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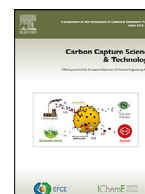
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Review

MINIMIZING CARBON FOOTPRINT VIA MICROALGAE AS A BIOLOGICAL CAPTURE



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ABSTRACT

The threatening crisis of climate change and pollution resulting from various anthropogenic interventions has attracted worldwide attention over the last few decades. However, carbon capture and storage (CCS) methods, once seen as a promising technology to mitigate this worrying scenario, are considered economically cumbersome, and their long term environmental implications are still unclear. Alternatively, biological capture of carbon dioxide (CO₂) using microalgae is considered an attractive medium for recycling the excess CO₂ generated from power plants, automobiles, volcanic eruptions, decomposition of organic matter, and forest fires. Furthermore, through microalgae, CO₂ can be captured and recycled into biomass, which in turn could be utilized as a carbon source to produce lipids for the production of bioenergy and other value-added products. In the future, these products are expected to sustainably replace petroleum-derived transport fuel without affecting the food supply chain and crops directly or indirectly. This review focuses on existing literature for biological capture via microalgae to minimize carbon footprint. It also highlights the molecular tools, methodologies and microalgae species currently utilized for CO₂ capture.

1.0. Introduction

Climate change is widely acknowledged as one of the top global threats with far-reaching consequences. These include; rising sea levels, elevation in the concentration of atmospheric greenhouse gases, elongated heat waves with more frequency, loss of mass in Greenland and Antarctic ice sheets and biodiversity loss (Dawson et al., 2011; Meinshausen et al., 2009; Biermann and Kim, 2020). Sadly, these resulting changes in natural phenomena have also negatively impacted human health (Xu et al., 2019; Ekwebelem et al., 2020). Such negative impacts are continuously exacerbated by the depletion of the ozone layer and increase in heat waves, which increases the number of heat-related illnesses and deaths directly or indirectly (Xu et al., 2019; Ekwebelem et al., 2020), and simultaneously increases the rates of infections spreading through the changes in temperature and precipitation patterns (Springmann et al., 2012; Huang et al., 2013; Costello et al., 2009). In a similar vein, global economies have not been spared by

climate change including consequences of its effects on agriculture (Springmann et al., 2016).

In some cases, cultures have been affected, while some coastal cities have been flooded due to rising sea levels affecting more than 150 million people (Xu et al., 2019; Ekwebelem et al., 2020). It is well known that carbon dioxide is a significant contributor to global warming and climate change (Singh and Dhar, 2019; Moreira and Pires, 2016). This is because carbon dioxide as a greenhouse gas (GHG) can trap and absorb sun rays within the atmosphere. The amount of carbon dioxide emitted from a power plant, transportation, industrial plants, and cement production is about 5 Gt per annum (Mondal et al., 2017). Furthermore, an increase in CO₂ emission into the environment due to fossil fuel combustion and anthropogenic activities has brought about sustainable and economical routes of chemical synthesis (Jajesniak et al., 2014).

Consequently, the massive investment in carbon dioxide capture is expected to contribute significantly to mitigating emissions mostly from power plants. Carbon utilisation involves the scavenging and transfor-

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mation of carbon dioxide into useful products or direct use. The areas of applications include enhanced oil recovery, syngas synthesis, extraction, mineralisation, carbonation, etc. However, these processes are energy-intensive, with harsh operational conditions involving safety and environmental concerns (Barati et al., 2021; Arun et al., 2020). Hence, a biological technique involving the application of microorganisms to capture and convert carbon dioxide to food, chemicals or fuel will be environmentally welcoming, greener, and cost-effective.

The need to develop sustainable, renewable industrial processes is crucial and one of the foremost global challenges facing humanity. Traditional manufacturing processes are unsustainable, utilising non-renewable feedstock and releasing large quantities of greenhouse gases and toxic side streams and waste products into the environment (Arun et al., 2020). The financial cost of emitting CO₂ is set to increase, with many countries implementing carbon taxes on companies that burn fossil fuels (Ricco et al., 2016; Kumar et al., 2017). Similarly, many cities have introduced the concept of a carbon tax and low emission zones within designated areas of the urban environment in which only specific types of vehicles are allowed to operate to limit carbon dioxide emissions (Kelly et al., 2011). Thus, there is a drive towards Carbon Capture and Storage (CCS) technologies. It is also apparent that utility-scale breakthroughs will need to be fast and cheap. There is, therefore, an opportunity to exploit technologies that use CO₂ as a cheap feedstock for the manufacture of key industrial chemicals, thereby creating a 'circular economy, which adds value, maximises efficiency and builds flexibility and security into the supply chain (Ekwebelem et al., 2020; Barati et al., 2021; Arun et al., 2020; Ricco et al., 2016; Kumar et al., 2017). Numerous carbon capture and conversion techniques have been proposed to ameliorate the CO₂ challenge. CO₂ can be separated from flue gas mixture through; i) solvent absorption, ii) physical adsorption, iii) membrane separation, and iv) cryogenic distillation (Singh and Dhar, 2019). The capture of CO₂ from the exhaust or reformed gases or power plants can be achieved through three major approaches: pre-combustion capture, post-combustion capture, and oxy-fuel combustion methods.

Presently, CO₂ is captured from flue gas through liquid absorption with solvents such as selexol, rectisol, and mono-ethanol-amine. This process is both capital and energy-intensive. The low-cost and easy regeneration alternative is physical adsorption with solid adsorbent, which selectively separate CO₂ from flue gas mixture (Hart and Onyeaka, 2020). The adsorbent materials commonly used in adsorption are activated carbon, zeolites, microporous/mesoporous silica, carbonates, carbon molecular sieves and metal-organic frameworks (MOFs). Also, direct air capture is used for the net removal of CO₂ released into the environment from the transportation sector. However, implementing a microalgae-based biological carbon-capture approach facilitates carbon footprint mitigation and bioenergy production, making the concept a CO₂-neutral substitute for fossil fuel (Choi et al., 2019). This is because the microalgae are utilized as a lignocellulosic biomass feedstock for biofuel production and value-added chemicals.

Interestingly, biological capture and sequestration of carbon using microalgae have been recognized as one of the world's most important and effective carbon sequestration methods (Moreira and Pires, 2016; Alami et al., 2021). In the long run, bio-capture of carbon using microalgae has been deemed environmentally friendly, economically feasible, and a sustainable technology (Basu et al., 2014). Microalgae is a type of micro-cell factory with an impressive ability to fix carbon dioxide efficiently. Some other key features of microalgae are the high photosynthetic efficiency (Moreira and Pires, 2016; Sayre, 2010). Microalgae have the ability to fix carbon dioxide 10-50 times more than other terrestrial plants (Batista et al., 2015). It has a rapid growth / multiplying rate (few hours), much greater than higher plants (Batista et al., 2015; Bennion et al., 2015; Brilman et al., 2013; Cheah et al., 2015).

Furthermore, microalgae have the potential to recycle CO₂ into bioenergy through photosynthesis, thus, highlighting that bioconversion of CO₂ using microalgae is an environmentally friendly and sustainable method (Brilman et al., 2013). Interestingly, bioconversion of

CO₂ using microalgae has shown strong environmental flexibility. Its ability to tolerate and adapt to a variety of extreme environmental conditions enhances applicability (Moreira and Pires, 2016). They do not occupy arable land, which makes them suitable for cultivation in coastal beaches, saline-alkali lands, and deserts (Moreira and Pires, 2016). Another noteworthy feature of microalgae is the capacity to convert flue gases into inorganic carbon sources from power plants and other industrial exhaust gas (Arun et al., 2020). Economic feasibility is another great advantage, as wastewater from industries, agricultural activities, and municipalities can be utilized as alternative nutrient sources to cultivate microalgae at a low cost. The simultaneous production of high added value products by microalgae is arguably the highest advantage in the biological utilization of microalgae. These high added value products can be used to prepare food, animal and aquaculture feed, cosmetics, pharmaceuticals, fertilizers, biologically active substances and bio-fuels such as biodiesel, bio hydrogen, aviation oil, methane (Cheah et al., 2015; Chung et al., 2011; Chung et al., 2013; Coasta et al., 2014).

