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Biochemical characterization of microalgal biomass from freshwater species isolated in Alberta, Canada for animal feed applications

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ABSTRACT

Biochemical composition of freshwater microalgae isolates from Alberta, Canada was determined. Growth rate (0.98 ± 0.07 d^{-1}), biomass production (0.35 ± 0.03 g DW L^{-1}) and daily productivity (0.14 ± 0.01 g DW L^{-1} d^{-1}) were the same for *Chlorella vulgaris* (AB02-C-U-BBM), *Nannochloris bacillaris* (AB03-C-F-PLM), *Tetracystis* sp. (AB04-C-F-PLM02) and *Micractinium reisseri* (AB05-C-U-BBM02). Whole algal biomass (WAB) contained low ash (~2 g 100 g DW^{-1}) and protein (13–15 g 100 g DW^{-1}), high esterifiable lipid (32–36 g 100 g DW^{-1}), carbohydrate (27–30 g 100 g DW^{-1}) and energy (26–28 MJ kg DW^{-1}). Oil fractionation was relatively ineffective for *C. vulgaris* and *M. reisseri* while oil was effectively extracted from *N. bacillaris* and *Tetracystis* sp. Accordingly, lipid-extracted biomass (LEB) from *N. bacillaris* and *Tetracystis* sp. contained higher protein (22 g 100 g DW^{-1}) and carbohydrate (43–44 g 100 g DW^{-1}) and lower residual esterifiable lipid (6–9 g 100 g DW^{-1}) than *C. vulgaris* and *M. reisseri* at 17–18 g 100 g DW^{-1} (protein), 34–36 g 100 g^{-1} DW (carbohydrate) and 28–32 g 100 g DW^{-1} (residual esterifiable lipid). Biomass had favorable essential amino acid (EAA) profiles with high EAA indices (0.9–1.1); rich in first-limiting EAA lysine (0.9–1.5 g 100 g DW^{-1}). Fatty acids (% of total) were predominantly monounsaturated fatty acids (MUFA; 40–53%), high in polyunsaturated fatty acids (PUFA; 27–40%) and low in saturated fatty acids (14–24%). *N. bacillaris* and *Tetracystis* sp. were particularly rich (9–12%) in α-linolenic acid (18:3n − 3), had attractive n − 3:n − 6 ratios (0.5–0.7:1), were rich in iron (800–1616 mg kg DW^{-1}), had attractively low Ca:P ratios (0.6–0.9:1) and were virtually absent of contaminating heavy metals.

1. Introduction

Microalgae are one of the most efficient organisms at transforming solar energy, carbon dioxide (CO_2) and inorganic elements into nutrient-rich biomass [1]. With a rapidly growing biofuel sector and expanding production of microalgae for other commercial purposes, it is expected that large quantities of algal products/co-products could become available in the near future [2]. Although algal oil for third-generation biodiesel production has been the subject of much research and a major driver for technological innovations in recent years, by all assessments it is not economically viable [3–5]. Utilization of the entire algal crop through a balanced biorefinery approach that effectively maintains the quality of various fractions has the potential to reduce the processing costs of each product and is likely the only feasible strategy to increase the viability of a microalgae industry [6–9]. At least in the near to mid-term, the livestock and aquaculture feed sectors appear to be among the most promising areas to focus for generating revenues [10,11]. The global animal feed market is currently valued at >$550 billion (b) USD annually (poultry, $69.9 b, swine, $89.3 b, cattle, $330.9 b, aquaculture, $60.5 b) and is continually seeking novel and sustainable sources of essential nutrients [12]. Depending on species/strain, environmental conditions and harvesting/processing methods, whole algal biomass and residual 'cake' after oil extraction may be highly attractive sources of essential dietary amino acids, fatty acids, sugars, vitamins, minerals, carotenoids and other health-promoting nutrients well suited as feeds or feed additives for terrestrial livestock and aquatic animals [1,13]. This potential for algal products/co-products for nutrition applications has long been recognized but commercial success has only been realized to a minor extent for a few species (e.g. *Spirulina*, *Chlorella*) occupying niche markets. The vast majority of this relatively small production (~15,000 t $year^{-1}$) is 'purpose-cultivated' as high value (>$10,000 t^{-1}) human nutritional supplements and pigments either in open cultivation ponds or open/closed hybrid systems [14]. Under the anticipated large-scale production of microalgae for biofuels and other commercial purposes, the residual biomass (e.g. defatted residues) will likely command a lower market value for animal feeds (<$1000 t^{-1}) than human nutritional supplements but, by sheer volume and potentially reduced processing costs, could dramatically increase the economic viability of industrial algaculture if marketed to the multi-billion dollar animal nutrition and aquaculture sectors [3,12].

