

**PRODUCTION AND CHARACTERIZATION OF BIODIESEL FROM
MICROALGAE *Spyrogyra spp.***

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In Partial Fulfillment
Of the Requirements for
SCIENCE RESEARCH 2

By

Jeff Lorenz U. Booc
JC Neal P. Villanueva
Patrick Andrew G. Bayona

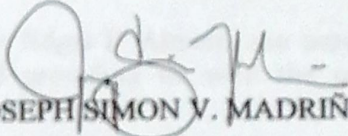
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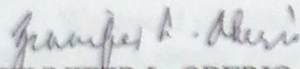
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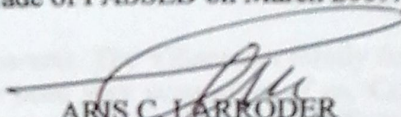
“PRODUCTION AND CHARACTERIZATION OF BIODIESEL FROM MICROALGAE,
Spirogyra spp.”

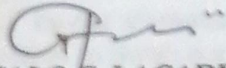
Prepared and submitted by Jeff Lorenz U. Booc, Patrick Andrew G. Bayona and JC Neal P. Villanueva in partial fulfillment of the requirements in Science Research 2 has been approved and is recommended for acceptance and approval.

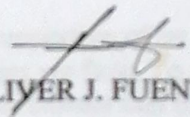

JOSEPH SIMON V. MADRIÑAN
Science Research 2 Adviser


Approved by the committee in oral examination with a grade of PASSED on March 2009.


ZENNIFER L. OBERIO
Member

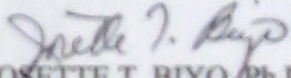

ARIS C. LARRODER
Member


MIALO C. LACADEN
Member


OLIVER J. FUENTESPINA
Member


EDWARD C. ALBARACIN
Member

Accepted in partial fulfillment of the requirements in Science Research 2.


JOSETTE T. BIYO, Ph.D.
Director III – PSHSWVC

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ABSTRACT

Biodiesel is biodegradable, less CO₂ and NO_x emissions. Continuous use of petroleum sourced fuels is now widely recognized as unsustainable because of depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide in the environment. Renewable, carbon neutral, transport fuels are necessary for environmental and economic sustainability. Algae have emerged as one of the most promising sources for biodiesel production. It can be inferred that algae grown in CO₂-enriched air can be converted to oily substances. Such an approach can contribute to solve major problems of air pollution resulting from CO₂ evolution and future crisis due to a shortage of energy sources.

Spirogyra is a filamentous alga which is common in freshwater habitats. It has the appearance of very fine dark-green filaments moving gently with the currents in the water, and is slimy to the touch. The slime serves to deter creatures which otherwise attach themselves to underwater plants, so *Spirogyra* under the microscope is usually spotless.

This study determined if biodiesel could be produced from the oil extracted from microalgae, *Spirogyra* spp.

The algae were cultivated and harvested in a natural pond. The methods of extraction used were a combination of the Hexane Solvent method and ultrasonication. The algae were mixed with hexane and sonicated for 30min. To separate the oil from the hexane, a rotary evaporator was used.

Oil extracted from the algae used were minimal in amount and thus cannot be transesterified and turned into biodiesel.

Therefore oil in microalgae, *Spirogyra* spp., cannot be turned into biodiesel.

This may not be true if very large amounts of *Spirogyra* spp were used. This may also not be true for all species of microalgae..

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CHAPTER I

INTRODUCTION

A. Background of the Study

Gas and oil products bring about comfort and ease to the world. These gas products originate from several sources, yet the most common is that from the oil depots from the grounds of the earth. The oil is converted into fuel for us to consume and enjoy. The comfort these fuels bring about is truly unquestionable. We may even have the tendency to make the most of the purpose it serves to the point of being abusive. Unfortunately these resources don't reproduce and as time passes, with constant exploitation, our oil reserves around the world are gradually depleting. On the contrary, several ways on how to extract energy have been introduced. These strategies proved to be effective and cheaper than petroleum products.

