INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 10, No. 2, p. 72-80, 2017

RESEARCH PAPER OPEN ACCESS

Protein quantification of mixed microalgae consortia under different concentration of Coal fired flue gas

Ambreen Aslam[*1,2], Tahira Aziz Mughal[1], Skye R. Thomas-Hall[2], Peer M. Schenk[2], Shazia Pervaiz[1]

[1]*Environmental Science Department, Lahore College for Women University (LCWU), Lahore, Pakistan*

[2]*Algae Biotechnology Laboratory, School of Agriculture and Food Sciences, University of Queensland, Brisbane, Queensland, Australia*

Key words: Microalgae cultivation, Outdoor photobioreactor, Protein content, Flue gas, Carbon dioxide (CO_2)

http://dx.doi.org/10.12692/ijb/10.2.72-80 Article published on February 20, 2017

Abstract

Microalgae have abundant ecological relevance because of its significant contribution in global carbon fixation. During last decade, microalgae became increasingly interesting for biotechnology as it provides many natural products. Microalgae are promising sources to enhance nutrition of food and animal feedstock as it contain high value biochemical compounds including fatty acids, protein etc. The present study evaluated the protein content from mixed microalgae consortia under different CO_2 concentrations (1%, 3% and 5.5%) from coal-fired flue gas. CB-X Protein Assay Kit (G Biosciences) was used for measurement of protein concentration from all microalgal samples. Under 1% CO_2 concentration, UQ-Lake (F) showed high protein content 39.3±1.89% which make algae attractive to be used as feed and nutrition supplement for animal. Mixed culture grown in nutrient rich media under air as control samples and in flue gas as CO_2 source as an attempt to reduce greenhouse gases in atmosphere.

* **Corresponding Author:** Ambreen Aslam ✉ ambreenaslam86@hotmail.com

Introduction

Microalgae are solar powered unicellular organisms with high photosynthetic rateand faster biomass yields as compare to terrestrial plants. Microalgae can utilize carbon dioxide from flue gas for photosynthesis and exhibit high efficiency in reducing greenhouse gas emissions which consequently remove carbon dioxide from industrial flue gasses by algae carbon biofixation (Wang *et al.,* 2008; Khan *et al.,* 2009). Microalgae are versatile feedstock with broader range of biofuel potential as algae species contain high lipid content which provide sustainable and cheaper source for biodiesel production as most interesting biotechnological application (Mata *et al.,* 2010; Wijffels and Barbosa, 2010; Georgianna and Mayfi, 2012; Oody *et al.,* 2014). The major advantage of biofuel production from microalgae is to minimize environmental impact, occupy little space for cultivation, no competition of space for food crops (Zhu *et al.,* 2014), and wastes (flue gas and waste water) can be used during the growth process (Mahdy *et al.,* 2015). Additionally, microalgae produce a wide range of natural value-added products i.e. fatty acids, vitamins, enzyme, pigments and high concentrations of natural proteins (Raja *et al.,* 2008; Buono *et al.,* 2014; Zhu *et al.,* 2014).

Protein quantity and quality are crucial factor while determining the nutritional value in microalgae foods, animal feeds and health products (Spolaore *et al.,* 2006; Becker *et al.,* 2013). Moreover, the crude protein content usually makes up a great fraction of their actively growing biomass and considered as alternative source of protein (Spolaore *et al.,* 2006; Becker, 2007; Lopez *et al.,* 2010; Becker, 2013). Interestingly, protein production had no link with cell concentration and mostly it produce during exponential phase. Higher protein production occurred concurrently with higher specific growth rates when substrate level were highest (level of substrate had started to drop off in the middle of exponential phase) (Kightlinger *et al.,* 2014). This quality of microlagal extracts might be useful in some industrial applications as prolonged protein expressions are essential to maximize batch cultivation before encountering the stationary phase, particularly during enzyme production or secondary metabolites where cell remain viable for long time (Kightlinger *et al.,* 2014).