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Dried whole algal biomass (WAB) can contain high levels of oil (generally up to 40%) that may be highly suitable as lipid supplements for animal feeds as a source of essential fatty acids (EFAs) and digestible energy (DE). Depending upon processing methods, the lipid-extracted biomass (LEB) remaining after oil extraction for other purposes (e.g., renewable energy) may contain significant levels of residual oil suitable for use in animal nutrition. Additionally, algal biomass from commercially-established species (e.g., *Chlorella*, *Spirulina*) generally contains all the essential amino acids (EAAs) in proportions more suitable to animal nutrition than many terrestrial plant-based crops in wide use such as corn, soybean, canola and wheat. As such, microalgae are highly attractive sources of essential nutrients and calories for animal feed applications [1,13]. Given good protein and lipid yields (both quantity and quality) of some algal species, good market potential and market price predictions, algal biomass could fall into the mid-value feed ingredient commodity sector (>$500 t^{-1}) presently dominated by terrestrial oilseed crops (e.g., soy, canola, corn) and could be sought after by the monogastric animal feed sector (e.g., poultry, swine, fish). Alternatively, if algal biomass is predominantly carbohydrate-rich, it will have a reduced market value similar to that of wheat and other cereal grain by-products (<$500 t^{-1}) and may be a better fit for the ruminant animal feed sector (e.g., cattle, sheep, deer). However, if it can be demonstrated that algal products have 'functional ingredient' properties (e.g., high response at low dose) their economic value may be considerably increased; provided that significant production tonnage can be economically achieved. In fact, it has been proposed that algal biomass could have certain market advantage over other terrestrial crops in terms of input costs (e.g. lower aerial foot-print, wastewater mitigation), carbon credits (e.g., industrial CO_2 conversion), nutritional value (e.g., more favorable EAA and EFA profiles) and raw ingredient sustainability [15]. As such, it is not surprising that algal products/co-products resulting from biofuel applications have been identified in Canada and elsewhere as a priority for further investigation as valuable commodities for sustainable development of terrestrial livestock and aquaculture feed inputs [16,17]. However, the nutritional content of algal biomass is poorly defined and for most species, including well-studied species like *Chlorella*, there is little consensus on their biochemical composition between and within species/strains and data on known and not-so-well known species isolated in Northern climates has not been reported, especially for LEB.

The present study is the first in a series of projects designed to evaluate the nutritional value of algal biomass produced from four freshwater species isolated in Northern Alberta, Canada for animal feed applications. Through an extensive microalgae isolation and screening program [18], these species have been identified as the most promising candidates for industrial carbon conversion in Northern climates, based on the following criteria: (1) freshwater and naturally-occurring in Alberta; (2) demonstrated high growth rate and esterifiable lipid productivity potential at flask level and (3) the inoculum was tolerant of simulated flue gas and grew well on treated wastewater at flask level. As such, they were mass cultivated in artificially illuminated 1000 L enclosed photobioreactors to produce sufficient biomass quantities for nutritional evaluation. The main objective of the present study was to generate novel primary biochemical composition data on whole algal biomass (WAB) and lipid-extracted biomass (LEB) including proximate, amino acid, fatty acid, carotenoid and elemental composition.

