



Recovery of microalgal biomass and metabolites: process options and economics

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Abstract

Commercial production of intracellular microalgal metabolites requires the following: (1) large-scale monoseptic production of the appropriate microalgal biomass; (2) recovery of the biomass from a relatively dilute broth; (3) extraction of the metabolite from the biomass; and (4) purification of the crude extract. This review examines the options available for recovery of the biomass and the intracellular metabolites from the biomass. Economics of monoseptic production of microalgae in photobioreactors and the downstream recovery of metabolites are discussed using eicosapentaenoic acid (EPA) recovery as a representative case study.

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1. Introduction

Microalgae can be used to produce numerous high-value bioactives (Borowitzka, 1986; Bubrick, 1991; Pulz et al., 2001; Li et al., 2001; Banerjee et al., 2002). Production of

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microalgae-derived metabolites requires processes for culturing the alga (Ben-Amotz and Avron, 1987; Molina Grima, 1999; Molina Grima et al., 1999; Sánchez Mirón et al., 1999; Tredici, 1999; Borowitzka, 1999; Pulz, 2001; Pulz et al., 2001), recovery of the biomass, and further downstream processing to purify the metabolite from the biomass. As with many microbial processes for producing bioactives, the downstream recovery of algal products can be substantially more expensive than the culturing of the alga. This review examines some commercially relevant options for recovering microalgal products. A case study is used to illustrate the economics of recovery of eicosapentaenoic acid (EPA), an essential fatty acid from microalgae. EPA is an established nutraceutical and evidence is emerging for its therapeutic benefits in disease management (Peet et al., 2001, 2002).

2. Production of microalgal biomass

Production of microalgal biomass can be carried out in fully contained photobioreactors or in open ponds and channels. Open-culture systems are almost always located outdoors and rely on natural light for illumination (Terry and Raymond, 1985). Closed photobioreactors may be located indoors or outdoors (Sánchez Mirón et al., 1999; Pulz, 2001), but outdoor location is more common because it can make use of free sunlight. Design and operation of the microalgal biomass production systems have been discussed extensively (Terry and Raymond, 1985; Borowitzka, 1996; Pulz and Scheinbenbogen, 1998; Pulz, 2001; Pulz et al., 2001; Molina Grima, 1999; Molina Grima et al., 1999; Sánchez Mirón et al., 1999; Tredici, 1999). Here, we focus exclusively on issues relating with the harvest of the biomass and its downstream processing to recover intracellular metabolites.

3. Recovery of biomass

Harvesting of biomass requires one or more solid–liquid separation steps. Biomass can be harvested by centrifugation, filtration or in some cases, gravity sedimentation. These processes may be preceded by a flocculation step. Recovery of biomass can be a significant problem because of the small size (3–30 μm diameter) of the algal cells. Culture broths are generally relatively dilute ($<0.5 \text{ kg m}^{-3}$ dry biomass in some commercial production systems) and hence large volumes need to be handled to recover the biomass. No single harvest method may be suited to every case. Recovery of the biomass from the broth has been claimed to contribute 20–30% to the total cost of producing the biomass (Gudin and Therpenier, 1986).

Any suitable harvest method must be able to process the large volumes typical of algal biomass production processes. Filtrative recovery may be unsatisfactory because filtration can be relatively slow. For extremely low value products, gravity sedimentation, possibly enhanced by flocculation, may be the method of choice. Sedimentation tanks or settling ponds are generally used in biomass recovery from sewage-based processes (Venkataraman, 1978). Flocculation–flotation are also used (Shelef, 1978). Centrifugal recovery of the

biomass is feasible for high-value products. Centrifuges can process large volumes relatively rapidly and the biomass can remain fully contained during recovery. A further consideration in selecting a suitable harvest method is the acceptable level of moisture in the product. Gravity sedimented sludge is generally more dilute than centrifugally recovered biomass. Too much moisture in the harvested biomass can substantially influence the economics of product recovery further downstream (Mohn, 1978), if dehydration of the biomass is required after harvest. Because thermal drying is more expensive than mechanical dewatering, thermal drying should be preceded by a mechanical dewatering step such as filtration or centrifugation. Some of the specific harvest methods are discussed next.

3.1. Flocculation

Various methods of flocculation can be used to aggregate the microalgal cells to increase the effective “particle” size and hence ease sedimentation, centrifugal recovery, and filtration (Elmaleh et al., 1991).

Microalgal cells carry a negative charge that prevents aggregation of cells in suspension. The surface charge can be neutralized or reduced by adding flocculants such as multivalent cations and cationic polymers to the broth. Ideally, the flocculants used should be inexpensive, nontoxic, and effective in low concentration. In addition, the flocculant should be selected so that further downstream processing is not adversely affected by its use.

Multivalent metal salts are effective flocculants or coagulants. The commonly used salts include ferric chloride (FeCl_3), aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$, alum) and ferric sulfate ($\text{Fe}_2(\text{SO}_4)_3$). The efficiency of electrolytes to induce coagulation is measured by the critical coagulation concentration, or the concentration required to cause rapid coagulation. Coagulation efficiency of metal ions increases with increasing ionic charge. Multivalent metal salts such as alum have been widely used to flocculate algal biomass in wastewater treatment processes (McGarry, 1970; Dodd, 1979; Benemann et al., 1980; Moraine et al., 1980; Koopman and Lincoln, 1983; Lincoln, 1985). Alum is an effective flocculant for *Scenedesmus* and *Chlorella* (Golueke and Oswald, 1965); however, flocculation by metal salts may be unacceptable if biomass is to be used in certain aquaculture and other applications.

Polyferric sulfate (PFS) is observed to be a better flocculant compared to the more traditional nonpolymerized metal salt flocculants (Jiang et al., 1993). Prepolymerized metal salts are effective over a wider pH range than nonpolymerized salts. Flocculation with prepolymerized metal salts is said to produce flocs that are easily dewatered. Efficiency of some flocculants for removing suspended cells of *Anabaena* and *Asterionella* is shown in Fig. 1.

An alternative to using metal salts is the use of cationic polymers (polyelectrolytes) (Tenney et al., 1969). In addition to reducing or neutralizing the surface charge on cells, the polymer flocculants can bring particles together by physically linking one or more particles through a process called bridging. Tenney et al. (1969) and Tilton et al. (1972) have demonstrated that the bridging mechanism also applies to flocculation of algal cells. Cationic polymers doses of between 1 and 10 mg ml⁻¹ can induce flocculation of freshwater algae; however, a high salinity of the marine environment can inhibit flocculation by polyelectrolytes (Bilanovic et al., 1988). Effective flocculation with polyelectrolytes has been reported

