Algal Research 16 (2016) 141–149

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Effect of temperature and nitrogen concentration on lipid productivity and fatty acid composition in three *Chlorella* strains

Vince Ördög [a,b], Wendy A. Stirk [b,*], Péter Bálint [a], Adeyemi O. Aremu [b], Ambrose Okem [b], Csaba Lovász [c], Zoltán Molnár [a], Johannes van Staden [b]

[a] Institute of Plant Biology, Faculty of Agricultural and Food Sciences, University of West Hungary, 9200 Mosonmagyaróvár, Hungary
[b] Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, P/Bag X01, Scottsville 3209, South Africa
[c] Central Agricultural Office, Food and Feed Safety Directorate, Feed Investigation National Reference Laboratory, Remény Street 42, 1114 Budapest, Hungary

ARTICLE INFO

Article history:
Received 21 July 2015
Received in revised form 26 February 2016
Accepted 1 March 2016
Available online xxxx

Keywords:
Carbohydrates
Fatty acids
Lipids
Nitrogen-deprivation
Proteins
Temperature

ABSTRACT

The effects of temperature and nitrogen (N) on lipid productivity and fatty acid composition in three *Chlorella* strains grown at 20 °C, 25 °C and 30 °C in modified Tamiya media with low (7 and 21 mg L^{-1} N) and moderate (70 mg L^{-1} N) N were investigated. Temperature and N influenced biomass accumulation with the largest biomass accumulation (1697, 1732 and 1809 mg DW L^{-1} for the three strains) at higher temperatures and N concentrations. Proteins decreased and lipids increased over time with N-deprivation. Strain, temperature and N concentration influenced lipid productivity with the highest productivity in cultures grown in 3% N at higher temperatures (68, 70 and 90 mg lipid L^{-1} day^{-1} for the three strains). The fatty acid methyl ester (FAME) profile was similar in the three strains with C16:0 > C18:1n9c > unidentified FAMEs > C18:2n6c > C18:3n3 > C18:0 comprising 90% of the total FAME. C18:1n9c and C16:0 showed the largest variation in response to culture conditions in *Chlorella* sp. MACC-438 (0.2–26.8% and 2.3–16.2% respectively) and *C. minutissima* MACC-452 (0.2–26.7% and 3.0–23.3% respectively); unidentified FAMEs (1.1–9.7%) and C18:2n6c (0.9–11.2%) were the most variable in *Chlorella* sp. MACC-728. Lower temperatures resulted in higher % FAME in *Chlorella* sp. MACC-438 (68.9%) and *C. minutissima* MACC-452 (91.7%). Nitrogen concentration had more influence on the specific FAME content compared to temperature. Thus, temperature tolerance is important when selecting strains to ensure high lipid productivity while the specific strains response to N-deprivation is important to ensure quality of the biofuel feedstock.

1. Introduction

Owing to limited fossil fuel reserves, alternative sources of renewable energy are being investigated. Oleaginous microalgae are able to accumulate high amounts of reserve lipids in the form of triacylglycerides (TAGs) when cultured in growth limiting conditions. TAGs are a suitable substrate for biodiesel production as well as a source of various long chain fatty acids such as C20:4 (arachidonic acid), C20:5 (eicosapentaenoic acid) and C22:6n3 (docosahexaenoic acid). These are valuable bioactive compounds for human and animal use [1,2]. Microalgal species, especially those grown in outdoor cultivation systems need a wide tolerance to environmental conditions. Geographical location and climatic conditions, especially temperature and solar radiation are the main environmental factors affecting lipid productivity [3,4].

Microalgae strains suitable for commercialization should have a fast growth rate and high lipid content. Promising strains have been identified from the genera *Chlorella*, *Nannochloropsis*, *Neochloris* and *Scenedesmus* [3,5]. Most commonly, nitrogen (N) deprivation is applied as the growth limiting factor as it causes a significant increase in the lipid content of many microalgae, is cost effective and is one of the more easily adjustable factors in both open and closed systems [6]. A survey of the available literature calculated the average lipid content in a variety of microalgal cultures grown in N-sufficient conditions as 23% dry weight (DW), which increased in N-limiting conditions to 41% DW [7]. However, fast growth rates (achieved in optimal growth conditions) and high lipid content (stimulated by growth limiting conditions) are mutually exclusive with high lipid yield often accelerating with the onset of the stationary growth phase in batch cultures [6,8].

