Microalgae as an alternative source for biodiesel and biogas production – A mini review

Khaled N. M. Elsayed[1,3]; Ahmed M. A. Mohamed[1,4]; Anja Noke[2]; Gerd Klöck[2]
[1] Department of Life Sciences and Chemistry, Jacobs University Bremen GmbH, Campus Ring 1, 28759 Bremen, Germany
[2] Institute of Environmental Biology and Biotechnology, University of Applied Sciences, 28199 Bremen, Germany.
[3] Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, 62511 Beni-Suef, Egypt.
[4] Department of Botany and Microbiology, Faculty of Science Helwan University, Egypt.
k.elsayed@jacobs-university.de, elyamany_bns@yahoo.com

Abstract: The incredible increase in world population, which could reach 9 billion by 2050, and the rapid progress of globalization in recent decades have put pressure on the food and energy sectors. The resources currently available for energy production are insufficient to meet future demand. These facts are pushing governments and scientific organizations all over the world to search for alternative renewable energy sources. Microalgae present an ideal, resurgent resource for the production of biofuel, especially biodiesel and biogas, because their lipid productivity is greater than that of other terrestrial food crops. However, from a biotechnological point of view, the use of microalgae requires further investigation and development to be economically viable, particularly in regard to cost and biomass production. The most important step in the use of microalgae for biofuel production is strain selection. The optimal strain must be able to withstand outdoor conditions and survive seasonality. From a practical perspective, only a few microalgae species have been investigated for pharmaceutical and industrial applications.
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1. Introduction

1.1. Energy crises and greenhouse gas reduction

Energy security is among the major issues that have been extensively studied and discussed in recent decades because of the global increase in energy demand and the twin crises of earth fossil fuel depletion and environmental degradation (Cobos et al. 2017; Liu et al. 2017; Su et al. 2017; Elsayed et al. 2012).

The indiscriminate extraction and consumption of fossil fuels due to overgrowth of the world population, urbanization and industrialization all over the world have led to a reduction in petroleum reserves (Bharathiraja et al. 2015; Cherubini et al. 2011).

Petroleum-based fuels are currently obtained from finite reserves that are highly concentrated in certain regions of the world. Furthermore, the quality and quantity of fossil fuels are diminishing day by day; consequently, countries not having these resources, as well as foreign countries will soon be facing energy crises (Sharma and Singh 2017; Show *et al.,* 2017; Singh and Singh, 2010).

Our basic sources of energy are currently petroleum, natural gas, coal, hydroelectric and nuclear energy. The need for energy is increasing continuously. The use of petroleum-sourced fuels is now widely recognized as unsustainable because of the depleting supplies and the contribution of these fuels to the accumulation of carbon dioxide (CO_2) in the environment, which contributes to global warming (Álvarez-Díaz *et al.,* 2017; Afify *et al.,* 2010).

Worldwide, oil reserves are quickly diminishing in the face of an ever-increasing demand for liquid transportation fuels. It has been predicted that most oil reserves will be exhausted in 50 years or less. This pending depletion has shifted global attention toward research and development endeavors to find alternative, renewable sources of fuels, offsetting the need for fossil fuels (Koutra *et al.,* 2017; Raehtz, 2009; Hossain *et al.,* 2008).

As the age of cheap petroleum is ending, and fossil fuel depletion is the major source of greenhouse gasses, which are mainly responsible for global warming. Therefore, renewable, carbon-neutral, economically viable alternatives to fossil fuels are urgently needed to avert the impending oil crises and the dramatic consequences of climate change (Atadashi *et al.,* 2012; Bertozzini *et al.,* 2011; Rodolfi *et al.,* 2009).

The combustion of fossil fuels emits tons of CO_2 into the atmosphere; As a result, the atmospheric CO_2 concentration has already reached the dangerously high level of 400 ppm. Other industrial activities are also contributing to this environmental catastrophe, which takes the form of the anthropogenic greenhouse gas (GHG) phenomenon, which increases global warming and leads to climate change (Aslam *et al.,* 2017; Mark, 2017).

Consequently, researchers all over the world are making great efforts to find proper solutions. They are trying to take appropriate action, such as capturing the CO_2 emitted by large sources (Abid *et al.,* 2017; Castro *et al.,* 2017; Veillette *et al.,* 2017; Sahay and Braganza 2016; Brennan and Owende, 2010).

Recent incentives to reduce greenhouse gasses, in particular CO_2, have enhanced the interest of researchers in vegetable-based fuels, because plants have an inherent ability to capture solar energy through photosynthetic pigments (via light reactions), while efficiently sequestering CO_2 from the atmosphere as their primary carbon source (via dark reactions) (Kuo *et al.,* 2016).

This carbon is then biologically converted to high-energy starches, celluloses, proteins and oils as storage and structural compounds. Some algae (e.g. Diatoms) are known to efficiently convert CO_2 to nearly 60–70% of their dry weight in the form of storage oils (Aslam *et al.,* 2017; Shirvani, 2012).

From an environmental perspective, the emissions produced by combustion engines burning biodiesel are greatly reduced compared with the emissions from conventional petroleum diesel (fossil fuels). The use of biodiesel results in reductions of up to 100 % in sulfur-dioxide, 48 % in carbon monoxide, 47 % in particulate matter, 67 % in total unburned hydrocarbon, and up to 90 % reduction in mutagenicity. Perhaps the most significant reduction, based on life cycle analysis, is the 78 % reduction in CO_2, which is considered the most important greenhouse gas in climatic models (Chisti, 2013; Brennan and Owende, 2010).

Researchers have also shown that biodiesel has much higher biodegradability than low-sulfur diesel fuel and the addition of biodiesel to diesel fuels actually promotes the biodegradability of diesel fuels, making the blends more environmentally attractive (Shirvani, 2012).

2. Biofuel production

Biofuel are defined as any sort of fuel that is derived from renewable organic biomass through physical and chemical processes with zero net CO_2 emission (Saber *et al.,* 2016; Luque *et al.,* 2008). Biomass is any organic material that can store chemical energy as a result of photosynthesis; this includes wood, wood waste, energy plants and many agricultural byproducts (Zhu *et al.,* 2014).