This review contains a survey of recent literature on the mechanisms and application of microalgae in carbon dioxide capture and sequestration. It highlights areas of active research in the field, such as progress made in the bio-capture of carbon dioxide, the conversion of this into energy using microalgae biomass, the technologies employed, the advantages and disadvantages of the method and areas of improvement. Consequently, the concept could potentially integrate carbon dioxide mitigation, bioenergy production and circular carbon economy. This suggests that the field of bioconversion and bioenergy is fast-evolving, as the prospects and applications in bio-refinery are explored.

Geologically stored carbon dioxide needs to be secured and monitored, in which uncertainties and leakage could be catastrophic. Additionally, the economics of operations and transportation, and long term environmental safety raises serious concern (Singh and Dhar, 2019). At the same time, the simplicity and potential for economic benefit emphasise the novelty of the microalgae approach for bio-sequestration of CO₂. The alternative, therefore, is to convert carbon dioxide into a stable liquid or solid such as carbonate. However, microalgae have emerged as a bio-sequestration of carbon dioxide, in which carbon dioxide is buried into biomass via photosynthesis, and the harvested microalgae could be used to produce bioenergy and other value-added products (Singh and Dhar, 2019). On the other hand, there has been growing interest in the development of microalgae to produce biofuels and value-added products. It is well known that the consumed CO₂ by the microalgae is a building block of macromolecules such as lipids, proteins, carbohydrates and pigment. Consequently, carbon dioxide capture efficiency as high as 90% has been reported in open ponds (Sayre, 2010). As a result, an integrated bio refinery could be developed to promote large scale production. In 2019, Singh and Dhar (2019) carried out a review on the microalga bio-refinery concept focusing on biofuels and value-added products. Mondal et al. (2016) reported a mini overview on the role of carbonic anhydrase on microalgae carbon capture. In the report, carbonic anhydrase, a zinc-containing metallo-enzyme was described as being responsible for the carbon concentrating mechanisms by catalysing the reversible hydration of CO₂ into bicarbonate and helping in fixation of atmospheric CO₂. The overview also highlighted the importance of the different types of carbonic anhydrase enzymes, their locations, mechanism of action and the various studies on biosequestration of CO₂ through microalgae. In a recent review by Mistry et al. (2019), they summarized the different microorganisms such as eukaryotes and prokaryotes that have the ability to assimilate CO₂, highlighting the metabolic pathway through which they sequester CO₂. However, this article focuses on the different approaches to bio-sequestration of CO₂ with microalgae, recent progress, challenges, and future prospects.

Furthermore, the mechanisms of photosynthesis to bio-sequester CO₂ and its integration into valuable biomolecules is also explored. Finally, it highlights and proposes genetic engineering and metabolic modifications as approaches for enhancing bio-sequestration of CO₂ us-

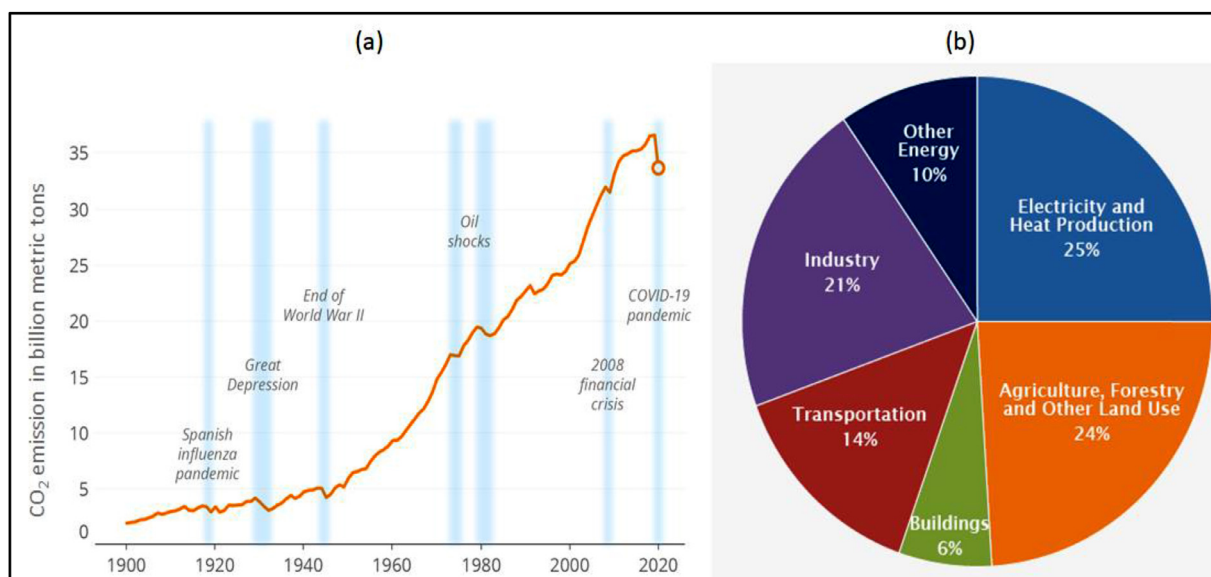


Figure 1. Global fossil emissions: (a) trends billion metric tons of CO₂ from 1900 to 2020 (Boden et al., 2017) and (b) emissions from several economic sectors (EPA, 2021).

ing microalgae. This provides insights and sets out the direction to drive and guide future research.

1.1. Overview of CO₂ emission

Global carbon dioxide emission continues to rise annually. Figure 1 shows the global carbon dioxide footprint from fossil sources (i.e., petroleum, coal, natural gas and cement production) in billion metric tons of CO₂ and also from different economic sectors. There has been a dramatic and progressive rise since the emergence of industrial evolution, from 9.34 billion metric tons in 1960 to 36.44 billion metric tons in 2019. This rising concentration of atmospheric carbon dioxide ascribed to fossil fuel burning has become a matter of environmental concern. The observed drops in worldwide CO₂ emissions, as indicated by vertical blue lines in the historical data, can be attributed to crisis such as pandemics, economic crisis and wars (Figure 1a). For instance, in 1918, the Spanish flu pandemic caused about 14% decrease in CO₂ emission, which later increased by 15 % in 1920. Likewise, in 2020, the global CO₂ emission fell by 6.4% (2.3 billion tonnes) because of COVID-19 pandemic (Tollefson, 2021), suggesting that drops in CO₂ due to crises are short-lived. According to the US Environmental Protection Agency (EPA), in 2010, the top three CO₂ emissions are electricity and heat production, agriculture, forest and land use, and industry, 14% of global carbon emissions was from the transportation sectors; whereas, carbon dioxide emissions from buildings contribute about 6% as shown in Figure 1b (EPA, 2021). The global lockdown which limited transportation sector orchestrated by the COVID-19 pandemic (Global Carbon Project, 2020), resulted in some direct, short-term, positive impacts on the environment, particularly in terms of air quality and emissions reduction. According to the most recent data from the global carbon project, the top five countries emitting the most CO₂ worldwide are China (10.06GT), USA (5.42GT), India (2.65GT), Russia (1.16GT), and Japan (1.16GT) (Global Carbon Project, 2020). The major processes of CO₂ emissions in these countries are electricity, particularly the burning of coal. Other major culprits of CO₂ emission are Germany (0.75GT), Iran (0.72GT), South Korea (0.65GT), Saudi Arabia (0.62GT), and Indonesia (0.61GT) (Global Carbon Project, 2020). Approaches to effectively reduce carbon dioxide from the atmosphere using physical and chemical methods have high limitations of cost and ecological consequences. There three ways in which carbon capture could generate

wealth include: 1) economic incentives within existing carbon markets, 2) preventing costly environmental and humanitarian disasters resulting from uncontrolled climate change, and 3) upgrading a damaging waste product into valuable products and renewable energy (Daneshvar et al., 2021).

