



Kerbala Journal for Engineering Science

<https://kjes.uokerbala.edu.iq/>



## Algae-assisted Microbial Fuel Cell for Electrical Energy Generation and Wastewater Treatment

Raghda Hamid Ali

Directorate of Education of Nineveh, Mosul, Iraq.

Email: [raghdahameed374@gmail.com](mailto:raghdahameed374@gmail.com)

Received: 31 March 2025; Revised: 02 September 2025; Accepted: 21 September 2025; Publish:30 September 2025.

### Abstract

This research investigates the potential of microalgae as a sustainable resource for bioenergy production using a microbial fuel cell (MFC)<sup>1</sup> technology. It explores the general taxonomy and characteristics of algae, outlines the different generations of biofuels, and highlights the benefits and limitations of algae-derived biofuels. Particular emphasis is placed on integrating bio-electrochemical systems that simultaneously treat wastewater and generate electricity. The study demonstrates that utilizing algae in MFCs not only supports organic biomass production but also enhances nitrogen recovery and energy efficiency. Through experimental trials with various electrode modifications, the research confirms that algal biomass can effectively function as both a biofuel substrate and a sustainable energy solution. These findings propose a closed-loop, self-sufficient energy system with minimal environmental impact, offering a viable alternative to fossil fuels.

**Keywords:** Algae; Microbial fuel cells; Biofuels; Renewable energy; Biomass recovery.

<sup>1</sup> Microbial Fuel Cell (MFC) is a bio-electrochemical system that uses the natural metabolic processes of microorganisms (usually bacteria) to convert chemical energy from organic matter directly into electrical energy.

## 1. Introduction

Algae are made up of several classes of organisms capable of photosynthesis, regardless of whether they are single cells or multiple cells. The sizes of these organisms range from very small and invisible to the naked eye (1  $\mu\text{m}$ ) to very large, reaching a length of more than 200 meters [1].

Algae are found in humid environments at a wide range of temperatures. Algae are a large part of the vegetation of oceans, lakes and other surface waters and algae are common in stagnant water, and some algae live on the surface of the water, forming an outer layer of green plankton (microflora that usually drifts in the upper layers of the ocean, and the phytoplankton consumes nutrients and light energy to produce biomass) [2].

Algae reproduce in a variety of ways, including vegetative reproduction expressed by simple fission, filamentous fragmentation, hormone formation, asexual reproduction, which is represented by the formation of mobile, non-motile and static blackboards, and sexual reproduction in different ways, including the union of similar gametes, the union of dissimilar gametes, and oogamy [1].

The classification of algae depends on several bases and criteria, the most important of which are: the presence or absence of a real nucleus, the types of pigments present within the pigment carriers, the structure of the cell wall, the type and number of flagella, methods of reproduction, and the type of life cycle. [3]

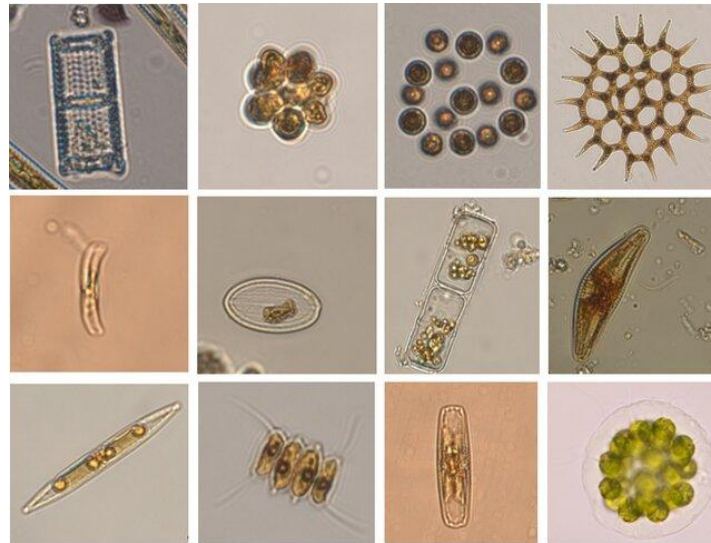
Algae have many benefits, as they are considered food for humans because they contain proteins, carbohydrates, fats, minerals, vitamins and hormones, so they are an important food source and are also used as animal feed, as algae play an important role in ecosystems, whether on land or in water, as they are the broad base of the food pyramid because they are considered the basic products in the environment, as well as algae are the basis in food chains in the aquatic environment, they are the first product of food, as they are food for aquatic life directly or Indirect, and used in industry and medicine, as many of the algae derivatives have been used in industries (a gelatinous natural substance used in playground food, and is also used in the hardness of the media Agar and medical uses such as agricultural microbiology), as well as extracted from algae some elements such as iodine extracted from brown algae and urn from the category of red algae, and algae have been used in many experiments and biological research such as photosynthesis, reproduction, geneti

cs and genetic engineering in order to shorten Its life cycle and ease of development, as well as used as biological evidence of pollution, where some spleen are evidence of water pollution [4], and algae are the main source of oxygen in nature, as their production of oxygen is comparable to the most dense plants on the planet as they are the main primary product of organic mass in the oceans and all water bodies on the globe, represented by more than three-quarters of them [3].

Algae take several forms, including unicellular form, multicultural form, filamentous forms, Siphonous forms, parenchymatous forms or Erect thallus form and there are many other forms. [5]

Despite the many benefits of algae, they may also cause harms Some algae may produce toxic substances that lead to the death of aquatic organisms, especially fish, and that the prosperity of some types of algae in the water causes an increase in the amount of nutrients, which is known as a phenomenon and leads to pollution in the water by changing the taste and smell, as well as the growth of some algae Eutrophication Food richness on the outer surfaces of boats and ships leads to damage and hindered their speed, and thus requires cleaning them a long time and economic losses, and also causes

some Algae harm to human health when eating fish that have previously fed on harmful algae, which secrete some toxins, which leads to damage to the digestive system and may cause some of the algae greens. And in small quantities in drinking water for diarrhea [4].



**Figure 1. Most morphological forms of algae [5]**

### **1.1. Search problem**

The problem with research in the manufacture of biofuels from algae is the low efficiency of converting biomass into energy. This technology faces costs associated with algae cultivation, wastewater modification, and efficient conversion technology. Moreover, there is the issue of environmental sustainability and dependence on limited water supplies, which necessitates the search for new solutions that ensure sustainable bioenergy production without harming ecosystems or natural resources.

### **1.2. The importance of the research**

The importance of research is to provide sustainable solutions to prepare bioenergy from algae, which helps reduce the need for fossil fuels and combat climate change. Using algae as an alternative source of energy can help collect CO<sub>2</sub> and reduce environmental impact. In addition, combining natural processes, such as the use of wastewater, improves process efficiency and reduces the financial and environmental burdens associated with biofuel production, making this technology more widely applicable in the future.

### **1.3. Research Objectives**

The research aims to identify the mechanism of using algae in the manufacture of bioenergy for renewable energy that helps reduce dependence on fossil fuels.

## **2. Background and related work**

### **2.1. Biofuels**

According to the US Energy Information Administration (EIA), the exponential growth of industry and population has resulted in a substantial rise in the world's energy demand, which in turn has increased the usage of fossil fuels [6].

Global energy consumption has increased over the past 20 years by about 44% and predicted that there will be an increase of 60% during the next 20 years because the majority of energy needs are satisfied by burning fossil fuels (coal, natural gas, and petroleum), which are readily available, affordable, and simple to use in comparison to alternative energy sources. However, the continued use of fossil fuels is undesirable because it accelerates the buildup of greenhouse gases like CO<sub>2</sub> [7]. This is what prompted many researchers and specialists in various countries of the world to seek new sources of renewable, sustainable, and environmentally friendly energy. Those alternatives include wind, sun, geothermal energy, hydropower, and biofuels. [6].