Biofuel are classified into three types (first, second and third generation biofuels) depending on their biomass source; the most popular biofuels are biodiesel, biogas and bioethanol (Chernova and Kiseleva, 2017; Chisti, 2013).

Increasing energy consumption, coupled with the widely fluctuating energy prices, has reflected negatively on natural resources (Show *et al.,* 2017).

This has motivated scientists and governmental representatives to look for alternative, renewable, carbon-neutral, non-conventional transport fuels that would be environmentally and economically sustainable (Naraharisetti *et al.,* 2017; Chisti, 2007).

The alternative fuel that has the most widely recognized potential to provide a sustainable energy system is biofuel, with biodiesel as its most popular exponent currently on the market, followed closely by bioethanol and biogas (Patil *et al.,* 2017; Mata *et al.,* 2010).

2.1. Biodiesel

Biodiesel is a type of renewable bioenergy fuel comprised of fatty acid methyl esters originating from vegetable oils, animal fats and microalgal oil (Chisti, 2007). It has a strong potential to replace petrodiesel. For low-cost production, however, the selection of the feedstock is very important (Wahidin *et al.,* 2014).

Two major steps are necessary in order to produce biodiesel from microalgae. The first step is the extraction of neutral lipids from microalgal cells, and the second step is the transforming the extracted oil into biodiesel (Che *et al.,* 2017; Wahidin *et al.,* 2014).

Biodiesel is obtained by the trans-esterification of triacylglycerols (TAGs). The process involves the reaction of the TAGs extracted from the different feedstocks with an alcohol, usually methanol or ethanol, in the presence of a catalyst (KOH, NaOH or H_2SO_4) at room temperature. This process produces mono-alkyl fatty acids esters or biodiesel (Hu *et al.,* 2008; Vasudevan and Briggs, 2008).

The trans-esterification reaction of TAGs involves three steps. In the first step, the TAG is converted to a diacylglycerol (DAG) and one molecule of fatty acid ester. In the second step, the DAG is converted to a monoacylglycerol (MAG) and one molecule of fatty acid ester. Finally, the MAG is converted to glycerol and one molecule of fatty acid ester. Therefore, the final products are one molecule of glycerol, as a by-product, and three molecules of fatty acid ester (Fig. 1) (Knothe and Razon, 2017; Sharma and Singh, 2017).

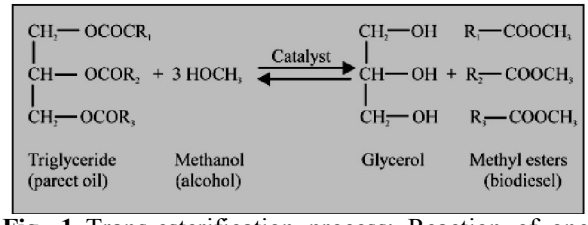

Fig. 1 Trans-esterification process: Reaction of one mole of triacylglycerol with three moles of alcohol. The alcohol, typically methanol or ethanol, reacts in the presence of a catalyst (Adopted from Pereira *et al.,* 2016).

Over the past few years, biodiesel has received considerable attention, as it is an environmentally friendly, renewable, bio-degradable, CO_2-neutral energy source and a non-toxic fuel. Biodiesel contributes no net CO_2 or sulfur to the atmosphere and emits less gaseous pollutants than normal diesel (petrodiesel) does (Abou-Shanab *et al.,* 2014). It has physical and chemical properties similar to petrodiesel, such as a higher flash point, a low sulfur concentration, and better lubricating efficiency and cetane number (Piloto-Rodríguеza *et al.,* 2017; Nabi *et al.,* 2006). Moreover, biodiesel contains no aromatic compounds and other chemical substances, which are harmful to the environment (Sharp, 1996).

2.2. Biogas

Biogas generation is a well-established technology that uses a wide range of residues as substrate. Biogas is produced from the anaerobic biodegradation of organic biomass, in which the organic matter is decomposed with the help of microbes in the absence of oxygen (Zhu *et al.,* 2014; Torres *et al.,* 2013). The biogas produced as a product of the metabolic action of methanogenic bacteria consists mainly of methane (55 – 75%) and CO_2 (25 – 50%) (Chen *et al.,* 2015; Zheng *et al.,* 2014).

Several methods have been proposed for the removal of CO_2 from biogas, among which the most common are solvent absorption, activated carbon adsorption, membrane filtration and cryogenic separation (Meier *et al.,* 2015).

Different feedstocks are used for biogas production. They include agricultural crops, sewage sludge, vegetable solid wastes, leaves, grass, seaweeds, animal wastes and recently microalgae; generally, the yield of biogas from different sources ranged from 0.15 to 0.65 m^3 per kg dry weight (Torres *et al.,* 2013).

Microalgae have a huge potential for biogas production (Meier *et al.,* 2017). During anaerobic digestion, biogas is produced and nutrients used for microalgae cultivation, such as nitrogen (N), phosphorus (P) and potassium (K) (Table 1) are recycled (Zhu *et al.,* 2014).

The production of biogas using microalgae as a feedstock is not yet economically viable because of the energy required to heat the digesters and the need for a larger land construction surface. Moreover, the retention time for biodigestion, which averages 20 – 30 days, increases the total costs, as does cell wall resistance (Sialve *et al.,* 2009).

The cell wall composition is crucial for anaerobic digestion because it prevents contact between anaerobic bacteria and the readily degradable content of algal biomass (Budzianowski, 2016). The ideal microalgal species for maximum biogas production should have some important characteristics, including a thin cell wall, large cells, high resistance against environmental contamination and high growth rates in non-sterile media (Meier *et al.,* 2015; Torres *et al.,* 2013).

Pretreatments can solve the limitations of cell wall degradability. They can decrease resistance to bacterial attack and increase both reaction speed and digestion efficiency. Therefore, different pretreatment techniques are usually applied to microalgae prior to biogas production; they are classified into enzymatic, chemical (acids, bases, ozonation), thermal, and mechanical (e.g. autoclaving, homogenizers, microwaves and ultra-sonication) pretreatments (Budzianowski, 2016; Torres *et al.,* 2013).

