



2020

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Recommended Citation

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RESEARCH ARTICLE

Comparative scoping study report for the extraction of microalgae oils from two subspecies of *Chlorella vulgaris*

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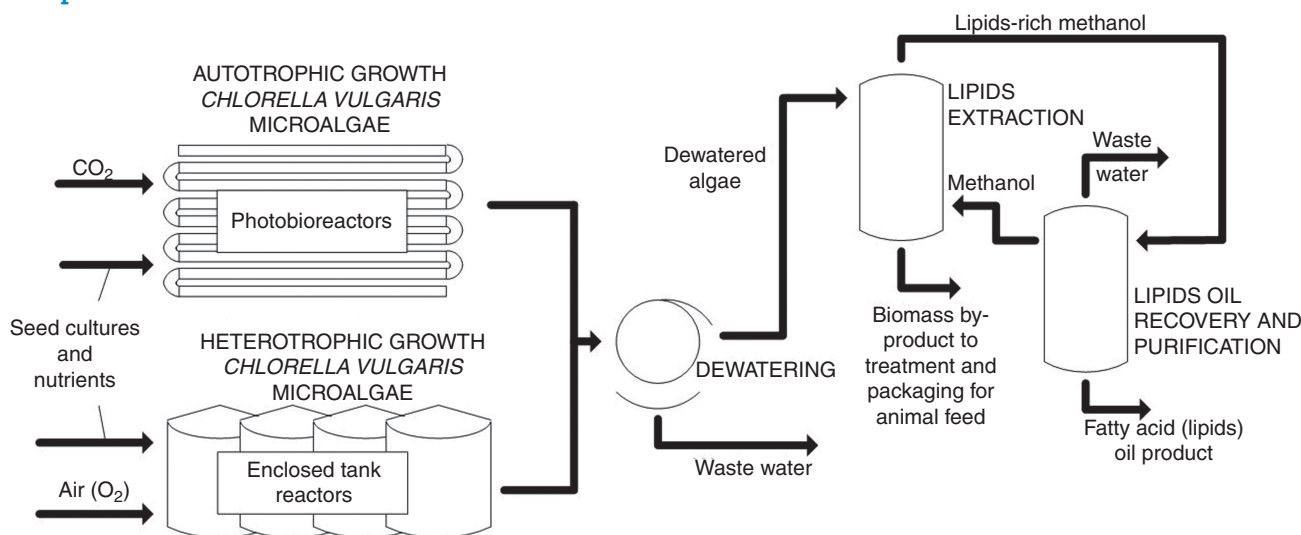
Abstract

The production of microalgae as a fatty acid oil resource for use in biofuels production is a widespread research topic at the lab scale. Microalgae contain a higher lipid content on a dry-weight basis compared to oilseeds such as soybeans. Additionally, the growth and cultivation cycle of microalgae is 15 days, in comparison to soybeans, for which the cycle occurs once or twice annually. However, to date, it has been uneconomical to produce microalgae oils in a world-scale facility due to limitations in cultivating microalgae at commercial scales. Recent developments suggest that the use of heterotrophic microalgae may be economically feasible for large-scale oil production. To assess this feasibility, a comparative scoping study was performed analysing the feasibility of an industrial-scale process plant for the growth and extraction of oil from microalgae. Processes were developed at the preliminary design level using heterotrophic subspecies and autotrophic subspecies of *Chlorella vulgaris*. AACE Class 4 cost estimates and economic analyses were performed. This study concludes that processes based on heterotrophic microalgae are more likely to reach economic feasibility than processes using autotrophic microalgae. However, a few barriers still remain to achieving free-market economic viability.

Received: 11 February 2020; Accepted: 12 June 2020

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Graphical Abstract



Keywords: algae oil; lipids; process design; heterotrophic microalgae; preliminary design; economic analysis

Introduction

Microalgae have been proposed by many as a potential source of fatty acid-based oils, in the form of lipids that can be converted into renewable replacements for a number of petroleum-derived fuels and chemicals [1–4]. By utilizing microalgae as the feedstock, the land area required to produce this oil is significantly reduced. Microalgae have a short 2-week growing and cultivation cycle. This maximizes the number of harvesting cycles per year compared to harvesting once or twice a year due to the lengthy growing season when using a cash crop [5].

Despite a decade of extensive research and development activities [6–9], currently, there are no world-scale facilities for the production of lipid-based oil extracted from microalgae. Research has been done to identify ideal microalgal strains to increase lipid production, growth rate and growth density, and to minimize nutrient consumption, environmental impacts, invasive biologicals and other external factors [10]. Yet, barriers to commercialization remain. One of these is the inability to effectively cultivate microalgae at large scales.

Recently, some researchers have explored transforming autotrophic microalgae to heterotrophic microalgae, negating the light dependence of the studied strains [11]. The transition to heterotrophic microalgae halts the photosynthesis process and requires an organic carbon source to provide energy. Heterotrophic microalgae are unable to produce energy using the same processes as autotrophic strains, which produce an energy source through photosynthesis [12]. However, the transition to heterotrophic conditions has been shown to increase the lipid content of the microalgae by replacing the chlorophyll cells produced during photosynthesis with lipids and, more importantly, eliminates one of the key scale-up barriers of autotrophic

microalgal cultivation [13, 14]. If the strain of microalgae used is non-light-dependent, it eliminates the requirement for industrial-scale, clear photo bioreactors or open ponds. Further, it has been shown that these heterotrophic strains can be grown efficiently using waste carbon resources, mitigating the need for more valuable sources [12, 15].

This paper documents a study conducted to evaluate the commercial potential for the production of fatty acid oils from the cultivation and extraction of lipids using a heterotrophic version of the microalgae strain, *Chlorella vulgaris*. In order to evaluate the heterotrophic strain completely, two process designs were developed: one based on the autotrophic version of the strain and the second based on the heterotrophic version. In-house lab-scale experimental data were generated when the data required to develop a preliminary process design of the required production facility were not readily available in the literature.

The *C. vulgaris* strain of microalgae has been proven to yield a high lipid content (15–35 wt%) and is one of the fastest-growing microalgal strains [16]. Additionally, this strain of microalgae has been found to be amenable to heterotrophic adaptation [12]. The heterotrophic strain of *C. vulgaris* should yield a higher lipid content that will generate a larger amount of oil when compared to the autotrophic strain. The microalgae would be grown in trains of reactors for heterotrophic or autotrophic growth. The reactors within each train increase in size and would be designed for microalgal growth to optimize the operating time of the plant.

The lipid-extraction method for both the heterotrophic and autotrophic processes can be similar with the only difference being minor variations in the flow rates of each process. The extraction process begins with the separation of the majority of the liquid growth media using a

vacuum filter. Subsequently, a press is utilized to remove the majority of the entrained water and to begin to break the cell walls of the microalgae. A grinder is then used to completely destroy the cell walls and expose the lipids. A solvent is used to leach the lipids out of the biomass. Methanol has been shown to be an effective solvent for this purpose [17]. The oil-lean biomass is collected and sold as a high-protein animal feedstock by-product [18]. The lipid/methanol mixture is separated using a multi-effect evaporator from which the fatty acid oils are collected as the primary product and the methanol is recycled as solvent in the oil-leaching portion of the process.

The preliminary process design was developed to produce 500 000 kg/yr of fatty acid-based oil from either the heterotrophic or autotrophic strains of the microalgae. This oil can be transformed into biodiesel and other high-value chemicals. However, the transformation of the oil was outside of this study and these processes were not developed.

1 Materials and methods

1.1 Experimental materials and methods

1.1.1 Solvent and microalgae-to-solvent-ratio selection: experimental

Methanol was chosen as the extraction solvent for the scoping study as a result of an in-house preliminary solvent-selection study for optimum fatty acid-oil extraction from a *C. vulgaris* strain of microalgae. The solvents utilized for the preliminary study were chloroform, methanol, hexane, acetonitrile, ethanol and deionized water (DI). The study was performed by mixing autotrophic microalgae, cultured from a strain obtained from the Scottish Association for Marine Science and Cultures, and each solvent in a 1:10 ratio (mass-to-volume) followed by filtration to separate the oils from the biomass.

