration curve was also precise, with a correlation coefficient of 0.9975. The calibration curve is presented in Figure 4.1.

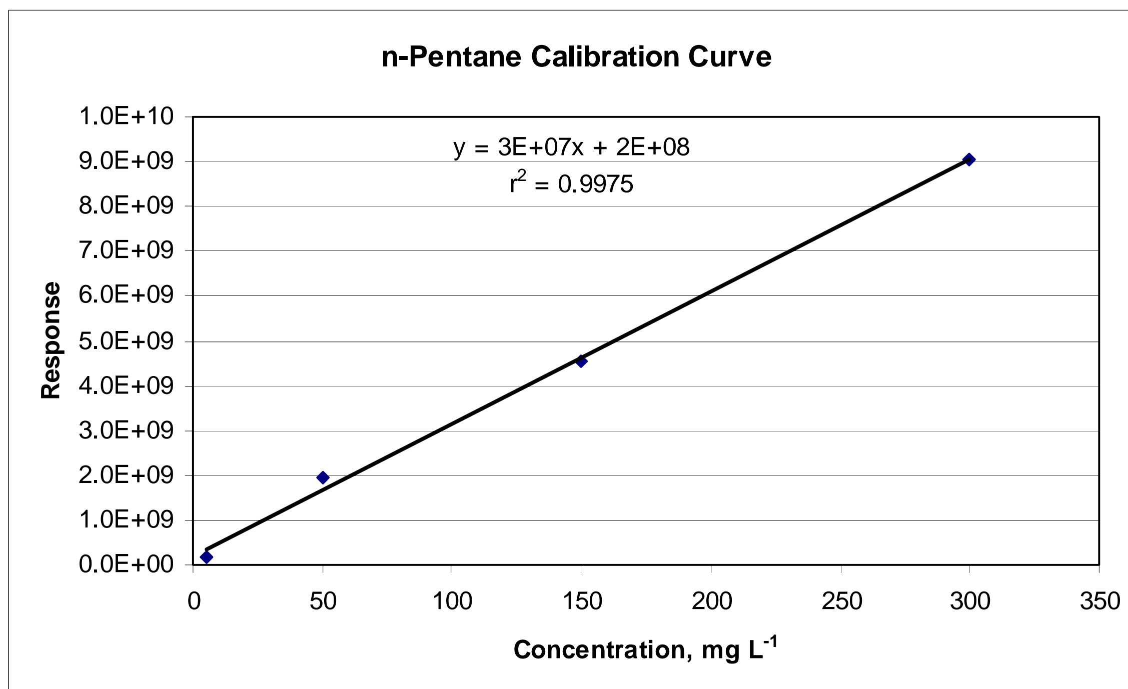


Figure 4.1: n-Pentane Calibration Curve. n-Pentane analysis was accurate and precise, resulting in a calibration curve correlation of 0.9975.

Mass closure analysis was accomplished through analysis and comparison of influent gas phase to combined effluent gas and liquid phases. Mass closure was performed to establish the ability of the membrane to transfer the contaminant and to ensure that no mass was escaping the system through leaks, cracks, or diffusion through the PVC outer shell. N-pentane mass closure accounted for 98.8 +/- 1.06% of the mass introduced into the system with a 95% confidence interval. This result gave good indication that no mass was escaping the system, and presence of n-pentane in the liquid samples indicated mass transfer was occurring across the membrane. N-pentane sampling data, calibration data, and mass closure analysis data is presented in Appendix B.

Despite accurate calibration and good mass transfer in the reactor, n-pentane proved to be too volatile for realistic use in this research. N-pentane is very volatile, with a vapor pressure of 420 mmHg (NIOSH, 2004). Due to the high volatility, the reactor system was eliminating approximately 1 L of n-pentane every 5 days, most of it passing through the reactor and not removed. The cost of the continued use of n-pentane prohibited further use in this project. Due to the large available supply in the AFIT laboratory, it was decided that cyclohexane would be used as another representative compound for JP-8. Cyclohexane was representative of the ring compounds in JP-8.

4.4 Cyclohexane Gas Chromatography Results

Chromatography of standard aqueous cyclohexane standards proved to be very similar to chromatography of n-pentane. Retention time for cyclohexane was 2.1 minutes. The largest variability between replicates was 7.7%, most likely due to human error of manual injections. The calibration resulted in a curve with a correlation coefficient of 0.99. The calibration curve is presented in Figure 4.2.

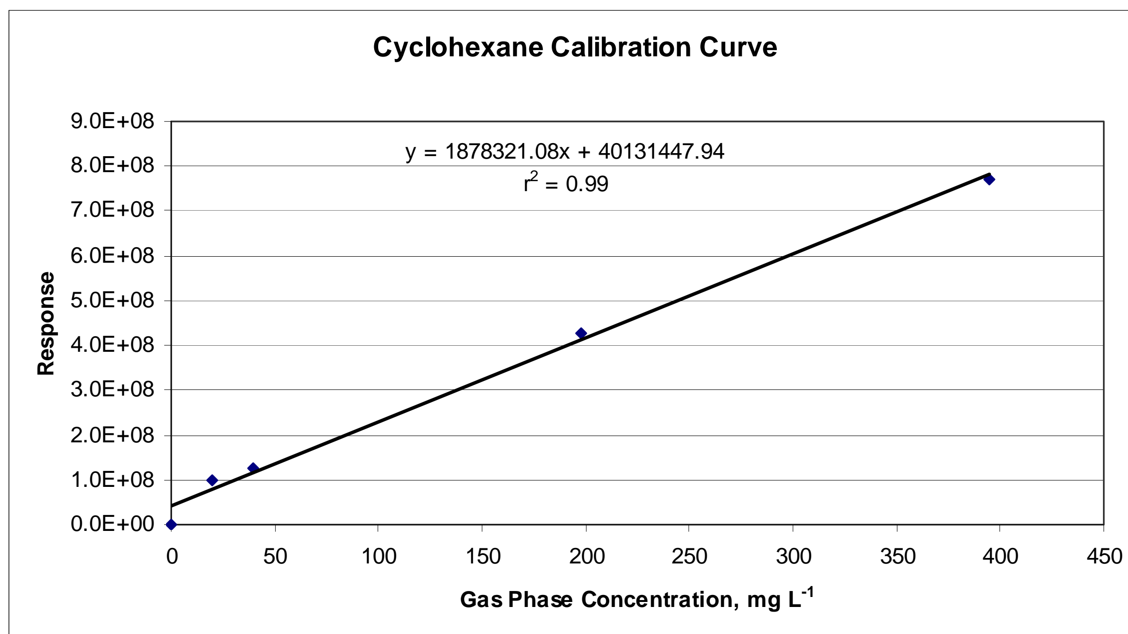


Figure 4.2: Cyclohexane Calibration Curve. Results were accurate and precise, with a correlation of 0.99 achieved.

The method limit of detection, calculated using equation 3.6, resulted in positive detection capable at a response of 3981 response units. The y-intercept of the calibration curve line was 40,131,447 response units, corresponding to a gas phase concentration of 0 mg L^{-1}. This value was used as the method limit of quantitation. All influent and effluent gas phase samples resulted in responses well above the method LOQ. All data used to determine the calibration curve for cyclohexane can be seen in Appendix C.

4.5 Cyclohexane Mass Closure

Mass closure analysis was performed using cyclohexane in the membrane bioreactor. Six replicate analyses each of the influent gas phase, effluent gas phase, and effluent liquid phase were run. In this mass closure analysis, 97.5 +/- 10.6% of the mass introduced into the reactor was accounted for in the effluent gas and liquid phases with a

95% confidence interval. The average influent concentration during the mass closure analysis was 114.2 mg L^{-1}, and the average effluent concentration was 111.1 mg L^{-1}. Liquid samples returned a detectable, but not a quantifiable result. Like the n-pentane mass closure, the result of the cyclohexane mass closure analysis gave good indication that no mass was escaping the system and that mass transfer was occurring across the membrane. All cyclohexane mass closure data can be seen in Appendix D.

