CHAPTER II
Literature Review

Chapter II discusses the literature review of microalgae, starting with an introduction to microalgae, including cultivation methods, their growth phases, and their role in wastewater treatment. Wastewater is also discussed as a source of microalgae growth, with a particular focus on *Dunaliella salina*. Furthermore, the next chapter discusses microalgae harvesting techniques using electrocoagulation (EC) and HHO gas production, including the basic principles of microalgae harvesting with this method, the stability of dissolved particles, as well as factors affecting coagulation-flocculation. At the end of the section, the nature and production of HHO gas, as well as the knowledge, awareness, acceptance, and willingness to pay for fuel derived from microalgae will be reviewed.

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2.1. Microalgae

Microalgae are unicellular photosynthetic microorganisms of small size (1-50 μm) with a high photosynthetic rate, so microalgae can convert sunlight into biomass (Martínez, 2016). Based on light utilization, microalgae can be either prokaryotes or eukaryotes. Eukaryotic microalgae contain membrane-bound organelles such as chloroplasts, mitochondria, and nuclei that contain genetic material. In contrast, prokaryotes do not contain chloroplasts, mitochondria, and nuclei but contain chlorophyll "a" and a high protein content. However, most species of microalgae belong to the eukaryotic group. Based on their carbon source, microalgae are classified as autotrophic microorganisms or heterotrophs. Autotrophs use inorganic carbon, such as atmospheric CO_2, and perform photosynthesis using light as an energy source. On the other hand, heterotrophic microalgae consume organic carbon to grow. Microalgae species that can use organic and inorganic carbon sources are called myxotrophs (Rashid, Rehman and Han, 2013). The most common species of microalgae are eukaryotic microalgae. Green microalgae species have a typical biochemical composition of $C_{106}H_{181}O_{45}N_{16}P$ (Martínez, 2016)

Microalgae have adapted to various conditions, including salty *(saline)*, freshwater, and terrestrial environments, hot and cold weather conditions, various mineral compositions, and low and high-intensity light conditions. Scientists have categorized microalgae in a classification system mainly distinguished by their pigmentation, life cycle, storage products, and cellular structure. The most abundant microalgae species have been classified into four main groups: (1) Diatoms *(Bacillariophyceae)*, (2) Green algae *(Chlorophyceae)*, (3) Blue-green algae *(Cyanophyceae)*, and (4) Golden algae *(Chrysophyceae)*.

2.2. Microalgae cultivation

Microalgae are the main raw material for biodiesel production with various benefits such as 1) higher growth rate and biodiesel production than other higher crops, 2) Can be cultivated without terrestrial land, 3) less water consumption, 4) Can be cultivated in fresh and brackish water, 5) can be used in biodegradation and detoxification (Safonova *et al.*, 2004). These unicellular organisms are widely cultivated in sunlight in the presence of carbon dioxide and water. Each type of microalgae requires different nutrients and energy sources so that different lipid content and productivity of microalgae biomass. The most common microalgae cultivation methods include photoautotrophic, heterotrophic, photoheterotrophic, and mixotrophic (Ananthi *et al.*, 2021).

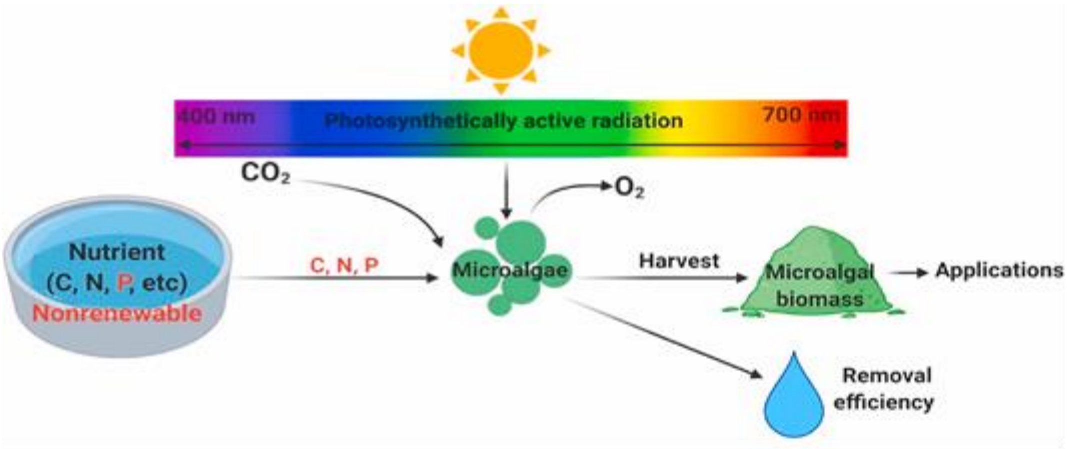

Figure 2. 1 Scheme of microalgae photosynthesis process (Su, 2021)

Microalgae use photosynthesis during cultivation, converting solar energy into chemical energy (Saadaoui *et al.*, 2021) (Figure 2. 1). During the growth phase, energy absorbed from the sun combined with water, CO_2 gas, nitrogen, and phosphorus produces biomass (Christenson and Sims, 2011). Although the photosynthetic mechanism of microalgae is identical to other plants, microalgae are more efficient in utilizing solar energy for cultivation (Cabanelas *et al.*, 2013). In addition, microalgae cultivation also serves to store abundant CO_2 from the atmosphere and reduce global warming because algae consume about 1.8 tons of CO_2 to produce 1 ton of biomass (Saadaoui *et al.*, 2021). Yen & Brune (Yen &; Brune, 2007) state that CO_2 absorption through microalgae is one or two times more effective when compared to land plants. Each type of microalgae requires different nutrients and energy sources so that different lipid content and productivity of microalgae biomass. Some environmental conditions, such as light, temperature, nutrient and oxygen concentration, etc., can affect microalgae growth, biomass production, algae species dominance, and microalgae concentration (Martínez, 2016). These environmental conditions include:

a. Light

Generally, light intensity will decrease exponentially as it passes through water because the concentration of biomass (microalgae cells) changes light intensity. The light entering the waters depends on the concentration of biomass. This impacts the rate and efficiency of photosynthesis and biomass production (Grobbelaar, 2009). In open aquaculture systems, light intensity is increased by modifying the light (e.g., reducing the depth of the pond) and reducing the hydraulic retention time (HRT) to reduce the concentration of biomass and allow light to penetrate the pond (Kroon et al., 1989). Nevertheless, recent studies reported an increase of up to 200% in microalgae biomass production by increasing the pond depth from 0.2 m to 0.4 m (Sutherland et al., 2014). At light intensities above saturation (about 200–400 µmol/m2s), the growth rate of microalgae will stabilize at its maximum level (Boelee et al., 2014). Excess light can cause photoinhibition, which is a decrease in photochemical efficiency experienced by microalgae in response to excessive lighting (Giacometti and Morosinotto, 2013)

b. Oxygen

The optimal dissolved oxygen concentration in microalgae culture is between 5-30 mg O_2/L (Mendoza *et al.*, 2013). High oxygen concentration (>35 mg O_2 / L) coupled with high exposure to sunlight for a long time can produce photooxidation of microalgae cells and reduce the efficiency of pollutant removal (Chisti, 2008). Photo oxidation is that high light intensity can oxidize chlorophyll, so chlorophyll loses its ability to photosynthesize (Jiao *et al.*, 2002). The oxygen concentration is four times that of saturated oxygen, causing toxins for microalgae species, thereby inhibiting the growth of microalgae (Lee and Lee, 2001). In this case, an open system works better than a closed reactor because oxygen does not accumulate in the open pool. As a result, closed systems require an air cycle.