The 1:10 (mass-to-volume) algae-to-solvent ratio was chosen as the extraction ratio for the scoping study as a result of an in-house preliminary solvent-selection study for optimum fatty acid-oil extraction from the strain of microalgae *C. vulgaris*. The ratios utilized for the preliminary study were: 1:3, 1:7, 1:11, 1:15 and 1:19. The study was performed by mixing autotrophic microalgae, cultured from a strain obtained from the Scottish Association for Marine Science and Cultures, and a solvent in each ratio (mass-to-volume) followed by gravity filtration to separate the oils for collection.

In both the extraction-solvent and the solvent-to-microalgae-ratio studies, both the filtered liquid product and the residual biomass were separately collected, heated to evaporate the solvent from the product and weighed. The ratio of dry products collected (extracted liquid/biomass) was utilized to determine extraction efficiency.

1.1.2 Design: simulation

A preliminary design was developed for each process option. This includes the identification and size approximation of

all equipment of pump or larger size, organized into the unit operations necessary to transform the raw material and other inputs into the product oil and by-products. In addition to equipment sizing, the design includes an estimate of all required utilities, chemicals and other resources required by the process. The design is primarily summarized on process flow diagrams (PFDs). Equipment sizing was performed using approximation methods from Ulrich [19] or using the ChemCad™ simulation program. The 'Results and discussion' section includes a description of the detailed design for each process, including the cultivation of each species of microalgae.

1.2 Economic analysis

A broad cost estimate (AACE category 4 [20]) of the project costs along with estimates of the manufacturing costs, raw-material costs and product revenues was generated at a nominal accuracy level of $\pm 40\%$ [19]. These cost elements were used to quantify the economic feasibility of the technology. The discounted cash-flow return of investment (DCFROR) and the net present value at a hurdle rate of 20% (NPV@20%) were estimated to evaluate the economic feasibility of the two process options at a basis date of October 2016.

The broad cost estimate is primarily based on the PFD and the preliminary equipment sizes of the design. The revenues of the process were calculated based on trend-price forecasts for product sales, by-product sales and operating-cost credits. To determine the overall potential profitability of each process, an economic cash-flow sheet consisting of the process revenues, operating cost, gross profit, depreciation, taxable profit, income tax, non-taxable charges, net profit and present value was developed based on a 20-year operating life.

Depreciation for tax calculations was based on the value of the fixed capital investment (FCI) written off over a period of 17 years with no salvage value using the Modified Accelerated Cost Recovery System method. The taxable income for the process designs was determined by subtracting all annual expenditures (except capital expenditures) and depreciation charges from the annual revenue. The income tax was calculated by multiplying the annual taxable income by the tax rate, which was assumed to be 35% (2017 US tax-law basis). The non-taxable charges included the FCI spread across the estimated project-completion time with an estimate for the initial inventory of chemicals plus working capital added to the final project year. The working capital was recovered in the final year.

The annual net profit was determined by subtracting the annual operating expenses, annual non-taxable charges and annual taxes from the annual revenue. The present value for each year was determined by discounting the annual net profit using a 20% discount rate to determine the value at the chosen basis date. The NPV@20% was then found as the sum of all of the present values over the life of

the project. The DCFROR was determined to be the hurdle rate at which the NPV was equal to zero.

1.3 Process-design assumptions

- (i) The process is designed as a grassroots project with a lifespan of 20 years.
- (ii) The designed process has an operating factor of 95%.
- (iii) The fatty acid-based oil product is of sufficient quality for processing into a biofuel in a downstream operation.
- (iv) The growth media in both the autotrophic and heterotrophic processes enters the growth system already mixed as a concentrated solution in an outside auxiliary area; 95% consumption of the input Bolds Basal Media and the Heterotrophic Basal Media was assumed in the autotrophic and heterotrophic reactors, respectively.
- (v) Each process has a 100% operating throughput to produce 500 000 kg per year of oil. The size used is 1/1000th of a typical US Midwest soybean/canola oil-processing facility and represents a likely minimum scale necessary for competitive oil production.
- (vi) 32% of the total heterotrophic-microalgae biomass and 15% of the total autotrophic microalgae biomass are extracted as lipids [16].
- (vii) The cell density of the heterotrophic and autotrophic microalgae achieved during the growth phase is 20 and 8 g/L, respectively.
- (viii) Any pigments or other unwanted polar compounds that are extracted by the solvent will partition into the aqueous waste streams during the separation and purification portions of the process.
- (ix) The CO₂ flow rate for autotrophic microalgae growth is 12 mL/min [21].
- (x) The seed (inoculant) microalgae concentration required for growth is 40 mg/L [22].
- (xi) 5% methanol is assumed to be lost on an annual basis and requires a make-up stream.
- (xii) Sucrose solubility in water is 200 g/100 mL [23].
- (xiii) NaNO₃ solubility in water is 91 g/100 mL [24].
- (xiv) CaCl solubility in water is 74 g/100 mL [24].
- (xv) MgSO₄ solubility in water is 341 g/100 mL [24].
- (xvi) NaCl solubility in water is 35 g/100 mL [24].

1.4 Equipment-design assumptions

- (i) A pressure drop of 35 kPa occurs across all unit operations unless otherwise specified [25].
- (ii) All pumps have an overall efficiency of 70% [25].
- (iii) All compressors have a polytropic efficiency of 65% [25].
- (iv) Surge drums are sized based on an overall length-to-diameter ratio of 4:1 [19].
- (v) Surge drums have a liquid holding time of 10 min.

- (vi) Conveyors are 0.61 m wide, 15 m long and doubled/redundant [25].
- (vii) 95% of the water entering a vacuum filter is removed, leaving 5% weight in the outlet solid [25].
- (viii) 95% of the water entering a filter press is removed [25].
- (ix) Heterotrophic reactors are sized with a height-to-diameter ratio of 2:3 [26].
- (x) Carbon steel is a sufficient material of construction for all process equipment and ancillary piping [19].
- (xi) The multi-effect evaporator operates with the first effect at 97 kPa and the final effect at 14 kPa. All effects will have an equal pressure drop over that range.
- (xii) All evaporators have the same heat-transfer area [19] and the same volume. The multi-effect evaporator system is small enough such that each separate effect is not individually optimized. The volume of the first effect is sized by utilizing the rule of thumb that a 30-min residence time will account for 75% of the total volume. For each evaporator effect, the bottom diameter is equal to the height divided by 5 and the top diameter is equal to 2× the bottom diameter. A 14-kPa pressure drop occurs across the heat-transfer area in each effect of each evaporator.
- (xiii) A 62 kPa pressure drop occurs for the process stream in the E-103 and E-1003 systems to account for the long piping run necessary to route the process stream to the beginning of the multi-effect evaporator system.
- (xiv) 4 wt% of the methanol entering the leacher will exit with the biomass stream from the leacher.

1.5 Utility assumptions

- (i) Low-pressure steam is generated in an auxiliary area of the facility and is available to the process at T = 160°C and P = 500 kPa [25].
- (ii) Process cooling water is generated in an auxiliary area of the facility and is available to the process at T = 30°C and P = 210 kPa [25].
- (iii) Moderately low-temperature refrigerated water is generated in an auxiliary area of the facility and is available to the process at T = 5°C and P = 210 kPa [25].
- (iv) CO₂ will be externally supplied to the process and priced as a consumable chemical cost [19].

1.6 Economic assumptions

- (i) Values of 400 [19], 543 [27] and 585 [27] are used as the 2004, 2016 and 2012 CEPCI values, respectively. The CEPCI index is used to bring all economic data to the same basis date of October 2016.
- (ii) The annual maintenance cost is estimated to be 6% of the FCI [19].

