Recovery of Lipids from Algae

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Abstract One of the crucial steps in generating biofuel from algae is the separation and recovery of lipids from algal biomass. These lipids are eventually converted into liquid biofuel after processing and refining. This chapter presents an overview of extraction techniques and some of the challenges in applying these techniques to industrial-scale algal biofuel production. Lipids are well-encased inside algal cell walls. The aqueous environment of the cells makes it even more difficult to extract the lipids. Hexane extraction is presently the most economical method. Cell-disrupting methods have been attempted as complementary techniques to hexane extraction. Other methods such as super-critical fluid extraction and microwave extraction that may prove better in future are still in developmental stages. The need to dry the algal biomass is a key challenge in hexane extraction. The development of on-site smaller-capacity technologies can be another vital step to enhance industrial-scale biofuel production.

Keywords Microalgae · Extraction · Lipid · Extraction efficiency · Hexane extraction · Supercritical carbon dioxide extraction · Extraction cost · On-site technology · Scale-up

Acronyms

DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid

1 Introduction

Fahy et al. (2009) define lipids as "hydrophobic or amphipathic small molecules that may originate entirely or in part by carbanion-based condensations of thioesters (fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and

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polyketides) and/or by carbocation-based condensations of isoprene units (prenol lipids and sterol lipids).” Algal lipids are mainly found as (a) lipid droplets inside the cytoplasm and (b) cell membrane-bound components. Lipid droplets are mostly in the form of triacyl glycerides and fatty acids. Membrane-bound lipids often tend to be phospholipids. They are intracellular components that are well-encased by the cell wall. Some algal species, such as *Botryococcus braunii* also produce straight chain hydrocarbon type of lipids (C8-C30 alkanes and alkenes) that are predominantly extracellular (Wolf et al. 1985).

2 Challenges in Algal Lipid Extraction

Typical vegetable oils are interspersed copiously inside the oilseed kernel. The seed kernel is ripe with oil (25–60 % by fresh weight) and even a slight press with fingers is enough to feel the expelled oil (Pramanik 2003; Akintayo 2004). Algal lipids, however, are well-bound by the thick cell walls of individual algal cells. Compared with oilseeds, algal lipid content is comparatively lower—typically 1.5–4 % by fresh weight or 15–40 % by dry weight (Chisti 2007)—but the lipid yield per unit area of cultivation is much higher. Vegetable oil inside oilseeds can often be efficiently extracted by mechanical pressing in a first step; only the remnant needs to be extracted by solvent extraction for complete oil recovery. For algal lipids, mechanical pressing is not effective and more intensive extraction procedures such as solvent extraction are needed from the beginning. The presence of sturdy cell wall and the sparse distribution in larger biomass load presents the major problem for the extraction of algal lipids.

Lipids are inside the aqueous environment of algal cell. This tends to resist the diffusion of non-polar solvents such as hexane, if such a solvent is employed straightaway. If a polar solvent such as methanol is employed before that, it may diffuse inside the cell first and facilitate the diffusion of less polar solvent later. Such polar solvents are not effective by themselves to extract the algal lipids. The algal cells may need to be dried first to facilitate effective extraction employing non-polar solvents directly. Even when the algal cells are dried, the interior cellular components are still hydrophobic; therefore diffusion of a polar solvent first may still be desirable. Also, the quantity of solvent needed to extract lipids from wet cells is normally much more than for dry cells.

The different types of lipids inside the algal cells show varying degrees of extraction effectiveness depending upon the type of solvents used. To effectively extract neutral lipids, non-polar solvents such as hexane are needed. To effectively extract phospholipids, polar solvents such as acetone are required (Halim et al. 2012; Vandana et al. 2001). At times a mixture of solvents with specific composition or a series of solvents may be necessary for effective extraction. Neutral lipids are hydrophobic and have weak van der Waals attraction between the molecules. A non-polar organic solvent such as hexane penetrates into the cell, interacts with

neutral lipid molecules forming the globular droplet through similar van der Waals force, and forms an organic solvent-lipid complex. This complex elutes out of the cell because of the concentration gradient. Some neutral lipids are found as a complex with polar lipids. This complex is in strong hydrogen bonding with proteins in the cell membrane. The van der Waals interaction between non-polar organic solvent and neutral lipids is too weak to disrupt the membrane-associated lipid-protein complexes. But a polar organic solvent such as methanol or iso-propyl alcohol can disrupt such lipid-protein associations through hydrogen bonds with polar lipids. This complex can then similarly elute out from the cell membrane by concentration gradient (Halim et al. 2012; Kates 1986; Medina et al. 1998).

