Biofuel production – tapping into microalgae despite challenges

Kamaljeet Sekhon Randhawa, Louise E Relph, Michael C Armstrong, Pattanathu K.S.M. Rahman*

School of Science and Engineering, Teesside University, Tees Valley, Middlesbrough, TS1 3BA, UK
*Corresponding author: p.rahman@tees.ac.uk

Abstract

Biofuels provided 2.7% of world's transportation fuel in 2015 which is expected to go up to 28% by 2050. However, most of the biofuel produced till date is from crops that can be used as food or feed. Microalgae or most famously the 3rd generation of biomass has the potential to overcome the problems associated with this food vs fuel debate. Microalgae are microscopic photosynthetic organisms which have the ability to fix CO_2. Thermochemical conversion via hydrothermal liquefaction is a favourable technology for recovering energy from algal biomass. Research is focused on discovering a viable and sustainable feedstock by cultivating and up-scaling the use of microalgae and then utilizing hydrothermal liquefaction to produce a workable biofuel. Synthetic biology and several genetic engineering techniques have also shown promising results in the production of biofuels. Plenty of research is being carried out in the field of using microalgae as biomass for biofuel generation; however, it still calls for a robust conversion technology to make the process commercially viable.

Keywords: Greenhouse gases, Biomass, Biofuels, Microalgae, Hydrothermal Liquefaction, Engineered algae, Transgenic algae, Synthetic biology

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1. Introduction

The world has witnessed the Paris Climate Conference (COP21) in December 2015. Over 195 countries adopted the first-ever universal, legally binding global climate deal. The agreement focusses on to avoid the dangerous climate change by limiting global warming to below 2^0C by reducing carbon emissions and mitigating other climate changes. Since many decades, there has been a global concern associated with higher fuel prices, climate variability and CO_2 emissions (Cuellar-Bermudez *et al.*, 2014). These are becoming more difficult to control due to population explosion and consequent rise in energy needs. A further major complication is the depletion of inexpensive non-renewable energy sources such as oil, diesel and ethanol (Cherubini and Stromman, 2011; Franco et al., 2015). Presently, transportation and energy sectors are responsible for a huge proportion of greenhouse gas emissions. The global energy crisis and political pressure to reduce greenhouse gas emissions has increased the volume of research being carried out to discover a sustainable alternative method of producing energy which is more economical and environment friendly (Cuellar-Bermudez *et al.*, 2014).

As a source of energy, biofuels are considered renewable through sustainable farming techniques and are associated with low production costs (Franco et al., 2015). The production of biofuels from renewable feedstock is nothing new and has been going on from many decades. The oil crisis in the 1970's led to an interest in producing and using biofuels as an alternative to fossil fuels for use in transportation in many countries (Timilsina and Shrestha, 2011). However, the use of vegetable sugars and oil for producing biofuels (sugar beet, sugar cane, corn and oily seeds) has been named as one of the main reasons for increasing food prices and disputes over land use; thus the global sustainability of such a process comes under scrutiny (Fung *et al.*, 2014; Franco et al., 2015). The possible environmental benefits that could be gained by replacing petroleum fuels with bioenergy and biofuels obtained from renewable biomass sources remain the primary motivators for advancing the manufacture and use of bioenergy and ls (Von Doderer and Kleynhans, 2014). **Table 1** shows the different types of biomass used and the conversion technologies adopted so far. Biomass viz. plant waste and microalgae is regarded as one of the most positive alternatives to conventional feedstock as it is the only renewable source of fixed carbon that converts into solid, gaseous heat and liquid, fuel and power (Jahirul et al., 2012). The carbon sequestration during biomass growth and ensuing release of carbon during the combustion process in the form of CO_2 can be regarded as a carbon neutral part of the bioenergy system (Von Doderer and Kleynhans, 2014).

Biofuels can be classified as natural biofuels, first generation, second generation and third generation biofuels (Noraini *et al.*, 2014).

Natural biofuels are usually obtained from organic sources such as animal waste, vegetables and landfill gas. These are used for heating, cooking, brick kiln or electricity production. The technologies used to produce biofuels are dependent on the type of feedstock used. The first generation group comprises of the technologies that utilize the starch or sugar elements of edible plants; sugar beet cereals, sugar cane and cascara being used as feedstock to produce ethanol and those that use rapeseed, sunflower, oilseed crops, palm oil and soy bean to make biodiesel (Timilsina and Shrestha, 2011; Noraini *et al.*, 2014). Second generation biofuels are produced using technologies that convert fervent lignocellulose biomass such as *Sterculia foetida*, *Ceiba pentandra*, *Miscanthus*, jatropha, switch grass, poplar forest and agricultural residues into usable biofuels (Peters, *et al.*, 2011; Noraini *et al.*, 2014). Biofuels produced from more advanced feedstock such as jatropha and microalgae are also included in the second generation group (Timilsina and Shrestha, 2011). Thermochemical conversion by fast pyrolysis is one of the most efficient methods of producing biofuels from the lignocellulosic biomass. The acquired pyrolysis oil is a high density and moderate heat value liquid that can be upgraded in a biorefinery into diesel and gasoline blend stocks (Peters et al., 2011). Third generation biofuels use macro and micro algae as feedstock and have been widely accepted as a potentially viable alternative energy source.

First generation biofuels are still commercially produced; however, despite the advantages of biomass that can be used for second generation biofuel production, higher yields and lesser requirement for land use, they are not presently being commercially produced due to lack of efficient technologies (Noraini *et al.*, 2014).

There is enormous research going on to examine the role of microalgae in biofuel production. In recent years, microalgae have become a popular feedstock for triacylglycerol, neutral lipid storage and biodiesel production (De Bhowmick, Koduru and Sen, 2015). By genetically engineering the microalgae used in biofuel production, the target product yield could be increased, improving the viability of the technology.