Table 1 Biogas production yields without pretreatments from different microalgae species (adopted from Chen *et al.,* 2015; Zhu et al. 2014)

Feedstock	Yield*	Methane content (%)
Chroococcus sp.	0.401–0.487 L CH_4 g^{-1} VS	52.0–54.9%
Scenedesmus obliquus	0.240 L CH_4 g^{-1} VS	-
Phaeodactylum tricornutum	0.360 L CH_4 g^{-1} VS	-
Chlorella vulgaris	0.375 L g^{-1} S	-
Blue algae from Taihu Lake, China	0.190 L g^{-1} VS	36.7
Chlamydomonas reinhardtii	0.587 L g^{-1} VS	-
Macrocystis pyrifera	0.180 L g^{-1} S	65.0
Durvillea antarctica	0.180 L g^{-1} S	65.0

*L g^{-1} VS, liters per gram of volatile solids; L g^{-1} S, liter per gram of solids

2.2.1. Basics of Anaerobic Bio-digestion

During anaerobic biodigestion, polymeric organic substances are broken down by a consortium of symbiotic effects of various strictly anaerobic and facultative anaerobic bacteria. These effects produce mainly methane and CO_2 (Fig. 2). The material remaining at the end of the digestion process, known as the decomposed substrate or the digestate, can be used as bio-fertilizer (Bajpai, 2017; FNR, 2013; Ziemiński and Frąc, 2012; Sialve *et al.,* 2009; De Mes *et al.,* 2003). The principal reaction sequences can be classified into the four major steps described below.

2.2.2. Enzymatic hydrolysis (Cleavage of substrate polymers)

In this step, insoluble polymeric organic compounds such as carbohydrates, proteins, fats and cellulose are hydrolyzed into monomers and dimers by extracellular enzymes, including amylases, proteases, lipases, cellulases, and others, produced by the hydrolyzing bacteria (Ziemiński and Frąc, 2012).

These monomers and dimers can subsequently be consumed by the hydrolyzing bacteria. This is a crucial, but relatively slow step and rate-limiting step that affects the overall rate of the anaerobic biodigestion process. The rate of enzymatic hydrolysis depends on the particle size, the pH of the reaction medium, and the production and activity of hydrolyzing enzymes (Barbot, 2014; De Mes *et al.,* 2003). The bacterial strains included in this step are *Butyrivibrio* spp., *Clostridium* spp., *Selenomonas* spp., and *Streptococcus* spp. (Björnsson *et al.,* 2001).

2.2.3. Acidogenesis (Acid forming step)

In this step, the acidifying bacteria convert the hydrolysis products to short chain organic acids (acetic, formic, lactic acid, propionic, butyric, pentanoic), fatty acids, alcohols (methanol, ethanol), aldehydes, $CO_2$and hydrogen. The bacterial strains included in this step are *Bifidobacterium* spp., *Selenomonas* spp., and *Flavobacterium* spp. (FNR, 2013; Barbot, 2014).

2.2.4. Acetogenesis (Acetic acid formation)

In this step, acetate bacteria convert the acid phase products into acetates and hydrogen, which are key intermediate products of the methane digestion process. The hydrogen released during this step inhibits the toxic effects of strains performing acetogenesis.

This hydrogen is also used by autotrophic methane-producing bacteria (hydrogen-scavenging bacteria) with a symbiotic relationship. The bacterial strains included during this step are *Acetobacterium* spp., *Sporomusa* spp., and *Ruminococcus* spp. (Bajpai, 2017; Ziemiński and Frąc, 2012).

2.2.5. Methanogenesis (Methane formation step)

In this step, methanogenic bacteria metabolize the organic acids formed in the previous steps (formic and acetic acids), methanol, CO_2 and hydrogen to methane (Fig. 2). Acetic acid is decomposed to methane and CO_2, and CO_2 is reacted with hydrogen to produce additional methane according to the following equations:

$CH_3COOH \rightarrow CH_4 + CO$ $\Delta G^{0'} = -31$ kJ/mol

$CO_2 + 4H_2 \rightarrow CH_4 + 2\,H_2O$ $\Delta G^{0'} = -131$ kJ/mol

The bacterial strains included in this step are *Methanosarcina* spp., *Methanothrix* spp., *Methanococci* spp., *Methanobacteria spp*., and *Methanomicrobia* spp. (Bajpai 2017; Barbot, 2014; FNR, 2013; Schink, 1988).

2. Microalgae

Microalgae comprise an extremely diverse group of heterogeneous prokaryotic and eukaryotic microscopic photosynthetic microorganisms that live in a wide range of habitats (Ogburn and Vogt, 2017; Suganya *et al.,* 2016; Mahmoud *et al.,* 2014a; Mahmoud *et al.,* 2014b; Razzak *et al.,* 2013; Ibraheem *et al.,* 2012; Chisti,2007).

They are chlorophyll-bearing organisms that are more photosynthetically efficient than higher plants and exist in all earth ecosystems. Unlike higher plants, they lack roots, stems, leaves, conducting vessels and complex sex organs. Their sizes range from a few micrometers (μm) to a few hundreds of micrometers. They live in different growth forms (individually, in chains or colonies, and in filamentous forms) depending on the species (Abomohra *et al.,* 2017; Ali and Watson, 2015; Bharathiraja *et al.,* 2015; Moreno-Garrido, 2008).

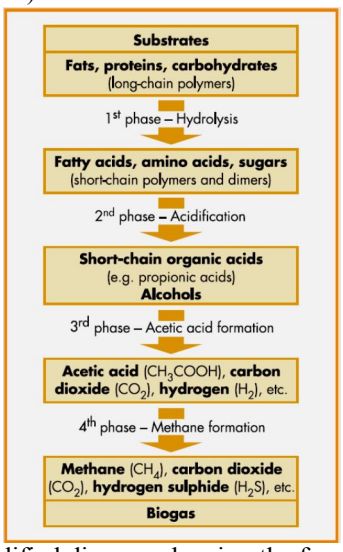

Fig. 2 Simplified diagram showing the four main steps of organic matter decomposition during anaerobic degradation for biogas production (adopted from FNR 2013; Barbot, 2014; Zheng *et al.,* 2014).