Extraction efficiency can be strain-specific. Some algal species such as *Dunaliella salina* and *Dunaliella tertiolecta* lack the sturdy primary cell wall that makes extraction of intra-cellular lipids difficult. They contain only the secondary cell membrane. Lipid extraction may be easier with such species. Extraction efficiency can also depend on the lipid content; efficiency is normally low for cells with low lipid content.

The kinetics of extraction of lipids out of the cells was reported to follow first order kinetics, attributed to diffusion based on concentration gradient. The solvent: (dry) biomass ratio depends on lipid content and the respective solvent-cellular interaction (strain-specific) (Halim et al. 2012). Increase in temperature has been reported to result in increase in lipid yield in many cases. But in some cases, beyond a certain temperature oxidative degradation may lower the yield (Halim et al. 2012). The extent of lipid extraction by a solvent is also limited by thermodynamic phase equilibrium (Wang and Weller 2006). Multiple washing may be needed to increase the yield beyond a certain limit.

High value metabolites such as astaxanthin, β-carotene, and omega fatty acids are recovered from crude extracts by various chromatographic methods.

3 Extraction Methods

3.1 Solvent Extraction

Solvent extraction involves using a solvent (usually non-polar organic, sometimes in combination with polar co-solvents) that permeates inside the algal cells through the cell wall, attempts to fill the cells inside and outside as a continuum, and helps partitioning lipids out of the cells. The algal biomass is then typically separated by gravity. Lipids are recovered by stripping the solvent off. Hexane, cyclohexane (Harun et al. 2010), and heptane (Horst et al. 2012) are commonly used for solvent extraction. Benzene, ether, acetone (Harun et al. 2010), and other polar co-solvents such as methanol (Bligh and Dyer 1959) have also been used.

Folch method (Folch et al. 1957) and Bligh-Dyer method (Bligh and Dyer 1959) employing methanol-chloroform mixtures are generally used as analytical methods.

These methods require large quantities of solvents that are expensive and toxic. Therefore they are not preferred for industrial-scale extraction. Soxhlet hexane extraction method refers to an extraction process where the solvent is heated, percolated, and repeatedly refluxed to extract lipids. This method, however, provides low lipid yields (Mercer and Armenta 2011; Halim et al. 2010).

Hexane extraction efficiencies have been rated around 95 % (Stephenson et al. 2010; Frank et al. 2011). Nagle and Lemke (1989) reported 90 % extraction efficiency for n-butanol and 78 % for hexane/2-propanol (40/60, vol %) (400 g wet biomass (15 % solids), 1200 g solvent, boiling temperature, 90 min). This technology is industrial-scale.

Martek Biosciences Corporation (2003) has employed hexane for extraction of the poly-unsaturated fatty acid docosahexaenoic acid (DHA) from *Schizochytrium* sp. for use as a food ingredient. The report states: "The de-oiled biomass is separated from the oil-rich hexane phase (miscella) by centrifugation and/or filtration. The miscella is chilled and held for a period of time to allow any saturated fats, or other high melting point components (stearins), to crystallize (winterization). The chilled miscella is centrifuged and/or filtered to remove the solid phase. Hexane is then removed from the miscella, leaving behind the winterized oil." Hexane extraction facilities are well-known for vegetable oil extraction (Lundquist et al. 2010).

The need to dry the algal biomass before extraction is the main challenge with solvent extraction. The solvent requirement is much higher (by an order of a magnitude) if algae are not dried. The Aquatic Species Program reported that direct solvent extraction was unlikely to be feasible for wet biomass (Sheehan et al. 1998). Solvents are toxic and pose handling concerns. Compromising the value of high-value byproducts such as nutraceuticals is another concern (Sahena et al. 2009). The options for benign solvents for industrial applications are limited. Also, energy is lost during residual solvent recovery from deoiled algal biomass.