The present review gives an overview of this field of research throwing light on the global concerns in bioenergy sector and if microalgae is the answer to the biomass required for biofuel generation. Biotechnological approaches, their challenges and prospective opportunities have been discussed in this review. Despite vast research in this field it seems an inexpensive and commercially viable technology to convert microalgae into biofuel is yet to be discovered.

2. Increasing energy demands – global concerns

The global transportation sector is responsible for producing approximately 15% of all greenhouse emissions, over 70% originating from road transportation (Soimakallio and Koponen, 2011). The use of diesel and gasoline fossil fuels is set to double in the next 25 years and, therefore, greenhouse gas emissions will certainly increase vastly unless preventative measures are put in place (Soimakallio and Koponen, 2011). The world energy needs can be predicted to increase by 44% from 2006 to 2030 (Cherubini and Stromman, 2011). This has been made evident by the 4th Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, which reveals that the growing use of fossil fuels coupled with the current population growth has resulted in a rapid increase in greenhouse gas emissions.

Environmental concerns are that every year the earth's atmosphere is subjected to more than 15 billion tonnes of CO_2. Fossil fuel combustion is a major contributor to the increase in the levels of CO_2, which is a direct cause of global warming (Kolar and Civas, 2013). Worldwide, oceans annually absorb approximately one-third of all CO_2 produced from human activity (Cuellar-Bermudez *et al.*, 2014). The continuous rise in the amount of CO_2 present in the atmosphere increases the amount that is absorbed into oceans, which is an environmental concern. This gradually changes the pH of the water making it more acidic and precipitates immediate losses to the ecosystem diversity of both marine life and coral reefs (Li and Gao, 2012). If CO_2 production continues at the present rate there are huge implications for ocean life and consequential effects to earth life (Cueller-Bermudez *et al.*, 2014).

Bioenergy has some advantages as it has an almost closed CO_2 cycle that produces only small amounts of greenhouse gas emissions. However, there are some disadvantages to using bioenergy as an alternative fuel source. The conversion processes are dependent on the addition of external fossil fuels to produce and harvest feedstock, to handle and process biomass, to fuel bioenergy plants and they are also required in the transportation of the feedstock and biofuels (Von Doderer and Kleynhans, 2014).

Biofuels have also been identified as one of the principal reasons behind the increased food prices. As the industrial use of biofuels has increased so has the need for agricultural land to produce the feedstock (Kim *et al.*, 2013). Even if the land usage problem is solved there are still key issues in relation to the use of crops to produce biofuels as a long-term alternative to petroleum including inadequate scalability, minimal net energy reductions and insignificant reduction in the production of greenhouse gases (Quinn *et al.*, 2014). These will need to be investigated thoroughly and be overcome if crop biofuels are to be a future sustainable option.

There also have been some feasibility studies conducted globally to encourage the use of biofuels. Brazil increased its national ethanol programme following a peak in oil prices in 1979. The US launched a corn-based ethanol programme at the same time but on a much smaller scale. China, Kenya and Zimbabwe were also motivated to try and produce biofuels but failed (Timilsina and Shrestha, 2011). An example of where this type of feasibility study has been implemented successfully is in Malaysia. Historically this is a country that produces huge amounts of palm oil that was exported and sold to other countries in order to generate an income from an otherwise surplus commodity. In 2011 a mandatory implementation of a programme to use its palm oil yield to produce biodiesel for the transportation sector was ordered to meet the countries contribution to carbon reduction and biofuel sustainability (Masjuki *et al.*, 2013).

Concerns over greenhouse gas emissions and the potential for rapid increases in petroleum prices due to the limitations in supply-demand has activated a global search for an alternative transport fuel and a high efficiency conversion technology that can achieve the maximum motive power out of chemical fuels (Bergthorsen and Thomson, 2015). To reduce and limit the levels of global greenhouse gases below the current 550 ppm CO_2 equivalent would require huge emission reductions and would result in a total phase out of all fossil fuel emissions in developed countries by 2050 (Ullah *et al.*, 2014).

All the data indicate that with the outburst in population, the demand for food and fuel is bound to increase in the coming years. Therefore, an out-and-out sturdy technology where the biomass such as microalgae can be inexpensively and conveniently converted into biofuel is the need of the hour.

3. Microalgae - the ultimate solution?

Since the 1950's attempts have been made to extract fuels from algae and there has been significant investment made worldwide, particularly by the military, the aviation industry and energy companies. Large scale commercial production is only just starting to emerge, the primary issue is whether the production of biofuels from algae is commercially viable (Benson *et al.*, 2014). Several life cycle assessments (LCA) have been performed to evaluate the microalgae biomass to biofuel and bio-product possibilities on a conceptual level, based on a range of different approaches and methods. LCA are focused on determining the severity of an environmental impact due to the production of microalgae-based biofuels (Benson *et al.*, 2014). LCA are critical to validate usable technological innovation, with lower energy intensities and improved environmental performance (Grierson *et al.*, 2013).

Microalgae are aquatic as well as terrestrial species and are photosynthetic microorganisms that convert water, sunlight and carbon dioxide into biofuels, feed, food and high-value bioactive compounds (Li and Savage, 2013; Chen *et al*., 2014a). Autotrophic algae cultivation can be performed in either enclosed photo-reactors or open pond raceways; however photo-reactors are usually seen as too expensive for large-scale production of biofuels (Handler *et al.*, 2014). The use of microalgae in large-scale production of biofuels is inhibited by expense and feasibility. Microalgae that store lipids are usually unicellular and found in suspensions with low densities making separation problematic (Rawat *et al.*, 2013). One solution could be to utilize all the constituent parts of the microalgae. The carbohydrate and lipid content is approximately 70% and has several applications including bio-oil, bio-hydrogen, bio-ethanol, bio-methane, plastics, fertilizers, nutrients, sorbents and animal feed (Rizwan *et al.*, 2015). The use of pure strains or cultures causes problems in industrial applications due to contamination therefore the utilization of mixed indigenous microalgae cultures could be a potential solution with commercial capability (Cea-Barcia *et al.*, 2014).