c. Nutrients

In addition to light sources, carbon, nitrogen, and phosphorus availability is essential for microalgae growth. The Redfield determines the molar ratio of carbon, nitrogen, and phosphorus. The ratio 106:16:1 (C: N:P) is the concentration of nutrients needed for optimal microalgae growth (Redfield, 1958). Generally, the C: N ratio of wastewater is between 2.5-4:1, which means a deficit of carbon sources for microalgae growth. Efforts to meet nutrients for microalgae can be done by adding CO_2 so that the C: N ratio of wastewater increases to 6: 1 (Park, Craggs and Shilton, 2013). Adding CO_2 to the system can lower the pH of the mixture and shift the ammonia equilibrium towards ammonium, where ammonium can be absorbed by microalgae (Martínez, 2016). The maximum amount of CO_2 microalgae can absorb from the open air is 33%. In addition, a new process has been initiated to capture CO_2 gas in flue gas and transfer it to microalgae culture (López *et al.*, 2009).

2.1.1. Microalgae growth phase

Microalgae growth can be determined by two main growth phases: (1) exponential growth phase (with high growth rate) and (2) stationary phase (with low growth rate) (Figure 2. 2). During the exponential growth phase, the growth rate of microalgae cells reaches maximum values (about 0.11/day). In this phase, intracellular metabolic rate, unicellular mobility, and cell growth kinetics become optimal. These optimal microalgae biomass growth and cell mobility increase repulsion between microalgae cells. This has been stated by (Danquah et al., 2009b); in the exponential phase of microalgae biomass, there is an increase in electronegative properties. In the stationary phase, the growth rate of microalgae is low (about 0.03/day), and cell mobility is reduced, resulting in low electronegativity and high cell interaction, causing agglomeration (cell union). Therefore, higher cell interaction and low microalgae growth are the best conditions for harvesting biomass (de Godos *et al.*, 2011).

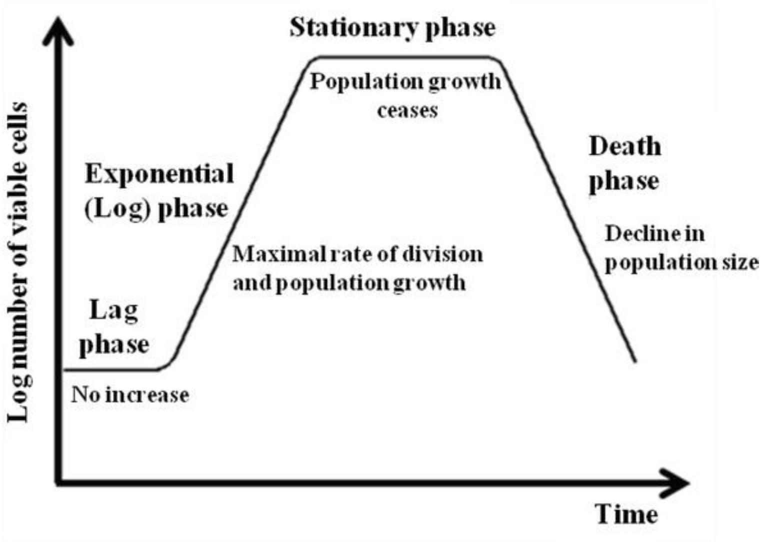

Figure 2. 2 Microalgae culture growth curve model representing the four phases of microalgae growth (Cruz *et al.*, 2018)

2.1.2. Microalgae for wastewater treatment

The use of microalgae in wastewater treatment has great potential because it can remove nitrogen, phosphate and toxic metal pollutants efficiently. Over the past few decades, studies have reported the success of urban, agricultural, and industrial wastewater treatment using microalgae (Aguirre *et al.*, 2011; Sforza *et al.*, 2014; Alcantara *et al.*, 2015). Microalgae-based wastewater treatment (WWT) systems can produce biomass products (Kouhia et al., 2015), and the scheme of wastewater treatment mechanisms using microalgae is shown in Figure 2. 3. Several stages are involved in the biomass production process. Initially, wastewater was treated in the preliminary treatment unit using a sand filtration system. Furthermore, wastewater is treated in the primary treatment unit and secondary treatment. Microalgae biomass is cultivated in the secondary treatment unit. Microalgae biomass is harvested and separated from waste. In some cases, the harvesting stage requires two consecutive steps if the final product requires a high concentration of solids.

Microalgae can lower the concentration of BOD and COD in wastewater in two ways. First, wastewater contains organic and inorganic materials that microalgae can use simultaneously using light energy (Mujtaba *et al.*, 2018). Second, microalgae growth produces oxygen during photosynthesis, thereby increasing the supply of oxygen in the water. Microorganisms use this oxygen smear to lower the concentration of BOD and COD (Lam *et al.*, 2022). *Chlorella* reduced the concentration of BOD and COD in domestic waste by 81.78 and 83.67% (Jaiswal *et al.*, 2022).

Li's research (L.-H. Li *et al.*, 2020) shows that the reduction rate in total ammonia and phosphorus nitrogen concentrations in black and odorous wastewater using *Spirulina* can reach 100%. Su & Jacobsen, (2021) showed that the rate of decrease in N and P in food industry wastewater using *Chlorella vulgaris* and *Scenedesmus obliquus* >54%. Mennaa et al., (2015) studied seven types of microalgae to treat urban wastewater. They found a decrease in total dissolved phosphorus and nitrogen was >80% and 87%, respectively.

The efficiency of wastewater treatment by microalgae with different nitrogen sources is also different. Microalgae in wastewater containing ammonia nitrogen have a slow rate of development and low pollutant removal effectiveness; this may be due to a decrease in pH value caused by the release of H^+ in the metabolic process of ammonia nitrogen microalgae, which inhibits microalgae activity (Song *et al.*, 2022).