The aim of the study is to assess protein content in mixed culture microalgal consortia. This research quantify total protein content in biomass before and after starvation period under three different concentration of carbon dioxide from coal fired flue as an attempt to reduce carbon emissions into atmosphere and get value-added products. Protein rich microalgae can be used in aquaculture feed and animal nutrition (Spolaore *et al.,* 2006). Specifically, several trails of utilizing microalgae as an alternative protein feed supplement have been performed on poultry and ruminants, and significantly higher growth rates and lower non-specific mortality rates were observed (Becker, 2013).

Materials and methods

Organism, culture conditions and inoculation protocols

Water samples were collected from two different places of Brisbane which is explained in the following points. Fresh water algae species were collected from UQ (University of Queensland) (27°30'01.98"S 153°00'58.53"E) at different points which seemed to contain some algal growth. Storm water algae samples were collected from Sun state Cement Factory (27°23'.304"S 153°9'.638"E). Sample collected from UQ Lake were freshwater and Storm Water. Mixed culture of UQ Lake (Freshwater algae) and Storm Water algae were treated with air (as control sample) and flue gas in triplet. For the preparation of outdoor experiment, microalgal cultures were inoculated in Bold Basal Medium (BBM). Samples having high specific growth rate were used as inoculum. UQ Lake (A) and Storm Water (A) treated with air whereas UQ Lake (F) and Storm Water (F) were treated with flue gas (1% & 3% CO_2). The two samples (UQ Lake+Storm Water species) were mixed together for treating 5.5% CO_2 (flue gas) with addition of 50mM phosphate buffer in order to combat with low pH. The Mixed C. with and without phosphate buffer (MC+P-F; MC+P-A) treated with flue gas and air respectively.

In this study, coal fired boilers smoke were used as flue gas sources from a beef processing industry Australian Country Choice (ACC) located at Cannon Hill, Brisbane, Australia. The fuel gas composed of SO_x (781.8mg/Nm^3), NO_x (423.9mg/Nm^3), CO (388.8mg/Nm^3), CO_2 (11.24%), O_2 (8.26%) and particulates (0.4 mg/Nm^3). The raw flue gas were transferred through 2 inch pipe (2-3m long) from smoke stack to algal broth. The outdoor plastic bags photobioreactor (conical transparent polyethylene) were bubble column connected to each other with 25 L microalgal culture capacity while 5L served as the gas space (Fig 1.). The flue gas produced from burning of coal in the flue gas generator is mixed with air to create different v/v mixtures of air-flue gas of desired concentration. Mixing in all bags was accomplished by sparging air introduced at the bottom through plastic tube at different rate to culture volume. Sunlight was used as natural illumination source corresponding to a photosynthetically active radiation (PAR) of 1650.3 µmol photon·m^{-2}·s^{-1} operated on a 12:12hours light/dark cycle.

After inoculation, samples were collected from all bags for the following analysis: pH, OD and nutrient (nitrate and phosphate). Sampling was done on 1st day, 4th day, 8th day, 12th day and 16th day after every inoculation. Samples collected until they run out of nutrient on day 8th and then depending on the period of starvation mostly experiment finished on day 16th. At end of experiment, biomass were centrifuged, freeze dried and store at -20^{o}C until it was being analysed.

Protein extraction from microalgal samples

Protein contents in the algal biomass were measured following the protocol described by López *et al.,* (2010) with modifications. In brief, freeze-dried biomass (10 mg) was milled and protein extraction was done by incubation in 10 mL lysis buffer (containing 0.0348 gL^{-1} of phenyl methyl sulfonyl fluoride, 0.3722 g L^{-1} of ethylenediaminetetraacetic acid disodium salt, 5 mL L^{-1} of Triton X-100). Shake well mixture by vortex and keep it for 30 mints. A 100µL of this solution was transferred in a 1.5 mL Eppendorf tube for analysis and remains can be stored in the freezer for repeat analysis. Protein standard had been prepared with concentrations: 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 of volume 200ml.

