Pulsed Electric Fields for Algal Extraction and Predator Control

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Abstract

Pulsed electric field (PEF) processing uses short, high-voltage electrical pulses and a specially designed treatment chamber to permeabilize cellular membranes. There are at least two distinct applications of PEF to algal growth and processing – extraction of intracellular material and microalgae predator population control. PEF processing has the potential to provide lower costs and higher productivity for the production of biofuels, human and animal food and feed supplements, and high-value specialty chemicals from large-scale algae farms, but it is not possible to assess either the applicability or costs of PEF processing in a commercially meaningful manner today. This paper describes two potential PEF applications – extraction enhancement and predator control.

Keywords

Pulsed electric field • Algae • Biofuels • Extraction • Predators • Pests

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Introduction

Microalgae products are sold primarily into commodity markets and must compete with alternative processes/products for market share. This puts the primary emphasis on cost reduction in order to grow market size – sales will follow lower prices (Rijffels 2014). Decreasing the cost of extraction has been recognized by the algal products community as one of the most significant challenges to the commercialization of microalgae products (Gendy and El-Temtamy 2013).

Pulsed electric field (PEF) processing is a low temperature, nonthermal, nonchemical, low impact process that induces electroporation of cell membranes in microbes, plant, and animal cells. PEF processing involves the application of short-duration (1–20 μs), very high-voltage pulses that create a high-voltage field (from 1 to 50 kV/cm) across a liquid. Depending on the desired effect, this field can open the cell membranes in plant cells (1–10 kV/cm) or kill bacteria, molds, and other microorganisms at higher field strengths Gaudreau et al. 2004. More recently, emphasis in industrial processing techniques has initiated the use of electroporation of plant and animal cells as a precursor to slicing, drying, extraction, etc., where PEF processing typically replaces heat treatments with lower impact on the product and lower energy usage

Multiple studies have shown that PEF processing can be successfully applied to both extraction of valuable products from algae and the control of potentially devastating predators in algal growth ponds and raceways. While these are wildly different applications of PEF, they both focus on increasing the cost-effectiveness of algal products in the marketplace. For extraction, PEF lyses the cell membranes, allowing the intracellular compound to be released into the surrounding solution, as well as allowing solvents to enter the cell itself (Kempkes et al. 2015; Fig. 1). In predator control, the differences in size and composition between algal cells and their predators provide the potential to selectively kill predators, while having little impact on the algae itself.

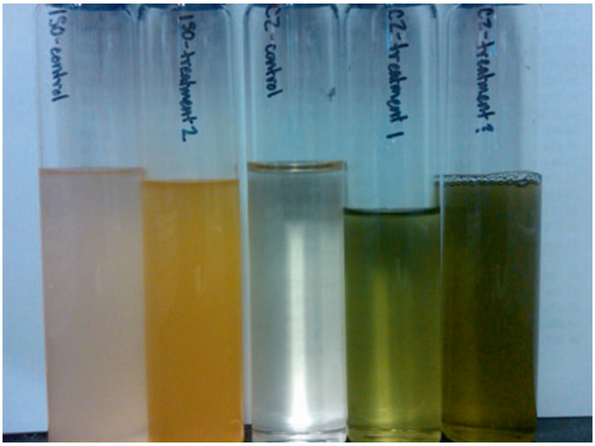

Fig. 1 Supernatant from PEF-treated *Isochrysis galbana* (*left two*) and *Chlorella vulgaris* (*right three*). The dark supernatant shows released intracellular material after PEF lysing. PEF processing could reduce the costs of extraction by over an order of magnitude compared to drying prior to solvent extraction

While sales of algal-based products have grown significantly in recent years, this growth is from a small base Thurmond 2011. Algal products still represents a very small niche, especially compared to other agricultural markets. PEF is not known to be part of any commercial algae process chain at this time. This is likely due to several factors, including the size of the algal products market today, the relative novelty of PEF in other applications,

Despite the many algal PEF treatments reported in the literature Rego et al. 2015, however, the field strengths, pulse shapes, treatment chamber geometries, and treatment times vary considerably, depending on the equipment used to collect the data. This is primarily due to inherent differences in the PEF systems used and the manner in which the results are analyzed, evaluated, and reported (chapter "▶ Industrial Pulsed Electric Field Systems," Kempkes). As a result, potential PEF adopters cannot consistently compare results and are far from being able to determine if PEF treatment is an economically viable proposition, without extensive research. This also makes PEF processing difficult to compare to other methods for cell lysing and extraction (high-pressure homogenization, sonication, microwaves, enzymes, etc., alone or in combination with solvents (Grimi 2015; Singh and Gu 2010). This lack of consistent data has become a barrier to the widespread adoption of PEF for algal products.