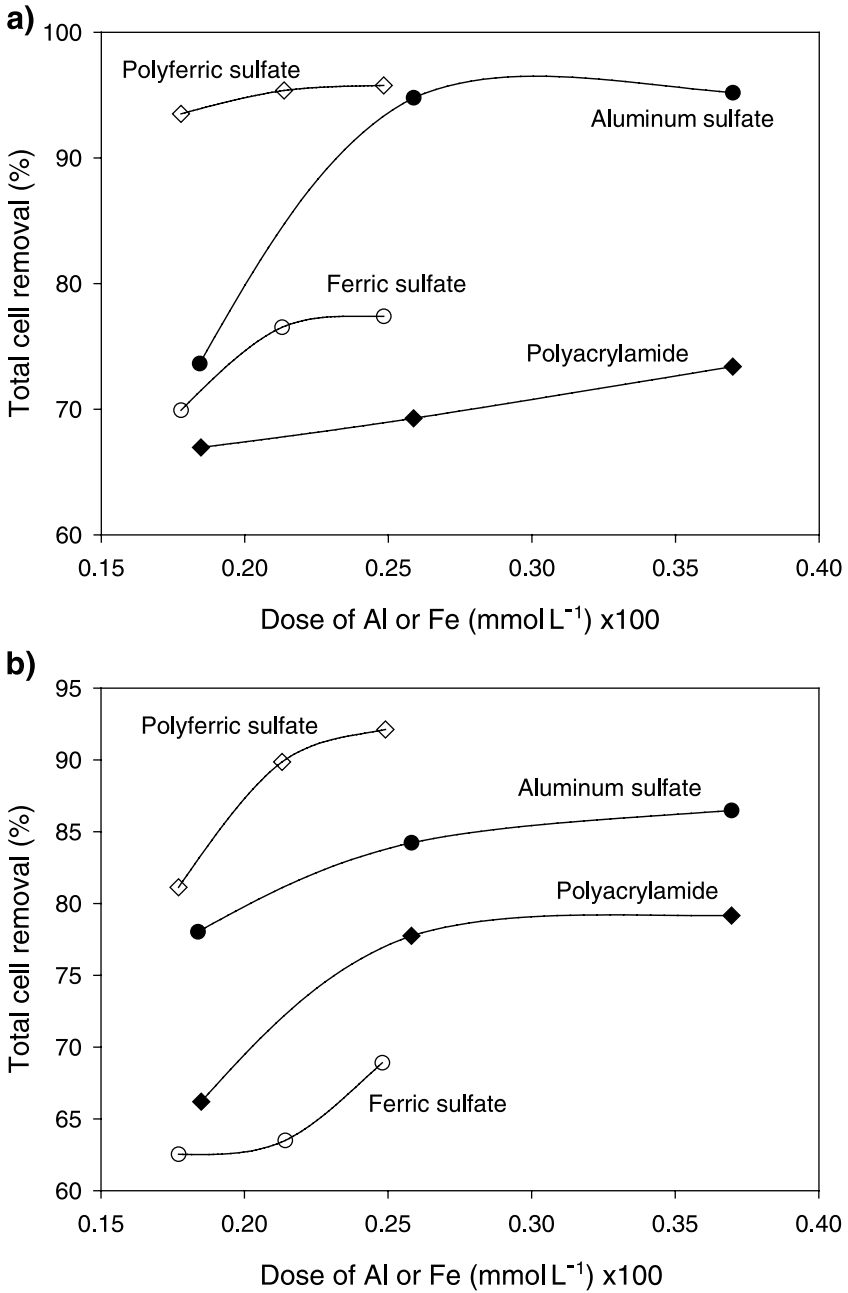


Fig. 1. A comparison of four coagulants for cell removal by flocculation of (a) *Anabaena* and (b) *Asterionella* (Jiang et al., 1993).

at salinity of less than 5 kg m^{-3} (cf. salinity of seawater is $\sim 37 \text{ kg m}^{-3}$). At high ionic strengths, cationic polymers tend to fold tightly and fail to bridge microalgal cells. The effect of ionic strength on flocculation of microalgae by some cationic polymers is shown in Fig. 2. In all cases, the flocculation was reduced by increasing ionic strength of the cell slurry.

The flocculation effectiveness of polyelectrolytes depends on many factors, including the molecular mass of the polymer, the charge density on the molecule, the dose used, the concentration of the biomass, the ionic strength and pH of the broth, and the extent of mixing in the fluid. Generally, high molecular weight polyelectrolytes are better bridging agents. Similarly, a high charge density tends to unfold the polymer molecule, improving its bridging performance and the ability to neutralize the surface charge on cells. A high cell concentration in the broth helps flocculation, because the cell–cell encounters are more frequent in concentrated suspensions. A certain low level of mixing is useful as it helps bring the cells together; however, excessive shear forces can disrupt flocs (Chisti, 1999).

Polymeric flocculants have been used extensively for recovering microalgal biomass. However, in comparison with salts such as aluminum sulfate, cationic polyelectrolytes may be less effective (Pushparaj et al., 1993). Studies with *Chlorella ellipsoidia* at biomass concentration of $0.05\text{--}3 \text{ kg m}^{-3}$ and polymer concentration of 1×10^{-5} to 1 kg m^{-3} have shown an absence of flocculation at polymer concentration up to 0.2 kg m^{-3} (Tilton et al., 1972). Cationic polyethyleneimine is an effective flocculant for *Chlorella*. In studies with *Chlorella*, the amount of polymer required to initiate flocculation decreased as the molar mass of the polymer increased from 800 to about 2000 Da; however, further increases in molar mass did not improve flocculation efficiency. Changes in pH over the range of pH 4–7, did

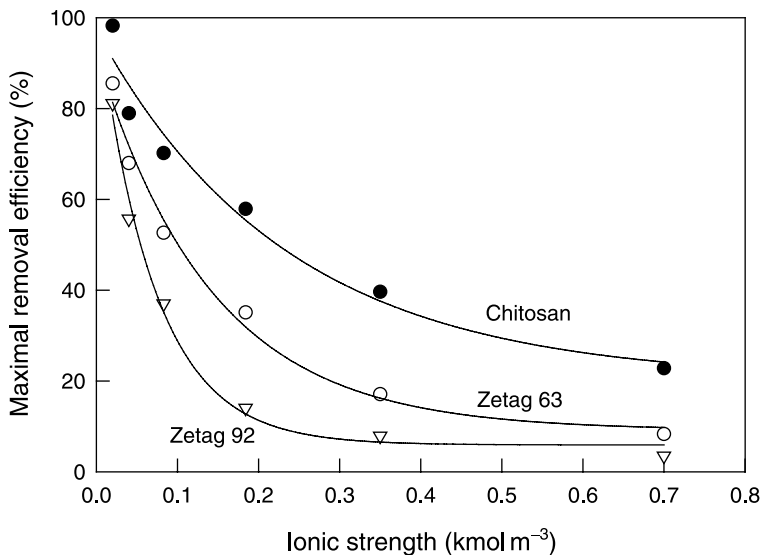


Fig. 2. The effect of ionic strength on efficiency of cell removal by flocculation with commercial polymers. The polymers tested included the cationic chitosan (400 kDa molar mass), Zetag 63 (a cationic polyacrylamide of 10,000 kDa molar mass) and Zetag 92 (a cationic polyacrylamide of 20,000 kDa) (Bilanovic et al., 1988).

not affect flocculation behavior (Tilton et al., 1972). In one study, *Chlorella* could be flocculated with a cationic polymer but not an anionic polymer (Cohen et al., 1957). Polyvalent organic polymers have been claimed as effective flocculants for *Scenedesmus* and *Chlorella* (Golueke and Oswald, 1965).

Chitosan, a polymer of acetylglucosamine, is an edible nontoxic flocculant that has proved effective with various microalgae (Nigam et al., 1980; Lavoie and de la Noue, 1983; Morales et al., 1985; Lubian, 1989). Chitosan is easily manufactured and requires low dosage rates (Richmond and Becker, 1986), but its flocculating power is reduced in salt water. Optimal flocculation dose of chitosan varies greatly. Optimal flocculation of *Tetraselmis chui*, *Thalassiosira pseudonana* and *Isochrysis* sp. has been observed at a chitosan dosage of 40 mg l⁻¹ (Heasman et al., 2000). In contrast, 150 mg l⁻¹ of chitosan was required for optimal flocculation of *Chaetoceros muellari* (Heasman et al., 2000). There appears to be no consistent correlation between the taxonomic group of the algae and the quantity of chitosan needed for optimal flocculation (Heasman et al., 2000). Although chitosan is generally regarded as nontoxic, oyster larvae fed with chitosan-flocculated microalgae have shown a dramatically reduced survival (Heasman et al., 2000). Chitosan can be used as an entrapment matrix to immobilize microalgal biomass for use in tertiary treatment of wastewater (Kaya and Picard, 1996).

