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LIQUID BIOFUEL FROM MICROALGAE : A REVIEW

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ABSTRACT

In accordance with present and projected economic, social, and societal needs, sustainable usage of renewable technology is thought to be a clean source of energy that has minimal negative effects on the environment. With the use of new technologies, fossil fuel can be replaced by biofuel. Biofuel extraction can be done from different feed stocks and microalgae proves to be one of the promising feedstocks. This review paper includes feedstock used as different generation of biofuels, different types of liquid biofuels that can be extracted from microalgae and steps included in extraction of biofuels from algae.

Keywords: Biofuel, Bioethanol, Biodiesel, Fermentation, Saccharification, Transesterification.

Introduction

One of the biggest challenges facing the global scientific community today is the search for renewable energy. Short-term solutions and recommendations are needed in order to address population increase, industrial activity, and the resulting high energy consumption. One of the biggest and most strategically significant sustainable fuel sources today is biofuels, which are also seen as a key step towards reducing greenhouse gas emissions, enhancing air quality, and developing new sources of energy (Jaecker-Voirol *et al.*, 2008). Sustainable biofuels that are both carbon neutral and renewable are essential for both the environment and the economy. Since people will always need fuel for heating and daily needs, the production from huge oil fields is dropping at a pace of 45% per year. As a result, the world's oil production is predicted to peak in the upcoming years. One new barrel of oil is discovered for every four that are consumed globally, which is a concerning number considering global gas and oil production is almost at its peak (Alekkett & Campbell., 2003). As a result, biofuels have been positioned as a major source of energy in the future that can replace fossil fuels and have the potential to increase supply stability, cut down

on car emissions, and give farmers a reliable source of income.

The term "biofuel" refers to fuels that are primarily made from biomass such as biochar, ethanol and biodiesel as well as gaseous fuels like biogas, biohydrogen and synthetic gas (Kothari and Gujral, 2013). Fuels that are made mostly from biomass are classified as biofuels and might be liquid, gaseous or solid. From biomass, a range of fuels including ethanol, methanol, and biodiesel can be created. Tropsch- Fischer hydrogen, methane, and diesel (Demirbas 2008). It has been determined that microalgae are a third-generation potential biofuel source that potentially take the place of transportation fuels made from fossil fuels. A lipid feedstock and an alcohol are used to create biodiesel, an alternative diesel fuel. The term "biodiesel" refers to a non-petroleum-based diesel fuel made of short-chain alkyl (methyl or ethyl) esters and commonly produced through transesterification (Krawczyk, 1996). Due to the current energy crisis and concerns about environmental safety, bioethanol has emerged as a promising source for the manufacturing of biofuels. This makes it a better alternative to fossil fuels. Broad groups of biomass resources, which can be divided into

sugar product, starch, and lignocellulosic biomass have been studied and tested for the generation of bioethanol. (Zabed *et al.*, 2017).

Biofuel: Food Vs Fuel

The issue over "food vs. fuel" and biodiversity must be taken into consideration when cultivating biofuels in order for them to be helpful. It is advised by Groom *et al* (2008) regarding the cultivation of biofuel crops with an eye towards biodiversity to promote low-impact, sustainable feed stocks like switchgrass, karanja, and jatropha. Additionally, vital native food crops should be preserved and carbon-neutral biofuel crops should be promoted in order to manage oil crop production in an environmentally responsible and resourceful manner. There will undoubtedly need for an alternate solution to be discovered because to the growing global population and the amount of motor vehicles on the road. To address this, the oil-producing crops karanja, jatropha, switchgrass, and prairie grasses are specifically mentioned in studies by Sharma *et al.* (2008) and Groom *et al.* (2008). These crops can be cultivated on agricultural waste land and require little in the way of inputs, such as fertiliser or waste water. *Jatropha* is a crop that may be planted once and continue to produce oil for at least thirty years, making it a perennial.

The utilisation of algae as an oil producer to create biodiesel is one idea that is presently being considered. Studies have revealed that algae might have an astounding 200 times higher oil content per acre compared to the most productive crop grown on land (algae are photosynthetic organisms that grow the fastest; they can generate 46 tonnes of oil per hectare year). Given that these may be grown on non-agricultural areas, this is a viable direction for next-generation biofuels without impacting the availability of food (Nigam & Singh., 2011). Few agriculturally and economically important plants that serve as feedstock for first and second generation of biofuel are enlisted in Table 1. Some important plants which can be used for biofuel production are given in fig.1.