Furthermore, in one of the broadest reviews of algal biofuel costs and technologies conducted for the US Department of Energy, the two highest-ranking research activities needing to be addressed were:

- "Obtain experimental data to validate the assumed harvesting and extraction performance and efficiency metrics, or to support switching to alternative operations
- Work with researchers and developers in the field to quantify performance metrics for novel harvesting/extraction operations, to set realistic future process and cost goals" (Davis et al. 2012).

Standardization and expansion of the research into the effects of PEF on algae is required to move this technology and its application forward.

Microalgae Products Market

The potential microalgae products market is conservatively estimated to be at least \$500M–1B annually today and is growing at a rapid pace. Estimates for individual market sizes vary wildly, however, because microalgae products are typically a small percentage of the total market for each commodity. This market consists of two major areas: biofuels and "everything else." "Everything else" includes food (human, fish, and pet), nutritional supplements (e.g., omega-3, spirulina, and astaxanthin), cosmetics, fertilizers, and specialty chemicals (Henrikson 2010). These products vary considerably in value, from specialty chemicals at the apex to fish food near the bottom (Table 1), with multiple products often available from a

Table 1 Selling prices for microalgae products (Rijffels 2014)

	Product	Price/ton ($)
Biofuel		
	Biokerosene	500
	Biochar	150
Biochemical		
	Biopolymer	2,500
	Biolubricant	2,000
	Biopolymer additives	3,000
	Coating	5,000
	Paint	10,000
	Bulk chemical	1,000
Food/feed		
	Protein	1,000
	Lipids	950
	Carbohydrates	750
Food additives		
	Polyunsaturated fatty acids	75,000
	Functional protein	3,000
	Pigments	1,100,000
Cosmetics		
	Antioxidants	30,000
	Glycolipids, phospholipids	6,000

single microalgae stream. Most microalgae producers focus primarily on the most valuable product and sell the remaining biomass/product as fish food or other low-value product.

Algae has the distinct benefit that it does not compete with food products for land and has up to two orders of magnitude higher productivity per acre than competitive crops (soybeans, corn, etc.) (Oilgae.com). Microalgae's ability to utilize wastewater and CO_2 emissions for growth adds to its potential value stream.

Biofuels, despite being the most publicized and visible market, do not represent a real market today. The existing markets for algal biofuels are artificially maintained to incentivize R&D (e.g., the Navy's Green Fleet program; http://greenfleet.dodlive.mil/energy/great-green-fleet/). Overall, biofuels are actually one of the least valuable algal products today, and cost estimates range from $200 to $500 per barrel (Table 1) – far above petroleum, especially with the price of oil at ~ $50 per barrel in mid-2016.

It is the potential scale of the biofuels market in the future, combined with concerns about the availability and detrimental impacts of fossil fuels, which has resulted in microalgae biofuels receiving significant R&D funding and investment. In the USA, this includes well over a billion dollars from the US Department of Energy, US Department of Agriculture, and US Department of Defense in the last

5 years. Europe has also invested over a billion Euros in this area (http://biofuelstp.eu/funding.html), with significant additional funding in India, China, Japan, Israel, and other countries. This is before adding in the private investment in dozens of algal product companies around the world. There is widespread expectation that the cost of algal biofuels will reach competitive levels within a decade, given sufficient investment in their production, and the expected long-term rise in petroleum prices.

This R&D and outside investment, motivated by the drive for biofuels, provides a significant benefit to other microalgae products, as enhanced techniques for growing and processing microalgae are developed and biofuel-focused companies look to near-term markets for revenues.