- (iii) No royalties or patent fees are required for this process [19].
- (iv) A rough planning schedule based on a rule-of-thumb of 30% design, 40% procurement and 30% implementation is used to estimate the project schedule with the longest procurement time used to dictate the schedule.
- (v) The FCI is depreciated over a 17-year period.
- (vi) The hurdle rate (minimum acceptable rate of return) is 20%.
- (vii) The revenue price for the bioproduct biomass is based on a trend price of soybean meal discounted by 20% to account for the residual methanol that must be removed prior to use.

2 Results and discussion

2.1 Process design

Preliminary designs were prepared to generate 500 000 kg/yr of the primary fatty acid-based oil product from either heterotrophic or autotrophic strains of the microalgae *C. vulgaris*. Figs 1 and 2 provide an overview of the heterotrophic- and autotrophic-process schemes, respectively, used in this evaluation. Each process is organized into three major areas: Area 01 is the growth and cultivation

area, Area 02 is the filtration and crushing area and Area 03 is the extraction and solvent-separation area. A more detailed display of the preliminary design for each area of the heterotrophic-microalgae option is provided on the PFDs in Figs S5–S13 in the online Supplemental Data. The comparable information for the autotrophic microalgae option is provided on the PFDs in Figs S19–S29 in the online Supplemental Data. A detailed process description is also provided in the Supplemental information to explain the PFDs.

The most substantial difference in the design occurs in Area 01. The growth reactors for the autotrophic case were based on the largest-scale commercially available reactor design that we could identify at the time of the study, which was 25 000 L. Based on the capacity of this reactor, the autotrophic case requires 792 reactors. The growth of the *C. vulgaris* is accomplished by using parallel trains of photo bioreactors, where each train contains three differently sized reactors, arranged sequentially. The microalgae from each reactor will be transferred into the next larger reactor in the sequence after a 14-day growing period. With each transfer, additional growth media is added to facilitate the growth process. The products from the final-stage 25 000-L reactors in each train are fed to a holding tank that will feed the rest of the process at a constant rate of 45 000 kg/hr.

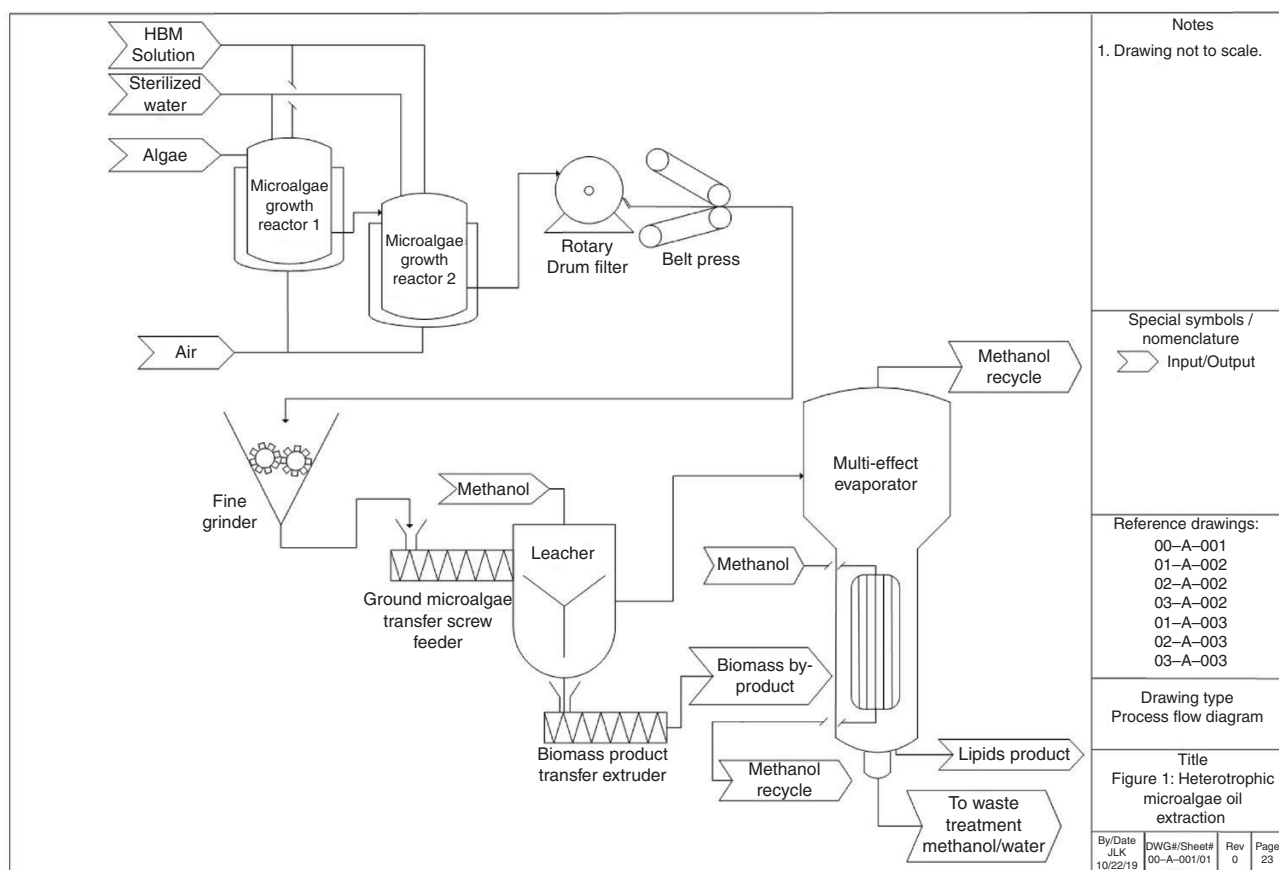


Fig. 1: Heterotrophic-microalgae fatty acid-based oil-extraction process flow schematic.