It has been estimated by the United Nations Environment Programme that for the world to prevent global warming from reaching more than 1.5 °C, the world would need to cut carbon emissions by roughly 7.6% annually for next decades (Tollefson, 2021). To achieve this set goal in the 2015 Paris climate agreement, a combination of carbon capture strategies is required in addition to the transition to low-carbon energies. Additionally, the global demand for energy has been forecasted to increase by 50% in 2030, with oil and gas the major feedstock for 90% chemicals, petrochemical products and energy (Hart and Onyeaka, 2020). Hence, carbon capture, also known as sequestration, is an effective strategy to scavenge carbon dioxide from the atmosphere, decarbonize industries and promote clean fossil energy production. It has become an effective approach to mitigate global warming and/or dangerous climate change. Furthermore, it has become a sustainable technology that could be used for long-term storage of CO₂.

One of the most sustainable approaches to capture and store CO₂ from the atmosphere is photosynthesis, and photosynthetic microorganism such as microalgae has exhibited the highest carbon fixing capabilities. It has been reported that microalgae can fix approximately 100 Gt of CO₂ into biomass annually (Jajesniak et al. 2014; Field et al. 1998).

In this way, Global Warming Gases (GWGs) released through natural and anthropogenic activities and accumulated in the ecosystem could be slowed down. While innovative technologies for utilizing CO₂ as chemical feedstock are being developed, as the use of this technology at an industrial level CO₂ utilization is still marginally limited (Kumar et al. 2017). Only a few industries use carbon scavenged from the environment to produce various chemicals such as methanol, propylene carbonate, urea, inorganic carbonates and pigments, and salicylic acid (Jajesniak et al. 2014). The impact of CO₂ utilization in mineralization, food and beverages, enhanced oil recovery, chemicals, and energy storage is still very limited; hence, technologies that capture, store and converted CO₂ into biomass and consequently transformed into bioenergy would produce significant mitigation. In addition to the utilization of microalgae, for the logistics and transportation sector, effective decarbonisation can be achieved by adopting renewable energy and green

vehicles. One option is to utilize the microalgae biomass for bioenergy production via bio-refinery for logistics and transportation, creating carbon neutrality.

2.0. Carbon capture and storage methods

Carbon dioxide capture is an indispensable technology in both fossil fuels- and biofuels-based power plants to lessen CO₂ emissions from power generation. Generally, there are three major methods to capture carbon dioxide from power plants: pre-combustion capture, post-combustion capture, and oxy-fuel combustion. Pre-combustion carbon dioxide capture method refers to the removal of CO₂ from the fossil fuel prior to the completion of combustion. With this technology, the fuel first undergoes a pre-treatment stage; for instance, gasification and reforming processes in which the feedstock such as coal/biomass is partially oxidized in steam and oxygen/air under high temperature and pressure to form synthesis gas before the actual combustion stage (Omogbe et al., 2020; Park et al., 2021). In other words, it is usually operated with Integrated Gasification Combined Cycles (IGCC), which involve gasification and partial oxidation of the fuel to produce CO₂ and hydrogen, which are then separated, normally using physical absorption processes (Park et al., 2021). However, the water-gas-shift (WGS) reaction and the pre-combustion capture schemes suggest efficiency penalties. Compared to post-combustion technology, which separates dilute CO₂ around 5% to 15% in concentration in the flue gas after the fossil fuel has undergone combustion, generating CO₂ in the flue gas (Song et al., 2019; Wu et al., 2020; Nessi et al., 2021). Though the pre-combustion capture normally is more efficient and the hydrogen produced can be utilised as a transportable fuel/product, but the capital costs of the base gasification process are often more expensive than conventional pulverized coal power plants. Conversely, the advantage of post combustion approach is that it can be applied to already existing power plant. The requirements for both technologies include large sizes of equipment due to the high volume of flue gases and the low partial pressure of CO₂, energy-intensive cooling systems, pre-treatment to remove impurities, and considerable cost of capture (Jiang et al., 2019; Omogbe et al., 2020; Nessi et al., 2021). On the other hand, oxy-fuel combustion technology involves the combustion of carbonaceous fuel in a stream of pure oxygen instead of air. Although, the comparative ease with which CO₂ can be separated is one of the merits, the challenges include pre-treatment requirement, the high cost of supplying oxygen could be a limiting factor in commercialisation and high temperatures of combustion process (Seddighi et al., 2018). Hence, the CO₂ capture ability of microalgae cultivation needs to be benchmarked against other post-combustion CO₂ capture methodologies. However, microalgae CO₂ carbon capture is dependent on the microalgae species, cultivation systems, and growth conditions such as temperature, pH, turbidity, salinity, light intensity, trophic modes, and culture nutrients (Cheng et al., 2021).

In 2019, the National Energy Technology Laboratory (NETL, USA) published a report recommending a carbon capture cost target maximum of \$40/tonne CO₂, which suggests that even the most well established CO₂ capture methods far exceed this cost margin (Daneshvar et al., 2021). This target can be achieved by adopting a novel cost-saving approaches, such as microalgae carbon capture on industrial scale. Despite the increasing investment, research and awareness of conventional carbon capture and storage technologies, their public acceptance is still low. On the other hand, to create a sustainable environment, there should be a considerable reduction of carbon dioxide emission from logistics operations, especially the transportation sector (Hart and Onyeaka, 2020). The logistics sector of the economy generates a large carbon footprint in the environment due to its dependence on fossil fuels (Ekwebelem et al. 2020). Consequently, it is challenging to implement conventional capture technologies suitable for power plants in the transportation sector. In light of this, the application of microalgae in carbon capture would prove viable for a sustainable environment,

particularly for the carbon footprint emanating from the transportation sector. However, CO₂ emission in the transportation sector can be reduced by capture and store CO₂ onboard. As regard this, Sharma and Marechal (2019) proposed a study using integration of an onboard CO₂ capture and storage unit with an internal combustion engine. This is applicable to various internal combustion or Stirling engines with emphasis on the transportation sector. The *adsorption-desorption on vehicle with CO₂ liquefaction* is one way in which CO₂ can be captured in the transportation sector. The study used truck transport for goods delivery as an example for onboard CO₂ capture and storage system design. The design includes the integration of temperature swing adsorption, Rankine cycle, heat pump and CO₂ compression and liquefaction on vehicle. According to the study, the process of capturing CO₂ in the transportation sector using the proposed design employed the conversion of waste heat available in the exhaust stream into mechanical power through Rankine cycle which helps to drive the heat pump and product compressors, as the system design is an energy self-sufficiency. During this process, the captured CO₂ can store renewable energy by the conversion of product of CO₂ capture into green fuel using co-electrolysis. Interestingly, the CO₂ capture system in the transportation sector (car, truck, bus, ship or train) can capture 90% of the emitted CO₂ without any energy penalty. Hence, the captured CO₂ can be recycled as a conventional liquid or gaseous fuel produced from renewable energy source (Sharma and Marechal, 2019). The other alternatives are bioenergy to create carbon neutrality and the transition to electric vehicles.

The captured CO₂ from the transportation section can be used to improve the algae cultivation system and growth design, which tends to contribute to the mitigation of CO₂ losses and improve consumption efficiency according to the report published by the US Department of Energy on bioenergy technology (None, 2017). In optimizing the growth, the following strategies must come into place; improving culture mixing, providing sufficient sunlight through the design of the reactor, installing control system for pH and temperature. The report also mentioned that CO₂ outgassing decline by two orders of magnitude as pH increases from 7.9 to 9.5. Therefore, the use of outgassing could also maximize by using high pH tolerance stream (None, 2017). On the other hand, sensors and machine learning for cultivation diagnostic and analysis which can improve cultivation diagnostic and analysis can improve cultivation efficiency and harvesting strategies.