4.6 Cyclohexane Removal with Active Biofilm

Following the mass closure analysis, the bioreactor was inoculated with 500 mL of a viable batch of cyclohexane degrading organisms. Sampling began 72 hours after inoculation. Initial results showed very little removal of cyclohexane in the system. Initially, the water phase of the reactor became supersaturated and the concentration gradient drove cyclohexane from the water phase to the gas phase. The result of undesirable concentration gradient was higher effluent gas phase concentrations than influent gas phase concentrations. After 5 days of operation, the organisms in the reactor mineralized enough of the cyclohexane in the system to permanently change the concentration gradient in the desired direction, from gas phase to liquid phase. From day 5 through day 38, the reactor continued to increase in removal performance as the biofilm was established on the membrane. The biofilm was slightly noticeable on day five, and by day ten, a clearly visible biofilm approximately 1-2 mm thick had grown on the membrane. Figure 4.3 presents the gas phase influent and effluent concentrations over the course of the research. Tabular concentration data is presented in Appendix E.

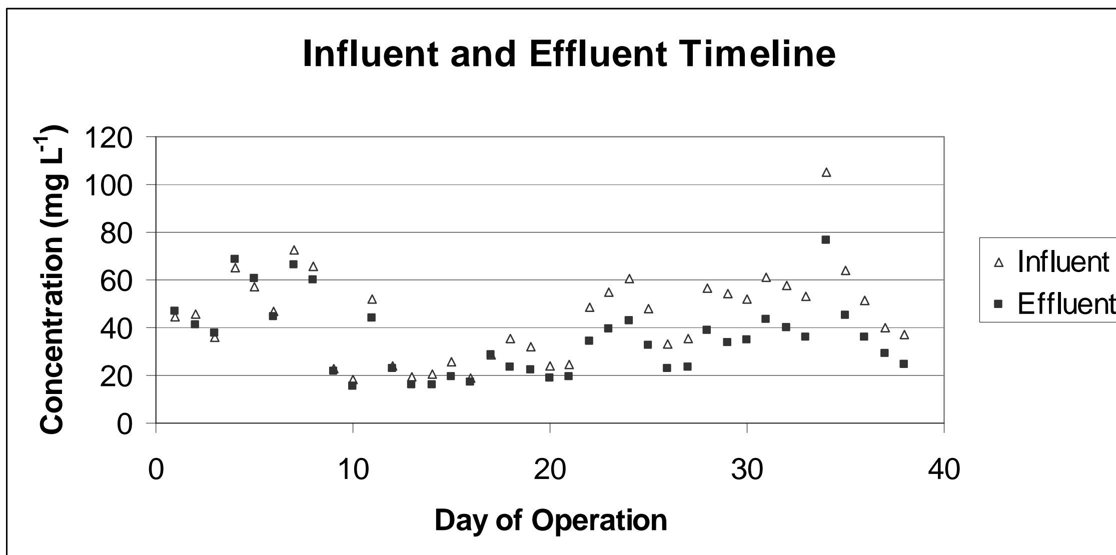


Figure 4.3: Influent and Effluent Timeline. Reactor performance improved throughout the course of the research.

In Figure 4.3, an overall trend of increasing removal is displayed. The graph also depicts the fluctuating influent concentrations. The average influent concentration was 44.5 +/- 5.8 mg L^{-1}, and the average effluent concentration was 35.4 +/-4.9 mg L^{-1}, both with a 95% confidence interval giving an average removal of 20.4%. The influent concentrations fluctuated as a result of the amount of liquid cyclohexane in the volumetric head space flask and the amount of bypass flow allowed in the system. As the liquid cyclohexane was depleted throughout the research and the flask was refilled, influent concentrations would rise as seen on days 7, 12, 18, 25, and 34. At day 22, the bypass flow in the system was considerably decreased driving more flow directly from the head space flask to the reactor, effectively increasing the influent concentration. The influent concentration was increased in an attempt to find the maximum removal capabilities of the reactor.

Another useful representation of any environmental remediation system's performance is to analyze the concentration leaving the reactor (C_{out}) in comparison to the concentration that entered the reactor (C_{in}). This data is presented over the course of the research in Figure 4.4, and depicts the improvement in performance observed over the course of the research.

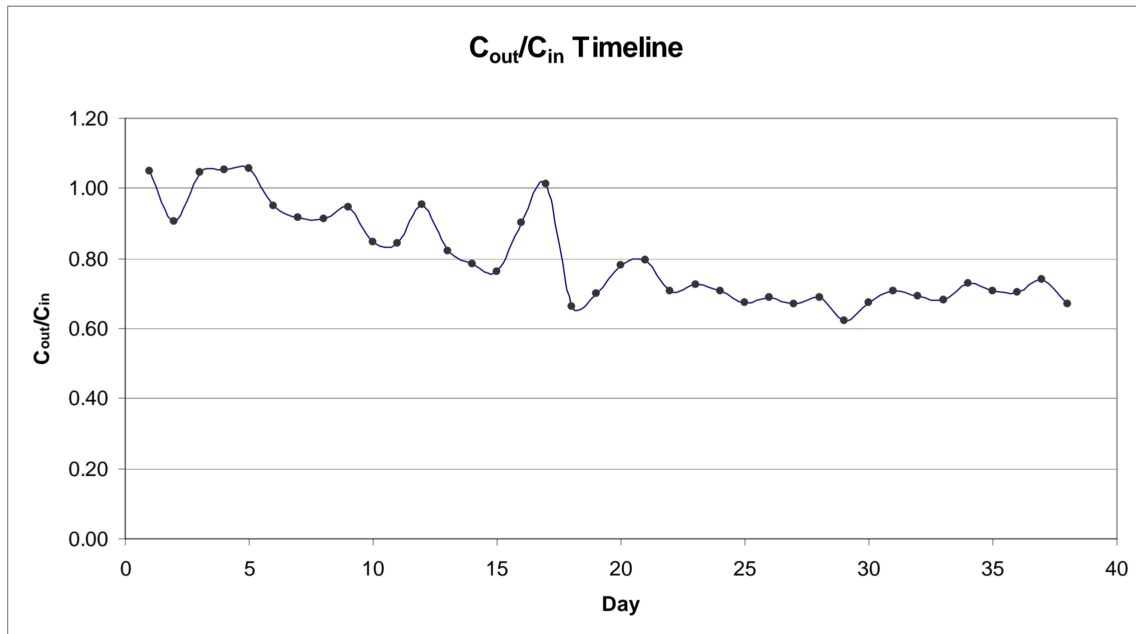


Figure 4.4: C_{out}/C_{in} Timeline. Displays decreases seen in reactor performance related to reactor draining and decreased liquid flow rate on days 9, 12, 17, and 37.

A difficulty encountered in the course of this research was keeping the liquid flowing correctly through the reactor. The peristaltic pumps being used to pump the liquid through the reactor often wore through the tubing used in the peristaltic portion of the pump. As the tubing was worn through, the liquid would leak out of the tubing, draining the reactor and often dislodging the biofilm from the surface of the membrane. This occurred on days 9, 12, 17, and 37. Figure 4.4 depicts the results of this occurrence on those days, with cyclohexane removal suffering significantly. As soon as the water

flow was started again, mass transfer was restored. This supports the findings of Rishell (2002), by demonstrating an increase in mass transfer with an increase in liquid flow in the reactor.

Reactor performance was primarily measured in mass removal rate, or mass removed per time per surface area of the membrane. Mass removal rate accounts for influent load, flow rate (thus residence time) in the reactor, and surface area of the membrane, and is a commonly used performance parameter for membrane bioreactors (Cole, 2001; England, 2003; Ergas, *et al*, 1999). Influent load, or the mass flow rate entering the reactor divided by the membrane surface area, ranged from 395.8 to 2189.4 mg min^{-1} m^{-2}. The average influent load was 1020.8 +/- 138.6 mg min^{-1} m^{-2}, with a 95% confidence interval. The average removal rate of the membrane bioreactor in this research was 202.3 +/- 47.9 mg min^{-1} m^{-2} with a 95% confidence interval. Figure 4.5 presents the mass removal rate of the reactor over the course of the experiment. A trend line was added to this figure using linear least squares analysis to depict the increase in mass removal rate observed over the course of the experiment.

