MODIFICATION OF MEDIA FORMULATION FOR ENHANCING PRODUCTION OF EXTRACELLULAR POLYSACCHARIDES BY *Porphyridium cruentum*

AMIRA SURIATY BINTI YAAKOP

A dissertation submitted in partial fulfillment of the
requirements for the award of the degree of
Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

JANUARY, 2013

A special dedication to my lovely parents,
family, fiancée and friends

ACKNOWLEDGEMENTS

I would like to take this opportunity to dedicate my endless gratitude, sincere appreciation and profound regards to my dearest supervisor, Dr. Haryati Jamaluddin for her scholastic guidance. She has been instrumental in guiding my research, giving me encouragement, guidance, critics and friendship. Without you this thesis will not be presented as it is today.

Furthermore, I would like to convey my appreciation toward Miss Katijah Mahat and Miss Dalila Mat Zaki for their guidance and support. Not forgetting the helpful lab assistant Mr. Khairul and Mr.Hafizi for all their assistances throughout my research. My sincere appreciation also extends to all my lab mates in special equipment laboratory who have provided assistance at various occasions.

Last but not least, my special gratitude to my lovely parents and family members for their support and concern, special thanks also to my love one that always had been there for me. Not to forget, my course mates and friends who had given their helps and guidance all the time with love. Their mentorship was truly appreciated.

ABSTRACT

The red microalgae *Porphyridium cruentum* produce many valuable compounds such as extracellular polysaccharides (EPS) that are extensively used in the industry. In this study modification of media formulation for optimal production of EPS from *Porphyridium cruentum* was carried out by varying nitrate, sulfate and glucose concentrations in the Jones 1962 media. Different concentration of each nutrient was used in the media formulation. For nitrate, the highest concentration provided in the medium at 50 mM showed the highest growth rate and EPS production, but by inhibiting the nitrate concentration, the growth is reduced while the production of EPS is increased as when a high concentration of nitrate was provided to the medium. For sulfate, increasing concentration in the media showed great decrease in growth rate, but increasing EPS production, the highest production of EPS was at 50 mM which was the highest concentration of sulfate provided in the media modification. Addition of glucose in the medium on the other hand substantially increased the growth rate and the biomass production but as the glucose concentration kept on increasing, it also inhibited the production of EPS by the algae. The growth rate of the algae did not correlate with the EPS production. The result showed that high concentration of glucose and low concentration of nitrate and sulfate inhibited the production of EPS by the microalgae and in order to increase the EPS production, medium without nitrate or a very high concentration of nitrate or and sulfate must be provided. Therefore, the optimum medium formulation for optimal growth and EPS production by *Porphyridium cruentum* is at either 20 mM of nitrate or without nitrate at all, 50 mM of sulfate and 0.5% (w/v) of glucose. The compositions of the EPS were also confirmed by using HPLC that showed the composition of glucose, galactose and xylose of the EPS.