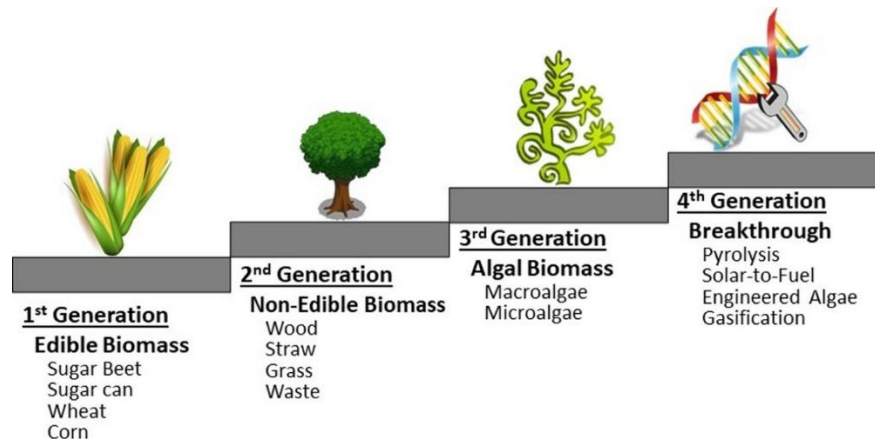
Biofuels are considered one of the renewable energy sources that constitute a successful alternative to traditional fossil fuels due to their advantages due to their positive characteristics, including biodegradability, low toxicity compared to fossil fuels, and low impact on the environment, especially the percentage of CO<sub>2</sub> gas released from combustion [8].

## **2.2. Biofuel Generations**

According to the raw materials and production method, biofuels are divided into different groups called biofuels of successive generations: I, II, III, and IV. The first creation of bioenergy uses edible biomass, including sugar and starch, which raises production costs and results in wasteful use of energy and resources used in crop cultivation. In particular, using edible biomass necessitates a lot of water and fertiliser, as well as a lot of agricultural land, and it competes with food crops. When using inedible lignocellulosic biomass, such as switch grass, sawdust, inexpensive lumber, crop wastes, and municipal trash, Generation II biofuels rely on more effective renewable alternatives. Even if this generation solves the drawbacks of the first generation, several steps have to be taken to create the appropriate biofuels at a competitive price. In recent years, numerous studies have been conducted to accomplish this goal through the use of thermal, biological, enzymetic, or chemical processes. All of these conversion strategies have run into problems. Nonetheless, the most adaptable processes are found to be chemical ones. Numerous combinations of processes were also examined, such as the chemical method followed by an enzymatic or biological phase in the manufacture of sugar solvent from biomass.

The third generation of biofuels uses feedstock like algae biomass. In addition to producing oil, algae and other photosynthetic plants, including seaweed, absorb a lot of CO<sub>2</sub>. The high cost of this biomass type and the fact that biofuels made from algae are less stable than those made from other sources are some drawbacks, though. This is mainly because the oil that algae produce is mostly unsaturated, which makes it more volatile, particularly at high temperatures, and more likely to decompose.

Fourth-generation biofuels, which are still in the early stages of development, use genetically modified microorganisms, such as genetically modified crops or algae, to absorb more CO<sub>2</sub> from the atmosphere than they release when burned. Ethanol, butanol, hydrogen, methane, vegetable oil, biodiesel, isoprene, petrol, and jet fuel are among the fuels that can be produced with bioenergy [9].



**Figure 2. Illustrates biofuel generations [9].**

### 2.3. The Future of Biofuels

Biofuel research shows that many plants can be grown and used in the production of biofuels, which do not need a lot of water and do not require large areas, In addition to the Safaf plant and hemp, other natural herbs that grow on the American continent include the switch plant, which is currently used as animal feed. According to this research, these plants are a clean, affordable source of energy that can meet a significant portion of the world's energy needs in the future [10]. That microalgae will become, in the near future, the most important, crucial source of biofuels, as the idea of using algae to produce fuel is not new, but it is given an interest in recent time for the sustainable energy researches [11].

Moreover, microalgae have great potential as they contain a large percentage of fat due to their high photosynthesis capacity, and the production capacity of fat per dry unit ranges from 15 to 300 times compared to conventional crops [12]. Algae biofuel feedstock can also be produced year-round, and oil productivity can exceed the production of the best oilseed crops [13]. Algae productivity is estimated at more than 50 tons of biofuels per hectare per year for a competing raw material such as Jatropha (Jatropha) Table (1) [14].

**Table 1. Comparison of the amount of oil in agricultural crops**

| Crop       | Oil production (l/dunum) |
|------------|--------------------------|
| Corn       | 18                       |
| soybeans   | 45                       |
| sunflower  | 78                       |
| Castor     | 141                      |
| coconuts   | 269                      |
| palms      | 595                      |
| Microalgae | 10000                    |

## 2.4. Biofuels

Several types of biofuel including:

- 1- Lethanol: One of the most significant alcohols, ethanol is used in several crucial chemical sectors, and the United States of America is one of the most producing and using countries that produce and use ethanol  $\text{CH}_3\text{-CH}_2\text{-OH}$ . Automotive fuel followed by Brazil during the period 2013-2007. Ethanol is produced from biomass through the fermentation process of waste with the help of microorganisms and the absence of air, knowing that ethanol is characterized by being an ideal fuel as it does not leave behind by-products harmful to the environment, and it is possible to utilise ethanol. In order to generate the hydrogen required for fuel cells.
- 2- Methanol: Despite the dangers of methanol to humans if exposed to it, it is used in many industries and applications. Uses include the manufacture of pharmaceuticals and the manufacture of plastics, which is a high-purity fuel, with applications in fuel cells.
- 3- Biodiesel: It is produced by the reaction of some vegetable oils with one of the basic compounds, where what is known as biodiesel is produced, which is currently witnessing wide applications and uses in both the United States of America and Europe [15].

## 2.5. The possibility of producing biofuels from algae:

Algae are a renewable resource that can generate enough biodiesel to satisfy the world's need for transportation fuels. Through thermochemical and biochemical reactions, microalgae can be grown in industrial reactors, such as open tanks and photobioreactors, in addition to closed systems, to produce biodiesel, bioethanol, biooil, biohydrogen, and biomethane. Algae can be cultivated practically anywhere, even in salt water or effluent. Algae need little energy to develop as they do not need fertile land or agricultural fertilizers. The growth rate of algae is much faster than the growth rates of agricultural crops, as approximately 20 to 80 thousand liters of algae oil can be produced annually from algae development per acre (0.1 hectare or 1000 m<sup>2</sup>), equivalent to 7 to 31 times the palm oil produced from the same area. Algal oil is used to form biofuels that can be used as fuel for cars, trucks, and aircraft, as the fat and fatty acid content of algae varies according to algae development conditions [16].

## 2.6. Algal Cultivation Systems

In the past ten years, microalgae have received specific attention, especially in Asia and North America. The main applications during that period of algae were abbreviated to food, animal feed, and only a few algal species, such as *Chlorella* and *Spirulina* were known. These species emerged with their acquisition and support for the manufacture of diet materials, and the cultivation of microalgae is an essential technique because it gives a significant return from oils or biofuels. Algae can grow in extreme conditions such as saline or continental lands (with a difference). Higher temperatures between night and day [17]. One of the most critical obstacles facing the manufacture of biofuels from algae is the low concentration of biomass produced due to the limited light penetration of algal growth, in addition to the relative increase in the cost of harvesting biomass, and the high cost of capital and high care required by algae farms compared to conventional farms is one of the strategic obstacles facing the implementation of bioenergy production from algae [11]. There are two main systems of photo reactors approved for the

development of microalgae, including open and closed systems. They reflect the diversity in the requirements of different algae growth and the continuous maintenance of the desired farms to achieve optimal productivity.

### A. Open system

One of the open systems is the open pool device, which is considered a large-scale economic solution for microalgae cultivation [18], but these algae cultivation systems were used in the fifties of the last century, and traditional outdoor farming methods consist of lakes, natural ponds, circular ponds, basins and waterway channels, and that outdoor systems are the most widespread growth systems and are huge farms and are considered one of the most essential commercial systems used in this type of production (Figure 4 and photo 2) [19].

