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Article

Approaches for sustainable production of third-generation biofuel from selected microbial strains

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ABSTRACT: Because of their renewable nature, low carbon impact, and ability to counteract climate change, biofuels have emerged as one of the most promising alternatives to traditional fossil fuels. Though several forms of biofuels, such as solid, liquid, and gaseous biofuels, have been created over the years, they have not always lived up to expectations in terms of efficiency and cost-effectiveness. Third-generation biofuels from algal biomass have recently come under scrutiny due to their potential as a viable alternative bio resource to the shortcomings of earlier generations of biofuels. Due to its high lipid content, rapid growth rate, and adaptability to a broad variety of conditions, including wastewater, brackish water, and saltwater, algal biomass is a promising source of biofuels. Biodiesel, bioethanol, biogas, and biohydrogen are just some of the biofuels that might be made from algae, and there are many more useful byproducts as well. Current studies using algal biomass for biofuel production aim to improve conversion efficiency and cut production costs. Several different methods, such as pyrolysis, gasification, fermentation, and hydrothermal liquefaction, have been developed for processing algal biomass.
When organic matter is heated to high temperatures in the absence of oxygen, a bio-oil is produced that may be further processed into a variety of liquid biofuels. Another method for transforming biomass into a form usable for generating power or liquid biofuels is gasification. Hydrothermal liquefaction turns wet biomass into a crude oil-like material, whereas fermentation employs microorganisms to transform carbohydrates into bioethanol or biogas. Despite its many benefits, algal biomass for biofuel production still faces a number of obstacles. The high cost of production is a big obstacle because of the high price of energy and the labour intensive nature of planting, gathering, and processing. Additional study is needed to optimise the conversion process and enhance the efficiency of algal biomass for biofuel production, and there is a shortage of large-scale commercial production facilities. The high lipid content, rapid growth rate, and adaptability of algal biomass make it a potentially useful source of biofuels. The entire potential of algal biomass for biofuel production may be realised via the development of efficient and cost-effective conversion technologies, which will ultimately lead to a sustainable and secure energy future.

Keywords: algae, microalgae, biofuels, bioethanol, biogas, biodiesel, biohydrogen.

INTRODUCTION

Because of its renewable quality, small carbon impact, and ability to slow global warming, biofuels are quickly becoming one of the most talked-about alternatives to traditional fossil fuels. Although several forms of biofuels, such as solid, liquid, and gaseous biofuels, have been created over the years, they have not always lived up to expectations in terms of efficiency and cost-effectiveness. Third-generation biofuels made from algal biomass are now receiving a lot of attention since they are seen as the greatest alternative bio resource for getting around the problems that plagued first- and second-generation biofuels. Due to its high lipid content, rapid growth rate, and adaptability to a variety of conditions, such as wastewater, brackish water, and saltwater, algal biomass is a promising source of biofuels. Biodiesel, bioethanol, biogas, and biohydrogen are just a few of the many useful byproducts that might be created from algae-based biofuels. Recent studies on algal biomass for biofuel production have focused on optimising the conversion process and lowering production costs. Algal biomass may be converted using a number of different processes, including pyrolysis, gasification, fermentation, and hydrothermal liquefaction.

When organic matter is heated to high temperatures in the absence of oxygen, a bio-oil is created that can be further processed into several types of liquid biofuels. Another method of converting biomass into a synthesis gas (syngas) that may be used to generate power or liquid biofuels is the gasification process. Using microorganisms, fermentation transforms carbohydrates into bioethanol or biogas, whereas hydrothermal liquefaction transforms wet biomass into a crude oil-like material. There are a number of obstacles that must be overcome before algal biomass can be widely used to produce biofuels, despite its many benefits. The high cost of production is a significant barrier to entry because of the high cost of energy and the high costs associated with growing, harvesting, and processing. Lack of large-scale commercial production facilities is another obstacle, as is the need for more research to optimise the conversion process and enhance the efficiency of algal biomass for biofuel generation. Considering its high lipid content, rapid growth rate, and adaptability to a variety of conditions, algal biomass is a viable source of biofuels. A more sustainable and secure energy future may be achieved via the

development of efficient and cost-effective conversion technologies that allow for the full potential of algal biomass for biofuel generation to be realised.

BIODIESEL PRODUCTION

FAMEs are used to make biodiesel, and they are monoalkyl esters of long-chain fatty acids that may be generated from a wide variety of renewable lipid feedstocks and biomass. It needs no modifications to be used in diesel engines. As a possible answer to the energy issue, studies on the use of microalgae for the manufacture of liquid fuels started in the 1980s. Due to its increased oil yield compared to traditional oil seed crops, algal biomass has showed potential as a feedstock for biodiesel synthesis. The generation of biodiesel from algal biomass vs. terrestrial plants is compared in Table 1. The rate of algal growth and the makeup of the species' biomass determine the oil productivity, or the quantity of oil generated per day per volume of microalgal broth. Ankistrodesmus fusiformis, Kirchneriella lunaris, Chlamydocapsa bacillus, and Ankistrodesmus falcate are some examples of microalgae with a high PUFA FAME concentration that are commonly favoured for biodiesel synthesis. During exponential development, the biomass of these plants may treble in size within 24 hours, and their oil content can account for more than 80% of their dry biomass weight. The annual biodiesel yield from one acre of algal biomass is estimated to be between 5,000 and 15,000. However, the American Society for Testing and Materials (ASTM) and the International Biodiesel Standard for Vehicles have set rules that must be met before algal-based biodiesel may be used as a fossil fuel alternative (EN14214). Due to their higher polyunsaturated fatty acid content, algal oils get oxidised more quickly in storage than vegetable oils do, reducing their usefulness. The high oil output and rapid biomass development of algae make it an attractive alternative to traditional oil crops for use in biodiesel manufacturing, as shown by a number of studies. Collecting the biomass, drying it, extracting the oil, and trans esterifying it are all necessary steps in making algal biodiesel.