Lately, use of biodiesel has been introduced. The idea of extracting oil from organic products like corn, soy beans, and even algae is truly innovative.

Biodiesel can be made from new or used vegetable oils and animal fats, which are non-toxic, biodegradable, renewable and a resource. Fats and oils are chemically reacted with an alcohol (methanol is the usual choice) to produce chemical compounds known as fatty acid methyl esters. Biodiesel is the name given to these esters when they're intended for use as fuel.

In today's world, one can hardly escape the subject of fuel prices and fuel supply. For a number of different reasons people have turned from standard petroleum based fuel sources and looked for reliable alternative fuel. Biodiesel is one such fuel that experts and enthusiasts have embraced not only as their idea of a fuel of the future, but it is also their choice for fuel for today.

Biodiesel is a fuel containing some of the traits as conventional diesel fuel. It is made from high quality vegetable oils through a manufacturing process that can be done on a large scale- such as a refinery, or on a small scale- such as a home biodiesel kit. The primary use for biodiesel right now is a substitute for petroleum based diesel fuel.

In different parts of the world, different plants are used as the source for the oil that is made into biodiesel. Theoretically, any vegetable grown can be broken down and turned into biodiesel, but right now most biodiesel producers use one primary crop as their source. In America, the primary crop grown for biodiesel production is corn, being one of the most common crops grown there, provides a readily available supply of vegetable matter for biodiesel manufacturers. In Southeast Asia, the primary plants grown for the manufacture of biodiesel are soybeans. After going through the manufacturing process, there is little difference in the properties of biodiesel made from one plant over the other.

The current uses for biodiesel are mainly limited to that of being a cleaner burning replacement for petroleum based diesel fuel. Biodiesel can be both economically viable and highly efficient for most mobile applications. There will be some performance and usage differences between diesel and biodiesel and they will vary from vehicle to vehicle. Currently biodiesel is marketed as a biodiesel/ethanol mix or a biodiesel/diesel mix. Still a young industry by any measure, biodiesel is on its way to becoming one of the brightest lights in the search for a viable alternative fuel.

Proponents say such renewable fuels could light a fire under our moribund economy, help extract us from our sticky dependence on the Middle East, and best of all, cut our ballooning emission of carbon dioxide (Bourne Jr., 2007).

While a number of bio-feedstock is currently being experimented for biodiesel production, algae have emerged as one of the most promising sources for biodiesel production. Though research into algae as a source for biodiesel is not new, the current oil crisis and fast depleting fossil oil reserves have made it more imperative for organizations and countries to invest more time and efforts into research on suitable renewable on suitable feedstock such as algae.

This particular study aims to collect oil from the micro algae, *Spirogyra sp.*, and to be able to produce and characterize the biodiesel produced from the algae oil through the orsat apparatus.

B. Statement of the Problem

Can biodiesel be produced using the oil extracted from the algae, *Spirogyra* spp.?

C. Objectives of the Study

This study aims to:

1. Extract oil from the algae, *Spirogyra* sp., using the combination of Hexane Solvent Method and ultrasonication;
2. Produce a biodiesel from the oil extracted;

D. Significance of the Study

Algae are very common in the Philippines. There are lots of species of algae that can be found in seas, ponds, and others. Although they are widespread, they are rarely utilized and are mostly used to feed the fishes in ponds.

By extracting the organic oil found in the algae, biodiesel may be developed to power cars and/or machines. This kind of biodiesel just might be a breakthrough in the oil industry for it is cheap because they are very easy to grow.

This biodiesel, if mass produced, can provide drivers with a cheap fuel to replace the expensive diesel. Air pollution can be reduced due to its ability to emit less toxic gases.

E. Definition of terms

Oil – any of various viscous, combustible, water-immiscible liquids that are soluble in certain organic solvents, as ether and naphtha; may be of animal, vegetable, mineral, or synthetic origin. (McGraw-Hill Dictionary of Scientific and Technical Terms. Fifth Edition.)