Freshwater macroalgae, a largely overlooked class of phototrophic microorganisms, can show high rates of areal productivity and usually form either substrate-attached turfs, or closely packed floating mats that could mean huge reductions in the cost of harvesting and dewatering compared to microalgae (Yun *et al.*, 2015). Typically macroalgal cultivation is synonymous with seaweed growth and harvesting, over 16 million dry tonnes are produced yearly worldwide (Yun *et al.*, 2015). Despite the economic and environmental advantages of using macroalgae biomass to produce biofuels there remains several challenges that need to be addressed. One such challenge is that macroalgae contains unique carbohydrates that means the conventional biomass conversion process to produce biofuel cannot be utilized (Jung *et al.*, 2013).

Autotrophic microalgae are able to use carbon dioxide and solar energy to synthesize proteins and lipids enabling them to grow. The production of biodiesel from autotrophic microalgae mostly occurs in indoor photo-bioreactors. However autotrophic microalgae depends heavily on light for photosynthesis resulting in higher energy outputs for illumination, a requirement for shallow cultivation systems with large surface areas (Kim *et al.*, 2015; Mohan *et al.*, 2015). In comparison there is a lot more flexibility in heterotrophic microalgae culturing as they can grow without the addition of a light source and are capable of storing higher lipid contents in their cells (Zhang *et al.*, 2013). In heterotrophic nutritional mode, microalgae use organic molecules as their main carbon and energy source which assists in high biomass yields and makes large-scale production much more feasible. The relative simplicity of operations, easy maintenance and cost effectiveness are the primary benefits to heterotrophic microalgae culturing (Mohan *et al.*, 2015).

A variety of biomass conversion technologies have been investigated in an effort to use microalgae to produce biofuels commercially. Technologies for the extraction and conversion of biomass include hydrothermal liquefaction (HTL), pyrolysis and lipid extraction. Two thermochemical technologies, slow pyrolysis and HTL have been successful experimentally in the conversion of microalgae into bio-oil. Both slow pyrolysis and HTL have the potential to be used but there has been limited assessment of industrial-scale feasibility and the environmental impact (Bennion *et al.*, 2015). HTL converts biomass into liquid fuels by thermal conversion, operating in heated pressurized water conditions for a long period to break down the hard polymeric structure into mostly liquid components. The process allows wet materials to be treated without having to dry them first and to achieve ionic reaction conditions by preserving a liquid water-processing medium (Elliot *et al.*, 2015).

Undoubtedly the algae-to-biofuel conversion is not an affordable and trivial process and has several challenges. It certainly has limited market as of now but the majority of research in this regard has shown promising consequences. There are several ongoing projects which when completed seem to have a game-changing influence in this area. An overview of research councils and companies which have invested in the micro and macro algae projects is shown in **Table 2**.

4. Complications and opportunities in converting microalgae to biofuel to its scale-up

Transportation fuels and energy industry are responsible for producing the majority of all energy related emissions. Currently renewable energy only contributes about 11% to global primary energy, although it is predicted that 60% of all energy will originate from renewable sources by 2070 (Ullah *et al.*, 2014). The first developments in discovering effective biofuels for transportation purposes were based on the established process of converting plant sugars into ethanol by fermentation and the upgrading of vegetable oils by trans-esterification (Bergthorson and Thomson, 2015). Globally it is expected that there will be a rise in the production and use of biofuels. But the overall contribution to the total energy demands, particularly in the transport sector, will continue to be limited. This is mainly due to the competition with fibre and food production for arable land use, lack of appropriately governed agricultural practices in emerging markets, regionally constrained market structures and the necessity for bio-diversity conservation (Noraini *et al.*, 2014).

Current research is focused primarily on discovering a viable sustainable feedstock to produce biofuels, upscaling the use of certain types of microalgae and then utilizing hydrothermal liquefaction to produce a workable biofuel. There are negative and positive factors to be considered for the upscaling of algal cultivation especially to the marine and coastal environments (Coelho *et al.*, 2014). Presently the technology required to make each stage of the process economically feasible viz. microalgae cultivation, harvesting, transport, pretreatment and successful conversion of biomass into high yield biofuels, has not yet been discovered (Coelho et al., 2014).

For the successful mass production of biofuel from microalgae cultivation the problems that would need to be resolved are locating the large amounts of fresh water needed, obtaining enough nutrient sources of nitrogen, phosphorus and trace elements and over-coming the shortage of cost effective and energy efficient procedures for the harvesting of algal biomass, oil extraction and conversion. There is also a need for fully developed and tested technologies to deal with CO_2 mitigation from microalgae and a system integration and evaluation (Zhou *et al.*, 2014). Algal cultivation could hypothetically provide a sustainable feedstock and has the potential for CO_2 remediation when the microalgae biomass reaches a higher CO_2 fixation than that of terrestrial biomass if these initial problems are addressed (Coelho et al., 2014).