DRYING AND HARVESTING OF ALGAL BIOMASS

FAMEs are used in the production of biodiesel; they are monoalkyl esters of long-chain fatty acids that may be generated from a wide variety of renewable lipid feedstocks and biomass. No modifications to diesel engines are necessary to use it. Back in the 1980s, scientists started investigating the feasibility of using microalgae as a source of liquid fuels to help alleviate the world's pressing energy problems. Due to its greater oil yield than traditional oil seed crops, algal biomass has showed potential as a feedstock for biodiesel generation. The generation of biodiesel from algal biomass is compared to that of terrestrial plants in Table 1. Microalgal oil production, measured in milligrammes of oil per millilitre of microalgal broth, is a function of algal growth rate and biomass composition. Ankistrodesmus fusiformis, Kirchneriella lunaris, Chlamydocapsa bacillus, and Ankistrodesmus falcate are some examples of microalgae with a high PUFA FAME concentration that are commonly selected for biodiesel synthesis. The biomass of these plants may treble in 24 hours of exponential development, and their oil content can be as high as 80% of their dry biomass weight. One acre of algae may potentially provide between $5,000 and $15,000 in biodiesel annually. The American Society for Testing and Materials (ASTM) and the International Biodiesel Standard for Vehicles have both set requirements that algae-based biodiesel must meet before it can be used as a fossil fuel alternative (EN14214). Since algal oils are higher in polyunsaturated fatty acids than vegetable oils, they oxidise more quickly in storage. The high oil output and rapid biomass development of algae make it an attractive alternative to traditional oil crops for biodiesel generation, as shown by a number of studies. Gathering the biomass, drying it, extracting the oil, and then trans esterifying it are all necessary steps in making algal biodiesel.

EXTRACTION OF OIL FROM ALGAL BIOMASS

The cell walls of unicellular microalgae contain lipids and fatty acids, setting them apart from higher animals and plants. In

Table 1 | Comparative study between algal biomass and terrestrial plants for biodiesel production.

Feedstock	Conditions	Biodiesel	Reference
ALGAE			
Spirulina platensis	Reaction temperature 55°C, 60% catalyst concentration, 1:4 algae biomass	60 g/kg lipid	Nautiyal et al. (2014)
	to methanol ratio, 450 rpm stirring intensity		
Nannochloropsis sp.	Oil extraction with n-hexane, acidic transesterification	99 g/kg lipid	Susilaningsih et al. (2009)
Scenedesmus sp.	Alkaline (NaOH), temperature of 70°C	321.06 g/kg lipid	Kim et al. (2014)

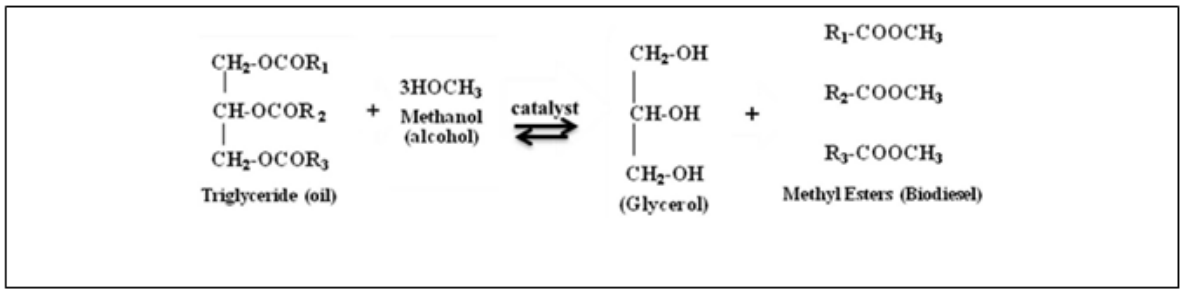
	Acidic (H_2SO_4) catalyst, temperature of 70°C	282.23 g/kg lipid	
Nannochloropsis salina	Freeze drying of biomass, extraction with chloroform–methanol (1:1 ratio),	180.78 g/kg lipid	Muthukumar et al. (2012)
	alkali transesterification		
Chlorella marina		100 g/kg lipid	
TERRESTRIAL PLANTS			
Madhuca indica	0.30–0.35 (v/v) methanol-to-oil ratio, 1% (v/v) H_2SO_4 as acid catalyst, 0.25 (v/v) methanol, 0.7% (w/v) KOH as alkaline catalyst	186.2 g/kg lipid	Ghadge and Raheman (2005)
Pongamia pinnata	Transesterification with methanol, NaOH as catalyst, temp. 60°C	253 g/kg lipid	Mamilla et al. (2011)
	Acid-catalyzed esterification by using 0.5% H_2SO_4, alkali-catalyzed transesterification	193.2 g/kg lipid	Naik et al. (2008)
Azadirachta indica	Reaction time of 60 min, 0.7% H_2SO_4 as acid catalyst, reaction temperature of 50°C, and methanol: oil ratio of 3:1	170 g/kg lipid	Awolu and Layokun (2013)
Soybean	Hydrotalcite as basic catalyst, methanol/oil molar ratio of 20:1, reaction time of 10h	189.6 g/kg lipid	Martin et al. (2013)

FAMEs are used to make biodiesel, and they are monoalkyl esters of long-chain fatty acids that may be generated from a wide variety of renewable lipid feedstocks and biomass. It needs no modifications to be used in diesel engines. As a possible answer to the energy issue, studies on the use of microalgae for the manufacture of liquid fuels started in the 1980s. Due to its increased oil yield compared to traditional oil seed crops, algal biomass has showed potential as a feedstock for biodiesel synthesis. The generation of biodiesel from algal biomass vs. terrestrial plants is compared in Table 1. The rate of algal growth and the makeup of the species' biomass determine the oil productivity, or the quantity of oil generated per day per volume of microalgal broth. Ankistrodesmus fusiformis, Kirchneriella lunaris, Chlamydocapsa bacillus, and Ankistrodesmus falcate are some examples of microalgae with a high PUFA FAME concentration that are commonly favoured for biodiesel synthesis. During exponential development, the biomass of these plants may treble in size within 24 hours, and their oil content can account for more than 80% of their dry biomass weight. The annual biodiesel yield from one acre of algal biomass is estimated to be between 5,000 and 15,000. However, the American Society for Testing and Materials (ASTM) and the International Biodiesel Standard for Vehicles have set rules that must be met before algal-based biodiesel may be used as a fossil fuel alternative (EN14214). Due to their higher polyunsaturated fatty acid content, algal oils get oxidised more quickly in storage than vegetable oils do, reducing their usefulness. The high oil output and rapid biomass development of algae make it an attractive alternative to traditional oil crops for use in biodiesel manufacturing, as shown by a number of studies. Collecting the biomass, drying it, extracting the oil, and trans esterifying it are all necessary steps in making algal biodiesel.