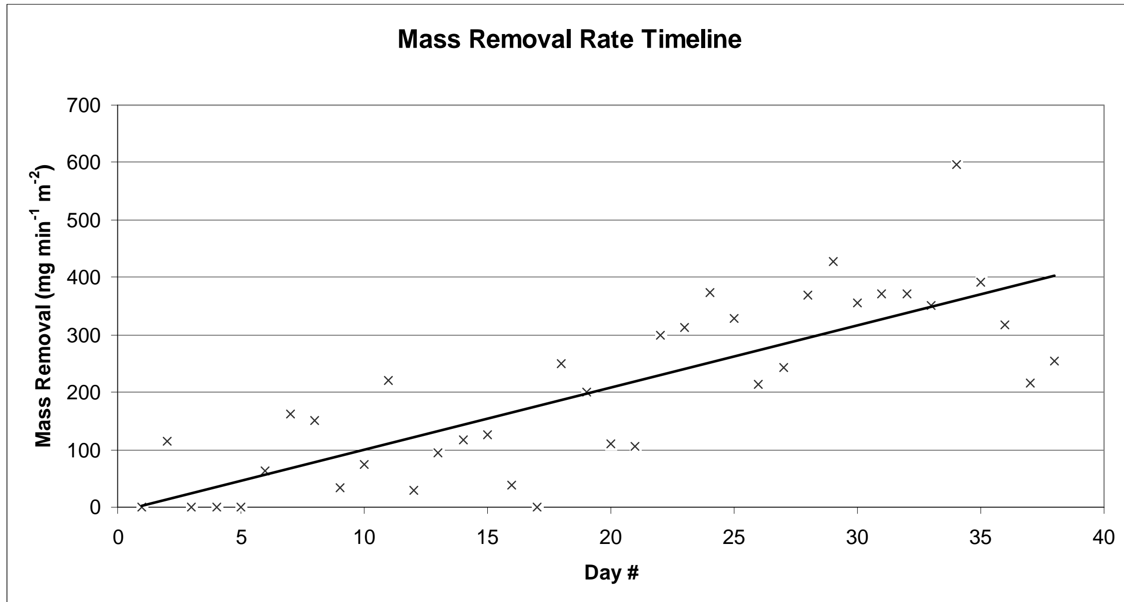


Figure 4.5: Mass Removal Rate Timeline. Cyclohexane removed based on membrane surface area. The trend line was added to depict the overall increase in performance over the course of the research.

Another measurement taken to represent reactor performance is elimination capacity, measured in units of mass per time per volume of reactor. Elimination capacity is very similar to mass removal rate, but uses the reactor volume instead of the membrane surface area as a dimensional measurement. Elimination capacity is often used as a performance descriptor in conventional bioreactor designs (DeVinny, *et al*, 1999). The average elimination capacity of this reactor was 321.4 +/- 76.2 g m^{-3} hr^{-1}, with a 95% confidence interval. Figure 4.6 presents the elimination capacity achieved by the reactor over time.

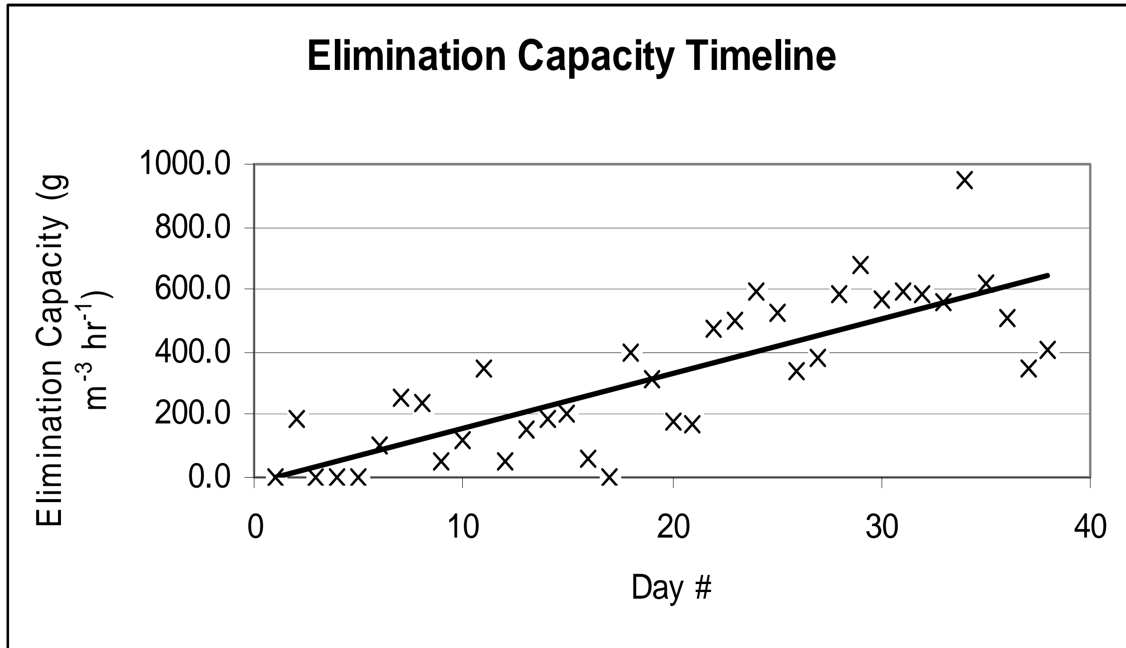


Figure 4.6: Elimination Capacity Timeline. Cyclohexane removal based on total reactor volume. Similar in appearance to Figure 4.3, differing only in y-axis scale and units.

The decrease in removal from draining the reactor can also be observed in Figures 4.5 and 4.6 on days 9, 12, 17, and 37. Also, fluctuation in mass removal rate can be observed in Figures 4.5 and 4.6, primarily due to fluctuation of influent concentration.

The results of the research showed that removal rate (thus elimination capacity) was proportional to the influent load rate. Increasing mass removal rates were observed with higher influent load rates. The maximum removal rate achieved (596.6 mg min^{-1} m^{-2}) was observed at the maximum influent load rate introduced to the system (2189.5 mg min^{-1} m^{-2}). Figure 4.7 presents this correlation graphically, and tabular data is presented in Appendix E.

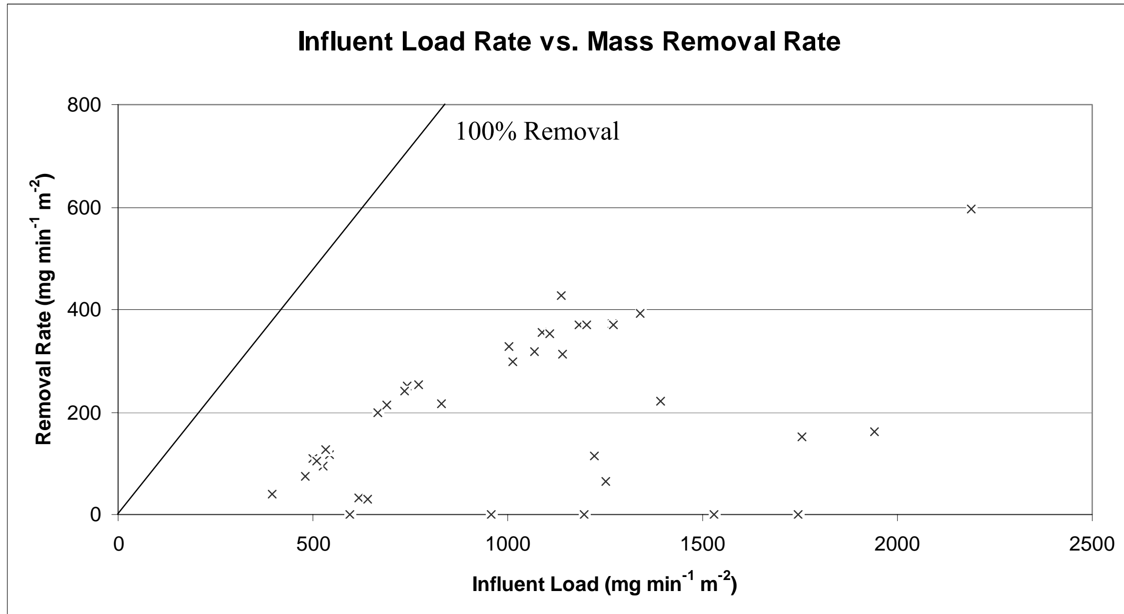


Figure 4.7: Influent Load Rate vs. Mass Removal Rate. Data based on surface area of membrane. Direct correlation between influent load and mass removal rate displayed through increased removal rate occurring at increase influent load rates.