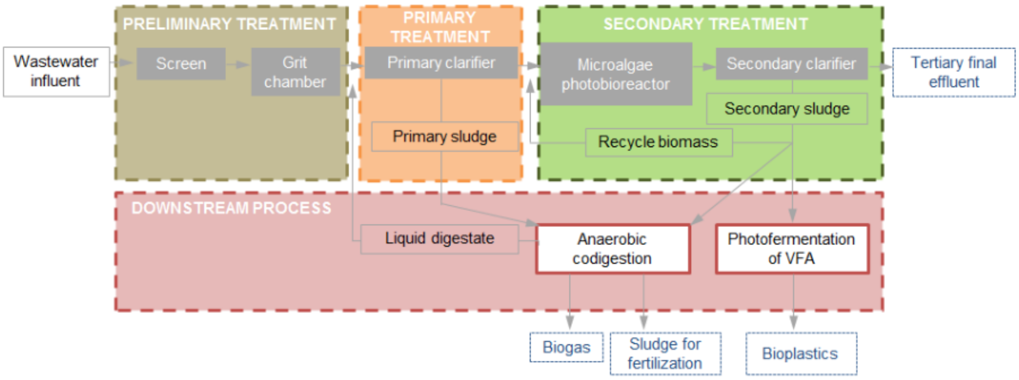

Figure 2. 3 Integrated microalgae-based WWT flow diagram consisting of wastewater influent treated using preliminary treatment, primary treatment, secondary treatment, tertiary treatment, and producing effluent (Martínez, 2016).

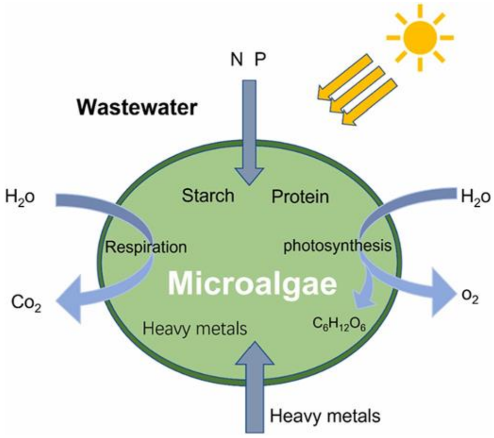

Figure 2. 4 Microalgae mechanism treats wastewater (Song *et al.*, 2022)

2.1.3. Wastewater as a source of microalgae growth

Microalgae in wastewater have a symbiotic effect on heterotrophic and autotrophic bacteria through substrate exchange (Figure 2. 5). During this symbiosis, microalgae produce oxygen (O_2) needed by heterotrophic bacteria to oxidize organic matter present in wastewater, and microalgae will use carbon dioxide (CO_2) released by heterotrophic bacteria.

Optimal microalgae growth requires sufficient nutrients (carbon, nitrogen, and phosphorus). Carbon comes from CO_2 (bacterial breathing and atmospheric exchange), nitrogen from NH_4^+-N, and phosphorus from PO_4^{3-}-P (NH_4^+-N and PO_4^{3-}-P are in wastewater). Hydrogen and O_2 come from water (Oswald, 1991).

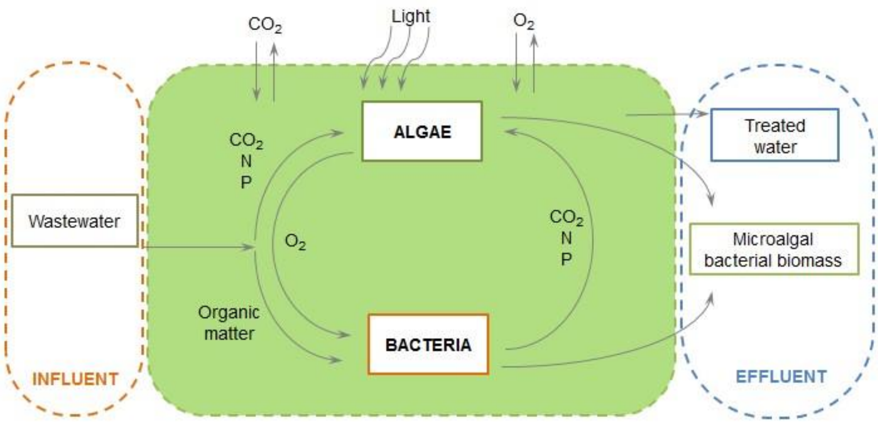

Figure 2. 5 Algae-bacterial symbiosis contained in the process of microalgae-based wastewater treatment

Microalgae generally require nitrogen and phosphorus concentrations of about 30 mg NH_4^+-N/L, and 5 mg PO_4^{3-}/L, respectively (Boelee et al., 2012). The biological removal process of nitrogen can be done in several different ways: (1) consumption by microalgae, (2) evaporation of ammonia, and (3) denitrification nitrification process. Under aerobic conditions, the nitrification process consists of two oxidation stages sequentially: ammonia is converted to nitrite by ammonia-oxidizing bacteria (equation 1), and nitrite is converted to nitrate by nitrite-oxidizing bacteria (equation 2).

$$NH_4^+ + 1{,}5O_2 \rightarrow NO_2^- + H_2O + 2\,H^+ \quad (1)$$

$$NO_2^- + 0{,}5\,O_2 \rightarrow NO_3^- \quad (2)$$

$$NH_4^+ + OH^- \leftrightarrow NH_3.H_2O \leftrightarrow NH_3 + H_2O \quad (3)$$

When the amount of carbon in wastewater is limited, microalgae photosynthesis can cause a rise in wastewater pH to 10-11. The increase in pH shifts the equilibrium of NH_4^+ towards NH_3, like equation (3) (P. Purwono *et al.*, 2017). Phosphate pollutants will be removed by depositing orthophosphate with Ca^{2+} ions (García et al., 2002; Nudogan and Oswald, 1995).

2.1.4. *Dunaliella salina*

Along with the development of the era, various research in various fields, including food and the environment, have been conducted, one of which is using various types of microalgae. *D. salina* is a microalgae species that has a role in various fields of life, so many studies examine abiotic factors that affect the growth and productivity of its cultivation.

Hosseini Tafreshi & Shariati (2019) stated that D. salina is the highest carotenoid-producing organism in the world, so many studies related to carotenoids on *D. salina* have been conducted. Research related to abiotic factors has been widely reported before. Mansouri & Soltani Nezhad (2021) reported in their research that the hormones auxin and gibberellin added to *D. salina* growth media can increase cell growth, the amount of chlorophyll, carbohydrates, starch content, glycerol, protein, and carotenoids in cells. Carotenoids are one of the ingredients in *D. salina cells.* The concentration of salts present in the culture medium influences the number of carotenoids contained in *D. salina* cells. The higher salt concentration due to *the condition of salinity stress* causes the production of carotenoids in *D. salina* cells to increase (Hosseini Tafreshi &; Shariati, 2019) Qin et al., (2021) added that the production of microalgae carotenoids increases when given salt.