TRANSESTERIFICATION

FAMEs, or fatty acid methyl esters, are monoalkyl esters of long-chain fatty acids that may be generated from a wide variety of renewable lipid feedstocks and biomass and used in the production of biodiesel. It needs no adjustments before being used in diesel engines. In the 1980s, scientists started investigating the feasibility of using microalgae as a source of liquid fuels to help alleviate the world's pressing energy problems. The high oil content of algal biomass makes it a promising fuel for biodiesel generation. Table 1 shows the benefits of using algae vs land plants to make biodiesel. Microalgal oil productivity, measured in terms of oil yield per unit of microalgal broth volume per day, is a function of algal growth rate and biomass composition. Biodiesel production often favours Ankistrodesmus fusiformis, Kirchneriella lunaris, Chlamydocapsa bacillus,

and Ankistrodesmus falcate because of their high PUFA FAME concentration. In only 24 hours of exponential development, the biomass of these plants may treble, and their oil content can surpass 80% of their dry biomass weight. It is estimated that 5–15 gallons of biodiesel may be extracted from an acre of algal biomass annually. But before it can be used as a fossil fuel alternative, algal-based biodiesel must meet criteria set by groups like the American Society for Testing and Materials (ASTM) and the International Biodiesel Standard for Vehicles (EN14214). Since algal oils have a higher concentration of polyunsaturated fatty acids than vegetable oils, they are more susceptible to oxidation during storage and hence have a shorter shelf life. Numerous scientific articles have lauded the efficacy of employing algal biomass for biodiesel production on account of its superior oil output and biomass growth to that of traditional oil crops. To make algae biodiesel, you first have to harvest the biomass, then dry it, extract the oil, and finally trans esterify it.



THE MANUFACTURE OF BIOETHANOL

According to certain studies, some species of algae that generate copious quantities of carbohydrates as reserve polymers may also make bioethanol. Since it contains less lignin and hemicellulose than lignocelluloseic biomass, algal biomass has been seen as more suitable for bioethanol synthesis (Chen et al., 2013). Recently, efforts have been undertaken to produce bioethanol from algae instead of traditional crops like maize and soya beans using a fermentation process (Singh et al., 2011; Nguyen and Vu, 2012; Chaudhary et al., 2014). Table 3 displays a contrast between the biomass of terrestrial plants and that of algae used to create bioethanol. Two such instances are substrate selectivity and regioselectivity.

Reduced waste water pollutants

However, sodium methoxide has replaced sodium hydroxide as the catalyst of choice (Singh et al., 2006).

Biodiesel was produced from Scenedesmus sp. by Kim et al. (2014) utilising an acid and alkali trans esterification technique. The biodiesel conversion rate was found to be 55.07 2.18 percent higher using NaOH as an alkaline catalyst compared to the rate attained using H2SO4 (48.41 0.21 percent) when calculating lipid by weight. Due to their versatility, lipases are superior to acids and alkalis when utilised as biocatalysts.

Standard reference number for extractive transesterification Direct transesterification in situ
Having a low thermal capacity

a) Value as a heat source is rather high.
b) Lower-than-expected output
c) A better end result
d) Complex and time-consuming procedure
e) Processing time is minimal and the procedure is straightforward.
f) Fat is lost throughout the procedure.

Lipases hydrolyze ester bonds in their secondary positions, while another class of enzymes hydrolyzes esters in both their primary and secondary forms. Another group of lipases, known as fatty acid selectivity lipases, can only hydrolyze a subset of fatty acids in ester linkages. Luo et al. (2006) cloned the lipase gene lipB68, and its expression in Escherichia coli BL21 was used as a catalyst in the creation of biodiesel. The trans esterification process was catalysed by LipB68 at 20°C, and 92% biodiesel was generated after 12 hours. It is possible that considerable energy savings will arise from the lipase enzyme's capacity to work at such low temperatures. Unfortunately, its high cost means it is seldom used (Sharma et al., 2001).

To get transesterification via extraction

Biodiesel is manufactured by a series of steps including drying, cell disruption, oil extraction, trans esterification, and refining (Hidalgo et al., 2013). The primary issues are caused by the biomass's high water content (over 80%), which raises the process's total cost.

Instantaneous transesterification

Direct transesterification is a promising strategy since it cuts out the middle man, or the process of extracting the oil. This method promotes membrane permeability by using ethanol as both an esterification catalyst and an extraction solvent. As a consequence, production levels are increased while waste is decreased compared to conventional methods. Extraction solvents are utilised in conventional biodiesel production procedures, adding to pollution and heat. Consequently, lowering the barriers to entry for this promising biofuel may be accomplished by streamlining the esterification procedure. Reduced chemical and energy inputs during biodiesel manufacturing are an appealing feature of single-step technologies like direct

transesterification (Patil et al., 2012). Efficient and sustainable biodiesel production may be achieved by simplifying the esterification processes. In Table 2, the benefits of direct transesterification as a technique for manufacturing biodiesel are laid forth in contrast to extraction transesterification. This new approach has the potential to completely reshape the biodiesel industry, making it a more viable and environmentally friendly replacement for petroleum products.