In 1.5mL tube, take 50µL of each concentration (Table 1). The mixture was then used for measurement of protein concentration following the protocol described in the CB-X Protein Assay Kit (G Biosciences). Add 100µL of SDS (0.05g of sodium dodecyl sulphate salt) to standard concentration and sample solution tubes. Mixing well by using vortex. Then 1mL of pre-chilled (-20^{o}C) CB-X added and mix well by vortex. Centrifuge the mixture for 5mins at 16,000g and remove supernatant without disturbing protein pallet. Now add 50µL CB-X Solubilization Buffer-I and 50µL of CB-X Solubilization Buffer II to the tube and dissolve protein pallet by vortex. Invert 2-3 times to mix CB-X Assay dye and 1mL added in all tubes. Vortex briefly and incubate at room temperature for 5 mints. Take sample solution of 200mL to each well in 96 wells plate. Make 3 well of each sample solution and standard. Read absorbance of sample at wavelength 595-600nm.

Analytical procedures

A microplate photometer (Glomax Multi Detection System, Promega) was used for sample processing in the dark to prevent degradation of the Folin reagent. The spectrophotometric absorbance was converted to protein concentrations using a linear calibration curve (correlation coefficient was 0.995) established by the absorbance range of 0.2-1 corresponding to the bovine serum albumin (BSA) standard concentration of 0-1 mg mL^{-1}. The protein content of the biomass was calculated using the following equation:

Protein (*% w/w*) = (*CVD/m*) ×100

Where *C* = protein concentration (mg/L) obtained from the calibration curve, *V* = volume (L) of the lysis buffer used to re-suspend the biomass, *D* = dilution factor, *m* = biomass (mg) (Lopez *et al.,* 2010). Protein productivity (mg/L/day) was calculated as the total protein content multiplied by the biomass productivity.

Statistical analysis

All data analysis was done in Microsoft office Excel 2010. The whole experiment was conducted in triplicate (n=3) and results are expressed as mean ± error bars.

Results and discussion

Key cultivational attributes of microalgae that contributed to its protein production for instance light density and photoperiod (Renaud *et al.,* 1994; Seyfabadi *et al.,* 2011), growth phase (Mata *et al.,* 2010), nutrient availability (Hu, 2013)are crucial considerations when evaluating protein content from different studies. Microalgae have high quantity of protein level range from about 19% to nearly 70% of cellular dry weight depending on growth stage (Gatenby *et al.,* 2003).Fast growing cells characterized by low CHO content and high protein, more carbon is incorporated into CHO and lipid when cells reached stationary phase (Piorreck and Pohl, 1984; Henderson and Sargent, 1989).

Table 1. Preparation of Stock solution and Buffer according to following concentration for Protein Extraction.

Concentration	Stock solution (µL)	Buffer (µL)
0	0	0
0.2	20	20
0.4	40	40
0.6	60	60
0.8	80	80
1.0	100	100

Protein is usually the key biochemical component of algae (Brown and Jeffrey, 1992; Wikfors *et al.,* 1992) however nitrogen concentration in medium affect growth and biochemical component (Leonardos and Lucas, 2000; Fidalgo *et al.,* 1998; Illman *etal.,* 2000). Nitrogen is fundamental element for synthesis photosynthetic pigments, nucleic acid and protein production in microalgae (Adams *et al.,* 2014).

Protein Content of Mix Culture Consortia Under 1% CO_2 at ACC

The amount of protein in dried samples of mix culture (UQ-Lake & Storm W.) varied considerably since they were treated under different concentration of CO_2 and air as control samples in ACC. Data for protein content in 12 mix algal samples are summarized and expressed in graphical form before and after 1 week starvation. While treating with air as control samples, the percentage of protein in UQ-Lake (A) range from 21-28% before starvation which decreases up to 6.9-17.7% after one week of starvation (Fig 2).

Similarly, Storm W. (A) species had 18.7-30.6% protein decline 11-13% after starvation. Under 1% CO_2 (Flue gas), the protein content increase 30-47%, 23-36% before starvation (Fig2) in UQ-Lake (F) and Storm W. (F) species which drop after starvation to 14-19%, 12-16% (Fig2) respectively.

Under different CO_2 concentration (flue gas), highest protein production was observed in UQ-Lake (F) with value 39.3±1.89% DW while treating with 1% CO_2 in this study.