According to different morphological, physiological, life cycle, biochemical and genetic criteria, microalgae are categorized into groups such as cyanophyta (cyanobacteria), chlorophyta (green algae), phaeophyta (brown algae), rhodophyta (red algae), bacillariophyta (diatoms), and others (Hoek et al., 1995). It is thought that 200,000–800,000 species exist, of which only 40,000–50,000 have been described and studied for different purposes (Suganya *et al.,* 2016; Zhang *et al.,* 2014; Varfolomeev and Wasserman, 2011).

During recent decades, microalgae culture collections have come to the forefront of the agendas of many scientific organizations around the world.

Some of the major microalgae culture collections are listed below: (Emami *et al.,* 2015; Mata *et al.,* 2010).

- Fresh Water Culture Collection at the University of Coimbra in Portugal, which includes more than 4000 strains representing 1000 species.
- SAG: Experimentelle Phykologie und Sammlung von Algenkulturen; Culture collection at the University of Gottingen in Germany, which includes 2213 strains representing 1273 species (https://www.uni-goettingen.de/en/culture-collection-of-algae-sag/184982.html).
- UTEX: Culture Collection of Algae at the University of Texas at Austin, USA, which includes 2300 strains (https://utex.org/).
- Culture collection at the National Institute for Environmental Studies (NIES) in Ibaraki, Japan, which includes 2150 strains representing 700 species.
- CSIRO: The Australian National Algae Culture Collection, with more than 1000 different strains (https://www.csiro.au/en/Research/Collections/ANACC/About-our-collection).
- CCAP: Culture Collection of Algae and Protozoa is located within the Scottish Association for Marine Science campus on the Dunstaffnage peninsula near Oban on the scenic west coast of Scotland. (http://www.ife.ac.uk/ccap). This is the most diverse collection of its kind in the world, with approximately 3000 strains of marine and freshwater algae, protista and seaweeds.
- CPCC: Canadian Phycological Culture Centre for Algae, Cyanobacteria and Lemma. (https://uwaterloo.ca/canadian-phycological-culture-centre/).
- NMCA: The Provasoli-Guillard National Center for Marine Algae and Microbiota, Maine. (https://ncmaccmp.bigelow.org/).
- PCC: Pasteur Culture Collection of Cyanobacteria, Pasteur Institute, Paris, France (http://cyanobacteria.web.pasteur.fr/).

Microalgae are single-celled molecular factories. Their uses for a variety of purposes, including the production of bioactive molecules and metabolites, pharmaceutical and cosmetic applications, and biofuels production have been investigated (Úbeda *et al.,* 2017).

2.2. Microalgae as a feedstock for biodiesel production

Microalgae have the inherent ability to capture solar energy from the surrounding environment through the light phase of photosynthesis (Hill reaction) and convert it to chemical energy and reducing equivalents. The efficient fixing of CO_2 from the atmosphere as their primary carbon source takes place through the dark phase of photosynthesis (Calvin cycle). This fixed carbon is converted to high-energy compounds like carbohydrates, proteins and lipids (Piloto-Rodríguez *et al.,* 2017; Borowitzka and Moheimani, 2013; Deng *et al.,* 2009).

A large number of scientific works have demonstrated the potential and feasibility of biodiesel production (Chiaramonti *et al.,* 2017; Del Río *et al.,* 2017; Chisti, 2007).

In comparison with other available feedstocks, microalgae have been extensively studied as lucrative and endurable sources for biodiesel production. Microalgae are easy to cultivate, can grow in extreme ecosystems, can using non-drinking water for growth, can be used in wastewater treatment to provide water for irrigation purposes, have no overlap with food crops, and limit GHG emission through CO_2 sequestration (Řezanka *et al.,* 2017).

Microalgae can double their biomass within 24 hours, as they can complete an entire growth cycle every few days (Angles *et al.,* 2017; Patil *et al.,* 2017; Řezanka *et al.,* 2017; Chisti, 2007).

Different microalgae species can be adapted to live under a variety of environmental conditions. Thus, it is possible to find species best suited to local environments or specific growth characteristics. This cannot be done with other current biodiesel feedstocks (e.g. Soybean, Rapeseed, Sunflower and Palm oil) (Liu *et al.,* 2008).

Microalgae have much higher growth rates and productivity, compared to conventional forest, agricultural crops, and other aquatic plants. They require much less land area than other biodiesel feedstocks of agricultural origin, up to 49 less than rapeseed and 132 times less that soybean crops. Algal biomass has 30% (w/w) oil content (Table 2 and Table 3) (Su *et al.,* 2017).

Table 2 Comparison of microalgae with other biodiesel feedstocks (Adopted from Chisti, 2007)

Raw Material	Oil Content (% in dry weight biomass)	Output (L oil/ha year)	Land used (M2 year/ Kg biodiesel)	Water Footprint (m2/ton)	Production Cost (US$/L)	Acid value of oil	Biodiesel yield
Soybean	18	636	18	4200	0,40-0,60	0,2	90%
Rapeseed	41	974	12	4300	0,99	2,0	87%
Sunflower	40	1070	11	6800	0,62	0,1	90%
Palm	36	5366	2	5000	0,68	6,1	95%
Castor	48	1307	9	24700	0,92-1,56	4,6	89%
Microalgae	50	97800	0,1	591-3276	3,96-10,56	8,9	60%

In addition, microalgae have emerged as a feedstock for several different types of renewable fuels such as biodiesel, methane, hydrogen, and ethanol, among others; microalgae biodiesel contains no sulfur and performs as well as petrodiesel (Wu *et al.,* 2012; Mata *et al.,* 2010).

Currently, multiple international companies are investing in microalgae biomass for biofuels production, with projects in the USA (Algenol, Green Fuel Technologies, Origin Oil), in Portugal (AlgaFuel), in the UK (Varicon Aqua Solutions), Finland (Neste Oil), Japan (Euglena), the Netherlands (Algae Link) and others (Saber *et al.,* 2016).

