ASTAXANTHIN PRODUCTION IN *Haematococcus pluvialis* UNDER LEAD NANOPARTICLE STRESS IN DIFFERENT pH AND SALINITY CONDITIONS

SHARDHAVI RAMBURUTH

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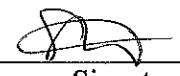
Shardhavi Ramburuth	
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Student ID

DATE/JAN/YEAR

Dr Wong Ling Shing
(SUPERVISOR)

Dr Cheng Wan Hee
(CO-SUPERVISOR)

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ABSTRACT

Haemaatococcus pluvialis is a unicellular fresh water microalga which has the highest amount of antioxidant compared to other microorganisms. It is also rich in astaxanthin, which is a carotenoid. Astaxanthin is extremely beneficial to food, pharmaceutical, nutraceutical and cosmetic industry. This is because of its very high antioxidant activity which is very much more than vitamin C and green tea etc. Since it is so desirable by so many companies, it should be produced in large amounts. In this experiment, a new method of accumulating astaxanthin from *H. puvialis* is tested which is the use of lead nanoparticle in order to find the ideal condition for maximum astaxanthin production. The *H. pluvialis* was cultured and then subcultured in different pH and salinity conditions. The culture at the condition where there has been maximum cell growth was obtained which is pH 7 and salinity 0.25%. The optimum culture condition obtained was used to grow the *H. pluvialis* and the culture was subjected to lead nanoparticle stress. The astaxanthin was then extracted using the HCl-ACE method and its concentration was measured using the spectrophotometer at 480 nm. There was maximum astaxanthin production at 10ppm and therefore lead nanoparticle can be used as stimulatory for growth.

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LIST OF ABBREVIATIONS

cm	Centimetre
e.g.	Example
mg/L	Milligram per Litre
etc.	et cetera (and so forth)
i.e	that is
Mm	Millimetre
HCl	Hydrochloric acid
CO_2	Carbon dioxide
O_2	Oxygen
Pb	Lead
NaCl	Sodium Chloride
rpm	Revolutions per minute
min	Minute
nm	Nanometer
M	Molar concentration
NP	Nanoparticle
H. pluvialis	*Haematococcus pluvialis*
ppm	Part per million
BBM	Bold Basal Medium

CHAPTER 1

INTRODUCTION

Astaxanthin is a powerful widely used carotenoid to provide the characteristic of red color in aquaculture feeds as well as provide numerous health benefits and as such it has become a commercial reality (Yu Ri Lee & Kyung Ho Row, 2016). Furthermore, it is highly desirable in the pharmaceutical and food companies because of its antioxidant properties which is roughly 100 times higher than that of β-tocopherol, and can be utilized as a potential specialist against tumor (Prachanart, Sorawit, Prasert, Artiwan, 2007).

Astaxanthin accumulation study in *H. pluvialis* in extreme conditions is extremely important as it is receiving huge commercial interest due to its potential application in worldwide industry and as source of pigment in feeds and aquaculture. Its tremendous positive health effects such as improving liver, immune system, heart functions and preventing muscular degradation has made it a powerful antioxidant. Studies have also revealed that it provides protection against cancer and cardiovascular disease, alleviate diabetes mellitus and endothelial dysfunction, prevent eye disease and reduce metabolic syndrome risks as well (Satoh, 2016).

The freshwater *H. pluvialis* is a biotechnologically important green algae known as ''the king of antioxidants'' with the richest source of astaxanthin (Minxi et al., 2014). However, the efficient extraction of this carotenoid is complicated by the thick cell wall of the mature red cyst (Yoon, Min & Sang, 2015). Though it is the most promising source having the immense potential to produce astaxanthin under abiotic stress (Yongteng et al., 2015), one of the primary obstacles in that process is inducing the accumulation of astaxanthin under stress conditions (Zewen Wena et al., 2015).

The high price of astaxanthin and its rapidly increasing demand raises multiple efficient systems of its production from *H.pluvialis*. There have been different approaches in cultivation and methods of producing astaxanthin, such as open or closed photobioreactors, batch or fed-batch, heterotrophic and phoroautotrophic (Kang et al., 2005). The induction of the synthesis of carotenoid together with the cultivation process in *H.pluvialis* is directly correlated to the content of astaxanthin in cells.