Hexane extraction is presently the most economical option for extracting algal lipids. Other methods may become less expensive in future after further research and development. A study conducted by Lundquist et al. (2010) showed that the cost of hexane extraction, modeled similar to the soybean oil extraction facilities, would depend on the scale of the system. The cost of extraction was calculated to be $60/barrel for a model based on a 100 T/d facility shared by five 100-hectare raceway pond systems. But the cost decreased to ~ $15/barrel for a model based on a 4,000 T/d facility shared by fifty 400-hectare systems. The cost was calculated based on the extraction plant capital cost, operating cost, and the cost to transport algae from harvest site to the extraction plant. The study stressed that development of on-site smaller capacity extraction technologies that could handle algal biomass grown locally were crucial to reduce the cost of algal lipid extraction. This would reduce the considerable cost of transporting harvested biomass to remote large extraction facilities.

3.1.1 Complementary Methods

Mechanical Methods

Extraction can be facilitated by physically rupturing or disrupting the algal cells. Such techniques usually complement solvent extraction. Mechanical methods include pressing, grinding with beads that agitate and collide with cell walls, and wet milling. Shen et al. (2009) compared different mechanical disruption methods and concluded that optimum methods would be strain-specific, depending on cellular characteristics. *Scenedesmus dimorphus* (big, bean-shaped and clustered cells) showed the best extraction (19% higher) with wet milling (15 mL concentrated algae, Straub, 100-g (Straub Co., Hatboro, PA), three times). *Chlorella protothecoides* (small and round cells) showed the best extraction (16% higher) with bead-beating (15 mL concentrated algae, bead-beater, Model 3110 BX with 1 mm glass beads (Biospec, Bartlesville, OK), 2 min, twice). Solvent system used was ethanol/hexane (1:1, V/V). Pure hexane resulted in better extraction (16 and 29.7% respectively). Wet milling has large-scale commercial application in starch processing industry, and has been reported to have easy and low-cost operation and maintenance. Bead-beating, even if efficient, has been reported to be limited to small-scale use. Shen et al. (2009) reported that the other methods tested (sonication and French press) were either too expensive or low in lipid recovery. Doucha and Livansky (2008) reported that the extent of disruption in bead milling depended on many variables that determined the nature of collisions between the beads and the algal cells, including shape, size, speed, spatial density, and material properties of the beads, as well as cellular characteristics. The aforementioned Martek process employed wet milling (algal suspension in hexane) as the complementary method (Martek Biosciences Corporation 2003).

Sonication

Extraction can also be ultrasonic-aided, with sonication-induced cavitation acting as an agent of cell disruption (Harun et al. 2010; Shen et al. 2009). Algal cell walls are damaged when cavitation happens near them. This enhances mass transfer and facilitates solvent access to the cell content (Cravotto et al. 2004). The effect has been reported to be much stronger at low frequencies (18–40 kHz) and negligible at 400–800 kHz. Cravotto et al. (2008) reported that ultrasonic assisted extraction proved to be the best when compared with microwave-assisted extraction and Soxhlet extraction for the marine microalga *Crypthecodinium cohnii* rich in DHA. The yield was 25.9% (based on algae dry mass) for ultrasound (8 g sample, 50 mL solvent, 45 °C, 0.5 h) compared with 4.8% for Soxhlet (15 g, 100 mL, reflux, 4 h) and 17.8% for microwave (2 g, 35 mL, 120 °C, pressurized, 0.5 h). Zheng and Hu (2007) reported that ultrasound not only reduced extraction temperature, time, and solvent quantity, but also increased the lipid extraction yield (79.5 and 81.5% extraction efficiencies of DHA and EPA by ultrasound (1 h, 1:4.5 algae mass:solvent

volume); 63.6 and 64.9% by solvent extraction (2 h, 1:5.5)). The method has already found application in the food industry (Cravotto and Cintas 2007). There are some concerns on uncontrolled destruction of biomass that may lyse the lipid molecules randomly, but there has not been much evidence of it. Question marks remain on the scale-up and cost of this method for biofuel production.

Enzymatic Extraction

Enzymes can be employed to degrade the cell walls (Shah et al. 2004). Cell walls can be limited to partial degradation if needed. Shah et al. (2004) reported a three phase partition system for extraction of oil from *Jatropha curcas* L. seeds using enzyme treatment as a complementary method. It involved simultaneous addition of t-butanol (1:1, v/v) and ammonium sulfate (30% w/v) to the seed slurry (5 g seed/30 mL water). The yield was 82% without enzymatic treatment. With commercial fungal protease enzyme, Protizyme (250 mg, 50 °C, stirring, 1 h, pH 9), the yield was 92%. The yield improved to 97% with sonication (5 min). Soto et al. (2007) reported that enzymatic treatment (0.25% E/S of Olivex–Celluclast (1:1) mixture) improved the oil yield of borage meal from 88 to 95% (45 °C, 20% moisture, 9 hours, double pressing (39.2 MPa), pre-heat (5 min, 70 °C)).