Though in all samples, the percentage reduction of protein content was observed under nitrogen starvation. The higher percentage reduction in protein content was 59.12% in UQ-lake (F) and 53.02% in Storm W. (F) under 1% CO_2 after one week nutrient starvation period. However, some of the variability in protein content was driven by different analytical methods (Kent *et al.,* 2015; Lee *et al.,* 2013; Lopez *et al.,* 2010). For example, the commonly used Kjeldahl method overestimates the protein content in microalgae with the nitrogen to protein conversion factor of 6.25 (Lee *et al.,* 2013; Lopez *et al.,* 2010).

Fig. 1. Experimental setup as outdoor photobioreactor in ACC.

Protein Content of Mix Culture Consortia Under 3% CO_2 at ACC

In another experiment when treated with 3% CO_2 (Flue gas), the protein content drop UQ-Lake (F) after starvation from 22.2-28.4% to 15-23.3%. Whereas the Storm W. (F) after 3% CO_2, decline occur in protein concentration after one week starvation from 26-27.2% (Fig3) to 20.6-25.2%. However the control sample, UQ-Lake (A) very little change took place from range 24.8-30.9% to 24.3-29.8% whereas the amount of protein reveal a minor difference in quantity from 21.9-23% to 16.4-22.1% when Storm W. (A) species treated with air. Under sufficient nitrogen conditions, microalgae will assimilate environmental nitrogen by accumulating in numerous nitrogen containing compounds, either inorganic forms (ammonium and nitrate) or organic forms (peptides, amino acids, RNA, protein and pigments).

Under 3% CO_2, the percentage reduction decreases to 22.13% for UQ-Lake (F) and 10.02% for Storm W. (F) as samples maintained good protein content at the end of starvation period. Right after nitrogen depletion, intermediate nitrogen reservoir (ammonium, amino acids, nitrate and peptides) is directly synthesis proteins in order to support growth. For the time being, cells degrade non-growth related protein by autophagy (Mc Glathery *et al.,* 1996).

Therefore, protein content decreases under nitrogen starvation (Chen *et al.,* 2015). In contrast, biomass productivity and protein content reached their maximal levels on 90% utilization of nitrogen, leading to maximal protein productivity. Taking this into consideration, microalgal biomass as protein source must be harvested at 90% nitrogen utilization (or just before nitrogen starvation occurs) (Chen *et al.,* 2015).

Protein Content of Mix Culture Consortia Under 5.5% CO_2 at ACC

As mentioned earlier that before treating with 5.5% CO_2 (flue gas), a samples aggregate generated by mixing UQ-Lake and Storm water species termed as Mixed Community (Mixed C.). A total 12 samples made with (Mixed C.+P) and without phosphate buffer to assess protein content after treating with CO_2 in flue gas and air as control samples in order to observe any significant difference.

The Mixed C. (A) tends to have higher percentage reduction after one week starvation from 18.2-32.9% to 7.4-12.1% (Fig4). However, the same community treated with phosphate buffer (Mixed C.+P-A), the protein content increased from 31.4-33.7% to 18.1-35.8%.

The results revealed that the amount of protein increased under high phosphorus (P) concentration. The protein contents of Mixed C. (F) varied from 20.3-44.1% to 13-23.9% when treated with 5.5% CO_2 in flue gas. Whereas, Mixed C.+P (F) tends to have decline in percentage range from 25-36.6% to 28.3-32.7% while treating with CO_2 under high phosphate concentration after one week starvation.

