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Purpose

This working paper describes an experimental program to assess the suitability of large-scale separation processing of algal biomass in aqueous culture. It assumes the possibility of successful large-scale growth of heterotrophic *Chlorella protothecoides* to produce algal lipid/free fatty acid (FFA) biodiesel precursors.

Introduction

Renewable fuels such as ethanol and biodiesel are increasingly important for transportation. Farm crops to produce plant-derived ethanol or biodiesel precursor oils require arable land in a suitable climate, irrigation or sufficient natural precipitation, farm labor and equipment, fertilizer, and pest and weed control to secure crop yield.[1] These resources are not uniformly distributed in the U.S. or the world at large, and food production competes for them, with economic implications for agriculture prices. A potential option to autotrophic photosynthesis of biofuel precursors is large-scale culture of heterotrophic biomass using abiotically synthesized reduced carbon.

The intensive culture of photosynthetic microalgae in ponds or photobioreactors does not require arable land and associated farming costs and materials. Such cultures can consume CO_2 and biomass wastes as nutrients.[2-4] The ultimate energy yield limit of autotrophic production is governed by the light energy input in the photosynthetic spectrum and the biochemical conversion efficiency, assuming no other nutrient or process limitation. To avoid these limitations of photosynthesis, heterotrophic culture of the microalga *Chlorella protothecoides* has been studied for algal lipid/FFA biodiesel precursor producution.[5] If suitable reduced carbon food can be synthesized abiotically from CO_2 or waste water, agricultural resource consumption will be minimized, with reduced net carbon emissions as a result.

Large-scale industrial bioreactors for heterotrophic microbes are used for production of commercial products such as antibiotics and enzymes. Bioreactor volumes over 100 m^3 are routine, with separation and purification operations to scale. In the late 1970's, ICI, Ltd. produced genetically-engineered single cell protein in a 1500 m^3 heterotrophic bioreactor.[6] Production of algal biofuel precursors at this scale is likely necessary for significant market replacement of fossil fuels.

Algal Lipid/FFAs from *Chlorella protothecoides* Bioreactor Production

A rough estimate of the production potential of *C. protothecoides* may be derived from laboratory *Chlorella* culture optimization studies. High density production of *C. pyrenoidosa* was optimized for biomass yield with a hybrid neural network in a 19L batch culture over 5 days, producing a maximum of 116 g L^{-1} dry cell weight; 250 mL flask culture prior to optimization produced 20 g L^{-1} maximum biomass. Protein content of the growth-maximized *C. pyrenoidosa* culture was about 30%.[7] This is in contrast to the reported 10.3% protein content of heterotrophic *C. protothecoides* in culture, with lipid/FFA content of 54.7% and moisture content of 5.4%.[8] Another report states *C. protothecoides* flask biomass production of 15.5 g L^{-1} for 55.2% lipid/FFA content.[5] Whether the latter two *C. protothecoides* cultures were optimized for biomass growth and lipid/FFA production using the HNN is not reported.

The production capacity for the ICI bioreactor was claimed to be 50 – 60 dry kt per year, or conservatively 50 x 10^6 kg yr^{-1}, assuming metric ton as the unit of measure implied.[6] The plant was authorized £40M (1976) for its construction. As a maximum upper bound for large scale *C. protothecoides* lipid/FFA production in the ICI-scale vessel, assume a 50% lipid/FFA content at a biomass level of 116 g L^{-1} for a 5-day batch process over 350 production days at 95% recovery efficiency. Under these assumptions, the annual lipid/FFA production of 5.8 x 10^6 kg per bioreactor unit is derived, or 1.8 x 10^6 gal. This means that the reactor produces 4.5 times its volume annually in lipid/FFA for conversion to biodiesel fuel. Alcohols may be produced from cell waste fermentation for more complete algal biomass utilization.

The NREL report[2] estimates that 1 quad (10^{15} Btu) of biodiesel could be produced annually from 772 mi^2 of outdoor algae ponds, or about 15,000 gal ac^{-1} yr^{-1}. A 5 ac bioreactor facility is estimated to produce 360,000 gal ac^{-1} yr^{-1} of biodiesel precursor. This analysis suggests that heterotrophic culture of microalgae may produce significantly higher quantities of biodiesel precursor per unit volume of aqueous culture or land used, with the added benefit of insensitivity to seasonal climate and sunlight variations.