The concerns arising from the production of biodiesel from food crops are inefficiency and unsustainability (Chisti, 2007). The oil content of oil crops is less than 5 % of the total biomass; hence, a large amount of crops is required to produce a significant amount of oil for biodiesel production (Elsayed *et al.,* 2017).

Moreover, extensive agricultural areas would be required to produce the amount of crops necessary; this leads to negative impacts on the environment. This situation could pit food against fuel, because many people around the world rely on food grown on a relatively small agricultural land area (Song et al. 2008). In addition, large-scale crop production for biodiesel requires large amounts of pesticide, fertilizer and water (*Groom et al.,* 2008).

Sustainability may be achieved only by minimizing the land area required for growth of sufficient amount of feedstock. This has led to the conclusion "Biodiesel are sustainable energy source only if feedstocks are grown sustainable" (Ben-Iwo *et al.,* 2016).

Microalgae are a promising source of oil for biodiesel production (Abomohra *et al.,* 2012; Csavina *et al.,* 2011).

Among the different terrestrial oil crops used as biodiesel feedstocks, microalgae show the greatest promise as bioenergy candidates because they contain more oil, grow faster and are easier to grow (Table 2) (Su *et al.,* 2017; Griffiths and Harrison, 2009; Hossain *et al.,* 2008).

Their growth doubling time is usually around 24 hours. During exponential growth, they can double their biomass in as little as 3.5 hours under ideal conditions of temperature, light and nutrients (Aslam *et al.,* 2017; Meng *et al.,* 2009; Song *et al.,* 2008). The yield of oil from microalgae is estimated at 20,000–80,000 liters per acre, approximately 7-31 times greater than the most productive oil crop (Table 2) (Demirbas, 2011).

2.3. Possible microalgal metabolic pathway for biofuels production

Modification of microalgal metabolic pathways can redirect and push cell metabolism toward the synthesis of certain desired constituents (i.e. lipids, carbohydrates and proteins) for further utilization.

This can be done using different techniques, such as physiological stimulation (i.e. nutrient limitation) (Elsayed *et al.,* 2017), or genetic manipulation/improvements (i.e. insertion or removing key genes) such as the trials of ACCase enzyme expression, which is considered the most important step during TAG biosynthesis in microalgae (Rosenberg *et al.,* 2008).

Table 3. Oil content in the dry biomass of various species of microalgae (Adopted from Borowitzka and Moheimani, 2013; Singh and Singh, 2010; Demirbas *et al.,* 2011; Chisti, 2007).

Microalgae species	Oil content (% dry weight)
Chlamydomonas reinhardtii	25-80
Botryococcus braunii	25-75
Chlorellavulgaris	14-22
Chlorella pyrenoidosa	46.7
Chlorella protothecoides	57.9
Chlorella emersonii	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella primolecta	23
Dunaliella salina	6
Dunaliella tertiolecta	35.6
Isochrysis sp.	25-33
Monallanthus salina	>20
Nannochloris sp.	20-35
Nannochloropsis sp.	31-68
Neochloris oleoabundans	35-54
Nitzschia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Tetraselmis suecica	15-23
Spirulina platensis	4-9
Spirulina maxima	6-7
Scenedemus obliqus	12-14
Scenedesmus dimorphus	16-40
Prymnesium parvum	22-38
Pleurochrysis carterae	30-50
Hormidium sp.	38
Euglena gracilis	14-20

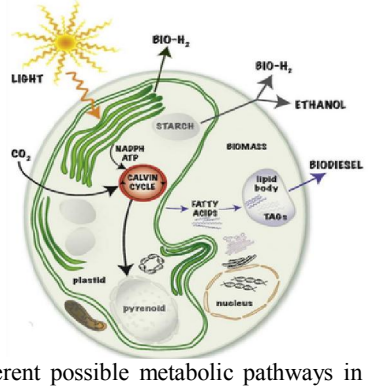

Fig.3 Different possible metabolic pathways in microalgae for biofuels production; also, pathways to other photosynthetic outputs for the synthesis of proteins, nucleic acids, carbohydrates, lipids and H_2 (Beer *et al.,* 2009).

2.4. Lipid biosynthetic pathway in microalgae

Lipids are generally considered the most valuable fraction of microalgal biomass. Different types of lipid are found in microalgae, but the neutral lipids or TAGs are the backbone of biodiesel production through the transestrification process. The basic pathways of fatty acid and TAG biosynthesis in algae are directly analogous to those in higher plants (Hulatt *et al.,* 2017; Maity *et al.,* 2014; Qiang et al., 2008).

A generalized scheme for the overall synthesis of TAG in cells (Fig 4) is composed of three major steps: (1) carboxylation of acetyl-CoA to form malonyl-CoA, the committing step for fatty acid biosynthesis; (2) acyl chain elongation; and (3) TAG formation (Nobusawa *et al.,* 2017; Sakthivel *et al.,* 2011; Courchesne *et al.,* 2009).

All TAGs are synthesized by a single set of enzymes in the chloroplast. Acetyl-CoA carboxylase is a key enzyme responsible for regulating synthesis. It is thought that fatty acid biosynthesis starts with glycolysis-derived pyruvate. Glycolysis and pyruvate kinase catalyze the irreversible synthesis of pyruvate, which is later converted to acetyl-CoA (Banerjee *et al.,* 2017).

Acetyl-CoA carboxylase (ACCase) catalyzes the conversion of acetyl-CoA to malonyl-CoA, which considered the committed step in the biosynthetic pathway used to create fatty acids (Sakthivel *et al.,* 2011; Hu *et al.,* 2008). Further evidence suggests that ACCase plays an integral role in TAG synthesis and the cellular response to environmental stress (Wirshing and Minocha, 2012).

The composition of TAGs is strain specific and is controlled mainly by the genetic make-up of each organism. TAGs don't perform any metabolic function inside the cell; instead, they act as a storage form of carbon and energy that, after being synthesized, is regularly deposited and packed together to form lipid bodies inside the cytoplasm of algal cells (Bagnato *et al.,* 2017).