The overall trend of data presented in Figure 4.7 shows increasing removal correlated to increasing influent load. The data resembles expected removal rates in conventional bioreactor systems as depicted in DeVinny, *et al*, (1999), exhibiting increased removal rate with increased influent load. Conventional bioreactors exhibit this same trend, reaching a maximum point at which the removal rate does not increase further with increased influent load (DeVinny, *et al*, 1999). Figure 4.8, adapted from the DeVinny text, displays the conventional bioreactor trend graphically.

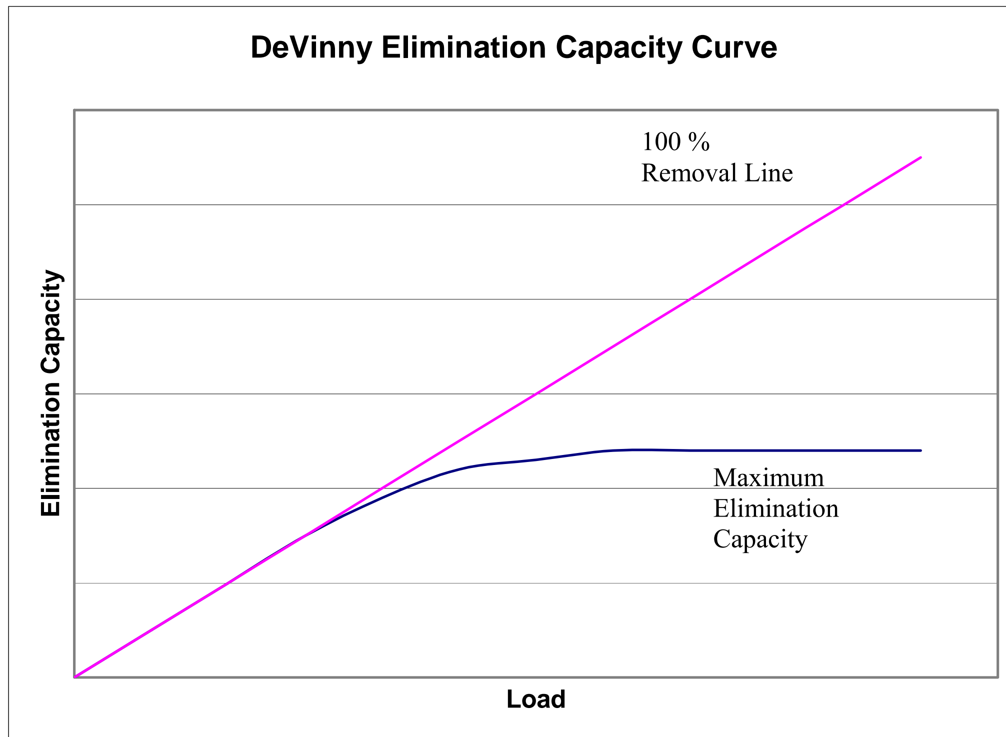


Figure 4.8: DeVinny Elimination Capacity Curve (DeVinny, et al, 1999). Displays trend for conventional bioreactors to exhibit 100% elimination capacity to a maximum elimination capacity, at which elimination capacity levels off.

Figure 4.7 also displays five points with no removal, occurring at influent loads of 1197, 957, 1744, 1530, and 593 mg min^{-1} m^{-2}. Four of these points (1197, 957, 1744, and 1530 mg min^{-1} m^{-2}) occurred during the first five days of the reactor's testing, prior to establishment of the biofilm. The fifth point (593 mg min^{-1} m^{-2}) occurred on day 17 following a drainage episode which completely removed the biofilm. There are also four points at influent loads (1939, 1755, 1222, and 1252 mg $min^{-1}m^{-2}$) which exhibited removal rates less than 200 mg $min^{-1}m^{-2}$. This low removal rate does not seem to fit the removal curve. These four data points occurred on days 6, 7, 8, and 9, as the biofilm in the reactor was initially establishing itself on the membrane and was not fully developed, perhaps explaining the decreased removal rates. Also presented in Figure 4.7 is a line representing 100% removal. The membrane bioreactor in this research never achieved

100% removal of the influent cyclohexane, possibly due to the gas phase flow rate set at a rate too high to allow for all of the cyclohexane to transfer through the membrane, or limited by the degradation rate of the microorganisms in the reactor. The inability to achieve 100% removal was also observed in other work (England, 2003, Rishell, 2002).

Reactor performance was higher than performance observed in conventional bioreactor designs, as reported by DeVinny, *et al* (1999), but not as efficient as some others listed in this report. In comparison to England's work (2003), the reactor in this research was loaded at a much higher influent load rate, which is a possible explanation for the increased removal observed in this research. England's (2003) highest load rate was approximately 550 g m^{-3} h^{-1}, while the reactor in this research was maximally loaded at almost 3,500 g m^{-3} h^{-1}. However, a much lower elimination capacity was observed in this research than in Ergas, et al, (1999) and Attaway, et al, (2001). The decreased elimination capacity could have been due to cyclohexane having lower membrane permeability in comparison to aromatics like BTEX compounds, the air flow rate set too high (resulting in a low residence time), the membrane thickness limiting diffusion, or resistance to biological degradation at low concentrations. Table 4.1 presents the results of this research in comparison to others.

Table 4.1: Comparison of Bioreactor Performance: While in the range of comparable conventional systems, the membrane bioreactor in this research achieved lower elimination capacity than others in the literature.

Study	Bioreactor Type	Contaminant	Max Elimination Capacity ($g\ hr^{-1}\ m^{-3}$)
DeVinny, *et al*, 1999	Conventional	VOC's	5-229
Ergas, *et al*, 1999	MBR, Polypropylene HFMB	Toluene	2520
Attaway, *et al*, 2001	MBR, Silicone	BTEX	2580
England, 2003	MBR, Silicone	Toluene	220
Roberts, 2005	MBR, Silicone	Cyclohexane	947.8

4.7 Cyclohexane Liquid Phase Results

The liquid phase of the reactor was sampled on 5 separate occasions throughout the course of the research. Each liquid sample resulted in a detectable, but not quantifiable amount of cyclohexane in the liquid phase. The average liquid response was 4287235 response units. The limited amount of cyclohexane in the liquid indicates that degradation is occurring by organisms suspended in the liquid culture. Periodic pH data was collected throughout the research. Liquid phase pH measurements varied slightly, all falling between pH's of 6.94 and 7.05. All data gathered to determine cyclohexane active biofilm and liquid phase results can bee seen in Appendix E.

5.0 Conclusion and Recommendations

5.1 Conclusion

The purpose of this research was to examine the ability of a membrane bioreactor to remove and degrade volatilized components from a JP-8 contaminated airstream. Cyclohexane was used as a representative compound of those volatile components of JP-8 jet fuel. The research questions answered throughout the course of this research were:

1. What is a membrane bioreactor and where is this technology being applied?
2. Would cyclohexane diffuse through the dense phase membrane?
3. If diffusion occurred, would the biofilm derived from the Paramount Refinery, composted soil, and activated sludge grow on the dense phase membrane and eventually degrade the cyclohexane?
4. If successful degradation occurred, what was the removal rate the membrane bioreactor could achieve?

Each question was answered. A summary of those answers, research limitations, recommendations for improvement, and suggestions for further research are presented in this section.

5.2 Research Questions Answered

Membrane bioreactors are systems which use porous hollow fiber membranes or dense phase membranes to supply either degradable substrate or vital nutrients or gases to an active biological community. Membrane bioreactors are being used widely throughout environmental remediation roles, to include wastewater, groundwater, and air pollution systems. Their advantages include separation of phases, large surface areas for

transport, and increased control over system parameters such as liquid and gas phase flow rates, pH, and pressure drop.