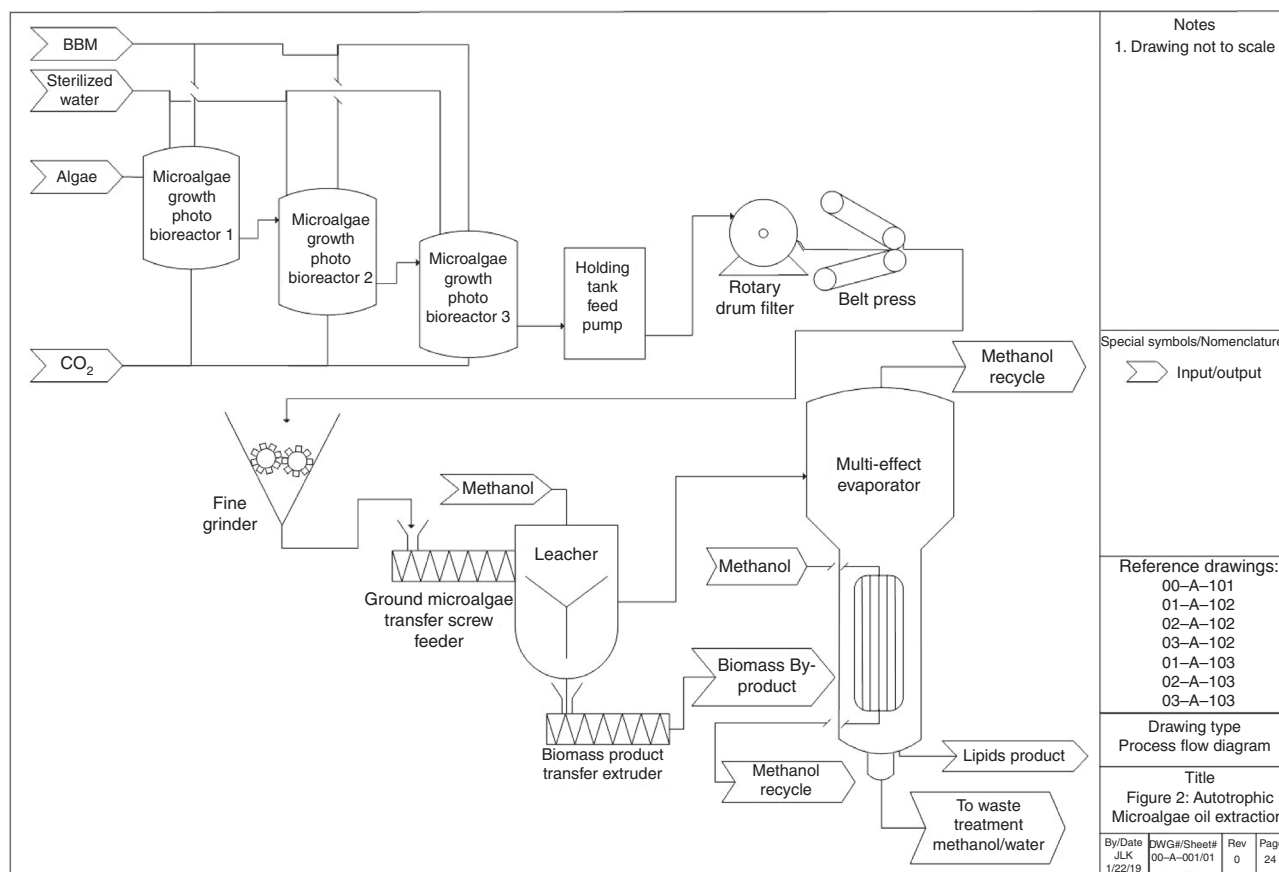


Fig. 2: Autotrophic microalgae fatty acid-based oil-extraction process flow diagram.

Table 1: Raw-material requirements

Raw material	Heterotrophic process (kg/yr)	Autotrophic process (kg/yr)
<i>Chlorella vulgaris</i>	2.1	0.45
Carbon dioxide	-	340 000 000
Sterile process air	20 000 000	-
Sucrose	3 200 000	-
Sterile process water	210 000	21 000 000

For the heterotrophic case, the growth reactors were based on the typical size of a world-scale corn-to-ethanol reactor that was estimated at 4 500 000 L. Based on the capacity of this reactor, the heterotrophic case requires seven reactors. The growth of the *C. vulgaris* is accomplished using two stages of reactors in sequence with three initial reactors operating in parallel feeding four final reactors operating in parallel. At the end of the 15-day growth period, each of the final reactors will contain enough microalgae to feed the rest of the process continuously for 5 days at 4200 kg/hr. This rate is substantially lower than for the autotrophic case due to differences in biomass density in the final reactors and the lower lipid content of the autotrophic strain. In both cases, these configurations provide a continuous production of biomass that can be fed to the rest of the process to generate the same quantity of oil product.

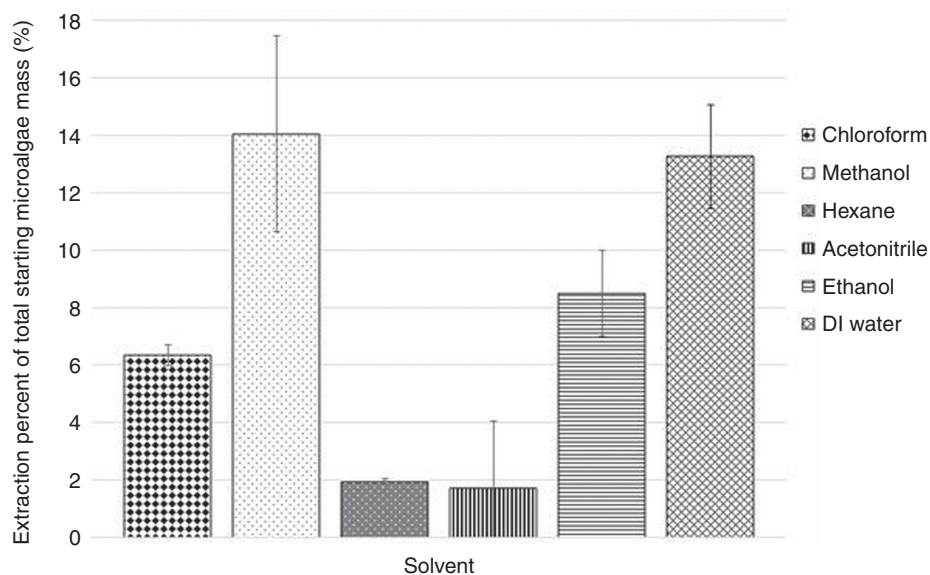
There were substantial differences in the inputs required to grow the two different microalgae strains. The estimated annual consumption of these inputs is summarized in Table 1 for both cases. The annual input into the autotrophic process will be higher in comparison to the heterotrophic process to yield the same amount of product due to the lower lipid content in autotrophic microalgae. Utility requirements, summarized in Table 2, are also substantially different due to the additional challenge of managing >790 reactors in the autotrophic design.

After growth and cultivation in Area 01, the microalgae are dewatered by filtration and then crushed to rupture the cell walls, making oil extraction more efficient in Area 02. These units were designed to operate continuously and are essentially the same for both feedstocks.

Area 03 was designed for the extraction and recovery of the oil from the biomass. The system was designed based

Table 2: Utility requirements and costs

Utility description	Heterotrophic process		Autotrophic process	
	Annual cost (\$/yr)	Requirement	Annual cost (\$/yr)	Requirement
Electricity	2200	Amount: 49 000 kWh/yr	12 000	Amount: 270 000 kWh/yr
Low-pressure steam	350 000	Amount: 12 000 000 kg/yr Supply temperature: 149°C Supply pressure: 450 kPa	1 800 000	Amount: 64 000 000 kg/yr Supply temperature: 149°C Supply pressure: 450 kPa
Carbon dioxide	–	–	–	Amount: 340 000 000 kg/yr Supply temperature: 25°C Supply pressure: 140 kPa
Process cooling water	560	Amount: 38 000 000 kg/yr Temperature: 10°C Pressure: 100 kPa	8700	Amount: 590 000 000 kg/yr Temperature: 10°C Pressure: 100 kPa
Heating water	–	–	210 000 000	Amount: 1 500 000 000 000 kg/yr Temperature: 43°C Pressure: 100 kPa
Moderately low-temperature refrigerated water	2100	Amount: 11 000 000 kg/yr Temperature: 5°C Pressure: 210 kPa	4500	Amount: 24 000 000 kg/yr Temperature: 5°C Pressure: 210 kPa
Sterile air	–	Amount: 20 000 000 kg/yr Temperature: 43°C Pressure: 100 kPa	–	Amount: 1 100 000 kg/yr Temperature: 43°C Pressure: 100 kPa
Methanol	17 000	Amount: 34 000 kg/yr Temperature: 43°C Pressure: 100 kPa	660 000	Amount: 1 300 000 lb/yr Temperature: 25°C Pressure: 100 kPa
Total	\$370 000/yr		\$210 million/yr	

**Fig. 3:** Extraction efficiency of fatty acid-based oils from autotrophic microalgae based on solvent performance.

on the use of methanol as the extracting solvent. The choice of methanol is based on lab-scale experiments performed with a number of different solvents. A summary of the results of the solvent-performance study for the autotrophic strain is shown in Fig. 3. Comparable results were obtained for the heterotrophic strain (results not shown). Additionally, a summary of the results of a study to optimize the solvent-to-microalgae ratio is provided in Fig. 4.

A detailed description and documentation of this work can be found in [17] and will be published in journal form in the near future.