Sometimes, effective flocculation is achieved simply by changing the pH of the algal broth. In one study, extensive flocculation was achieved at pH values between 11.8 and 12, without other added flocculants (McCausland et al., 1999). This method removed 95% of the algal biomass from suspension and the sludge obtained had good settling characteristics and mechanical resistance. In other instances, extreme pH values can cause adverse effects. In a

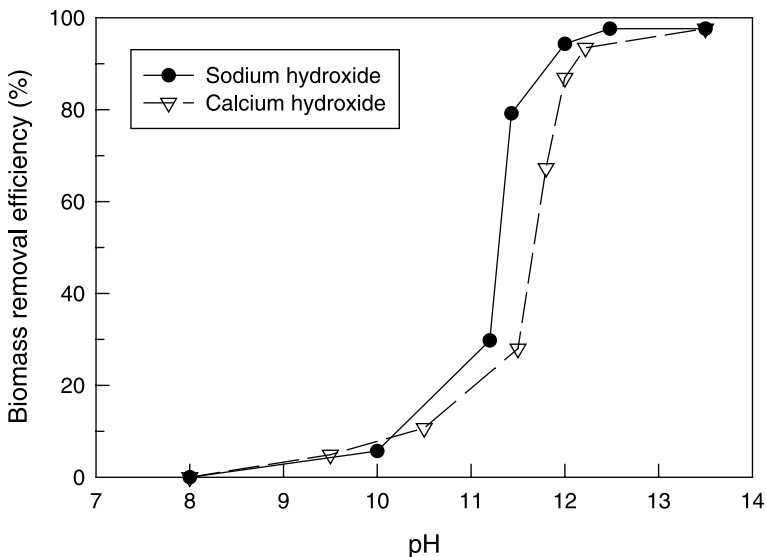


Fig. 3. Efficiency of cell removal by flocculation with alkalis. Data shown is for *Chlorella* at an initial biomass concentration of 180 mg l⁻¹ (Yahi et al., 1994).

study of EPA recovery from *Skeletoma costatum*, a pH of 10.2 sedimented 80% of the suspended cell mass but the cells lysed and released the intracellular contents (Blanchemain and Grizeau, 1999). Effect of two alkalis on cell removal by flocculation from suspensions of *Chlorella* is shown in Fig. 3 (Yahi et al., 1994).

Autoflocculation produced by modifying the culture medium, has been investigated for algal cultures in both fresh and seawater systems (Cannell, 1990; Suh et al., 1997; Lee et al., 1998). Also, bioflocculant produced by nonalgal microbial cultures have been assessed for flocculating microalgal cells (Hee-Mock et al., 2001). The bacterium *Paenibacillus* sp. AM49 is known to produce a bioflocculant that has proved effective for harvesting *Chlorella vulgaris* (Hee-Mock et al., 2001).

3.2. Centrifugal recovery

Most microalgae can be harvested from suspension by centrifugation. Centrifugal recovery methods are commonly treated in textbooks (Belter et al., 1988), but practical guidelines are rarely given. The guidelines provided by Chisti and Moo-Young (1991) for selection and use of centrifuges are especially relevant to recovery of microalgal biomass. Centrifugal recovery can be rapid, but it is energy intensive. Nevertheless, centrifugation is a preferred method of recovering algal cells (Benemann et al., 1980; Mohn, 1980; Richmond, 1986), especially for

Table 1
Comparison of some centrifugal methods of harvesting of microalgae (based on Mohn, 1980)

Machine and make	Operational mode	Concentration method	Alga	Suspended solids (%) in concentrate (concentration factor in parentheses)	Energy consumed (kW h) per m ³	Relative harvesting cost ^a	Reliability
Self-cleaning, disc-stack centrifuge; Westfalia	Suspension continuous; concentrate discontinuous	One step	<i>Scenedesmus</i> , <i>Coelastrum proboscideum</i>	12 (120)	1	1	Very good
Nozzle discharge centrifuge; Westfalia	Continuous	For final concentration or for preconcentration	<i>Scenedesmus</i> , <i>C. proboscideum</i>	2–15 (20–150)	0.9	0.72	Good
Decanter bowl centrifuge; Westfalia	Continuous	For final concentration only to ca. 2	<i>Scenedesmus</i> , <i>C. proboscideum</i>	22% (11)	8	–	Very good
Hydrocyclones; AKW	Continuous	For preconcentration	<i>C. proboscideum</i>	0.4 (4)	0.3	9	Poor

^a Does not include labor. Relative harvesting costs are calculated on the basis of operational cost of a self-cleaning plate separator being 1.0.

producing extended shelf-life concentrates for aquaculture hatcheries and nurseries (D'Souza et al., 2000; Heasman et al., 2000).

The recovery of the biomass in a sedimenting centrifuge depends on the settling characteristics of the cells, the residence time of the cell slurry in the centrifuge, and the settling depth. Settling depth can be kept small through the design of the centrifuge. The residence time of the slurry in the centrifuge can be controlled by controlling the flow rate. Heasman et al. (2000) evaluated the extent of cell recovery and the effects of cell viability at different conditions of centrifugation. Nine different strains of microalgae were assessed (Heasman et al., 2000). A cell harvest efficiency of >95% was obtained only at $13,000 \times g$. The harvest efficiency declined to 60% at $6000 \times g$ and 40% at $1300 \times g$. Cell viability depended significantly on the algal species and the method of centrifugation (Heasman et al., 2000). Information on comparative performance of some centrifugal methods of recovering microalgal biomass is given in Table 1 (Mohn, 1980). Of the methods shown (Table 1), only hydrocyclone proved unsatisfactory for the application shown. The other methods were quite effective for biomass recovery (Table 1).

3.3. Filtration

Filter presses operating under pressure or vacuum are satisfactory for recovering relatively large microalgae such as *Coelastrum proboscideum* and *Spirulina platensis* but fail to recover organisms approaching bacterial dimensions (e.g., *Scenedesmus*, *Dunaliella*, *Chlorella*) (Mohn, 1980). Using a chamber filter press, a concentration factor of 245 could be attained for the large microalgae *C. proboscideum* to produce a cake with 27% solids (Mohn, 1980). Rotary drum vacuum or pressure filters can be used also. A rotary drum precoat filtration process has been described for recovering microalgal biomass (Gudin and Therpenier, 1986; Gudin and Chaumont, 1991). Diatomaceous earth or cellulose (i.e., filter aid) was first filtered through the filter cloth to form a cake or precoat of the filter aid. The biomass slurry was then filtered through the precoat layer. The settled biomass was recovered by scraping off together with a thin layer of the filter aid. Recovery by precoat filtration is not suitable if contamination of the biomass with filter aid cannot be tolerated. This would generally be the case if the biomass is intended for use as aquaculture feed, or further processing is required for extracting intracellular products from the biomass.

For the small cells of *Dunaliella*, filtration through sand filters, cellulose fibers and other filter materials has not proved practical (Ben-Amotz and Avron, 1987). One exception was filtration through diatomaceous earth. *Dunaliella* grown in salt ponds in Australia could be recovered by passing diluted culture broth through diatomaceous earth. The filtered alga was then directly extracted with organic solvent to recover β -carotene (Ruane, 1977).

Table 2 provides information on some pressure and vacuum filters that have been used to recover microalgae (Mohn, 1980). Of the filters listed in Table 2, the pressure belt filter and the vacuum filter thickener are not recommended for use with the microalgae tested.