General Separation Process Considerations

Consider a two-stage batch growth and harvest process for *C. protothecoides* biomass under optimal nutrient conditions to near maximum cell density. This is followed by nitrogen nutrient deprivation to induce lipid/FFA synthesis. Process and product conditions are given in Table 1. (Values for cell density in the lipid/FFA-rich stage and the cell mean diameter are estimated.) Note that these two values support calculations of separations efficiency, and a priority for separations process development is determination of these values at the lipid-rich harvest stage.[9]

Process Parameter	Value
Reactor process volume	1500 m^3
Cell mean diameter[9]	20 - 40 mm (est.)
Batch cycle time	5 days
Cell specific gravity	unknown
Biomass dry weight yield	116 g L^{-1}
Lipid/FFA fraction of biomass	0.5
Total biomass to process	1.7 x 10^5 kg
Total lipid/FFA to recover	8.7 x 10^4 kg
Lipid/FFA volume, r = 0.85	~ 100 m^3
pH	6.5
Temperature	28 C
Dissolved O_2 saturation	50%
Annual lipid/FFA yield at 0.95 recovery efficiency	5.8 x 10^6 kg 1.8 x 10^6 gal 43,000 bbl

Table 1. Estimated full-scale bioreactor process conditions, approximate

An advanced supercritical methanol transesterification (SCMT) reaction is being considered for algal biodiesel synthesis within the Sandia Biofuel Program.[10] The proposed SCMT process will convert both algal lipids and FFAs to biodiesel by identifying a catalyst and reaction conditions for large-scale SCMT at elevated temperature and pressure. Algal oil can contain 40 – 60% free fatty acids. Since the process conditions for the improved SCMT route are not yet determined, a detailed separations process cannot be proposed. However, candidate approaches can be identified for experimentation. These experiments will require pilot scale (20 L or larger) culture of lipid/FFA-rich *C. protothecoides* to generate data on separation characteristics and suitability for SCMT reaction conditions.

Specific separation methods must be considered in the context of the process synthesis route as a whole. There are several possible routes that can rely on feasible combinations of biomass and lipid/FFA separations processes, or omit them entirely (Figure 1).

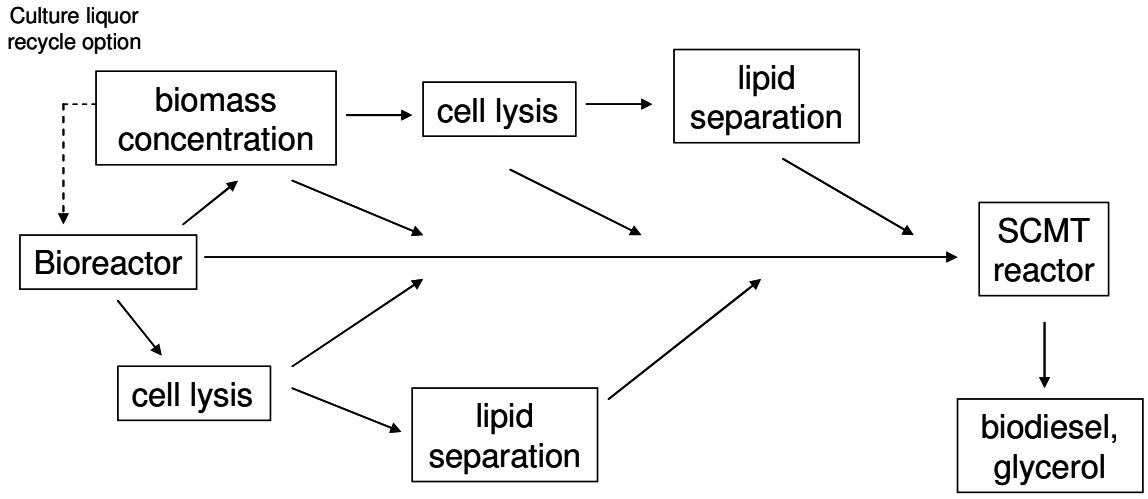

Figure 1. Potential routes to large-scale algal biodiesel synthesis by SCMT

The SCMT reactor feed may be composed of one of the following materials:

1. Raw, unprocessed bioreactor culture liquor
2. Biomass concentrate slurry of whole cells
3. Lysed biomass concentrate slurry of lipid/FFA/FFA, mixed with cell debris
4. Separated lipid/FFA/FFA