PEF-Assisted Extraction

In general, algal products are commodities, competing with alternative sources and processes; the cost of the final product is the critical element in achieving commercial viability. There are many steps required to produce economically valuable products from microalgae, with the most expensive being growing the microalgae itself. The second largest cost is typically extracting the desired products from the microalgae (Gendy and El-Temtamy 2013). Most algal products today rely on drying and solvent extraction processes (or freeze-drying and separation using supercritical CO_2) to reach a commercial end product. These extraction processes are inherently energy intensive and expensive, limiting the market for microalgae products.

The major impediment to extraction is the algae cell itself, which has evolved specifically to protect the contents of the cell Grimi and Barba 2014. A rapid, efficient approach to lysing the cell would simplify the process of creating biofuels and extracting other chemicals, lowering the cost of these products. PEF has been shown by multiple researchers to lyse a variety of microalgae species through electroporation, which releases their intracellular contents into the surrounding solution (Figs. 2 and 3). The primary benefit of lysing the algal cells through PEF is to make those intracellular materials, which may include lipids, proteins, and other chemicals, available for downstream extraction, separation, and purification into specific products, as shown in Table 1. PEF will not significantly assist the extraction/separation of compounds found within the cell walls themselves, however.

One of the more comprehensive survey papers (Joannes et al. 2015b) lists PEF field strengths reported by various researchers to lyse nine different microalgae strains. Numerous other researchers have confirmed that PEF treatment lyses a range of common algal species (Frey et al. 2012; Kempkes et al. 2012; Lai et al. 2014; Luengo et al. 2015; Rego et al. 2014; Roth 2011; Zbinden et al. 2013; and many others). The specific PEF treatment parameters, however, vary significantly across these published reports, even for the same algal species.

Fig. 2 Laboratory PEF System from Diversified Technologies, Inc. (DTI), installed at Arizona State University (ASU) in September 2014. This PEF system operates at up to 20 kV and 10 kW average power

Commercial Application

PEF treatment is one step in the overall process of extraction of commercially valuable products from algal solutions. Typically, such extraction requires a combination of concentration (to remove the water from the algal growth media), drying, and chemical treatments. The drying step is extremely energy intensive and therefore costly. Eliminating this drying step, and enabling wet extraction, is one of the primary benefits of PEF. Quantifying the benefits, therefore, requires comparing the energy costs of drying versus PEF, as well as the costs of subsequent extraction processes for each alternative.

PEF processing clearly lyses microalgae cells. The majority of published research, unfortunately, does not allow evaluation of the energy required (cost) versus the level of electroporation achieved (benefit). Very little of this data addresses the impact of lysing on downstream processes for the extraction of valuable compounds, and even where data does exist, the results for a given algal species vary widely. Without this information, it is not yet possible for microalgae processors to determine if PEF treatment is a commercially attractive proposition, since the literature contains such a wide range of treatment conditions. A definitive