Membrane microfiltration and ultrafiltration are possible alternatives to conventional filtration for recovering algal biomass. Microfiltration is suitable for fragile cells (Petrusevski et al., 1995), but large-scale processes for producing algal biomass do not generally use

Table 2
Performance of some pressure and vacuum filters used in harvesting of microalgae (based on Mohn, 1980)

Type	Machine and make	Operational mode	Concentration procedure	Alga	Suspended solids (%) in concentrate (concentration factor in parentheses)	Energy (kW h) consumed per m ³	Relative harvesting cost ^a	Reliability
Pressure filter	Netsch chamber filter; Netsch	Discontinuous	One step	<i>C. proboscideum</i>	22–27 (245)	0.88	0.4	Very high
	Belt press; Bellmer	Continuous	Needs preconcentration to ~4% total suspended solids, or flocculation	<i>C. proboscideum</i>	18 (180)	0.5	1.1	–
	Suction filter; Seitz	Discontinuous	Original and preconcentrated suspension with 1.5% total suspended solids	<i>C. proboscideum</i>	16 (160)	–	–	Good
	Cylindrical sieve rotators; Engelsmann	Continuous	One step and for preconcentration	<i>C. proboscideum</i>	7.5 (75)	0.3	1.9	Sufficient
Vacuum filter	Filter basket; Seitz Dinglinger	Discontinuous	For preconcentration	<i>C. proboscideum</i>	5 (50)	0.2	0.48	Good
	Non-precoat vacuum drum filter; Dorr Olliver	Continuous	One step	<i>C. proboscideum</i>	18 (180)	5.9	3.9	Low
	Potato starch precoat vacuum drum filter; Nivoba, Walther	Continuous after precoating	Needs 2- to 15-fold preconcentration	<i>C. proboscideum</i> , <i>Scenedesmus</i>	37 (2–18.5)	–	–	Good
	Suction filter	Discontinuous	One step	<i>C. proboscideum</i>	8 (80)	0.1	4.5	Satisfactory
	Belt filter; Dinglinger	Continuous	For preconcentration	<i>C. proboscideum</i>	9.5 (95)	0.45	0.88	Good
Filter thickener; Schenck	Discontinuous	For preconcentration	<i>C. proboscideum</i> , <i>Scenedesmus</i>	5–7 (50–70)	1.6	3.2	Satisfactory	

^a Does not include labor. Relative harvesting costs are calculated on the basis of operational cost of a self-cleaning plate separator being 1.0.

membrane filtration. Small aquaculture farms do frequently use membrane technology for recovering algal cells for feeding shellfish larvae (Borowitzka, 1997).

Rossignol et al. (1999) investigated the use of polymer membranes for continuous recovery of two marine microalgae (*Haslea ostrearia* and *Skeletonema costatum*). Eight commercial membranes were evaluated. A polyacrylonitrile ultrafiltration membrane of 40-kDa molecular weight cutoff was found satisfactory for recovering the cells (Rossignol et al., 1999). These membranes could be used on a long-term basis under conditions of a low imposed transmembrane pressure and a relatively low cross-flow velocity (to keep shear rates low for the fragile cells). Although microfiltration membranes provided higher initial fluxes than the ultrafiltration membranes, the former tended to clog up more readily. Flux values of between 15 and 60 l m⁻² h⁻¹ were attained with the ultrafilter membranes.

Membrane replacement and pumping are the major cost contributors to membrane filtration processes. Generally, microfiltration can be more cost-effective than centrifugation if only small volumes (e.g., <2 m³ day⁻¹) are to be filtered. For larger scale of production (e.g., >20 m³ day⁻¹), centrifugation may be a more economic method of recovering the biomass (MacKay and Salusbury, 1988).

4. Biomass processing for metabolite recovery

4.1. Dehydration of biomass

Harvesting generally results in a 50- to 200-fold concentration of algal biomass. The harvested biomass slurry (5–15% dry solids) must be processed rapidly, or it can spoil within a few hours in a hot climate. The specific postharvest processing necessary depends strongly on the desired product. Dehydration or drying of the biomass is commonly used to extend the shelf-life of the biomass especially if biomass is the final product. Drying methods that have been used for microalgae include spray drying, drum drying, freeze-drying and sun drying. Spray drying, freeze-drying and drum drying of β -carotene-rich *Dunaliella* all produce satisfactory results in terms of the uniformity of the biomass powder and stability of β -carotene in the biomass (Ben-Amotz and Avron, 1987).

Spray drying is the method of choice for high-value products (>\$1000 ton⁻¹), but it can cause significant deterioration of some algal components such as pigments. The expense of drying can be a significant impediment to producing microalgal biomass powder for use in food and feeds. Freeze-drying, or lyophilization, has been widely used for drying microalgae in research laboratories; however, freeze-drying is too expensive for use in large-scale commercial recovery of microalgal products. In some cases, solvent extraction of dry biomass has proved much more effective for recovery of intracellular metabolites than the extraction of wet biomass.

Intracellular products such as oils can be difficult to solvent extract from wet biomass (Belarbi et al., 2000) of undisrupted cells, but are extracted readily if the biomass has been freeze-dried. Lipids have been extracted directly from freeze-dried biomass of *Isochrysis galbana* (Molina Grima et al., 1994).

4.2. Cell disruption

Cell disruption is often necessary for recovering intracellular products from microalgae (Ruane, 1977; Mendes-Pinto et al., 2001). Most of the cell disruption methods developed for use with nonphotosynthetic microorganisms (Chisti and Moo-Young, 1986; Middelberg, 1994) can be applied to microalgae. High-pressure homogenisers are widely used to disrupt *Haematococcus* cells for use as fish feed. Disruption greatly enhances the bioavailability of and the assimilation of the pigments from the cells. Agitation of microalgal biomass in presence of glass and ceramic beads (~ 0.5 mm bead diameter) in bead mills (Chisti and Moo-Young, 1986) has been used to disrupt cells (Hedenskog et al., 1969) of *Scenedesmus obliquus*, *S. platensis* and *Monodus subterraneus*. Ultrasonication of suspended microalgal cells can be used to disrupt small amounts of biomass (Dunstan et al., 1992; Bermejo Román et al., 2001) but this method is not applicable to large-scale use.

Different cell disruption processes have been assessed for recovering astaxanthin from encysted cells of *Haematococcus pluvialis* (Mendes-Pinto et al., 2001) (Fig. 4). Biomass that had been autoclaved or mechanically disrupted in a high pressure homogenizer, yielded three times as much astaxanthin as biomass treated with other methods (Fig. 4). The latter included treatment with acid, alkali, and enzymes. Bubrick (1991) used cryogenic (-170 °C) grinding of dried *Haematococcus* biomass to extract astaxanthin with butylated hydroxytoluene. This method is not realistic for large-scale commercial use.

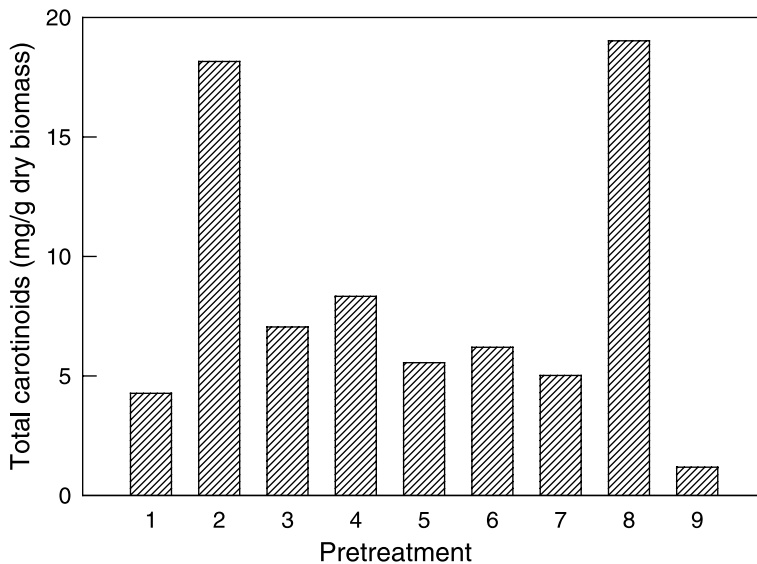


Fig. 4. Effect of various biomass pretreatments on recovery of carotenoids in acetone extracts of *Haematococcus*: 1. No pretreatment; 2. Autoclave; 3. Hydrochloric acid, 15 min; 4. Hydrochloric acid, 30 min; 5. Sodium hydroxide, 15 min; 6. Sodium hydroxide, 30 min; 7. Enzyme; 8. Mechanical disruption; 9. Spray drying (Mendes-Pinto et al., 2001).