Water itself has been used as a solvent (Institute for Applied Environmental Economics 1995). The enzyme was SP 311, developed by Novo Nordisk, based on *Aspergilus niger*. Lipids suspended in water were easily recovered. Downstream separation of lipids and proteins was much easier than solvent extraction, and their quality was also higher. Enzymatic extraction was much costlier than hexane extraction, and recovery of lipids was lower by 8%.

Simultaneous Extraction and Transesterification

Simultaneous extraction and transesterification of algal lipids has also been pursued (Belarbi et al. 2000; Lewis et al. 2000) and has been reported to be 15–20% more efficient than the separate extraction and transesterification processes. Molina Grima et al. (2003) showed that the cost of simultaneous extraction-transesterification of a high-value product, omega fatty acids, using a strain with lipid content of 10% (dry basis) was $360/kg of esterified oil for a smaller-scale on-site 8 kg/d oil production facility (80 kg/d biomass). The method has been demonstrated at pilot scale. The cost may be correspondingly lower for larger-scale biofuel system using strains with higher lipid content, eventually approaching numbers similar to those indicated by Lundquist et al. (2010). Similarly, simultaneous extraction and saponification has also been carried out to extract the free fatty acids from wet algal biomass using a mixture of potassium hydroxide and ethanol (Gimenez Gimenez et al. 1998; Robles Medina et al. 1995).

Chromatography

Chromatographic methods are employed to recover high-value metabolites from crude extracts. High pressure liquid chromatography and gas chromatography methods are employed to recover astaxanthin, β-carotene, and omega fatty acids (Molina Grima et al. 2003). Omega fatty acids are recovered by methods such as reverse phase chromatography, silica gel adsorption chromatography, and argentated silica gel chromatography (Belarbi et al. 2000; Gimenez Gimenez et al. 1998; Robles Medina et al. 1995). Supercritical fluid chromatography has been employed to recover astaxanthin, β-carotene, and omega fatty acids (Lim et al. 2002). Membrane-based selective enrichment has been reported to be another alternative (Molina Grima et al. 2003). The chromatographic methods are already in commercial use. For the smaller-scale on-site facility referred to by Molina Grima et al. (2003), argentated silical gel chromatography was used, and the estimated cost of recovering EPA from crude extract was $4,205/kg. This number, however, can be expected to be lower for larger scale as reported by Lundquist et al. (2010). Martek Biosciences Corporation has been marketing DHA as a food ingredient. At present one can obtain omega fatty acid for $75–110 per kg retail (www.vitamins.org).

The scale-up of the cell disrupting complementary methods is yet to be ascertained for viable biofuel production.

3.2 Super-Critical Carbon Dioxide Extraction

This method refers to extracting lipids using carbon dioxide above its critical point (31 °C, 74 atm) (Cooney et al. 2009; Sahena et al. 2009). A supercritical fluid is rated to have excellent extraction ability because it combines solvation contact property of liquids with high diffusion constants of gases. Other advantages of this method are non-toxicity, chemical inertness, and the simplicity of separation of lipids from gaseous carbon dioxide after extraction. Use of a co-solvent such as ethanol has been reported to increase the solvating property of carbon dioxide (Mendiola et al. 2007a; Mendes et al. 2006). The polarity of the solvent is increased and its viscosity is changed. Temperature and pressure are also lowered. One of the advantages of supercritical fluid extraction is the tunable solvent power (Halim et al. 2012). This is a function of density and can be changed by temperature and pressure. Co-solvent and flow rate are the other extraction variables.

Supercritical fluid extraction has been reported to result in higher yields than organic solvents and has higher selectivity for triglycerides (~99 %) (Mendes et al. 2006; Cheng et al. 2011). Supercritical carbon dioxide is non-polar, and addition of a polar co-solvent can aid the yield of polar lipids (Halim et al. 2012). With 20 % ethanol, more than 80 % of phospholipids were reported to be recovered from salmon roe (Tanaka et al. 2004).