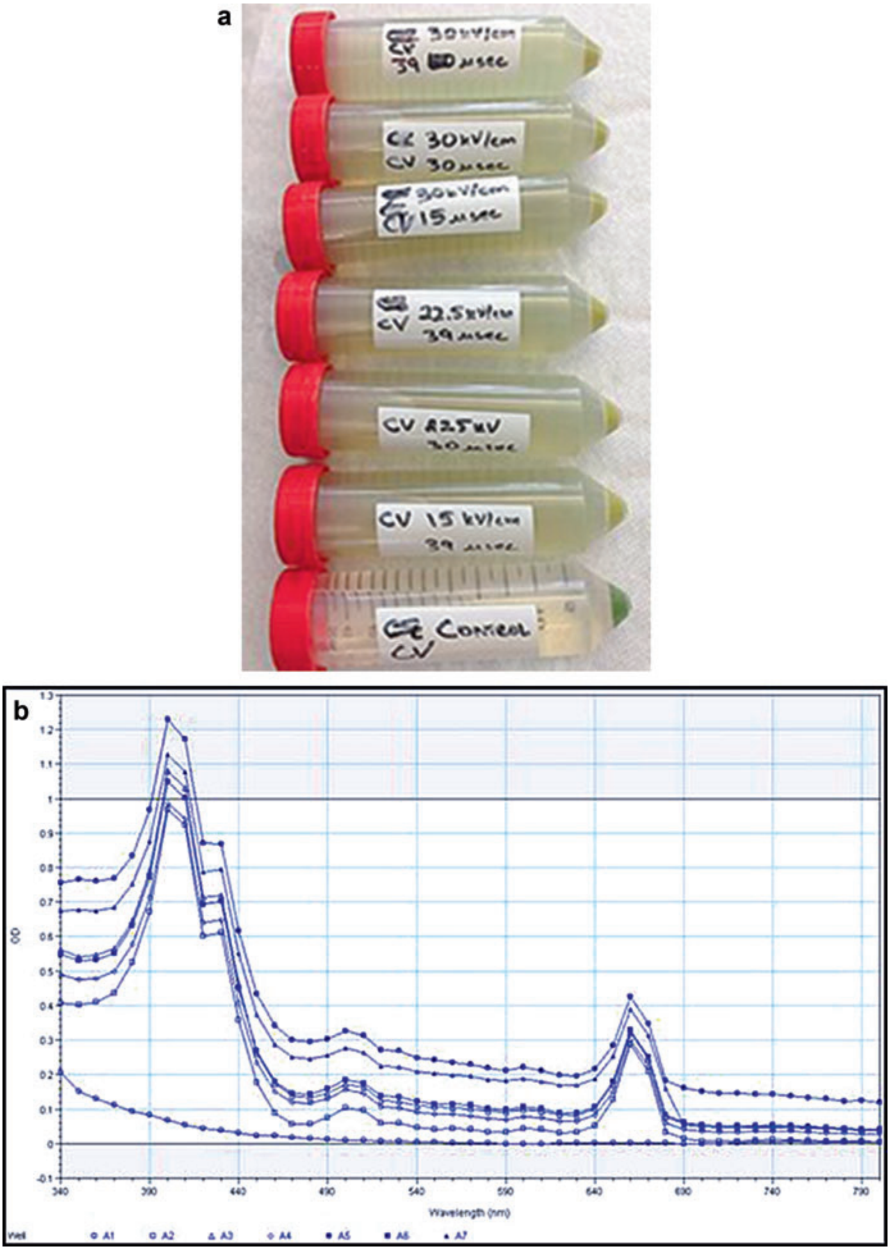

Fig. 3 *Chlorella vulgaris* treated at different field strengths and durations at ASU on the DTI Lab PEF System (Dempster 2014). The control sample (*bottom, left*) is optically clear after centrifuging and exposure to Triton X for 3 min, and the chlorophyll can readily be seen in the biomass pellet (*bright green*). The PEF-treated samples clearly show intracellular material released into the supernatant and a reduction of the chlorophyll in the biomass pellet (*yellow-green*). Spectrophotometric scans (R) correlate vertically to the samples (L) and show the differentiated levels of cell lysing that occur at each treatment condition, with the control at the *bottom*

assessment of the applicability of PEF, and the required process conditions for algal cell lysing, across a range of microalgae species, must be conducted.

PEF does appear to have substantial promise, even with the limited data available. One calculated cost was \$2.69 USD for PEF lysing of a volume of *C. zofingiensis* algae containing one barrel of lipids (at a treatment condition of 9.6 kV/cm, 29 μs into a 3.5 mS/cm concentrated solution – this cost is prior to extraction) (Roth 2011). This is over an order of magnitude lower than the cost of drying a concentrated microalgae solution prior to extraction, which was calculated at ~ \$40 USD/barrel under the same assumptions. The drying cost alone is comparable to the current price of petroleum oil. If the extraction costs are similar after either PEF or drying (which is still a subject of demonstration and confirmation), PEF would be over an order of magnitude cheaper than drying.

The key to commercial adoption of PEF for extraction of lipids and other compounds from microalgae, therefore, will be the overall cost of PEF and downstream extraction processing relative to the alternatives. The cost calculations themselves are straightforward. The energy per volume w (in J/cm^3) is deposited in a slurry by PEF treatment given by

$$w = \sigma E^2 t \tag{1}$$

where σ is the conductivity of the feedstock being treated, E is the electric field, and t is the total treatment time. By calculating this cost for a volume of end product (so that the effect of different algal concentrations and feedstock conditions is removed), we can compare PEF costs to other extraction techniques.