In order to recover the oil out of the methanol, a multi-effect evaporator is used to separate the solvent from the desired lipid product. Although this method is relatively energy-intensive, it allows us to use a proven method in this comparison study. This is an area in which future

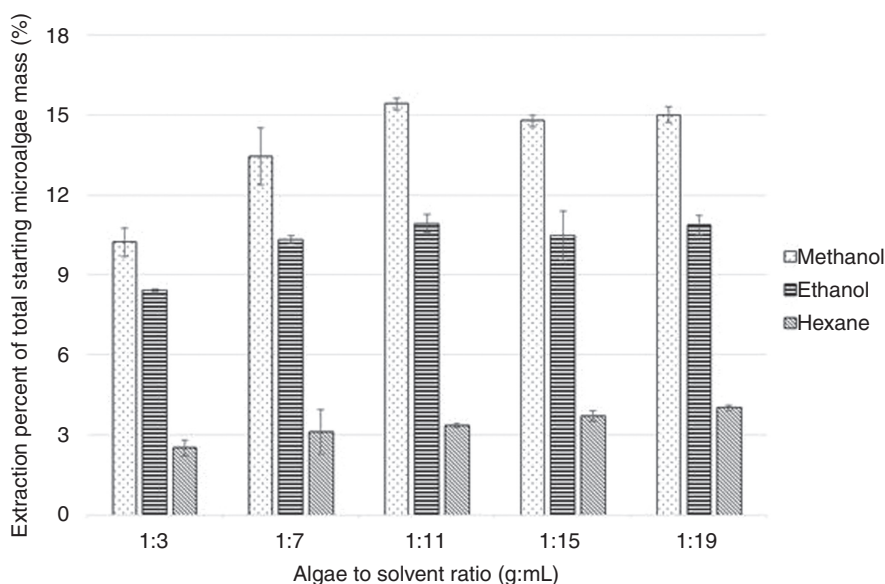


Fig. 4: Extraction efficiency of fatty acid-based oils from autotrophic microalgae based on solvent-to-microalgae ratio.

Table 3: Product fatty acid-oil compositions from each process

Product	Heterotrophic process (kg/yr)	Autotrophic process (kg/yr)
Free fatty acids	500 000	500 000
Components (wt %)		
Palmitic acid	29%	29%
Palmitoleic acid	2%	2%
Stearic acid	1%	1%
Oleic acid	18%	18%
Linoleic acid	27%	27%
Alpha linolenic acid	23%	23%

technology development is likely to improve the efficiency of this process. Annual solvent losses are estimated to be 5% of the recirculating solvent. This section of the process generates 500 000 kg/yr of fatty acid-based oil with a composition assumed to match one commonly reported in the literature from each strain, as summarized in Table 3. One million and 2 800 000 kg/yr of residual microalgal biomass are also produced from the heterotrophic- and autotrophic-process designs, respectively. This biomass is assumed to have value as a high-protein animal feedstock [18]. Additional clean-up to remove residual methanol is assumed to occur in an area not covered by the design presented herein. Costs for this removal is assumed to be 20% of the most likely product value.

2.2 Broad cost estimates

A broad estimate of the capital costs for both heterotrophic- and autotrophic-process designs was completed based on the equipment listed in Tables 4 and 5, respectively. A condensed version of the estimated capital costs for each process are also reported in these tables and include an approximate cost for each piece of equipment, as

well as the total capital investment required for the project using an October 2016 basis date. Detailed estimated capital cost tables are included in the supplementary information in Tables S3 and S4 in the online Supplemental Data.

The cost estimates for the conveyors and fine grinder for this process were determined by acquiring a vendor cost estimate. The remaining equipment was estimated by utilizing the cost charts published by Ulrich and Vasudevan [19]. The Ulrich Cost Data estimate the purchased costs of the equipment using a basis date of 2004. These costs were adjusted to the project's basis date using CEPCI values for 2004 [19] and 2016 [27]. The total capital investment for the heterotrophic and autotrophic processes were estimated to be \$13 million \pm 40% and \$84 million \pm 40%, respectively.

Area 01 for each process is where the two process designs differ and account for most of the difference in the total capital investment of the two processes. The growth and cultivation area for the autotrophic process requires a much higher initial capital cost due to the large number (792) of photobioreactors coupled with the use of a more expensive (polypropylene) material for construction than for the heterotrophic bioreactors (seven carbon-steel reactors). The designs for Area 02 and Area 03 are nearly identical, except for slightly larger equipment in the autotrophic process due to a higher throughput of raw materials.

2.3 Operating-cost estimates

An itemized analysis of the estimated yearly operating costs for the heterotrophic and autotrophic processes is reported in Table 6. More details of the operating costs are included in the supplementary information in Tables S5 and S6 in the online Supplemental Data. The total operating costs for the two processes are estimated to be

Table 4: Equipment table for the heterotrophic process

ID#	Equipment description	Number of units	Total BMC
C-101 A/B	Fine grinder	2	\$440 000
D-101	Leacher	1	\$34 000
D-102	Surge drum	1	\$11 000
E-101–105 A/B	Heat exchangers	12	\$180 000
G-101	Gas compressor and drive shaft	1	\$160 000
H-101 A/B	Vacuum filter	2	\$320 000
H-102 A/B	Belt press	2	\$380 000
J-101–105 A/B	Conveyors/screw feeders	10	\$100 000
L-101–113, 201–202 A/B	Pumps	66	\$680 000
T-101	Water-recycle tank	1	\$23 000
R-101 A-C, R-102 A-D	Algae-growth reactor	7	\$4 900 000
V-101–107	Evaporator	7	\$63 000
Total bare modular cost		CTBM »	\$7 200 000
Contingency and fee		CTM	CTBM * 0.18 =
Total module cost		CTM »	\$8 500 000
	Auxiliary facilities	CAUX	CTM * 0.30 =
Fixed capital investment		FCI »	\$11 000 000
	Working capital	CWC	FCI * 0.15 =
	Chemicals and catalysts		\$340 000
Total capital investment		TCI »	\$13 000 000

C_{TBM} , total bare modular cost; C_{TM} , total module cost; C_{AUX} , auxiliary-facilities cost; FCI, fixed capital investment; C_{WC} , working capital; TCI, total capital investment.

Table 5: Equipment table for the autotrophic process

ID#	Equipment description	Number of units	Total BMC
C-1001 A/B	Fine grinder	2	\$440 000
D-1001	Leacher	1	\$34 000
D-1002	Surge drum	1	\$15 000
E-1001–1005 A/B	Heat exchangers	12	\$280 000
G-1001	Gas compressor and drive shaft	1	\$220 000
H-1001 A/B	Vacuum filter	2	\$580 000
H-1002 A/B	Belt press	2	\$380 000
J-101–105 A/B	Conveyors/screw feeders	10	\$100 000
L-101–113, 201–202 A/B	Pumps	792	\$7 900 800
T-1001 A-C, T-1002	Holding tank	4	\$260 000
R-101 A-C, R-102 A-D	Algae-growth reactor	691	\$37 000 600
V-101–107	Evaporator	10	\$110 000
Total bare modular cost		CTBM »	\$47 000 000
Contingency and fee		CTM	CTBM * 0.18 =
Total module cost		CTM »	\$56 000 000
	Auxiliary facilities	CAUX	CTM * 0.30 =
Fixed capital investment		FCI »	\$73 000 000
	Working capital	CWC	FCI * 0.15 =
	Chemicals and catalysts		\$160 000
Total capital investment		TCI »	\$84 000 000

C_{TBM} , total bare modular cost; C_{TM} , total module cost; C_{AUX} , auxiliary facilities; FCI, fixed capital investment; C_{WC} , working capital; TCI, total capital investment.

\$3.7 million and \$240 million per year for the heterotrophic and autotrophic processes, respectively, using an October 2016 basis date. These costs include raw-materials costs, chemical and catalyst costs, operating labour, maintenance costs and utilities. These costs are based on a plant-operating factor of 95%.

The heterotrophic process requires four raw materials: *C. vulgaris*, an organic carbon source, water and air. For the purposes of this study, sucrose is used as the organic carbon source. The autotrophic process requires three raw materials: *C. vulgaris*, process water and carbon dioxide. The requirements for raw materials are reported in Table 1.

Table 6: Operating-costs summary (\$/yr)

Process	Year	Raw materials	Chemicals and catalysts	Operating labour	Maintenance	Utilities	Royalties and patent fees	Other expenses	Yearly total
Heterotrophic	1–20	880 000	180 000	1 600 000	660 000	370 000	–	28	3 700 000
Autotrophic	1–20	7 400 000	2 300 000	14 000 000	4 400 000	210 000 000	–	60	240 000 000

Table 7: Nutrient cost chart and media requirements [30].