ABSTRAK

Mikroalga merah *Porphyridium cruentum* menghasilkan banyak sebatian berharga seperti rembesan polisakarida luar sel (EPS) yang digunakan secara meluas di dalam pelbagai industri. Dalam penyelidikan ini, pengubahsuaian kepekatan nutrisi nitrat, sulfat dan glukosa dilakukan terhadap formulasi media Jones 1962 untuk memperoleh pengeluaran optimum EPS dari *P. cruentum*. Bagi nitrat, semakin tinggi kepekatannya, semakin tinggi kadar pertumbuhan dan penghasilan EPSnya namun dengan tidak membekalkan nitrat di dalam medium, kadar pertumbuhan mikroalga semakin berkurang tetapi penghasilan EPS meningkat seperti jika dibekalkan kepekatan nitrat yang tinggi. Pengubahsuaian kepekatan sulfat pula menunjukkan penurunan yang amat banyak dari segi kadar pertumbuhan mikroalga apabila kepekatan sulfat yang tinggi di bekalkan pada medium. Bagi penghasilan EPS, kepekatan sulfat yang tinggi (50 mM) menunjukkan penghasilan EPS yang tertinggi. Penambahan glukosa dalam medium menunjukkan peningkatan kadar pertumbuhan dan pengeluaran biojisim yang sangat tinggi namun ia menyekat pengahasilan EPS dari alga dengan menunjukkan penghasilan EPS yang sangat rendah. Kadar pertumbuhan bagi *P. cruentum* tidak berkolerasi dengan pengeluaran EPSnya. Hasil kajian menunjukkan bahawa kepekatan glukosa yang tinggi dan kepekatan nitrat dan sulfat yang rendah menghalang pengeluaran EPS oleh *P. cruentum*. Justeru, untuk meningkatkan pengeluaran EPS, bagi kepekatan nitrat, medium boleh disediakan dengan tidak meletakkan nitrat ataupun dengan meletakkan kepekatan nitrat dan sulfat yang sangat tinggi. Formulasi medium yang optimum bagi pertumbuhan dan penghasilan EPS optima oleh *Porphyridium cruent*um ialah diantara tanpa kandungan nitrat ataupun pada 20 mM nitrat, 50 mM sulfat dan 0.5% (w/v) glukosa. Komposisi EPS juga dikenalpasti mengandungi glukosa, galaktosa dan xylosa melalui HPLC.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ASW	-	Artificial Seawater
$(NH_4)_6Mo_7O_{24}$	-	Ammonium heptamolybdate
$CaCl_2.2H_20$	-	Calcium Chloride Dihydrate
CO_2	-	Carbon dioxide
$CoCl_2.6H_20$	-	Cobalt (II) chloride hexahydrate
$CuCl_2.2H_2O$	-	Copper Chloride dihydrate
EPS	-	Extracellular polysaccharides
$FeCl_3.4H_20$	-	Ferric Chloride
Fect	-	Ferric citrate
Glc	-	Galactose
GlcA	-	Glucoronic acid
Glu	-	Glucose
H_2O	-	Water
H_3BO_3	-	Boric acid
HCl	-	Hydrochloric acid
HCO_3	-	Bicarbonate ion
HPLC	-	High Performance Liquid Chromatography
KCl	-	Potassium cloride
KH_2PO_4	-	Monopotassium phosphate
KNO_3	-	Potassium nitrate
KOH	-	Potassium hydroxide
$MgCl_2.6H_20$	-	Magnesium Chloride Hexahydrate
$MgSO_4.7H_20$	-	Magnesium Sulfate heptahydrate
$MnCl_2.4H_2O$	-	Manganese(II) chloride
N_2	-	Nitrogen

Na_2- $B_4O_7.10H_2O$	-	Sodium Borate Decahydrate
Na_2EDTA	-	Sodium Ethylenediaminetetraacetic acid
NaCl	-	Sodium Chloride
NaHCO	-	Sodium bicarbonate
$NaNO_3$	-	Sodium nitrate
NaOH	-	Sodium hydroxide
O_2	-	Oxygen
OD	-	Optical density
P. cruentum	-	*Porphyridium cruentum*
U.S. $	-	U.S Dollar
v/v	-	Volume per volume
w/v	-	Weight per volume
w/w	-	Weight per weight
Xyl	-	Xylose
$ZnCl_2$	-	Zinc Chloride

LIST OF SYMBOLS

%	-	Percentage
°C	-	Degree celcius
d	-	Day
g	-	Gram
g/ L	-	Gram per liter
g/g	-	Gram per gram
h	-	Hour
h^{-1}	-	Per hour
lx	-	lux
m	-	Meter
min	-	Minute
ml	-	Mililitre
mm	-	Milimeter
mM	-	Milimolar
nm	-	Nanometer
rpm	-	Revolutions per minute
µl	-	Microlitre
µm	-	Micrometer