The captured carbon dioxide by the microalgae can be converted into biofuel upon harvesting for the transportation sector. This cyclic process is in line with the carbon neutrality concept. The biological and physicochemical strategies for carbon capture, which has been uniquely dubbed carbon capture and storage (CCS) methodologies, are categorized into three major steps. These are as follows: i) Carbon capture, ii) Carbon transportation, and iii) Carbon storage (Singh and Dhar, 2019; Kumar et al., 2017). The separation and capture of carbon via chemical absorption, physical adsorption; membrane separation; and cryogenic distillation are suitable for large point sources such as power plants, cement manufacturing plants and other exhaust components (Huang et al., 2013; Figueroa et al., 2008; Pires, 2011; Pires, 2017; Rao et al., 2021; Pires et al., 2012). After separation and capture, the highly concentrated carbon is then compressed and transported through pipelines and ships (Svensson et al., 2004; McCoy and Rubin, 2008).

Subsequently, reservoirs such as geological storage, oceanic storage are used to store the captured CO₂. In the storage mechanism, the carbon is directly injected deep into the ocean, saline formation, aquifers or depleted oil/gas wells (Lackner, 2003; Power et al., 2013). Notably, some setbacks still exist with CCS, despite its remarkable storage capacity. The operational needs of CCS are expensive, and other operational issues related to transportation, the environmental threat of long term CO₂ leakage and other uncertainties still exists (Lam et al., 2017; De Silva et al., 2015). However, storage of CO₂ using physicochemical methods have shown to be practically successful and a preferable approach for storage of CO₂ from point sources producing high concentrations of CO₂ (Nouha et al., 2015). Besides physicochemical CCS, biological meth-

Table 1

Biological CCS mechanism, their mechanisms and limitations (Cheah et al., 2016; Williamson et al., 2012; Harun et al., 2010; Singh et al., 2014; Kao et al., 2014).

Method	Mechanisms	Advantages	Limitations
Forestation	Ø Afforestation, reforestation, and the farming of crops and livestock	Ø No hazards of chemicals	Ø Long time requirement Ø Large area requirement Ø Affect biological diversity Ø Compete with food crops for arable land
Oceanic fertilization	Ø Fertilizing oceans with iron and other nutrients promoting increased carbon dioxide uptake by the phytoplankton's	Ø Significant potential for CO ₂ capture	Ø Cost intensive Ø May have uncertain and unintended impacts Ø May affect marine biodiversity
Microalgae-based carbon capture and utilizations	Ø Bioconversion CO ₂ into bioenergy and other valuable products via photosynthesis	Ø Highly efficient in a wide range of CO ₂ concentration Ø Faster growth rate than plants Ø Co-production of food, feed, biofuel and value-added products	Ø Economically cumbersome culture systems and downstream processing, mainly harvesting Ø Sensitive to other flue gas components (NO _x , SO _x), predation, contamination and extreme culture conditions (pH, temperature, salinity)
<i>Escherichia coli</i> -based carbon capture and utilization	Ø Bio-assimilation of CO ₂ instead of carbohydrate or other organic molecules.	Ø Readily tweaked or optimized via genome-editing Ø production of food, feed, biofuel and value-added products at much lower CO ₂ emissions	Ø Still at the laboratory stage

ods such as natural sinks, including forestation, afforestation, reforestation, and farming crops and livestock, can capture CO₂ (Farrelly et al., 2013; Cheah et al., 2016). Another biological CCS are ocean fertilization, which involves fertilizing oceans with iron and other nutrients resulting in an increased carbon dioxide uptake by the phytoplankton (Williamson et al., 2012) and through microalgae cultivation (Lam et al., 2012; Cheah et al., 2016; Yadav and Sen, 2017; Zhou et al., 2017). However, oceans that are over cultivated with algae results to algal blooms and low biochemical oxygen demand (BOD), which means low oxygen is present in water that have tendency to kill fish and other aquatic life, thereby reducing essential microorganisms and macroscopic plants and animals, that inhabit water bodies. Furthermore, it decomposes plant matter, producing large amounts of carbon dioxide resulting to lower pH of seawater, that is, ocean acidification (NOAA, 2017).

It is well known that carbon dioxide flows between natural carbon sinks, including the atmosphere, land biosphere, and oceans. However, transformation of natural to managed ecosystems (i.e., agroecosystems, urban lands, and mined lands) and agriculture diminishes ecosystem carbon stocks, magnifying gaseous emissions (Lal et al., 2018). Table 1 shows the biological methods applied in carbon capture, their mechanisms, merits and limitations. This demonstrates that the ecosystem plays a critical role in mitigating global warming through carbon assimilation. In Figure 1b, land-use, forestry and agriculture account for about 24% carbon footprint and a major contributor to climate change; hence, forestation can mitigate climate change by absorbing carbon. It carbon sequestration is generally acknowledged as both an ecological and economically viable instrument to help mitigating climate change, offering permanent CO₂ sink and storage by capturing carbon from the environment (Cunha-e-Sá et al., 2013; Fleischman et al., 2021). This is because terrestrial plants assimilate CO₂ through photosynthesis to support respiration and development. For instance, atmospheric CO₂ can be transformed into plant biomass through photosynthesis.

3.0. Different molecular tools and approaches to carbon sequestration

One of the scopes of this review was to expose researchers dealing with minimizing carbon footprint using microalgae as a biological capture. However, it would be necessary to details the molecular tools and approaches that involve carbon sequestration, which this section pro-

vides in Table 2. First, sequestration of carbon involves capturing carbon from the fossil fuel before it reaches the environment. Secondly, the enhancement of terrestrial or ocean ecosystem also be an approach to capture and sequester CO₂ (Singh and Ahluwalia, 2013).

Having looked at the different tools and approaches of CO₂, these approaches brought about the successful development of microalgae as a promising biological capture of CO₂. However, the authors sought it right to compare these approaches of carbon sequestration under the following heading; permanence, volatility, capacity time scale and cost. This is presented in Table 3.

4.0. Bio-capture of carbon by microalgae

The conventional CCS technologies are faced with the following drawbacks high energy consumption, challenges of transportation, immediate utilization of capture and the possibility of leakage to the atmosphere during storage. However, microalgae are 3rd generation bioenergy feedstock with high CO₂ fixation efficiency eliminating the aforementioned challenges. The concept concurrently promotes economies of higher scale by reducing capital and operation costs relative to traditional CCS technologies. Consequently, the approach is eco-friendly as the conversion process occurs under ambient temperature and pressure. It is obvious this microalgae technique is a promising approach of recycling CO₂ into biomass feedstock through photosynthesis which is in turn utilized for the production of bioenergy such as biogas (fermentation, gasification, hydrothermal liquefaction and anaerobic digestion), bio-oil (pyrolysis and hydrothermal liquefaction), and other value-added products such as syngas and biochar (Choi et al., 2019). In addition, comparing terrestrial plant and microalgae as regards to carbon capture, Valdovinos-Garcia et al. (2020) mentioned that microalgae have a greater capacity, which makes them as an attractive capture system. Furthermore, the current concept of integrated bio-refinery would promote the scale-up of a microalgae-based biological carbon-capture approach to increase the availability of biomass feedstock. In addition to biomass feedstock for bioenergy production through an integrated bio-refinery, microalgae could become a source for producing valuable products such as pharmaceuticals, cosmetics, nutrition, fine chemicals, food, and feed (Singh and Dhar, 2019; Choi et al., 2019).