Component	Cost (\$/kg)	Heterotrophic Basal Media		Bolds Basal Media	
		kg/yr	Cost (\$/yr)	kg/yr	Cost (\$/yr)
Sodium nitrate [31]	0.10			590 000	290 000
Calcium chloride [32]	0.024	110	10.00	59 000	7100
Magnesium sulphate [33]	0.13	1400	860.00	180 000	110 000
Dipotassium hydrogen phosphate [34]	0.41	1400	2700.00	180 000	350 000
Potassium dihydrogen phosphate [35]	0.41	3200	6400.00	420 000	840 000
Sodium chloride [36]	0.045	110	20.00	59 000	13 000
Trace element solution ^a [37]	0.81	4500	18 000.00	43 000	170 000
Sucrose [28]	–	3 000 000	–	–	–
Yeast extract [38]	1.60	18 000	150 000.00		
EDTA [37]	16			24 000	1 900 000
Acidified iron stock solution [39]	0.000054			43 000	12
Boric acid [40]	0.31			43 000	65 000
Distilled water	–	4 500 000	–	24 000 000	–
Total		7 700 000	180 000.00	56 000 000	3 700 000

^aTrace Element Solution priced as 5% EDTA.

The large quantity of sterile water needed for the autotrophic process accounts for the major difference in cost between the heterotrophic and autotrophic processes. The autotrophic process requires more sterile water because:

- (i) The cell density of the autotrophic strain under optimum growth conditions is much lower than the cell density of the heterotrophic strain.
- (ii) The autotrophic strain of *C. vulgaris* has a lower lipids content under optimum conditions compared to the heterotrophic strain; therefore, to produce the same quantity of oil, more microalgae must be grown, requiring more water.
- (iii) The larger number of reactors results in a higher consumption of sterile water during the cleaning and sterilization steps of the bioreactor batch cycle.

Sucrose used in the heterotrophic process is priced using commodity-trend pricing [28]. The water is priced using a commonly accepted cost [25]. Carbon dioxide used in the autotrophic process is priced using a spot price [29]. The yearly cost for the four raw materials for the heterotrophic process is estimated to be \$880 000 per year. The yearly cost for the three raw materials for the autotrophic process is estimated to be \$7.4 million per year, with most of the difference due to the differences in sterile-water costs.

The chemicals required for the heterotrophic process are the nutrients required for the Heterotrophic Basal Media (HBM), while those required for the autotrophic

process are the nutrients required for the Bolds Basal Media (BBM). The nutrient requirements and costs are reported in Table 7. The costs associated with the media are priced based on bulk prices commercially available for each component. The bulk price of ethylenediaminetetraacetic acid (EDTA) is obtained from a vendor [37]. The HBM is estimated to cost a total of \$180 000 per year. The BBM is estimated to cost a total of \$2.3 million per year. The difference in the cost of chemicals for the two processes is due to the larger quantity of chemicals required to generate the BBM per litre in comparison to the quantity of chemical to generate the HBM per litre.

The estimate for the cost of labour is based on the number of pieces of equipment that each process contains. The heterotrophic-process design requires an estimated five operators per shift with an additional board operator, yielding a total of 21 operators across 4.5 shifts to obtain a 95% operating factor. Due to the large number of bioreactors required, the autotrophic-process design requires 36 operators per shift with an additional board operator, yielding a total of 166 operators across 4.5 shifts. The labour-estimation requirement is determined by utilizing the method found in Ulrich [19]. The average hourly wage for a plant operator in Texas of \$25.86 [41] is used. Due to the number of operators needed per day, a supervisor is also estimated to be required. The supervisory labour cost is estimated to be 15% of the operating labour costs [19]. The total yearly labour cost for the heterotrophic design

is estimated at ~\$1.6 million, whereas the costs for the autotrophic-design case are estimated to be ~\$40 million.

The maintenance cost for the heterotrophic- and autotrophic-process designs are estimated by utilizing the rule of thumb that the cost of maintenance is 6% of the FCI [19], as shown in Table 6. The yearly cost for maintenance for the heterotrophic-process design is ~\$660 000, whereas the yearly cost for maintenance for the autotrophic-process design is ~\$21 million. The autotrophic-process design has a higher maintenance cost due to the large number of photobioreactors required in Area 01.

The required utilities and chemicals for the heterotrophic-process design are electricity, low-pressure steam, process water, cooling water, low-temperature refrigerated water and methanol, whereas the required utilities and chemicals for the

autotrophic-process design are electricity, low-pressure steam, medium-pressure steam, cooling water, heating water, moderately low-temperature refrigerated water and methanol. The annual requirement for each utility is reported in Table 2. The price of electricity was found using trend-price data. The costs for low-pressure steam, medium-pressure steam, heating water, moderately low-temperature refrigerated water, process water and cooling water are estimated using commonly accepted utility prices [25]. The price of methanol is determined to be \$0.23 per pound [42], with an estimated 5% annual make-up.

The difference in the costs of utilities and chemicals for the two processes is due to the difference in water and steam consumption. The autotrophic process requires significantly more steam and cooling water due to the

Table 8: Revenue projection for the heterotrophic and autotrophic processes

Products	\$ /kg	Heterotrophic		Autotrophic	
		Amount (kg/yr)	Revenue (\$/yr)	Amount (kg/yr)	Revenue (\$/yr)
Lipids	0.40 [27]	500 000	970 000	500 000	970 000
Biomass	0.059 [12]	1 000 000	300 000	2 800 000	2 100 000
		Total	1 300 000		3 100 000

Note: Numbers may not sum to total due to rounding.

Table 9: Economic cash-flow sheet for the heterotrophic process (\$ millions)

Job Title: Oil Extraction from Heterotrophic Microalgae

Location: Texas

Basis Date: October 2016

Year	Revenues	Operating cost	Gross profit	Depreciation	Taxable profit	Income tax	Non-taxable charges	Net profit	Present value @20%
-1							(2.2)	(2.2)	(2.6)
0							(11)	(11)	(11)
1	1.3	(3.7)	(2.4)	1.3	3.7	1.3		(1.1)	(0.92)
2	1.3	(3.7)	(2.4)	1.1	3.5	1.2		(1.2)	(0.81)
3	1.3	(3.7)	(2.4)	1.0	3.4	1.2		(1.2)	(0.70)
4	1.3	(3.7)	(2.4)	0.89	3.3	1.2		(1.2)	(0.60)
5	1.3	(3.7)	(2.4)	0.78	3.2	1.1		(1.3)	(0.52)
6	1.3	(3.7)	(2.4)	0.69	3.1	1.1		(1.3)	(0.44)
7	1.3	(3.7)	(2.4)	0.61	3.0	1.1		(1.3)	(0.38)
8	1.3	(3.7)	(2.4)	0.54	2.9	1.0		(1.4)	(0.32)
9	1.3	(3.7)	(2.4)	0.48	2.9	1.0		(1.4)	(0.27)
10	1.3	(3.7)	(2.4)	0.45	2.8	1.0		(1.4)	(0.23)
11	1.3	(3.7)	(2.4)	0.45	2.8	1.0		(1.4)	(0.19)
12	1.3	(3.7)	(2.4)	0.45	2.8	1.0		(1.4)	(0.16)
13	1.3	(3.7)	(2.4)	0.45	2.8	1.0		(1.4)	(0.13)
14	1.3	(3.7)	(2.4)	0.45	2.8	1.0		(1.4)	(0.11)
15	1.3	(3.7)	(2.4)	0.45	2.8	1.0		(1.4)	(0.090)
16	1.3	(3.7)	(2.4)	0.45	2.8	1.0		(1.4)	(0.080)
17	1.3	(3.7)	(2.4)	0.45	2.8	1.0		(1.4)	(0.060)
18	1.3	(3.7)	(2.4)	-	2.4	0.84		(1.6)	(0.060)
19	1.3	(3.7)	(2.4)	-	2.4	0.84		(1.6)	(0.050)
20	1.3	(3.7)	(2.4)	-	2.4	0.84	1.7	0.14	0.0037
								NPV@20%=	(20)

Note: Numbers in parentheses represent negative values.