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background Information

Polysaccharides are natural polymers that have properties which may be translated into significant commercial applications. Polysaccharides are extensively used in industry. Some significant uses of polysaccharides in industry are as thickening agents, gelling agents, suspension, flocculation, binding, coating and emulsification. For chemical use, polysaccharides can have application in oil recovery, food, and pharmaceutical fields. In natural environments, the production of extracellular polysaccharides is often related to the formation of biofilms on surfaces, in which the polymer-exuding micro-organisms grow, multiply, and produce extracellular material, mostly polysaccharide. Extensive research have shown that marine organisms particularly algae and microalgae are a source for many well-known polysaccharides. For algae, its polysaccharides serve mainly as storage and structural molecules (Adda *et al.*, 1986). In seaweeds, the structural cell wall polysaccharides usually consist of an outer amorphous mucilage matrix, commonly made by linear sulfated galactan polymers (carrageenans, agarans, and alginates) and an inner rigid component, cellulose fibrils (Gasljevic *et al.*, 2009). In the red microalgae, the cell walls lack this cellulose microfibrillar component but they were encapsulated with a sulfated polysaccharide in the form of a gel. The unicellular nature of this algae itself has made it a useful experimental system and an attractive organism for studies of its high content of polysaccharide.

Red microalgae produced valuable extracellular polysaccharides (Pulz *et al.*, 1995). *Porphyridium cruentum* is one of the most studied red microalgae. These microalgae possess nutritional and therapeutical value. These biochemicals include a high content of polysaccharides, long-chain polyunsaturated fatty acids, carotenoids such as zeaxanthin, and fluorescent phycobiliproteins. The polysaccharides of *Porphyridium* have also been shown to possess impressive antiviral activity (Huleihel *et al.*, 2001, 2002; Huang *et al.*, 2005). Therefore, the extracellular polysaccharide was extensively studied. The EPS is an acidic heteropolymer composed of xylose, glucose, galactose and sulfate esters. It forms ionic bridges through divalent cations, thus reaching a very high molecular weight and forming a thermodynamically stable structure. *Porphyridium* cells can be solitary or massed together into irregular colonies held together by mucilage, which is constantly secreted by the cells, forming a capsule around it. The thickness of the polysaccharide capsule varies according to the phase of growth and with growth conditions. Its outer part dissolves into the medium, which increases the viscosity of the medium (You and Barnett, 2004).

Culture condition is well known to affect the production of the biomass of *P. cruentum* as well as the quality of the extracellular polysaccharides (EPS) produced. There have been a few reports about the effects of different culture and medium conditions on its growth and production. For culture condition a few condition were proposed in term of light quality (You and Barnett, 2004), high light intensity and low gas flow rates (Eteshola *et al.*, 1998), pH, stirring and mineral nutrients in the flat plate glass reactors (Singh *et al.*, 2000), aeration and agitation (Iqbal and Zafar, 1993) and renewal rate of the culture volume (Fabregas *et al.*, 1999). Therefore a few optimum conditions were developed. The effects of media formulation on the EPS production of microalgae have not yet been well studied.

Growth medium have been said to play a role in polysaccharide production (Thomas *et al.*, 1984), thus modification of medium formulation in terms of crucial nutrient for the microalgae would facilitate in the understanding of the importance of different nutrients on extracellular polysaccharide production. Many studies have previously been carried on growth optimization of algae based on its nutrient medium nutrient compositions, the result showed a tendency for carbohydrate accumulation under certain nutrient starvation such as nitrate starvation in *Chlorella* and *Chlamydomonas* (Kroen and Rayburn, 1984). This carbohydrate accumulation apparently takes place at the expense of protein production. Thepenier and Gudin (1985) show that in immobilized cells of *Porphyridium cruentum*, the depletion of nitrate from the mineral medium caused a sharp increase in the viscosity of the medium, indicating an increase in polysaccharide production.

1.2 Objectives of Research

i) To compare different media formulation to grow *P. cruentum* and optimized the selected media formulation in terms of nutrient composition toward EPS production.

ii) To measure the extracellular polysaccharides yield extracted from different media formulation.

iii) To quantify and characterise the extracellular polysaccharides produced by *P. cruentum* by phenol-sulfuric assay and High Performance Liquid Chromatography (HPLC).