Generally, microalgae is the term used to denote cyanobacteria (prokaryotic blue-green algae) and eukaryotic forms such as green al-

Table 2
Different approaches to carbon sequestration

Terrestrial carbon sequestration	Ocean carbon sequestration	Deep ocean injection sequestration	Geological carbon sequestration
According to Singh and Ahluwalia (2013), carbon sequestration by terrestrial is a natural biological scrubber for CO ₂ from fossil fuel emission. The principle processes or strategy for the terrestrial carbon sequestration as reported by Lal et al. (2018) includes: enhancement net primary productivity (NPP) and net ecosystem productivity (NEP) as well as increasing the storage in the soil as SOC and SIC (soil inorganic carbon). The terrestrial approach requires land in order to sequester any volume of carbon and these may or may not be available over very large areas (Sheps et al., 2009)	The development of microalgae as a good CO ₂ capture can be seen in the application of nutrients and fertilizers to the ocean. This enables the growth of microalgae (Singh and Ahluwalia, 2013). From Sheps et al. (2009), the carbon sequestration by ocean carbon naturally removes large amount of carbon from the atmosphere at shallow depth in the photic zone. Hence, this process stresses carbonate and other organisms as a result of the dissolved CO ₂ which increases the acidity of the seawater.	The main attractive feature of the deep injection is that CO ₂ can be injected as a flue gas instead of pure CO ₂ , which tends to reduce cost (Sheps et al., 2009). Although the deep injection has received attention, however the addition of CO ₂ and other gases causes the coal and carbonaceous sediment to swell. As a result of this, the porosity and permeability are reduced (Karacan and Swelling, 2007). The deep ocean sequestration is said to have the depth of greater than 1000 m, which facilitate the storage of the liquefied CO ₂ (Singh and Ahluwalia, 2013)	This involves the pumping of CO ₂ either in liquid or critical fluid into subsurface reservoir (aquifers or oil and gas reservoir) provided porosity and permeability exist (Sheps et al., 2009). It is interesting to state that capacity of different sequestration mechanism to hold carbon contribute much in determining its applicability. In the light of this, the storage capacity of geological carbon as one of the approach for carbon sequestration remain unknown. Hence, this type of approach is not an option in the absence of suitable reservoir within a transport distance of range of allowable cost (Sheps et al., 2009)

Table 3
Comparison of different carbon sequestration approaches (Sheps et al., 2009)

Approaches of carbon sequestration	Permanence	Volatility	Capacity	Time scale (years)	Cost
Terrestrial	Low	High	Require land	5 – 25	Low/medium
Ocean	Low	Low	Uncertain	Unknown	Uncertain
Deep injection	High	Very low	Large	Infinite	High
Geological	Variable	Low	Uncertain	Unknown	High

gae, red algae, and diatoms (Singh and Dhar, 2019). They are also being considered as an alluring bio-system with a potential described as CO₂ concentrating mechanism (CCM) because of their ability to efficiently photosynthesize by acquiring inorganic carbon even from very low atmospheric CO₂ concentrations (Costello et al., 2009; Whitton, 2012; Ward et al., 2012). Despite the potential of microalgae to capture carbon, the production of microalgae as regards to low value bulk products for example proteins for food application and fatty acid for nutraceuticals is not economically feasible (Zhou et al., 2017). Recent studies on the techno-economic analyses and assessment of microalgae based production system has shown that the use of biomass in an integrated bio-refinery is the only way of realizing its potential production, especially where valuable component is extracted and processed (Chew et al., 2017). To capture CO₂ from combustion gases, *Chlorella* is referred to as the most promising species. Interestingly, *Chlorella* species are characterized to grow in an atmosphere containing 40% (v/v) CO₂ (Brennan and Owende, 2010), with a fixation CO₂ rate of 0.77 to 2.22 g/L/day (Cheah et al., 2015). Although NO_x and SO_x usually present in the CO₂ stream do not affect the performance of the production of *Chlorella* (microalgae biomass) (Cheah et al., 2015; Pires et al., 2012; Singh and Singh, 2014). In Kao et al. (2014) and Duarte et al. (2016) studies, the cultivation of microalgae such as the *Chlorella* species serves as a bioremediation option for CO₂ in combustion gases from power plant. However, the study reported other components such as the NO_x and SO_x content in the gas are also reduced. The dissolution of NO_x in water which forms nitric acid, is beneficial to microalgae in their metabolism, which helps to save nutrient during cultivation. This observation is an evidence that microalgae (*Chlorella* species) would be of a great potential as a bioremediation option not only for CO₂ but also for greenhouse gases.

Bio-capture of CO₂ with microalgae in industries was conducted by Anguselyi et al. (2019). Freshwater algal was isolated and identified for CO₂ capture using *Oscillatoria* (blue green algae with uniserial arranged cells that are not constricted at the cross walls). In the study, photo-bioreactor acts as a closed pond system used for the cultivation of the microalgae, as it has the capacity to reduce contaminated risk from unwanted alga, mold and bacteria, as well as reduction of carbon dioxide losses and minimizes water evaporation. The microalgae strains

were subjected and screened based on the different parameter such as fast growth rate, high rate of photosynthesis, strong tolerance to trace constituent of gases (NO_x, SO_x and gaseous hydrocarbon), high temperature tolerance and possibility to produce high value products. It was reported in the study that higher CO₂ capture occurred from 16 to 32 h at optimum pH and temperature of 7 – 9 and 25 – 30 °C respectively. The study further relates to integrated methods and systems utilizing 90 – 99% CO₂ from natural gas processing industry as well as 13 – 15% of CO₂ from flue gas as carbon nutrient source. These results indicate that microalgae such as *Oscillatoria* as a freshwater algal is ideal and capable for CO₂ capture.

The efficiency of CO₂ bio-capture by microalgae varies depending on their algal physiology, pond chemistry, and temperature. A study conducted by Keffer and Kleinheine (2002) revealed that CO₂ capture efficiencies are as high as 80% to 99% under normal conditions, with gas residence time obtained within two seconds. For instance, considering a 200 MWh natural gas-fired power plant, Herzog and Golomb (2004) reported that a microalgal pond of 3600 acres is estimated to capture 80% of the CO₂ from the plant during the daylight hours. This statistic assumes that microalgae real biomass productivity rate is 20 g dry weight per square meter per day.

In 2020, a comprehensive quadric surface-based logistic model developed by Zhao and Su, found that, theoretically, a maximum of 2.35 Gt CO₂ can be sequestered in 100,000 km² microalgae culture area, accounting for 8.01–5.31% of global CO₂ emission reductions (Zhao and Su, 2020). On average, annual 0.5393 Gt CO₂ sequestration and 324.33 million tons of microalgal-biomass yields would be achieved using 53,000 km² culture area. In other words, microalgae with both open and closed culture modes could convert in theoretical terms 513 tons of CO₂ into 280 tons of dry biomass per hectare per year using about 9% light energy to sequester (Zhao and Su, 2020).