In this study oil refers to the organic fats extracted from the microalgae *Spirogyra sp.*

Biodiesel – refers to a diesel-equivalent, processed fuel derived from biological sources (such as vegetable oils), which can be used in unmodified diesel-engine vehicles.

In this study, biodiesel refers to the target product of our study.

Hexane Solvent Method –

In this study, the Hexane Solvent method refers to the method of extracting the algal oil from the microalgae *Spirogyra sp.*

Microalgae – a vast group of photosynthetic, heterotrophic organisms which have an extraordinary potential for cultivation as energy crops. They can be cultivated difficult agro-climatic conditions and are able to produce a wide range of commercially interesting byproducts such as fats, oils, sugars and functional bioactive compounds.

(<http://www.micrographia.com/specbiol/alg/filamer>)

In this study, microalgae refers to the kind of algae used.

Spirogyra spp. – is a genus of filamentous green algae of the order Zygnematales, named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is commonly found in freshwater areas, and there are more than 400 species of *Spirogyra* in the world. *Spirogyra* measures to around 10 to 100µm in width and may stretch centimeters long.

(<http://en.wikipedia.org/wiki/Spirogyra>)

In this study, *Spirogyra spp.*, is the microalgae in which its organic oil is extracted and formed into biodiesel.

Gas emission - A substance discharged into the air, especially by an internal combustion engine. (<http://dictionary.reference.com/browse/emission>).

In this study, gas emissions refers to the amount of different gases emitted by the biodiesel.

Performance - process or manner of functioning or operating

(<http://dictionary.reference.com/browse/performance>).

In this study, performance refers to the gas emissions of the biodiesel.

CHAPTER II

REVIEW OF RELATED LITERATURE

A. Biodiesel

Biodiesel refers to a diesel-equivalent, proceed fuel derived from biological sources (such as vegetable oils), which can be used in unmodified diesel-engine vehicles. It is thus distinguished from the straight vegetable oils (SVO) or the waste vegetable oils used as fuels in some vehicles.

It is a domestically produced, renewable fuel that can be manufactured from vegetable oils, animal fats, or recycled restaurant greases. Biodiesel is safe, biodegradable, and reduces serious air pollutants such as particulates, carbon monoxide, hydrocarbons, and air toxics.

Biodiesel is biodegradable and non-toxic, and typically produces about 60% less net carbon dioxide emission than petroleum-based diesel, as it is itself produced from atmospheric carbon dioxide via photosynthesis in plants.

Biodiesel is produced currently from plant and animal oils, but not from microalgae. This is likely to change as several companies are attempting to commercialize microalgal biodiesel. Biodiesel is a proven fuel. Technology for producing and using biodiesel has been known for more than 50 years (Knothe et al., 1997; Fukuda et al., 2001; Barnwal and Sharma, 2005; Demirbas, 2005; Van Gerpen, 2005; Felizardo et al., 2006; Kulkarni and Dalai, 2006; Meher et al., 2006). In the United States, biodiesel is produced mainly from soybeans. Other sources of commercial biodiesel include canola oil, animal fat, palm oil, corn oil, waste cooking oil (Felizardo et al., 2006; Kulkarni and Dalai, 2006), and jatropha oil (Barnwal and Sharma, 2005). Any future production of biodiesel from microalgae is expected to use the same process. Production of methyl esters, or biodiesel, from microalgal oil has

been demonstrated (Belarbi et al, 2000) although the product was intended for pharmaceutical use.

It has a viscosity similar to petrodiesel, the current industry term for diesel produced from petroleum. It can be used as an additive in formulations of diesel to increase the lubricity of pure Ultra-Low Sulfur Diesel fuel, which is advantageous because it has virtually no sulfur content. Much of the world uses a system known as the "B" factor to state the amount of biodiesel in any fuel mix, in contrast to the "BA" system used for ethanol mixes.

Advantages of deriving biodiesel from algae include rapid growth rates, a high per acre yield, and an algae biodiesel contains no sulfur, is non-toxic, and is highly biodegradable. Some species of algae ideally suited to biodiesel production due to their high-oil content- in some species, topping it 50%.