Classification of Biofuel

The term "primary biofuels" refers to unprocessed, natural biomass such as wood chips, firewood, and pellets, when the organic material is mostly used in its unaltered, natural state. Direct combustion occurs with primary fuels typically to meet the needs of small and large-scale industrial applications for heating, cooking fuel, or energy production. Secondary fuels refer to refined primary fuels that have undergone processing and production. These can be solids like charcoal, liquids like ethanol,

biodiesel, and bio-oil, or gases like hydrogen, biogas, and synthesis gas. Secondary fuels are useful for a variety of tasks such as transportation and high-temperature industrial operations. According to the 2008 study The State of Food and Agriculture, more sophisticated and effective conversion technologies are now available for the extraction of biofuels in solid, liquid, and gaseous forms from materials including wood crops and waste material (FAO, 2008).

The type and source of biofuels are also factors in classification. They may come from municipal wastes, forest, agricultural, or fishery goods, as well as by-products and wastes from the food, agro-industry, and food services. Biofuels can be liquid (such as ethanol, biodiesel, and pyrolysis oils); gaseous (such as biogas; methane); or solid (such as fuelwood, charcoal, and wood pellets). (Nigam & Singh, 2011).

Transformation of Biofuels

1. First generation feedstock: Starch and sugar crops (for bioethanol) and oil seeds (for biodiesel) are the conventional feedstocks for first-generation biofuels. 40 million gross hectares (2.5 percent of the world's cropland) (FAOSTAT, 2011) are utilised to produce bioenergy crops, namely bioethanol, biodiesel and biogas from arable food crops. The fermentation of sugar-rich plants (such as sugarcane, sugar beetroot, and sweet sorghum) or a sequence of hydrolysis/fermentation stages for starchy plants (such as corn, wheat, and cassava) produces the first generation of bioethanol. Ethanol made from corn is leading the market with around 60 billion litres produced in 2012, with the US being the biggest producer, followed by ethanol made primarily from sugarcane in Brazil at 20 billion litres. (REN21, 2013). Transesterification, a chemical process that produces biodiesel, is a method of producing oil from seeds and oil-rich nuts when combined with an alcohol. Crops like rapeseed, soyabean, palm and coconut are commonly used biodiesel extraction (Balat & Balat; 2010). The types of fatty acids attached in triacylglycerols (TAG) which affect degree of saturation/unsaturation and molecular structure are the main variation between various oil feedstocks. (Ramos *et al.*, 2009).

2. Second generation feedstock: In the transition to low carbon economies, lignin-rich biomass is anticipated to play a significant role under the challenge of food security vs rising global energy demand. The non-food lignocellulosic materials that make up the second-generation feedstocks can be broken down into three main categories: (i) homogeneous, like wood chips from energy crops; (ii)

quasihomogeneous, like agricultural and forest residues; and (iii) non-homogeneous, like municipal and industrial solid wastes (Lee & Lavoie, 2013). Perennial grasses (such as miscanthus, switchgrass, and reed canary) and short rotation forestry (such as willows and poplar) are examples of energy crops that have been designed and grown exclusively for fuel. Other potential herbaceous crops include giant reed, lucerne, and reed canary grass, banagrass, napiergrass, and johnsongrass are tropical and subtropical plants that are adapted to temperate climates. Other species with biodiesel potential include jatropha, pongamia, mahua, castor and linseed. Agricultural residues consist of wheat straw, maize stove (leaves, stalks and cobs), and bagasse (waste from sugarcane processing) whereas forestry residues are made up of logging byproducts, fuel wood taken from forested areas and primary as well as secondary wood-processing mill waste. (Eisentraut, 2010). The majority of MSW is biodegradable and has a high calorific (heat) value which makes it viable for energy recovery operations despite its extremely varied composition. Scraps and peelings are examples of solid wastes. Fruits, vegetables, leftover meat and poultry, coffee grounds, pulp and fibre from glucose and starch extraction and more can all be used as a source of energy.