Also, during the conversion process organic nitrogen transforms into ammonia in a reducing environment and NO_X in an oxidising/combustible environment. During the production of biogas the substantial levels of nitrogen biomass content causes ammonia toxicity throughout the anaerobic process and may impede the bacterial decomposition of algal biomass. A prime concern regarding this process is that nitrogen in biomass will also produce NO_x molecules throughout the gasification process, which is performed with a limited oxygen supply, resulting in NO_x release into the atmosphere. This is an environmental concern as it has greenhouse gas properties and thus the requirement for the implementation of rigid emission regulations (Garcia-Moscosa *et al.*, 2013). Initial problems with developing the microalgae industry at large scale are the massive installation and continuous operating costs, robustness of the strains, quality of lipid for the production of biodiesels, the loss in lipid content during scale-up and the difficulty managing the conditions of cultures, particularly outdoor cultivation (Ahmad *et al.*, 2013; Yen *et al.*, 2014).

Figure 1 shows an experimental new alternative to traditional conversion methods. Thermochemical conversion via hydrothermal liquefaction (HTL) has a strong potential for commercial production as it seamlessly merges with existing petroleum refining infrastructure (Liu *et al.*, 2013). During the HTL process drenched algae biomass with a 85-90% water content is transformed through temperature reactions and high pressure into four process streams; non-aqueous bio-crude, made mainly of fatty acids, long chain alkanes and phenolic compounds, an aqueous phase comprising of organic acids and nearly all of the phosphorous and nitrogen in the biomass (30 – 50% wt.), a gas phase that contains CH_4 and CO_2 and the other volatile compounds (1 – 8% wt.) and a solid phase comprising mainly of bio-char (-3% wt.) (Liu *et al.*, 2013). The main advantage of this technique is that it is not a threat to food crop production as it is a simple cultivation process in open sea (Anastasakis and Ross, 2015).

Most biomass can be processed by HTL due to its hydrothermal nature and the adequate ease in producing water slurries from biomass particles at pump able concentrations usually about 5-35% dry-solids (Elliot *et al.*, 2015). High-moisture microalgae biomass often requires some dewatering which helps in lowering costs of processing excess water. Using HTL to process microalgae has various advantages over conventional methods as it can tolerate low cell concentrations and allows conversion of low-lipid strains that usually have higher growth rates than those optimized to acquire high lipid levels (Jazrawi *et al.*, 2015). The long-term environmental and societal effects of biofuel and bioenergy production have certain concerns associated with them that need to be overcome if a more sustainable global energy and fuel source is to be discovered (Seay and Badurdeen, 2014).

There are a significant number of economic and technical challenges associated with the usage of microalgae in the biofuels industry. Harvesting microalgae is a major problem. The unicellular algae that stores lipids have low densities and are located in suspensions making separations laborious. The extraction processes used for large-scale production are particularly complex and are still in the early development stages (Rawat *et al.*, 2013). Microalgae cultivated in open pond systems are prone to contamination. Bacterial contamination aggressively competes for nutrients and oxidises the organic matter, which can lead to culture putrefaction. They are also susceptible to protozoa and zooplankton grazers that consume microalgae and may destroy the concentrations of algae in a short time (Rawat *et al.*, 2013). In open pond systems there is also loss of water through evaporation and in order to maintain a fixed volume and salinity in the culture it is necessary to add large quantities of freshwater (Das *et al.*, 2015).

Other challenges that inhibit the commercialization of algal based biofuel production include; difficulties in finding rapid growing algae strains with high oil content, photosynthetic efficiency, simple algae culture harvesting systems, infrastructure, operation and maintenance costs and the ability to develop economical photo-bioreactor designs (Adenle *et al.*, 2013).

5. Genetics to Synthetic Biology – approaches and their challenges

The use of microalgae in biotechnology has the potential to revolutionise the field, this potential increases with the utilisation of transgenic or genetically modified algal strains (Rosenberg *et* *al.*, 2008). Both genetic engineering and lately synthetic biology techniques have been deployed to produce biofuels from microalgae.

How genetic engineering is applied in various processes can be polarising. Many genetic engineering processes are considered the norm; such as therapeutic protein production, however processes such as genetically modified crops or laboratory grown meat are much more controversial. With the current demands for food and fuel for a growing population, the economical production of algal biomass to be used in the fuel industry has placed focus on the use of engineered algae (Henley *et al.*, 2013).

Transgenic or engineered algae can be produced through various methods. The well-documented ones are transformation using electroporation and using *Agrobacterium tumefaciens*. The second highly used approach is biolistics.

Previously the lipid production of *Phaeodactylum tricornutum* has been improved through genetic modification; specifically the enhanced expression of *Phaeodactylum tricornutum* Malic enzyme (PtME) by Xue *et al.*, (2015). Transformation in the Xue *et al.*, (2015) study was accomplished through the use of electroporation. The process involved running a pulse of electricity though the host cell to disrupt the cell membrane to allow for the introduction of new genetic information. The resulting lipid yields increase 2.5-fold to a record 57.8% of dry cell weight. Furthermore the growth rate of the cells is similar to that of the wild type. Neutral lipid content increases by 31% in a nitrogen-deprived environment; a 66% improvement when compared to the wild type. This could prolong the production of lipids in an environment where a wild type algal species would reduce its lipid production due to nutrient restrictions. The study commented on the ability to optimise electroporation by the management of plasmid amount, concentrations of osmosis solution, the duration of the pulse and the voltage used to create the pulse (Guo *et al.*, 2013), essential for the creation of effective genetically engineered microalgae.

