Microalgal Biodiesel Production

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Introduction

Fossil fuel reserves are diminishing.[1] At the current consumption rate, estimated oil and natural gas reserves will be exhausted in 40 and 64 years, respectively. Biodiesel is a renewable fuel that can be produced from vegetable oil and waste processing oils.[2] Commercially, it is produced from oil-crops such as rapeseed and soybean.[3] Alternatively, microalgae can produce higher oil content than oil-crops and are also photosynthetically more efficient.[4] Although production of biodiesel from microalgae is technically possible, it is not economically competitive with petroleum diesel due to high cost of cultivation and downstream processing.[4] This seminar will focus on reducing the costs associated with cultivation and downstream processing.[5]

Common downstream processing methods involve extraction of lipids from the biomass followed by fatty acid derivatization and purification to yield fatty acid methyl esters (i.e. FAME or biodiesel). Separate extraction and derivatization processes are time-consuming and utilize large volumes of solvents which make downstream processing expensive. Alternatively, fatty acids can be converted into biodiesel in situ and extracted simultaneously to reduce the length and cost of the procedure.[6] In this alternate method, biodiesel is produced through esterification of fatty acids by a two step reaction involving basic and acidic catalysts in the presence of methanol followed by extraction of the biodiesel in situ. Efficiency of this method depends on the following factors: catalyst type (sodium methoxide, sulfuric acid), use of non-polar solvents, reaction temperature and duration of reaction, amount of solvents used for extraction, ability of solvents to penetrate into the cells and dissolve the lipids, biomass amount, and type of the lipids found in biomass.[6]

In this study, we investigated strategies for reducing costs associated with cultivation of microalgae and with downstream processing. We present the effect of nitrogen and carbon source on improving growth and lipid biosynthesis rate from the green microalgae Chlorella vulgaris. In addition, the above conditions for optimizing the in situ transesterification/extraction method were investigated.

Experimental Methods

A) Culture Maintenance and Effect of Nutrients on Biomass and Lipid Production:
Chlorella vulgaris (UTEX# 259) was obtained from the Culture Collection of Algae at the University of Texas, Austin. For maintenance, cells were grown in Bold’s Basal Medium (BBM),[7] maintained at 26±2°C, illuminated at a light intensity of 350 – 450 µEm²s⁻¹ with 16 hours light/8 hours dark cycle, and shaken at 110 rpm on an orbital shaker. To assess nutrient limitations on fatty acid biosynthesis, C. vulgaris cells were cultivated under the following medium conditions: 1) nitrogen-deficient versus nitrogen-rich conditions: BBM, modified BBM containing 3-fold higher sodium nitrate concentrations (3N-BBM), and diluted BBM containing half the nutrients present in the BBM (1/2xBBM), 2) organic versus inorganic carbon source: BBM supplemented by 10 g/L glucose or BBM supplemented with 2 % CO₂. Cultures were grown for 25 days, sampled every 4 to 5 days, and harvested 2 to 4 days for biomass measurement and fatty acid analysis. All measurements were performed with two biological replicates.

B) Optimization of Lipid Extraction and Analysis Method:
Optimum conditions for extracting fatty acids using the two step transesterification reactions with in situ extraction were investigated. The biomass for these studies was C. vulgaris cells grown in 1/2XBBM for 25 days to obtain high lipid-containing biomass. Fatty acids were chemically derivatized into methyl esters with freeze-dried biomass (~25 mg) by varying the following parameters: 1) basic catalyst concentration (0.5 or 1 N methanolic sodium methoxide), 2) basic reaction temperatures (25, 60 or 80°C), 3) duration of basic reaction (10, 60 or 90 min), 3) duration of the acidic reaction at 60°C (30 or 60 min), and 4) hexane volume used in the extraction step (2, 3, 5 or 7 mL). Furthermore, toluene, chloroform or hexane was added to change polarity of the reaction medium prior to reactions to facilitate extraction of non-polar lipids by increasing their solubility. The internal standard for the quantification of fatty acids was heptadecanoic acid (C17:0; 20mg/mL in hexane solution) which was added to the biomass prior to derivatization reaction. The hexane layer containing the
FAMEs were analyzed with GC-MS (HP 6890) equipped with 30m DB-WAX (J&W, Agilent) capillary column (0.25mm ID and 0.25um film thickness). All measurements were performed in three replicates.

Results and Discussion

A) Effect of Nutrients on Growth and Lipid Production Rate:
C. vulgaris cultures were grown in 3 different levels of nitrogen. Cells grown in regular BBM and 1/2XBBM produced higher levels of lipids; however, the biomass concentration was significantly less than under nitrogen-rich conditions (3N-BBM; Figures 1a). Since similar growth rates were observed between all 3 different levels of nitrogen, growth rate may be limited by carbon rather than nitrogen availability while nitrogen deprivation is required to increase the lipid content of the cells. To investigate the role of carbon source on growth rate, C. vulgaris cells were grown in BBM, BBM supplemented with 10 g/L glucose, or BBM supplemented with 2% CO₂ in the headspace of the cultures (Figure 1b).

![](image1.png)

Figure 1. Growth of C. vulgaris (a) in high nitrogen content, regular and diluted BBM (b) in regular, 10g/L glucose or 2% CO₂ supplemented BBM

Results showed that both glucose and CO₂ supplemented cultures grew faster and reached a higher cell density than the cells grown in regular BBM (Figure 1b). Preliminary results also showed that glucose supplementation promotes lipid production compared to cells grown photosynthetically.

B) Optimization of FAME Derivitization and Extraction:
Basic transesterification is faster compared to acidic transesterification, however, acidic reaction is required for esterification of free fatty acids. Initially, basic reaction was optimized by varying the concentration of the catalyst, and temperature and duration of the reaction, while the acidic reaction conditions were kept the constant. Increasing the concentration of sodium methoxide, or eliminating the basic reaction step significantly decreased the extraction efficiency of the procedure. The organic solvents, hexane and chloroform used for solubilizing the non-polar lipids did not have a significant affect at the conditions studied, while toluene slightly decreased the extraction efficiency. A basic reaction with 0.5N methanolic sodium methoxide carried out at 60°C for 60 min combined with acidic reaction with 5% methanolic sulfuric acid at 60°C for 30 min resulted in the highest extraction efficiency (430±15 mg/g DW). Increasing the basic reaction time above 60 min did not cause any significant change in reaction efficiency, while short reaction times resulted in incomplete transesterification. When the reaction temperature was increased to 80°C, basic reaction carried out for 10 min resulted in comparable extraction efficiency (424±19 mg/g DW). Increasing the volume of hexane for extraction also did not affect the total amount of FAMEs recovery from microalgal biomass under the conditions studied.