3. Third generation feedstock: Production of biofuel from microorganisms as a source of lipid and polysaccharides make up the third-generation feedstock. Microalgae can be found in diverse range habitat like hot water spring, estuaries, salt water lake, agricultural fields, tree bark, buildings, etc. (Kant *et al.*, 2004a-b, 2005, 2006a-b, 2020; Tiwari *et al.*, 2007, 2009, 2013; Kesharwani *et al.*, 2008; Kant, 2011, 2012a-b; Singh *et al.*, 2008; Tandon *et al.*, 2014; 2021a-b; Neha *et al.*, 2021; Saini *et al.*, 2022, 2023; Sarma *et al.*, 2022 a-b; Kumar and Kant, 2023; Singh *et al.*, 2023). Algae are photosynthetic microorganism capable of converting light and carbon dioxide into stable higher complex compounds like polysaccharides, protein and lipids. These compounds have application in cosmetics, pharmaceuticals, food industry, nanoparticles, and many more. (Kant, 2012a; Sarma, *et al.*, 2023a-c; 2024a-b). Species like *Chlorella*, *Botryococcus*, *Spirogyra*, *Trentepohlia* and *Microchaete* are known to be high biofuel producing species (Costa & de Morais, 2011; Ugwu *et al.*, 2008; Sarma *et al.*, 2020) and used in biofuel production. Mixotrophic growth in nature of some important micro-algal species and their morphological details are given in Fig.2 (a-i) and Fig.3 (a-j). There is a good likelihood of increased biomass output since algae are often defined to have a biomass production yield that is 5–10 times higher than that of land-based plants (Hill

and Feinberg, 1984). The increased output rate is due to a number of factors. The improved photosynthetic efficiency is one. Microalgae have an intrinsic solar-energy conversion efficiency (measured as energy released by complete combustion of biomass divided by the solar energy absorbed) of up to 9%, whereas land-based C3 crops (such as switchgrass) and C4 crops (such as maize) have theoretical maximums of 2.4% and 3.7%, respectively. The unicellular morphology and hence significantly shorter reproductive cycles - hours to many days- are one explanation for this. (Dismukes *et al.*, 2008). The microalgal biomass yield can be further increased by altering light quality and nitrogen source (Doli *et al.*, 2023; Sarma *et al.*, 2024b.)

4. Fourth generation feedstock: New types of microalgae have emerged as a result of the desire to improve or optimise the production of specific items. As a result, the conversion processes required for the manufacture of biofuel grow as more new types of microbes are discovered. Genes may now be synthesised, cloned, and transformed into living organisms thanks to the development of molecular techniques in the last two decades. Genetically modified organisms (GMOs) or genetically enhanced organisms (GEOs) are organisms that have undergone transformation in laboratory, including microalgae. Recombinant DNA technology, which uses these methods to produce new creatures with altered or novel features, is generally known. (Sicard, 2009). The aforementioned transformation techniques have been used to effectively turn over twenty marine microalgal strains to date. Using the trans-conjugation approach (Sode *et al.*, 1992) or natural transformation (Jiang *et al.* 2003), the genetic transformation of marine cyanobacteria was effectively demonstrated in 5 strains of *Synechococcus*, *Synechocystis* and *Pseudanabaena*.

Limitations with Different Biofuel Generations

Over the past two decades, the conflict between food and fuel has become more heated. The main ingredients of first-generation biofuels are grains, sugar, and oilseeds, which are also important ingredients in food production. The conflict between food and fuel has its roots in the growing struggle for these resources between the biofuel, food, and food animal industries. Utilising these priceless resources for food production, about one-third of the grain used to make ethanol is converted into concentrated energy and nutrient sources that have good nutritional and financial value in animal feeds. (Lywood and Pinkney, 2012).