Ideally, we would be able to calculate this cost from the literature available. In all of the reported efforts examined, however, the scope was very narrow in terms of both microalgae strains and PEF parameters examined, and considerable ranges of effective field strength, treatment time, energy, and effectiveness have been reported, even for the same species. These disparities can, in part, be explained by differences in algae growth phase, PEF systems, voltage measurements, treatment times, assessment of lysing, etc. They do not, however, point to definitive treatment protocols that microalgae producers can adopt.

There are a number of other factors that influence the reported treatment conditions, shown in Table 2. A clear conclusion is that different algal species react differently to PEF and have varying levels of sensitivity. There does not (yet) seem to be a simple model to predict how a given species will react to a given PEF treatment. Cell size, cell wall thickness, and other first-order characteristics have been examined, but have not proved predictive.

Differences in the equipment used to provide the high-voltage pulses for PEF treatment onto the algal solution probably account for the second largest source of variations. Different pulse widths, treatment chambers, and even methods of measuring the treatment applied can lead to widely divergent reported results, even for ostensibly similar treatments. For example, the energy required may vary considerably as a function of pulse width (Luengo et al. 2015), to achieve the same levels of electroporation.

Table 2 Factors influencing PEF treatment of algae

Fluid conductivity
Field strength (kV/cm)
Treatment time
Pulse width
Pulse shape
Treatment chamber design
Flow rate/turbulence in chamber
Inlet/outlet temperature
Time between treatment and analysis
Algal species
Concentration
Growth phase
Extraction method
Compound(s) of interest

Complicating these comparisons, many researchers report only energy per volume or weight (i.e., kJ/kg), without information on the conductivity, pulse shape, and treatment time, initial temperature, or even algal concentration. Without these additional parameters, it is very difficult to translate these results into consistent PEF treatment protocols and realistic costs. In addition to the PEF treatment protocol itself, the time between PEF treatment and extraction/analysis may have a significant impact on the level of extraction reported (Luengo et al. 2014).

All of these factors make reported PEF results difficult to verify and nearly impossible to translate into the usable PEF system parameters of use to a commercial entity exploring the costs and benefits of PEF. A more controlled approach is required, where the species are known, the treatment conditions (pulse shape, treatment chamber, treatment time, temperature, conductivity, concentration, etc.) are controlled and consistent, and the resulting levels of cell lysing are recorded (and potentially over some time after treatment). Since this data is not typically included in published papers, the algal processor must collect it directly, adding to the cost of adopting PEF. Only with this data, however, will it be possible to assess the applicability and cost-effectiveness of commercial PEF processing for microalgae products.

Marine microalgae present an additional complication. The electric field and duration of the PEF treatment is severely limited by the temperature rise that occurs due to the very high conductivity of salt water (~40–60 mS/cm, vs. 2–4 mS/cm for freshwater). Boiling can occur even with relatively low PEF doses at these conductivities. Without remediation, this is a significant limitation on the application of PEF processing to saltwater algae – not because the boiling itself is detrimental to extraction, but because PEF treatment is a very expensive way to boil water. Potential approaches to PEF processing of saltwater microalgae require that the salt concentration be reduced, either through concentration and dilution with freshwater followed by reconcentration, or by other means. For saltwater species, without remediation/dilution, the cost would increase by a factor of 20–30, making PEF more expensive than drying. Reducing the conductivity by at least an order of magnitude is required. Using PEF for saltwater algae, at reasonable energy levels, represents a significant challenge.

Finally, while PEF can help release intracellular components into solution (which is recognized as a significant challenge), this is only the first step. The use of PEF alone, or with methanol or other solvents, has been reported, but not the overall cost of extraction. The compounds of interest (lipids, proteins, etc.) must still be separated from the solvent and purified before they represent a viable product – and it is the cost of this entire processing chain that is critical, not the cost of the individual steps.