Cyclohexane successfully diffused through the dense phase silicone membrane used in the membrane bioreactor, as indicated by the mass closure experiment. As the concentration gradient was increased, cyclohexane transfer across the membrane increased as well. The concentration gradient was increased through increased biological degradation, increased liquid flow rate in the reactor, and increased influent load rate introduced into the reactor.

The biological culture grown to degrade cyclohexane successfully formed a biofilm on the silicone membrane and established a favorable concentration gradient in the reactor system within 5 days of inoculation. Throughout the course of the research, the biofilm proliferated and cyclohexane removal in the system continued to improve. The biofilm was essentially destroyed four different times due to the reactor draining, but each time the biofilm re-established itself on the membrane and continued to remove cyclohexane from the airstream.

The maximum removal rate achieved (596.5 mg min^{-1} m^{-2}) was observed at the maximum influent load rate introduced to the system (2189.4 mg min^{-1} m^{-2}). Though the maximum removal was only attained one day, it indicated that this membrane bioreactor system was capable of removing and successfully degrading considerable amounts of cyclohexane. As influent load rate increased driving the concentration gradient higher, removal rate increased as well. Maximum elimination capacity achieved was 947.8 g hr^{-1} m^{-3}. The elimination capacity achieved was higher than England's (2003) due to significantly higher load rates applied to the reactor during this research. However, the

elimination capacity was significantly lower than those achieved by Ergas, *et al.* (1999) and Attaway, *et al.* (2001). The elimination capacity could have been lower due to less efficient mass transfer through the silicone membrane or less efficient degradation of cyclohexane by the biofilm.

5.3 Research Limitations

This research was limited primarily by the use of cyclohexane as a representative compound of JP-8 jet fuel. The results of this research should not be directly applied to a JP-8 remediation scenario without further research. The difficulty in maintaining an efficient water flow through the system presented a major limitation. Each time the water flow was interrupted, removal suffered and the biofilm was often dealt a major setback. Another limitation to this research was the lack of complete understanding of the metabolic pathways and degradation rates of the microorganisms. Metabolic pathways should be determined to ensure no harmful byproducts were being formed, and degradation rates should be determined to predict a quantifiable concentration gradient in the system to understand its loading and removal capabilities. Also, this research was limited in the ability to adequately supply enough influent load to test the maximum removal capabilities of the reactor. Only one data point was obtained at an influent load above 2000 mg min^{-1} m^{-2}, and the highest removal rate was consequently attained at this high load rate. This result indicates that the reactor was capable of more removal, and was limited by the load rates tested.

5.4 Suggestions for Improvement

To improve future research on this membrane bioreactor, the reactor should be configured with a failsafe liquid pump. Each time the liquid pump developed an air bubble, that air bubble was transported into the reactor. To remove the air bubbles, the reactor had to be tipped up in the direction of the effluent water port. Each time the reactor was tipped and the air bubble traveled along the length of the reactor tube, the bubble removed large amounts of the biofilm attached to the silicone membrane. This removal required the biofilm to re-establish itself periodically, essentially starting the growth process over each time. The biofilm was also removed by draining of the reactor as mentioned in section 4.5. The draining was also due to the liquid peristaltic pump. These problems could have been eliminated by using a submersible jet drive pump.

Another suggestion for improvement is to configure the reactor in a vertical arrangement, as configured by England (2003). The horizontal arrangement used in this research allowed air bubbles that entered into the reactor from the liquid pump to stay in the reactor. When dealing with volatile chemicals that readily partition from aqueous solution to gas phase, it is likely that any air bubbles in the reactor could form pockets where the cyclohexane could partition into the gas phase, making it unavailable for the suspended organisms to degrade. With the reactor configured vertically, air bubbles would simply pass through the reactor and out the top without staying in the reactor for a long period of time.

5.5 Recommendations for Future Research

Future research on this membrane bioreactor system should include determination of biological degradation rates and mass transfer rates of the silicone membrane. Knowledge of these parameters would allow the researcher to model the system, taking a step toward accurate prediction of the system's capabilities at given influent load rates.

In pressing on toward the goal of determining the ability of a membrane bioreactor to remove and degrade JP-8 from a contaminated air stream, I would suggest this same project be attempted using the dense phase membrane bioreactor's ability to remove a complex polynuclear aromatic hydrocarbon present in JP-8, like naphthalene. Past research has shown the ability of similar reactors to remove BTEX compounds, straight chain compounds, and now cyclic structured compounds. The successful removal of a complex PAH would complete the body of research for all classes of volatile compounds present in JP-8.

Another future research focus should be on testing a porous hollow fiber membrane instead of a dense phase silicone membrane for JP-8 removal. Hollow fiber membranes more efficiently transfer bulk materials, and do not rely on the ability of the contaminants to diffuse into and through the membrane like silicone membranes.

5.6 Significance

This research filled a vacant niche in the body of knowledge regarding membrane bioreactor applications: transport and removal of cyclic ring structured compounds. By demonstrating the ability of a dense phase membrane to successfully transport the cyclic structured cyclohexane to an active biofilm, future researchers and engineers can have

confidence in applications involving many different ring structured compounds, including those found in JP-8 and many solvents used throughout the Air Force. This research has completed another step toward application and field deployment of a simple and effective air pollution control system that could be successfully tailored to a number of different applications.

Appendix A: Gas Chromatography Procedures

Part I: Daily Operating Instructions

1. Open Chemstation from Desktop of GC Computer

2. Load Method (###.M) File, Cyclohexane.M
   -If no method exists for operation, see Part II, Establishing a Method

3. Turn on gases in Gas Storage Closet nearest GC (turn valves counter clockwise until regulator shows pressure)
   -Set Compressed Air to 60 psi
   -Set Hydrogen to 25 psi
   -Set Helium to 40 psi
   -Set Nitrogen to 60 psi
   -These flows set to work with Cyclohexane.M method, pressure may need to be tailored for other methods

4. Allow GC to warm to operating temperatures and light the FID
   Monitor flows and temperatures from control panel on "Instrument #1" window

5. From "Instrument #1" window, click on "Method" dropdown

6. Select "Run"
   -Instrument #1 MSTop/Enhanced window will pop up

7. Choose Data File Path for results to be directed to

8. Name Data File (###.D) → DateEffluent/Influent#x.D
   Example: 15 Jan Effluent #1.D

9. Ensure "Data Acquisition" and "Data Analysis" are checked

10. Click on "Run Method"

11. Machine will cycle, ask you to press "Prep Run" and then "Start". Instructions will pop up on the computer screen.

12. When GC readout (on the GC itself, above the key pad) says "Ready for Injection", press "Prep Run" key

13. Obtain sample, inject into appropriate inlet

14. Press "Start" key to start the run

15. Observe run from "Instrument #1" Window

-At completion of run, auotintegrated results file (xxx.txt) will automatically pop up

16. Save results file as in/out#.txt on flash drive for Excel import (example, out1.txt)

17. Return to step # 5 and repeat until all samples are completed

18. When all samples are completed, load "Sleepmode.M" file

19. Turn off all gases (tighten all valves clockwise until you can't turn them anymore)

Part II: Establishing a Method

1. Click on "Instument #1" Window

2. Choose Method Pulldown

3. Click on "Edit Entire Method"

4. Edit all three choices, Method Information, Instrument/Acquisition, and Data Analysis

5. Enter any notes you want in the Method Comments section

6. Hit OK

7. Inlet and Injection Parameters Screen:
    a. Choose Inlet
    b. Choose Source
    c. Check "Use MS" if you want to use the Mass Spectrum Detector

8. Hit OK
   -Control Panel will pop up

9. Click on each section to change that parameter
   -As you review settings and go to next parameter, notice a blue check comes up in the box you just checked if you didn't change anything. If you changed something in the last section, a blue x will appear

10. Injector
   -only applicable if you're using the autoinjector

11. Values

-Not Applicable

12. Inlets
    -Set Front and Back inlets to desired values (flows, temps, splits, gases)

13. Columns
    -Ensure correct inlet and detector for each column
    -Changing flow rates in this screen can affect flow rates in inlet screen, be sure to double check if changes are made