1.3 Problem Statement

There is an increasing market demand for natural polysaccharides in the food, cosmetics and pharmaceutical industries for red and brown microalgae. Natural polysaccharides usually harvested from their natural habitats are being depleted due to extensive harvesting and detrimental environmental conditions. The alternative way may be found in EPS by red microalgae which offer vast range of potential application such viscoelasticity, stability in the body that can be used as water soluble lubricant and other biolubricating fluid and additives to the synovial fluid joints (Shoshana Arad *et al.*, 2006). Therefore it is worth developing methods to increase the production of EPS. Currently there is very limited research on the effect of different medium culture condition for growth so far. The main conditions that had been studied are light quality (You and Barnett, 2004), high light intensity and low gas flow rates (Merchuk *et al*., 2000), pH, aeration and agitation (Iqbal and Zafar, 1993).

1.4 Research Significance

EPS produced by *P. cruentum* has been proven as very valuable polysaccharides that were extensively used in many field of industry. Many medium formulations were used by many researchers in order to increase the growth and EPS production of *P. cruentum*. Up until now there were no conclusive study were conducted in determining which medium formulation and nutrients concentration will produce the highest EPS and growth production in red microalgae. Current studies only provided optimum condition to grow the algae. The optimum growth conditions of *P. cruentum* have been determined in several studies (Catherine *et al.*, 1985; Wang *et al*., 2007; Gasljevic *et al*., 2009) and assumption was made that the highest extracellular polysaccharides production is at the optimum condition. Thus, by studying the effect of the medium modification at optimum conditions, it should facilitate understanding the relationship of nutrients that were required for the algae for growth and polysaccharide production.

1.5 Scope of Research

The type of microalgae used is a freshwater red microalgae *Porphyridium cruentum,* comparison of four medium formulations were used to determine the best medium formulation. Jones medium were selected and varying source of nitrogen, sulfate and glucose in the medium were used to optimize the EPS production of the microalgae. The cultures were cultivate at optimum condition for 20 days. The growth rate and total polysaccharide production were determined by reading the optical density at 690 nm and phenol-sulfuric assay (Dubois *et al*., 1979). Analysis of EPS composition and content were carried out by HPLC. .

REFERENCES

Adda, M., Merchuk, J. C. and Arad, S. (1986). Effect of nitrate on growth and production of cell-wall polysaccharide by the unicellular red alga Porphyridium. *Biomass,* 10**,** 131-140.

Agustini, N. W. S., Kabinawa, I. N. K., Hirata, K., and Miyamoto, K. (2002). Development of the polyunsaturated fatty acids from microalgae. *Biotechnol Sustain Util Biol Resour Trop, 16*, 397-400.

Allan G, G., Lewin, J. and Johnson P, G. (1972). Marine Polymers. IV Diatom Polysaccharides. *Botanica Marina.*

Arad, S., Friedman, O. and Rotem, A. (1988). Effect of Nitrogen on Polysaccharide Production in a *Porphyridium* sp. *Applied and Environmental Microbiology,* 54**,** 2411-2414.

Arad, S. (1988). Production of sulfated polysaccharides from red unicellular algae, *Algal Biotechnology*. *Elsevier Applied Science, London*, pp. 65–87.

Arad, S., Lerental, Y. and Dubinsky, O. (1992). Effect of nitrate and sulfate starvation on polysaccharide formation in rhodella reticulata. *Bioresource Technology,* 42**,** 141-148.

Arad, S., Keristovesky, G., Simon, B., Barak, Z. E. and Geresh, S. (1993). Biodegradation of the sulphated polysaccharide of Porphyridium by soil bacteria. *Phytochemistry,* 32**,** 287-290.

Arad, S., Kolani, R., Simon-Berkovitch, B. and Sivan, A. (1994). Inhibition by DCB of cell wall polysaccharide formation in the red microalga Porphyridium sp. (Rhodophyta). *Phycologia,* 33**,** 158-162.

Arad, S., Lerental, Y. and Dubinsky, O. (1992). Effect of nitrate and sulfate starvation on polysaccharide formation in *Rhodella reticulata*. *Bioresource Technology,* 42**,** 141-148.

Bold, H.C. and Wynne, M.J. (1985). *Introduction to the Algae*: Structure and ReproductionPrentice Hall Inc Carbohydrate Analysis: a Practical Approach. Oxford, IRL Press.55-96.