The utilization of *Agrobacterium tumefaciens* as a transformation method is a common approach and is the most efficient method to transform plant cells (Sanitha *et al.*, 2014). This transformation can occur in nuclear DNA or chloroplast DNA (Cheng *et al.*, 2012). The tumour inducing (Ti) plasmid found in *Agrobacterium tumefaciens* is empirical to the introduction of any gene of interest into the genome, specifically the segment of DNA known as the T-DNA and its accompanying flanking regions (Lee *et al.*, 2012). T-DNA, can be replaced with the gene of interest; such as a gene involved in lipid or carbohydrate synthesis for biofuel production, and be used to manipulate the algal species used in biofuel production. A binary plasmid approach; whereby the genetic information is split over two plasmids (Lee *et al.*, 2012), a T-DNA plasmid and a helper plasmid can be used to overcome issues such as limited restriction sites and difficulty in recovery due to the size of the engineered plasmid (Cheng *et al.*, 2012). The expression can be tailored through the use of promoters. An inducible promoter will allow the lipid expression to be linked to a specific action such as a metabolic function. The inclusion of a constitutive promoter allows for continuous expression of the gene of interest. **Figure 2** provides an example of how a binary plasmid, genetically engineered to provide desirable traits, can be introduced via *A. tumefaciens*.

Biolistic particle delivery system or a gene gun provides an approach that circumvents the need for marker genes. It is used for selection in other methods of genetic engineering (Bertalan *et al.*, 2015). This process involves microscopic beads of an inert metal such as gold coated with the genetic information that is to be incorporated into the genome. Biolistics nullifies the obstacles of the cell wall and cell membrane as the gold is fired through these barriers at high velocity into the cytoplasm of the cell, allowing for incorporation into the chloroplast or nuclear DNA through the inclusion of homologous regions of DNA sequence (Martin-Ortigosa *et al.*, 2012b).

The use of genetically engineered microalgae to enhance the production of biofuel has been an area of interest for scientists from a long time. The process of genetically enhancing algae improves the yield of the final product however; it has its challenges which must be addressed to assess the commercial viability of its use in biofuel production (Rawat *et al.*, 2013).

Studies have also been carried out to maximise photosynthetic ability of microalgae by reducing the size of chlorophyll antenna; which has been shown to result in more efficient use of light resulting in increased productivity (Sutherland *et al.*, 2015). However genetic engineering can come with drawbacks, in this case the reduction in antenna size causes a reduced ability for the cell to dissipate any excess photon energy which can cause susceptibility to photo-damage (Simionato *et al.*, 2013). The processes involved in the reduction in chlorophyll antenna involve gene knockout, however the addition of genes of interest through methods such as *A. tumefaciens* also has difficulties; such as gene silencing or little to no expression of target gene. This can be as a result of the compatibility with the host genome; including usage of codons not reflecting the plants bias, premature poly-andenylation sites or mRNA interference and the stresses that factors like these induce (Moshelion and Altman 2015).

The approaches known as the "omics" have made a significant contribution to the understanding of the molecular processes of microalgae. Furthermore, the discoveries that omics studies have made; such as the identification of genes involved in specific processes, may be vital to engineering of enhanced microalgae (Winck, Melo and Barrios, 2013). To assess the expression levels of the genes involved transcriptomics can be utilised, this involves the sequence information gathered from reverse transcribed mRNA that is extracted from the algal sample (Vanwonterghem *et al.*, 2014). The results will show gene expression *in situ* and provide an understanding of expression rates and allow for optimisation of the target product. Through the understanding of the levels of transcription and the gene activation data gathered from transcriptomics, the effectiveness of the genetic alteration can be measured. Should the new gene insert be operating at its optimum then the transcriptomics data should show an increase in the mRNA of the target gene when compared to the wild type (if an increase in output is the aim).

Apart from genetics, microalgae are commonly put under stress conditions such as temperature, nutrient starvation or pH to enhance production of a target product such as lipids or carbohydrates (Ho *et al.*, 2014). The result of the introduction of stress conditions is the alteration of lipid synthesis pathways in many microalgae (Rawat *et al.*, 2013), a feature of great interest to biofuel production. Omics techniques can again provide valuable insight into this process. Metabolomics assesses the low molecular metabolite end products and are indicative of response to stresses (Jamers, Blust and De Coen, 2009). A combination approach would allow for optimisation of algal engineering, as the data gathered from transcriptomics should show an increase in transcription in the gene of interest that coincides with a reduction in metabolism caused by stress such as nutrient limitation; highlighted by metabolomics, should the expression of the gene of interest be linked to a metabolism process. The application of omics studies can not only ascertain the effectiveness of any genetic modification but can also be used to optimise the scale up process. With the use of spatial and temporal omics studies of systems such as raceways, used for algal growth, a deeper understanding of how algae will perform in varying areas of the raceway can be gained allowing for process optimisation.

The technology for small-scale commercial cultivation of microalgae to produce nutraceutical products and animal feed is already available, however the commercial production of biofuel from algae still seems like a farfetched dream. There are many doubts and technical challenges associated with large-scale algae biofuel manufacturing. As well as the long-term physical impact on ecosystem health by the commercialization of open pond cultivation, the use of genetically modified algae for biofuel production can affect the sustainability of the regional ecosystem. This is particularly important in developing countries as the introduction of invasive foreign species can endanger biodiversity. The appropriate experiments and clear independent assessments should be used to evaluate genetically modified algae opportunities and risks, especially in regard to regulatory issues and biosafety (Adenle *et al.*, 2013).

In the recent years, synthetic biology has made biology easier for genetic engineers. Using the tools of synthetic biology the algal strains have been designed as per the environmental conditions and yield requirements. Synthetic biologists are assembling genetic materials and working on the manipulation of lipid content of the microalgae, along with the biomass accumulation and increasing biofuel production. The promising results are surely going to change the fate of biofuels industry from microalgae for better in the near future.