Wu et al., (2016) conducted a study to test the factors of light intensity, temperature, and nutrients on the growth rate and accumulation of pigments in *D. salina.* The results showed that the optimal light intensity and temperature for the growth of *D. salina* were 135.3 mol m-2 s-1 and 22 °C, while conditions of 245.6 mol m-2 s-1 and 22 °C resulted in the highest level of beta-carotene production (117.99 mg L-1).

Regarding light factors, Kwan et al., (2021) the addition of light to culture in the laboratory can be used LEDs that produce the best wavelength for biomass production, while the production of other components, namely lipids, can be used blue and green LEDs. Another abiotic factor that affects the growth of *D. salina* is the acidity level of the growing environment. Sui, YixinSui, Y., &; Vlaeminck, S. E. (2019) examined related pH factors and *D. salina* salt concentration. As a result, the highest protein productivity of *D. salina* occurs when the salt concentration is 2 M and pH 7.5.

In Indonesia, D. *salina* cultivation research has been conducted by Putri et al., (2020). In this study, researchers focused on cultivating *D. salina on a* laboratory scale to prevent all contaminants and facilitate the control of various parameters. The treatment applied by Putri et al., (2020) in conducting laboratory-scale cultivation is by lighting treatment with fluorescent lamps, environmental temperature of 24°C, culture media using seawater, and added nutrients in the form of Guillard's fertilizer. Hadiyanto et al., (2022) researched the ability of *D. salina* to degrade microplastics through treatment starting with the cultivation stage. Cultivation is carried out in bioreactors with environmental pH conditions ranging from 8-9, temperatures ranging from 15-35 ° C, media with salt concentrations ranging from 30-35 ppt, and Walne fertilizer (1 ml / L) and other nutrient mixtures.

2.3. Microalgae harvesting using Electrocoagulation (EC) and HHO gas production

2.3.1. Microalgae harvesting

In efforts to utilize microalgae for various applications, microalgae need to be separated/harvested from the culture media. Microalgae are usually 5-50 μm in diameter and have a density equivalent to water (1020 kg/m3). So the separation/harvesting of microalgae cells from growth media is one of the most expensive stages that contributes up to 30% of the total production cost (Fasaei *et al.*, 2018). The main challenges of microalgae harvesting are their small cell size, low biomass concentration, and electrostatic repulsion between cells (Barros *et al.*, 2015). Microalgae harvesting methods are selected based on the density, size, and desired product quality (Brennan and Owende, 2010). In general, microalgae harvesting techniques are grouped into two, namely thickening and dewatering. A schematic of microalgae harvesting techniques is shown in Figure 2. 6. The thickening technique requires more steps and energy than other harvesting techniques. About 2–7% of solids can be separated using sedimentation, flotation, and flocculation. The main purpose of the dewatering technique is the separation of microalgae biomass from the microalgae substrate. The dewatering process includes centrifugation and filtration (Ananthi *et al.*, 2021).

Recent advances in microalgae technology have resulted in many efficient harvesting techniques to improve microalgae harvesting. Techniques used to harvest microalgae include centrifugation, coagulation, ultrasonic, pH change, filtration, etc., (Nguyen *et al.*, 2019). The ultrasonic process uses ultrasonic waves to create high-frequency sound waves (20 kHz) that generate cavitation bubbles in the liquid medium. Cavitation is the process of formation, growth, and collapse of small bubbles in liquids due to ultrasonic waves. When these bubbles collapse (collapse), the energy released generates very high pressure, creating a strong mechanical force around the bubbles. Ultrasonication leads to the clumping or aggregation of microalgae cells, thus facilitating their separation from water (Lee, Show, Ling, & Chang, 2017).

The most frequently used methods for harvesting large-scale microalgae cultures are centrifugation and membrane filtration. However, centrifugation and membrane filtration methods require complex maintenance. Therefore, other techniques are needed to simplify the maintenance process and reduce harvesting costs without reducing the speed of harvesting and the purity of biomass (Guldhe *et al.*, 2016).

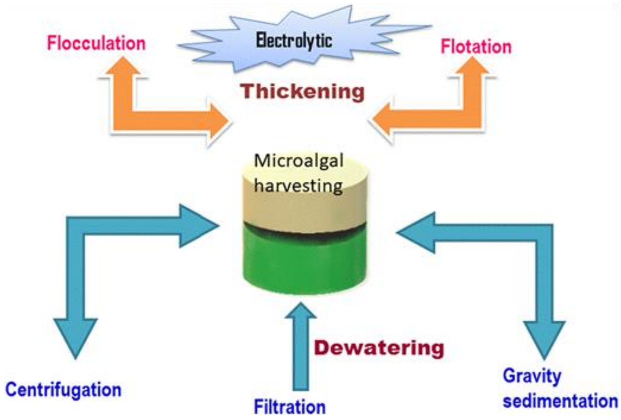

Figure 2. 6 Scheme of microalgae harvesting techniques in general (Ananthi *et al.*, 2021)

Table 2. 1 Comparison of common microalgae harvesting methods that researchers have carried out

Harvesting system	Solids Concentration (% w/w)	Required energy (KWh/m^3)	Cost
Centrifugation	2-22	0,7 – 8	High
Filtering	5-27	0,5 – 6	High
Sedimentation	0,5-3	0,1 – 0,3	Low
Flotation	2,5-7	0,015 – 1,5	Low
Electrocoagulation	3-5	0,8 – 1,5	Medium

The harvesting process has different purposes. Harvesting microalgae biomass is to separate biomass from culture media using low energy during thickening or drying. The next goal is to ensure that wastewater generated from the harvesting process meets the required quality standards or reuse wastewater for recultivation. Gravity sedimentation and flotation are considered cheaper ways to harvest microalgae biomass (Khan, et al., 2022; Martínez, 2016). Other approaches, such as electrocoagulation, can increase high harvesting efficiency but should be investigated further (Barros et al., 2015). A comparison of microalgae harvesting methods is shown in Table 2. 1 (Christenson and Sims, 2011; Gerardo et al., 2015; Henderson et al., 2008; Shen et al., 2009; Uduman et al., 2010).

1. Sedimentation

Sedimentation involves the separation of suspended microalgae cells through the process of gravitational deposition by gravity. Among other harvesting techniques, sedimentation is a low-energy harvesting process and one of the simplest ways to harvest microalgae. The different properties of microalgae species affect the speed of biomass deposition. Therefore, the shape of the sedimentation basin varies depending on the species of microalgae to be separated. The deposition rate of one spherical microalgae cell (such as *Chlorella sp.*) of 0.1 m/day, while the deposition velocity of the most complex microalgae is 0.4 and 2.2 m/day (Peperzak et al., 2003). Conventional sedimentation has a low harvesting efficiency (60-70%) (García et al., 2000a), so it can be improved by preconcentration steps such as flocculation.