14. Oven
    -Set temperatures and ramps

15. Detectors
    -Front → Flame Ionization Detector
    -Back → Electron Capture Detector
    -Set temperature and flow rates
    -Rules of thumb/starting values:
        -Temperature of FID must be at least 20 degrees higher than maximum oven temperature in method
        -Hydrogen flow should be around 40.0 mL/min
        -Compressed Air should be around 400-450 mL/min
        -Make up flow (Nitrogen) should be around 25.0 mL/min
        -Make sure flame is "On"

16. Signals
    -Will determine what graphs you see on the instrument panel

17. Auxilliary
    -Not Applicable

18. Runtime
    -Leave at set mode

19. Options
    -Allows you to change units, lock down keyboard, and change column compensation

20. When done with all changes, click "Apply"
    -Notice that all blue checks and x's disappear

21. Hit OK

22. GC Real Time Plot
    -Will determine what graph you will see in real time on the instrument panel (from the signals chosen in step 16)

23. Select Reports
    -Percent Report Gives Areas under Peaks
    -LibSearch Report is only used with Mass Spectrum Detector (MSD), searches results against known values
    -Quant Report is also only used with MSD, quantifies values

24. Hit OK

25. Report Options
    -For each report you picked in step 23, allows you to pick and choose options

26. Hit OK

27. Select Printer

28. Hit OK

29. Save Method As
    -Establish Data File Path
    -Name Method File (.M) files

30. Hit OK

31. Return to Part I to continue running samples

Appendix B: n-Pentane Information

Calibration Data:

Run #	Response	Response	Response	Response
Aqueous Conc (mg L^{-1})	5	50	150	300
1	172983114	1960149573	4605500438	9103424231
2	174303328	1959600423	4568703101	9138530880
3	168580620	1919557795	4552170107	9049095238
4	169721500	1933585497	4489907354	9068328846
5	170087392	1932552434	4469592567	8958060852
6	168368112	1933364268	4475031898	8965313164
Average	170674011	1939801665	4526817578	9047125535
% Error	3.41	2.07	2.95	1.97
Standard Deviation	2427128.08	16422390.97	56403835.67	72943433.15
95% UCL	173221540	1957038711	4586019402	9123687426
95% LCL	168126482	1922564619	4467615753	8970563644

Mass Closure Data:

Influent				
	Run	Area		
	1	609292927		
	2	620819184		
	3	586161228		
	4	612434020		
	Ave	607176840		
	stdev	14831108		
	95% UCL	626457280		
	95% LCL	587896400		
Effluent	1	618559995		
	2	590317506		
	3	583349535		
	4	610073186		
	Ave	600575056		
	stdev	16488465		
	95% UCL	622010060		
	95% LCL	579140051		
Water Effluent	1	3890590		
	2	1623462		
	3	1178153		
	4	828402		
	Ave	1880151.8		
	stdev	1379218.5		
	95% UCL	3673135.9		
	95% LCL	87167.644		
	Effluent Total Response			Conc (mg L^{-1})
	95% UCL	625683196		14.42381
	Ave	602455207		13.63559
	95% LCL	579227219		12.84738
	Influent			
	95% UCL	626457280		14.45008
	Ave	607176840		13.79582
	95% LCL	587896400		13.14156
	Mass Closure (%)			
	95% UCL	99.818218		
	Ave	98.838612		
	95% LCL	97.761466		

Appendix C: Cyclohexane Calibration Data

Run#	Area	Area	Area	Area	Area
Gas Phase Conc (mg L^{-1})	0	19.75	39.5	197.5	395
Aqueous Conc (mg L^{-1})	0	2.5	5	25	50
1	8515	95523015	126415455	415286259	740332314
2	8523	98938355	125740717	433013667	780888326
3	8289	97570866	127472032	429769399	778112075
4	11428	101917746	133466935	436381354	760609761
5	7763	98351634	124811217	430329410	788754199
6	6812	97145215	123070298	428700755	776465707
Average Response	8555	98241139	126829442	428913474	770860397
% Difference	40.39202	6.2744039	7.7896724	4.8340963	6.1390336
Standard Deviation	1548.73	2147290	3578556.6	7225849.9	17560144
Minimum Detection Limit	3981.785				
Limit Of Quantitation	40131448				
95% UCL	10180.56	100494948	130585518	436497772	789291635
95% LCL	6929.443	95987329	123073367	421329176	752429159

Appendix D: Cyclohexane Mass Closure Data

Run #	Influent Response	Effluent Response	Liquid Response
1	269052329	259670081	856939
2	244624918	251385333	1154289
3	252237878	264261301	7076210
4	260932543	261231705	7121045
5	243931030	244431985	6742811
6	257205135	187504545	1169928
Average Response	254663972	244747492	4020204
Standard Deviation	9743120	28969353	3246868
95%UCL	264890412	275153909	7428137
95% LCL	244437532	214341074	612270
	Influent Total		Effluent Total
95% UCL	264890412	95% UCL	282582046
Ave	254663972	Ave	248767695
95% LCL	244437532	95% LCL	214953345
	Influent Conc (mg L^{-1})		Effluent Conc (mg L^{-1})
95% UCL	119.6595073	95% UCL	129.0783626
Ave	114.2150491	Ave	111.0759281
95% LCL	108.7705909	95% LCL	93.07349362
	Mass Closure (%)		
95% UCL	1.078713807		
Ave	0.972515697		
95% LCL	0.855686201		

Appendix E: Active Removal Information

Day #	Influent Average Response	Effluent Average Response	C_{in} (mg L^{-1})	C_{out} (mg L^{-1})
1	124210452	128200981	44.76	46.89
2	125926797	117824335	45.68	41.36
3	107350278	110504226	35.79	37.47
4	162579238	169233015	65.19	68.73
5	147562211	153507345.2	57.20	60.36
6	128060603	123584796	46.81	44.43
7	176327008	165024908	72.51	66.49
8	163372092	152745783	65.61	59.95
9	83511303	81218672	23.10	21.87
10	73941756	68772669	18.00	15.25
11	137921980	122395929	52.06	43.80
12	85170596	83107808	23.98	22.88
13	77158286	70555164	19.71	16.20
14	78249807	69991909	20.29	15.90
15	88003314	76612051	25.49	19.42
16	75757225	72238050	18.97	17.09
17	93554808	94221336	28.44	28.80
18	106889102	84389151	35.54	23.56
19	100102472	82114709	31.93	22.35
20	85000382	75151011	23.89	18.64
21	86086971	76594090	24.47	19.41
22	131398884	104544881	48.59	34.29
23	142848833	114619366	54.69	39.66
24	154113962	120484303	60.68	42.78
25	130372893	100776082	48.04	32.29
26	102278842	82984695	33.09	22.81
27	106401586	84615475	35.28	23.68
28	146688810	113443580	56.73	39.03
29	142412295	103897129	54.45	33.95
30	138168028	106090718	52.19	35.12
31	154573027	121193315	60.93	43.16
32	148348712	115005746	57.61	39.86
33	139913644	108258296	53.12	36.27
34	237159245	183476282	104.90	76.32
35	160718158	125458608	64.20	45.43
36	136431596	107780362	51.27	36.02
37	114845350	95338390	39.78	29.39
38	109437481	86569182	36.90	24.72
Average	123759159	106645377	44.52	35.41
Standard Deviation	34762233.7	29300763.1	18.5	15.6
95% UCL	134811949.3	115961671.9	50.4	40.4
95% LCL	112706367.8	97329083.1	38.6	30.5