While treatment with alkali is an effective method of lysing the cell wall, it is not generally suitable for sensitive products such as proteins. However, alkaline lysis can be used to isolate free fatty acids from microalgae. Free fatty acids of *Porphyridium cruentum* (Giménez Giménez et al., 1998) and *Phaeodactylum tricornerutum* (Robles Medina et al., 1995; Cartens et al., 1996; Molina Grima et al., 1996; Ibáñez González et al., 1998) have been extracted by direct saponification of wet biomass with KOH–ethanol mixture.

4.3. Extraction and purification of metabolites

Solvent extraction of algal biomass is widely used to extract metabolites such as astaxanthin, β -carotene and essential fatty acids. Hexane, ethanol, chloroform and diethyl ether can extract fatty acids such as EPA, docosahexaenoic acid (DHA) and arachidonic acid (AA) from various microalgae. Fatty acid extraction from *P. cruentum*, *P. tricornerutum*, *I. galbana*, *M. subterraneus* and other microalgae has been described (Robles Medina et al., 1995; Giménez Giménez et al., 1998; Belarbi et al., 2000). Extraction with aqueous buffers has been used to obtain phycobiliproteins from *P. cruentum* (Bermejo Román et al., 2001, 2002) and lutein from *C. vulgaris* (Li et al., 2001). Extraction of many proteins requires biomass that has not been dried previously (Bermejo Román et al., 2002).

Crude extracts are generally filtered and purified by various chromatographic methods to obtain the metabolite of interest. Supercritical fluid chromatography has been used to recover astaxanthin (Lim et al., 2002), polyunsaturated fatty acids and other compounds. Some other chromatographic methods that have been used for recovering pure fatty acids (or equivalent esters) have included reverse phase chromatography, silica gel adsorption chromatography and argentated silica gel chromatography (Robles Medina et al., 1995; Giménez Giménez et al., 1998; Belarbi et al., 2000). Proteins are usually purified using ion exchange chromatography (Bermejo Román et al., 2002).

5. Process economics: a case study of EPA production

Here, we discuss the economics of producing EPA from the marine microalga *P. tricornerutum*, as a representative case study for producing high-value intracellular products from microalgae. The case study is useful in identifying potential bottlenecks to commercializing microalgae-derived products.

5.1. The EPA production process

The process for producing high-purity EPA from *P. tricornerutum* is shown in Fig. 5. The process was developed at the University of Almería, Spain. All aspects of the process have been demonstrated at a pilot production scale. Modifications of the process have been also proved for recovering EPA from another microalga, *M. subterraneus*, and fish oil (Belarbi et al., 2000). The process consists of three distinct parts: (1) production and recovery of the microalgal biomass; (2) one-step extraction and esterification of oils in the wet biomass paste;

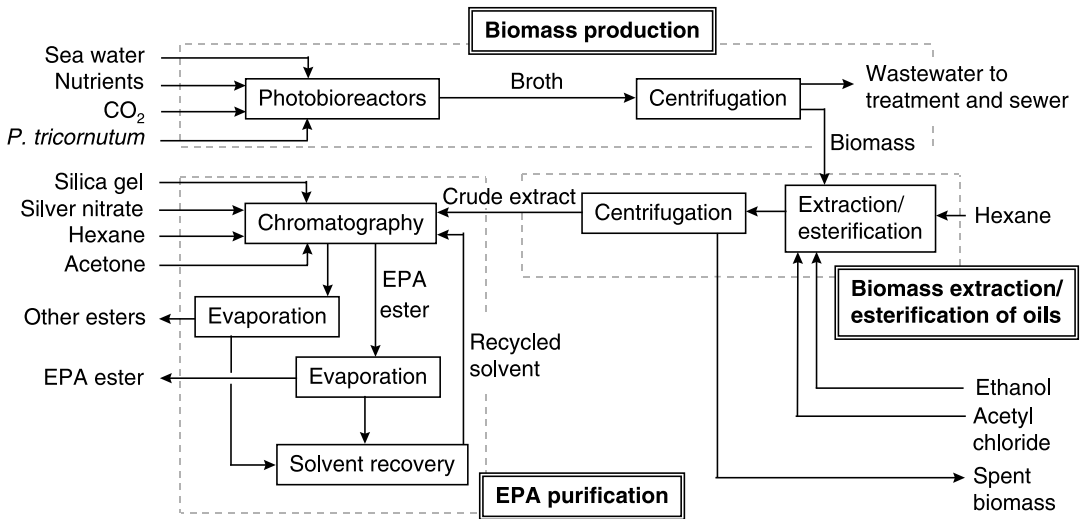


Fig. 5. Production of pure EPA from the microalga *P. tricornutum*.

and (3) chromatographic purification of the crude extract to yield highly pure (>96% pure) EPA ester and other fatty ester co-products (Fig. 5). The optimal processing conditions and the scale up of recovery have been described by Belarbi et al. (2000).

Outdoor-placed tubular photobioreactors are used to produce the alga in continuous culture (Fig. 6) (Ación Fernández et al., 1998, 2001; Molina Grima et al., 1999). Typically, the dilution rate used is one-third bioreactor volume per day during the daylight hours. The *P. tricornutum* biomass concentration in the broth from the photobioreactors is 3.8 kg m^{-3} on average. The broth is centrifuged in a continuous flow disc stack centrifuge to recover a biomass paste with $\sim 80\%$ moisture content. The spent broth is treated and discharged to sewer. The biomass contains $\sim 2.5\%$ (w/w) EPA of which about 70% is recoverable. There is $\sim 10\%$ total oil in the dry biomass. The recovered biomass paste is processed through a one-step extraction–esterification operation that yields a crude extract of the fatty esters. This one-step process eliminates several intermediate processing steps that would be required if extraction and esterification of the oil are carried out in separate steps (Rodríguez-Ruiz et al., 1998). Esterification–extraction requires hexane, ethanol (for obtaining ethyl esters) and acetyl chloride (Fig. 5). Although, *P. tricornutum* biomass is a potentially suitable animal feed, the solvent extracted alga that has been depleted of its fat contents and contains residual solvents can be reasonably disposed off only by landfilling or incineration.

The biomass-free esterified crude extract is purified by silver silica gel chromatography to obtain a highly pure (>96% pure) EPA ester and esters of the other fatty acids present in the alga. The solvents used in chromatography (i.e., hexane and acetone) are recovered by evaporation of the eluted fractions. The solvents are recycled. Possible alternatives or adjuncts to using silver silica gel chromatography include membrane-based selective enrichment of EPA, supercritical chromatography, kinetic resolution, and winterization. Processes such as urea complexation and preparative HPLC are of limited value in the commercial purification of EPA.