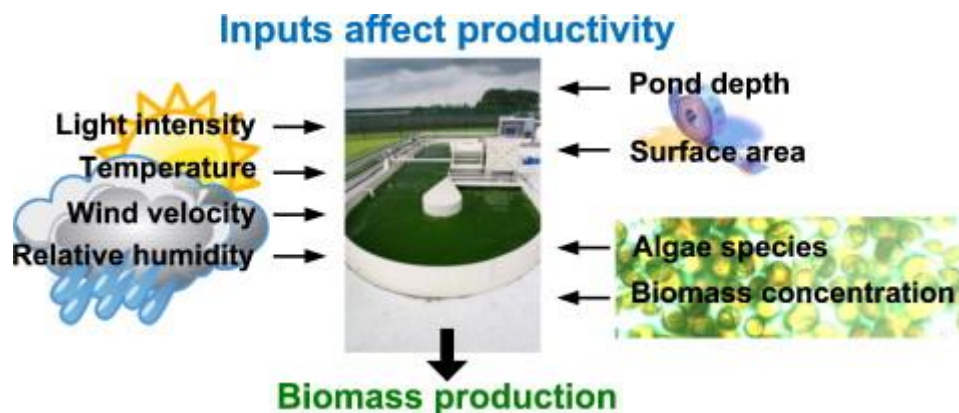


Figure 3. Open ponds system [11].

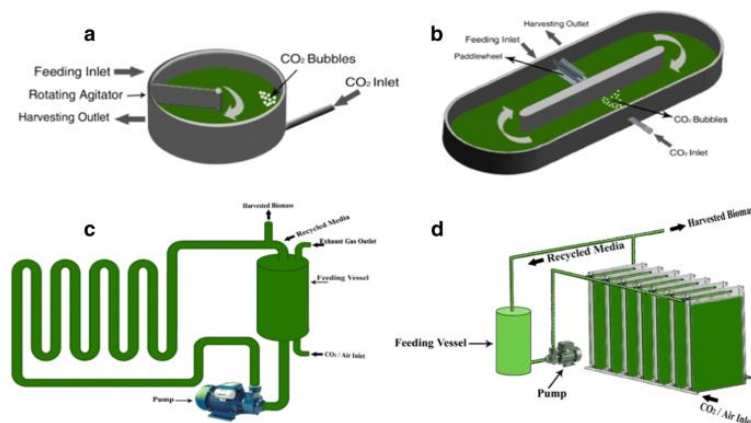


Figure 4. Open sink system sewer duct model. Source: [20]

## B. Closed system

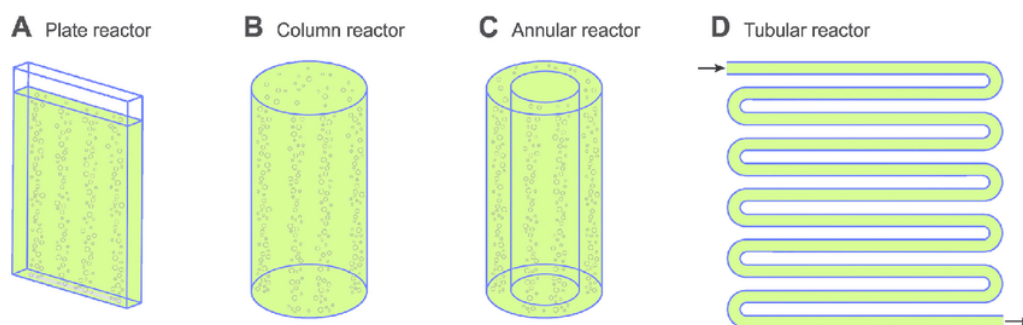
The advantages of this type of system are the ease of controlling pollution and environmental conditions, expanding the scope of production, and the concentration of biomass for this class of systems is higher than open systems, thus reducing the cost of harvesting [21]. These systems receive sunlight either directly through the transparent container walls or via optical fibers or tubes that guide them from sunlight collectors. Despite the relative success of open systems, recent developments in large microalgae farms require Closed systems. For example, microalgae and their products must be highly valuable for use in various applications such as the pharmaceutical and cosmetic industry and be free of expected contaminants such as heavy metals and other microorganisms [19]. There are several types of closed photovoltaic reactors: -

### Tubular photobioreactor (TPR)

Tubular photovoltaic reactors have a very high ratio of area to volume, depending on the diameter (tube, but the biomass transport through them is very low, which increases the accumulation of O<sub>2</sub> and the depletion of CO<sub>2</sub>, which leading to photorespiration, cell damage by oxidation ,and pH oscillation). The pipes of this system are generally made of polyethylene or glass, and the most critical parameters of their construction materials are transparency to allow good penetration of light and low cost, which are available in several vertical, horizontal, or spiral shapes. Figure 5 additional challenges include photoinhibition, temperature control, and calcifications due to cells adhering to the tube walls, resulting in low light penetration [21].

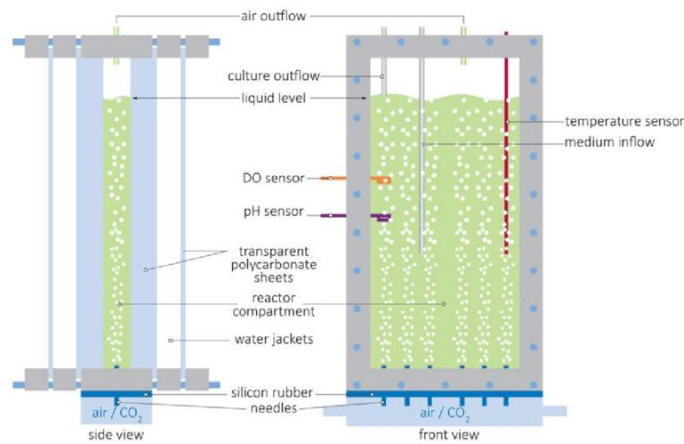
### Flat photobioreactor (FPR)

This type is one of the oldest forms of closed systems, which is the flat that has received significant attention [19]. These reactors are usually made of transparent materials that allow large areas of lighting to ensure high efficiency of photosynthesis and accumulation of low dissolved oxygen concentration [11]. These panels generally consist of narrow-thick channels with walls made of tempered glass or plastic, and the increased output must be replaced to retain the same volume from the farm and are usually with working volumes of up to 1000 liters and can be installed either vertically or horizontally, flat or undulating (Figure 6) [21].

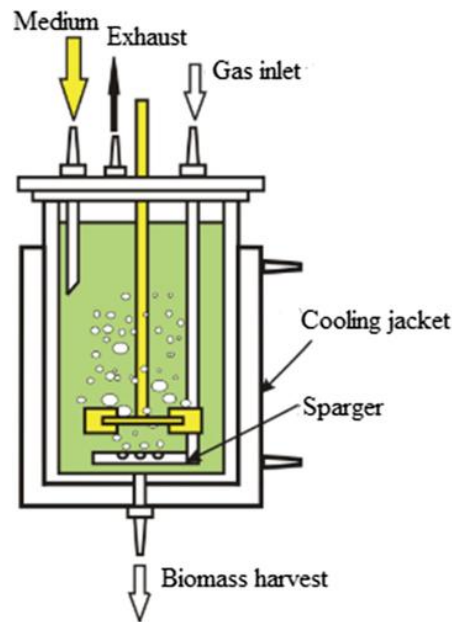


**Figure 5. Tubular biophotoreactor shapes [22]**





**Figure 6. Flat biophotoreactor shapes [23]**



**Figure 7. Mobile fermentation tank [24]**

## 2.7. Microalgae Growth Requirements

Microalgae, like other organisms, require a certain group with the conditions in which they can grow and flourish, and algae evolve significantly whenever the optimal conditions for them are similar to one of their large natural habitat, and that the crop finally achieves the growth rate and productivity and to enable control we need to provide several factors [25], namely:

### 1- Nutrients: Nutrients

Microalgae require for their growth key nutrients called macro-elements such as K, P, N, C ga, and need micronutrients such as: Co, S, F, Cu, Mn, Si, Zn and some types require in their growth to

vitamins such as vitamin B1 (Thianine) (Cobalamin) B12, and (Blotin) These nutrients and nutrients are usually supplied as inorganic or organic salts dissolved in the nutrient medium or added separately [26]. The optimal ratios of these nutrients have many benefits for algae such as improving cell integrity, fat storage capacity and cell diffusion rate, and it is also known that a certain nutrient deficiency can be the key that radically changes the cell structure that can be useful for biotechnology purposes, for example, low nitrates often lead to the accumulation of fat in algae [27]

## 2- Light Light:

One of the most significant factors influencing biomass productivity in photoautotrophic farms—those that rely solely on CO<sub>2</sub> as a carbon source—is light. Light gives algae energy to grow, and light intensity and nitrate concentration have an impact on biomass production and fat accumulation in *Tetraselmis suecica* algae. It should be mentioned that light intensity needs to be raised in order to penetrate high cell densities [6].