Methanol as reactant and supercritical solvent would be added to each feed, increasing volume significantly. Processing times must be very short in comparison to bioreactor cycle times, and metabolism of lipid by algae in process storage must be minimized. The SCMT reaction compatibility with each of these feeds and the relative benefits of recycling culture liquor are critical aspects of the process integration that must be understood through pilot testing and economic analysis. An ideal process sequence would minimize capital equipment and utility needs and the number of separations required prior to SCMT reaction while maximizing lipid/FFA product recovery and ultimate conversion efficiency to biodiesel. A comparison of some relative performance characteristics of SCMT reactor feeds is presented in Table 2; these are potential conditions, subject to evaluation of the SCMT reaction conditions for each feed type.

Reactor Feed Type	Advantages	Disadvantages
Raw culture liquor	No separations costs No cell lysis cost Complete lipid/FFA/FFA use Lysis may occur in SCMT	Volume large, throughput time Heating costs for SCMT No recycle potential No downstream alcohols Reaction inhibition potential
Biomass concentrate	Lower volume Recycle potential Downstream alcohols Lysis may occur in SCMT	Reaction inhibition potential Volume, throughput time
Biomass concentrate, lysed	Lower volume Recycle potential Downstream alcohols	Reaction inhibition potential Volume, throughput time Lipid/FFA separation follows
Separated lipid/FFA	Lowest volume SCMT reaction confident	Separations and cell lysis costs May lose recycle potential May lose downstream alcohols

Table 2. Potential peformance considerations of SCMT reactor feed types

Direct SCMT Processing Routes: In these routes, the biomass is fed to the SCMT reactor without separate cell lysis or lipid/FFA separation steps. The most direct route supplies bioreactor effluent and methanol to the SCMT unit without any separation. This may be possible if the high water content and cell debris are compatible with the SCMT reaction.[11] Costs of heating a large volume of water can be avoided by biomass concentration and moisture reduction. Direct processing routes assume that efficient cell lysis for lipid/FFA release will occur in pumping, methanol mixing, or the SCMT reactor itself. Biomass may be concentrated to a pumpable slurry for SCMT reactor feed, and culture liquor may be recycled as an optional route.

Indirect SCMT Processing Routes: For indirect routes, the biomass requires some level of cell lysis or lipid/FFA separation for efficient SCMT processing. Biomass concentration may be required, but complete drying is not neeeded, as the process has at least a high tolerance for moisture. A pumpable slurry with some moisture content may yield faster SCMT processing.

Candidate Biomass and Precursor Separation Unit Operations

There are a wide variety of methods capable of separating algal biomass from aqueous suspension efficiently at small scales, so process compatibility and economics of energy input, equipment, and raw materials prices will guide selection of full-scale methods. The 1984 SERI literature review on microalgae harvesting and processing summarizes these issues concisely:[12]

> *"There is no unique answer to the question, which of the various methods and technologies of microalgae harvesting would be the most suitable. The decision of the preferable harvesting technology depends on a few variables: algae species, growth medium, algae production, end product and production cost benefit."*

The extensive SERI review is a good reference for understanding the basic physical and chemical processes involved in microalgal separations. The following sections present discussion of separation processes that have been applied to cultivation of microalgae in the context of the proposed large-scale algal lipid/FFA/FFA recovery process.

Separations Economics: The capital equipment and operating costs of separations equipment can be substantial contributors to total biodiesel production costs.[13] If a relatively inefficient separation can be performed quickly with inexpensive equipment, this may be more economical than a highly efficient separation that requires more time and expensive equipment. For example, a flotation or flocculation operation to separate biomass can be conducted in the bioreactor itself. The choice for lipid/FFA separation, if any, must be weighed against the SCMT process requirements of reactant purity and throughput.

Biomass Concentration: Key considerations for large-scale algal biodiesel production are whether recycling of some culture liquor is technically feasible for a given biomass concentration method, and whether the economic savings of nutrient and water recycling justify any level of process risk this introduces. Recycling of some viable biomass may give shorter culture process cycle times, since non-destructive concentration methods should leave a minor (2% - 5%) fraction of algal cells in the clarified liquor for regrowth. Minimization of cell lysis during biomass concentration is desirable, since lipid/FFA can be lost to the aqueous phase. Highly efficient separation processes at laboratory scales may be difficult or uneconomical to employ at large scales, and an optimal process solution involves multidimensional technical and economic trade-off analysis. Multiple concentration operations in series may be needed to meet currently unknown SCMT process compatibility or throughput considerations.

