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# Analytical evaluation of different carbon sources and growth stimulators on the biomass and lipid production of *Chlorella vulgaris* – Implications for biofuels

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ABSTRACT

The key challenges in lipid production from marine microalgae include the selection of appropriate strain, optimization of the culture conditions and enhancement of biolipid yield. This study is aimed at evaluating the optimal harvest time and effect of chlorella growth factor (CGF) extract, carbon sources and phytohormones on the biomass and lipid production in *Chlorella vulgaris*. CGF, extracted using hot water from *Chlorella* has been reported to possess various medicinal properties. However, in the present study, for the first time in *C. vulgaris*, CGF was found as a best growth stimulator by enhancing the biomass level (1.208 kg $m^{-3}$) significantly on day 5. Gibberellin and citrate augmented the biomass by 0.935 kg $m^{-3}$ and 1.025 kg $m^{-3}$. Combination of CGF and phytohormones were more effective than CGF and carbon sources. Analysis of fatty acid methyl esters indicated that the ratio of saturated to unsaturated fatty acids is higher in cytokinin, abscisic acid and CGF, and are also rich in short chain carbon atoms, ideal criteria for biodiesel. Nitrogen starvation favoured synthesis of more unsaturated fatty acids than saturated. This study shows that CGF enhances the biomass and lipid significantly and thus can be used for large scale biomass production.



*Abbreviations:* CGF, chlorella growth factor; SFA, saturated fatty acids; UFA, unsaturated fatty acids.

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## 1. Introduction

Heterotrophic and mixotrophic culture conditions have shown significant increase in microalgal biomass and lipid production earlier [1,2]. It was also reported previously that the addition of phytohormones to *Chlamydomonas reinhardtii* increased the growth rate and lipid production in the organism [3]. Recent data concerning the pathways of phytohormone biosynthesis suggest their connection with chloroplasts and plastids [4,5]. Thus, it can be hypothesized that phytohormones, whose synthesis and functioning is immediately connected with plastids, should display their regulatory activity in algae as well. At present, our knowledge on the algal hormonal system is still rather fragmentary [6,7]; although previous reports shows that the addition of phytohormones to *Chlamydomonas reinhardtii* increased the growth rate and lipid production [3,8]. Nevertheless, the roles of phytohormones on the biomass and lipid production in *Chlorella vulgaris* (*C. vulgaris*), have not been reported elsewhere, which forms one of the major aim of this study.

Nutrient availability, on the other hand, has a significant impact on growth and propagation of microalgae as well as on their lipid and fatty acid composition. In general, when algal growth reduces and there is no requirement for the synthesis of any additional membrane compounds, the cells instead divert and deposit fatty acids [9–12]. However, under nitrogen depletion condition, the growth rate was very low leading to higher lipid productivity. Therefore, increasing the biomass level is one of the serious issues that require major attention, which is also an important objective of this study.

Most interestingly, a physiologically activating substance which accelerates the growth and reproduction of chlorella cells called the chlorella growth factor (CGF) derived from *Chlorella* sp. was found to possess wide pharmacological properties [13,14]. In the 1950s, the People's Scientific Research Centre in Tokyo isolated a substance from hot water extract of chlorella by electrophoresis and found that the extract promoted the healthy growth of children as well as young animals. Several lines of evidence have earlier documented that CGF enhances immune function and possess potent anti-tumour effect, which is currently in the clinical trial [15–18]. Importantly, it has been reported that CGF consists of nucleic acid derivatives, including RNA (up to 10%) and DNA (up to 3%), which govern cell growth, reproduction, and repair. It is produced during intense photosynthesis, and is mainly responsible for the reproduction of the chlorella cells [19]. This prompted us to examine whether exogeneous supplementation of hot water extract of chlorella (crude CGF extract) would have any effect on the growth and biolipid content of *C. vulgaris*. In addition, an attempt was also made to explore and evaluate the effect of crude CGF, phytohormones and carbon sources on the biomass and lipid yield of *C. vulgaris* along with the combinatorial effect of CGF with carbon sources and phytohormones in the present study, and thereby to assess for its biodiesel production efficiency.

## 2. Experimental

### 2.1. Strain and culture conditions

Seawater samples (1000 $cm^3$) were collected from Pondicherry coast (11° 54′ 27.54″ N; 79° 49′ 35.70″ E), filtered in Whatman filter paper (0.2 μm) and plated in nutrient rich agar plates. Single colonies were picked up, monocultures were developed and stored in agar slant. One of the isolates that was identified as *C. vulgaris* by standard microscopic observation, physiological and biochemical methods (pH, temperature, salinity, nitrate reduction, starch hydrolylsis and mannitol utilization properties) and partial sequencing of 18S rRNA (NCBI, Accession Number: JF894249) was used for the study. This strain has been deposited in a public repository domain at National Facility for Marine Cyanobacteria, Bharathidasan University, India, under the accession number, BDU GD 003. The culture was maintained in $f^2$medium at 297.15 K, 24 h light periods with a light intensity of 200–400 μmol $m^{-2}$ $s^{-1}$.

### 2.2. Extraction of crude CGF

Crude CGF extract was prepared from *C. vulgaris* using the hot water extraction method, as described [17]. The cells during the exponential phase were harvested by centrifugation at relative centrifugal force (RCF) of 4000 g for 10 min, washed once with sterile filtered (0.2 μm filter) seawater. The cells were dried using a freeze dryer (Benchtop "K" Manifold Freeze Dryer, Virtis), and to about 10 g of freeze dried biomass, 100 $cm^3$ of nuclease free water was added. The algal suspension was then boiled at 373.15 K for 20 min followed by centrifugation at 10,000 g for 20 min. The supernatant, reported to contain CGF was used for the study [14,19].

### 2.3. Experimental setup for mixotrophic culture

Experiments were conducted in triplicates in 1 L conical flask with 500 $cm^3$ of culture. Groups I and II served as control except that Group II was subjected to nitrogen starvation after 7th day. Group III was given 5 $cm^3$ of crude CGF extract. Groups IV, V, VI and VII were given 1 g $m^{-3}$ of plant growth hormones such as auxins, cytokinin, gibberellin and abscisic acid, respectively. Groups VIII, IX and X were supplemented with 1 kg $m^{-3}$ glucose, 50 g $m^{-3}$ sodium citrate and 50 g $m^{-3}$ sodium acetate. Groups XI and XII were the combination groups, wherein Group XI was given CGF, glucose, citrate and acetate, while Group XII was given CGF, auxins, cytokinin, gibberellin and abscisic acid, respectively at the above mentioned concentrations. The combination groups were included basically with an idea to figure out to which combination of sources does the CGF has the maximum efficacy on biomass and lipid, i.e. either with carbon sources (Glucose, citrate and acetate) or with phytohormones (Auxins, cytokinin, gibberellins and abscisic acid). After addition of respective concentrations of carbon sources and phytohormones into axenic algal culture at exponential growth phase (6–9 day of the culture), 50 $cm^3$ of inoculums