6. Applications of microalgae

Due to the increase in consumer concerns over the use of chemicals as ingredients in cosmetics there has been a higher demand for more natural and environmentally sustainable products. Microalgae biomass has a considerable market value as researchers have recently discovered that compounds derived from algae, particularly those that express immune response, anti-inflammatory and antibiotic potency, can be utilized in the production of cosmetics such as anti-aging supplements and colouring pigments (Koller *et al.*, 2014; Wang *et al.*, 2015). The phylogenetically archaic cyanobacteria produce material containing polyunsaturated fatty acids (PUFA), anti-oxidative agents, heat induced proteins or immunologically effective and viro-static compounds that could also be used in the production of cosmetics (Wang *et al.*, 2015). Marine algae have recently attracted attention in the search for natural tyrosinase inhibitors that have skin whitening properties. Tyrosinase catalyses two separate reactions in the synthesis of melanin; the hydroxylation of L-tyrosine to 3,4-dihydroxy-L-phenylalanine (L-dopa) and the oxidation of L-dopa to dopaquinone, following further conversion into melanin. Exposure to the sun increases the synthesis of both melanosomes, which mature into melanin and tyrosinase. Melanin is transported to keratinocytes and degradation occurs to encourage skin melanisation and tanning. Therefore the depletion of melanin by desquamation can remove a tan (Wang *et al.*, 2015).

The rising cost of fodders has resulted in the use of microalgae in poultry aquaculture by adding a specific amount into poultry rations for the commercial production of animal feed. Microalgae biomass is suitable for food and feed as it is rich in proteins and minerals; it also contains beneficial compounds such as enzymes, pigments, lipids that contain high value fatty acids, sugars, vitamins (riboflavin, thiamine, niacin, pantothenic acid, *inter alia* β-carotene, biotin, folic acid and pyridoxine) and sterols (Koller *et al.*, 2014).

Scenedesmus almeriensis is currently used to feed farmed sea bream and can be used to partly replace fishmeal in practical diets (Zhu, 2015). The nutritional value of some microalgae species is rich due to the high quality of their intrinsic proteins that are often of a better quality than some common vegetable proteins (Das *et al.*, 2015). In addition to these proteins microalgae also contain other cell components including simple sugar carbohydrates, peptides, lipids, vitamins, pigments, minerals and trace elements (Das *et al.*, 2015).

Recent developments and findings from a life cycle assessment (LCA) have shown that microalgae have a huge potential for producing and overcoming a lot of the problems associated with long-term bioenergy production (Quinn *et al.*, 2014). The potential for microalgae to be used to produce certain biofuels comes from the organisms' high efficiency, productivity and the capacity for CO_2 fixation maximising production (Gerde *et al.*, 2013). They also have ten times greater photosynthetic efficiency than land plants and produce larger lipid and biomass content which equates to 5-50% of dry biomass (Ahmad *et al.*, 2013). Microalgaes' lipid content is very important as this is used to produce biodiesel through transesterification (Garcia-Moscosa *et al.*, 2013). The nitrogen content of microalgae is approximately 4-8 wt% depending on nutrient availability and the algae's physiological state.

Concluding Remarks

Biofuels remain the most environment friendly and practical solution to the global fuel crisis however further research is needed to discover an effective, cheap, sustainable biomass and a method of conversion that does not produce harmful emissions and is not reliant on the addition of fossil fuels.

Microalgae have the potential to be used to produce certain biofuels without the controversial issues associated with land use, the environment and sustainability. There is lot of focus on the possibility of thermal conversion using hydrothermal liquefaction (HTL) to transform microalgae biomass into usable biofuels. The feasibility of up-scaling a microalgae cultivation system requires testing especially in terms of economic viability and product yield. The use of microalgae in biotechnology certainly has the possibility to transform the field, this potential increases with the utilisation of transgenic algal strains (Rosenberg *et al.*, 2008). The success stories w.r.t. synthetic biology and genetically engineering microalgae indicate a bright future for the biofuels industry. There already are several big players in the business of generation of biofuels from microalgae in the USA i.e. Solazyme, Sapphire Energy, PetroSun, Joule Unlimited, Green Fuel Technologies Corporation, Global Green Algae, Gevo, Algenol. In Europe, there are: Powerfuel.de (Germany), Alpha Biotech (France), Algae-farms (Greece), AlgaeLink (Spain), and Varicon Aqua Solutions Ltd and British Algoil Ltd (UK). All these companies are already producing commercial scale biodiesel, bioethanol, algal oil, hydrogen, and aviation fuel from algae. There is massive amount of research going on in this field, but relaxing the legislation w.r.t growing genetically modified algae in open ponds and attracting more innovative projects is the need of this field. Coming up with vigorous and cost-competitive conversion technologies would be staggeringly beneficial to the biofuel industry and to humankind in the long run.