Day #	Delta C	C_{out}/C_{in}	Flow Rate (L min^{-1})	Membrane Area (m^2)
1	-2.1	1.05	1.41	0.0527
2	4.3	0.91	1.41	0.0527
3	-1.7	1.05	1.41	0.0527
4	-3.5	1.05	1.41	0.0527
5	-3.2	1.06	1.41	0.0527
6	2.4	0.95	1.41	0.0527
7	6.0	0.92	1.41	0.0527
8	5.7	0.91	1.41	0.0527
9	1.2	0.95	1.41	0.0527
10	2.8	0.85	1.41	0.0527
11	8.3	0.84	1.41	0.0527
12	1.1	0.95	1.41	0.0527
13	3.5	0.82	1.41	0.0527
14	4.4	0.78	1.41	0.0527
15	6.1	0.76	1.1	0.0527
16	1.9	0.90	1.1	0.0527
17	-0.4	1.01	1.1	0.0527
18	12.0	0.66	1.1	0.0527
19	9.6	0.70	1.1	0.0527
20	5.2	0.78	1.1	0.0527
21	5.1	0.79	1.1	0.0527
22	14.3	0.71	1.1	0.0527
23	15.0	0.73	1.1	0.0527
24	17.9	0.70	1.1	0.0527
25	15.8	0.67	1.1	0.0527
26	10.3	0.69	1.1	0.0527
27	11.6	0.67	1.1	0.0527
28	17.7	0.69	1.1	0.0527
29	20.5	0.62	1.1	0.0527
30	17.1	0.67	1.1	0.0527
31	17.8	0.71	1.1	0.0527
32	17.8	0.69	1.1	0.0527
33	16.9	0.68	1.1	0.0527
34	28.6	0.73	1.1	0.0527
35	18.8	0.71	1.1	0.0527
36	15.3	0.70	1.1	0.0527
37	10.4	0.74	1.1	0.0527
38	12.2	0.67	1.1	0.0527
Average	9.1	0.80		
Standard Deviation	7.8	0.1		
95% UCL	11.6	0.8		
95% LCL	6.6	0.8		

Day #	Influent Load (mg m^{-2} min^{-1})	Mass Removal Rate (mg m^{-2} min^{-1})	Reactor Volume (m^3)	Elimination Capacity (g m^{-3} h^{-1})
1	1197.6	0.0	0.00199	0.0
2	1222.1	115.4	0.00199	183.4
3	957.5	0.0	0.00199	0.0
4	1744.2	0.0	0.00199	0.0
5	1530.3	0.0	0.00199	0.0
6	1252.5	63.8	0.00199	101.3
7	1940.0	161.0	0.00199	255.8
8	1755.5	151.4	0.00199	240.5
9	617.9	32.7	0.00199	51.9
10	481.6	73.6	0.00199	117.0
11	1392.9	221.2	0.00199	351.4
12	641.5	29.4	0.00199	46.7
13	527.4	94.1	0.00199	149.5
14	543.0	117.6	0.00199	186.9
15	532.0	126.6	0.00199	201.1
16	395.9	39.1	0.00199	62.1
17	593.7	0.0	0.00199	0.0
18	741.8	250.0	0.00199	397.3
19	666.4	199.9	0.00199	317.6
20	498.6	109.5	0.00199	173.9
21	510.7	105.5	0.00199	167.6
22	1014.2	298.4	0.00199	474.2
23	1141.4	313.7	0.00199	498.5
24	1266.6	373.7	0.00199	593.8
25	1002.8	328.9	0.00199	522.6
26	690.6	214.4	0.00199	340.7
27	736.4	242.1	0.00199	384.7
28	1184.1	369.4	0.00199	587.0
29	1136.6	428.0	0.00199	680.1
30	1089.4	356.5	0.00199	566.4
31	1271.7	370.9	0.00199	589.4
32	1202.6	370.5	0.00199	588.7
33	1108.8	351.8	0.00199	558.9
34	2189.5	596.6	0.00199	947.9
35	1340.0	391.8	0.00199	622.6
36	1070.1	318.4	0.00199	505.9
37	830.3	216.8	0.00199	344.4
38	770.2	254.1	0.00199	403.8
Average	1020.8	202.3		321.4
Standard Deviation	436.1	150.8		239.6
95% UCL	1159.4	250.2		397.6
95% LCL	882.1	154.3		245.2

BIBLIOGRAPHY

ACGIH (American Council of Government Industrial Hygienists). "Threshold Limit Values," 2004 Edition.

Acid Rain, EPA. U.S. EPA Website, "Acid Rain", found at www.epa.gov/airmarkets/acidrain/index.html#what, 29 August 2004.

AFIOH (Air Force Institute of Occupational Health). "Typical Base Fuel Storage Tanks," Information obtained from data collected by Captain Vincent Falls, Air Branch Chief, AFIOH/RSEA. 12 Mar 2004.

AFRL (Air Force Research Laboratory). Unpublished data obtained through DNA sequencing of biofilm by 2Lt Tracy Denaro, Research Chemist, Fuels Branch, AFRL/PRTG, 20 July 2004.

Ahn, K.H., K.G. Song, I.T. Yeom, and K.Y. Park. "Performance Comparison of Direct Membrane Separation and Membrane Bioreactor for Domestic Wastewater Treatment and Water Reuse," *Water Science Technology: Water Supply,* 1: 315-324 (2001).

Army Fuel Guide. "Fuel Users Guide 2000." September 2000. Prepared by the U.S. Army Tank-Automotive RD&E Center, Fuels & Lubricants Team, AMSTA-TR-D/210, Warren MI 48397-5000. Found at http://usapc.army.mil/miscellaneous/2000%20Fuel%20Users%20Guide%20(Sep%202000).pdf.

ATSDR (Agency for Toxic Substance and Disease Registry). "Public Health Statement for JP-5 and JP-8," Statement published at http://www.atsdr.cdc.gov/toxprofiles/phs121.html. August 1998.

Attaway, H., C.H. Gooding, M.G. Schmidt. "Biodegradation of BTEX vapors in a silicone membrane bioreactor system," *Industrial Microbiology and Biotechnology,* 26: 316-325 (May 2001).

Benjamin, M. M. *Water Chemistry.* Boston: McGraw Hill, 2002.

Benner, M.L., S.M. Stanford, L.S. Lee, R.H. Mohtar. "Field and numerical analysis of in-situ air sparging: A case study," *Journal of Hazardous Material,* 72, 217-236 (2000).

BP Fuels. "History of Jet Fuel," Online article, no page numbers given, no author given. 13 Dec 2004. http://www.airbp.com/airbp/public/generalinterest/jethistory.html.

Bruce, M.O., and E.D. Schroeder. "Hydrogenotrophic denitrification in a microporous membrane bioreactor," *Water Research,* 36: 4683-4690 (November 2002).

CERCLA Overview. "CERCLA Overview." Web Page prepared by the Environmental Protection Agency, downloaded 13 December 2004. http://www.epa.gov/superfund/action/law/cercla.htm.

Chang, K., Chungsying L., Min-Ray L. "Treatment of Volatile Organic Compounds from Polyurethane and Epoxy Manufacture by a Trickle-Bed Air Biofilter," *Journal of Bioscience and Bioengineering,* 92: 126-130 (2001).

Chiang, C.Y., P.K. Petrovsky, S.J. Rouse. "Bioremediation and clean site-closure of a contaminated aquifer," *6th International Conference on Radioactive Waste management and Environmental Remediation*, pp. 655-658 (1999).

Christian, G.D. *Analytical Chemistry, 6th Edition.* Boston: John Wiley and Sons, Inc., 2003.

Clean Air Act, EPA. "The Plain English Guide to the Clean Air Act." April 1993. Report prepared by the EPA on provisions of the Clean Air Act, downloaded 14 Dec 2004 from http://www.epa.gov/oar/oaqps/peg_caa/pegcaain.html#index.

Cole, S.A. "Membrane Biofiltration Treatment of Contaminated Airstreams." Opportunities for Undergraduate Research Experience Report, Department of Civil Engineering, University of Missouri-Rolla. January, 2001.

Cyclohexane, EPA. "Chemical Summary for Cyclohexane." Prepared by the Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, September 1994. Found at www.ess.co.at/RISK/MSDS/cycloh0.html.

Deshusses, Marc A., G. Hamer, I.J. Dunn. "Transient-State Behavior of a Biofilter Removing Mixtures of Vapors of MEK and MIBK from Air," *Biotechnology and Bioengineering,* 49: 587-598 (1996).

DeVinny, J.S., M.A. Deshusses, T.S. Webster. *Biofiltration for Air Pollution Control.* Boca Raton: CRC Press, 1999.