Second generation biofuel deals issues with the production, logistics, and availability of feedstock such

as the seasonal and yearly variability of feedstock, as well as the price of pre-processing, storage, and transportation. The second generation of biofuels currently face limited market acceptability and competition as a result of high production costs and a lack of enabling regulations and mandates (IEA, 2010). Cultivation method, photobioreactor design, treatment selection and production modes are the main difficulties associated with third generation algal biofuel production. The ideal criteria for selecting algal strains for commercial biofuel production are high biomass yields with high carbohydrate and fat contents. However, cell expansion is necessary to increase the production of lipids and photosynthesis are frequently impaired, which causes a drop in overall production. In-depth basic research on genetic modification and the alteration of lipid and cellulose synthesis pathways to increase productivity may be necessary to solve this challenge. Algal biofuels would also be more commercially viable if downstream processing, conversion, and extraction procedures were made more effective. (Day *et al.*, 2012; John *et al.*, 2011).

Extractable Liquid Biofuel

1. Bioethanol

- Depending on the type of feedstock, bioethanol output varies. (Fig. 4).
- By pulverising the material, later followed by addition of yeast for fermenting, bioethanol can be extracted from sugar.
- Fermentation and saccharification procedures are the major components in the manufacture of bioethanol from starch.
- Enzymatic hydrolysis is used to break long chains of glucose molecules in starchy material into shorter chains during the saccharification process. It takes a lot of work to produce ethanol from cellulosic biomass. Lignin, hemicellulose, and cellulose are present in significant amounts in cellulosic materials. Prior to the saccharification and fermentation procedures, these cellulosic materials must undergo an additional pre-treatment procedure to produce bioethanol. Enzymatic hydrolysis or acid hydrolysis are both used in the pre-treatment process to turn cellulosic material into glucose molecules. The crystalline structure of cellulose is disturbed, and the lignin structure is broken down. (McMillan, 1994).

2. Biodiesel

- Biodiesel can be derived mono-, di- and triacylglycerides (TAGs) or any triglyceride feedstock by transesterification. When an alcohol and the triglyceride molecules react, the process is

known as transesterification. (Azean and Yilmaz, 2012).

- Transesterification can be done via two methods i.e. direct in situ method and conventional method.
- Direct in situ transesterification, produces biodiesel without disrupting cells or removing oil from biomass, combining the steps of lipid (oil) extraction and transesterification. It also involves the esterification of free fatty acids and the transesterification of triglycerides from microalgae. (Umar, 2014). This process includes adding pure methanol and the acid catalyst at the same time to microalgal biomass, which is typically in the form of dry powder. (He & Bi, 2014)
- Under conventional method, algal biomass can be used to make biodiesel (FAME) by first extracting the oil, which is then transesterified from the biomass. The recovery of green crude from microalgae often necessitates several solid-liquid separation phases in the standard procedure. These procedures include cell wall disruption, drying and extraction using solvents. As toxic organic solvents like hexane, chloroform, and methanols are typically used in the extraction of green crude, these procedures are both very energy- and environmentally-intensive. (Li *et al.*, 2014).
- Base catalysts, acid catalysts, enzyme catalysts, and supercritical transesterification are all forms of catalyst.
- Base catalysts include sodium hydroxide and potassium hydroxide. The production of oil largely uses the base catalyst method. It cannot be recycled or used again. Acid catalysts include hydrochloric acid and sulphuric acid. It has been discovered that acid catalysts are slower than base catalysts and are best suited for oils with a high lipid content. The employment of enzyme catalysts such as lipase is a labor-intensive and costly procedure that is not appropriate for the synthesis of biodiesel for commercial usage. Process variables such processing duration, temperature, methanol/algae ratio, and catalyst concentration are critical for direct transesterification. (Chamola *et al.*, 2020).
- Species of *Chlorella* and *Botryococcus* have been found as high oil yielding microalgae beneficial in biofuel industry (Ruangsomboon *et al.*, 2020).

Algal Biomass Cultivation Process

1. **Strategically planning:** This step includes establishing the whole plan of experiment starting with selection of species, culturing method,

treatment selection and arrangement of resources (equipments, chemicals, glass wares).