Oleaginous microalgae produce small amounts of TAG under favorable environmental conditions, but TAG synthesis can be greatly induced by either chemical or physical environmental stimuli. The major chemical stimuli are nutrients starvation, pH of the growth medium, and salinity; the major physical stimuli are temperature and light intensity (Hu *et al.,* 2008).

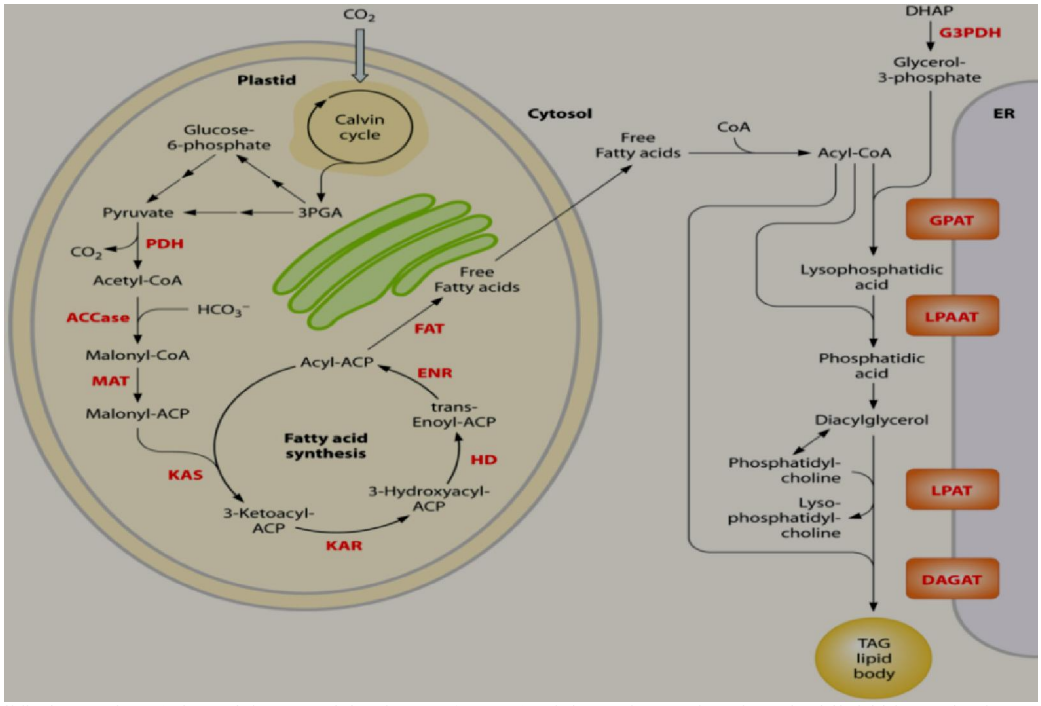

Fig.4 Simplified overview (adopted from Radakovits *et al.,* 2010) of the pathways in microalgal lipid biosynthesis. Intermediates are shown in black and enzymes shown in red. Free fatty acids are synthesized in the chloroplast, while TAGs may be assembled in the ER. ACCase, acetyl-CoA carboxylase; ACP, acyl carrier protein; CoA, coenzyme A; DAGAT, diacylglycerol acyltransferase; DHAP, dihydroxyacetone phosphate; ENR, enoyl-ACP reductase; FAT, fatty acyl-ACP thioesterase; G3PDH, gycerol-3-phosphate dehydrogenase; GPAT, glycerol-3-phosphate acyltransferase; HD, 3-hydroxyacyl- ACP dehydratase; KAR, 3-ketoacyl-ACP reductase; KAS, 3-ketoacyl-ACP synthase; LPAAT, lyso-phosphatidic acid acyltransferase; LPAT, lyso-phosphatidylcholine acyltransferase; MAT, malonyl-CoA: ACP transacylase; PDH, pyruvate dehydrogenase complex; TAG, triacylglycerols.

2.5. Challenges and prospects for biofuels production from microalgae

Although, microalgae have received considerable attention as fascinating feedstocks for biofuel production, upstream and downstream processes are still in various stages of development. There is incomplete information regarding production costs and a lack of understanding of the full details of lipid metabolism needed to manipulate the process physiologically and genetically (Wang *et al.,* 2017).

In addition, there is a lack of resources to develop pilot-scale production facilities at suitable locations to enhance lipid productivity.

Some biological challenges and opportunities for a more sustainable society are listed below (Baudry *et al.,* 2017; Chernova and Kiseleva, 2017; Chiaramonti *et al.,* 2017; Lim and Schenk, 2017; Sharma and Singh, 2017; Wang *et al.,* 2017; Sahay and Braganza, 2016; Wu *et al.,* 2014; Chisti, 2013; Hu *et al.,* 2008).

- Developing sophisticated techniques to cultivate selected microalgae strains for biomass and lipid productivity in non-sterilized wastewater, which contains sulfur, phosphorus and carbon sources in addition to various heavy metals and biotic contaminations such as bacteria, fungi and zooplankton. These techniques must be cost-effective in large-scale production.
- The TAG biosynthesis pathway is not fully understood. Fatty acids are common precursors for both TAGs and another polar membrane lipids, and the mechanisms by which the cells regulate the distribution of the fatty acids to different forms of lipids is not known.
- How algal cells control the flux of atmospheric CO_2during the light phase of photosynthesis and portioning into various major molecules i.e. carbohydrates, proteins and lipids remains unclear.
- The relationship between cell cycle and TAG formation also remains unclear. Understanding this relation will enable the genetic manipulation of selected strains, which will enable rapid growth and high TAG accumulation simultaneously.
- Isolation and characterization of robust microalgal strains from unusual habitats requires extensive further investigation.
- Genetic engineering strategies such as cloning and transforming genes that enhance lipid productivity per dry weight or improve robustness of strains will improve the overall performance and sustainability of TAG production.
- The development of innovative and breakthrough approaches to cost-effective, large-scale cultivation systems that allow selected microalgal strains to achieve high growth rates and high lipid productivity is essential to develop a microalgae-based biofuels industry. It is essential to avoid the problems of open pond and closed cultivation systems.
- Costs and energy consumption during downstream processes for harvesting and de-watering microalgal biomass, lipid extraction, and transesterification must be reduced.
- Screening protocols for strains that can withstand extreme and variable environmental conditions such as high salinity, high pH, and high temperature fluctuations must be developed.
- Technologies for the utilization of byproducts and residual biomass must be developed.