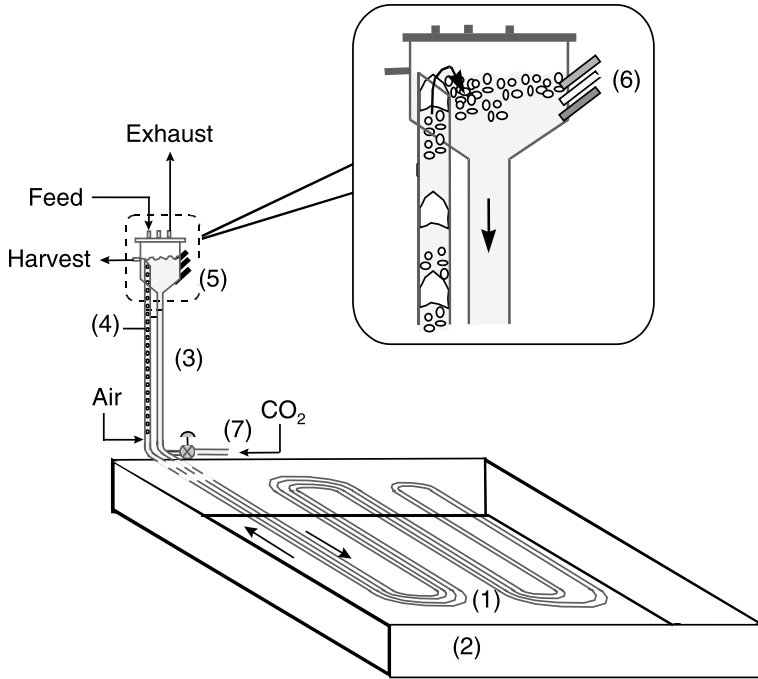


Fig. 6. A tubular photobioreactor used for culturing the alga. The tubular solar receiver (1) is held in a thermostated pool (2) for temperature control. The airlift device (3) is used to circulate the culture broth through the solar loop. Only the riser zone (4) of the airlift device is injected with air. The injected gas separates from the culture broth in the degassing zone (5) before the broth returns to the solar loop. The degasser zone contains sensors (6) for pH, dissolved oxygen and temperature. The carbon dioxide required for photosynthesis is injected at the entrance (7) of the solar loop (Acién Fernández et al., 1998, 2001).

5.2. Process economics

5.2.1. Basis

The basis of the economic analysis was a facility producing 430 kg of 96% pure EPA per annum. The biomass and the crude extract required for the EPA were produced within the integrated facility. Producing 430 kg of EPA required a biomass production capacity of 26.2 tons (dry basis) per annum and this translated to 2620 kg of crude esterified extract. The integrated production facility was costed for a coastal “green field” site in the south of Spain. This provided ready access to sea water and the irradiance levels required to attain an average biomass productivity of $\sim 1.25 \text{ kg m}^{-3} \text{ day}^{-1}$ in continuous culture.

The processes for producing the biomass, the crude esterified extract, and the pure EPA from the extract, were evaluated separately to gain a detailed insight into the major contributors to the final price of producing the EPA. This approach allowed us to separately estimate the cost of the biomass paste, the crude esterified extract and the EPA purified from the crude extract. For the economic evaluation, the components of costs taken into account included the charges related to capital investment and all operating expenses. The investment

Table 3
Major equipment list and costs for algal biomass production

Item	Delivered cost (\$)	No. of units	Total cost	% of MEC
1. Photobioreactors (0.8 m ³)	3524	75	264,300	30.0
2. Centrifuge (24 bowl, solids discharge, s.s., 2.99 m ³ /h)	123,949	2	247,898	28.1
3. Medium filter unit (5.99 m ³ /h)	18,014	1	18,014	2.0
4. Medium feed pumps (0.04 m ³ /h)	349	75	26,175	3.0
5. Medium preparation tank (19.96 m ³)	34,814	3	104,442	11.9
6. Harvest broth storage tank (19.96 m ³)	34,814	3	104,442	11.9
7. Centrifuge feed pumps (2.99 m ³ /h)	841	2	1682	0.2
8. Air compressors (240 m ³ /h)	26,103	3	78,309	8.9
9. Harvest biomass conveyer belts	7100	2	14,200	1.6
10. Seawater pump station (5.99 m ³ /h)	13,661	1	13,661	1.6
11. Carbon dioxide supply station (27.4 kg/h)	3006	1	3006	0.3
12. Weighing station	2366	1	2366	0.3
13. Biomass silos (0.07 m ³)	1370	2	2740	0.3
Total MEC (\$)			881,235	

Table 4
Total and annual fixed capital for biomass production

Item	Cost (\$)	% of A
1. Major purchased equipment (MEC)	881,235	29.2
2. Installation costs (at 0.3 MEC)	264,371	8.8
3. Instrumentation and control (at 0.1 MEC)	88,124	2.9
4. Piping (at 0.3 MEC)	264,371	8.8
5. Electrical (at 0.1 MEC)	88,124	2.9
6. Buildings (at 0.3 MEC)	264,371	8.8
7. Yard improvements (at 0.1 MEC)	88,124	2.9
8. Service facilities (at 0.2 MEC)	176,247	5.8
9. Land (at 0.06 MEC)	52,874	1.8
10. Engineering and supervision (at 0.25 MEC)	220,309	7.3
11. Construction expenses (at 0.1 \sum items 1–9)	216,784	7.2
12. Contractor's fee (at 0.05 \sum items 1–9)	108,392	3.6
13. Contingency (at 0.06 total fixed capital investment)	180,888	6.0
Total fixed capital investment, A (\$)	3,014,803	96.0
Item	Cost (\$)	% of B
Depreciation (at (\sum items 1–8, 10–13)/10 years)	284,134	85.6
Property tax (at 0.01 depreciation)	2841	0.9
Insurance (at 0.006 depreciation)	1705	0.5
Debt service (none, 100% equity capital)	0	0.0
Purchase tax ((at 0.16 of items 1–12)/10)	43,413	13.1
Total fixed capital per year, B (\$)	332,093	

Table 5
Direct costs of biomass production

Raw materials	Total quantity (kg)	Cost (\$)	% of C
1. Culture medium (at \$0.5883/kg)	65,500	38,534	34.9
2. Carbon dioxide (at \$0.4706/kg)	96,940	45,620	41.3
3. Media filters (at \$70.59/unit)	210 units	14,824	13.4
4. Air filters (at \$94.12/unit)	105 units	9883	9.0
5. Other consumables (at \$117.65/kg)	13	1529	1.4
Total raw materials cost, C (\$)		110,390	
Utilities	Total quantity	Cost (\$)	% of D
6. Cooling water (included in pumping station)	408,196	0	0
7. Power (at \$0.05883/kW h)	99,822 kW h	5873	100
Total, D (\$)		5873	
Others	Total quantity	Cost (\$)	% of E
8. Labor (at \$16/h, 1 shift)	3 persons	140,160	35.6
9. Supervision (at 0.2 labor)		28,032	7.1
10. Payroll charges (at 0.25 (labor + supervision))		42,048	10.7
11. Maintenance (at 0.04 MEC)		35,249	8.9
12. Operating supplies (at 0.004 C)		442	0.1
13. General plant overheads (at 0.55 (labor + supervision + maintenance))		111,893	28.4
14. Tax (at 0.16 items 1–7, 11 and 12)		24,312	6.2
15. Contingency (at 0.05 items 1–7)		5813	1.5
16. Marketing (not included)		0	0.0
17. Wastewater treatment (at \$0.59/m ³)	10,480 m ³	6183	1.6
Total, E (\$)		394,133	
Total production cost, F (B (Table 4) + C + D + E) (\$)		842,488	
Unit cost of producing biomass (\$/kg)		32.16	

Table 6
Major equipment list and costs for production of crude esterified algal oil

Item	Delivered cost (\$)	No. of units	Total cost	% of MEC
1. Ethanol tank (1.5 m ³ , carbon steel)	2404	1	2404	4.6
2. Acetyl chloride tank (1 m ³ , carbon steel)	1881	1	1881	3.6
3. Reactor (2 m ³ , 66 psig, jacketed)	5242	1	5242	10.0
4. Hexane storage tanks (60 m ³)	11,613	2	23,226	44.3
5. Holding tank (2.5 m ³ , carbon steel)	3434	1	3434	6.5
6. Evaporator (50 ft ²)	14,622	1	14,622	27.9
7. Condenser (shell and tube, 5 m ²)	1678	1	1678	3.2
Total MEC (\$)			52,487	