The compatibility of any additive with the SCMT process is an important factor to weigh. In conventional water treatment, inorganic coagulants or organic flocculant aids may be added to promote formation of larger particle aggregates for rapid biomass sedimentation. Changes in solution pH and ionic strength may also promote flocculation and settling. Small-scale jar tests are standardized methods for evaluating additive performance and

energy requirements for mixing as well as evaluation of incidental cell lysis. Other biomass concentration methods used for algal and cell product recovery are dispersed or dissolved air floation (DAF) or sedimentation, with or without flocculant aids.[12, 19] The bioreactor itself may be used as the separations unit in these methods if suitable for the product recovered.[6] Chemical aids can be selected for suitability for further use of remainder protein and carbohydrate cell fractions for animal feed or fermentation to alcohol fuels. Many different filtration approaches are available, but vary in suitability and performance. High-speed disk centrifugation was recommended by a key NREL scientist for full-scale process modeling of algal biodiesel production, based on Israeli studies of open pond harvesting.[2, 14] Some cell lysis and lipid/FFA release during centrifugation is a potential risk of this approach that should be balanced against advantages of speed, efficiency, and reproducibility. Acoustically-induced aggregation and rapid sedimentation was effective at laboratory scale; more research is needed for assessment in large-scale use.[15]

Cell Lysis: Mechanical or nonmechanical methods of cell lysis are possible; raw culture liquor or biomass concentrate can be treated. The large scale of biodiesel production likely rules out liquid shear methods efficient at the small scale. Sonication and high-speed homogenizers are likely unsuitable for the rapid throughput needed unless these can be shown effective as in-line processes. A 10 m^3 hr^{-1} sonication unit is currently advertised.[16] Osmotic shock may prevent culture liquor recycle, and dessication and freeze-thaw are likely to be economically impractical at scale. Chemical or enzymatic lysis is possible, but may affect recycle potential of culture liquor; concentrated biomass may be treatable by these methods. Pressure homogenization is suited for large-scale use and could be used on biomass concentrate or raw culture liquor. Pressurized liquid is passed through a pressure relief valve to rupture the cells, and multiple passes may be required. Since the SCMT reaction occurs at high pressure and temperature, lysis may not be necessary. Pressure homogenization may be inexpensively integrated into the process by pumping the feed to a higher pressure prior to supply to the SCMT reactor at high pressure.

Lipid/FFA Separation: After cell lysis, a lipid-rich phase will separate that can be decanted at low cost, without need for solvent washing or liquid-liquid centrifugation. The recovery efficiency of this method for both lipids and FFAs and SCMT reaction compatibility must be tested at lab scale for the precursors of *C. protothecoides*. The moisture tolerance of the SCMT process suggests only crude lipid separation is needed, if any, but this must be balanced against the total recovery of lipid/FFA precursor. High capacity centrifugal separation is commonly employed in biodiesel precursor recovery and processing from crops, and large-scale process units are commercially supplied.[17] Since methanol is the SCMT diluent, its extraction performance should be evaluated. Solvent washing has been used to extract algal lipids and FFAs with reasonable efficiency using 1-butanol, hexane, and other alcohol mixtures.[18]

Suggested Studies

Biodiesel production must succeed economically in a competitive commodity diesel market with alternative precursor sources. The process design goal is to achieve the lowest cost per unit yield for a market-relevant product volume. This goal may rule out technically elegant and efficient separations in favor of crude and inexpensive processes. Thorough process economic modeling is required to identify a technically and economically viable design. As emphasized in the preceding discussions, the reaction characteristics and economics of the SCMT reaction will likely govern the separations process design. In conjunction with any cost and technical advantages of culture liquor recycle, a process design can be developed to satisfy the constraints. The following experiments and analyses are suggested for further process technology development and integration.