Microalgae uses photosynthetic process to capture solar energy and store it in the form of chemical fuels (Mussgnug et al., 2007). Figure 2 shows that based on the mechanism of photosynthesis, microalgae utilise water from the environment, light energy gotten from the sun and carbon dioxide from the atmosphere as a carbon source to produce oxygen and energy which is stored in the form of starch and fatty acids inside the cell. However, the fatty acids can be recovered from

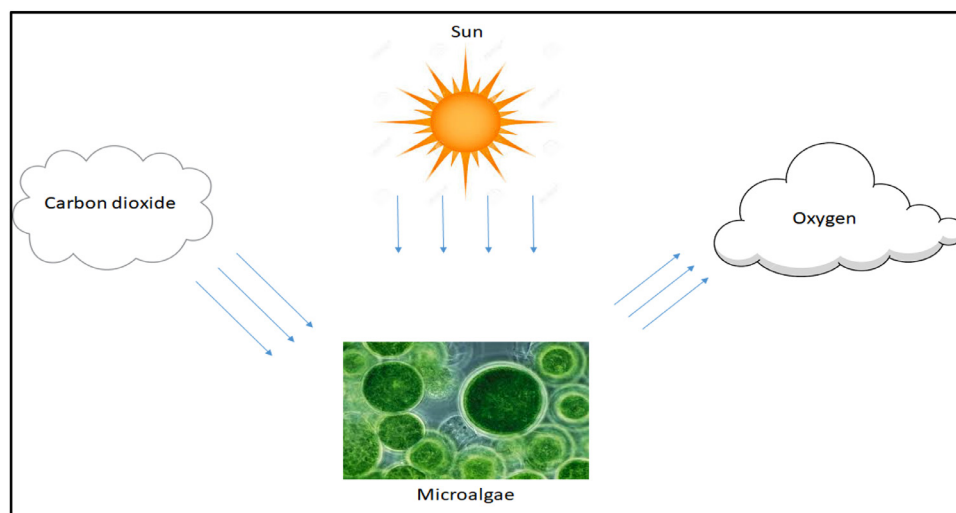


Figure 2. Schematic overview of the proposed mechanism towards sustainable microalgae biological carbon capture.

microalgae and transesterified to produce a carbon neutral biodiesel (Mondal et al., 2016). As a result, both atmospheric and industrial CO_2 can be captured and converted biomass and bioenergy using microalgae ponds. The three major pathways for carbon acquisition in microalgae cultivation include; active, direct uptake of HCO_3^- , an active CO_2 transport mechanism, and a carbonic anhydrase (CA) enzyme external to the plasma membrane (Shahid et al., 2020). The key factors influencing microalgal photosynthetic efficiency and CO_2 assimilation include heat, light, and mass transfer of CO_2 and nutrients (Daneshvar et al., 2021). Others are the properties of flue gas such as CO_2 concentration, temperature and toxic compounds.

Generally, a simple diffusive uptake of dissolved CO_2 in microalgae is relatively rare, even though, microalgae cells have a high affinity for CO_2 molecules (Daneshvar et al., 2021). It has been reported that most microalgae species growth is favoured at a pH range between 7.0 and 8.4 (Daneshvar et al., 2021), conditions under which majority of inorganic carbon is available as bicarbonate ions (HCO_3^-). The absorbed CO_2 is utilized as a source inorganic carbon for microalgae cultivation instead of being stored, converting the inorganic carbon to organic carbon-based compounds such as lipids, proteins, carbohydrates, pigments, and phenols via photosynthesis. The carbon in the CO_2 molecule is thereby fixed to the molecular skeleton of these substances, where it is used to perform different biological functions.

The microalgae cultivation systems could be open-pond or closed bioreactor which can be improved by developing low-cost growth media or using wastewaters as growth media to reduce the cost (Shahid et al., 2020). Figure 3 shows the stages involved from microalgae cultivation pond via photosynthetic fixation of CO_2 into microalgae biomass to biomass processing into bioenergy and value-added products. The generated CO_2 from the combustion of the biofuels closed-loop in a circular carbon bio-economy. The physico-chemical factors influencing microalgae cultivation include pH, temperature, salinity, culture medium and turbidity.

4.1. Why microalgae as an alternative carbon capture

Having looked at different approaches to carbon sequestration, microalgae is a promising alternative for carbon sequestration because of their unique features (Herzog et al., 1997). It is interesting to note that microalgae exist in temperate, tropical and Polar Regions as a habitat. They flourish in aquatic habitats and grow in soil, deserts, oil field, bare rocks and hot springs (Singh and Ahluwalia, 2013). Microalgae are referred as the primary energy conversion with its simplicity to adapt to the prevailing environmental condition in the long term. Furthermore, the intensity of light of typical microalgae is relatively low

compared to other higher plants. For instance, the light intensity of *Chlorella* and *Scenedesmus* (microalgae) is in the order of $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Rodolfi et al., 2009). There is high aerial productivity in microalgae compared to other photosynthesis organisms. In addition, microalgae can tolerate saline water. Report has it that 70% of the total global freshwater are from the use of agriculture due to scarcity of fresh water in many parts of the world. This allows greater potential utilization of microalgae in algaculture. Hence, microalgae do not require herbicides or pesticides application, as mentioned by Rodolfi et al. (2009). Previous studies revealed that Nitrous Oxide is present in the atmosphere as a result of the use of Nitrogen (fertilizer) to produce crops for biomass and biofuel tends to contribute to global warming terms. This can be compared with the quasi cooling effect of saving emission of fossil fuel-derived CO_2 which could add to more global warming than cooling by fossil fuel saving (Crutzen et al., 2007). These serious hazards were looked into or solved by applying fast-growing microalgae for carbon sequestration. Others relatively choice of microalgae for CO_2 capture in respective of others biological captures such as forestation and ocean fertilization as earlier mentioned deals with biomass productivity, photosynthesis efficiency, flue gas utilization, waste water use, and biomass application.

Above all, despite the simple yet versatile nutritional needs, the ability of microalgae to survive in an environment with extreme conditions puts them ahead of other feedstocks. Furthermore, waste gases from flue gas and various pollutants from agricultural, industrial and sewage wastewater sources such as CO_2 and NO_x , SO_x , and inorganic and organic carbon, N, and P can meet the nutritional requirements of microalgae as shown in Figure 4. Meeting these nutritional needs provides us with opportunities to convert this waste into bioenergy, valuable products and to forms less harmful to the environment (Pires et al., 2012; Mofijur et al., 2019; Ho et al., 2014; Singh and Thakur, 2015).

Figure 4 provides a detailed illustration involving the concept of integration of microalgae for carbon capture and sequestration and the paths to bioenergy production. The concept deals with the use of nitrogen and phosphorus-rich wastewater, which replaces the artificial media that support microalgae growth and nutrients. During this process, CO_2 is recycled through a refining process, producing renewable bio-products and bioenergy formed by converting harvested algae biomass (Zhou et al., 2017). This enhances the economic viability as well as the environmental friendliness of using a microalgae-based CO_2 system.

Compared to terrestrial plants, the simple cellular makeup and growth rate of microalgae have enhanced their CO_2 fixation potential to as high as 10-50 folds (Li et al., 2008; Alam et al., 2012; Khan et al., 2009). After fixation, the supplied carbon ends up becoming an integral component of lipids, proteins, sugars and pigments. In addition to its

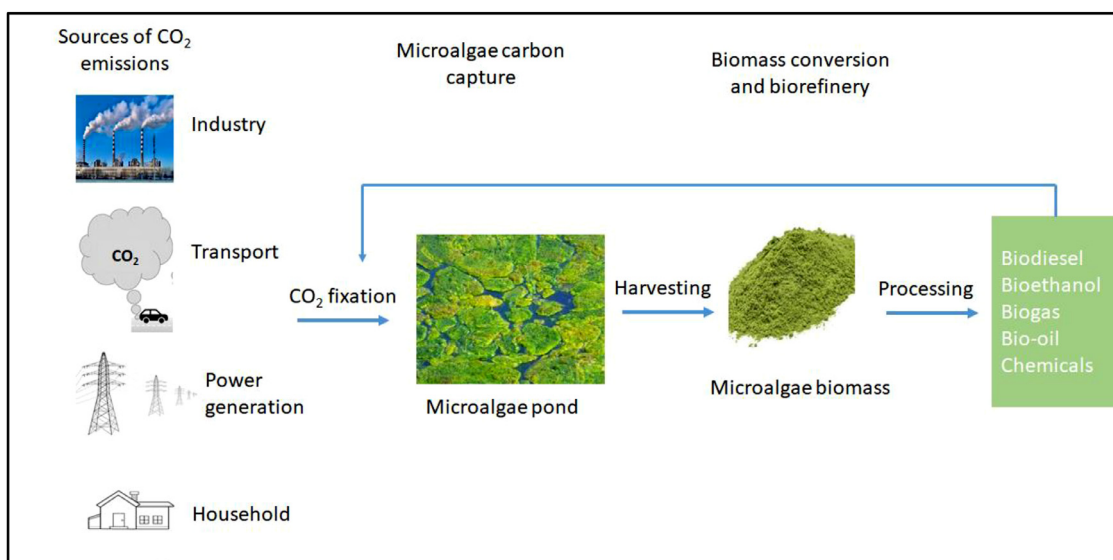


Figure 3. Schematic representation of microalgae cultivation, photosynthetic fixation of CO₂ into microalgae biomass and biomass processing.