2. Flocculation

Flocculation is a harvesting process in which solute particles are separated from a solution through the formation of aggregates known as floc (Uduman et al., 2010). According to Ananthi et al. (Ananthi *et al.*, 2021) microalgae cells are negatively charged, so they can be neutralized by the addition of positively charged flocculants such as cationic polymers and multivalent cations present in the substrate. The method of harvesting microalgae by flocculation occurs in four mechanisms that can be combined or alone:

1. A charge neutralization process in which the surface of a charged microalgae cell will be adsorbed by oppositely charged ions, polymers, or colloids,

2. Electrostatic mechanism whereby the surface charge of a microalgae cell is bound by oppositely charged polymers so that the cell surface and polymer stick together and cause flocculation
3. The *bridging* process in which a colloidal or charged polymer binds together the surface of two different microalgae cells to form a bridge
4. The flocculation process is when microalgae cells are trapped by the deposits formed (Vandamme, 2013).

Microalgae harvesting using electrocoagulation can save up to 89% of energy compared to centrifugation alone (Fayad *et al.*, 2017). Electrocoagulation techniques combine particles and stable suspensions in solution using salt polymers or polyelectrolytes. Bubbles of hydrogen gas (H_2) and oxygen gas (O_2) produced by the electrodes help lift coagulated floc through the electro-flotation process (Apshankar and Goel, 2018). Figure 2. 7 shows the schematic of the electrocoagulation reactor and the mechanism of harvesting microalgae through the electrocoagulation process shown in Figure 2. 8. The main operational parameters affecting the electrocoagulation process are electrode type, distance between electrodes, solution pH, electrical power, and electrode configuration. As a result, in any electrocoagulation study, these operational parameters must be optimized (Rahman *et al.*, 2021). The effects of electrolysis are responsible for chemical and physiochemical phenomena in electrocoagulation. This shows that electricity is needed for coagulant production, particulate destabilization, and floc formation at the destabilization stage (Sadik, 2019). The electrocoagulation process is based on electrochemical phenomena, where oxidation or removal of electrons occurs at the cathode while reduction or addition of electrons occurs at the surface of the anode (Syam Babu *et al.*, 2020). Because coagulants are produced *in situ* during the electrocoagulation process, the electrocoagulation process does not require the use of additional chemicals (Brillas and Martínez-Huitle, 2015).

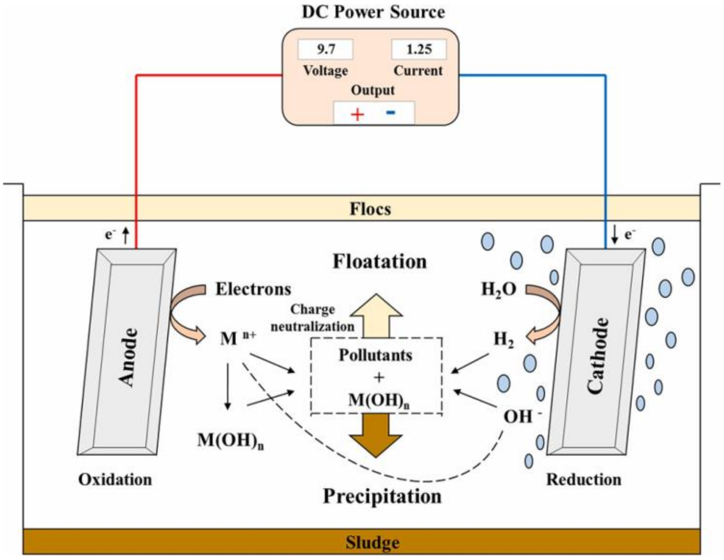

Figure 2. 7 Scheme of the electrocoagulation reactor (Das, Sharma and Purkait, 2022)

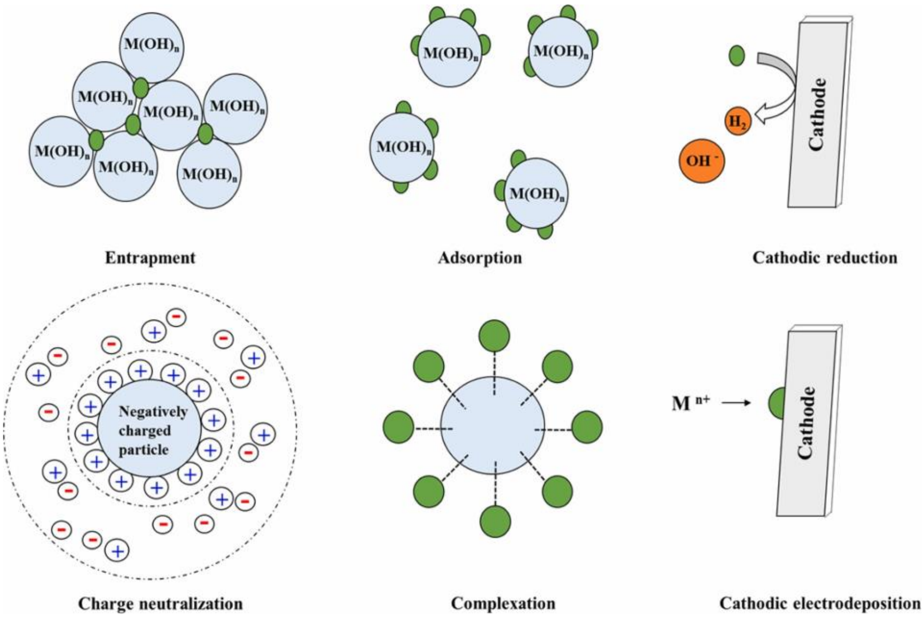

Figure 2. 8 Mechanism of pollutant degradation through electrocoagulation process (Das, Sharma and Purkait, 2022)

The electrocoagulation process is widely used to destabilize particles present in the form of dissolved or suspended particles in solution by adding an electric current. The electrocoagulation configuration consists of an electrolytic cell and a series of metal electrodes (victim, Fe, or Al) connected to a direct DC (Direct current) power source. When connected with an external power source, the anode material will corrode electrochemically due to oxidation, while the cathode will be reduced (Vandamme *et al.*, 2014). The cathode and anode used during the EC process are made of the same or different materials (Dindaş *et al.*, 2020). There are seven important stages in the electrocoagulation mechanism, namely:

1. The formation of metal cations due to the supply of electric current to the anode;
2. Production of hydroxyl ions due to cathode hydrolysis;
3. The interaction of metal cations with hydroxyl ions to form metal hydroxides;
4. Oxidation of pollutants into harmless intermediate products;
5. Neutralization of pollutant charges as a result of reacting with metal hydroxides;
6. Adsorption of charged neutralized pollutants on metal hydroxides, followed by their removal through coagulation sweeps and
7. Gas formation (H_2 gas) (Figure 2. 7) at the cathode lifts the resulting floc to the surface of the solution via flotation (An *et al.*, 2017).