2.6. Strain selection and improvement

The most crucial and cumbersome step in the development of reliable biotechnological applications of microalgae in biofuels production and other industrial processes is appropriate strain selection (Angles *et al.,* 2017; Del Río *et al.,* 2017; Piloto-Rodrígueza *et al.,* 2017; Zhang *et al.,* 2014; Rawat *et al.,* 2016).

A vast number of microalgae are thought to exist in earth's ecosystems, but only around 3000 species have been described in detail by researchers. Not all of them are suitable for biofuel applications (Borowitzka and Moheimani, 2013). The best microalgal strains for different applications can be obtained by screening naturally existing isolates using different strategies, and then using the available tools to improve the initial strains (Singh and Singh, 2010).

After collecting samples from different habitats, several techniques are used for isolating and purifying uni-algal strains (strains that still contain bacterial contaminations) or axenic strains (pure strains). These techniques include single-cell picking, sequential dilution, plating on solid media, antibiotic selection. Recently, florescence activated cell sorting (FACS) has emerged as a powerful technique for selecting microalgal strains from among thousands of cells with desired properties (e.g. high lipid productivity) (Lim and Schenk, 2017; Pereira *et al.,* 2016).

There are many characteristics that have been used by screening strategies to distinguish promising strains from others, including high CO_2 utilization rate (%), high photosynthetic efficiency, maximum biomass production (g L^{-1}), specific growth rate (d^{-1}), doubling time (h), biomass productivity (mg L^{-1} d^{-1}), lipid content (%), lipid productivity (mg L^{-1} d^{-1}), ability to grow in open systems, ability to grow in wastewater and pollutant removal, high tolerance and adaptation to various changes in harsh environmental conditions (temperature fluctuations, pH, light), robustness, production of valuable co-products, and low-cost downstream processing (Del Río *et al.,* 2017;

Elsayed *et al.,* 2017; Pereira *et al.,* 2016; Benvenuti *et al.,* 2015; Ho *et al.,* 2014; Wu *et al.,* 2014; Bahadar and Khan, 2013).

Each of these criteria has been studied separately under laboratory conditions, but no strains exhibit all of these characteristics under full outdoor conditions. Moreover, each of these parameters differs from one strain to another (Benemann, 2013; Rawat *et al.,* 2016).

Subsequently, a process-oriented screening strategy indicated that the microalgal strains to be selected for biodiesel production should meet different requirements, including high growth rate, high tolerance to growth in outdoor environmental conditions (temperature, light intensity) and high lipid productivity (Winckelmann *et al.,* 2015; Bleeke *et al.,* 2014).

In the case of open pond cultivation systems, the selected strains should be isolated from an area close to the site of production, which they can easily adapt to the climatic conditions (Rawat *et al.,* 2013).

Furthermore, it is very important to study the biology and the eco-physiological parameters of the selected strains for optimum production of desired characteristic, such as lipid productivity. The desired characteristic may be strain specific, as some strains, like *Chlorella* sp., can easily grow under outdoor conditions with low contamination from other non-target microalgae, but other strains cannot (Lim and Schenk, 2017; Rawat *et al.,* 2016).

There are several techniques and methodologies to improve certain strains characteristics, such as high biomass, broad environmental tolerance, and high lipid productivity rate. These techniques and methodologies include nutrient starvation, genomics, lipidomics, proteomics and metabolomics (Sun *et al.,* 2014; Nigam and Singh, 2011).

2.7. Genetic engineering strategies to increase microalgae lipid production

Several genetic engineering tools have been used to manipulate microalgal genes to increase lipid productivity, such as selection markers, homologous recombination, RNA silencing, electroporation, and biolistic transformation (Ghosh *et al.,* 2016; Fulbright *et al.,* 2014).

Tremendous interest has been invested in using genetic engineering strategies to obtain desirable traits, such as triggering lipid accumulation in different microalgae species (Boyle *et al.,* 2012; Merchant *et al.,* 2011; Siaut *et al.,* 2011; Courchesne *et al.,* 2009).

Of particular relevance are some of the key enzymes in the fatty acid biosynthesis pathway, including acetyl-CoA carboxylase (ACCase), which was first isolated from the microalga *Cyclotella cryptic* (Roessler, 1990). However, several attempts to utilize ACCase over-expression to increase lipid content in various microalgal species have been disappointing (Dunahay *et al.,* 1995).

Interestingly, the effect of ACCase over-expression in potato tubers, a tissue that normally is very starch-rich and lipid-poor, resulted in a 5-fold increase in TAG content (Dehesh *et al.,* 2001).

From this can be concluded that ACCase is a limiting step in lipid biosynthesis mainly in cells that do not normally store large amounts of lipid.

Another attempt to increase expression of a protein involved in fatty acid synthesis, 3- ketoacyl-acyl-carrier protein synthase III (KASIII), was also not successful in increasing lipid production (Radakovits *et al.,* 2010). Subsequently, ACCase was successfully inserted into the diatoms *C. cryptica* and *Navicula saprophila*; however, the over-expression of ACCase yielded only a 2- to 3-fold enhancement in enzyme activity (Roessler, 1990).

These experiments demonstrated that ACCase could be inserted efficiently into microalgae, but with no significant increase in lipid accumulation in the transgenic diatoms (Dunahay *et al.,* 1995).

It should be pointed out that, at present, genetically modified (GM) strains of microalgae can only be used in small-scale closed controlled bioreactors and are very strictly regulated; this may increase the total cost of production when compared with non-GM algae in open pond systems or raceways (Lim and Schenk, 2017).

