



### The Effect of Radiation and Environmental factors on Biofuel production by certain Bacteria and Microalgae

## Thesis Submitted for Partial Fulfillment of Master Degree in Microbiology

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#### **Approval sheet**

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# This dissertation has not been previously submitted for any degree at this or at any other university

Habiba Nagah Sadek Elsayed

## Acknowledgement

Praise and Thanks be to ALLAH, the most merciful for assisting and directing me the right way.

I would like to express my deepest and sincere gratitude to my lovely supervisor Dr. Abeer E. M. Zakaria, Assistant Professor of Microbiology, National Center of Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA) for her sponsorship, for giving me the necessary scientific and incorporeal support, for constructive criticism, for continuous interest, fruitful discussion, direct supervision and for providing invaluable guidance throughout the entire research, and for always treating me like her daughter. Her dynamism, vision, sincerity and motivation have deeply inspired me in my life. She always encourage me and push me for success. It was a great privilege and honor to work and study under her guidance.

I am cordially indebted to my supervisor **Dr. Khaled Zakaria El-Baghdady**, Assistant Professor of Microbiology, Department of Microbiology, Ain Shams University, for his help, encouragement, continuous advice, valuable suggestion in all steps of this thesis and his expert supervision.

I gratefully and sincerely thank my supervisor **Dr.Hesham**Mohamed Abd El Fatah, Assistant Professor of Botany,

Department of Botany, Ain Shams University, for his continuous help, warm encouragement, understanding and facilities offered by him throughout this work.

I am cordially indebted to my advisor **Prof. Dr. Lotfy Abdelreheem Ali Mousa,** Prof. of Radiation Microbiology,

National Center for Radiation Research and Technology

(NCRRT), for continuous interest, direct supervision and valuable guidance throughout this work.

Many thanks to all my colleagues (Amany, Naglaa, Aya and Ahmed) in Microbiology Department in the Center (NCRRT) for their help. facilities offered and continuous encouragement in various ways.

A very special thanks and deepest love to my lovely husband Abdelrazq I. Sallam for his love, support, patience and for providing invaluable guidance, sincerity and motivation in my life. I want to make you always proud and happy and I wish for you all the success, the good health and the happiness. An extremely grateful and Thanks to Allah for my coming baby. "I am waiting to see you".

My deep love and extremely grateful to the most important people in my life and never being able to say thank you enough for everything they have done to me, to the reason why I am here and why I am able to make any success or achieve any goal in my life, to my mother and my father. I just want to make you happy and proud like you always make me happy and proud.

Also a great love and grateful to my sisters (samar, Alshimaa, Sama and Malak) and to my brothers (Mohamed and Islam).

Finally, I would like to record my sincere thanks to all who directly and indirectly have lent their help to me.

Habiba Nagah Sadek

#### Aim of the work

The aim of this work was to isolate powerful biodiesel-producing cyanobacteria and green microalgae from Egyptian environment. In addition, optimizing growth and lipid productivity for the selected isolates to enhance biodiesel productivity. Furthermore, using the available wastewater as microalgae growth media for biodiesel production.

#### **Abstract**

Microalgae biodiesel is a green and renewable energy resource. In this study, ten microalgae isolates were isolated from different samples collected from Egypt. The purified isolates were identified microscopically as: Lyngbya confervoides, Phormidium bohneri. Oscillatoria pseudogeminata, Amorphonostoc sp., Nostoc paludosum, Anabaena sphaerica related to cyanobacteria (blue green algae) and Chlorella vulgaris, Chlorella ellipsoidea, Scenedesmusacutus acutus, Chlamydomonas globosa related to green algae. Lipid production of the ten organisms was examined using the selected solvent system (Hexane-ethanol 1:1). Oscillatoria pseudogeminata and Chlamydomonas globosa were selected for optimization due to their high lipid / dry weight percentage (14% and 13%) respectively. The optimal microalgal growth lipid productivity were found to be on BG-11 medium with the following incubation conditions: temperature 30°C, incubation period one week, pH 8.5, light intensity 1000 lux, inoculum size 20 ml, 150 ml with static incubation for growth media volume and aeration, zero KGy for gamma radiation, 45 for N/P ratio and finally 0.05 g/l, 1 g/l for nitrogen concentration and 0.00174 g/l, 0.045 for phosphorus concentration for Chlamydomonas globosa and Oscillatoria pseudogeminata respectively. **Optimal** conditions resulted in 39% and 53% increase in the lipid/Dwt%

of BG-11 medium for Chlamydomonas globosa and Oscillatoria pseudogeminata respectively, on the other hand modification of BG-11 using N/P: 45 resulted in 71% and 92% increase in the lipid/Dwt % for Chlamydomonas globosa and Oscillatoria pseudogeminata respectively. Lipid yield by Chlamydomonas and Oscillatoria pseudogeminata using agricultural globosa wastewater was found to be 59% and 55% of that is produced using modified BG-11 respectively. The fatty acid profile obtained by Gas-Chromatography (GC) illustrated that palmitic, stearic, oleic, linoleic and linolenic acids were the most common fatty acids. Although Chlamydomonas globosa and Oscillatoria pseudogeminata are not considered as native isolates for biodiesel production, They can be considered as a promising lipid producing microalgae. Modified BG-11 and agricultural wastewater are considered as a promising source of growth media for production of good biodiesel quality.

*Key words:* Cyanobacteria, Microalgae, biodiesel, Lipid productivity, Modified BG-11, Agricultural wastewater, *Chlamydomonas globosa, Oscillatoria pseudogeminata*.