Thus, microalgae harvesting in the EC process is mainly associated with three phenomena, namely adsorption, coagulation, and flotation. Coagulants are generated in the electrocoagulation chamber due to the reaction at the anode (Equation (1)); at the same time, the cathode H_2 gas is formed (Equation (2)) and hydroxyl ions at the anode (Equation (3)). This resulting coagulant is responsible for forming floc surrounded by metal hydroxide, which serves as an efficient adsorbent.

Reaction at the anode:

$M_{(s)} \rightarrow M^{3+}{}_{aq} + ne^-$ .... (4)

$H_2O \rightarrow 4H^+{}_{aq} + O_{2\,(g)} + 4e^-$ .... (5)

Reactions at the cathode:

$nH_2O + ne^- \rightarrow H_{2\,(g)}\ nOH_{aq}$ .... (6)

On the other hand, water electrolysis produces microbubbles (O_2 and H_2 gases), which are highly recommended for further investigation (Das et al., 2022). According to equation (7), the combination of O_2 and H_2 gas is called HHO gas or Brown's gas (Sudrajat *et al.*, 2020).

$$2H_2O \rightarrow 2H_2 + O_2 \quad (7)$$

Metal ions released from the electrode (e.g., Fe^{3+}) will combine with hydroxyl ions to form metal-hydroxides or polyhydroxides, such as $Fe(OH)_3$, which function as coagulants in the coagulation process. On the other hand, water electrolysis produces microbubbles. This is an advantage of the EC process because it is more efficient in harvesting microalgae, environmentally friendly, and produces HHO gas which can be used as fuel gas.

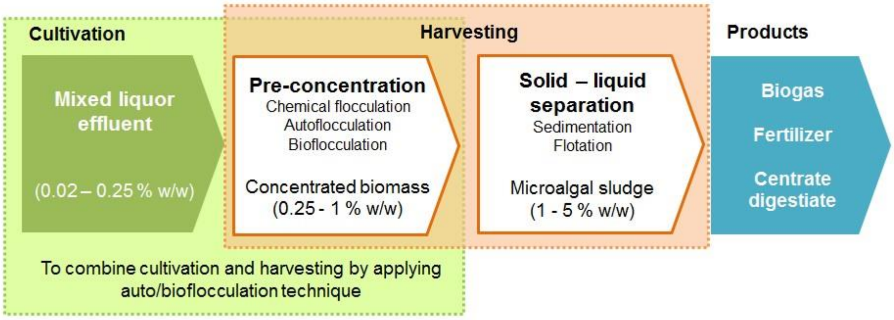

Figure 2. 9 Solution scheme for large-scale microalgae harvesting applications using a combination of low-cost preconcentration techniques (auto flocculation, bio-flocculation, chemical flocculation) followed by solid/liquid separation such as gravity sedimentation.

In the context of wastewater treatment, low-cost harvesting techniques are expected to be able to treat wastewater and produce large amounts of biomass. Nevertheless, the energy requirement for the harvesting stage should be low. Therefore, combining low-cost preconcentration techniques such as auto flocculation, bio-flocculation, and chemical flocculation (with organic and inorganic flocculants) followed by solid/liquid separation such as gravity sedimentation can be a viable and cost-effective option. The solution scheme for implementing large-scale microalgae harvesting is shown in Figure 2. 9. Basic principles of microalgal harvesting using electrocoagulation

Three main reasons make biomass recovery of microalgae difficult to: (i) small microalgal cells (usually within 3-30 μm, although smaller cells do exist, for example 0.8-1.5 μm for Synechococcus), (ii) concentrations of culture pots are usually low (from < 0.5 g/L in open outdoor pools), (iii) like most biological materials, microalga's usually carry a negative surface charge, which gives them colloidal stability in the suspension (Visigalli *et al.*, 2021a). The surface load density is a function of microalgae species, medium ionic strength, pH and other environmental conditions (Shelef, Sukenik and Green, 1984). The cell walls of microalgae consist of polysaccharides, proteins and lipids (Monteiro, Castro and Malcata, 2012), which contain many function groups, such as carboxyl (–COOH), hydroxyl (-OH), phosphate (–PO_3), amine (–NH_2) and sulfhydryl (–SH) (Sun *et al.*, 2015). Carboxyl and amine groups are very relevant because they can produce surface loads and, consequently, surface potential. Indeed, under a typical neutral pH/base, this function cluster is deprotoned, producing a clean negative surface charge, coherent with its negative zeta potential (Ozkan and Berberoglu, 2013).

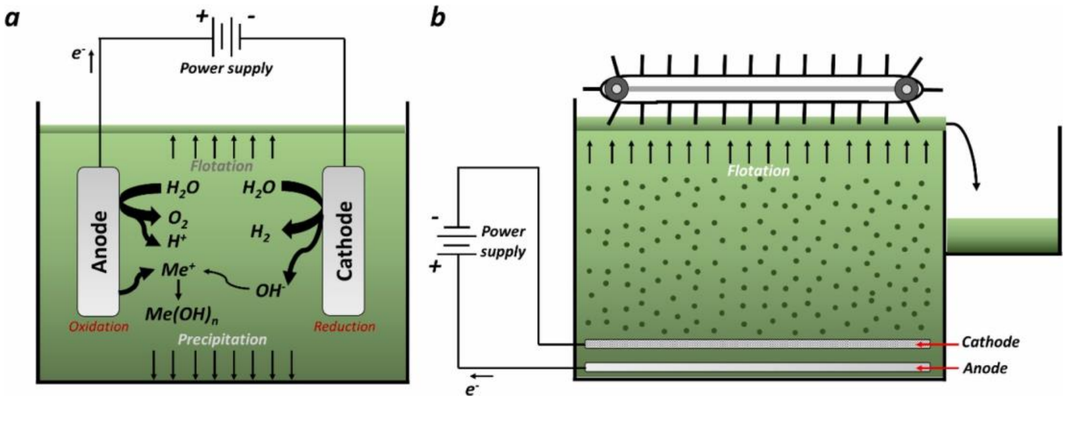

Figure 2. 10 Scheme (a) electrocoagulation and (b) the electroflotation process for microalgae harvesting (Visigalli *et al.*, 2021a)

Therefore, the suspension behavior of microalgae is similar to the colloidal suspension in which the load-related propulsion (increasing with the decrease in particle size) overcomes the Van der Waals attraction (decrease with decreasing particle sizes) and the microalgal cell remains in a stable dispersed state. To support algae separation, the propulsion should be reduced by allowing the aggregation of microalgae cells and facilitating their separation from water (Moussa *et al.*, 2017a).