Table 7

Total and annual fixed capital for production of crude esterified algal oil

Item	Cost (\$)	% of <i>A</i>
1. Major purchased equipment (MEC)	52,487	24.9
2. Installation costs (at 0.4 MEC)	20,995	10.0
3. Instrumentation and control (at 0.15 MEC)	7873	3.7
4. Piping (at 0.4 MEC)	20,995	10.0
5. Electrical (at 0.1 MEC)	5249	2.5
6. Buildings (at 0.45 MEC)	23,619	11.2
7. Yard improvements (at 0.12 MEC)	6298	3.0
8. Service facilities (at 0.2 MEC)	10,497	5.0
9. Land (at 0.06 MEC)	3149	1.5
10. Engineering and supervision (at 0.3 MEC)	15,746	7.5
11. Construction expenses (at 0.1 \sum items 1–9)	15,116	7.2
12. Contractor's fee (at 0.05 \sum items 1–9)	7558	3.6
13. Contingency (at 0.08 total fixed capital investment)	16,852	8.0
Total fixed capital investment, <i>A</i> (\$)	210,648	98.0
Item	Cost (\$)	% of <i>B</i>
Depreciation (at (\sum items 1–8, 10–13)/10 years)	20,329	85.8
Property tax (at 0.01 depreciation)	203	0.9
Insurance (at 0.006 depreciation)	122	0.5
Debt service (none, 100% equity capital)	0	0.0
Purchase tax ((at 0.16 of items 1–12)/10)	3033	12.8
Total fixed capital per year, <i>B</i> (\$)	23,687	

capital was assumed to be 100% venture capital and, therefore, no debt charges were included. All prices were for the year 2001.

5.2.2. Biomass production

Producing the specified 26.2 tons of *P. tricornutum* biomass annually requires an array of 75 tubular photobioreactors each having a volume of 0.8 m³. The cost of photobioreactors is based on local experience. The biomass is harvested using a continuous flow disk stack centrifuge. The major process equipment required for such a facility and the delivered cost of the equipment are listed in Table 3. The costing of the photobioreactors and the seawater pumping station is based on direct experience. Other costs have been estimated using standard process engineering data (Humphreys, 1991; Peters and Timmerhaus, 1991) or actual quotes from vendors. The fixed capital investment required for the biomass paste production facility is itemized in Table 4. In keeping with standard bioprocess engineering practice (Humphreys, 1991; Peters and Timmerhaus, 1991), the fixed costs are generally estimated as factors of the major equipment costs (MEC). The fixed investment, depreciated over 10 years, contributes \sim 39% to the cost of producing the biomass. The remaining 61% of the production cost originates in the direct production expenses itemized in Table 5. The direct production costs account for the raw materials consumed, utilities charges, labor, general overheads, and waste treatment. Marketing expenses are not included, as the biomass

Table 8
Direct production costs of crude esterified algal oil

Raw materials	Total quantity	Cost (\$)	% of C
1. Algal biomass, kg (at \$32.16/kg)	26,200	842,592	95.0
2. Ethanol, m ³ (at \$784.70/m ³)	33	25,895	2.9
3. Acetyl chloride, m ³ (at \$1533/m ³)	7	10,731	1.2
4. Hexane, m ³ (at \$364.7/m ³)	20	7294	0.8
Total raw materials cost, C (\$)		886,512	
Utilities	Total quantity	Cost (\$)	% of D
5. Cooling water, m ³ (\$0.0294/m ³)	5790	170.226	43.2
6. Steam, kg (at \$0.0049/kg)	39,300	192.57	48.9
7. Power, kW h (at \$0.05883/kW h)	524	31	7.8
Total, D (\$)		394	
Others	Total quantity	Cost (\$)	% of E
8. Labor (at \$16/h, 1 shift)	1 person	46,720	36.4
9. Supervision (at 0.2 labor)		9344	7.3
10. Payroll charges (at 0.25 (labor+supervision))		14,016	10.9
11. Maintenance (at 0.04 MEC)		2099	1.6
12. Operating supplies (at 0.004 C)		3546	2.8
13. General plant overheads (at 0.55 (labor+supervision+maintenance))		31,990	24.9
14. Tax (at 0.16 \sum items 2–7, 11 and 12)		7993	6.2
15. Contingency (at 0.1 \sum items 2–7)		4431	3.5
16. Marketing (not included)		0	0.0
17. Spent biomass (kg) disposal (at \$350/ton)	23,318	8161	6.4
Total, E (\$)		128,302	
Total production cost, F (B (Table 7)+C+D+E) (\$)		1,038,894	
Unit cost of producing esterified oil (\$/kg)		396.52	

produced is “sold” internally to the extraction–esterification part of the overall integrated process.

The cost of producing the biomass is estimated at \$32.16/kg (dry basis) (Table 5). This value is derived by summing *B* (Table 4), *C*, *D* and *E* (Table 5) and dividing the result by 26.2 tons, the total annual biomass production. Raw materials (excluding utilities) and direct process labor contribute $\sim 13\%$ and $\sim 17\%$, respectively, to the production cost. The labor input and raw materials consumption cannot be reduced significantly. Depreciation charges contribute $\sim 34\%$ to annual production cost, but the present technology does not permit a significant reduction in capital expenses. One significant option for reducing cost is through enhancing biomass productivity by engineered design of photobioreactors. A 30% enhancement in productivity can reduce the cost of producing the biomass by $\sim 20\%$. If the alga can be selected, engineered or otherwise coaxed into increasing the EPA content in the biomass, the production economics can be improved substantially.

Table 9
Major equipment list and costs for producing purified EPA

Item	Delivered cost (\$)	No. of units	Total cost	% of MEC
1. Ethanol tank (1.5 m ³ , carbon steel)	2404	1	2404	0.5
2. Acetyl chloride tank (1 m ³ , carbon steel)	1881	1	1881	0.4
3. Reactor (2 m ³ , 66 psig, jacketed)	5242	1	5242	1.0
4. Hexane storage tanks (60 m ³)	11,613	2	23,226	4.6
5. Holding tank (2.5 m ³ , carbon steel)	3434	1	3434	0.7
6. Acetone storage (4 m ³ , carbon steel)	5016	1	5016	1.0
7. Chromatography system (stainless steel 304)	260,247	1	260,247	51.1
8. Slurry tank with mixer (1.5 m ³ , carbon steel)	4447	1	4447	0.9
9. Silica gel/ethanol slurry tank with mixer (1 m ³ , carbon steel)	2846	1	2846	0.6
10. Activated silica gel storage silo (2.5 m ³ , carbon steel)	3638	1	3638	0.7
11. Evaporator (150 ft ²)	33,331	1	33,331	6.5
12. Evaporator (1000 ft ²)	131,762	1	131,762	25.9
13. Condenser (shell and tube, 50 m ²)	9435	1	9435	1.9
14. Condenser (shell and tube, 10 m ²)	2989	1	2989	0.6
15. Dryer (100 lb/h)	19,808	1	19,808	3.9
Total MEC (\$)			509,706	

Table 10
Total and annual fixed capital for producing purified EPA

Item	Cost (\$)	% of A
1. Major purchased equipment (MEC)	509,706	24.9
2. Installation costs (at 0.4 MEC)	203,882	10.0
3. Instrumentation and control (at 0.15 MEC)	76,456	3.7
4. Piping (at 0.4 MEC)	203,882	10.0
5. Electrical (at 0.1 MEC)	50,971	2.5
6. Buildings (at 0.45 MEC)	229,368	11.2
7. Yard improvements (at 0.12 MEC)	61,165	3.0
8. Service facilities (at 0.2 MEC)	101,941	5.0
9. Land (at 0.06 MEC)	30,582	1.5
10. Engineering and supervision (at 0.3 MEC)	152,912	7.5
11. Construction expenses (at 0.1 \sum items 1–9)	146,795	7.2
12. Contractor's fee (at 0.05 \sum items 1–9)	73,398	3.6
13. Contingency (at 0.08 total fixed capital investment)	163,650	8.0
Total fixed capital investment, A (\$)	2,045,620	
Item	Cost (\$)	% of B
Depreciation (at (\sum items 1–8, 10–13)/10 years)	197,413	85.8
Property tax (at 0.01 depreciation)	1974	0.9
Insurance (at 0.006 depreciation)	1184	0.5
Debt service (none, 100% equity capital)	0	0.0
Purchase tax ((at 0.16 of \sum items 1–12)/10)	29,457	12.8
Total fixed capital per year, B (\$)	230,028	

5.2.3. Production of esterified crude oil extract

Recovering 430 kg/annum pure EPA requires 2620 kg of esterified algal oil. The major equipment required for the one-step esterification–extraction facility and the delivered cost of the equipment are shown in Table 6. The total annual fixed capital charges are calculated in Table 7, as \$23,687. The capital charges contribute <2.5% to the cost of the final crude extract. The latter is obtained by summing *B* (Table 7), *C*, *D* and *E* (Table 8) and dividing the result by 2620 kg of crude oil produced. All the direct production costs are identified in Table 8. Of the direct costs, raw materials (excluding utilities) contribute >85% of the cost of the crude extract oil. Much of this cost, i.e. ~81% of the final cost of crude oil, is contributed by the microalgal biomass. Direct operating labor contributes only ~4.5% to the final cost of the crude oil. Landfill disposal of spent biomass costs \$350/ton, or <1% of the final oil cost.