Borowitzka, M. A. (1986). Microalgae as sources of fine chemicals. *Microbiol. Sci.*, 3, 372-5

Borowitzka, M. A. (1997). Microalgae for aquaculture: opportunities and constraints. *Journal of Applied Phycology*, *9*(5), 393-401.

Brody, M. and Emerson, R. (1959). The Quantum Yield of Photosynthesis in Porphyridium cruentum, and the Role of Chlorophyll a in the Photosynthesis of Red Algae. *The Journal of General Physiology,* 43**,** 251-264.

Cerning, J., Renard, C.M.G.C., Thibault, J.F., Bouillanne, C., Landon, M., Desmazeaud, M. and Topisirovic, L. (1994). Carbon source requirements for exopolysaccharide production by Lactobacillus casei CG11 and partial structure analysis of the polymer. *Appl. Envir. Microbiol.* 60,3914–3919.

Chaplin, M. F. (2006). Carbohydrate Analysis. *Encyclopedia of Molecular Cell Biology and Molecular Medicine.* Wiley-VCH Verlag GmbH & Co. KGaA.

Chen, B., You, W., Huang, J., Yu, Y. and Chen, W. (2010). Isolation and antioxidant property of the extracellular polysaccharide from *Rhodella reticulata*. *World Journal of Microbiology and Biotechnology*, 26**,** 833-840.

Cheirsilp, B. and Torpee, S. (2012). Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresource Technology,* 110**,** 510-516.

Cho, D., Chae, H. and Kim, E. (2001). Synthesis and characterization of a novel extracellular polysaccharide by *Rhodotorula glutinis*. *Applied Biochemistry and Biotechnology,* 95**,** 183-193.

Christi, Y. Biodiesel from Microalgae. (2007). *Biotechnology Advances*, *25*, 294-306.

Cohen, E., Koren, A. and Arad, S. (1991). A closed system for outdoor cultivation of microalgae. *Biomass and Bioenergy,* 1**,** 83-88.

Cook, C. M. and Colman, B. (1987). Some characteristics of photosynthetic inorganic carbon uptake of a marine macrophytic red alga. *Plant, Cell & Environment,* 10**,** 275-278.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry,* 28**,** 350-356.

Dubinsky, O., Simon, B., Karamanos, Y., Geresh, S., Barak, Z. and Arad, S. (1992). Composition of the cell wall polysaccharide produced by the unicellular red alga *Rhodella reticulata*. *Plant physiology and biochemistry,* 30**,** 409-414.

Durack, P. J., Wijffels, S. E. and Matear, R. J. (2012). Ocean Salinities Reveal Strong Global Water Cycle Intensification During 1950 to 2000. *Science,* 336**,** 455-458.

Eteshola, E., Karpasas, M., Arad, S. and Gottlieb, M. (1998). Red microalga exopolysaccharides: 2. Study of the rheology, morphology and thermal gelation of aqueous preparations. *Acta Polymerica,* 49**,** 549-556.

Fabregas, J., GarcíA, D., Fernandez-Alonso, M., Rocha, A. I., Gómez-Puertas, P., Escribano, J. M., Otero, A. and Coll, J. M. (1999). In vitro inhibition of the replication of haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. *Antiviral Research,* 44**,** 67-73.

Fedorov, A., Kosourov, S., Ghirardi, M. and Seibert, M. (2005). Continuous Hydrogen Photoproduction by Chlamydomonas reinhardtii. *In:* Davison, B., Evans, B., Fikelstein, M., and Mcmillan, J.(eds.) *Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals.* Humana Press.

Gantt, E. (1981). Phycobilisomes.Annual Review of Plant Physiology,32, 327±347.

Gasljevic, K., Hall, K. A., Oakes, S., Chapman, D. J. and Matthys, E. F. (2009). Increased production of extracellular polysaccharide by Porphyridium cruentum immobilized in foam sheets. *Engineering in Life Sciences,* 9**,** 479-489.

Gavrilescu, M. and Chisti, Y. (2009). Biotechnology, a sustainable alternative for chemical industry. *Biotechnol* Adv, 23:471–99.

Geresh, S., Shefer, A. and Arad, M. S. (1997). A chemically cross-linked polysaccharide from a red microalga as a potential ion exchanger and adsorbant of metal ions. *J Carbohydr Chem* 16:703-708.