## 3- Carbon and pH:

The amount of CO<sub>2</sub> in seawater is less than 1% at pH 8, and over 90% of it forms carbonic acid, or HCO<sub>3</sub>, so the conversion of bicarbonate to [6] CO<sub>2</sub> can raise the medium's pH. Light, water, and carbon dioxide are all necessary for photosynthesis, and a lack of available carbon dioxide can reduce photosynthesis, which impacts biomass productivity. Algal growth and biomass production have been shown to significantly increase at pH values above 7.5 due to a variety of factors [28].

## 4- Temperature

Temperature regulation is crucial for all physiological activities carried out by the organism, including algae, as high temperatures can block photosynthesis pathways [26]. Temperature is perhaps one of the most important factors studied in lipid formation and fatty acid synthesis in algae [29], in microalgae *Chlorella vulgaris* and *B. braumii*. The increase in temperatures led to a decrease in the relative content of unsaturated fatty acids within the cells. In contrast, the composition of the secreted fatty acids in the medium did not change [30]. Changes in structure were observed. Chemical algae in reaction to differences in high and low temperatures vary from one type to another, which leads to a difference in the amount of bioaccumulation of compounds within cells. [6].

## 2.8. The composition of biofuels from algae

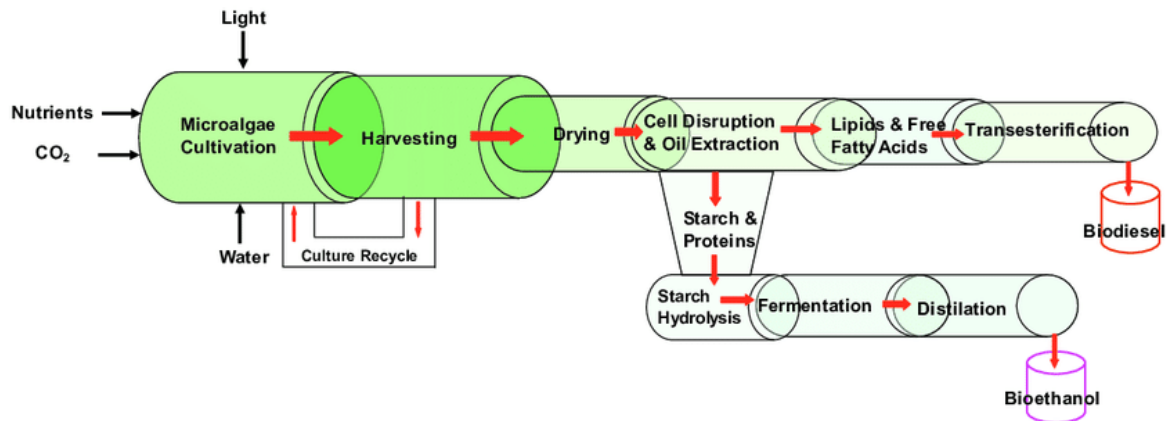
Biofuel manufacturing is a complex process. Shown in Figure 8 is a graph of the steps for producing biofuels from microalgae. The process consists of:

I Stage I: Growing Algae

II Second stage: harvesting, drying, and cell damage. Isolation of cells from the growth medium

III Stage III: Fat Extraction for Biodiesel Production through Esterification

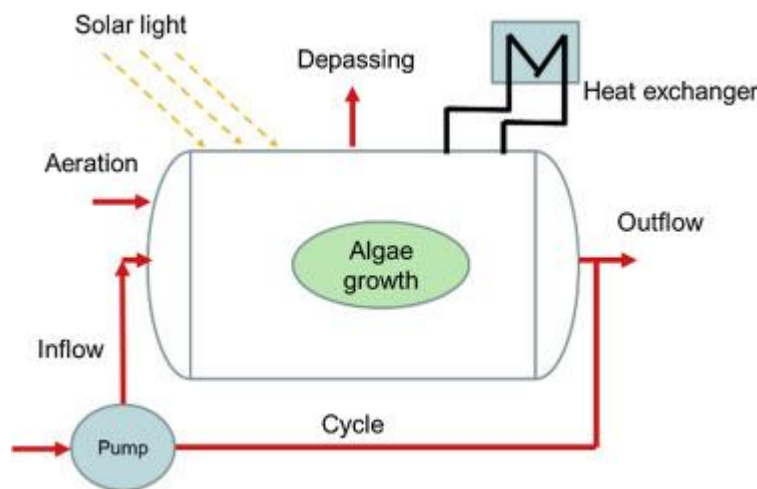
IV Stage IV: hydrolysis, fermentation, and distillation to produce bioethanol [31].



**Figure 8: Biodiesel and bioethanol production processes from microalgae [19].**

## 2.9. Study the use of algae as biofuels

Algae have been used in many studies related to the field of biofuel production. In one study on the production of biofuels from *Botryococcus braunii* algae isolated from Syrian waters to obtain biomass to convert it into biofuels, the photobioreactor was used and designed with local expertise, as shown in Figure 6.

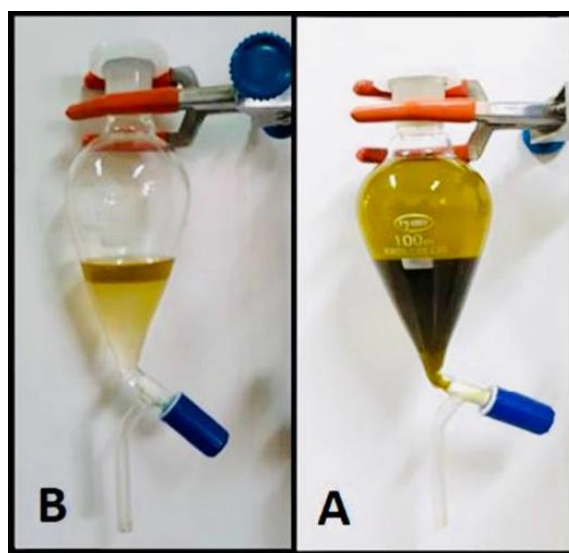


**Figure 9: Photobioreactor [14].**

Local strains of *B. braunii* algae isolated from the Rum Dam Lake in Sweida showed good biofuel productivity compared to international strains and a high lipid content of 45% of the dry weight of biomass. The performance of the photobioreactor manufactured with local expertise, which is the basis for this study, showed an excellent performance of 1.2 g/l dry biomass of algae with a biofuel production of about ml/g0.45 Dry weight, which means that the technology of producing biodiesel from algae on a large industrial scale and with local expertise is available at low economic cost, as well as examples of production conditions to obtain the most significant amount of biofuels [14].

In another research, three types of microalgae, *Dimorphus*, *Scendesmus*, *Chlorella vulgaris*, and *Chlorococcum humicola* were grown in a 5-liter photoreactor, and their productivity of dry biomass was

estimated and then chemical elements were analyzed to determine their total fat content and diagnose fatty acids. The results were that the highest biomass productivity was in *S. dimorphus*. It amounted to 1.58 g l<sup>-1</sup> for dry biomass. In contrast, the results of the fat estimation indicated that *C. vulgaris* obtained the highest total fat productivity, which amounted to 29.6%. As for the results of diagnosis using GLC technique, it was found that the highest percentage of saturated fatty acids from *S. dimorphus* oil was for mercetic acid ester (C14:0) by 47.105% and lincoseric acid ester (C24:0) by 7.194%. While the moss showed *vulgaris*, and moss *Chlo humicola* has a lower level of saturated fatty acids, indicating the superiority of algal oil produced from *S. dimorphus* in the production of fatty acids important in the formation of biofuels. Where the total fat content of microalgae species was estimated: - All fats were separated from the other chemical components of algae cells by suppressing separation using organic solvent solution Hexane/isopropanol as shown in Figure (10). The results of the determination of total lipids showed that the highest fat content (0.296 g L<sup>-1</sup>) of algae Studied was in the moss *vulgaris*. This constituted about 29.6% of the dry weight of the algae cells, while the fat content (0.242 g liter<sup>-1</sup>) of the algae was *Chlo humicola*, which constituted about 24.2% of the dry weight of the algae. *S. dimorphus* came in third place with a value of fat content (0.234 g liter<sup>-1</sup>) in its cells, which constitutes about 23.4% of the dry weight [25].