Making Bioethanol

Some studies suggest that algae with the capacity to synthesise vast quantities of carbohydrates as reserve polymers may also be capable of producing bioethanol. As it contains less lignin and hemicellulose than lignocelluloseic biomass, algal biomass has been seen as more suitable for bioethanol synthesis (Chen et al., 2013). Alternatives to traditional crops like maize and soya beans have been sought for, and recent efforts have focused on producing bioethanol through fermentation utilising algae as the feedstocks (Singh et al., 2011; Nguyen and Vu, 2012; Chaudhary et al., 2014). In Table 3, we see a contrast between the biomass of terrestrial plants and that of algae used to make bioethanol. Selectivity with respect to substrate and regiochemistry are two such examples.

BIOETHANOL PRODUCTION

Some species of algae that produce large amounts of carbohydrates as reserve polymers may also produce bioethanol, according to certain research. Algal biomass has been considered to be more suited for the production of bioethanol because it contains less lignin and hemicellulose than lignocelluloseic biomass (Chen et al., 2013). Lately, attempts have been made to generate bioethanol using the fermentation process and algae as the feedstocks as an alternative to conventional crops like maize and soyabeans (Singh et al., 2011; Nguyen and Vu, 2012; Chaudhary et al., 2014).

For the manufacture of bioethanol, many micro- and microalgae, including Chlorococcum sp., Prymnesium parvum, Gelidium amansii, Gracilaria sp., Laminaria sp., Sargassum sp., and Spirogyra sp., have been employed (Eshaq et al., 2011; Rajkumar et al., 2014). For these algae to create significant amounts of polysaccharides like starch and cellulose, they typically need light, nutrients, and carbon dioxide. As demonstrated in Figure 3, these polysaccharides may be hydrolyzed to yield fermentable sugars, then fermented again to yield bioethanol, before being purified by distillation.

PRE-TREATMENT AND SACCHARIFICATION

As a result, sodium methoxide has replaced sodium hydroxide as the preferred catalyst (Singh et al., 2006).
Scenedesmus sp. was employed in the acid and alkali trans esterification method, developed by Kim et al. (2014), for the production of biodiesel. They discovered that the conversion rate of biodiesel using NaOH as an alkaline catalyst was 55.07 2.18 percent higher than the rate reached with H2SO4 (48.41 0.21 percent) based on lipid by weight. Lipases, as versatile biocatalysts, offer advantages over acids and alkalis.

This has been deemed implausible by some. Environmental degradation is a direct result of wastewater discharge.
Pollutant levels in wastewater have been decreased.

However, lipases only hydrolyze ester bonds in their secondary positions, whereas another class of enzymes may break down esters in either their primary or secondary forms. Some lipases are selective for the fatty acids they hydrolyze, allowing them to only break esters containing those acids. Since its cloning by Luo et al. (2006), the lipase gene lipB68 has been produced in Escherichia coli BL21 and used as a catalyst in the creation of biodiesel. After 12 hours, LipB68 catalysed the trans esterification process at 20°C to yield 92% biodiesel. Possible substantial energy savings might be realised due to the lipase enzyme's low-temperature functionality. Nonetheless, it is seldom used owing of its high cost (Sharma et al., 2001).
This process is called extractive transesterification.

Biodiesel is made by a series of steps, including drying, cell disruption, oil extraction, trans esterification, and biodiesel refining (Hidalgo et al., 2013). The primary challenge is the high expense of the process, which is caused by the high water content of the biomass (over 80%).

Transesterification in situ

Direct transesterification is a promising technology since it bypasses the first stage of oil extraction. In this method, alcohol is used in two capacities: as an esterification catalyst and as an extraction solvent. It increases productivity and decreases wastage in comparison to conventional methods. Common extraction solvents used in conventional biodiesel production are a contributor to environmental degradation and climate change. To lessen the negative aspects of this useful biofuel, the esterification procedure should be made easier. To reduce the total quantity of chemicals and energy consumed in biodiesel manufacturing, single-step techniques like direct transesterification might be appealing (Patil et al., 2012). The manufacture of biodiesel may be made more productive and ecologically friendly by simplifying the esterification processes involved. Table 2 provides a comparison of direct transesterification and extraction transesterification, illustrating why the former is preferable as a biodiesel production technique. This new method may significantly transform the biodiesel industry, making this fuel source more viable and environmentally friendly.

PRODUCTION OF BIOETHANOL

Researchers have found that certain algae species that generate a lot of carbohydrates as reserve polymers may also be able to make bioethanol. Because it contains less lignin and hemicellulose than lignocelluloseic biomass, algal biomass has been seen as more suitable for bioethanol synthesis (Chen et al., 2013). The fermentation process and algae as feedstocks have recently been explored as potential alternatives to traditional crops like maize and soya beans in the production of bioethanol

(Singh et al., 2011; Nguyen and Vu, 2012; Chaudhary et al., 2014). Table 3 displays a contrast between the biomass of terrestrial plants and that of algae used to make bioethanol. Examples include substrate selectivity and regioselectivity.

Table 2 | Comparative study between algal biomass and terrestrial plants for bioethanol production.