Geresh, S. and Arad, S. (1991). The extracellular polysaccharides of the red microalgae: Chemistry and rheology. *Bioresource Technology,* 38**,** 195-201.

Geresh, S., Mamontov, A. and Weinstein, J. (2002). Sulfation of extracellular polysaccharides of red microalgae: preparation, characterization and properties. *Journal of Biochemical and Biophysical Methods,* 50**,** 179-187.

Geresh, S., Arad, S., Levy-Ontman, O., Zhang, W., Tekoah, Y. and Glaser, R. (2009). Isolation and characterization of poly- and oligosaccharides from the red microalga *Porphyridium* sp. *Carbohydrate Research,* 344**,** 343-349.

Geresh, S., Dawadi, R. P. and Arad, S. (2000). Chemical modifications of biopolymers: quaternization of the extracellular polysaccharide of the red microalga *Porphyridium* sp. *Carbohydrate Polymers,* 43**,** 75-80.

Geresh, S., Adin, I., Yarmolinsky, E. and Karpasas, M. (2002). Characterization of the extracellular polysaccharide of *Porphyridium* sp.: molecular weight determination and rheological properties. *Carbohydrate Polymers,* 50**,** 183-189.

Gloaguen, V., Ruiz, G., Morvan, H., Mouradi-Givernaud, A., Maes, E., Krausz, P. and Strecker, G. (2004). The extracellular polysaccharide of *Porphyridium* sp.: an NMR study of lithium-resistant oligosaccharidic fragments. *Carbohydrate Research,* 339**,** 97-103.

Grobbelaar, J., Nedbal, L. and Tichý, V. (1996). Influence of high frequency light/dark fluctuations on photosynthetic characteristics of microalgae photoacclimated to different light intensities and implications for mass algal cultivation. *Journal of Applied Phycology,* 8**,** 335-343.

Guedes, A. C., Amaro, H. M. and Malcata, F. X. (2011). Microalgae as Sources of Carotenoids. *Marine Drugs,* 9**,** 625-644.

Hamerick, G. (1973). *Handbook of phycological methods: culture methods and growth measurements,* Cambridge University Press, 260.

Hodge, J. E. (1962). Determination of reducing sugars and carbohydrates. *Methods in carbohydrate chemistry,* 1**,** 380-394.

Huang, J., Chen, B. and You, W. (2005). Studies on separation of extracellular polysaccharide of Porphyridium cruentum and its anti-HBV in vitro. *Chin J Mar Drugs* 24, 18–21

Huleihel, M., Ishanu, V., Tal, J. and Arad, S. (2001). Antiviral effect of red microalgal polysaccharides on Herpes simplex and *Varicella zoster* viruses. *Journal of Applied Phycology,* 13**,** 127-134.

Huheihel, M., Ishanu, V., Tal, J. and Arad, S. (2000). Activity of *Porphyridium* sp. polysaccharide against herpes simplex viruses in vitro and in vivo. *Journal of Biochemical and Biophysical Methods,* 50**,** 189-200.

Iqbal, M. and Zafar, S. (1993). Strategies toward optimization of cultural conditions of *Porphyridium cruentum* for higher polysaccharide production. *Acta Microbiologica Polonica,* 42**,** 71-82.

Jones, R. F., Speer, H. L. and Kury, W. (1963). Studies on the Growth of the Red Alga *Porphyridium cruentum*. *Physiologia Plantarum,* 16**,** 636-643.

Kapdan, I. K. and Kargi, F. (2006). Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology,* 38**,** 569-582.

Kost, H. P., Senser, M. and Wanner, G. (1984). Effect of Nitrate and Sulphate Starvation on *Porphyridium cruentum* Cells. *Zeitschrift für Pflanzenphysiologie,* 113**,** 231-249.

Kroen, W. K. and Raynburn, W. R. (1984). Influence of growth status and nutrients on extracellular polysaccharide synthesis by the soil alga *Chlarnydomonas mexicana* (Chlorophyceae). *Journal of Phycology,* 20, 253-257.