**Figure 10: Separation of total fats and free separation of fatty acids, A /separation of all fats from other chemicals, B / separation of free fats by the method of sobation [25].**

There is also the potential to combine the removal of nutrients from wastewater and the creation of biofuels through algae treatment. The fact that microalgae can be produced efficiently with little freshwater input, in contrast to many plant biofuel products, is one of their appeals as a raw material for biofuel. This makes the process viable and sustainable in terms of freshwater resource conservation. For instance, microalgae can be cultivated close to the sea to benefit from brackish or salty water. As a result, there was a lot of interest in growing microalgae for biofuels in salty environments. Using

wastewater to do so, Since the main issue with most wastewater is extremely high concentrations of nutrients, particularly the total concentration of N and P, in addition to toxic metals that require expensive chemical treatments to remove during wastewater treatment, it has been estimated for several years that microalgae can be used to deal with wastewater in a low-cost and environmentally friendly way compared to other treatment processes that are more commonly used, The reviewed microalgae growing in wastewater shows that there is real potential in using these high nutrient resources to produce biofuels at an affordable price. Microalgae are a very appealing way to treat wastewater because of their ability to grow effectively in nutrient-rich environments and efficiently assemble nutrients and minerals from wastewater.

### **2.10 Some algae produce biofuels:**

There are some algae whose suitability for the production of biofuels is being studied, and they may be available in many parts of the world are These genera that produce high oils, such as the following algae [17]: -

- Botryococcus brauni
- Chlorella sp.
- Dunaliella tetraiolecta
- Gracilaria sp.
- Pleurochrysis
- Sargassum sp.

## **3. Experimental work**

### **3.1. Materials and methods of work**

#### **3.1.1. Materials and Experimental Procedure**

MFC reactors consist of anode chambers with a capacity of 25 ml and cathode chambers with a capacity of 25 ml, separated by a cation exchange membrane. The electrode material was carbon fiber cloth with a total area of 270 cm<sup>2</sup> (20 g/m<sup>2</sup>), which was used in cathode and anode chambers. The carbon cloth sheets were folded into rectangular cubes and attached to the outer circuit using nickel-chrome wire that was 0.45 mm thick. Five distinct experimental groups (Table 1) had modified cathode electrodes, including two control states: one group had no electrode modification, and the other was an abiotic control group (algae water). Four experimental groups had the following components: a) an unmodified cathode electrode (algae); b) a cotton thread (2 mm thick) wrapped around the electrode (algae filament); c) a layer of cellulose (1 mm thick) covering the electrode (algae cellulose); and d) stainless steel wire (type 316, thickness 0.45). The changes were made to collect current and encourage the growth of algal biofilm on the cathode electrode. A total of 15 vital algal cells were obtained from three tests of each experimental scenario [2]. There was no usage of pre-chemical treatment, growing medium, or pH control.

**Table 2: Electrode modifications in the biotic and abiotic environment of the cathode.**

| <b>Cathode Type</b>                                       | <b>Catholet</b>                                     | <b>Cathode Name</b> |
|-----------------------------------------------------------|-----------------------------------------------------|---------------------|
| <b>Carbon cloth (control)</b>                             | (initially) abiotic – (later)<br>turned into biotic | Algae water         |
| <b>Carbon cloth</b>                                       | Vital                                               | Algae               |
| <b>Carbon cloth wrapped in<br/>cotton thread</b>          | Vital                                               | Algae Series        |
| <b>Carbon cloth coated with<br/>cellulose coating</b>     | Vital                                               | Cellulose algae     |
| <b>Carbon cloth wrapped with<br/>stainless steel wire</b> | Vital                                               | Algae wire          |

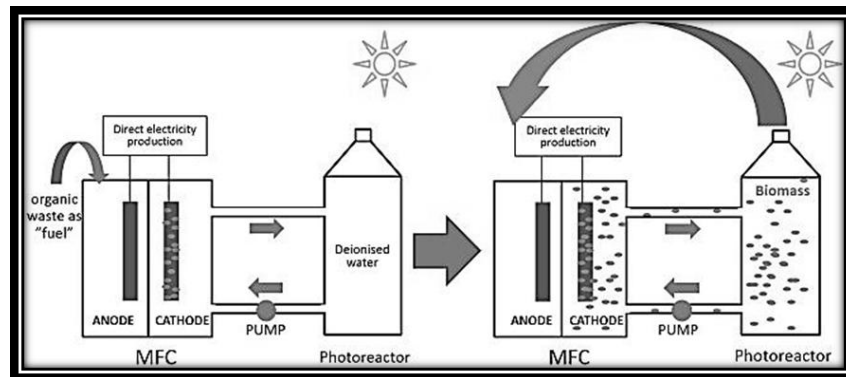
**Table 3: Shows the tools and their manufacturers.**

| <b>Instrument / Material</b>                           | <b>Manufacturer / Brand (Suggested or Stated)</b>               |
|--------------------------------------------------------|-----------------------------------------------------------------|
| <b>Axiostar plus light microscope</b>                  | Carl Zeiss, Germany                                             |
| <b>Spectrophotometer 6300</b>                          | Jenway (now part of Cole-Parmer), UK                            |
| <b>Analytical balance (HR120)</b>                      | Mettler Toledo, Switzerland                                     |
| <b>Vacuum filtration system with 47 mm<br/>filters</b> | MilliporeSigma (Merck), USA / Germany                           |
| <b>Sterile membrane filters (0.2 µm, 47<br/>mm)</b>    | Sartorius or MilliporeSigma                                     |
| <b>Peristaltic pump (16-channel)</b>                   | Watson-Marlow, UK or Heidolph, Germany                          |
| <b>Photoreactor bottles (0.5 L Schott<br/>bottles)</b> | Duran (formerly Schott Duran), Germany                          |
| <b>Scanning Electron Microscope (SEM)</b>              | Philips XL30 SEM, Netherlands (Philips Electron Optics)         |
| <b>Gold sputter coater (Emscope SC500)</b>             | Emscope Laboratories, UK (acquired by Thermo Fisher Scientific) |
| <b>ADC-24-16 Data Logger</b>                           | Pico Technology, UK                                             |
| <b>Software: Microsoft Excel</b>                       | Microsoft Corporation, USA                                      |
| <b>Software: GraphPad Prism</b>                        | GraphPad Software (now part of Dotmatics), USA                  |
| <b>Carbon cloth electrodes</b>                         | Fuel Cell Earth or AvCarb Material Solutions                    |
| <b>Nickel-chrome wire (0.45 mm)</b>                    | Omega Engineering or McMaster-Carr (commonly supplied)          |
| <b>Stainless steel wire (Type 316, 0.45<br/>mm)</b>    | Loos & Co. or Precision Brand                                   |

### 3.1.2. Fetal stem cell vaccination and operation

Two months before the start of the trials, fresh pond water was cultivated in a well-lit laboratory setting for the cathodic vaccine sample to facilitate the growth and development of algae. In order to guarantee voltage colonisation and biofilm formation, the sample was utilised as a cathode half-cell grafting sample and run in batch mode once it turned noticeably green [17].

In control, tiny fuel cells, sterile deionised water served as a catalyst. Before being employed for periodic feeding, the anodes were inoculated with anaerobic clay from the laboratory and combined with 0.1 mol sodium acetate (pH 7.2). Each cathode chamber was attached to a 0.5L Schott photoreactor bottle after 40 days, and the algae were then floated in fresh pond water. As seen in Figure 11 the photoreactor bottles were linked to MFCs by closed-loop recirculation using a 16-channel peristaltic pump with a flow rate of 123 ml/h. A temperature and light-controlled incubator with two tubes of cold white daylight (3500 lux) and a programmable timer was used to keep microscopic fuel cells and photoreactor bottles at 22°C for 14 hours of light and 10 hours of darkness [19].



**Figure 11: Microbial fuel cell (MFC) with photoreactor for biomass harvesting. The fuel source was organic waste (sludge + 20 mmol of sodium acetate) or biomass harvested from the cathodic photoreactor.**

For eight months, microscopic fuel cells were subjected to external resistance loads of 8.2 k $\Omega$ , and half-cathode cells and photoreactors demonstrated distinctly formed green communities. A dominant population of green algae and other species, including cyanobacteria, heterotrophic bacteria, and primary parasites, were observed under a microscope. All minuscule fuel cells had a growing photodynamic film in half of the cell on the cathode side, and control cathodes (abiotic) unintentionally became essential during long-term operation. As seen in Figure 11, this occurred when all photoreactors were filled with deionised water and the development of photovoltaic organisms was monitored and assessed.