Domenico, P.A., and F.W. Schwartz. *Physical and Chemical Hydrogeology, 2nd Edition.* New York: John Wiley & Sons, Inc., 1998.

England, E.C. "Modeling and Operation of Single and Multiple Tube Membrane Bioreactors," Dissertation performed at the University of Missouri Rolla, Department of Civil Engineering, 2003.

EPA (Environmental Protection Agency). National Pollutant Emissions Standards for Hazardous Air Pollutants, 1990 Clean Air Act Amendments, Title I, Section 112, Part 63 found at http://www.epa.gov/region4/air/airtoxic/mnmain.htm. 1990.

Ergas, S.J., L. Shumway, M.W. Ritch, J.J. Neemann. "Membrane process for biological treatment of contaminated gas streams," *Biotechnology and Bioengineering,* 63: 432-441 (May 1999).

Fox, S.I. *Human Physiology, 8th Edition.* Boston: McGraw Hill, 2004.

Freitas dos Santos, L.M., U. Hommeric, A.G. Livingston. "Dichloroethane Removal from Gas Streams by an Extractive Membrane Bioreactor," *Biotechnology Prog.,* 11: 194-201 (1995).

Gilbert, R.O. *Statistical Methods for Environmental Pollution Monitoring*. New York: John Wiley and Sons, Inc., 1987.

Godish, T. *Air Quality, 4th Edition.* New York: CRC Press, 2004.

Hartley, J.D., L.J. Furlow, W.N. Zwiren. "Cycled Air Sparging: Field Results in a High-Velocity Aquifer," *5th International Symposium on In-Situ and On-Site Bioremedation,* pp. 77-81 (1999).

Hazardous Air Pollutants, EPA. "NEI Hazardous Air Pollutant Names, Page 6 of 10." List of HAPs listed in the National Emissions Inventory, downloaded 14 Dec 2004 from http://www.epa.gov/air/data/help/hneihaps6.html.

Madigan, M.T., J.M. Martinko, and J. Parker. *Brock Biology of Microorganisms.* New Jersey: Prentice Hall, 2003.

Maier, R.M., I. Pepper, and C. Gerba. *Environmental Microbiology*. San Diego: Academic Press, 2000.

Masters, G.M. *Introduction to Environmental Engineering and Science, 2nd Edition.* New Jersey: Prentice Hall, 1998.

McClave, J.T., P.G. Benson, and T. Sincich. *Statistics for Business and Economics 8th Edition.* New Jersey: Prentice Hall, 2001.

Min, K.N., S.J. Ergas, J.M. Harrison. "Hollow-fiber membrane bioreactor for nitric oxide removal," *Environmental Engineering Science,* 19: 575-584 (Nov-Dec 2002).

Newell, C.J., P.E. Haas, J.B. Hughes, T.A. Khan. "Results from two direct hydrogen delivery field tests for enhanced dechlorination," *Proceedings of the 2nd International Conference on Remediation of Chlorinated and Recalcitrant Compounds,* vol. 2, pp. 31-37 (2000).

NIOSH, 2004. National Institue of Occupational Safety and Health (NIOSH) Pocket Guide to Chemical Hazards, 2004 Edition. Found online at http://www.cdc.gov/niosh/npg/npg.html, downloaded 7 February 2005.

OSHA (Occupational Safety and Health Administration). "Permit Required Confined Spaces," *29 CFR 1910.146*. 14 January 1993.

OSHA (Occupational Safety and Health Administration). "Health Guidelines for Cyclohexane," Downloaded from www.osha.gov/SLTC/healthguidelines/cyclohexane/recognition.htm. 5 Jan 2004.

Parvatiyar, M.G., R. Govind, D. F. Bishop. "Biodegradation of toluene in a membrane biofilter," *Journal of Membrane Science,* 119: 17-24 (1996).

Pleil, J.D., L.B. Smith, S.D. Zelnick. "Personal Exposures to JP-8 Jet Fuel Vapors and Exhaust at Air Force Bases." *Environmental Health Perspectives,* 108: 1-7 (2000).

Reij, M.W., J.T.F. Keurentjes, S. Hartmans. "Membrane bioreactors for waste gas treatment," *Journal of Biotechnology,* 59: 155-167 (1998).

Rishell, S. "Scale Up Studies Using a Mixed Methanotrophic Culture in a Membrane Biofilm Reactor." Doctoral Thesis at the Department of Chemical Engineering, University College, Dublin, Ireland. November 2002.

Roggy, D.K., P.J. Novak, R.M. Hozalski, L.W. Clapp, M.J. Semmens. "Membrane Gas Transfer for Groundwater Remediation: Chemical and Biological Fouling," *Environmental Engineering Science,* 19: 563-575 (November 2002).

Semmens, M.J., K. Dahm, J. Shanahan, and A. Christianson. "COD and nitrogen removal by biofilms growing on gas permeable membranes," *Water Research,* 37: 4343-4350 (November 2003).

Skoog, D.A., F. J. Holler, and T.A. Nieman. *Principles of Instrumental Analysis, 5th Edition.* Philadelphia: Saunders, 1998.

Smith, L.B. "Exposure to Fuel Vapors During Aircraft Fuel Systems Maintenance Operations, Little Rock Air Force Base, Arkansas." Air Force Institute for Environmental Risk Assessment (AFIERA) Consultative Letter IERA-RS-BR-CL-1999-0070, 18 August 1999.

Thalasso, F., E. Razo-Flores, R. Ancia, H.P. Naveau, E.J. Nyns. "Pressure-drops control strategy in a fixed-bed reactor," *Journal of Hazardous Materials,* 81: 115-122 (2001).

UFC (Unified Facilities Criteria) 3-460-03. *Operation and Maintenance: Maintenance of Petroleum Systems.* 21 January 2003, 83-84.

Weber, F.J. and S. Hartmans. "Prevention of clogging in a biological trickle-bed reactor removing toluene from contaminated air." *Biotechnology and Bioengineering,* 50: 91-97 (April 1996).

Westbrook, S.R., D.W. Naegeli, K.H. Childress. "JP-8 Volatility Study," Research funded by AFIOH performed by the Fuels and Lubricants Technology Department, Engine and Vehicle Research Division, Southwest Research Institute, San Antonio, TX. Project No. 03-3148, March 2001.

Yeom, S.H., and A.J. Daugulis. "Development of a novel bioreactor system for treatment of gaseous benzene." *Biotechnology and Bioengineering,* 72: 156-165 (Jan 2001).

Yeow, M.L., R.W. Field, K. Li, W.K. Teo. "Preparation of divinyl-PDMS/PVDF composite hollow fiber membranes for BTX removal," *Journal of Membrane Science,* 203: 137-143 (2002).

Zerbonia, R. , J.A. Spivey, S.J. Agarwal, A.K. Damle, C.W. Sanford. "Survey of Control Technologies for Low Concentration Organic Vapor Gas Streams." May, 1995. Report prepared for the Environmental Protection Agency's Information Transfer and Program Integration Division, Office of Air Quality Planning and Standards. Downloaded 15 December 2004 from http://www.epa.gov/ttn/catc/products.html.

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14. ABSTRACT

The purpose of this research was to determine the ability of a dense phase membrane bioreactor to remove cyclohexane, a volatile organic compound in JP-8 jet fuel, from a contaminated air stream using a biologically active film for degradation. The research answered questions regarding applications of membrane bioreactors, the ability of cyclohexane to diffuse through a dense phase membrane, growth of a viable microbial culture, and determination of the performance capabilities of the reactor. To answer these questions, a literature review was conducted and laboratory experiments were performed. Through the design, construction, and testing of the dense phase membrane bioreactor used for this research, it was determined that the reactor removed cyclohexane from a contaminated air stream at an average elimination capacity of 321.4 +/- 76.2 g m^{-3} hr^{-1} with a 95% confidence interval.

The successful removal of cyclohexane with the dense phase membrane bioreactor in this research effort filled a vacant niche in the scientific body of knowledge surrounding membrane bioreactor technology. Current technology applications, laboratory techniques, and data analysis are discussed.

15. SUBJECT TERMS

Membrane Bioreactor, Air Pollution Remediation, Cyclohexane, Biofilter

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