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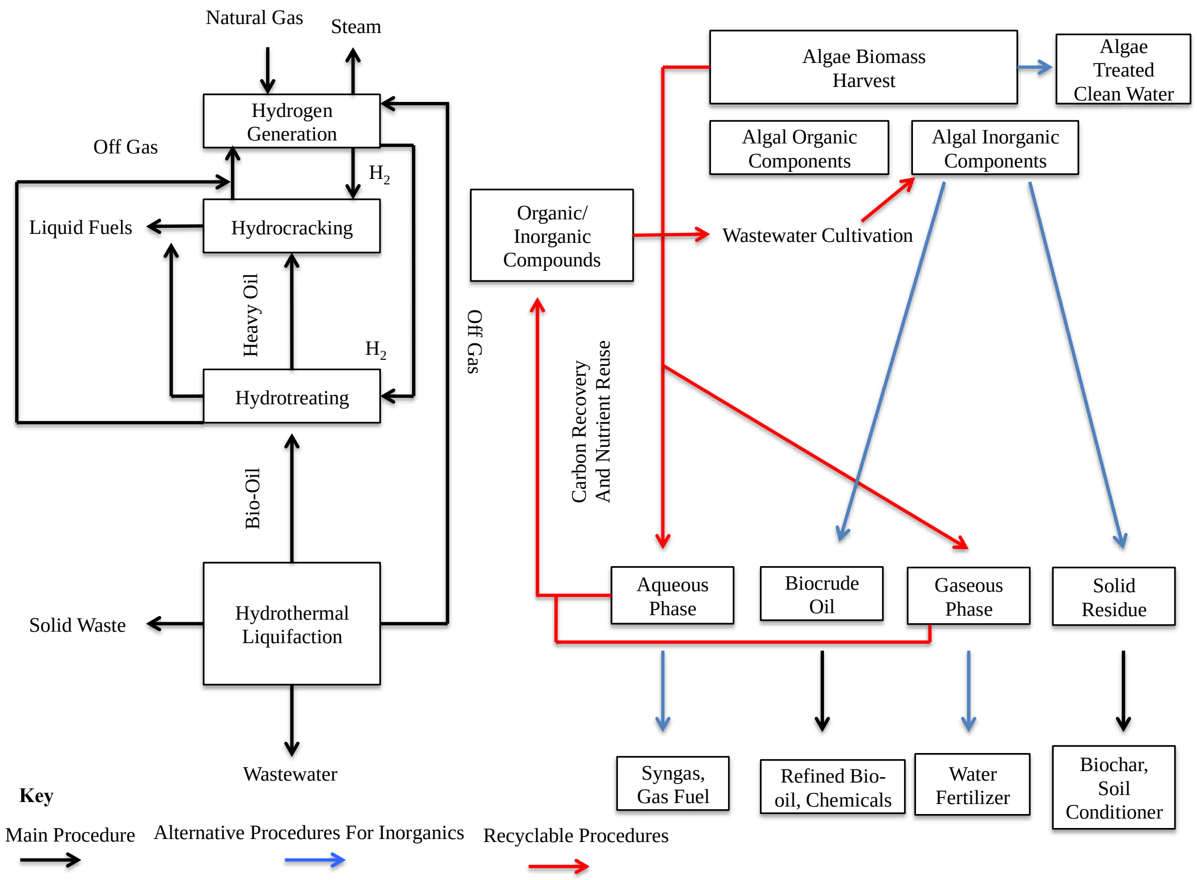

Figure 1. The main procedures involved in hydrothermal liquefaction (HTL) conversion of algal biomass into usable biofuels (Li *et al.*, 2014a; Tian *et al.*, 2014; Zhu *et al.*, 2013).