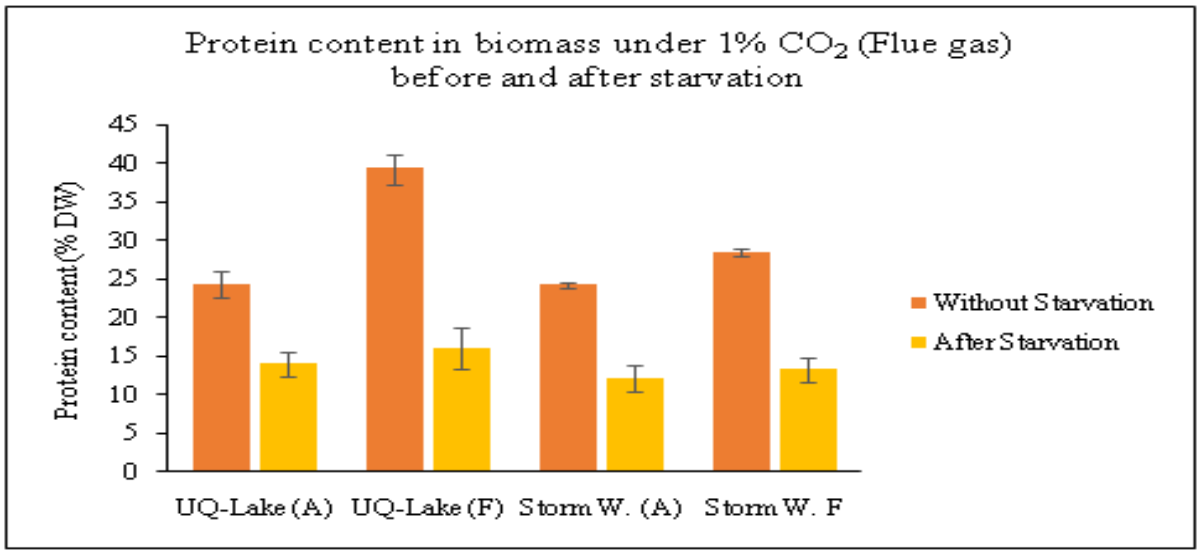

Fig. 2. Protein Content in samples before and After Starvation (1% CO_2) at ACC and shown as mean values (±SE) from three replicates.

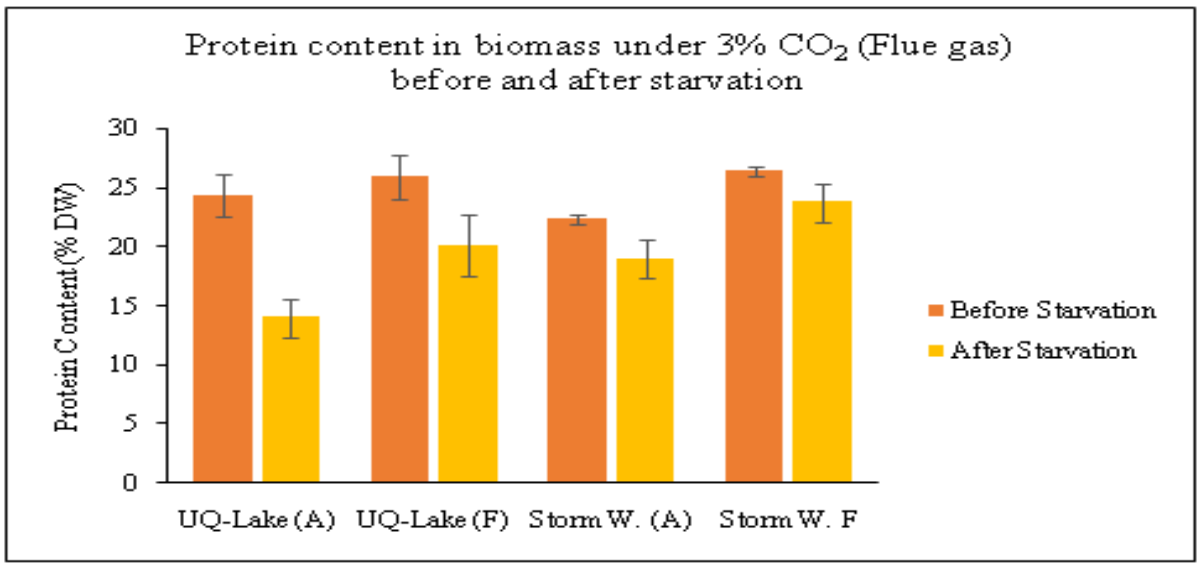

Fig. 3. Protein Content in samples before and after starvation (3% CO_2) at ACC and shown as mean values (±SE) from three replicates.

Protein rich microalgae can be used in animal nutrition and aquaculture feed (Spolaore *et al.,* 2006). Specifically, several trails of utilizing microalgae as an alternative protein feed supplement have been performed on poultry and ruminants, and significantly higher growth rates and lower non-specific mortality rates were observed (Becker, 2013).