A. Pilot-scale (20L) cultivation of lipid/FFA-rich *C. protothecoides* at high biomass concentrations

    a. Verify yield and cycle time obtained in literature and determine properties of culture liquor and cell fraction

    b. Evaluate nondestructive separations to concentrate biomass
        i. Air flotation and skimming
        ii. Sonication
        iii. Continous solid-liquid centrifugation
        iv. Flocculation using benign chemical additives

    c. Evaluate culture liquor recycle after biomass concentration

    d. Evaluate lipid separations methods following lysis
        i. Crude (decanting supernatant)
        ii. Continous liquid-liquid centrifugation
        iii. Solvent-assisted extraction using methanol

B. Experiment to study SCMT process yields, varying factors of

    a. In-line pressure homogenization lysis vs. no lysis
        i. Raw culture liquor
        ii. Biomass concentrate at different moisture levels
        iii. Methanol dilution quantity
            1. In-line mixer contribution to lysis

    b. Crude lipid fraction vs. refined lipid fraction SCMT yield

C. Process Design Integration and Economic Study Estimate (+/- 30%)

Candidate Process Design Concept

This design concept is intended to illustrate a process that might be suitable for biodiesel precursor production from heterotrophic algae. It allows culture liquid recycle and intends to minimize the capital equipment costs for separation and the volume input to the SCMT reactor (Figure 2).

Step 1: Cultivate algae to maximum biomass and lipid content in bioreactor.
After about 4 days of culture to near peak biomass concentration, nitrogen deprivation induces lipid formation. Subsequent processing should be rapid to avoid lipid metabolism losses.
Step 2: Employ dissolved air floation in bioreactor, skim algae froth to concentrate
This step may be possible without a chemical additive, permitting recycle of remaining culture liquor.
Step 3. Centrifuge bioconcentrate to obtain solid-rich fraction as pumpable slurry
Step 4. Waste necessary portion of bioreactor liquor and restart culture process
Step 5. Blend methanol to bioconcentrate slurry to comprise SCMT reactor feed
Option: Pressure homogenize slurry: cell lysis by step up/down to SCMT reactor.
Step 6. SCMT reaction to convert lipd/FFAs
Step 7: Standard biodiesel ester-glycerol separation process

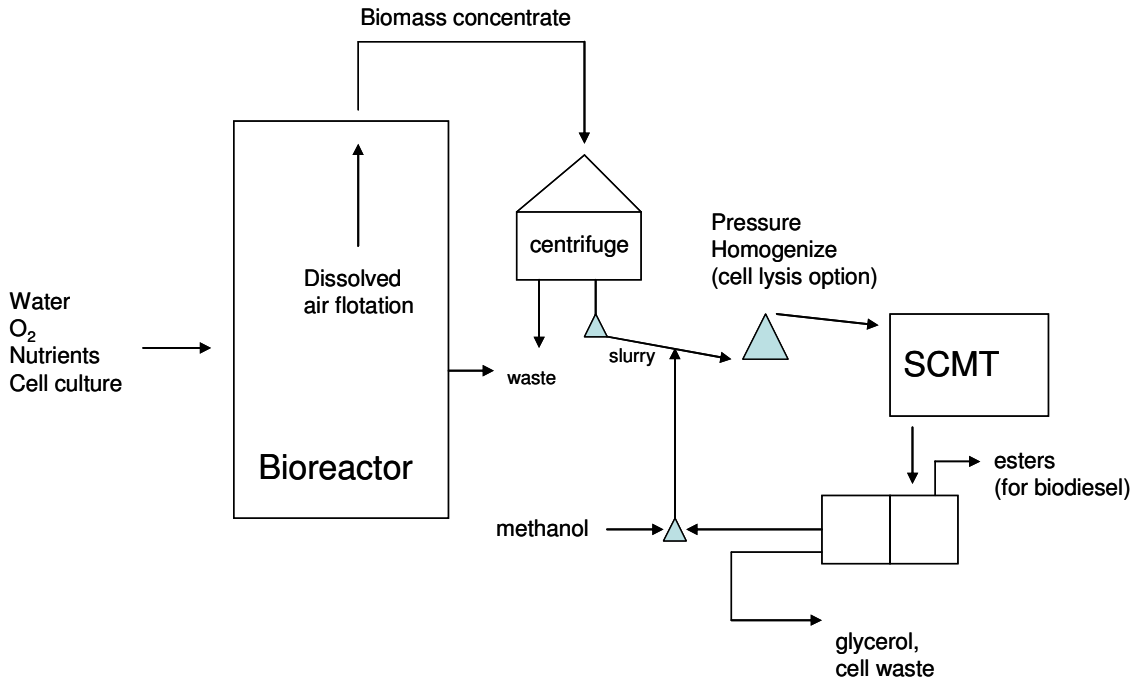

Figure 2. Candidate large-scale algal biodiesel process concept

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