#### Introduction

Fossil fuels are the major energy sources that are being used in the world today. Extracting petroleum (crude oil) from the ground is not only a costly process but can be environmentally damaging. Since petroleum (crude oil) is toxic and not biodegradable, any type of spill has a harmful effect on the environment. Oil spills are responsible for destroying numerous ecosystems around the world, One of the major problems to arise from burning fossil fuels, especially coal, is acid rain that comes from the high sulfur content of these fuels. (Sahadeva dasa, 2008).

Because of dwindling fossil fuel supplies, high oil prices, climate change, greenhouse gas emissions and the need of energy is increasing continuously, and increases in industrialization and population. Biofuels have resolved some of the greatest challenges of today. Biofuels have the potential to stimulate economic development, and can provide cheap, renewable, locally sourced, carbon neutral fuel (Kulkarni and Dalai, 2006; IEA 2011; Nuffield Council on Bioethics 2011; UNEP 2012). Biofuels and other renewable energy sources are now back on the political agenda, and there is much talk and activity about biofuels from many players in many sectors.

Biofuels powered cars before fossil fuels became the dominant fuel; Henry Ford designed his original 1908 Model T to run on ethanol, and Rudolph Diesel intended to power his engine with vegetable oil (**Schubert**, **2006**; **English**, **2008**).

Unlike fossil fuels, biofuels are renewable energy source, theoretically unlimited which help to reduce the adverse effects of the frequent oil supply crisis, environmentally friendly, biodegradable. Biofuels can completely eliminate sulfur and thus can eliminate this component of acid rain, reduce greenhouse gas emissions. Biofuels can be produced locally, which decreases the nation's dependence on foreign fuel sources (**Hu** *et al.*, **2011**; **Brennan and Owende, 2010**).

Microalgae are photosynthetic microorganisms that are able to rapidly generate biomass from solar energy, CO<sub>2</sub> and nutrients in bodies of water. This biomass consists of important primary metabolites such as sugars, Proteins, oils and lipids, for which process path-ways exist for the production of high-value products including human and animal feed supplements, transport fuels, industrial chemicals and pharmaceuticals. (Sheehan *et al.*, 1998). Lipids can be used as a source for biofuels, as building blocks in the chemical industry and edible oils for the food and health market. (Radakovits et al., 2010; http://www.oilgae.com/algae/pro/eth/eth.html).

Microalgae have long been recognized as potentially good sources for biofuel production because of their high oil content and rapid biomass production. In recent years, use of microalgae as an alternative biodiesel feedstock has gained renewed interest from researchers, entrepreneurs, and the general public. (Indira and Biswajit, 2012).

#### 1. <u>Literature Review</u>

Microalgae are widely recognized as a promising source for biofuel production as a renewable source of energy. They are exceedingly rich in oil, which can be converted to biodiesel. Renewable energy is considered as an important resource in many countries around the world (**Lund**, 2007). Some of the species of microalgae already possess high oil content and they can be manipulated to produce more oil (**Gao** *et al.*, 2010).

#### 1.1 Fossil Fuels

#### **1.1.1 History**

Fossil fuels are the major energy sources that are being used in the world today. Fossil fuels as the name are derivatives of plant and animal fossils that are millions of years old. These are primarily formed from the remains of the decayed plants and animals of the carboniferous era. The three fuel sources i.e. coal, natural gas and oil/petroleum help to meet the energy and electricity demands of today's world. Because of the long time it takes to create these materials, they are non-renewable, meaning that more will not be made during anyone's lifetime, or even during modern civilization of humanity on the Earth. The demand for energy will never be in the declining graph. Industrial revolution has shown the way and it's still going on (Ribeiro, 2015).

#### 1.1.2 Production

To obtain the raw materials that converted into fossil fuels, it takes a lot of team work to find them and then extract them from the ground. Coal is preserved by the earth in a rock layer called a coal seam, mining is the only way to remove it whether it's near the surface or deep underground. Oil and natural gas are preserved inside sedimentary rocks which contain and trap these fluids in earth. Seismic reflections are used to locate these fluids in which vibrations reflect off layers within the earth, and show where there are light fluids such as oil and gas then access to them through drilling and then set up wells to draw them up to the surface. Once all these raw materials are gathered, they are sent to processing plants to become fossil fuels that are usable by consumers (Melton et al., 2015).

#### **1.1.3** Uses

In order to use the petroleum (crude oil), it must be refined. Once refined, it can be used in a variety of items including gasoline and diesel fuel. Changing petroleum (crude oil) into gasoline is done in large refineries. In a refinery, the petroleum (crude oil) is chemically altered so the gasoline will burn in predictable ways. Basically, the chains of carbon atoms with attached hydrogen atoms are forced to change shape into specific forms. Fuels are the most common use of petroleum (crude oil). However, petroleum is also used in plastics, wax, asphalt, and many other products. Also, In order to use natural gas as fuel, it

must undergo some type of processing. Once refined, it can be used for a variety of things including electricity generation, heating, and cooking. Additionally, natural gas is used in the manufacture of many common products including fabric, glass and paper. It's also used as a raw material in paints, plastics, and fertilizer (Ronald and Colwell, 2009).

Natural gas in its raw form is a greenhouse gas. It's currently considered as an important fuel source, but it's still a substantial contributor to global warming, as it's the most evolved fossil fuel compared to coal and oil, it has fewer emissions looking for natural gas deposits and drilling for them is harmful to habitats on both land and sea. New horizontal and directional drilling procedures are trying to maximize the amount of gas obtained from a single source and minimize the impact on the surrounding environment. If released into the atmosphere in its raw form, natural gas can be much more potent and dangerous than carbon dioxide. Unfortunately, small leaks can sometimes occur in the storage and transportation infrastructure such as wells, tanks or pipelines and lead to an explosion. (Melton et al., 2015).

#### 1.1.4 Disadvantages of fossil fuels

Drilling on land requires a lot of land use which can disturb local habitats. Off shore drilling impacts the surrounding marine