The overall protein production for food or food substitutes have not been fully exploited, despite its high nutritious content (Becker, 2007).

An extended knowledge of the protein quality and functional properties in microalgae hydrolysates would be useful in understanding their use as potential additives for food items.

However, despite a variety of biological functions of microalgae, effective application of microalgae is still limited because of their inefficient cultivation and high production cost. To further investigate the nutritional quality of protein, the content and quantity of individual amino acids, especially essential amino acids should be analyzed and compared to FAO/WHO guidelines.

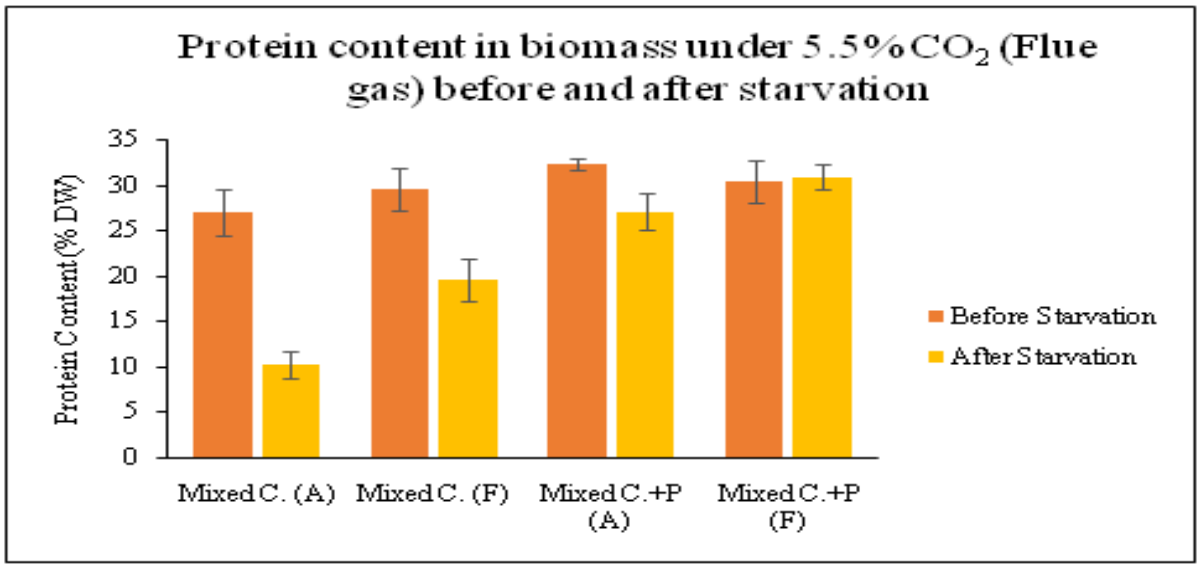

Fig. 4. Protein Content in samples before and After Starvation (5.5% CO_2) at ACC and shown as mean values (±SE) from three replicates.

Conclusion

Microalgae have higher potential to be used as protein production as feed and nutritious supplement because of high protein content, productivity and fast settling. The finding of this study revealed that highest percentage of protein content was observed in Storm W. (F) species while treating with 1% CO_2. Whereas, Mixed C. (F) showed highest percentage reduction in protein content without phosphate buffer under 5.5% CO_2 (flue gas). The protein content in Mixed C.+P (F) with phosphate buffer (5.5% CO_2) increased 1.85% even after one week nutrient starvation is the major outcome of this research. This study provides an overview on prospects and advantage of microalgal protein productivity as value added product while cultivation. These procedures are most economical when combined with CO_2 sequestration from flue gas emissions, with extraction of high value products for application in other industrial processes.

Acknowledgement

This work was financially supported by Higher Education Commission (HEC) Pakistan under International Research Support Initiative Program (IRSIP) and Australian Research Council (LP0990558). The author would like to pay deepest gratitude to Professor Dr. Peer Schenk and Dr. Skye Thomas-Hall for their continuous help and support in data collection and compilation of research work. The author is also thankful to Professor Dr. Tahira Aziz Mughal for proofreading to improve the manuscript.