2.8. Mutagenesis as a recent strategy for lipid induction in microalgae

Induced cell mutagenesis and mutant selection have been suggested as a technique for microalgal strain improvement (Hlavova *et al.,* 2015; Choi *et al.,* 2014; Lee *et al.,* 2014; Doan and Obbard, 2012).

The mutagens fall into two main categories: physical (e.g. UV, gamma and x-rays) and chemical (e.g. ethyl methane sulfonate EMS and nitrosomethyl guanidine NTG) (Gupta *et al.,* 2017; Schneider *et al.,* 1995).

The acquisition of mutants with the desired phenotype following mutagenesis can be challenging, because individual cells must be screened within a short time (Meireles *et al.,* 2003).

Many classical screening methods rely on metabolite antibiotic responses or chromatographic methods, but these methods take long time (Wang *et al.,* 2010). FACS represents a powerful technique to isolate target cells from a complex population within few seconds, based upon specific fluorescence cell properties (Radakovits *et al.,* 2010). FACS has been used to successfully isolate desired mutants of yeast, filamentous fungi, and bacteria, but has not yet been reported for the isolation of high lipid-producing mutants of microalgae (Lim and Schenk, 2017; Natarajan *et al.,* 2013).

Another strategy could be the production of new GM microalgal strains with low antenna pigment content, which will allow more light to pass into the medium. A study done with a mutant of *Synechocystis* PCC6714 and green alga *Chlamydomonas perigranulata* showed that these strains can efficiency capture light even in low light conditions during the early hours and the evening and in deeper water and that the mutation minimizes photo-inhibition (Borowitzka and Moheimani, 2013; Cagnon *et al.,* 2013).

2.9. Microalgae cultivation and scale-up production

Cultivation of microalgae on the laboratory scale is only 140 years old, but the commercial cultivation of microalgae for industrial applications is less than 60 years old (Řezanka *et al.,* 2017).

This should be compared with the farming of other plants, which was started thousands of years ago (Borowitzka and Moheimani, 2013; Mata *et al.,* 2010). The basic requirements for microalgal cultivation differ from one species to another, but microalgae typically require basic nutrients to grow, including carbon, nitrogen and phosphorus sources; CO_2; and appropriate temperature (°C) and light intensity (Smith-Baedorf, 2012).

Several factors affect the cultivation of microalgae; among them are the physical and biotic factors, such as temperature, light quality and quantity, invading bacteria or viruses; and the operational factors, such as mixing, aeration, CO_2 concentration, harvesting, and depth of the designed pond system (Zhu *et al.,* 2014).

Large-scale production involves transitioning the production volume from small laboratory flasks to laboratory-scale photobioreactors to pilot-scale photobioreactors, and finally to industrial-scale photobioreactors. Scale-up poses many challenges, including the selection of strains with high growth rates and high lipid productivities (Chen *et al.,* 2011).

Basically, microalgae can be cultivated in different cultivation systems, including open ponds (raceways) and controlled photobioreactors. Open ponds are the oldest and cheapest type of commercial microalgae cultivation because the required energy and carbon source are freely available from the surrounding atmosphere, the cultures are easy to manage and the ponds are easy to construct (Rawat *et al.,* 2016; Cong, 2012; Grobbelaar, 2011).

On the other hand, only a few microalgal strains can be successfully cultivated in open ponds because they must maintain a high growth rate, despite the uncontrolled environmental conditions, to cut down on other microbial contamination that can damage the culture (Cuello *et al.,* 2016; Rodolfi *et al.,* 2009).

The limitations to the use open raceways include the loss of too much water due to evaporation; contamination by competing microorganisms such as bacteria, fungi, viruses, other algal species; insects; and the loss of biomass due to direct susceptibility to environmental conditions and large area occupation (Benemann, 2013).

Despite all the previously mentioned limitations for cultivation in open ponds, there have been successful stories about the cultivation of certain microalgal species in open ponds, either in batch mode (e.g. *Haematococcus pluvialis*) or in longtime continuous cultivation (e.g. *Phaeodactylum tricornutum*, *Pleurochrysis carterae* and *Nannochloropsis* sp.) without any contamination problems (Bahadar and Khan, 2013).

Closed systems or photobioreactors offer highly controllable production conditions. They also provide other advantages, including higher efficiency biomass productivity, shorter harvest time, reduced risk of contamination, and high surface to volume ratios. These closed systems can cultivate a variety of microalgal strains and ensure production stability over time. However, these systems are much more expensive than open systems (Hulatt *et al.,* 2017; Bahadar and Khan, 2013).

Closed systems involve panels of transparent tubes or plates placed vertically or horizontally and provided with CO_2 cylinders; there are several types, including flat -plate, tubular-column and biofilm photobioreactors. Gas exchange and mixing take place through the use of pumps. *Spirulina* sp., *Aphanothece microscopic*, *Chlorella* sp. and *Porphyridium purpureum* are examples of strains that are cultivated in closed systems (Pulz *et al.,* 2001; Li *et al.,* 2007).

The combination of wastewater and seawater with flue gasses emitted from industrial stations offers a great opportunity to cultivate microalgal biomass. This would eliminate the need to increase the pressure on fresh water resources and, in the meantime, reduce environmental burdens due to emission of greenhouse gasses into the atmosphere (Nigam and Singh, 2011).

3. Conclusion

Although, promising steps towards economical feasibility of using microalgae as candidates for different biofuels production such as biodiesel and biogas, but the production process to be economic feasible, sustainable, and competitive to other feedstocks further investigations and analysis are needed. Consequently, the calculations of total energy-input and energy-output from the whole production process in regard to strain selection, cultivation, harvesting of microalgae biomass, inducing more lipid productivity, and trans-estrification are required.

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Conflict of interest

The authors declare that they have no conflict of interest.

Corresponding author:
Name: Khaled N. M. Elsayed
Email: k.elsayed@jacobs-university.de
elyamany_bns@yahoo.com
ORCID Identifier: 1234-5678-2811-1900
Phone number: 0049(0)15219455400
Fax: 0049(0)42159054250

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