PEF Control of Microalgae Predators

The second potential area of PEF applicability to algal products is predator control in microalgae cultivation. There are a number of potential algal predators, impacting different species of algae (Table 3). These range from bacteria and amoeba to multicellular animals, such as rotifers. Growing large quantities of microalgae is the single largest cost in the microalgae product stream (Brennan and Owende 2009). Open pond cultivation represents the lowest-cost approach to growing large volumes of microalgae biomass for downstream processing (Davis et al. 2012). These ponds, however, are subject to a variety of threats, including microalgae predators. Chemical treatments are not attractive, since they may have deleterious impacts on the microalgae or its intended products. Microalgae predators, such as amoeba, ciliates, flagellates, rotifers, and *Poterioochromonas* (Figs. 4, 5, and 6), can devastate a large microalgae pond in hours, causing extensive losses and production delays for commercial microalgae farms.

Table 3 shows one list of potential predators and their prey (Carney and Lane 2014). Chemical approaches to predator control have shown dismal results, given the range of both predator species and algae. The potential for wind-borne infection by these predators in open raceway ponds, and the frequency of contamination events, has led microalgae producers to accept the high costs of photobioreactors, invest in greenhouses and air filtration systems, change microalgae species, and/or adopt other countermeasures. At commercial scale, these can be cost prohibitive for all but the most valuable products.

Preliminary investigations by researchers on amoeba in France (Vernhes et al. 2002) and rotifers in Portugal (Rego et al. 2014) demonstrate that it is possible to apply PEF treatment to microalgae at intensities which are strong enough to kill predators without causing damage to the microalgae cells themselves. Both amoeba and rotifers appear to be susceptible to PEF at <1 kV/cm field strength, versus the 4–40 kV/cm field strengths typically required to kill commercial microalgae strains. The selective destruction of predators, but not microalgae, may be due to the absence of cell walls (amoeba), or their larger size (e.g., rotifers). There may also be a lingering impact of PEF treatment on larger predators, such as rotifers, in which

Table 3 Parasites reported for microalgae, including common group names and phyla, and the type of system the relationship was reported for (Carney and Lane 2014)

Group/taxonomy	Species	Microalgal host	System	Citation
Amoebae/ *Endomyxa*	*Vampyrella* sp.	Various	Natural systems	Hess et al. 2012
Aphelid/ *Cryptomycota*	*Amoeboaphelidium protococcarum*	*Scenedesmus* sp.	Open raceways for mass cultivation	Letcher et al. 2013
Aphelid/ *Cryptomycota*	*Amoeboaphelidium protococcarum*	*Scenedesmus* sp.	Laboratory culture	Gromov and Mamkaeva 1970
Chytrid/ *Blastocladiomycota*	*Paraphysoderma sedebokerensis*	*Haematococcus pluvialis*	Laboratory culture	Hoffman et al. 2008; Gutman et al. 2009
Chytrid/ *Chytridiomycota*	*Rhizophydium algavorum*	Various	Laboratory culture	Gromov et al. 1999
Chytrid/ *Chytridiomycota*	*Chytriomyces* sp. and *Zygorhizidium* sp.	Various diatoms	Laboratory culture and natural systems	Canter and Jaworski 1979; Beakes et al. 1988; Bruning 1991; Grami et al. 2011; Kagami et al. 2011
Chytrid/ *Chytridiomycota*	*Entoplyctis apiculata*	*Chlamydomonas* sp.	Natural system	Shin et al. 2001
Chytrid/ *Chytridiomycota*	*Phlyctidium scenedesmi*	*Scenedesmus* sp.	Open raceways for mass cultivation	Fott 1967; Ilkov 1975
Chytrid/ *Chytridiomycota*	*Rhizophydium* sp.	*Scenedesmaceae*	Closed photobioreactors for mass cultivation	Carney and Lane 2014
Labyrinthulid/ *Labyrinthulomycota*	*Labyrinthula* Cienk.	*Cyanobacteria*	Natural system	Raghukumar 1987
Oomycete/ *Oomycota*	*Ectrogella* sp.	*Pseudo-nitzschia*	Natural system	Hanic et al. 2009
Oomycete/ *Oomycota*	*Lagenisma coscinodisci*	*Coscinodiscus centralis*	Natural system	Gotelli, 1971
Sindinids/*Alveolata*	*Amoebophrya* sp.	*Dinoflagellate*	Natural system	Guillou et al. 2008; Chambouvet et al. 2011
Sindinids/*Alveolata*	*Amoebophrya ceratii*	*Dinoflagellate*	Natural system	Chambouvet et al. 2008

