Algae: an essential link between our past and future

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Interest in the replacement of imported fossil-based fuels with home-grown, renewable and cheap sources of energy spikes each time we are paying over $1.50/litre for petrol. This time, however, the danger of an energy shortage coincides with an increasing level of atmospheric CO_2 and an approaching global food shortage. This all dictates an urgency for the development of a new generation of feedstocks that will not only produce biofuels or their various components, but that can also be used for feeding animals and humans and at the same time will reduce a level of atmospheric CO_2. These feedstock organisms have to be grown on marginal lands not used for food crops and require low inputs of fresh water and fertiliser. Further adding to this wish list is that these feedstocks could also grow in sea water, can utilise animal and human waste and be used as fertilisers for crops. All of this will finish a portrait of algae, a group of aquatic, photosynthetic, organisms ranging in size from few microns for microalgae to over 100 metres in the case of *Macrocystis pyrifera*.

There are over 40,000 algal species that can be grouped into several eukaryotic phyla; including the Rhodophyta (red algae), Chlorophyta (green algae), Phaeophyta (brown algae), Bacillariophyta (diatoms), and dinoflagellates, as well some in the prokaryotic phylum Cyanobacteria (blue-green algae). In order to adapt to their real estate, algae have evolved an extraordinary diverse ability to grow: autotrophically, utilising light energy and carbon dioxide to produce hydrocarbons; heterotrophically utilising energy from a range organic carbon sources dissolved in the media in absence of light, and mixotrophically utilising both types of energy sources, light and organic molecules. Because of their high levels of proteins, lipids, hydrocarbons, carbohydrates, small valuable molecules and pigments, algae have been used by humans for centuries as food supplements, animal feed, food for aquatic organisms, cosmetics and pigments[1,2].

Despite the wide range and long history of applications of algal-based products, it is an energy shortage that has spiked a renewed interest in algal research. This interest was first triggered by the energy crisis in the 1970s, in response to which the US government initiated the "Biodiesel from Algae" program. The second wave of interest in algal-produced biofuel has been accompanied by massive investments from government and private funds including the multi-international airline companies and has triggered significant recent progress in microalgal functional genomics and lipidomic research.

Algae represent a potential third-generation biofuel. The first-generation biofuels, bioethanol and biodiesels were fermented, distilled or chemically converted from the seeds of food crops such as corn, sugar cane, soybeans and oil palms. Significant use of fresh water and fertilisers together with associated controversial "food versus fuel" issues are serious limitations of these technologies. Extensive research in plant cell wall biochemistry has shown the huge potential for photosynthetic energy accumulation in the form of lignocellulosic polymers within plant cell walls[3]. This biomass-to-fuel strategy is largely based on use of non-food plants, trees or industrial and agricultural residues. However, one of the main limitations of this approach is the fact that bioalcohols (ethanol and butanol), the main products of cell wall fermentation, are not the most energy dense molecules, having only 70% of petroleum's energy. They, therefore, are of little or no use to run heavy road vehicles, rail vehicles, or aircraft[4].

Compared with other energy crops, algae have obvious advantages in biofuel production:

(i) Some algae can accumulate large amounts of oil, (in the case of *Botryococcus braunii* up to 80% of dry weight), producing up to 150–200 times more bio-lipid than any other known feedstock on a per-acre per-year basis. In addition, algal biomass also can be processed into other biofuels such as syngas, methane, hydrogen, ethanol and butanol.