References

Adams C, Bugbee B. 2014. Nitrogen retention and partitioning at the initiation of lipid accumulation in nitrogen-deficient algae. Journal of Phycology **50(2),** 356-365.
http://dx.doi.org/10.1111/jpy.12167

Becker EW. 2007. Micro-algae as a source of protein, Biotechnology Advances **25(2),** 207-10.
http://dx.doi.org/10.1016/j.biotechadv.2006.11.002

Becker EW. 2013. Microalgae for human and animal nutrition, in A Richmond & Q Hu (eds), Handbook of microalgal culture, John Wiley & Sons, Ltd., p. 461-503.
http://dx.doi.org/10.1002/9781118567166.ch25

Brown MR, Jeffrey SW. 1992. Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 1. Amino acids, sugars and pigments. Journal of experimental marine biology and ecology **161(1),** 91-113.
http://dx.doi.org/10.1016/0022-0981(92)90193-E

Buono S, Langellotti AL, Martello A, Rinna F, Fogliano V. 2014. Functional ingredients from microalgae. Food and Function **5(8)**, 1669-1685. http://dx.doi.org/10.1039/c4fo00125g

Chen CY, Lee PJ, Tan CH, Lo YC, Huang CC, Show PL,... Chang JS. 2015. Improving protein production of indigenous microalga Chlorella vulgaris FSP-E by photobioreactor design and cultivation strategies. Biotechnology journal **10(6),** 905-914. http://dx.doi.org/10.1002/biot.201400594

Fidalgo Paredes P, Cid Blanco A, Torres E, Sukenik A, Herrero López C. 1998. Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga" *Isochrysis Galbana*. Aquaculture **166(1-2),** 105-116. http://dx.doi.org/10.1016/S0044-8486(98)00278-6

Gatenby CM, Orcutt DM, Kreeger DA, Parker BC, Jones VA. Neves RJ. 2003. Biochemical composition of three algal species proposed as food for captive freshwater mussels. Journal of Applied Phycology**15(1)**, 1-11. http://dx.doi.org/10.1023/A:1022929423011

Georgianna DR, Mayfield SP. 2012. Exploiting diversity and synthetic biology for the production of algal biofuels. Nature **488,** 329–335. http://dx.doi.org/10.1038/nature11479

Henderson RJ, Sargent JR. 1989. Lipid composition and biosynthesis in ageing cultures of the marine *Cryptomonad*, *Chroomonas salina*. Phytochemistry **28(5),** 1355-1361. http://dx.doi.org/10.1016/S0031-9422(00)97745-8

Hu Q. 2013. Environmental effects on cell composition, in A Richmond & Q Hu (Eds), Handbook of microalgal culture, John Wiley & Sons, Ltd., p. 114-22. http://dx.doi.org/10.1002/9781118567166.ch7

Illman AM, Scragg AH, Shales SW. 2000. Increase in Chlorella strains calorific values when grown in low nitrogen medium. Enzyme and microbial technology **27(8),** 631-635. http://dx.doi.org/10.1016/S0141-0229(00)00266-0

Kent M, Welladsen HM, Mangott A, Li Y. 2015. Nutritional evaluation of Australian microalgae as potential human health supplements, PLoS One **10(2),** p. e0118985. http://dx.doi.org/10.1371/journal.pone.0118985

Khan SA, Rashmi Hussain MZ, Prasad S, Banerjee UC. 2009. Prospects of biodiesel production from microalgae in India. Renewable and Sustainable Energy Reviews **13(9),** 2361-2372. http://dx.doi.org/10.1016/j.rser.2009.04.005

Kightlinger W, Chen K, Pourmir A, Crunkleton DW, Price GL, Johannes TW. 2014. Production and characterization of algae extract from *Chlamydomonas reinhardtii*. Electronic Journal of Biotechnology **17(1),** 3-3. http://dx.doi.org/10.1016/j.ejbt.2013.12.003

Lee Y-K, Chen W, Shen H, Han D, Li Y, Jones HDT, Timlin JA, Hu Q. 2013. Basic culturing and analytical measurement techniques', in A Richmond & Q Hu (Eds), Handbook of microalgal culture, John Wiley & Sons, Ltd., 37-68. http://dx.doi.org/10.1002/9781118567166.ch3