Moisture can reduce the effectiveness of supercritical fluid extraction; therefore samples are dried before extraction. Moisture has been reported to impart pasty

consistency to the biomass, and presents a barrier to diffusion of carbon dioxide into biomass and diffusion of lipids out of the cells (Dunford and Temelli 1997; Sahena et al. 2009).

Increase in supercritical carbon dioxide flow results in better contact between the solvent and the lipids, but it can also result in uneven fluid penetration and dead volumes in the vessel (Pourmortazavi and Hajimirsadeghi 2007; Halim et al. 2012). The density of biomass packing as fixed bed is another important factor in extraction. Packing density is related to biomass powder particulate size and volumetric ratio of packing material to biomass powder. Higher packing increases the lipid elution rate, but it can also compromise extraction kinetics by fluid channeling (Pourmortazavi and Hajimirsadeghi 2007).

The cost of supercritical fluid extraction is relatively high because of the high capital costs and the high operating pressures. The cost is likely to come down with time and as more development work is done on this technology. The benign nature of the solvent and the simplicity it offers in downstream processing make this process attractive. The method is still viewed to be only at its initial stages of development given the cost-competitiveness required for biofuel production. Scale-up process design is a key area of development needed for the method. Lowering pressure by using co-solvent may be explored. The method has so far found industrial applications such as

- extraction of
  - tea and coffee (decaffeination)
  - natural products
  - pharmaceuticals
  - herbs
  - essential oils, fats
  - flavors
  - natural colorants (from carrot, alfalfa leaf, sweet potato, tomato, red grape)
  - anti-oxidants
  - oil from nuts (almond, peanut, walnut, hazelnut, pistachios)
  - cholesterol (from egg yolk, meat, milk fat)
- nutrition labelling related fractionated modification
- dealcoholization of alcoholic beverages
- degreasing and dry cleaning

(Mendiola et al. 2007b; Bell 2009; Stewart 2003; Sahena et al. 2009).

Prempiyawat et al. (2011) report that for 93.4% removal of hydrocarbon contaminants (10% wt) from solids (9,000 kg of solids/year, 8 h/d operation), the total capital investment was $ 115,310, the operating cost was $ 5,020/year, and the energy requirement was 3.21 kW. Another study (Gifford et al. 2001) compared supercritical carbon dioxide extraction and hexane extraction for peanut oil as 1.8 GWh/year vs 4.6 GWh/year energy input, $ 6.2M/y vs $ 14M/y operating cost (peanut feed 10 million lb/y; yield 30%; extraction conditions: 550 bar, 55 °C; separation condition: 270 bar).

3.3 Microwave Extraction

When algal cells are mixed in a non-polar solvent such as hexane and subjected to microwave treatment, the polar moisture-laden algal cells are preferentially heated and punctured by the increasing temperature and pressure (Balasubramanian et al. 2011; Cravotto et al. 2008). The lipids inside the cells then partition toward the more preferred lipophilic medium, hexane. Thus the entire mixture (predominant portion as solvent) does not need to be heated, and the extraction energy is efficiently and preferentially transferred to the moisture-laden algal cells. An attractive feature of this method is the ability to treat wet biomass. The method has been reported to be rapid, relatively safer (Paré et al. 1997), and needs reduced solvent quantities (Cravotto et al. 2008).

Balasubramanian et al. (2011) reported a 1.2 kW, 2,450 MHz resonant continuous microwave system, designed and optimized for lipid extraction from *Scenedesmus obliquus*. Extraction efficiency of 76–77 % (31 % based on algae dry mass) was reported at 95 °C after 20–30 min (algae:water 1:1 w/w). Cravotto et al. (2008) reported 17.8 % oil yield (based on algae dry mass) for marine microalga, *Crypthecodinium cohnii*, by microwave extraction (2 g sample, 35 mL hexane, 120 °C/pressurized, 0.5 h). Microwave treatment was carried out in a commercial multimode oven operating at 2.45 GHz (Microsynth, Milestone, Italy). The yield was comparable to ultrasound extraction (25.9 %) and Soxhlet extraction (4.8 %) as mentioned earlier. Microwave extraction, along with ultrasound, was mentioned to be promising methods for algal lipid extraction.