2. **Culturing:** Algae are present in nature in mix culture and due to their microscopic nature, they are required to be isolated in pure form prior to any other process. It can be done by the process of culturing and sub-culturing in BG-11 media (Stanier *et al.*, 1971) also described by Kant *et al.* (2005). Next step should be treatment selection that involve the selection of stress under which algae will be cultured to enhance the quantity and quality of product under regulated growth factors.
3. **Harvesting:** This step involves the harvesting of cultured algae by the process of dewatering i.e filtering out algal biomass from water or by drying algal biomass in sun or in lab conditions in drying oven.
4. **Quantitative and qualitative analysis:** Main component of microalgae are protein, polysaccharides (carbohydrates), lipids (fats and oil).
5. **Product extraction:** Extracted components from algal biomass can be turned into different useful product like charcoal, ethanol, butanol, diesel, hydrogen gas, syngas via different process like fermentation, anaerobic digestion, gasification, pyrolysis, transesterification. Lipids are used in extracting biodiesel through the process of transesterification. Similarly, carbohydrates are used in extraction of bioethanol via process of fermentation (Chowdhury & Loganathan, 2019). Detailed process in given in Fig. 5.

Challenges of Biofuel Production

Beyond their many advantages, the development and application of biofuels present a number of difficulties. The primary challenge for an enhanced biomass waste collection network and its storage is stability of a commercial biofuel facility. To gather organic waste and blend biofuels more quickly, a robust policy is required. The production of biofuels will increase thanks to subsidies for the construction of biofuel facilities, and the market for biofuels will be created via tax credits for their use. Technological advancement may contribute to increased system efficiency and value-added coproducts, lowering production costs. The main challenges faced during biofuel production. (Hoekman, 2009) are given in Fig. 6.

Conclusion

When compared to fossil fuels, the production and use of biofuels often offer a number of environmental, energy, and social benefits, including reduced greenhouse gas emissions, increased supply security, and rural development. Biofuels made from specific plants or plant leftovers that don't directly compete with land used to grow food and feed crops. Microalgae can quickly and in vast quantities create proteins, lipids, and carbohydrates. These goods can be processed to produce lucrative co-products as well as biofuels. Feedstock may differ in terms of starch and lignocellulosic content throughout generations. To increase production, the technological and financial the following challenges of bioconversion must be taken into consideration: 1. Cost effective process; 2. Maximum yield production; 3. Minimum carbon emission.

Table 1: Agriculturally and economically important plants that serve as feedstock for first and second generation of biofuel.

Sr. No.	Common name	Scientific name	Family	Generation
1.	Sugar beetroot	<i>Beta vulgaris</i>	Amaranthaceae	First generation biofuel
2.	Palm	<i>Phoenix dactylifera</i>	Arecaceae	
3.	Rapeseed	<i>Brassica napus</i>	Brassicaceae	
4.	Cassava	<i>Manihot esculenta</i>	Euphorbiaceae	
5.	Soyabean	<i>Glycine max</i>	Fabaceae	
6.	Sugarcane	<i>Saccharum officinarum</i>	Poaceae	
7.	Sweet sorghum	<i>Sorghum bicolor</i>	Poaceae	
8.	Corn	<i>Zea mays</i>	Poaceae	
9.	Wheat	<i>Triticum aestivum</i>	Poaceae	
10.	Coconut	<i>Cocos nucifera</i>	Arecaceae	Second generation biofuel
11.	Jatropha	<i>Jatropha curcas</i>	Euphorbiaceae	
12.	Castor	<i>Ricinus communis</i>	Euphorbiaceae	
13.	Lucerne	<i>Medicago sativa</i>	Fabaceae	
14.	Pongamia	<i>Pongamia pinnata</i>	Fabaceae	
15.	Linseed	<i>Linum usitatissimum</i>	Linaceae	
16.	Miscanthus	<i>Miscanthus sinensis</i>	Poaceae	

17.	Switchgrass	<i>Panicum virgatum</i>	Poaceae	
18.	Reed canary	<i>Phalaris arundinacea</i>	Poaceae	
20.	Giant reed	<i>Arundo donax</i>	Poaceae	
21.	Reed canary grass	<i>Phalaris arundinacea</i>	Poaceae	
22.	Napiergrass	<i>Cenchrus purpureus</i>	Poaceae	
23.	Johnsongrass	<i>Sorghum halepense</i>	Poaceae	
24.	Willows	<i>Salix sp.</i>	Salicaceae	
25.	Poplar	<i>Populus deltoides</i>	Salicaceae	
26.	Mahua	<i>Madhuca longifolia</i>	Sapotaceae	