Leonardos N, Lucas IA. 2000. The nutritional value of algae grown under different culture conditions for *Mytilus edulis* L. larvae. Aquaculture **18(3-4),** 301-315. http://dx.doi.org/10.1016/S0044-8486(99)00269-0

Lopez CV, Garcia Mdel C, Fernandez FG, Bustos CS, Chisti Y, Sevilla JM. 2010.Protein measurements of microalgal and cyanobacterial biomass. Bioresource Technology **101(19),** 7587-7591. http://dx.doi.org/10.1016/j.biortech.2010.04.077

Mahdy A, Mendez L, Ballesteros M, Fernandez CG. 2015. Algal culture integration in conventional wastewater treatment plants: anaerobic digestion comparison of primary and secondary sludge with microalgae biomass. Bioresource Technology 184, 236-244. http://dx.doi.org/10.1016/j.biortech.2014.09.145

Mata TM, Martins AA, Caetano NS. 2010. Microalgae for biodiesel production and other applications: a review. Renewable and Sustainable Energy Reviews **14(1),** 217-232.
http://dx.doi.org/10.1016/j.rser.2009.07.020

McGlathery KJ, Pedersen MF, Borum J. 1996. Changes in intracellular nitrogen pools and feedback controls on nitrogen uptake in *Chaetomorpha linum* (Chlorophyta).Journal of Phycology **32(3),** 393-401.
http://dx.doi.org/10.1111/j.0022-3646.1996.00393.x

Oody JW, Mc Ginty CM, Quinn JC. 2014. Global evaluation of biofuel potential from microalgae. Proceeding of the National Academy of Sciences **111(23),** 8691-8696.
http://dx.doi.org/10.1073/pnas.132165211

Piorreck M, Pohl P. 1984. Formation of biomass, total protein, chlorophylls, lipids and fatty acids in green and blue-green algae during one growth phase. Phytochemistry **23(2),** 217-223.
http://dx.doi.org/10.1016/S0031-9422(00)80305-2

Raja R, Hemaiswarya S, Kumar NA, Sridhar S, Rengasamy R. 2008. A perspective on the biotechnological potential of microalgae. Critical Review Microbiology **34(2)**, 77-88.
http://dx.doi.org/10.1080/10408410802086783

Renaud SM, Parry DL, Thinh LV. 1994. Microalgae for use in tropical aquaculture I: Gross chemical and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia. Journal of Applied Phycology **6(3),** 337-345.
http://dx.doi.org/10.1007/BF02181948

Seyfabadi J, Ramezanpour Z, Amini Khoeyi Z. 2011. Protein, fatty acid, and pigment content of Chlorella vulgaris under different light regimes. Journal of Applied Phycology **23(4)**, 721-726.
http://dx.doi.org/10.1007/s10811-010-9569-8

Spolaore P, Joannis-Cassan C, Duran E, Isambert A. 2006. Commercial applications of microalgae. Journal of Bioscience and Bioengineering**101(2)**, 87-96.
http://dx.doi.org/10.1263/jbb.101.87

Wang B, Li Y, Wu N, Lan CQ. 2008. CO_2 bio-mitigation using microalgae. Applied Microbiology and Biotechnology **79(5),** 707-718.
http://dx.doi.org/10.1007/s00253-008-1518-y

Wijffels RH, Barbosa MJ. 2010. An outlook on microalgal biofuels. Science **329(5993),** 796-799.
http://dx.doi.org/10.1126/science.1189003

Wikfors GH, Ferris GE, Smith BC. 1992. The relationship between gross biochemical composition of cultured algal foods and growth of the hard clam, *Mercenaria mercenaria* (L.). Aquaculture **108(1-2),** 135-154.
http://dx.doi.org/10.1016/0044-8486(92)90324-E

Zhu LD, Hiltunen E, Antila E, Zhong JJ, Yuan ZH, Wang ZM. 2014. Microalgal biofuels: flexible bioenergies for sustainable development. Renewable Sustainable Energy Review **30,** 1035-1046.
http://dx.doi.org/10.1016/j.rser.2013.11.003