Blends like B2 (2% biodiesel and 98% diesel) and B5 (5% biodiesel and 95% diesel) are becoming increasingly common as drivers become more aware of the many benefits. Biodiesel is manufactured chemically reacted vegetable oils, recycled cooking grease, or animal fats with alcohol.

B. Producing Biodiesel from Algae

The algae itself contains vast amounts of natural oils thus making it a perfect candidate for making the fuel. Biodiesel from algae can also be grown and harvested very quickly, meaning, the production is sped up dramatically (Kiong, 2006).

Shay reported that algae were one of the best sources of biodiesel. In fact algae are the highest yielding feedstock for biodiesel. It can produce up to 250 times the amount of oil per acre as soybeans. In fact, producing biodiesel from algae may be only the way to produce enough automotive fuel to replace current gasoline usage.

Algae produce 7 to 31 time greater oil than palm oil. It is very simple to extract oil from algae. The best algae for biodiesel would be microalgae. Microalgae are an organism capable of photosynthesis that is less than 2 mm in diameter. Macroalgae, like seaweed, is not as widely used in the production of biodiesel. Microalgae has much more oil than macroalgae and it is much faster and easier to grow.

Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass biodiesel derived from microalgal oil and photobiologically produced biohydrogen. The idea of using microalgae as a source of fuel is not new but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning fossil fuels. No literatures are found regarding present research such as biodiesel from macroalgae having species *Oedogonium* and *Spirogyra*. That is why we have done the research to know the proper transesterification, amount of biodiesel production (ester) and physical properties (yield of biodiesel, glycerine and sediments) of biodiesel from algae (Sharif Hossain et al., 2008).

Studies have shown that algae can produce up to 30 times more biodiesel than the crops that are currently used. Biodiesel made from algae is also biodegradable and is non-toxic (Kiong, 2006).

Microalgae comprise a vast group of photosynthetic, heterotrophic organisms which have an extraordinary potential for cultivation as energy crops. They can be cultivated under difficult agro-climatic conditions and are able to produce a wide range of byproducts such as fats, oils, sugars and functional bioactive compounds. Certain microalgae are effective in the production of hydrogen and oxygen through

the process of biophotolysis while others naturally manufacture hydrocarbons which are suitable for direct use as high-energy liquid fuels.

All algae primarily comprise of the following, in varying proportions: proteins, carbohydrates, fats and nucleic acids. While the percentages vary with the type of algae, there are algae types that are comprised up to 40% their overall mass by fatty acids. It is these fatty acids that can be extracted and converted to biodiesel (http://www.castoroil.in/reference/plant_oils/uses/fuel/sources/algae/biodiesel_algae.html)

Algae	Protein	Carbohydrates	Lipids	Nucleic Acids
<i>Scenedesmus obliquus</i>	50-56	10-17	12-14	3-6
<i>Scenedesmus quadricauda</i>	47		1.9	
<i>Scenedesmus dimorphus</i>	8-18	21-52	16-40	
<i>Chalymodonas rheinhardii</i>	48	17	21	
<i>Chlorella vulgaris</i>	51-58	12-17	14-22	4-5
<i>Spirogyra sp.</i>	6-20	33-64	11-21	
<i>Dunaliella bioculata</i>	49	4	8	
<i>Dunaleilla salina</i>	57	32	6	
<i>Euglena gracilis</i>	39-61	14-18	14-20	
<i>Prymnesium parvum</i>	28-45	25-33	22-38	1-2
<i>Tetraselmis maculata</i>	52	15	3	
<i>Porphyridium cruentum</i>	28-39	40-57	9-14	
<i>Spirulina plantensis</i>	46-63	8-14	4-9	2-5
<i>Spirulina maxima</i>	60-71	13-16	6-7	3-4.5

<i>Synechococcus sp.</i>	63	15	11	5
<i>Chlorella pyrenoidosa</i>	57	26	2	
<i>Anabaena cylindrical</i>	43-56	25-30	4-7	

Table 1. Chemical Composition of Algae Expressed on a Dry Matter Basis (%)
(http://www.castoroil.in/reference/plant_oils/uses/fuel/sources/algae/biodiesel_algae.html)

Algae contain anywhere between 2% to 4% of lipids/oils by weight. There are three well-known methods to extract oil from oil seeds and these should work equally well with algae too: 1. Expeller/press 2. Hexane solvent oil extraction 3. Super critical fluid extraction.