Table 10: Economic cash-flow sheet for the autotrophic process (\$ millions)

Job Title: Oil Extraction from Autotrophic Microalgae

Location: Texas

Basis Date: October 2016

Year	Revenues	Operating cost	Gross profit	Depreciation	Taxable profit	Income tax	Non-taxable charges	Net profit	Present value @20%
-3							(15)	(15)	(30)
-2							(19)	(19)	(30)
-1							(19)	(19)	(23)
0							(30)	(30)	(30)
1	3.1	(240)	(240)	8.6	(250)	(90)		(150)	(130)
2	3.1	(240)	(240)	7.6	(240)	(90)		(150)	(110)
3	3.1	(240)	(240)	6.7	(240)	(90)		(150)	(88)
4	3.1	(240)	(240)	5.9	(240)	(80)		(150)	(73)
5	3.1	(240)	(240)	5.2	(240)	(80)		(150)	(61)
6	3.1	(240)	(240)	4.6	(240)	(80)		(150)	(51)
7	3.1	(240)	(240)	4.1	(240)	(80)		(150)	(43)
8	3.1	(240)	(240)	3.6	(240)	(80)		(150)	(36)
9	3.1	(240)	(240)	3.2	(240)	(80)		(150)	(30)
10	3.1	(240)	(240)	3.0	(240)	(80)		(150)	(25)
11	3.1	(240)	(240)	3.0	(240)	(84)		(150)	(21)
12	3.1	(240)	(240)	3.0	(240)	(84)		(150)	(17)
13	3.1	(240)	(240)	3.0	(240)	(84)		(150)	(14)
14	3.1	(240)	(240)	3.0	(240)	(84)		(150)	(12)
15	3.1	(240)	(240)	3.0	(240)	(84)		(150)	(10)
16	3.1	(240)	(240)	3.0	(240)	(84)		(150)	(8.3)
17	3.1	(240)	(240)	3.0	(240)	(84)		(150)	(6.9)
18	3.1	(240)	(240)	-	(240)	(83)		(150)	(5.8)
19	3.1	(240)	(240)	-	(240)	(83)		(150)	(4.8)
20	3.1	(240)	(240)	-	(240)	(83)	11	(140)	(3.7)
								NPV@ 20% =	(850)

Note: Numbers in parentheses represent negative values.

heating and cooling of the photobioreactors to ensure the microalgae is grown at a consistent temperature all year round. Additionally, low-pressure steam is utilized to sterilize the tank reactors and photobioreactors after each use. A greater amount of low-pressure steam is required for the autotrophic process due to the increased number of reactors required.

2.4 Revenues

The revenue earned by the heterotrophic- and autotrophic-process designs are generated by the sale of the extracted lipids as a fatty acid oil that can be utilized in renewable-fuel production, such as the production of biodiesel and/or the production of renewable chemicals and from the sale of the lipid-lean biomass. The value for the fatty acid oil is assumed to be \$0.40/kg based on a commonly reported value for these types of oils [43]. The lipid-lean biomass is assumed to generate revenue as a high-protein animal feed [18]. Table 8 reports the analysis of the revenues from the product and by-product. The total yearly revenues for the heterotrophic and autotrophic processes, respectively, are ~\$1.3 million and ~\$3.1 million. No tax credits associated with 'green'-products production are added to these revenues. In order to drive each process towards

non-subsidized economic viability, the total revenue for each process needs to increase. The current economic analysis indicates that each process generates less revenue than is required to cover the operating costs. The total revenue would increase if either the fatty acid-based oil could be sold at a higher value or if a higher-value co-product was also produced.

2.5 Overall profitability

The cash-flow sheets for the heterotrophic- and autotrophic-process designs are reported in Tables 9 and 10, respectively, and indicate the overall profitability of each option. The FCI required for each project is spread out over a 15-month preliminary schedule. Over the 20-year lifetime of the heterotrophic project, a NPV@20% of -\$20 million \pm 40% is estimated, whereas the autotrophic project has a NPV@20% of -\$850 million \pm 40%. Based on this economic assessment, both options are expected to be unprofitable. If a tax credit of \$11.0/L is added to the revenues, the heterotrophic process will rise to the breakeven point.

Adjusting the revenue price of the primary product, the breakeven point for the heterotrophic process corresponds to an oil-products price of \$14/kg (\$3.30/gal), whereas the comparable sales price for the autotrophic process is \$240/

kg (\$126/gal). In 2014, a process design with economics produced by the National Renewable Energy Laboratory (NREL) [44] indicated that the breakeven point for a microalgae oil was \$4.35/gallon gasoline equivalent (GGE). The goal is to advance microalgae-oil extraction to a cost of \$3/GGE [44]. For the designed heterotrophic process, the oil price is determined to be \$3.89/GGE. These results suggest that, if one or more of the key steps in the process can be made more cost-efficient, heterotrophic-microalgae production may become a competitive source for renewable biofuel/chemical-oil feedstock.

3 Conclusion

The objective of the scoping study was to determine whether a process for the growth and extraction of lipids from the heterotrophic-microalgae strain of *C. vulgaris* would be more economically attractive than a process based on the autotrophic version of the same microalgae. A process design was developed for the growth and extraction of lipids from the heterotrophic strain of *C. vulgaris* and the autotrophic strain of *C. vulgaris*. Using the heterotrophic strain was clearly more cost-effective than using the autotrophic strain, although, currently, neither the heterotrophic- nor the autotrophic-process designs are economically feasible. However, the heterotrophic-based process was close to the breakeven point, suggesting that this strategy had the potential, with additional advances, of providing a commercially viable industrial microalgae-oil generation and extraction facility.

Several recommendations to improve the economic feasibility of this technology could be concluded from the design. The two areas that appeared to have the most room for improvement were the growth phase and the fatty-acid solvent-extraction phase. During the heterotrophic-microalgae-growth phase, the media required a large quantity of chemicals and an organic carbon for production. If a lower-cost growth media was identified and/or if an alternative organic carbon source, such as a wastewater stream routed from another industrial process, were utilized, the cost of growing the microalgae would decrease greatly. Additionally, the cell density of the microalgae in the growth media was very low, resulting in a large water requirement. The large water requirement caused the dewatering of the microalgae to be energy-intensive. If a method of increasing cell density during growth was developed, the cost of the growth phase would decrease.

The fatty acid solvent extraction required a low ratio of microalgae to solvent to efficiently extract the oils resulting in an oil-rich solvent with only 3.9 wt% methanol. This low concentration led to the selection of a multi-effect evaporator to most efficiently separate the two miscible liquids. If a more efficient solvent-extraction step were developed, the cost of the solvent recovery would decrease and the

separation step would be simplified, pushing the economics of the process towards profitability. Further, adding a less energy-intensive pre-concentration step for the oil-in-methanol solution, such as using a pervaporation membrane, may also further reduce costs.

Supplementary data

Supplementary data is available at *Clean Energy* online.

Acknowledgements

The authors would like to thank the following for their contributions to this work: Edward Kolodka, Yun Ji, Ian Foerster and Maram Al-Sayaghi. Funding for the experimental work was provided by the US National Science Foundation International Research Experiences for Students programme. This work was supported by the National Science Foundation, Grant No. 1458962. Any opinions, findings and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the NSF.

Conflict of Interest

None declared.