2.3.2. Stability of suspended particles

Particles suspended in water can be free and colloidal particles of very small size, between 0.001 microns (10-6 mm) to 1 micron (10-3 mm). Particles found in this range include (1) inorganic particles, such as asbestos, clay, and silt/silt fibers, (2) coagulant precipitates; and (3) organic particles, such as microalgae, humic substances, viruses, bacteria, and plankton. Colloidal dispersions have light-illuminating properties. This luminescence property is measured as a unit of turbidity. Suspended particles are very difficult to settle directly naturally. This is due to the stability of colloidal suspension. Colloidal stability occurs due to:

1. Van der Waals force: This force is a force of attraction between two masses, the magnitude of which depends on the distance between them.
2. Electrostatic force. The electrostatic force is the main force that keeps the colloidal suspension in a stable state. Most colloids have an electric charge. Metallic oxides are generally positively charged, while nonmetallic and metallic sulfides are generally negatively charged. Colloidal stability occurs due to the repulsive force between colloids with the same charge. This force is known as zeta potential.
3. Brownian motion. This motion is the random motion of a colloidal particle caused by the small mass of the particle.

Van der Waals force and electrostatic force cancel each other out. Both forces are closer to zero with increasing distance between colloids. The resultant of both forces generally results in a greater repulsive force. This causes particles and colloids to be in a stable state. The forces on the particles are shown in Figure 2. 11.

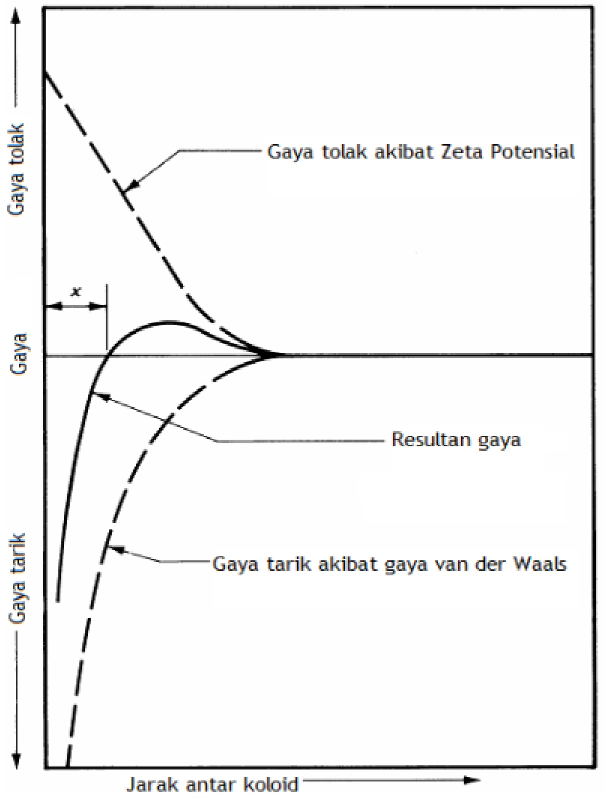

Figure 2. 11 Forces present in particles

2.3.3. Factors affecting coagulation-flocculation

An optimal coagulation-flocculation process requires regulating all interrelated factors that influence it. These factors include current density, the distance between electrodes, pH, temperature, coagulant concentration, and turbidity.

1. Current density

Operational variables that affect electrocoagulation efficiency include current density. The current density or current applied per effective electrode surface area is considered a significant parameter determining electrocoagulation performance. During the EC process, the current density regulates the rate of electron release, which occurs due to the dissociation of metal ions from the electrodes. The current density required in the sewage treatment process varies depending on the type of effluent. This variation in current density is largely due to changes in ionic interactions caused by the types of contaminants in the waste

(Moussa *et al.*, 2017b). In most cases, the current density applied in the EC process usually varies from 0.01 to 880 Am^2 (Larue *et al.*, 2003).

Excess current can reduce the performance of the EC process because it causes an in-situ secondary reaction and floc formation (coagulant overdose). Such a phenomenon can potentially shorten the service life of the electrodes while lowering the removal efficiency. Excess current also creates a thin film on the electrode surface, which greatly decreases the efficiency of the process. The current density must be precisely optimized based on the treated waste. A larger electrode surface area and lower current density can overcome such challenges. Thus, current density is an important parameter in EC processes that must be optimized to achieve the desired processing performance (Tahreen, M. Jami and Ali, 2020).

2. Distance between electrodes

The distance between the electrodes is considered an important parameter in the EC process since it regulates the electric field between the cathode and the anode. A decrease in the distance between the electrodes eventually increases the electric field. At shorter distances between electrodes, the efficiency of the EC process increases significantly (Khandegar and Saroha, 2013). Too wide an electrode distance also decreases the efficiency of the process, requiring high electrical power to release metal ions between the cathode and anode. Thus, it is very important to operate the EC process at optimal electrode spacing (Verma, Khandegar and Saroha, 2013).

3. pH and temperature

The coagulation process can run optimally when the pH of the solution is within the optimal coagulant range. If the pH is not optimal, it can result in the failure of the floc formation process and low pollutant removal efficiency. pH significantly impacts EC because pH affects the ratio between positive and negative ions in solution (Lucakova *et al.*, 2021a). This ratio of ions is key to neutralizing the charge of the cell surface and causing the cell to stick and clump. For example, the effect of pH on the oxidation of iron electrodes, where this oxidation process is complex. The graph of Fe (III) solubility at 25°C is shown in Figure 2. 12; under acidic conditions, Fe^{3+} and Fe $(OH)_4^-$ predominate.

The coagulation process can be reduced at low temperatures due to increased viscosity and changes in aggregate structure to be smaller, while at high temperatures, there is a decrease in viscosity (smaller density). At low temperatures, the reaction speed is slower, and the viscosity of water is greater so that floc is more difficult to settle (Risdianto, 2007)

4. Coagulant concentration and turbidity level

The dose of adding a coagulant to treat wastewater must be calculated precisely to optimize the coagulation-flocculation process. The concentration of coagulants will affect particle collisions and floc formation, so the addition of coagulants must be by needs. If the coagulant concentration is lower, it results in reduced collisions between particles, consequently complicating floc formation. Vice versa, if the coagulant concentration is too much, then floc is not formed properly and can cause turbidity again (Susanto, 2008).

Turbidity reduces water clarity caused by pollutants in the water, plates, soil particles, and other colloidal pollutants that usually cause turbidity. The degree of turbidity depends on the particles' fineness and concentration. Turbid water tends to be easier to treat than clear water because the more turbid the water, the larger the particles in the water are, so it is easier to be bound by coagulants. At low turbidity levels, the process of distillation will be difficult. Conversely, the destabilization process will take place quickly at a high level of water turbidity. But floc formation is less effective if the condition is used low coagulant dose.