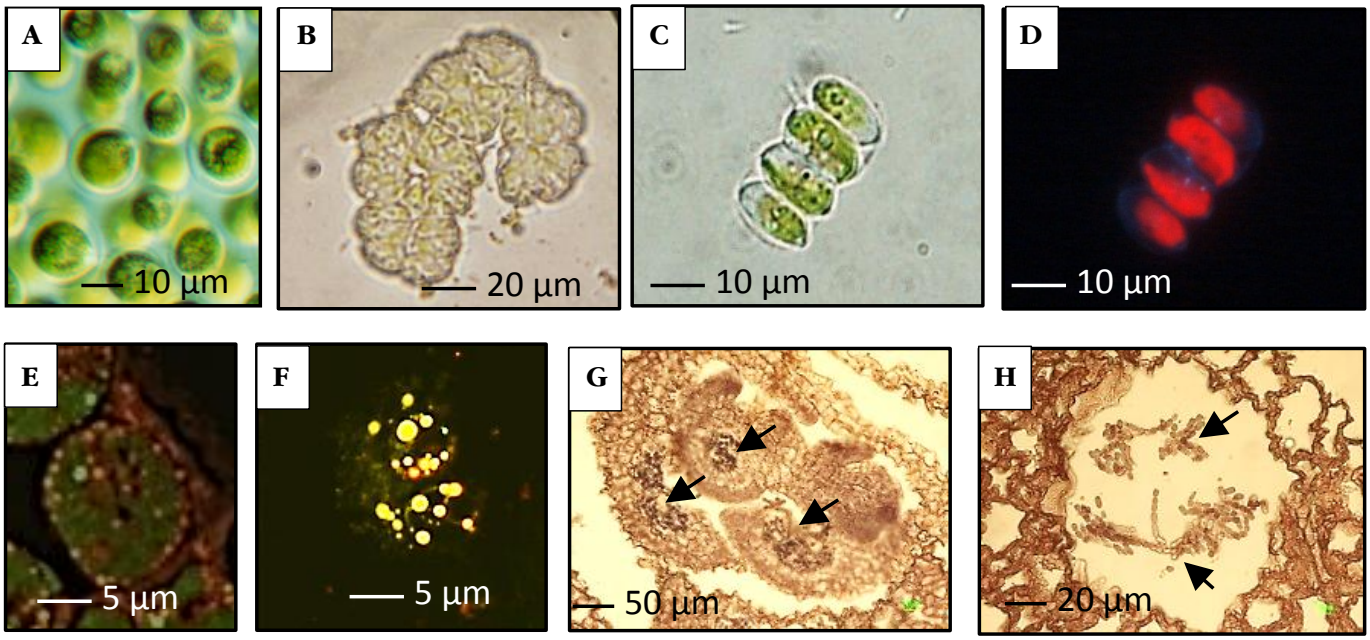
(ii) Algae are one of the fastest growing photosynthetic organisms on earth, some algal strains can double biomass every 8–12 hours, thereby producing oil products all year round, unlike most seasonal crops.

(iii) Algae can be grown on marginal lands and can utilise saline, brackish or in some cases waste water. A sewage-to-fuel strategy can in some cases potentially make the price of algal-driven biofuels competitive with gasoline and diesel fuel.

(iv) Being photosynthetic organisms they can efficiently sequester carbon dioxide reducing emissions of this major greenhouse gas in atmosphere.

Despite the advantages offered by microalgae and progress in understanding of molecular aspects of fatty acid and lipid biosynthesis in algae, microalgal biofuel is not yet commercially viable[5]. The most expensive steps in production involve large-scale biomass growth and harvest, lipid extraction and chemical transesterification for formation of biodiesel. In some cases, fatty acid composition also needs to be optimised, for example for applications such as biofuel for aviation. To overcome some of these challenges an ideal microalga for biofuel production needs to have accumulated a few key features: (i) a high oil yield (preferably with enhanced levels of saturated C8-C12 fatty acids) grown under both normal and stressed conditions; (ii) be insensitive to photo-oxidation; (iii) to excrete rather than accumulate oil or have thin cell walls for easier extraction; (iv) aggregate forming easy-to-collect flocks; and (v) be resistant to pathogens and predators. Genetics and transgenic technologies are very useful during the development of these traits within algal species. Commonly used strategies for the enhancement of cellular lipid yield include redirection of energy storage compounds from starch to lipid metabolic pathways in starch mutants and/or decreasing lipid catabolism such as β-oxidation that consumes triacylglycerols (TAGs)[6]. Another successful strategy for biomass enhancement has been increasing photosynthetic efficiency through enhanced resistance to photo-oxidative damage[7]. Tailored composition of fatty acids has also been successfully achieved through expression of a family of plant acyl-ACP thioesterases from species such as *Umbellularia californica* and *Cinnamomum camphorum*. Transgenic algae expressing these genes also showed enhanced levels of C8-C12 fatty acids[8].