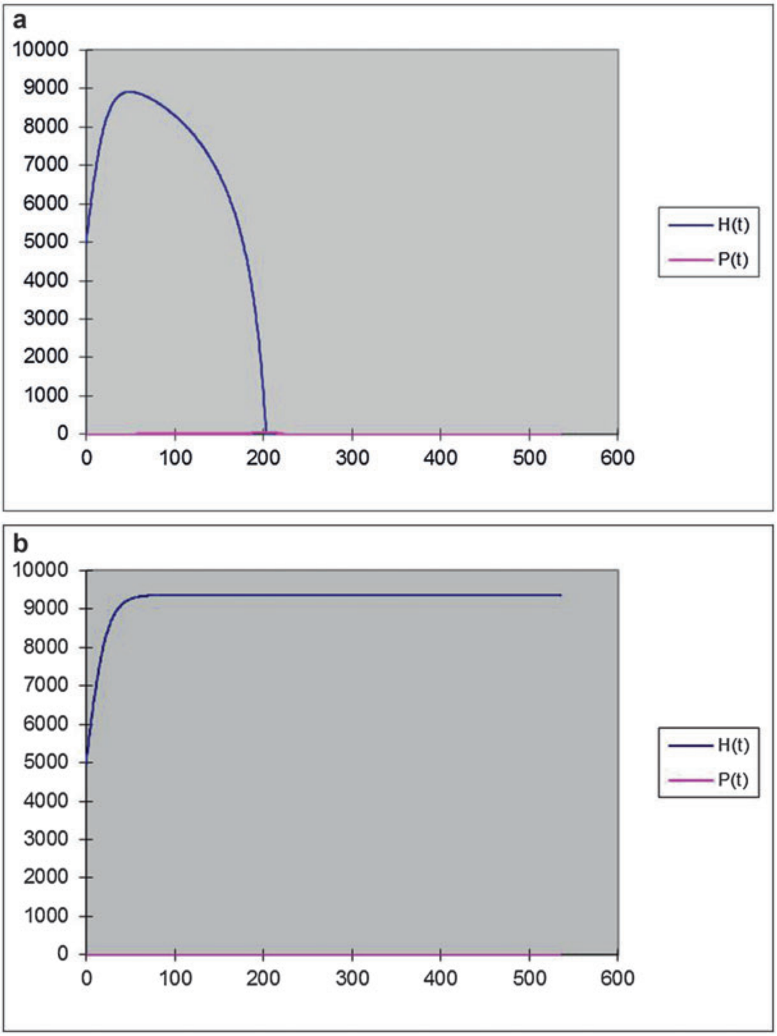

Fig. 4 Two simple predator prey models showing microalgae and predator population versus time, with representative growth rates and predation levels. The first shows the crash of a microalgae pond (L) with no predator control, and the second (R) shows the impact of low levels of predator control in the same pond (one complete treatment of the pond volume every 200 time units). All other parameters in this model were identical. Adapted from models in Kretschmer et al. 2003.

their population continue to diminish for days after PEF treatment (Rego et al. 2014). This distinction in lethality may not hold true for all predators (especially bacterial predators) and microalgae species.

For predator control, the basic parameters required to size the PEF system are the field strength and treatment time required for effective predator kill and the treated