While many studies have reported on ways to optimize microalgal biomass and lipid productivity, there are fewer reports on the quality of the lipids produced [reviewed in 6,7]. Commercialization of biodiesel has led to the formulation of specific biodiesel standards and guidelines

Abbreviations: FAME, Fatty acid methyl ester; HCA, Hierarchical Cluster Analysis; MACC, Mosonmagyaróvár Algal Culture Collection; MUFA, Monounsaturated fatty acid; PUFA, Polyunsaturated fatty acid; SFA, Saturated fatty acid; TAGs, Triacylglycerides.

* Corresponding author.
E-mail address: stirk@ukzn.ac.za (W.A. Stirk).

http://dx.doi.org/10.1016/j.algal.2016.03.001
2211-9264/

with the properties of biodiesel directly determined by the fatty acid composition of the microalgal feedstock [6,9]. The two most important factors are i) the length of the carbon chain with the long chain C16-C18 TAGs being the best substrate for biodiesel production and ii) the number of double bonds which affect other biodiesel properties such as cetane number (ignition quality), cold-flow properties, viscosity and oxidative stability [9]. To meet the specific technical biodiesel standards and to achieve the best balance between these properties, the most desirable TAGs are those containing the monounsaturated fatty acids (MUFAs) C18:1 (oleic acid) and C16:1 (palmitoleic acid). High concentrations of saturated fatty acids (SFAs) negatively affect the cold-flow properties and the polyunsaturated fatty acid (PUFA) C18:3 (α-linolenic acid) content is required to be below 12% as higher concentrations result in poor oxidative stability [9]. Thus the fatty acid composition is important in determining the quality of the microalgal feedstock for biodiesel production.

While the fatty acid composition in microalgae can be used as a taxonomic biomarker at the generic level, e.g., separating a true *Chlorella* clade from the *Parachlorella* clade in indigenous *Chlorella* strains from Malaysia [10], environmental variables can also alter the fatty acid composition of a species [6,9]. Physical cultivation parameters such as temperature and light intensity as well as media characteristics such as the source and concentration of N, salinity and pH influence the fatty acid composition and hence both the quantity and quality of lipids produced [11]. For example, when the arachidonic acid- producing *Parietochloris incisa* (Trebouxiophyceae) was grown in high light conditions to facilitate rapid growth, there was a low C20:4 content, while N-deprivation increased the C20:4 content in proportion to the total fatty acid content, allowing the cultures to be manipulated to achieve a higher C20:4 content [2].

Lipid production of specific strains in different culture conditions is required to predict overall lipid productivity in outdoor systems [4]. By investigating the relationship between fatty acid composition and environmental factors in microalgae, suitable strains can be selected to take into account the effect of uncontrollable environmental changes such as seasonal trends. As temperature is one of the main environmental factors, which influence fatty acid profiles in microalgae [12], the aim of the present study was to investigate the effects of temperature and N concentrations in three fast growing, oleaginous *Chlorella* strains. Growth, protein, carbohydrate, lipid content, and fatty acid composition were quantified, and lipid productivity calculated. These results should provide insight on lipid productivity and fatty acid composition in microalgae in response to variable ambient temperatures.

2. Materials and methods

2.1. Microalgal cultures and growth conditions

Three fast growing axenic *Chlorella* strains – namely *Chlorella* sp. MACC-438, *Chlorella minutissima* MACC-452 and *Chlorella* sp. MACC-728 isolated from soil samples collected in Brazil, were selected from the Mosonmagyaróvár Algal Culture Collection (MACC), Hungary. Culture suspensions were initiated from agar–agar stock cultures and grown in modified Tamiya medium [13,14] where the nitrogen concentration in the media was reduced from 700 mg L^{-1} N (100% N) to 70 mg L^{-1} N (10% N). This nitrogen concentration was selected for the present study as previous experiments using the same culture apparatus and *C. minutissima* MACC-452 showed that the highest lipid productivity was obtained with 10% N [14]. Culture conditions were 25 ± 2 °C, 14:10 h light:dark photoperiod and illumination from below at 130 μmol m^{-2} s^{-1}. Cultures were aerated with 20 L h^{-1} 1.5% CO_2-enriched sterile humidified air during the light periods.