Feedstock	Conditions	Bioethanol	Reference
ALGAE			
Chlorococcum infusionum	Alkaline pre-treatment, temp. 120°C, *S. cerevisiae*	260 g ethanol/kg algae	**Harun et al. (2011)**
Spirogyra	Alkaline pre-treatment, synthetic media growth, saccharification	80 g ethanol/kg algae	**Eshaq et al. (2010)**
	of biomass by *Aspergillus niger*, fermentation by *S. cerevisiae*		
Chlorococcum humicola	Acid pre-treatment, temp. 160°C, *S. cerevisiae*	520 g ethanol/kg	**Harun and Danquah (2011a)**
		microalgae	
TERRESTRIAL PLANTS			
Madhuca latifolia	Strain *Zymomonas mobilis* MTCC 92, immobilized in *Luffa*	251.1 ± 0.012 g ethanol/kg	**Behera et al. (2011)**
	cylindrical sponge disks, temp. 30°C	flowers	
Manihot esculenta	Enzyme termamyl and amyloglucosidase, 1 N HCl,	189 ± 3.1 g ethanol/kg flour	**Behera et al. (2014)**
	Saccharomyces cerevisiae, ca-alginate immobilization	cassava	
Sugarcane bagasse	Acid (H_2SO_4) hydrolysis, *Kluyveromyces* sp. IIPE453, Fermentation at 50°C	165 g ethanol/kg bagasse	**Kumar et al., 2014**
Rice straw	Cellulase, β-glucosidase, solid state fermentation, strain	93 g ethanol/kg pretreated	**Sukumaran et al. (2008)**
	***Trichoderma reesei* RUT C30, and *Aspergillus niger* MTCC 7956**	**rice straw**	

There are several polymers found in the cell walls of algae, including alginate, mannitol, and fucoidan. These polymers, like starch, need further processing, such as pre-treatment and saccharification, before they may be fermented. Laminarin is a unique sort of storage carbohydrate found in many brown seaweeds and microalgae and may be degraded by lamininases or 1,3-glucanases (Kumagai and Ojima, 2010). Many laminarinases, including as exo- and endo-glucanases, hydrolyze their substrates to yield glucose and smaller oligosaccharides, and so may be divided into two groups. Each enzyme plays a critical role in breaking down the laminarin polymer (Lee et al., 2014b).

Markou et al. (2013) obtained the greatest possible ethanol production of 16.32 and 16.27 percent (gethanol/gbiomass) from scarified spirulina (Arthrospira platensis) biomass by fermenting the hydrolyzate after pre-treatment with 0.5 N HNO3 and H2SO4, respectively. Sea lettuce (Ulva pertusa), chigaiso (Alaria crassifolia), and agar weed (Agaris globosa) were all examined by Yanagisawa et al. (2011) for the presence of polysaccharide components (Gelidium elegans). Due to the lack of lignin in these seaweeds, it is likely that their polysaccharides may be broken down without any pretreatment. Bioethanol was produced from spirogyra biomass using Zymomonas mobilis and Saccharomyces cerevisiae, as shown in a 2013 research by Singh and Trivedi. First, they acid pretreated algal biomass before saccharifying it using an Aspergillus niger strain that produces -amylase. To avoid this step, the biomass might be saccharified straight away. When comparing the alcohol yield from pretreated and scarified algal biomass with that from direct saccharification, the latter yielded a 2 percent (w/w) increase. Research found that Spyrogyra may be used directly in the ethanol manufacturing process without any prior chemical pre-treatment, indicating its potential commercial use. Saccharification of algal biomass containing cellulose has also been accomplished with the help of the enzyme cellulase. This enzyme system is more costly than amylases and glucoamylases, despite the fact that significantly greater dosages are sometimes necessary for successful cellulose saccharification. When using many cellulases for saccharification of the green alga Ulva, Trivedi et al. (2013) discovered that cellulase 22119 had the highest conversion efficiency of biomass into reducing sugars. According to the results, the optimal sugar yield was 206.82 14.96 mg/g when the enzyme loading was 2% (v/v) and the reaction time was 36 hours at 45°C.

FERMENTATION

A variety of polymers, including alginate, mannitol, and fucoidan, are found in the cell walls of algae. As with starch, these polymers need to go through additional steps before they can be fermented. These steps include pre-treatment and saccharification. Laminarin is a unique storage carbohydrate found in many brown seaweeds and microalgae; it may be degraded by lamininases or 1,3-glucanases (Kumagai and Ojima, 2010). Exo- and endo-glucanases are two examples of laminarinases that hydrolyze cellulose to release glucose and smaller oligosaccharides as their final products, respectively. The polymer laminarin can only be broken down into its component subunits by using both enzymes (Lee et al., 2014b).

Markou et al. (2013) obtained the highest feasible ethanol production of 16.32 and 16.27 percent (gethanol/gbiomass) from scarified spirulina (Arthrospira platensis) biomass by fermenting the hydrolyzate after pre-treatment with 0.5 N HNO3 and H2SO4, respectively. Sea lettuce (Ulva pertusa), chigaiso (Alaria crassifolia), and agar weed were examined by Yanagisawa et al. (2011) for the presence of polysaccharide components (Gelidium elegans). Polysaccharides in these seaweeds may be broken down directly due to the lack of lignin, which would normally need a pretreatment step. In 2013, Singh and Trivedi employed Zymomonas mobilis and Saccharomyces cerevisiae to ferment spirogyra biomass into bioethanol. They used an acid pretreatment of algal biomass followed by saccharification using an Aspergillus niger strain that produces -amylase. The biomass might also be saccharified immediately, without any pretreatment. When comparing the alcohol yield from pretreatment and scarified algal biomass with that from direct saccharification, the latter yielded a 2 percent (w/w) increase. Finding that Spyrogyra may be used directly in the ethanol manufacturing process without any prior chemical pre-treatment is an economic bonus. Saccharification of cellulose-containing algal biomass using the enzyme cellulase has also been attempted. This enzyme system is more costly than amylases and glucoamylases, despite the fact that much greater dosages are often needed for efficient cellulose saccharification. Cellulase 22119 exhibited the highest biomass conversion efficiency into reducing sugars when compared to viscozyme L, cellulase 22086, and cellulase 22128 during saccharification of the green alga Ulva by Trivedi et al., 2013. Sugar yielded a maximum of 206.82 14.96 mg/g when enzymes were loaded at a concentration of 2 percent (v/v) for 36 hours at a temperature of 45 degrees Celsius.

Table 3 | Comparative study between algal biomass and terrestrial plants for biogas production.