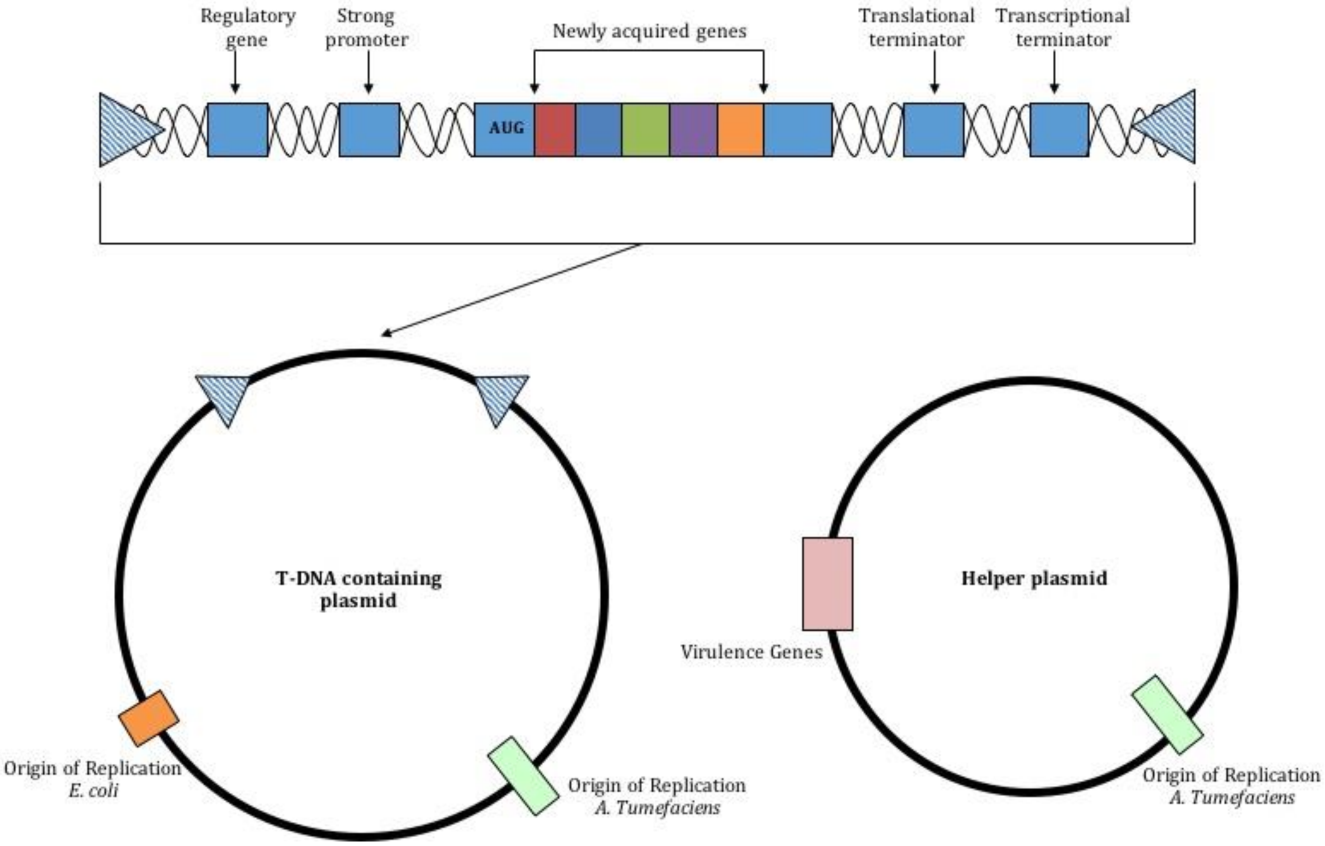

Figure 2. A schematic diagram of how a plasmid containing T-DNA can be modified to accomplish genetic engineering of microalgae. The AUG start codon proceeds the region of newly acquired genes (genes of interest/target genes). Regulatory genes allow for the linking to a cellular function such as metabolism, and the inclusion of a strong promoter will increase transcription rates, termination sequences must also be included. The use of an origin of replication in *E. coli* allows for the use of this organism as a vector due to its ease of culturing. *vir* genes are found in the genome of *A. tumefaciens* and allow for the incorporation of T-DNA into the host genome.

Table 1. Types of biomass and conversion technologies researched so far.

Biomass	**Conversion**	**Reference**
Firewood	Combustion	Guo *et al.*, 2015
Wood Chips	Combustion	Esteban *et al.*, 2015; Guo *et al.*, 2015
Charcoal	High Pressurised Palletisation	Mwampamba *et at.*, 2013; Guo *et al.*, 2015
Microalgae	Microalgae Fermentation	Chen *et al.*, 2015
Municipal Solid Waste	Hydrothermal Conversion	Zhao *et al.*, 2014
Microalgae	Transesterification	Chen *et al.*, 2015
Non-edible Oilseed Jatropha	Heat Conversion and Palletisation	Doshi *et al.*, 2014
Non-edible/ Edible Vegetable Oils, Waste Cooking Oils and Animal Fats	Direct Use and Blending Transesterification/Micro-emulsions and Pyrolysis	Adewale *et al.*, 2015
Kananja Defatted Residue	Heat Conversion and Palletisation	Doshi *et al.*, 2014
Microalgae	Hydrothermal Liquefaction	Chen *et al.*, 2015
Lignocellulosic Materials	Acid Hydrolysis/Pre-Treatment and Enzymatic Hydrolysis	Guo *et al.*, 2012
Sweet Sorghum	Advanced Solid State Fermentation	Li *et al.*, 2014b; Yu *et al.*, 2014
Sugar Cane, Sugar Beet, Sweet Sorghum, Corn Wheat, Barley, Potato Yam and Cassava	Fermentation, Distillation and Dehydration Process	Guo *et al.*, 2015
Landfills and Wastewater Treatment Plants	Anaerobic Digestion of Organic Waste	Surita and Tansel, 2015
Coal Derived from Wood Pellets and Sawdust	Pyrolysis or Gasification and Torrefication	Dudynski *et al.*, 2015; Guo *et al.*, 2015
Microalgae	Anaerobic Digestion	Allen *et al.*, 2015

Table 2. An overview of the micro and macro algae projects underway at various institutes.

Research Councils/Companies	Research Institutes	Research Area	Reference
Netherlands- based AkzoNobel and US bioproduct company Solarzyme (2014)	Partnership research	A multi-year supply deal of up to 10,000 tonne/year of tailored algal oils. Oil will replace petroleum and palm-oil derived chemicals	Chemistry and Industry (London), 2014
BBSRC	Durham University and the Institute of Chemical Technology	Investigating the use of Green macro-algae found along UK coastlines to convert into usable biofuel. Harnessing the natural processes by which seaweeds are broken down in order to make use of enzymes and microbes that are capable of converting the seaweed biomass into advanced biofuels	BBSRC (2015)
	The University of Sheffield and Bharathidasan University	Smaller water dwelling 'microalgae' to convert solar energy and carbon dioxide into the precursors of fuel	
BBSRC/DBT	Sustainable bioenergy and Biofuels (SuBB) initiative funding £4m	Renewable and sustainable fuel alternatives using microalgae/macroalgae	Algae Industry magazine (2013)
RCUK/BBSRC	University College London	Genetic engineering of the algal chloroplast to produce therapeutic proteins	UCL Algae Biotechnology (2015)
		Development of genetic strategies to improve biofuel production from cyanobacteria and algae	
		Development of synthetic biology tools for metabolic engineering of algae	
		Regulation of organelle gene expression by nuclear-encoded factors	
Natural Environment Council (NERC)	Algal Bioenergy Special Interest Group (AB-SIG)	To understand the opportunities and risks of the quality of freshwater and marine environments of using algal biomass as a source of renewable energy	NERC (2015)
Innovate UK	Cardiff University (School Of Biosciences), University of Southampton (water Engineering Group)	Development of a hybrid culture system for biomass production of "premium quality microalgae" for aquaculture and agriculture industry using wastewater in desert coastal areas	Innovate-UK-GOV.UK (2015)
PHYCONET (BBSRC NIBB)	Institute of structural & Molecular Biology, London	From January 2014 continuing over the next five years their focus is on producing high value products from microalgae and cyanobacteria industrially cultured in a controlled and intensive system using photobioreactor and fermenter-based technologies	PHYCONET (2015)
Australian Energy Market Operator (AEMO)	Clean Energy Council (CEC)	Investigated two possible futures in 2030 and 2050 by investigating the potential expense and feasibility of fuelling the electricity generation system using renewable fuels only.	Azad *et al.*, 2015