### 3.1.3. Data Capture

Polarization experiments were carried out using the resistance tool in the range of 30,000 to 10 ohms, and the time constant for each resistance value was 3 minutes. Data recorded using ADC-24-16 data logger. Data processed using Microsoft Excel, and GraphPad Prism software packages [22] [24].

### 3.1.4. Biomass assessment

Four weeks after the experiment began, microalgae samples were collected using a bright linear hemattometer and a carrier light microscope (Axiostar plus, Carl Zeiss) for direct microscopic counting. The 6300 light spectrometer was used to measure the optical density at 678 nm, which is the highest absorption of chlorophyll A. Using an analytical scale (HR120, Metler Toledo) and a vacuum filtration equipment, the dry weight of microalgae (mg/L) was measured using 47 mm sterile membrane filters with 0.2  $\mu\text{m}$  pores. Calibration was carried out using dilutions in the range of dilution factor (DF) from 1 to 0.1 after the filter papers had been dried for 24 hours at room temperature and 1 hour under a 100 W lamp to achieve a constant weight [25].

#### 3.1.5. Cathode surface analysis

Scanning electron microscopy (SEM) was used to view the morphological image of the cathode electrode surface with the attached photobiofilms. Contact adhesive was used to adhere the dry samples to the aluminium. The Philips XL30 SEM scanner magnifying glass was used to take and monitor the images. Gold spray plating was done on the samples using an Emscope SC500 to further prepare them for microscopic inspection [19].

### 4. Results and discussion

#### 4.1. MFC Power Performance

The algae-based cathode outperformed the abioalgae fuel cell (control group) with a maximum power output of 128  $\mu\text{W}$ , followed by algae water with 81  $\mu\text{W}$ , filament with 74  $\mu\text{W}$ , wire with 67  $\mu\text{W}$ , and cellulose with 61  $\mu\text{W}$ . The highest absolute power output is displayed in Fig. 12. The growth of biomass in solar reactors connected to algae-generating fuel cells emerges based on the charge transfer in algae-generating fuel cells and can be directly linked to the energy generated. Algae have been demonstrated to enhance system energy performance and longevity. Thus, it is hypothesised that the more power generated by algae-generating fuel cells, the more biomaterial their cathodic photoreactor can produce. The biomaterial that was obtained revealed that the algal cathode's cell density reached  $31 \times 10^7$  litres [25].

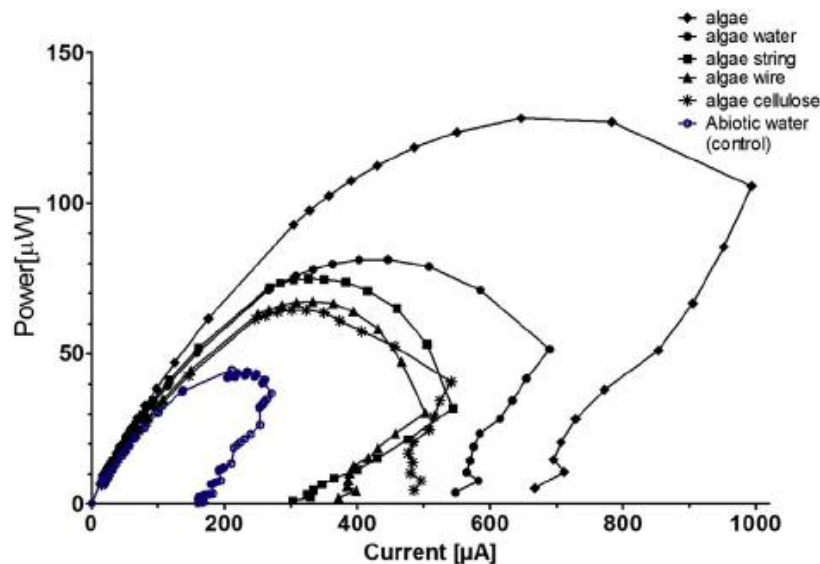
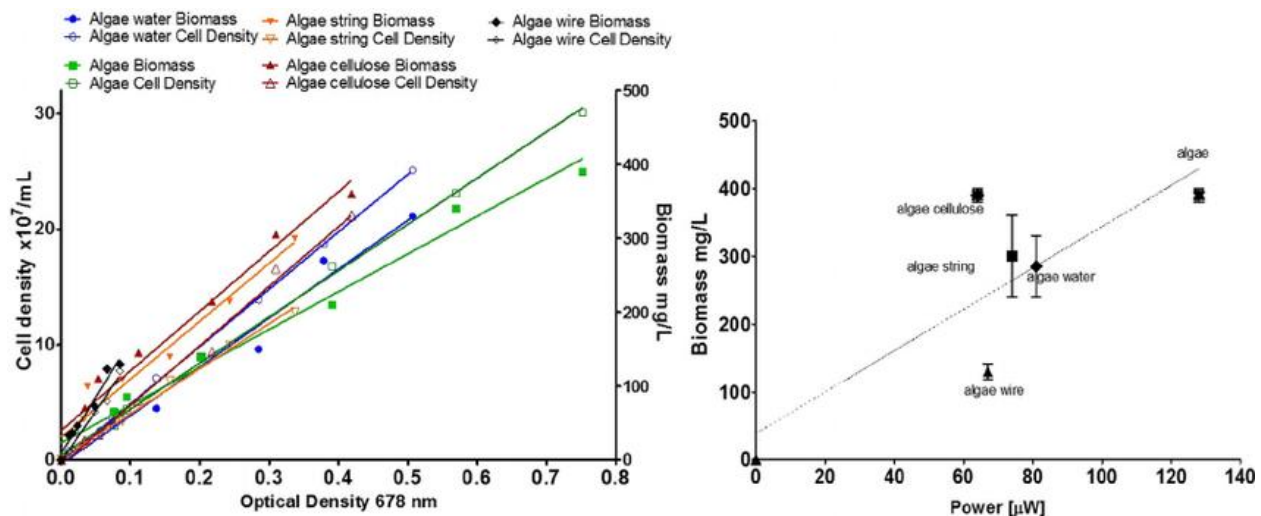


Figure 12: Power performance derived from MFC polarization experiments.

#### 4.2. Algae growth inside the reactor



The relationship between optical density, cell density, and dry weight was determined by linear regression, and Figure 13 (left) is shown. When sampling from photoreactor bottles, it was observed that algae, algae wires, and algae water showed uniform cell density. In contrast, a number of agglomeration was observed in filament and cellulose units. A Conglomerate in algae fuel cells with filaments and cellulose beside optical density measurement is represented as a reliable tool for biomass assessment. Therefore, dry weight was selected for correlation with maximum energy performance (Figure 13) [28]. The biomass growth of microalgae at the cathode was evaluated for its correlation with the output force. It has been shown that the most positive composition of the algae cathode is the uncoated carbon cloth matrix to allow better diffusion of dissolved oxygen to the electrode.