Fig. 1: (a). *Jatropha curcas*, (b). *Saccharum officinarum*, (c). *Madhuca longifolia*, (d & e). *Ricinus communis*, (f). *Populus deltoides*, (g & h). *Pongamia pinnata*, (i) *Salix sp.*

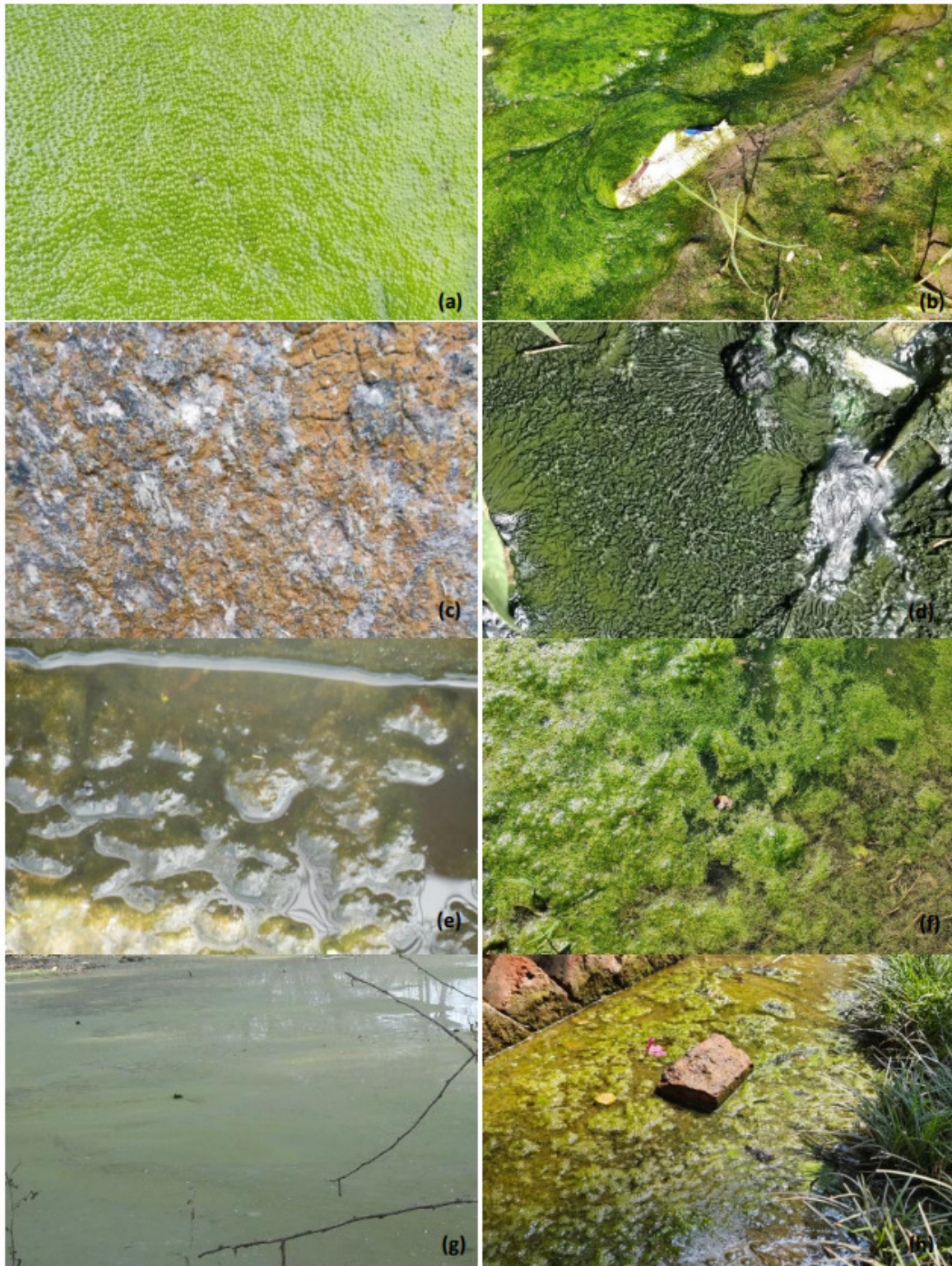


Fig. 2 : Mixotrophic growth of different algae in nature: (a). *Euglena* sp., (b). *Spirogyra* sp., (c). *Trentepohlia* sp., (d). Green algae mixed growth, (e). *Microchaete* sp., (f). Green algae filamentous growth, (g). Algal bloom, (h). *Ulothrix* sp.