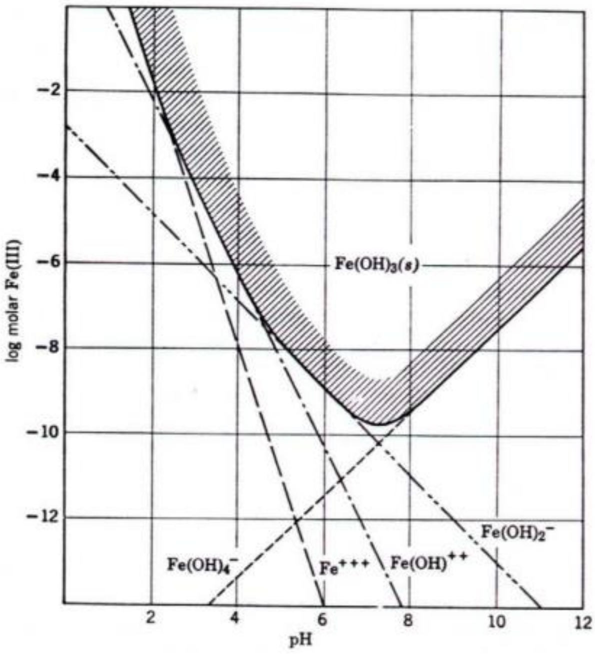

Figure 2. 12 The effect of pH on the solubility of Fe(III) at a temperature of 25°C (Fair, Geyer and Okun, 1968)

2.4. Properties and Production of HHO gas

The properties of HHO gas as a fuel are similar to hydrogen gas (Yilmaz, Uludamar and Aydin, 2010; Subramanian and Ismail, 2018). Hydrogen is a colorless and odorless gas and has a liquid-to-gas expansion ratio of 1:848 under atmospheric conditions. The melting point and boiling point are -259.14°C and -252.87°C, respectively. The presence of hydrogen gas and oxygen in HHO gas produces a high octane number (Subramanian and Ismail, 2018). Santilli (2006) proposed that HHO gas is composed of H and O atomic groups, H-O bond, H_2, O_2 molecules and water vapor. Santilli further suggested that HHO contains a 'magnecular' bond and a conventional molecular bond. Magnecular bonds are a concept proposed by the Italian-American physicist, Ruggero Santilli, as a new type of bond that differs from covalent, ionic and other chemical bonds. According to Santilli's theory, magnecular bonding occurs when molecules or atoms bind to each other through magnetic interactions between magnetic nuclei or electrons. In this theory, bonds do not depend entirely on the exchange or co-charge of electrons, but on the magnetic field produced by subatomic particles. However, the concept of magnecular bonds has not been widely accepted in the scientific community (Calo, 2007; Kadeisvili, 2008).

HHO gas is made by electrolysis of water (Subramanian and Ismail, 2018). During the electrolysis process, direct current is passed through the water. H_2O electrolysis reaction scheme into hydrogen (H_2) and oxygen (O_2) gases show in Figure 2. 13. The resulting ionization reaction is a reaction to decompose water into hydrogen and oxygen gases. A mixture of hydrogen and oxygen gases in almost stoiciometric proportions known by various names such as HHO, hydroxy gas, oxy-hydrogen and Brown gas. The earliest use of this gas was reported by Brown (Brown, 1977) who investigate welding applications. The reaction that occurs in the electrode is shown in the following reaction equation.

Cathode : $2H_2O + 2e^- \rightarrow H_2 + 2OH^-$ .... Eq. 1

Anode : $4OH^- \rightarrow O_2 + 2H_2O + 4e^-$ .... Eq. 2

Overall reaction : $2H_2O \rightarrow 2\,H_2 + O_2$... Eq. 3

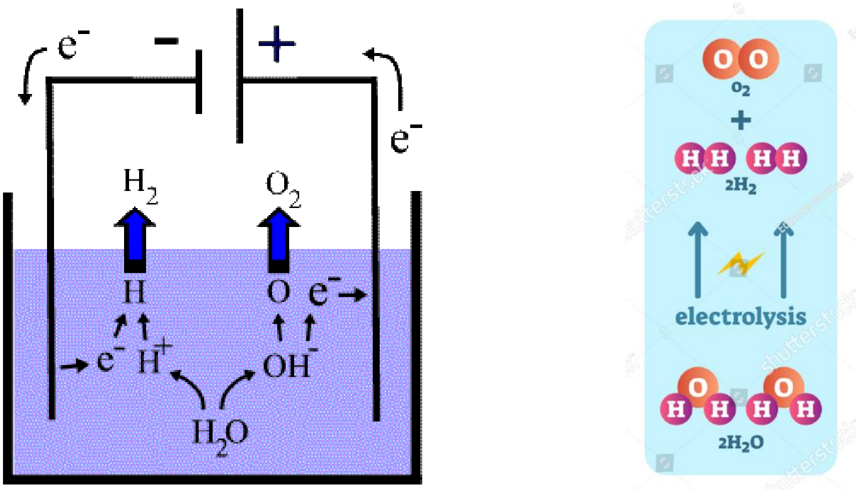

Figure 2. 13 H_2O electrolysis reaction scheme into hydrogen (H_2) and oxygen (O_2) gases

2.5. Consumer Knowledge, Awareness, Acceptance, and Willingness to Pay Regarding Fuels Derived from Microalgae

Something new (technology or product) will be accepted when people feel more benefits than risks. Perceptions of the benefits and risks of new products are sometimes subjective (Kang and Park, 2011). In product development, knowledge is needed about people's perception of the attributes of product factors. The community can consider these priority factors in accepting new products (Amini, 2022). According to Wüstenhagen et al. (Wüstenhagen, Wolsink and Bürer, 2007), there are three approaches to better understanding social acceptance, namely:

a. Socio-political acceptance refers to the social acceptance of policies by stakeholders and policymakers.
b. Community acceptance refers to the acceptance of local communities, such as community members and local authorities.
c. Market acceptance refers to acceptance by market participants on the supply side and consumers on the demand side.

Consumer awareness of alternative fuels, such as biodiesel, shows mixed results. Education campaigns and policy interventions need to be carried out to promote the sustainable use of biodiesel as an alternative fuel source (Gupta, 2023) Studies in Finland show that customers have a limited understanding and are worried about biofuel production (Moula, Nyári and Bartel, 2017) Consumers in India have low awareness due to reasons such as the availability of raw materials and prices (Gupta, 2023)Consumer awareness is influenced by price, suitability with vehicles, and availability of information (Radics, Dasmohapatra and Kelley, 2016) These studies highlight the need for public knowledge about the benefits and sources of bioenergy to increase the acceptance and support of sustainable fuels, thereby driving consumer behavior and people's purchasing decisions towards a greener energy future.

Consumers' willingness to pay for alternative biofuels varies depending on their level of awareness, education, number of family members, and price. Consumers are interested in alternative fuel vehicles and are ready to pay for vehicle feature modifications, especially among certain consumer segments (Hackbarth and Madlener, 2016; Obayelu, Jimoh and Agulanna, 2020) The willingness of users to pay for biofuels is influenced by the functional values, emotional values, and novelty of raw materials, while social values play a small effect. Consumers' reluctance to pay for biodiesel is related to concerns about its impact on food prices and a lack of support from car manufacturers (Zailani *et al.*, 2019) .