HETEROTROPHIC PRODUCTION OF THE MICROALGAE *Cyclotella cryptica*; FEED FOR AQUACULTURE

by

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"All truths are easy to understand once they are discovered;
the point is to discover them."

Galileo Galilei, (1564 – 642)

ABSTRACT

Feed cost and feed availability is a major bottleneck for the aquaculture industry and has prompted significant research into identifying new microalgal strains and/or growth conditions that reduce production costs, increase the ease of cultivation and improve the nutritional value of the cultivated biomass. The nutritional value of microalgal biomass depends on several physiological and biochemical attributes including microalgal cell size and shape, digestibility, non-toxicity and biochemical composition. The growth rate and biochemical composition of microalgal biomass can be influenced by culture and environmental conditions.

Heterotrophic cultivation systems are a potential alternative to the more traditional photoautotrophic cultivation systems, which typically suffer from low biomass concentrations, high production costs and unexplained '*crashes*'. This thesis presents the investigations undertaken into the heterotrophic cultivation of the diatom *Cyclotella cryptica* (UTEX 1269). *C. cryptica* was chosen as a model organism as this species is capable of heterotrophic growth, has previously been used within aquaculture and was recommended as a species worthy of further investigations. The effects of major nutritional and environmental factors on the growth and biochemical characteristics were studied in 250 mL Erlenmeyer flasks, 500 mL Schott bottles and 19 L carboys. The results from this investigation are unique as the optimal heterotrophic growth conditions were previously either not known or reported.

The major contributions from this research are the increased knowledge of the effects of the concentration of glucose, sodium metasilicate and nitrogen source (sodium nitrate, urea and ammonium chloride) on the growth dynamics and biomass productivity of *C. cryptica* and the effects of the nitrogen availability (sodium nitrate, urea and ammonium chloride) on the fatty acid composition and total fatty acid content. In addition, there is now an increased knowledge of the effects of the cultivation temperature, salinity, pH and degree of mixing or agitation on the growth dynamics and biomass productivity of *C. cryptica* and the effects of the cultivation temperature and salinity on the fatty acid composition and total fatty acid content. The calculated specific growth rates in

heterotrophic culture were in the order of 0.05 h^{-1}, which are comparable to the photoautotrophic growth rates reported in the literature. The most predominant fatty acids synthesised by *C. cryptica* under heterotrophic growth conditions were palmitic acid (16:0), palmitoleic acid (16:1 *n*-7), stearidonic acid (18:4 *n*-3, SDA), eicosapentaenoic acid (20:5 *n*-3, EPA) and docosahexaenoic acid (22:6 *n*-3, DHA). These fatty acids are similar to the fatty acids synthesised under photoautotrophic conditions. An economic assessment confirmed that the biomass production costs were sensitive to the biomass concentration. The cost of producing *C. cryptica* biomass under heterotrophic cultivations in a 100 L commercial bioreactor, at a steady state biomass concentration of 30 $g.L^{-1}$, was estimated at approximately AU $180 per kg. This cost, although high, is reasonable given photosynthetic microalgal production costs in small hatcheries is up to AU $750 - $1000 per kg. Unit production costs were sensitive to the steady-state biomass concentration and a steady-state biomass concentration of 2 $g.L^{-1}$ resulted in the unit production cost increasing to approximately AU $2,675 per kg.

STATEMENT OF ORIGINALITY

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Stephen Luke Pahl and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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SIGNED: DATE:

Stephen Luke Pahl

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LIST OF PUBLICATIONS

The publications produced during this research are listed below:

Journal papers

Pahl SL, Lewis DM, Chen F, King KD (2010) Growth dynamics and the proximate biochemical composition and fatty acid of the heterotrophically grown diatom *Cyclotella cryptica*. Journal of Applied Phycology 22: 165-171.

Pahl SL, Lewis DM, Chen F, King KD (2010) Heterotrophic growth and nutritional aspects of the diatom *Cyclotella cryptica* (Bacillariophyceae): Effect of some environmental factors. Journal of Bioscience and Bioengineering 109: 235-239.

Pahl SL, Lewis DM, Chen F, King KD (2010) Heterotrophic growth and nutritional aspects of the diatom *Cyclotella cryptica* (Bacillariophyceae): Effect of nitrogen source and concentration. Journal of Bioscience and Bioengineering (In Review).

Conference papers

Pahl SL, Lewis DM, Schwarz, M. Chen F (2003) Advantages of heterotrophically grown microalgae for aquaculture – economic and environmental. Environmental Engineering Research Event (EERE). Melbourne, Victoria, Australia. December 2003, CD-ROM.

Pahl SL, Schwarz M, Chen F, Lewis DM (2003) Experimental techniques used to determine the nutritional content of microalgae. CHEMECA 2003. Adelaide, South Australia, Australia. October 2003. CD-ROM.

Conference abstracts

Pahl SL, Lewis DM, Chen F, King KD (2006) Heterotrophic growth *of Cyclotella cryptica* for aquaculture. Skretting Australasian Aquaculture Conference, 27 – 30 August 2006. Adelaide, South Australia, Australia. CD-ROM.

Pahl SL, Lewis DM, Chen F (2005) Proximate biochemical composition and fatty acid profile of the diatom *Cyclotella cryptica* under heterotrophic growth conditions. Abstracts and Program of the 10th International Conference on Applied Phycology, 24 – 28 July 2005. Kunming, China. pp. 50-51.

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