Lapidot, M., Shrestha, R. P., Weinstein, Y. and Arad, S. (2010). Red Microalgae: From Basic Know-How to Biotechnology. *Red Algae in the Genomic Age,* Springer Netherlands. 205-225.

Lecclerc. J., Coute. A. and Et Hoarau. J. (1981). Pigments and photosynthetic activity adaptations in some algae living in low light places.*Photosynthesis VI. Photosynthesis and productivity, photosyn thesis and environment,* 443-453.

Mata, T. M., Martins, A. A. and Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, 14**,** 217-232.

Matsui, M., Muizzuddin, N., Arad, S. and Marenus, K. (2003). Sulfated polysaccharides from red microalgae have antiinflammatory properties in vitro and in vivo. *Applied Biochemistry and Biotechnology,* 104**,** 13-22.

Merchuk, J. C., Gluz, M. and Mukmenev, I. (2000). Comparison of photobioreactors for cultivation of the red microalga Porphyridium sp. *Journal of Chemical Technology & Biotechnology,* 75**,** 1119-1126.

Metting, B. and Pyne, J. W. (1986). Biologically active compounds from microalgae. *Enzyme and Microbial Technology,* 8**,** 386-394.

More, R. E. (1981). Constituents of blue-green algae. In *Marine Natural Products,* Vol. IV. Academic Press, New York, 1-52.

Namikoshi, M. and Rinehart, K. L. (1996). Bioactive compounds produced by cyanobacteria. *Journal of Industrial Microbiology,* 17**,** 373-384.

Norton, T. A., Melkonian, M. and Andersen, R. A. (1996). Algal biodiversity*. *Phycologia*, 35**,** 308-326.

Pazur, J. H., Reed, A. J. and Li, N.Q. (1994). An immunological method for the determination of the d and l configurations of monosaccharides. *Carbohydrate Polymers,* 24**,** 171-175.

Perkins, R., Underwood, G., Brotas, V., Snow, G., Jesus, B. and Ribeiro, L. (2001). Responses of microphytobenthos to light: primary production and carbohydrate allocation over an emersion period. *Marine Ecology Progress Series*, 223**,** 101-112.

Pulz, O., Koehler, E. and Sandau, P., (1995) *In The Proceedings of the International Symposium on Plant Polymeric Carbohydrates*, Behrs: Hamburg, Germany, 137–138.

Pohl, E (1982). Lipids and fatty acids of microalgae. *In Handbook of Biosolar Resources*, Vol. I, Part 1, ed. A.Mitsui and C. C. Black Jr. CRC-Press, Boca Raton.

Ramus, J. (1972). The production of extracellular polysaccharide by the unicellular red alga *Porphyridium aerugineum*. *Journal of Phycology,* 8**,** 97-111.

Ramus, J. (1985) Rhodophyte unicells: *biopolymer, physiology and production. In Algal Biomass Technologies*: An Interdisciplinary Perspective. Edited by Barclay WR, McIntosh RP. Cramer J51-55.

Roessler, P. G., Brown, L. M., Dunahay, T. G., Heacox, D. A., Jarvis, E. E., Schneider, J. C., Talbot, S. G. and Zeiler, K. G. (1994). Genetic Engineering Approaches for Enhanced Production of Biodiesel Fuel from Microalgae. *Enzymatic Conversion of Biomass for Fuels Production.* American Chemical Society.

Shoshana Arad, Rapoport, L., Moshkovich, A., Van Moppes, D., Karpasas, M., Golan, R. and Golan, Y. (2006). Superior biolubricant from a species of red microalga. *Langmuir,* 22**,** 7313-7317.

Singh, S., Arad, S. and Richmond, A. (2000). Extracellular polysaccharide production in outdoor mass cultures of Porphyridium sp. in flat plate glass reactors. *Journal of Applied Phycology,* 12**,** 269-275.

Smith, I. H. and Pace, G. W. (1982). Recovery of microbial polysaccharides. *Journal of Chemical Technology and Biotechnology,* 32**,** 119-129.

Sommerfeld, M. R. and Nichols, H. W. (1970). Developmental and cytological studies of Bangia fuscopurpurea in culture. *American Journal of Botany***,** 640-648.