5.2.4. EPA recovery from mixed esters

Argentated silica gel chromatography is used to recover pure EPA (>96% pure) from the crude oil. The relevant major process equipment needed and the costs are shown in Table 9.

Table 11
Direct costs of producing purified EPA

Raw materials	Total quantity	Cost (\$)	% of <i>C</i>
1. Crude algal oil, kg (at \$396.52/kg)	2620	1,038,882	89.4
2. Silica gel, kg (at \$6.6/kg)	1100	7260	0.6
3. Silver nitrate, kg (at \$529.4/kg)	100	52,940	4.6
4. Acetone, m ³ (at \$419.4/m ³)	20	8388	0.7
5. Hexane, m ³ (at \$364.7/m ³)	150	54,705	4.7
Total raw materials cost, <i>C</i> (\$)		1,162,175	
Utilities	Total quantity	Cost (\$)	% of <i>D</i>
6. Cooling water, m ³ (\$0.0294/m ³)	69,600	2046	34.3
7. Steam, kg (at \$0.0049/kg)	670,421	3285	55.0
8. Power, kW h (at \$0.05883/kW h)	10,897	641	10.7
Total, <i>D</i> (\$)		5972	
Others	Total quantity	Cost (\$)	% of <i>E</i>
9. Labor (at \$16/h, 1 shift)	2 persons	93,440	16.1
10. Supervision (at 0.2 labor)		18,688	3.2
11. Payroll charges (at 0.25 (labor+supervision))		28,032	4.8
12. Maintenance (at 0.04 MEC)		20,388	3.5
13. Operating supplies (at 0.004 <i>C</i>)		4649	0.8
14. General plant overheads (at 0.55 (labor+supervision+maintenance))		72,884	12.6
15. Tax (at 0.16 ∑ items 2–8, 12 and 13)		24,688	4.3
16. Contingency (at 0.1 ∑ items 2–8)		12,927	2.2
17. Marketing (at 0.02 ∑ items 1–15)		304,912	52.5
Total, <i>E</i> (\$)		580,607	
Total production cost, <i>F</i> (<i>B</i> (Table 10)+<i>C</i>+<i>D</i>+<i>E</i>) (\$)		1,978,783	
Unit cost of producing esterified oil (\$/kg)		4602	

The annual fixed capital charges are calculated in [Table 10](#). The direct production expenses are itemized in [Table 11](#). At a cost of production of \$396.52/kg, the crude oil accounts for ~53% of the cost of producing the pure EPA ester. All raw materials, excluding utilities, but including crude oil, contribute ~59% to the final EPA cost. The annual fixed capital charge contributes only ~12% to the final cost. The contribution of the direct operating labor is relatively small, at ~5% of final EPA cost. The latter works out to \$4602/kg ([Table 11](#)), i.e. the sum of *B* ([Table 10](#)), *C*, *D* and *E* ([Table 11](#)) divided by the 430-kg production.

Cost of the biomass, the extraction–esterification of crude microalgal oil, and final purification contribute 43%, 53%, and 4%, respectively, to the cost of producing the final EPA product. Overall, the downstream parts of the process (i.e., the two processes downstream of paste biomass production) contribute 57% to production cost of the final product. Typically, the downstream recovery of microbial products accounts for 70–80% of the total cost of production ([Chisti, 1998](#)). This suggests that the microalgal biomass for producing EPA is significantly more expensive than the norms for bioprocesses. Clearly, monoseptic culture of microalgae in outdoor photobioreactors is expensive compared to heterotrophic growth of most commercial bacteria, yeast and fungi.

Processes for producing the biomass, crude algal oil, and purified EPA are compared in [Fig. 7](#) to see how differently some of the major factors contribute to the costs of the final products of these processes. In comparison with biomass production, the raw materials costs for producing the crude oil and pure EPA are high ([Fig. 7](#)). These two latter processes require biomass and crude oil, respectively, and these raw materials are the overwhelming contributors to final production costs. At current market prices of high-purity EPA sourced from fish oil, microalgal sourced EPA is expensive even if we allow a significant credit for the byproducts. The cost of production of microalgal EPA needs to be reduced by at least 80% for the product to be

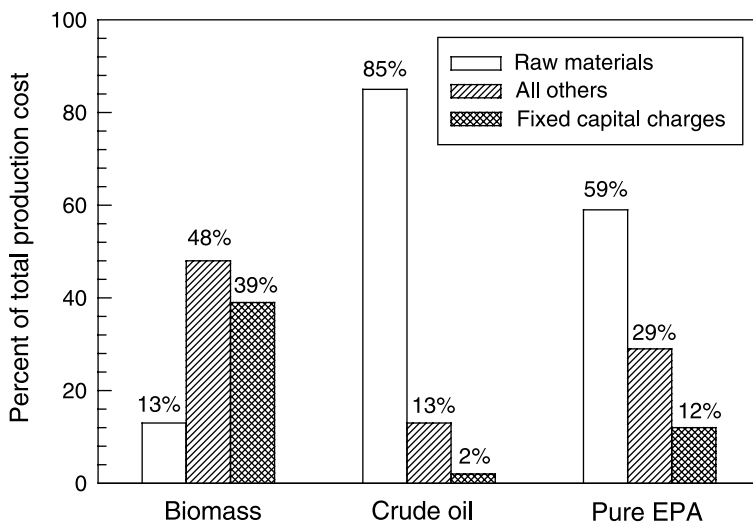


Fig. 7. Comparison of the major cost contributions to the cost of production of biomass, crude microalgal oil, and purified EPA.

competitive. Attaining this level of process improvement is difficult but not insurmountable. Enhanced EPA content in the alga in combination with better performing photobioreactors, can potentially lead to economically feasible processes for microalgal sourced EPA.

6. Conclusion

Several options exist for recovering and processing microalgal biomass to obtain intracellular metabolites produced by microalgae. For commercial recovery of high-value products, centrifugation appears to be the preferred method of recovering the biomass from the broth. Centrifugation may be preceded by a flocculation step to improve recovery. When centrifugal recovery is not feasible, for example when the alga being recovered is fragile, microfiltration can be a suitable alternative. To the extent possible, it is preferable to use moist biomass paste in the metabolite recovery scheme because a prior drying step significantly adds to costs. A concentrated slurry of algal biomass may in some cases require a cell disruption pretreatment for extracting intracellular metabolites. Cell disruption by bead milling is a generally suitable method for most microalgae. High-value, high-purity products almost always require some kind of chromatography for recovery from crude extracts. The economics of metabolite production are influenced significantly by the cost of producing the biomass, the metabolite content in the biomass, and the cost of purification. For a product such as EPA, 60% of the costs arise from the recovery process. The biomass contributes ~40% to the cost of EPA.

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