Micro algae are quite simply remarkable and efficient biological factories capable of taking a waste form of carbon dioxide (CO₂) and converting it into high density liquid form of energy (Cornell, 2007).

Converting algae into biodiesel uses the same process as that in vegetable oils. But the cost of producing algae oil is hard to pin down because nobody's running the process start to finish other than in a laboratory (Karnowski, 2007).

Algae's advantages include growing much faster and in less space than conventional crops. An acre of corn can produce about 20 gallons of oil per year compared with a possible 15000 gallons of oil per acre of algae (Karnowski, 2007).

Algae grow voraciously (measured by day), algae can proliferate at heinous growing conditions (saltwater and extreme conditions), and certain species contain up to 60% oil (measured by weight) (Cornell, 2007).

An algae farm could be located almost anywhere. It wouldn't require converting cropland from food production to energy production. It could use sea water. And algae can gobble up pollutants from sewage and power plants.

C. Spirogyra spp.

Spirogyra is a filamentous alga which is common in freshwater habitats. It has the appearance of very fine dark-green filaments moving gently with the currents in the water, and is slimy to the touch. The slime serves to deter creatures which otherwise attach themselves to underwater plants, so Spirogyra under the microscope is usually spotless.

Nearly of the numerous species belong to the low, quiet waters of the ponds and ditches, where they often form large, flocculent green mats nearly covering the surface of the water. A few species occur in running water. The mats are very slippery to the touch.

Most metals are poisonous to Spirogyra, even the small amount taken up by the water while standing in the water pipe being detrimental.

D. Hexane Solvent Extraction

In biodiesel production, hexane, a colorless, flammable liquid derived from petroleum, is traditionally used to extract oil. But hexane is an air pollutant regulated by the U.S. Environmental Protection Agency.

(<http://www.ars.usda.gov/is/AR/archive/apr05/diesel0405.htm>).

Solvent Extraction is a process which involves extracting oil from oil-bearing materials by treating it with a low boiler solvent as opposed to extracting the oils by mechanical pressing methods (such as expellers, hydraulic presses, etc.) The solvent extraction method recovers almost all the oils and leaves behind only 0.5% to 0.7% residual oil in the raw material. In the case of mechanical pressing the residual oil left in the oil cake may be anywhere from 6% to 14%. The solvent extraction method can be applied directly to any low oil content raw materials. It can also be used to extract

pre-pressed oil cakes obtained from high oil content materials. Because of the high percentage of recovered oil, solvent extraction has become the most popular method of extraction of oils and fats

(http://www.oilgae.com/ref/glos/hexane_solvent_oil_extraction.html).

Solvent extraction is used almost exclusively on delicate plants and produce higher amounts of essential oils at a lower cost than could be achieved by enflauge. In this process, a chemical solvent such as hexane is used to saturate the plant material and pull the essential oils out. The plant material is then removed and this renders a solvent.

The solvent is then boiled off under a vacuum or in a centrifugal force machine to help separate it from the oil. Because the solvent has a lower boiling point than the essential oil it evaporates and the oil is left. The solvent is cooled back into liquid and recycled. Along with the essential oil, the fats, waxes and heavier oils are extracted producing a substance known as a concrete. The process is continued by dissolving oils into warm alcohol. The alcohol is removed under a vacuum and an absolute is left. (<http://www.itselixir.com/Extraction-Methods-p-22.html>).

