Production of Biodiesel from the Microalgae *Chlorella vulgaris*

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Microalgae produce an alternative renewable source of oil known as biodiesel. In this thesis, *Chlorella vulgaris* was used as a model system to 1) develop and optimize a quick and reliable analytical method to evaluate fatty acid profile and lipid productivity and 2) investigate the effects of nitrate limitation and carbon supplementation and identify potential bottlenecks to biomass and oil production.

In the first aim, an *in situ* transesterification method was optimized where lipid extraction and fatty acids conversion to methyl esters occurred within the cells. Under the optimized conditions, the conversion efficiencies of 40% by dry weight were obtained with sodium methoxide reaction (0.5 N $NaOCH_3$ in methanol, 60˚C, 15 min) followed by sulfuric acid catalysis (5% v/v H_2SO_4 in methanol, 60˚C, 30 min).

One of the most effective strategies for inducing oil accumulation in microalgae is nutrient limitation, particularly nitrate. *C. vulgaris* were cultivated under low (1.47 mM), medium (2.94 mM), and high nitrate levels (8.82 mM) to determine the effect of nitrate on growth and lipid productivity. The highest lipid content (40% by dry weight) and highest lipid productivity (14.3 mg/L-day) were obtained with low nitrate in spite of the decreased biomass productivity.

Carbon supplementation (2% CO_2 or 10 g/L glucose) increased specific growth rate and biomass and lipid productivities. Similar lipid contents (~42%) were achieved in CO_2 and glucose-supplemented cultures; however, oil production rate in CO_2-supplemented cells was half the rate in glucose-supplemented cells, suggesting that lipid production in CO_2-supplemented cells was limited by CO_2 fixation or glucose biosynthesis rates. Although carbon-supplemented cultures reached similar cell densities and cellular oil content, the glucose-supplemented cultures reached higher ***dry*** weight concentrations, suggesting that the availability of non-limiting glucose levels in the cell diverted carbon towards the synthesis of other storage compounds like starch. Carbon supplementation resulted in similar high lipid content as *C. vulgaris* grown under low nitrate conditions ***and*** in higher lipid productivities (70.5 mg/L-day) due to increased biomass growth.

Our results suggest that the limitation to biomass and oil production is at the level of *de novo* CO_2 fixation during photosynthesis and suggests a target that can be enhanced by metabolic engineering strategies.