

Metabolic and physiological regulation of *Chlorella* sp. (Trebouxiophyceae, Chlorophyta) under nitrogen deprivation

YONG Wai-Kuan^{1,2}, LIM Phaik-Eem¹, VELLO Vejeysri^{1,3}, SIM Kae-Shin³, ABDUL MAJID Nazia³,
MUSTAFA Emienour Muzalina⁴, NIK SULAIMAN Nik Meriam⁵, LIEW Kan-Ern⁶, CHEN Brenna Jia-Tian⁷,
PHANG Siew-Moi^{1,3}

¹ Institute of Ocean and Earth Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia; ² Institute of Graduate Studies, University of Malaya, 50603 Kuala Lumpur, Malaysia; ³ Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia; ⁴ School of Fisheries and Aquaculture Sciences, University of Malaysia, Terengganu, T145, 21300 Kuala Terengganu, Terengganu, Malaysia; ⁵ Department of Chemical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia; ⁶ Airbus Group Malaysia, Menara HLA, 3, Jalan Kia Peng, 50450 Kuala Lumpur, Malaysia; ⁷ Aerospace Malaysia Innovation Centre, Level 1, MIGHT Building, 3517, Jalan Teknokrat 5, 63000 Cyberjaya, Selangor, Malaysia

Abstract A freshwater green microalgae *Chlorella* sp., UMACC344 was shown to produce high lipid content and has the potential to be used as feedstock for biofuel production. In this study, photosynthetic efficiency, biochemical profiles and non-targeted metabolic profiling were studied to compare between the nitrogen-replete and deplete conditions. Slowed growth, change in photosynthetic pigments and lowered photosynthetic efficiency were observed in response to nitrogen deprivation. Biochemical profiles of the cultures showed an increased level of carbohydrate, lipids and total fatty acids, while the total soluble protein content was lowered. A trend of fatty acid saturation was observed in the nitrogen-deplete culture with an increase in the level of saturated fatty acids especially C16:0 and C18:0, accompanied by a decrease in proportions of monounsaturated and polyunsaturated fatty acids. Fifty-nine metabolites, including amino acids, lipids, phytochemical compounds, vitamins and cofactors were significantly dysregulated and annotated in this study. Pathway mapping analysis revealed a rewiring of metabolic pathways in the cells, particularly purine, carotenoid, nicotinate and nicotinamide, and amino acid metabolisms. Within the treatment period of nitrogen deprivation, the key processes involved were reshuffling of nitrogen from proteins and photosynthetic machinery, together with carbon repartitioning in carbohydrates and lipids.

Keywords: Metabolic profiling, *Chlorella* sp., nitrogen stress, lipids, fatty acids

1 INTRODUCTION

Microalgae are a potential feedstock for triacylglycerol (TAG) and neutral lipids which are used in biofuel production due to their fast growth rate, high photosynthetic efficiency, high lipid yields and ability to grow in a broad range of environmental conditions. Various strategies, such as nutrient deprivation (e.g. nitrogen starvation), temperature, salinity, pH, high concentration of metal ion stress, and irradiation, have been developed to improve the biomass yield and the lipid content of microalgal cells (Sharma et al., 2012).

Nitrogen is an important building block for amino acids, nucleic acids, enzymes and proteins. It is assimilated into microalgal cells in the form of nitrate or ammonium, and is involved in multiple metabolic networks. While it is widely known that the deprivation of nitrogen leads to lipid accumulation in microalgae, it also triggers many other metabolic responses in the organisms. Nitrogen deprivation inhibits photosynthesis