E. Ultrasonication

Ultrasonication offers great potential in the processing of liquids and slurries, by improving the mixing and chemical reactions in various applications and industries. Ultrasonication generates alternating low-pressure and high-pressure waves in liquids, leading to the formation and violent collapse of small vacuum bubbles. This phenomenon is termed cavitation and causes high speed impinging liquid jets and strong hydrodynamic shear-forces. These effects are used for the deagglomeration and milling of micrometer and nanometer-size materials as well as for the disintegration of cells or the mixing of reactants. In this aspect, ultrasonication is an alternative to high-speed mixers and agitator bead mills. Ultrasonic foils under the moving wire in a paper machine will use the shock waves from the imploding bubbles to distribute the cellulose fibers more uniform in the produced paper web, which will make a stronger paper with more even surfaces, see more on Ultra Technology. Furthermore, chemical reactions benefit from the free radicals created by the cavitations as well as from the energy input and the material transfer through boundary layers. For many processes, this sonochemical (see sonochemistry) effect leads to a substantial reduction the reaction time, like in the transesterification of oil into biodiesel. Ultrasonication can easily be tested in lab scale for its effect on various liquid formulations. Within the past five years equipment manufacturers like Hielscher developed a number of larger ultrasonic processors of up to 16 kW power. Therefore volumes from 1mL up to several hundred gallons per minute can be sonicated today in order to achieve all kinds of results from the link that is shown below (<http://en.wikipedia.org/w/index.php?title=Ultrasonics&printable=yes>).

G. Rotary Evaporator

A rotary evaporator (or rotavap) is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation.

The main components of a modern rotary evaporator are: (1) a motor unit which rotates the evaporation flask or vial containing one's sample; (2) a vapor duct which acts both as the axis for sample rotation, and as vacuum-tight conduit for the vapor being drawn off of the sample; (3) a vacuum system, to substantially reduce the pressure within the evaporator system; (4) a heated fluid bath, generally water, to heat the sample being evaporated; (5) a condenser with either a coil through which coolant passes, or a "cold finger" into which coolant mixtures like dry ice and acetone are placed; (6) a condensate-collecting flask at the bottom of the condenser, to catch the distilling solvent after it re-condenses; and (7) a mechanical or motorized mechanism to quickly lift the evaporation flask from the heating bath.

The vacuum system used with rotary evaporators can be as simple as a water aspirator with a trap immersed in a cold bath (for non-toxic solvents), or as complex as a regulated mechanical vacuum pump with refrigerated trap. Glassware used in the vapor stream and condenser can be simple or complex, depending upon the goals of the evaporation, and any propensities the dissolved compounds might give to the mixture (e.g., to foam or "bump", see below). Various commercial instruments are available that include the basic features, and various designs of traps are manufactured to insert between the evaporation flask and the vapor duct. In addition, modern equipment often adds features such as digital control of vacuum, digital display of temperature and rotational speed, and even vapor temperature sensing.

Vacuum evaporators as a class function because lowering the pressure above a bulk liquid lowers the boiling points of the component liquids in it. Generally, the component liquids of interest in applications of rotary evaporation are research solvents that one desires to remove from a sample after an extraction, for instance, following a natural product isolation or a step in an organic synthesis. Use of a "rotavap" therefore allows liquid solvents to be removed without excessive heating of what are often complex and sensitive solvent-solute combinations.

Rotary evaporation is most often and conveniently applied to separate "low boiling" solvents such as n-hexane or ethyl acetate from compounds which are solid at room temperature and pressure. However, careful application also allows removal of a solvent from a sample containing a liquid compound if there is minimal co-evaporation (azeotropic behavior), and a sufficient difference in boiling points at the chosen temperature and reduced pressure.