The method has already found application in the food industry (Cravotto and Cintas 2007) and in the extraction of essential oils (Lucchesi et al. 2004). Scale-up development is needed for the method to become viable for biofuel industry (Mercer and Armenta 2011). The faster extraction feature of the method has been cited for better economics (Joshi et al. 2010). Nurdin (2007) reported that for the extraction of essential oils from *Mesua ferrea* L. leaves, microwave extraction was about 8 times faster, used 13–18 times less energy, and reduced cost from Malaysian currency RM 11.77 to RM 3.71–5.23 (dry distillation, wet distillation) compared with conventional method (hydro distillation, steam distillation).

3.4 Electro-Mechanical Pulsing

In this method, an electric field is applied to aqueous algal suspension in a pulsed fashion. The algal cells are stretched back and forth as the electric field is pulsed. This permeates and punctures the cell wall expelling the intracellular components outside. Lipids can thus be recovered from the intracellular and membrane components (Hebner et al. 2011; Guderjan et al. 2007).

For this method, non-corrosive electrodes need to be used; otherwise metals may leach into solution, potentially contaminating the algal biomass and lowering the by-product value of de-oiled algal biomass. The energy balance of the process is

affected by (a) electrical conductivity of the medium and cellular components, (b) the type of electrode material, (c) spacing and surface area of electrodes, and (d) the type of algae strain.

3.5 Extracellular Lipids

Extracellular lipids found in some algae such as the *Botryococcus* species are much easier to extract. Nevertheless, growing these species has proved to be difficult. Their growth rate is slower (about one-fourth the growth rate of *Nannochloropsis oculata*) (Metzger and Largeau 2005). One way to address this is believed to be avoiding colony formation while growing such algae. Also, these types of algae prefer freshwater and brackish water for growth, and may therefore not be able to exploit the abundant cheaper water source, sea water, for mass cultivation.

3.6 Live Extraction (Milking)

Researchers have developed a method to extract value components such as β-carotene from the algal strain *Dunaliella salina* while the cell is alive in its growth medium (Hejazi et al. 2004; Mojaat et al. 2008). A separate biocompatible organic phase such as decane and dichloromethane-decane mixture is mixed with the aqueous algal growth phase. The biocompatible organic component extracts products of interest by attaching to the wall of algal cell. The method avoids killing of algal cells and hence maximizes cellular density for continuous production of compounds of interest. It has been reported that the extraction ability of solvent depends on its affinity with the product extracted and on its concentration incorporated in the cellular membrane (Bligh and Dyer 1959). This intriguing extraction technology is in its nascent stages and may take a few years to realize the full potential.

3.7 Solventless Extraction Process

Recently Martek Biosciences Corporation (Ruecker et al. 2010) reported a "Solventless Extraction Process." The method refers to the use of an aqueous solvent which contains less than 1% of organic solvent. The method does not require the algae to be dried (50% water). The example reported by Ruecker et al. (2010) refers to 400 gallon batches in which 20 g of 45% KOH were added to hydrolyze proteinaceous compounds in algae. Algal broth was heated to 130 °C for 30 min for cell rupture. It was reported that cell rupture methods could be mechanical or chemical. Thermal method has been reported to be particularly useful for cell walls composed of proteins. The lipids are recovered by successive water washing and centrifuging (light lipid layer and heavy aqueous layer). The crude oil extraction

efficiency ranged from 75–89 % (based on original oil content). The final product was reported to be substantially equivalent to hexane-extracted lipids. The method is believed to be already employed by Martek Biosciences Corporation for the commercial production of DHA (as food ingredient).

4 Summary and Concluding Remarks

A variety of techniques exist for extracting lipids from algae, in various stages of development. The most common method currently used is solvent extraction. One of the main challenges with organic solvent extraction is the need to dry the algal biomass before extraction; otherwise the quantity of solvent needed is much higher, which increases processing costs. Development of on-site smaller-capacity processing technologies is crucial for reducing the cost of this technology. To aid solvent extraction process, cell-disrupting complementary methods can be used. Some of the other technologies are in developmental stages and need viable scale-up design. Care must be taken in choosing the extraction method to ensure there is no negative effect on the value of by-products by denaturing, decomposing, or contaminating them. For algae to become a viable biofuel feedstock (a) the energy required to extract and process algal lipids must be much lower than the energy value of the biofuel produced and (b) the capital cost and processing cost must be lower.

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