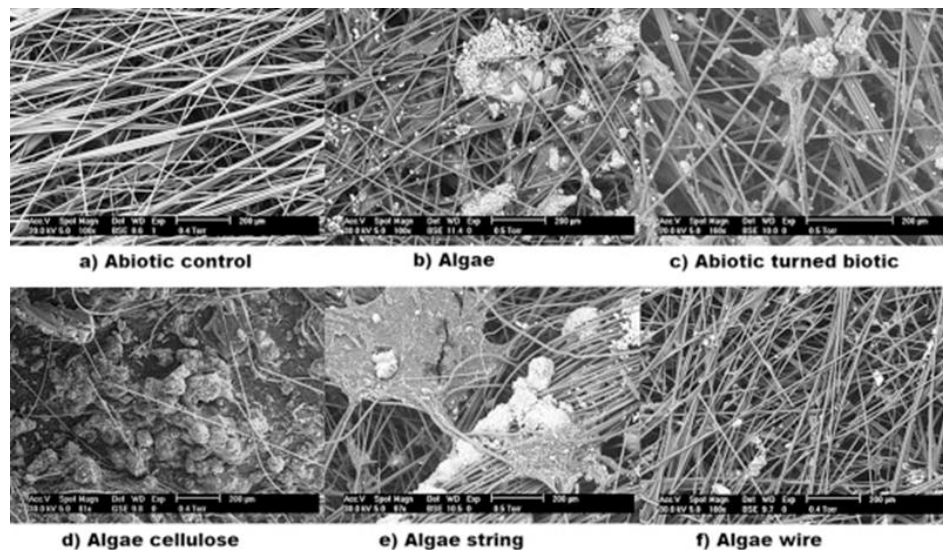


**Figure 13: Calibration curves of optical density at A678, cell density, and biomass (left).**

**Relationship in biomass harvested from photovoltaic reactors and fuel cell energy nanoscale performance (right)**

#### 4.3. Bio-photofilms

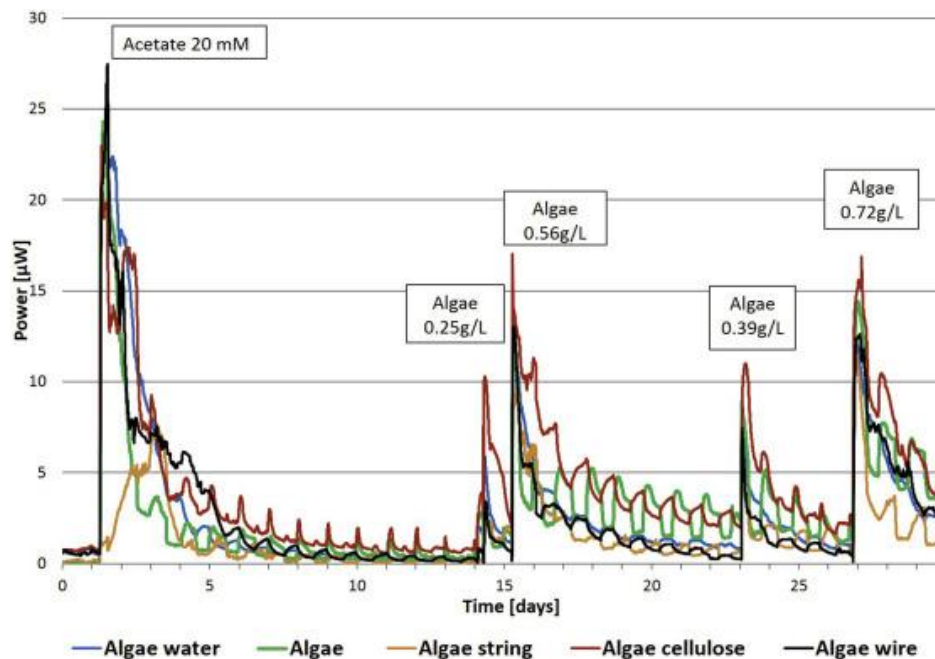
Images from scanning electron microscopy. Comparing all tested cathode electrodes to the abiotic control, Figure 14 demonstrates the formation of an algal biolayer. On the surface of the electrode, a layer of microorganisms contained in an extracellular polymeric substance (EPS) matrix was visible through the filament and cellulose coating. These two scenarios may result in a thicker biolayer with reduced oxygen transport and MFC performance. This implies that biomass production and energy generation are preferred up until a specific photobiofilm thickness reaches the cathode [27].



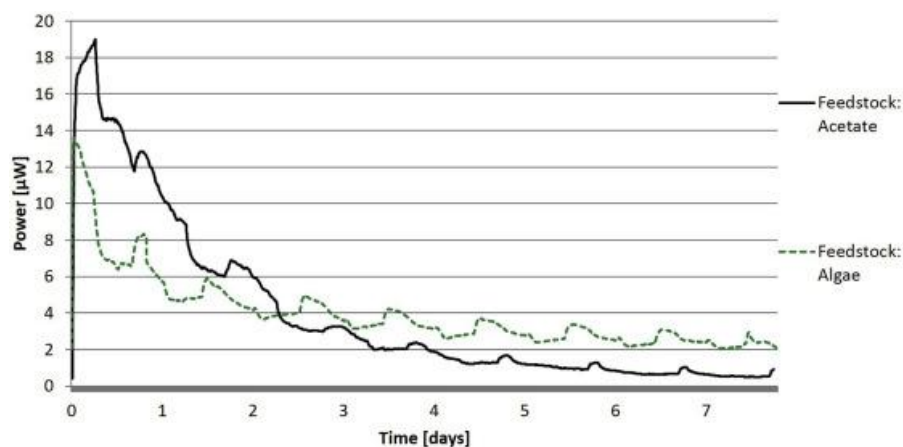
**Figure 14: Scanning electron microscopy images of cathode electrodes with advanced biofilm.**

#### **4.4. Algae used as raw materials**

Depending on the kind and amount of biomass, the catholite extracted from solar reactors was harvested and sorted into four ore solutions: unassembled ore, which had a biomass content of a) 0.25 g/l, b) 0.39 g/l, and mixed ores: Dry mass (c) 0.56 g/l and (d) 0.72 g/l. Without any prior processing, biomass was utilised as a direct raw resource. In comparison to the algal raw materials used, the diagram in Figure 15 displays the average capacity of all five types of algae-generating fuel cells when fed with sludge acetate +0.1 molar. It demonstrates that the algae-generating fuel cell cathode's algal raw materials may be effectively employed as an anodic substrate. The more biomass fed to half of the anode cell, the better the performance. The slow-release nature of these basic materials (Figure 16) in comparison to acetate implies that the mineral makeup of algal biomass makes it appear to be a more complicated substrate [28].



**Figure 15: Algae biomass used as raw material in comparison with acetate used at the beginning of the test.**



**Figure 16: Overview of raw material comparison.**

In this experiment, powdered algae, pre-treated microalgae, and high algae developed by photobiofilms in response to light, carbon dioxide, and inorganic nutrients—nutrient availability influencing the sort of biofilms formed—were utilised as raw materials for MFCs. The argument between food and fuel is resolved by the third generation of biofuels made from algae cells cultivated on non-arable land.

The current effort seeks to contribute to the development of algal biofuels through self-sustainable microbial fuel cell systems, as wastewater is the best alternative to lessen the environmental load resulting from the growing of algae biomass. One of the most popular methods for lowering CO<sub>2</sub> in the

development of carbon sequestration and renewable energy sources is the use of photosynthetic organisms, such as cyanobacteria and algae, which transform CO<sub>2</sub> into biomass. By incorporating such photoassistive cathodes, active oxygen is supplied to microbial fuel cells (MFCs) for a simultaneous carbon capture and oxygen reduction cycle [31].

## 5. Conclusions and Recommendations

Concluding from the use of microalgae in the synthesis of biofuels, this technology is arguably one of the sustainable and innovative options to meet our future fuel needs. Algae are a natural source of oils and sugars that can be converted into biofuels effectively and environmentally friendly. Moreover, algae are characterized by their ability to grow very quickly and in diverse environmental conditions, making them a sustainable and reliable option for fuel production. The use of algae also contributes to reducing greenhouse gas emissions and reducing dependency on fossil fuels. In addition, technologies to create fuel from algae can be developed continuously to increase the efficiency of procedures and reduce costs, making the industry more important and competitive in the long run. The use of algae in the production of biofuels is an important step towards achieving environmental sustainability and energy self-sufficiency.

To maximize algal biofuel production, adapt the following recommendations. Research and development should be prioritized to enhance algal fuel production efficiency and lower production costs. Manufacturing procedures should improve algae oil and fuel extraction and reduce waste. To prevent environmental damage, monitor the influence of algae cultivation and use on the surrounding ecosystem. Implement laws to boost algae use in biofuel production and sector development. To accelerate algal biofuel development and expansion, business, government, and research institutions should cooperate.

## Declaration of generative AI and AI-assisted technologies in the writing process

-None

## Disclosures

The authors have no conflicts of interest to declare in relation to this manuscript.

## REFERENCES

1. A. M. Adhabi, Scientific Algae. Basra, Iraq: Univ. of Basra, College of Education for Pure Sciences, Directorate of Publishing and Publishing House, 2014
2. R. Salman, M. Khalil, and A. Ibrahim, "Self-renewing water technology using algae," *National Center for Excellence*, no. 62, pp. 1–40, 2015.