A key disadvantage in rotary evaporations, besides its single sample nature, is the potential of some sample types to bump, e.g. ethanol and water, which can result in loss of a portion of the material, intended to be retained. Even professionals experience periodic mishaps during evaporation, especially bumping, though experienced users become aware of the propensity of some mixtures to bump or foam, and apply precautions that help to avoid most such events. In particular, bumping can often be prevented by taking homogeneous phases into the evaporation, by carefully regulating the strength of the vacuum (or the bath temperature) to provide for an even rate of evaporation, or, in rare cases, through use of added agents such as boiling chips (to make the nucleation step of evaporation more uniform). Rotary evaporators can also be equipped with further special traps and condenser arrays that are best

suited to particular difficult sample types, including those with the tendency to foam or bump (http://en.wikipedia.org/w/index.php?title=Rotary_evaporator).

References

1. Wikipedia.org

2. The instructions

1. Safety
2. Setup
3. Assembly
4. Rotary Evaporator Basics
5. Rotary Evaporator
6. Rotary Evaporator
7. Rotary Evaporator

CHAPTER 3

Methodology

A. Materials and Equipments:

◦ For Extraction:

1. Hexane
2. Algae
3. Aluminum foil
4. 250ml Erlenmeyer flasks
5. Pyrex funnel
6. Rotary evaporator
7. Ultrasonicator

B. Study site and Study Period

First phase of this study was conducted in Brgy. Lanas, Barotac Nuevo. Second and third phases were conducted in the PSHS Research Lab along with the said equipments above.

Harvesting and drying of the algae begun in June 2008. Extraction shall begin as soon as the alga has dried.

C. Overview

First, cultivation of *Spirogyra spp.* was performed in Brgy. Lanas, Barotac Nuevo, which was then harvested and dried. Once the algae were dry, they were then extracted of oil using the combination of Hexane Solvent Method and ultrasonication. Extraction of oil was undertaken repeatedly until ml of oil was produced.

D. Phase I: Cultivation, Harvesting, and Drying of algae

A 5x8 meter pond was prepared for the algae, *Spirogyra spp.* The pond is adjacent to a creek where brackish water penetrates during high tide. The area was cleared of weeds and kangkong plants. The algae were grown naturally with no fertilizers used in cultivation. Maintenance involved irrigation to control water level. A person was hired to inspect and clean the area daily. After about three months, when the alga matured, when the alga turns greenish brown, collection was done by hand.

Harvested algae were air-dried by hanging the harvest on suspended wire boxes or screens or net bags to drain out water. As soon as the *Spirogyra* dried up, turning brown and hard, they were ready for extraction of algae oil.

E. Phase II: Extraction Process

After the alga is dried and harvested, they underwent the extraction process. The combination of Hexane Solvent Method of extraction and ultrasonication was used. Using a kitchen weighing scale, 100g of algae was weighed. Two 1L beakers were filled with algae. The beakers containing the algae were then mixed with 300mL Hexane. The beakers containing the mixture of Hexane and algae were then put into the ultrasonicator. The mixture was then sonicated for 30min. After sonicating, the mixture inside the beakers was then filtered using filter paper and funnel. The mixture collected is a mixture of algae oil and hexane.

CHAPTER IV

RESULTS AND DISCUSSION

A. Results

The study aims to produce a biodiesel through the oil extracted from the microalgae *Spirogyra spp.* The algae were cultivated in a brackish water pond for three months before being harvested. The harvested algae were sun dried for one day.

The extraction methods used in this research is a combination of the Hexane Solvent method and the use of the Ultrasonicator. A rotary evaporator is used to separate the oil from the Hexane. The algae is mixed with the hexane and sonicated. Then the solution is put into the rotary evaporator.

The first method was the use of the Hexane Solvent method alone. This resulted in very small amount of oil collected (barely a milliliter) and easily evaporated when boiled. This was a failure.

The next method is combining the Hexane Solvent method with the ultrasonicator. The process was sped up through the use of the sonicator. In the limited time we performed the extraction, 600g of green microalgae, *Spirogyra spp.*, and 1623ml of Hexane were used. The mixtures of hexane and algae oil measured 1350ml were collected. Every 100g of algae were mixed with 270.5ml of hexane and were sonicated for 30min.