Feedstock	Conditions	Biogas	Reference
ALGAE			
Blue algae	pH-6.8, microcystin (MC) biodegradation	189.89 mL/g of VS	Yuan et al. (2011)
Chlamydomonas reinhardtii *Scenedesmus obliquus*	Drying as the pre-treatment, batch fermentation, temp. 38°C	587 mL/g of VS 287 mL/g of VS	Mussgnug et al. (2010)
Ulva sp. *Laminaria digitata* *Saccorhiza polyschides* *Saccharina latissima*	Batch reactor, Co-digestion with bovine slurry, temp. 35°C	191 mL/g of VS 246 mL/g of VS 255 mL/g of VS 235 mL/g of VS	Vanegas and Bartlett (2013)
TERRESTRIAL PLANTS			
Banana stem	Pre-treatment: 6% NaOH in 55°C for 54 h. 37 ± 1°C for 40 days, batch	357.9 mL/g of VS	Zhang (2013)
Saline creeping wild ryegrass	35°C for 33 days, batch	251 mL/g of VS	Zheng (2009)
Rice straw	Pre-treatment: ammonia conc. 4% and moisture content 70%, temp. 35 ± 2°C, 65 days,120 rpm, batch	341.35 mL/g of VS	Yuan (2014)
Date palm tree wastes	Pre-treatment: alkaline, particle size 2–5 mm, temp. 40°C	342.2 mL/g of VS	Al-Juhaimi (2014)

Table 4 | Comparative study between algal biomass and terrestrial plants for biohydrogen production.

Feedstock	Conditions	Biohydrogen	Reference
ALGAE			
Gelidium amansii	Hydrolysis at 150°C	53.5 mL of H_2/g of dry algae	**Park et al. (2011)**
Laminaria japonica	Mesophilic condition (35 ± 1°C), pH of 7.5, anaerobic sequencing batch reactor, hydraulic retention time (HRT) of 6 days	71.4 mL H_2/g of dry algae	**Shi et al. (2011)**
TERRESTRIAL PLANTS			
Bagasse	Strain *Klebsiella oxytoca* HP1, temp. 37.5°C, pH-7	107.8 ± 7.5 mL H_2/g bagasse	**Wu et al. (2010)**
Corn stalk	Temp. 55°C, pH-7.4	61.4 mL/g of cornstalk	**Cheng and Liu (2011)**
Pretreated wheat straw	Strain *Caldicellulosiruptor saccharolyticus*, Temp. 70°C, pH-7.2	44.7 mL/g of dry wheat straw	**Ivanova et al. (2009)**
Wheat straw	Acid pre-treatment, simultaneous	141 mL/g VS	**Nasirian et**

	saccharification and		**al. (2011)**
	fermentation (SSF)		

Many algae have polymers in their cell walls, such as alginate, mannitol, and fucoidan. These polymers, like starch, need further processing like pre-treatment and saccharification prior to fermentation. Laminarin is a unique sort of storage carbohydrate found in many brown seaweeds and microalgae; it may be degraded by lamininases or 1,3-glucanases (Kumagai and Ojima, 2010). Depending on the hydrolysis method used, laminarinases are either exo- or endo-glucanases, with the former often yielding glucose and the latter smaller oligosaccharides. In order to break down the laminarin polymer entirely, both enzymes are required (Lee et al., 2014b).

Spirulina (Arthrospira platensis) biomass was scarified and fermented by Markou et al. (2013), who found that pre-treatment with 0.5 N HNO3 and H2SO4 resulted in the maximum practicable ethanol production of 16.32 and 16.27 percent (gethanol/gbiomass), respectively. Sea lettuce (Ulva pertusa), chigaiso (Alaria crassifolia), and agar weed were all examined by Yanagisawa et al. (2011) for the presence of polysaccharide components (Gelidium elegans). In these seaweeds, lignin is absent, suggesting that polysaccharides may be broken down directly. In 2013, Singh and Trivedi conducted research on the conversion of spirogyra biomass into bioethanol using Zymomonas mobilis and Saccharomyces cerevisiae. They acid pretreated algal biomass before saccharifying it using an Aspergillus niger strain that produces -amylase. Saccharifying the biomass straight away, without any kind of pretreatment, was another option. When alcohol yields were compared between algal biomass that had been pretreated and scarified and that which had been subjected to the direct saccharification procedure, the latter yielded a 2 percent (w/w) increase. This research found that Spyrogyra may be used directly in the ethanol manufacturing process without any prior chemical pre-treatment, indicating its potential commercial use. Similarly, cellulose-rich algal biomass has been saccharified using the enzyme cellulase. Even though amylases and glucoamylases are cheaper, cellulose saccharification sometimes requires substantially greater concentrations of this enzyme system. When using several cellulases for saccharification of the green alga Ulva, Trivedi et al. (2013) discovered that cellulase 22119 had the highest conversion efficiency of biomass into reducing sugars. The greatest sugar yield in this study was 206.82 14.96 mg/g when the enzyme loading was 2% (v/v) for 36 hours at 45°C.

BIO-OIL AND SYNGAS PRODUCTION

When subjected to high temperatures under anaerobic conditions, algal biomass is converted into bio-oil in the liquid fermentation. However, they found that 1 gramme of dried algae could produce 53.5 mL of H2, for a hydrogen production rate of 0.518 L H2/g VSS/day. The scientists showed that 5-hydroxymethylfurfural (HMF), formed during acid hydrolysis of G. amansii, is an inhibitor that reduces hydrogen generation by around 50%. Therefore, improving the pre-treatment process is crucial to enhancing biohydrogen generation, which is useful for the future (Park et al., 2011; Shi et al., 2011). Utilizing optical fibre as an internal light source, Saleem et al. (2012) sped up the process of hydrogen production using the microalgae Chlamydomonas reinhardtii. An increased maximal hydrogen generation rate of 6 mL/L culture/h was observed in this investigation. Exogenous glucose and optical fibre both contributed to this pace. In the cells of certain microalgae, such blue-green algae, glycogen takes the role of starch. In this unusual case, hydrogen is produced by the oxidation of ferrodoxin in the absence of oxygen through the action of the hydrogenase enzyme. This enzyme also helps in the process of electron release. Making microalgal pho- tobiohydrogen has prompted studies into the enzyme activities that interact with ferrodoxin and other metabolic pathways. They are also trying to alter these links genetically to increase biohydrogen production (Gavrilescu and Chisti, 2005; Hankamer et al., 2007; Wecker et al., 2011; Yacoby et al., 2011; Rajkumar et al., 2014).