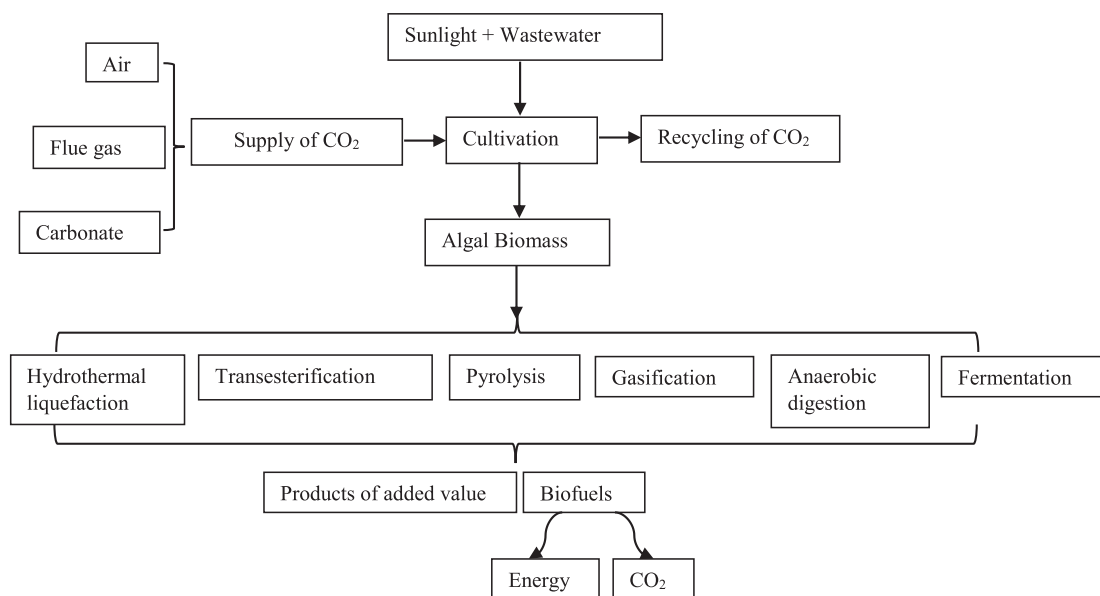


Figure 4. Schematic diagram of microalgae-mediated CO₂ bio capture and system integration (Modified from Zhou et al., 2017).

potential for scavenging carbon dioxide for bioenergy production, some microalgae can be utilised as single-cell protein, reducing the need for extensive livestock production, which has been shown to be a significant contributor to greenhouse gas emissions (Zhou et al., 2012; William and Laurens, 2010). As clearly highlighted by Chew et al. (2017), one of the most promising strategies to maximize the potential production is to use the biomass in an integrated bio-refinery set-up completely. This strategy will make it possible for every valuable component to be extracted, processed, and valorized (Chew et al., 2017).

In the aquatic capture of carbon using algal ponds and biomass production systems, the ability to capture CO₂ in a nongaseous form is considered another advantage. Once captured, this can be used as bicarbonate to improve microalgae growth (Sayre, 2010). As microalgae can concentrate bicarbonate in the cell through their active bicarbonate pumps, the bicarbonate is used to cultivate microalgae at moderate pH (> pH 7) and temperatures (below 30 °C) are subsequently dehydrated, spontaneously or by carbonic anhydrase (Mondal et al., 2017; Sayre, 2010). The resulting CO₂ is then captured through Calvin-cycle activity in the

form of algal biomass. Comparatively, anthropogenic sources of CO₂ are more concentrated and have more contaminated molecules than industrial sources of CO₂ (Xu et al., 2019; Arun et al., 2020; Sayre, 2020; Singh and Thakur, 2015). These attributes influence the design of CO₂-delivery systems for microalgae ponds. Typically, the concentration of CO₂ in flue gases from fossil fuel power plants are high, ranging from 10% to 20% (Sayre, 2010; Allah et al., 2016). Interestingly, these gases also contain significant amounts of biological gases, nitrous and sulfur oxides (NO_x and SO_x). When flue gases are injected into algal ponds, it leads to a threefold elevation of algal biomass, yields increasing productivity (Gillis and Hwang, 2003).

The physiological state of the algae, pond chemistry and temperature play a key role in the efficiency of microalgae to capture CO₂. If these conditions are optimally maintained, CO₂ capture efficiencies as high as 80% to 99% and gas residence rates as short as two seconds are highly achievable (Keffer and Kleinheinz, 2002). As it is not considered feasible to capture CO₂ in the dark, it is vital to develop a method that will serve as an integrated solution for capturing, concentrating, storing, and

Table 4
CO₂ tolerance of microalgae species

Microalgae species	Maximum CO ₂ tolerated (%v/v)	References
<i>Cyanidium caldarium</i>	100	Seckbach et al., (1979)
<i>Scenedesmus</i> sp.	80	Kandimalla et al., (2016); Sun et al., (2016)
<i>Chlorococcum littorale</i>	60	Ota et al., (2015)
<i>Synechococcus elongatus</i>	60	Miyairi, (1995)
<i>Euglena gracilis</i>	45	Nakano et al., (1996)
<i>Chlorella</i> sp.	40	Kao et al., (2014); Marin et al., (2018)
<i>Eudorina</i> sp.	20	Hanagata et al., (1992)

(Note: The data obtained are constant though few old references)

transporting CO₂ from sources to pond during the day. This method will help ensure CO₂ is captured as much as possible (Kadam, 1997).

5.0. Microalgae strains for carbon capture

The study has revealed that CO₂ capture using microalgae seems to be a promising technology to minimize or eliminate environmental problems. The concern came about as a result of the increase of GHGs concentration present in the atmosphere. Having established this, identifying the microalgae species, strains, and specific growth condition is of the essence. Some of these conditions include temperature, light intensity, nutrient concentration, water quality, and physiochemical water analysis. This condition maximizes the microbial growth rates. However, microalgae species capable of tolerating CO₂ concentration greater than 20% are classified as CO₂ tolerant microalgae groups, while CO₂ sensitive can only withstand 2-5% CO₂ concentration (Prokop et al., 2015; Morales et al., 2015). Owing to the increasing amount of CO₂ in the atmosphere, the ability of microalgae to tolerate a high concentration of CO₂ is becoming a key factor for selecting suitable microalgae species for carbon capture purposes.

As a result, one of the significant constraints in improving CO₂ capture processes is finding adequate microalgae strains capable of tolerating high CO₂ concentrations (Vuppalladadiyam et al., 2018). Due to the high concentration of CO₂ witnessed in flue gases in a similar vein, CO₂ sensitive microalgae strains are not preferred for CO₂ capture processes, but rather CO₂ tolerant microalgae strains, capable of tolerating high CO₂ concentrations and high temperatures (Bhati et al., 2019). Other attractive characteristics of CO₂ tolerant microalgae strains are high CO₂ fixation rates and the capacity to grow in the presence of toxic compounds such as NO_x, SO_x, and H₂S (Cheah et al., 2016; Yen et al., 2015). As shown in Table 4, the following species *Chlorella* (Kao et al., 2014; Marin et al., 2018), *Scenedesmus* (Kandimalla et al., 2016; Sun et al., 2016), *Chlorococcum* (Ota et al., 2015), have effectively been used for the capture of CO₂ present in effluents and those emitted from industrial activities.