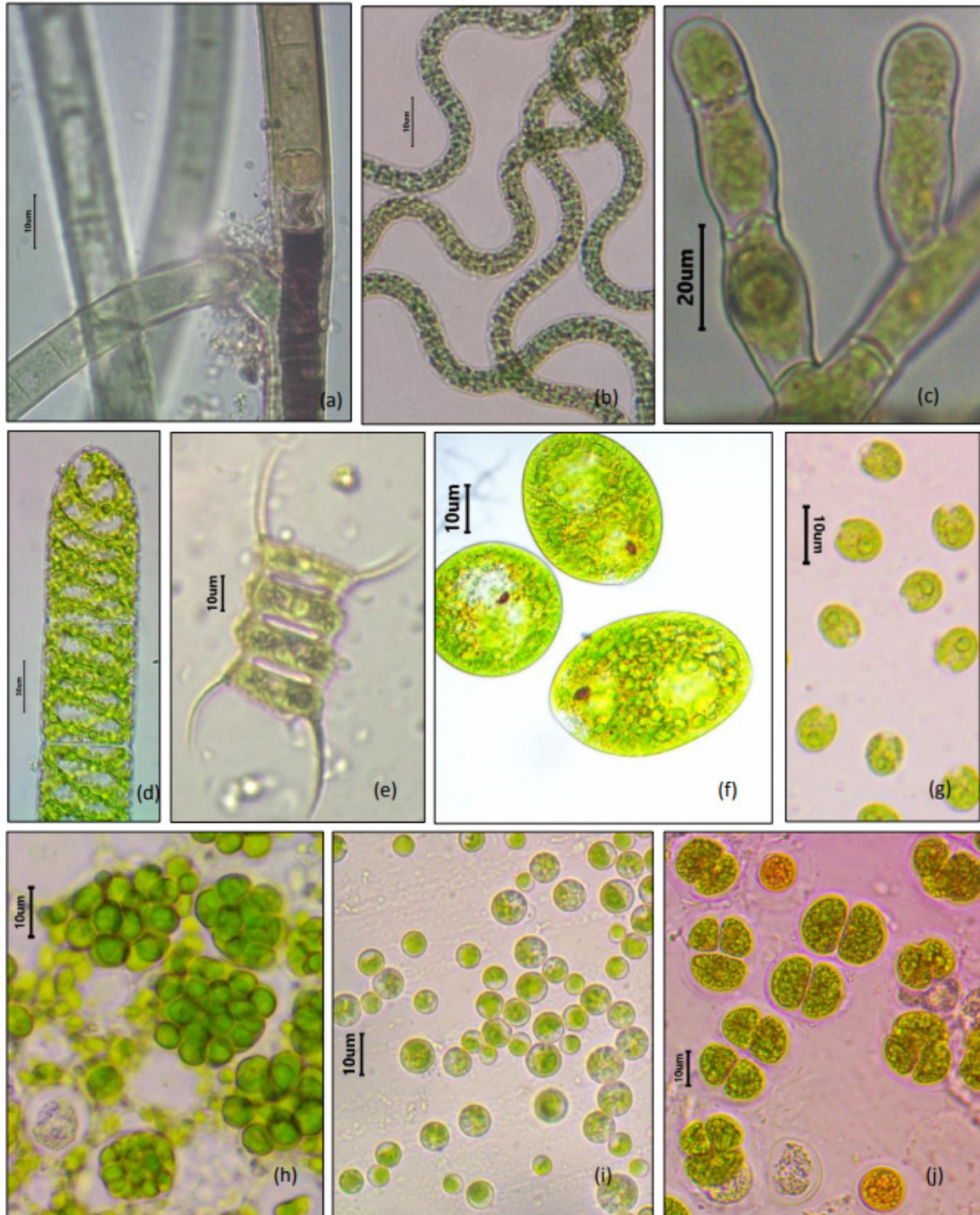


Fig. 3 : (a). *Microchaete* sp. (b). *Arthrospira* sp. (c). *Trentepohlia* sp. , (d). *Spirogyra* sp. , (e). *Scenedesmus* sp., (f). *Euglena* sp., (g). *Chlamydomonas* sp., (h). *Botryococcus* sp., (i). *Chlorella* sp., (j) *Chlorococcum* sp.