The mixture of algae oil and hexane need to be separated for the oil to be fully extracted. Two batches of the mixture were put in the rotary evaporator. The rotary evaporator was run at 30 rotation speed at 35°C for one hour. The second batch was run for 30min only. The hexane collected was less than what was used for the

extraction. The solution left after the hexane was separated was a 10 ml mixture of algae oil and water.

The amount of oil extracted was barely discernible to continue in the transesterification process. The extraction of oil from algae failed thus there was not enough oil to really determine if a biodiesel could be produced.

A. Discussion

The extraction process involves the combination of the Hexane Solvent oil extraction method and ultrasonification.

Solvent Extraction is a process which involves extracting oil from oil-bearing materials by treating it with a low boiler solvent. The solvent extraction method can be applied directly to any low oil content raw materials. Because of the high percentage of recovered oil, solvent extraction has become the most popular method of extraction of oils and fats.

Ultrasonication offers great potential in processing of liquids and slurries, by improving the mixing and chemical reactions in various applications and industries. Ultrasonication generates alternating low-pressure and high-pressure waves in liquids, leading to the formation and violent collapse of small vacuum bubbles. These effects are used for the deagglomeration and milling of micrometer and nanometer size materials as well as for the disintegration of cells or the mixing of the reactants. In this aspect, ultrasonication is an alternative to high speed mixers and agitator bead mills.

The rotary evaporator is used to separate Hexane from algae oil. Rotary evaporation is most often and conveniently applied to separate "low boiling" solvents

such as n-hexane or ethyl acetate from compounds which are solid at room temperature and pressure.

The key advantages in use of a rotary evaporator are (1) that the centrifugal force and the frictional force between the wall of the rotating flask and the liquid sample result in the formation of a thin film of warm solvent being spread over a large surface, and (2) the forces created by the rotation suppress violent, unpredicted boiling. The combination of these characteristics and the conveniences built into modern rotary evaporators allow for quick, gentle evaporation of solvents from most samples, even in the hands of the most inexperienced users.

Oil was extracted from the algae. However, due to minimal amount of oil extracted, the transesterification process was not able to take place. Flaws in the methodology were the main reason in the minimal amount of oil extracted.

Reasons to the failure of the study occur during the extraction method. Although hexane is an effective solvent, the amount may not have been enough. Through the addition of the ultrasonication process, more oil should've been extracted. The ultrasonic waves cause the cells to burst thus releasing the lipids or oil into the hexane. Failure may be due to insufficient experience in the use of the ultrasonicator. The time used in sonicating may not be enough for the cells to burst. The algae, *Spirogyra spp.*, could have been the reason for the failure. The algae may not have contained enough oil for biodiesel production. Oil content of the algae may have been minimal and the amount algae used for extraction may not have been enough for the production of biodiesel.

CHAPTER V

Summary, Conclusion and Recommendation

Can biodiesel be produced using the oil extracted from the algae, *Spirogyra spp.*?

This study specifically aimed to:

1. Extract oil from the algae, *Spirogyra spp.*, using the Hexane Solvent method and ultrasonication;
2. Produce a biodiesel from the oil extracted.

I. Summary

The findings of this study are:

The algae, *Spirogyra spp.*, produced very minimal oil. Methods of extraction included the Hexane solvent method and ultrasonication of algae. Oil was extracted using the hexane solvent and the ultrasonicator. Though oil was extracted, it couldn't be used for the production of biodiesel. The oil was separated from the hexane through the rotary evaporator. The algae oil extracted was very minimal in amount. Thus the transesterification process could not be done and the oil could not be turned to biodiesel.

II. Conclusion

Production of biodiesel from the extracted oil from algae, *Spirogyra spp.*, was a failure due to the minimal amount of algae extracted and flaws in the methodology.

III. Recommendations

Recommendations for success in the production of biodiesel from algae:

1. prolong the exposure of the algae to ultrasonic waves to ensure that the cells burst, thus the release of more oil;
2. use large amounts of algae for extraction to ensure enough oil is extracted;
3. prolong the exposure of algae to the hexane.

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