The goal of biofuels research is to develop methods of extracting energy from biological (mostly plant) sources and transforming them into usable fuels such alcohols (primarily ethanol, but also propanols and butanols, as well as propane and butane diols), diesel, hydrogen, and biogas. German scientists began studying how to extract ethanol from plants as early as 1898, and their work was maintained in the United States during World War I. Glucose was extracted from wood by acidification, and then fermented by anaerobic microbes. Research on biofuels and the creation of explosives during World War I both made advantage of the capacity of anaerobic microbes to convert carbohydrates to alcohols and ketones during this time period. Scientific studies conducted in the middle of the twentieth century definitively shown that certain fungi and bacteria can break down cellulose and other plant polymers. As there were already plenty of fossil fuels available, all that study had little practical application. Research in this field was intensified, and several possibilities for its commercialization were explored, in response to the oil shock of 1973–1974, when oil prices increased dramatically. Rising oil prices over the last two years and the expected growth in global oil consumption from growing countries like China and India have made this a topic of intense attention among scientists and policymakers. Burning biofuels (alcohols, hydrogen) produces far lower (if any) carbon emission to the atmosphere than burning fossil fuels, hence they are seen as more ecologically benign sources of energy. In conclusion, the United States and other industrialised, oil-importing nations have an abundance of the raw materials for biofuels (crops, perennial plant materials), making biofuel research a politically correct topic and regarded as a method to reduce or eliminate reliance on foreign oil.

This article presents a high-level summary of the biofuels research being conducted at both the laboratory and industrial sizes at the present time, with a focus on the economic feasibility of the different methodologies presently being used. This is a vast and dynamic field where regular reports detail the latest findings. Recent work by Wackett [1] has developed a list of resources available on the web for anyone interested in biofuels. Biofuel scientists can also check out the American Society for Microbiology's new, comprehensive book [2].

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Currently, there are two primary methods employed in biofuel research: direct fermentation and indirect fermentation, both of which attempt to produce alcohol. Many plant resources are converted into biofuels, mostly ethanol, by direct fermentation. There are two basic steps: first, plant matter is broken down into fermentable sugars, and then, sugar is converted to alcohol. Less frequent than direct fermentation, indirect fermentation involves first converting the initial plant material to gas (Syngas, a combination of mostly carbon monoxide, hydrogen, and carbon dioxide) by pyrolysis (burning), and then converting the gas to ethanol using acetogenic bacteria [3].

Indirect fermentations

Direct fermentation, which uses plant resources as a starting point, produces ethanol. In order to efficiently convert plant material to sugar monomers, one must first determine the proper starting plant material, isolate and establish suitable bacterial and fungal strains, and devise suitable procedures. Yeasts or manipulated bacterial strains ferment the sugars into ethanol (see below). The most vital and active aspect of biofuel research is the first phase, which entails changing plant material into sugar. Molasses from sugar cane, starch in maize kernels, and varying amounts of cellulose, hemicellulose, and lignin polymers in plant tissues are only some of the accessible beginning plant components. Thus, successful depolymerization calls for a wide range of microbes, enzymes, incubation conditions, and engineering methods. Sugars may be quickly and readily digested from plant components that are homogeneous in character (e.g. molasses from sugar cane, starch from corn kernels). Materials that are cheaper but more difficult to decompose are termed lignocellulolytic material and include things like agricultural waste, grasses, weeds, and other non-crop plants [4].

There may be two distinct generations of biofuel research, distinguished by the initial material used to produce the sugars. The simple sugars found in crops are used to make the first generation of biofuels, which are then transformed into ethanol. The second generation of biofuels utilises natural, permanently growing plants that do not need to be cultivated, or the whole flora of the prairie system, to produce sugar and, ultimately, ethanol. Third-generation biofuels often refer to processes that utilise photosynthetic algae to create biodiesel (see below).

Traditional biofuels

Brazil is the only nation in the world that produces ethanol on a large enough scale to be economically competitive, and it does it by using sugar cane as an energy crop. Over 40 percent of Brazil's total fuel consumption in 2005 was met by the 3.8 billion gallons of ethanol generated in the nation [5]. Many factors that are specific to this nation account for this: (1) Beginning in the 1970s, significant resources were allocated to this field, resulting in the development of extensive academic and business knowledge. (2) Sugar cane is special because its end product, sucrose, is a disaccharide rather than a polysaccharide and hence does not need the processing of complicated polymeric plant components. (3) The availability of immense swaths of land that were formerly a part of the Amazon jungle but have since been destroyed for massive sugar cane plantations due to their very rich soil and plentiful rainfall. Reason #4: near proximity of manufacturing facilities to processing facilities and availability of low-cost labour.

The high concentration of sucrose in sugar cane syrup makes the extraction of sucrose from sugar cane a straightforward procedure that doesn't need the use of microorganisms or enzymes. Here, sugar cane is crushed and milled to release its juice, which is high in sucrose; the liquid is then concentrated by evaporation and fermented [6].

The United States, Japan, and other industrial nations lack Brazil's favourable climate and topography for sugar cane cultivation. The lack of accessible, low-cost labour and arable land is a serious issue. Furthermore, sugar cane cannot be grown in frosty regions. This is why maize is used in the United States instead of sugar cane, and the starch in corn kernels is used as a base for ethanol. Methods like this include removing the chaff from ears of corn before grinding them into a coarse flour. The starch-rich flour may be milled either dry or wet to produce sugars. These processes need the usage of a glucoamylase enzyme to break down -1,4-glucosidic bonds in starches and dextrins, freeing glucose and maltose for fermentation. The specifics of these processes are detailed elsewhere [6, 7].