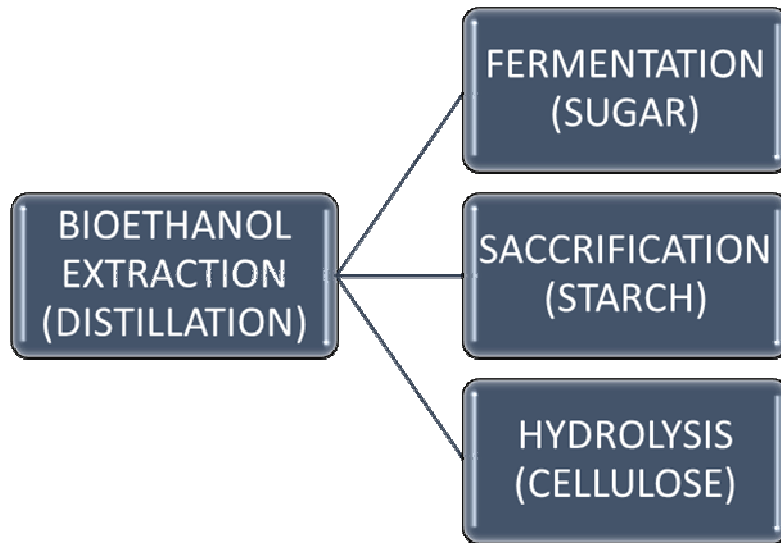


Fig. 4 : Bioethanol extraction from different sources

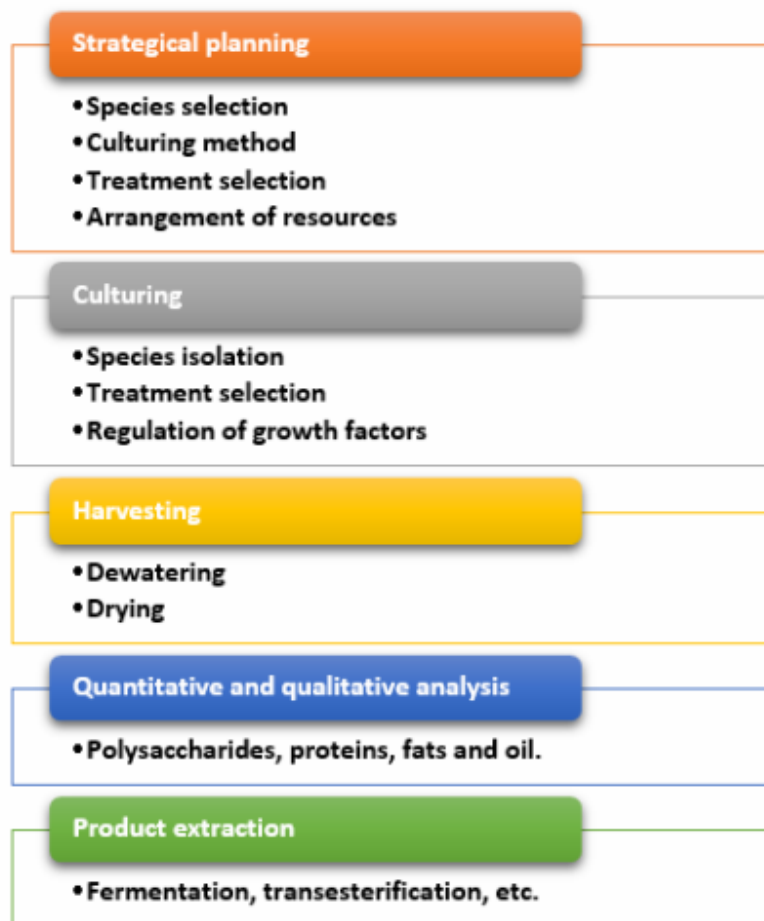


Fig. 5: Diagram showing different steps included in the process of product extraction from microalgae.



Fig. 6 : Challenges in biofuel production.

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