It is not surprising that microalgal-produced biofuel has attracted



Representatives of microalgal collection: (A) *Chlamydomonas reinhardtii*, CC-125; (B) *Botryococcus braunii,* race L; (C) *Scenedesmus sp*, bright field and (D) UV filter. Red colour represents chloroplasts; (E) Lipid bodies stained with Nile Red in *C. reinhardtii* wild type and (F) starch mutant, CC-4334 under nitrogen limited condition; (G,H) Symbiotic association between water fern *Azolla filiculoides* and nitrogen-fixing cyanobacteria *Anabaena azollae.* Arrows show *A. azollae* inside leaf cavities of *A. filiculoides*

significant interest and investment from commercial airlines. Continental, Virgin Atlantic, Air New Zealand, British Airways and Japan Airlines are already testing improved biofuel fuel mixtures and British Airways has recently signed an agreement with a UK biofuels firm to produce jet fuel from municipal wastes. Qantas will purchase a minimum of 200–400 million litres of jet fuel per year from Solazyme company from 2012. Solajet™, the world's first 100% algal-derived jet fuel produced by Solazyme, has application for both military and commercial aviation and has been assessed in a US Navy testing and certification program[9]. The USS Ford, a US Navy Frigate fleet ship, journeyed from Everett, in Washington State to San Diego in California powered with 25,000 gallons of Soladiesel HRF-76®[9].

A largely untapped reservoir of novel and valuable compounds, microalgae can also serve as the basis for the next generation of fine chemicals and healthy food ingredients. For example, the market for petrochemicals and oleochemicals (derived from fats and natural oils) was estimated at $611 billion in 2009[10]. In the same year about 9–10 million metric tons of palm kernel and coconut oils were consumed for food and industrial applications such as laundry detergent, soap, and shampoo. The $2 trillion packaged food industry is also actively seeking more natural, sustainable sources of fibre and healthier fats without compromising value, quality, taste or nutrition[9]. The World Health Organization (WHO), the National Institutes of Health and UNESCO have stressed an urgent need for a new generation of low-cost, needle-free, heat-stable vaccines to promote vaccination programs in the poorest regions of the world[11]. It was convincingly demonstrated that microalgae represent a viable platform for the expression of a range of therapeutic proteins and oral-based vaccines against a wide range of diseases.

Our group is actively establishing efficient transformation pipelines for the expression of recombinant proteins in microalgae and microalgal communities. We are targeting metabolic reprogramming of fatty acid biosynthesis pathways through expression of recombinant enzymes to tailor oil composition; are screening for algal-produced metabolites, and are also examining strategies for the production of therapeutic proteins and antigens. The projects include engineered secretion of a variety of recombinant enzymes, such as cellulases, invertases and fructan exohydrolases, recombinant antigens for algae-based vaccines against flaviviruses, including Dengue and Japanese encephalitis. With a breadth of disciplines, from biotechnology, aquaculture, chemical and analytic sciences and environmental sciences the School of Applied Sciences at RMIT presents a unique environment to investigate the production and use of sustainable algal biofuels for automotive, aviation, biomonitoring, food and health applications.

Conclusion

There will be no life on earth without algae. Colonisation of our planet 2 billion years ago by the first photosynthetic algae led to the reduction of the level of atmospheric nitrogen, CO_2 and increased the level of oxygen to 21%, the level required for life. Following this, the algal escape from water 450 million years ago and the subsequent evolution into a half million terrestrial plant species represent two of the key milestones of the evolution of life on earth. Whilst an important part of our evolutionary history, algae may also offer "green" and smart solutions for some of the serious challenges we face today, representing an essential link between our past and future.

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Biographies

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