Spoehr, H. A. and Milner, H. W. (1949). The Chemical Composition of Chlorella; Effect of Environmental Conditions. *Plant Physiol,* 24**,** 120-49.

Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering,* 101**,** 87-96.

Starr, R. C. and Zeikus, J. A. (1993). UTEX - The Culture Collection of Algae at the University of Texas at Austin. Journal of Phycology, 29, 1-106.

Sun, L., Wang, C., Shi, Q. And Ma, C. (2009). Preparation of different molecular weight polysaccharides from *Porphyridium cruentum* and their antioxidant activities. *International Journal of Biological Macromolecules,* 45**,** 42-47.

Sun, L., Wang, L. and Zhou, Y. (2012). Immunomodulation and antitumor activities of different-molecular-weight polysaccharides from *Porphyridium cruentum*. *Carbohydrate Polymers,* 87**,** 1206-1210

Sutherland, T. F., Grant, J. and Amos, C. L. (1998). The effect of carbohydrate production by the diatom *Nizschia curvilineata* on the erodibility of sediment. *Limnol. Oceanogr*. 43, 65–72

Tannin-Spitz, T., Bergman, M., Van-Moppes, D., Grossman, S. and Arad, S. (2005). Antioxidant activity of the polysaccharide of the red microalga *Porphyridium* sp. *Journal of Applied Phycology,* 17**,** 215-222.

Thomas, W. H., Seibert, D. L. R., Alden, M., Neori, A. and Eldridge, P. (1984). Yields, photosynthetic efficiencies and proximate composition of dense marine microalgal cultures. II. Dunaliella primolecta and Tetraselmis suecica experiments. *Biomass,* 5**,** 211-225.

Thepenier, C. and Gudin, C. (1985). Studies on optimal conditions for polysaccharide production by *Porphyridium cruentum*. *MIRCEN journal of applied microbiology and biotechnology,* 1**,** 257-268.

Ucko, M., Cohen, E., Gordin, H. and Arad, S. (1989). Relationship between the Unicellular Red Alga Porphyridium sp. and Its Predator, the Dinoflagellate *Gymnodinium* sp. *Applied and Environmental Microbiology,* 55**,** 2990-2994.

Ucko, M., Shrestha, R. P., Mesika, P., Bar-Zvi, D. and Arad, S. M. (1999). Glycoprotein moiety in the cell wall of the red microalga *Porphyridium* sp. (Rhodophyta) as the biorecognition site for the *Crypthecodinium cohnii*-like dinoflagellate. *Journal of Phycology,* 35**,** 1276-1281

Velea, S. and Filipescu (2007). Optimization of *Porphyridium Purpurem* culture growth using two variables experimental design: light and sodium bicarbonate. *Science Bulletin.* 11, 234-238

Vu, B., Chen, M., Crawford, R. and Ivanova, E. (2009). Bacterial Extracellular Polysaccharides Involved in Biofilm Formation. *Molecules,* 14**,** 2535-2554.

Wen, S., Zhang, T. and Tan, T. (2005). Optimization of the amino acid composition in glutathione fermentation. *Process Biochemistry,* 40**,** 3474-3479.

Wang, J., Chen, B., Rao, X., Huang, J. and Li, M. (2007). Optimization of culturing conditions of *Porphyridium cruentum* using uniform design. *World Journal of Microbiology and Biotechnology,* 23**,** 1345-1350.

Whiteley, N. M., Scott, J. L., Breeze, S. J. and Mccann, L. (2001). Effects of water salinity on acid-base balance in decapod crustaceans. *Journal of Experimental Biology,* 204**,** 1003-1011.

You, T. and Barnett, S. M. (2004). Effect of light quality on production of extracellular polysaccharides and growth rate of *Porphyridium cruentum*. *Biochemical Engineering Journal*, 19**,** 251-258.

Yamasaki, S. and Hirata, H. (1995). CO2 concentration change in *Nannochloropsis* sp. culture medium. *Aquacultural Engineering,* 14**,** 357-365.

Zhang, W. (1999). Biochemical technique of glycoconjugates. *Zhejiang University Press*, Hangzhou, 11–12.