3. Y. J. Al-Shahari, O. Al-Katib, and Z. W. Al-Jader, *Algae and Scientific Archaeon*. Mosul, Iraq: Univ. of Mosul, College of Education for Pure Sciences, 2009.
4. A. N. Al-Tamimi, *Lectures in Algology*, pp. 115–118, 2019.
5. B. A. Hassan and T. M. Ibrahim, *Algae and Archaeonids*, 2020.
6. A. Almutatier, “Production of biofuels from the green alga *Tetraselmis*,” Ph.D. dissertation, Univ. of Sheffield, Sheffield, U.K., 2015.
7. D. Ahuja and M. Tatsutani, “Sustainable energy for developing countries,” *SAPI EN. S. Surveys and Perspectives Integrating Environment and Society*, vol. 2, no. 1, 2009. [Online]. Available: <http://journals.openedition.org/sapiens/823>.
8. T. M. Al-Akeely, “The possibility of stimulating some locally isolated algae to produce biofuel,” Ph.D. thesis, College of Education for Pure Sciences Ibn Al-Haitham, Univ. of Baghdad, Iraq, 2016, p. 256.
9. H. A. Al-Alwan, A. H. Al-Munshed, and H. Al-Jaafari, “Promising development of biofuel generations: A review of the topic,” *Renewable Energy Focus*, vol. 28, pp. 127–139, 2019.
10. A. A. Al-Masry, Q. M. Ghassan, and H. B. Sunbli, *A Study on the Production and Manufacture of Biofuels*. Syrian Arab Republic: Al-Baath Univ., General Diploma, 2015, p. 59.
11. B. Al-Mashat, “Biofuel production and sustainable development,” *Saudi Journal of Biological Sciences*, vol. 18, no. 5, p. 15, 2011.
12. H. A. Alwan, A. H. Alminshid, and H. A. Aljaafari, “Promising evolution of biofuel generations: Subject review,” *Renewable Energy Focus*, vol. 28, pp. 127–139, 2019.
13. K. Dutta, A. Daverey, and J. G. Lin, “Evolution retrospective for alternative fuels: First to fourth generation,” *Renewable Energy*, vol. 69, pp. 114–122, 2014.
14. M. Siraaous and A. A. Nizam, “Use of a local strain of *Botryococcus braunii* in biofuel production,” *Al-Baath University Journal*, vol. 37, no. 6, pp. 103–125, 2015.
15. I. A. Al-Ajouza, “The efficiency of using petroleum energy resources in the light of the theory of exhaustible resources,” *Scientific Journal of Business and Environmental Studies*, vol. 6, no. 2, pp. 277–308, 2016.
16. H. B. Mashat, “Biofuel production and sustainable development,” *Saudi Journal of Biological Sciences*, vol. 18, no. 5, pp. 51–65, 2011.
17. H. Abu Al-Naja, *Biofuel Production, Features, Risks, Uses, Environmental and Developmental Impacts*. Cairo, Egypt: Academic Library for Publishing, Egyptian Joint Stock Company, p. 82. 2011,

18. B. Abdullah, S. A. F. A. S. Muhammad, Z. Shokravi, S. Ismail, K. A. Kassim, A. N. Mahmood, and M. M. A. Aziz, "Fourth generation biofuel: A review on risks and mitigation strategies," *Renewable and Sustainable Energy Reviews*, vol. 107, pp. 37–50, 2019, <https://doi.org/10.1016/j.rser.2019.02.018>.
19. G. Dragone, B. D. Fernandes, A. A. Vicente, and J. A. Teixeira, "Third generation biofuels from microalgae," 2010.
20. W.K. Lee, Y.K. Ryu, W.Y. Choi, T. Kim, A. Park, Y.J. Lee, Y. Jeong, C.G. Lee, and D.H. Kang, "Year-round cultivation of *Tetraselmis* sp. for essential lipid production in a semi-open raceway system," *Marine Drugs*, vol. 19, no. 6, p. 314, 2021, doi: 10.3390/md19060314..
21. T. A. Al-Sumaidaie, "Investigation of biofuel from some microalgae using protoplast isolation and integration technology," Ph.D. thesis, Univ. of Mosul, Mosul, Iraq, 2020, p. 176.
22. Y. del S. Matabanchoy-Mesias, Y. A. Rodríguez-Caicedo, and M. A. Imués-Figueroa, "Population growth of *Chlorella* sp. in three types of tubular photobioreactors under laboratory conditions," *Aquaculture, Aquarium, Conservation & Legislation*, vol. 13, no. 4, pp. 2094–2106, 2020. [Online]. Available: <http://www.bioflux.com.ro/aac1>
23. F. M. Yusoff, N. Nagao, Y. Imaizumi, and T. Toda, "Bioreactor for microalgal cultivation systems: Strategy and development," in *Prospects of Renewable Bioprocessing in Future Energy Systems*, pp. 117–159, 2019, doi: 10.1007/978-3-030-14463-0\_4.
24. M. Płaczek, A. Patyna, and S. Witczak, "Technical evaluation of photobioreactors for microalgae cultivation," *E3S Web of Conferences*, vol. 19, p. 02032, 2017, doi: 10.1051/e3sconf/20171902032.
25. T. A. K. Al-Sumaidaie, Y. J. Al-Shaher, and Q. S. Al-Naam, "Investigation of important fatty acids for biofuel production from a number of microalgae," *Journal of Education & Science*, vol. 30, no. 2, 2021.
26. K. Bangert, "Photo-bioreactor modelling and development of methods for the optimisation of micro-algal biodiesel production," Ph.D. dissertation, Univ. of Sheffield, Sheffield, U.K., 2014.
27. M. I. Khan, J. H. Shin, and J. D. Kim, "The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products," *Microbial Cell Factories*, vol. 17, pp. 1–21, 2018.
28. O. M. S. Al-Obaidi, "Testing the physical and chemical properties and their relationship to the qualitative variation of algae distribution in Duhok Dam Lake - Duhok," M.S. thesis, College of Science, Tikrit Univ., Iraq, p. 118 2018.

29. L. Harwood and I. A. Guschina, "The versatility of algae and their lipid metabolism," *Biochimie*, vol. 91, no. 6, pp. 679–684, 2009.
30. N. N. Sushchik, G. S. Kalacheva, N. O. Zhila, M. I. Gladyshev, and T. G. Volova, "A temperature dependence of the intra- and extracellular fatty-acid composition of green algae and cyanobacterium," *Russian Journal of Plant Physiology*, vol. 50, pp. 374–380, 2003.
31. R. Harun, M. K. Danquah, and G. M. Forde, "Microalgal biomass as a fermentation feedstock for bioethanol production," *Journal of Chemical Technology & Biotechnology*, vol. 85, no. 2, pp. 199–203, 2010.

#### خلية وقود ميكروبية بمساعدة الطحالب لتوليد الطاقة الكهربائية ومعالجة مياه الصرف الصحي

**الخلاصة:** يبحث هذا البحث في إمكانية استخدام الطحالب الدقيقة كمورد مستدام لإنتاج الطاقة الحيوية باستخدام تقنية خلايا الوقود الميكروبية (MFC). يستعرض التصنيف العام والخصائص الأساسية للطحالب، ويبرز أجيال الوقود الحيوي المختلفة، كما يسلط الضوء على فوائد ومحدوديات الوقود الحيوي المستخلص من الطحالب. يركز البحث بشكل خاص على دمج الأنظمة الكهروكيميائية الحيوية التي تعالج مياه الصرف الصحي وتولد الكهرباء في الوقت نفسه. وتُظهر الدراسة أن استخدام الطحالب في خلايا الوقود الميكروبية لا يدعم فقط إنتاج الكتلة الحيوية العضوية، بل يُعزز أيضًا من استعادة النيتروجين وكفاءة استخدام الطاقة. ومن خلال تجارب عملية تضمنت تعديلات متعددة على الأقطاب الكهربائية، تؤكد النتائج أن الكتلة الحيوية الطحلبية يمكن أن تعمل بفعالية كمادة أولية للوقود الحيوي وكحل مستدام للطاقة. وتُقدّم هذه النتائج نظامًا مغلقًا مكتفياً ذاتيًا لإنتاج الطاقة بحد أدنى من التأثير البيئي، مما يوفر بديلًا قابلاً للتطبيق عن الوقود الأحفوري.

**الكلمات المفتاحية:** الطحالب؛ خلايا الوقود الميكروبية؛ الوقود الحيوي؛ الطاقة المتجددة؛ استعادة الكتلة الحيوية.