Lead nanoparticle stress is a method to induce the desired product, astaxanthin, production in *H. pluvialis*. Microalgae can sometimes experience negative effect such as blockage of cell division or of photosynthetic mechanism and enzyme inhibition in its growth from nanoparticles might have an influence on the morphology of the cells. Nevertheless, as reports suggest, the nanoparticles can stimulate growth or cause mechanical damage also because of the metal ions which are released by the nanoparticles itself and due to interactions with media growth components. In addition, they might as well have an enhancing effect in the microalgae on the pigment content (Miazek et al., 2015).

Among all the different environment stresses, pH is one of the most essential factor to maximize the production of carotenoids and to exceed cell growth as it is known to have the greatest effect in carotenoid formation and on morphological changes of cells of *H. pluvialis*. Moreover, pH determines the minerals solubility and CO_2 in the culture and also influences the metabolism of the algae either indirectly or directly (Lee & Zhang, 1999). Though *H pluvialis* shows autotrophic growth under favourable growth conditions, it however initiates carotenogenesis under unfavourable growth conditions undergoing a morphological transformation to deep red astaxanthin from green vegetative cells (Harker et al., 1996). Salinity is another stress factor that represents unfavourable growth conditions that induce the accumulation of astaxanthin (Kobayashi et al., 1993). It is worthy to note that astaxanthin accumulation is induced and most expected in *H. pluvialis* when the cells are actively growing or when cell divisions have stopped as a response to stress-inducing conditions (Borowitzka et al., 1991).

Many research was carried out with new methodologies that could lead to the development of more specialized techniques for the production of astaxanthin. This course of action is important because right now, there are not enough attempts made to boost up the production of this widely demanded and used carotenoid. Nanoparticle testing is something new and this research will prove whether this proposal will enhance the production of astaxanthin or reduce it. The benefits of this research would be a giant step in medical science as it will encompass a number of health aspects for the population at large including its use in the treatment of tumors and eradication of the disease cancer.

The objective of this present investigation is to find the optimum condition for the maximum production of astaxanthin in *H. pluvialis* under Pb nanoparticle stress at salinity 0.25% and pH 7.

CHAPTER 2

LITERATURE REVIEW

2.1 *PLUVIALIS*

2.1.1 Overview

Microalgae are unicellular microscopic algae that generally are found in marine systems as well as in freshwater. The size of microalgae varies depending on their species and unlike other plants, they do not have stems, leaves or roots. Being the base of the food web, microalgae have the ability to photosynthesize and provide energy to all trophic levels. Microalgae represent an abundant resource as they are capable of producing a variety of unique products such as fatty acids, antioxidants, toxins, carotenoids etc (Lorenz, 2000).

There are more than 7000 different species of green microalgae that exists in a large variety of different habitats. *H. pluvialis* (Chlorophyceae, Volvocales) is a unicellular microalga which is unicellular found worldwide in fresh water. Furthermore, it is known as the main producing organism of the carotenoid astaxanthin which is of great commercial benefit (Lorenz, 2000). Astaxanthin is found in most of our favoured food such as red sea bream, salmon, lobster, shrimp, trout and salmon and it is mostly synthesized by microalgae, bacteria, yeast and plants. It belongs to the same family as lutein, lycopene and β-carotene and additionally, it is a red secondary carotenoid with the structure (3, 3′-dih 4). It normally has three different kinds of stereoisomers namely (3R, 3′R); (3S, 3′S) and (3R, 3′S) and contains two chiral centers (Yang et al., 2013).

Astaxanthin is synthesized through a biochemical reaction namely the carotenoid pathway from the substrates glyceraldehyde-3-phosphate and pyruvate. Depending on different cultivation conditions, these compounds are usually the products of glycolysis or photosynthesis. It has the capacity of free radical scavenging and due to this it is greatly used in aquaculture, pharmaceutical industries, feed, food and the nutraceutical industry. Its antioxidant activity is more powerful than Vitamin C by 65 times. Also it is more potent by 10 times than β-carotene (Borowitzka, 2013). Presently, there are above 95% synthetic astaxanthin in the market whereas only less than 1% derived from natural