2. Materials and methods

2.1. Microalgae cultivation

This study investigated four microalgae species isolated from freshwater samples in Alberta, Canada [18] (Table 1). The isolates were identified by DNA sequence analysis of 18S, ITS1 and ITS2 regions (Eurofins Genomics, Huntsville, AL, USA) [19]. Species included *Chlorella vulgaris*, *Nannochloris bacillaris*, *Tetracystis* sp. and *Micractinium reisseri*. Unicellular cultures from our Canadian algae species collection were maintained immobilized on agar slants with standard Bold's basal medium [20] at 18–20 °C in continuous low light (50–75 μmol photons m^{-2} s^{-1} photosynthetically active radiation [PAR]). These algal library stocks are turned over onto fresh agar media on a monthly basis. Starter cultures were grown for one week on filter-sterilized (0.22 μm) f/2 media [20] in 250 mL flasks at 25 °C under 142 μmol photons m^{-2} s^{-1} PAR and agitated at 175 rpm on an orbital shaker. Aliquots of the flask cultures were diluted into 19 L carboys containing filtered (0.35 μm), pasteurized (85 °C for 6 h) and UV-sterilized freshwater. Filter-sterilized f/2 media was added to the carboys and cultivation was carried out at 22 °C and 100 μmol photons m^{-2} s^{-1} PAR for an additional week under aeration with filter-sterilized air. Final cultivation occurred in duplicate, proprietary enclosed 'Brite-Box' photobioreactors (PBRs) [21]. These are 1000 L internally illuminated PBRs enclosed by a fiberglass shell. Continuous light (250 μmol photons m^{-2} s^{-1} PAR) was provided by forty, F32T8/TL765 PLUS (32 W) fluorescent bulbs (Alto^{II}™ Technology, Philips Lighting, Markham, ON, Canada) arranged in eight horizontal rows of five. Approximately 980 L of membrane ultrafiltered (500 kDa nominal cut-off size) and UV-sterilized freshwater and filter-sterilized f/2 media were added to each PBR. Cultures were inoculated with microalgae from carboy cultures to an initial cell density of 45×10^4 cells mL^{-1} and maintained for 9–12 days at 22 °C with titanium heat-exchange cooling loops. Automated on-demand CO_2 injections maintained cultures at pH 7.0 and mixing was provided by aeration with a turbulent flow of sterilized air introduced into the cultures through perforations in two T-shaped air-lines situated medially at the bottom of the PBR. Cell densities were monitored daily, using a particle multi-sizer equipped with a 100 μm aperture tube (model MS3, Beckman-Coulter Inc., Miami, FL, USA). Growth rates (d^{-1}) were calculated as $\mu = ([\ln\{t_2\} - \ln\{t_1\}] / [t_2 - t_1])$ and biomass productivity as the product of the growth rate (μ) and the standing biomass density at time *t*.

Table 1
Microalgae species isolated from freshwater in Alberta, Canada[a].

Species	ID	Origin	Location
Chlorella vulgaris	AB02-C-U-BBM	Sylvan Lake	N52°18.867/W114°05.721
Nannochloris bacillaris	AB03-C-F-PLM	Athabasca River	N54°43.555/W113°17.109
Tetracystis sp.	AB04-C-F-PLM02	Pigeon Lake	N52°58.776/W114°02.151
Micractinium reisseri	AB05-C-U-BBM02	Gregoire Lake	N56°29.239/W111°10.833

[a] Stocks maintained immobilized on BBM agar media (18–20 °C; 50–75 μmol $m^{-2}s^{-1}$ light) in our Canadian algae species collection (Conviron environmental chamber, model PGR15, Winnipeg, MB, Canada).

2.2. Harvest and sample preparation

Cultures were harvested 6 days into stationary phase using a process centrifuge (model Z101, CEPA Carl Padberg Zentrifugenbau GmbH., Lahr, Germany) equipped with a 10 L collection chamber at 15,760 ×*g* and immediately frozen at −20 °C. Frozen paste (~25% solids) was lyophilized for 72 h at a low shelf temperature (<5 °C) in a large-capacity freeze-dryer (model 35EL, The Virtis Company, Gardiner, NY, USA) to a final moisture content of 3–4%. Freeze-dried whole algal biomass (WAB) was pulverized (to pass through a 0.5 mm screen) at 10,000 rpm using a laboratory ultra-centrifugal mill (Retsch model ZM200, Retsch GmbH., Haan, Germany) equipped with a pneumatic auto-feeder (Retsch model DR100) and stored at −80 °C. Lipid-extracted biomass (LEB) was produced by solvent extraction of WAB on a Soxtec™ automated system (model 2050, FOSS North America, Eden Prairie, MN, USA) using HPLC-grade $CHCl_3$:CH_3OH (2:1 v/v) at 150 °C for 82 min [22]. Residual solvents and moisture were eliminated

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