References

- [1] Bwapwa JK, Anandraj A, Trois C. Possibilities for conversion of microalgae oil into aviation fuel: a review. *Renew Sust Energ Rev* 2017; 80:1345–4.
- [2] Ray M, Kumar N, Kumar V, et al. Chapter 12—microalgae: a way forward approach towards wastewater treatment and bio-fuel production. *Appl Microbiol Bioeng* 2019; 229–43.
- [3] Milano J, Ong HC, Masjiki HH, et al. Microalgae biofuels as an alternative to fossil fuel for power generation. *Renew Sustain Energ Rev* 2016; 58:180–97.
- [4] Bhattacharya M, Goswami S. Microalgae—a green multi-product biorefinery for future industrial prospects. *Biocatal Agric Biotechnol* 2020; 25:101580.
- [5] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. *Renew Sustain Energ Rev* 2010; 14:217–32.
- [6] Bhwimick G, Sarmah A, Ramkrishna S. Performance evaluation of an outdoor algal biorefinery for sustainable production of biomass, lipid and lutein valorizing flue-gas carbon dioxide and wastewater cocktail. *Bioresour Technol* 2019; 283:198–206.
- [7] Enamal M, Enamala S, Chavali M, et al. Production of biofuels from microalgae—a review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. *Renew Sustain Energ Rev* 2018; 94:49–68.
- [8] Chandra R, Iqbal HM, Vishal G, et al. Algal biorefinery: a sustainable approach to valorize algal-based biomass towards multiple product recovery. *Bioresour Technol* 2019; 278:346–59.
- [9] Khoo K, Chew KW, Yew GY, et al. Recent advances in downstream processing of microalgae lipid recovery for biofuel production. *Bioresour Technol* 2020; 304:122996.
- [10] Fishmans D, Majumdar R, Morello J, et al. *National Algal Biofuels Technology Roadmap*. College Park, MD: US Department of Energy: Biomass Program, 2010.

- [11] Ghidossi T, Marison I, Rosaleen D, et al. Characterization and optimization of a fermentation process for the production of high cell densities and lipids using heterotrophic cultivation of *Chlorella protothecoides*. *Indust Biotechnol* 2017; 13:253–9.
- [12] Ye Y, Huang Y, Xia A, et al. Optimizing culture conditions for heterotrophic-assisted photoautotrophic biofilm growth of *Chlorella vulgaris* to simultaneously improve microalgae biomass and lipid productivity. *Bioresour Technol* 2018; 270:80–7.
- [13] Parsons S, Chuck CJ, McManus MC. Microbial lipids: progress in life cycle assessment (LCA) and future outlook of heterotrophic algae and yeast-derived oils. 2018; 172:661–72.
- [14] Liu J, et al. Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: assessment of algal oils for biodiesel production. *Bioresour Technol* 2011; 102:106–10.
- [15] Hu J, Nagarajan D, Zhang Q, et al. Heterotrophic cultivation of microalgae for pigment production: a review. *Biotechnol Adv* 2018; 36:54–67.
- [16] Rattanapoltee P, Kaewkannetra P. Cultivation of microalga, *Chlorella vulgaris* under different auto-hetero-mixotrophic growths as a raw material during biodiesel production and cost evaluation. *Energy* 2014; 78:4–8.
- [17] Olesik J. A study of the feasibility of growth, extraction, and industrial scale up processing of microalgae lipids. Master's Thesis. University of North Dakota, April 2020.
- [18] NASDAQ. Soybean Meal Trend. 2017. Trend price for soybean meal. <https://www.nasdaq.com/market-activity/commodities/zm> (March 2017, date last accessed).
- [19] Ulrich GD, Vasudevan PT. *Chemical Engineering Process Design and Economics: A Practical Guide*, 2nd edn. Durham, NH: Process Publishing, 2004.
- [20] Cost Estimate Classification System—as Applied in Engineering, Procurement, and Construction for the Process Industries. Morgantown, WV: AACE International Recommended Practice No. 18R-97, 2005.
- [21] Zuniga C, Li C-T, Huelsman T, et al. Genome-scale metabolic model for the green alga *Chlorella vulgaris* UTEX 395 accurately predicts phenotypes under autotrophic, heterotrophic, and mixotrophic growth conditions. *Am Soc Plant Biol* 2016; 172:589–602.
- [22] Vaiciulyte S, Padovani G, Kostkeviciene J, et al. Batch growth of *Chlorella vulgaris* CCALA 896 versus semi-continuous regimen for enhancing oil-rich biomass productivity. *Energies* 2014; 7:3840–57.
- [23] Information, N.C.f.B. Sucrose. <https://pubchem.ncbi.nlm.nih.gov/compound/sucrose> (March 2017, date last accessed).
- [24] Table of Solubility. 2017. <http://periodic-table-of-elements.org/SOLUBILITY> (March 2017, date last accessed).
- [25] Turton R, Bailie RC, Whiting WB, et al. *Analysis, Synthesis, and Design of Chemical Process*, 4th edn. New York, NY: Pearson, 2013.
- [26] Shuler ML, Kargi F. *Bioprocess Engineering: Basic Concepts*. Upper Saddle River, NJ: Prentice Hall PTR, 2010.
- [27] *Chemical Engineering Essentials for the CPI Professional*. <https://www.chemengonline.com/>.
- [28] Group N.-C. Sugar Commodity Price. 2017. <https://www.indexmundi.com/commodities/?commodity=sugar>.
- [29] Luckow P, Stanton E, Fields S, et al. 2015 Carbon Dioxide Price Forecast. Cambridge, MA: Synapse Energy Economics, Inc., 2015.
- [30] Biller P, Ross AB, Skill SC, et al. Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. *Algal Res* 2012; 1:70–6.
- [31] Sodium Nitrate. 2017. <https://xsolid.manufacturer.globalsources.com/si/6008825535787/pdtl/Industrial-chemical/1151269759/Sodium-nitrate.htm> (March 2017, date last accessed).
- [32] Calcium Chloride. 2017. <https://www.globalsources.com/Inorganic-chemical/Calcium-Chloride-1175436804p.htm> (March 2017, date last accessed).
- [33] Magnesium Sulfate. 2017. <https://www.7springsfarm.com/epsom-salt-technical-grade-5-lb-bag/> (March 2017, date last accessed).
- [34] Dipotassium Hydrogen Phosphate. 2017. <https://detail.en.china.cn/provide/p113405303.html> (March 2017, date last accessed).
- [35] Potassium Dihydrogen Phosphate. 2017. <https://detail.en.china.cn/provide/p152661124.html> (March 2017, date last accessed).
- [36] Sodium Chloride. 2017. https://m.alibaba.com/mobile/padvpc.html?refer=https%3A%2F%2Fwww.alibaba.com%2Fproduct-detail%2F99-5-min-sodium-chloride-price_60754596797.htm%3Fspm%3Da2700.7735675.2017115.9.SqddyO%26s%3Dp (March 2017, date last accessed).
- [37] Law J. Personal Communication to obtain Quote for EDTA. March 15, 2017.
- [38] Dry Yeast Powder. 2017. <https://shop.chemicalstore.com/index.asp?MySessionID=332-899555602> (March 2017, date last accessed).
- [39] Acidified Iron Stock Solution. 2017. <https://shop.chemicalstore.com/index.asp?MySessionID=332-899555602> (March 2017, date last accessed).
- [40] Boric Acid. 2017. https://www.alibaba.com/product-detail/cas-10043-35-3-99-5_60765411777.html?spm=a2700.icbuShop.prewdfa4cf.2.26c27fece0pySb (March 2017, date last accessed).
- [41] Statistics, U.S.B.o.L. Chemical Plant and System Operators. 2017. <https://www.bls.gov/oes/2017/may/oes518091.htm> (March 2017, date last accessed).
- [42] Methanex. Methanol Prices. 2017. <https://www.methanex.com/our-business/pricing> (March 2017, date last accessed).
- [43] Stewart GW. Bio-Oil Commercialization Plan. Bluffton, SC: Cole Hill Associates, 2004.
- [44] Davis R, Kinchin C, Markham ECD. Process design and economics for the conversion of algal biomass to biofuels: algal biomass fractionation to lipid and carbohydrate-derived fuel products. National Renewable Energy Laboratory, 2014.