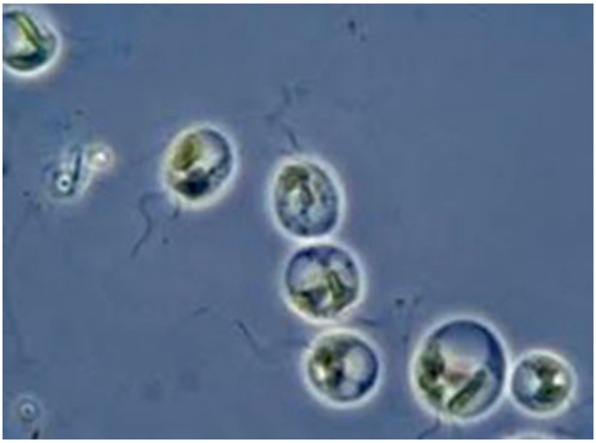

Fig. 5 Poterioochromonas

Fig. 6 Amoeba

volume required per hour as a percentage of the total pond volume. The low electric field reported for predator lethality makes predator control a very low energy application of PEF, since energy is a function of voltage squared (e.g., 10 % of the field strength requires only 1 % of the energy, with all other parameters constant).

With a PEF system continually treating a small fraction of the microalgae cultivation system volume (similar in concept to the operation of a swimming pool filter), it appears possible to prevent the population of predators from ever reaching concentrations required to "crash" cultivation systems (Fig. 7), at very low energy costs. In a 500,000 l raceway pond, for example, treating 10,000 l/h (2 % of the volume, or complete treatment every 2 days) at 1 kV/cm and 20 μs would require approximately 8 kWhrs of electricity, or less than $10 USD per month at $0.08/kWh.

Even if all of these factors were doubled to achieve the desired results (e.g., 2 kV/cm, 40 μs treatment time, and 20,000 l/h), the electricity cost would still be only ~ $150 USD/month – inconsequential compared to the cost of a single pond crash. The PEF equipment required at this scale would be much smaller than the Laboratory PEF

Fig. 7 Rotifers

System shown in Fig. 2. The same PEF system could also be used to treat feed water/cultures, to ensure that predators are not introduced via these avenues.

Conclusions

Algal products represent a very small market today, but their potential is enormous. The recent focus on, and of investment in, R&D for algal biofuels has created a new excitement around the cultivation and extraction of products from algae. This investment has led to substantive improvements in cultivating, harvesting, and processing algae. With this activity, the value of algal products for other markets has received close attention, and the recent drop in petroleum prices has shifted substantial activity from biofuels into high-value products. These factors, in turn, have spurred a level of commercial activity, with hundreds of companies involved around the world. It remains to be seen if this wave will recede in the face of continued lower petroleum prices (as occurred in the 1980s), or will be continued into the future.

Multiple researchers around the world have demonstrated that PEF processing lyses algal cells and that this can simplify the extraction of compounds from the algae itself. This research, however, has been limited to a small set of algal species and yields a wide range of different conclusions as to the energy required to lyse each species. Extending and consolidating this research, with consistently applied and reported PEF processes, would provide significant benefits to algal product manufacturers in assessing the utility of PEF in their operations. It may also open products that are cost-prohibitive under conventional processes to algal cultivation and extraction.

Characterizing the interaction of PEF processing with downstream extraction/separation technologies is in its infancy. Clearly, the elimination of a drying step prior to extraction yields significant energy savings. This is highly beneficial in some extraction processes, but irrelevant to others, since some extraction solvents and processes also lyse the cells. This appears to be the major missing element in determining the commercial applicability of PEF to the production of algal products. PEF treatment must be critical to the entire extraction and separation process to attract commercial interest from algal producers.

Finally, the use of PEF as a means of predator control is a very interesting application, which remains to be proven in practice, and at large scale. This technique clearly shows promise for some predators, but others (especially microbes) may not be amenable to PEF at nonlethal field strengths for the algae. Clearly, this is an area requiring additional research.

PEF is just one of many technologies being examined for algal cultivation and production/extraction. Its full applicability to this market has yet to be demonstrated, but it has the promise to lower the cost of algal products significantly. The range of variables that must be assessed to determine the actual cost of PEF treatment, and its overall benefit in algal compound production, is very broad, making simple conclusions about both cost and benefit impossible. Only after cost savings can be clearly demonstrated will commercial algal products companies adopt PEF in their operations. There is a lot of work required to make the promise of algal PEF a reality.

Finally, all of this depends on the growth and profitability of an algal products market – whether for biofuels, specialty chemicals, or protein/feed. This growth, in turn, depends on the work of a wide range of researchers, across dozens of fields – including PEF.

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