After 7 days, cultures of each strain were inoculated into flasks containing 250 mL modified Tamiya medium (10% N) to give an algal starting density of 10 mg L^{-1} DW. After 7 days, cultures were re-inoculated in the same way to obtain sufficient inoculum for the experiment. For the experiment, the Tamiya medium was modified to give two low nitrogen concentrations – 7 and 21 mg L^{-1} N (1% and 3% N respectively of the total N content in Tamiya media) and a moderate nitrogen concentration – 70 mg L^{-1} N (10% N). The flasks were inoculated to give an algal starting density of 10 mg L^{-1}. These cultures were grown at three temperatures, namely 20 °C, 25 °C and 30 °C. For each algal strain and treatment, 18 and 10 flasks were harvested on day 3 and day 6 respectively. Samples were taken from three randomly selected flasks for biomass quantification. Thereafter, the biomass from all the flasks was combined to give a single sample for each strain and for each sampling time (days 3 and 6) to ensure sufficient biomass for the biochemical analysis. Samples were lyophilized and stored at −70 °C until analysis.

2.2. Dry weight quantification

Samples (5–20 mL) were filtered and dried as previously described [14] and the density of the suspension cultures calculated as mg DW L^{-1}. As all cultures had the same initial biomass (10 mg L^{-1} DW), increase in biomass accumulation was used as a measure of growth. There were three replicates per treatment. Three-way analysis of variance (ANOVA) comparing the effect and interaction of temperature, N concentrations and time of harvest was performed on the biomass data. Statistical computations were done using SPSS for Windows (SPSS®, version 10.0 Chicago, USA).

2.3. Estimation of protein content

The N content of the microalgae samples (250 mg DW) was determined using a Kjeldahl method and the protein content calculated from the N content as previously described [14]. Results were expressed as g protein 100 g^{-1} DW (%).

2.4. Carbohydrate determination

The carbohydrate content of the samples was measured using a method based on the MSZ 6830/26 Hungarian Standard (Determination of feed nutritive value: Determination of sugar content). Microalgal samples (150–500 mg DW) were extracted in 50 mL hot (50 °C) deionized water after which 5 mL HCl (37% w/w) was added and the flasks heated to 100 °C for 45 min. During this extraction, the flasks were gently shaken on a horizontal shaker. The extracts were cooled and clarified with the addition of 5–10 mL Carrez I and Carrez II solutions (see above MSZ 6830/26 Standard Method) and deionized water. After 15 min, the solutions were filtered on filter paper. The glucose content of the filtrate was determined by the Luff-Schoorl method with titration (see above MSZ 6830/26 Standard Method). Various amounts of filtrate (5–25 mL) made up to volume with deionized water and a blank prepared with 25 mL deionized water were prepared and 2 mL NaOH (20% w/w) and 25 mL Luff-Schoorl reagent solution added. The solutions were heated to reflux and refluxed for an additional 10 min. The reagent mixture was cooled with cold water and 10 mL potassium iodide solution (30% w/w) added, followed by 25 mL 3 M sulfuric acid. The mixture was titrated with 0.1 M sodium thiosulfate solution with the addition of starch solution as an indicator. Based on the titration, the carbohydrate content in the microalgal samples was calculated.

2.5. Lipid determination and productivity

Lipids were quantified using two parallel samples (150 mg DW) that were hydrolyzed with 3 M HCl at 95–100 °C and then extracted using methanol, hexane and diethyl ether as previously described [14]. The resulting extract was dried and weighed as a measure of lipid yield that was expressed as g lipid 100 g^{-1} DW (%). Lipid productivity on day 3 and day 6 was calculated using the DW and lipid content as previously described [14].