In the United States, a few commercial ethanol-from-corn factories have begun to appear. Ethanol produced in this fashion will always be more costly than oil, as has been shown conclusively. Massive government subsidies are the sole reason these commercial ethanol generating facilities are being constructed. To produce the ethanol the United States needs as an oil alternative would require a massive quantity of land, more than the whole continent of North America. Ethanol made from maize is more of a feel-good way to improve local economies, but it doesn't provide much in the way of alternatives.

Biofuels of the second generation

In 2008, there was a major pushback against converting crops into biofuels. Several staple foods have seen price increases, which have been attributed to farmers shifting their focus from food and animal feed to energy crops. Moreover, the widespread use of fertilisers in 2008 in the United States to cultivate energy crops had a significant influence on the ecosystem, as seen by an uptick in the dead zone in the Gulf of Mexico, through which the Mississippi River runs.

Thus, researches are trying to find ways to extract energy from plants that grow naturally in non-arable grounds with low fertility (A process that has been termed cellulosic ethanol production). Stover, straws, hulls, stems, and stalks are all examples of agricultural wastes that may be used as energy crops [8]. Many weeds in the southern United States can also be used in this way. For instance, Oklahoma in the United States has begun a significant push to convert switch grass, a widespread plant there, into ethanol. The low-impact high-diversity (LIHD) strategy, which involves employing a variety of

plants from tall grass prairie ecosystems to generate electricity, is being considered by other US states (such as Minnesota) [9]. Although the exact percentages of each component may vary, lignocellulosic biomass typically consists of 35%-50% cellulose, 20%-35% hemicellulose, and 10%-25% lignin [10].

Second-generation biofuels offer advantages over utilising crops for biofuel production but also have their own problems. It's common knowledge that switch grass and other non-crop plants intended for usage are really invasive species, or weeds, rather than native plants. If these plants were grown or allowed to proliferate, it might have catastrophic consequences for the whole environment. However, compared to other well-studied energy crops, these plants are notoriously difficult to degrade, making their use in ethanol production a formidable challenge [11].

Cellulose, hemicellulose, and lignin must be degraded so that the complete plant may be utilised. As ethanol can only be created by fermentative or facultative bacteria in the absence of oxygen, lignin, which makes about 10–25% of plant biomass, is removed during pretreatment. In order for microorganisms and/or enzymes to degrade cellulose and hemicellulose, the surface area of the exposed cellulose and hemicellulose must be increased by pretreatment. Alkaline peroxidases, concentrated acids, dilute acids, alkali, alkali peroxidases, wet oxidation, steam explosion, ammonia fibre explosion, liquid hot water, and organic solvent treatments are only some of the pretreatment methods that may be applied. Readers interested in this topic should read Wyman's [12] great critique.

There are 1,4--glucosidic linkages between the d-glucose units in cellulose, making it a linear homopolymer. Between 4000 and 8000 monomers is a common chain length. Endo-1,4—gluconase, exo-1,4—gluconase, and beta-galactosidase all work together to convert cellulose into glucose. The first enzyme cleaves the chain of -glucosidic links at random. Cellobiose is converted to glucose by removing cellobiose units from the non-reducing ends of the chain, a process catalysed by enzymes 2 and 3. Fungal, aerobic, and anaerobic bacteria all have cellulase systems (including all three enzymes) that are actively working [13]. Cellulase enzymes are produced in two different ways, either extracellularly, as is the case in most aerobic fungi, or as a cellulosome, a complex structure bound to the cell membrane in anaerobic bacteria (such as Clostridia) and members of the Neocallimastigales, anaerobic fungi found in the gut of ruminants and other herbivores [14]. Trichoderma and Aspergillus, two species of aerobic fungi, are now the most popular sources for industrial enzymes [6].

Pentoses, hexoses, and sugar acids form the heteropolymer that is hemicellulose. The most prevalent kind of hemicellulose, xylans, are heteropolysaccharides with a backbone consisting of a relatively short chain (about 200 units) of 1,4-linked -d-xylopyranose units. In addition, xylan may include trace amounts of arabinose, glucuronic acid, acetic acid, ferulic acid, and p-coumaric acid. Hemicellulose's precise make-up is context-specific. Hemicellulases are a group of enzymes necessary for the depolymerization of hemicellulose. Endo—1,4-xylanase, which targets the xylan backbone, is essential for the complete breakdown of xylan. The xylooligosaccharides are then broken down by -xylodase into xylose. Other components and substitutions within the xylan polymer need the use of certain auxiliary enzymes for breakdown. The goal of biofuels research is to develop methods of extracting energy from biological (mostly plant) sources and transforming them into usable fuels such alcohols (primarily ethanol, but also propanols and butanols, as well as propane and butane diols), diesel, hydrogen, and biogas. German scientists began studying how to extract ethanol from plants as early as 1898, and their work was maintained in the United States during World War I. Glucose was extracted from wood by acidification, and then fermented by anaerobic microbes. Research on biofuels and the creation of explosives during World War I both made advantage of the capacity of anaerobic microbes to convert carbohydrates to alcohols and ketones during this time period. Scientific studies conducted in the middle of the twentieth century definitively shown that certain fungi and bacteria can break down cellulose and other plant polymers. As there were already plenty of fossil fuels available, all that study had little practical application. Research in this field was intensified, and several possibilities for its commercialization were explored, in response to the oil shock of 1973–1974, when oil prices increased dramatically. Rising oil prices over the last two years and the expected growth in global oil consumption from growing countries like China and India have made this a topic of intense attention among scientists and policymakers. Burning biofuels (alcohols, hydrogen) produces far lower (if any) carbon emission to the atmosphere than burning fossil fuels, hence they are seen as more ecologically benign sources of energy. In conclusion, the United States and other industrialised, oil-importing nations have an abundance of the raw materials for biofuels (crops, perennial plant materials), making biofuel research a politically correct topic and regarded as a method to reduce or eliminate reliance on foreign oil.

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CONCLUSION AND FUTURE PERSPECTIVES

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