6.0. The current state of the art

Here, the section focus on the current knowledge about the biological capture of carbon footprint via microalgae. This section is accomplished through similar analysis or related published work in the field. Attention will be drawn to providing a comprehensive overview of previous works and future studies to be undertaken on the subject matter. Prior, there have been successful studies on the utilization of microalgae biomass for bio-products production in bio-refinery. However, economic feasibility and microalgae bio-refinery are costly and unrealized, respectively (Zhou et al., 2017). Feasibility and sustainability of the methodology can be achieved provided upstream and downstream processing is simplified and integrated. For, the efficiency of the upstream processing is determined by microalgae strain selection (see the previous section), nutrient supply (CO₂, N, and P) and culture conditions such as temperature and light intensity (Vanthoor-Koopmans, 2013). In addition to the microalgae strains for carbon capture discussed earlier, this can be im-

proved by inducing acclimation through manipulation of different environmental stresses (Chen et al., 2017; Schuler et al., 2017). Considering the findings obtained by Aslem et al. (2017), it was emphasized that an unfiltered coal-fired power plant containing 11% CO₂ was adapted over several months to survive in 100% flue gas as a result of the mixed diverse microalgae community primarily dominated by *Desmodesmus* spp. Improving the efficiency of microalgae strains by genetic and metabolic engineering, as earlier mentioned in the study. However, lately, genome editing tools such as clustered regularly interspaced short palindromic repeat and transcription activator like effector nucleases can be used in microalgae gene alteration.

Interestingly, studies focusing on microalgae genetic and metabolic engineering were reviewed by Ng et al. (2017) and Jagadevan et al. (2018). On microalgae genetic and metabolic engineering, Yang et al. (2017), conducted a similar study on the genetic engineering of Calvin cycle of *Chlorella vulgaris* to enhance its photosynthesis capacity by 1.2 fold. This process is facilitated by introducing the cyanobacterial fructose 1, 6-bisphosphate aldolase, which is guided by a plastid transit peptide. Moving forward, screening an alkali-tolerant was done by Kuo et al. (2017). This approach was developed by *Chlorella* sp. ATI mutant strain by NTG mutagenesis that survived with 10% CO₂ for the prospective CO₂ sequestration. The cultivation of microalgae on a large scale and the supply of nutrient provides an economic hindrance. However, this is based on biofilm-based attached cultivation instead of aqueous suspend methods, which requires low biomass productivity, energy-intensive and absence of scaled-up (Wang et al., 2017). Besides the carbon footprint reduction through microalgae production, Pires et al. (2012) and Singh and Thakur (2015), mentioned that microalgae production with the aid of wastewater from industrial and agricultural sources is a promising technology used to reduce ecological footprints in a substantial manner. For effective, successful employment of microalgae for carbon footprint minimization, the centrifugation method is recommended as it provides up to 95% efficiency method used to harvest microalgae. However, the challenge of using centrifugation has to deal with its high cost-intensive and not suitable for large scale systems. With the limitations of the centrifugation method, the use of flocculation as an alternative for microalgae harvesting was proposed. Flocculation is less expensive but usually influences the toxicity of the biomass and output water (Ryan et al. 2009). Filamentous fungal spores were added to the microalgae culture under optimized conditions in a study conducted by Zhou et al. (2012). As a result, there was a development of pellets formed after 2 days, which was harvested by simple filtration. Other technologies for the harvesting of microalgae for the reduction of carbon footprints include conventional disruption methods and physical disruption methods (pulsed electric field). The methods of cultivation are suspended, bioreactor and attached. In the attached method, high density microalgae paste is attached on the surface of artificial substratum material to create biofilm that is photosynthetically active, making it easy to supply microalgae biomass for bio-refinery. This cultivation strategy for microalgae offer many superiorities over the conventional aqua-suspend approaches such as pen pond, which require huge water, easy contamination and challenges with scale-up. A detailed review

on biofilm based attached cultivation method for microalgal has been reported elsewhere (Wang, Liu and Liu, 2017).

7.0. Current challenges in the bio-capture of carbon using microalgae

The attractive potential of microalgae to capture carbon, which can be utilized in the production of biofuels and other high value-added products, had gained more and more attention in recent times. However, some challenges still exist in microalgae carbon sequestration technology, which has brought its industrial application to a relative standstill (Xu et al., 2019; Li and Kang, 2011).

- 1 Cultivating microalgae for bio-capture of carbon is an outdoor process, and various conditions in the outdoors could play a role in the process. Most research and developmental activities on carbon capture using microalgae are currently in the laboratory phase, and all cultivation requirements are experimentally controlled (Xu et al., 2019; Li and Kang, 2011). Nonetheless, only a few outdoor large-scale demonstration devices are available. The full cooperation of engineering technicians and theoretical researchers is needed to ensure maximum control of these uncertain factors and maintain the stability of culture conditions (Li and Kang, 2011). There is also a need to study the adaptability and growth of algae species in the natural environment and the possibility of producing photoreaction systems (Li and Kang, 2011).
- 2 According to Li et al. (2011), for complete fixation of carbon by microalgae to take place, the following step must be completed i) The CO₂ must transfer from the gas phase to liquid phase; ii) followed by the transfer of the liquidized gas into the intracellular algal environment; and iii) the growth, conversion, and utilization of CO₂ by the microalgae. The first and second steps are associated with fluid flow and mass transfer equipment; hence, they are considered physical processes. Also, the low solubility of CO₂ in water could contribute to mass transport limitations (Song et al., 2019), and this needs further attention. On the other hand, the third step is entirely a bio-transformational process. Current investigations on algae species seek to understand the effects of CO₂ concentration, temperature, pH value, and light on CO₂ fixation by microalgae by developing tools to measure the rate of CO₂ fixation microalgae under various biological and physical transformation conditions. Furthermore, these studies aim to understand the effects of CO₂ concentration, temperature, pH value and light on the rate of CO₂ fixation by microalgae by exploring the micro-mechanism of CO₂ fixation by microalgae. However, all these studies have focused on measuring the apparent rate of carbon capture of the above three main processes. Research methods need to be improved to help understand algae growth law and make the selection of efficient and suitable algae species less misleading (Li and Kang, 2011).
- 3 The culturing process of microalgae involves mass and heat transfer, lighting conditions, and biological reactions. These processes make the photoautotrophic culture of microalgae a complex process (Xu et al., 2019; Singh and Dhar, 2019; Mondal et al., 2017). At present, research and development activities aiming to smooth these processes have faced setbacks due to limited direct experience and a lack of theoretical research. In addition, there is a lack of theoretical calculations and a system design basis for flow and mass transport evaluations. Therefore, optimizing the theory and structuring the runaway pool and plate reactor to be low-cost, simple, and easy-to-operate will help minimize the high cost, complex structure, high productivity, and complex operation involved with photoautotrophic cultivation microalgae for carbon capture purposes (Li and Kang, 2011; Zhou and Ruan, 2014).
- 4 There is a dearth of theoretical research offering insights into the CO₂ absorption process around the cell membrane. Empirical operation or random selections are the primary mechanisms through

which CO₂ absorption takes place (Xu et al., 2019; Sayre, 2010). Currently, there limited data available in the literature on thermodynamic and kinetic research on the CO₂ absorption process. This striking fact highlights the need to design experiments that provide data to support the industrial production process. Such experiments will help overcome the difficulties involved in realizing quantitative control of the industrial process (Li and Kang, 2011).

- 5 The photosynthetic efficiency of microalgae is high. While this is a considerable advantage, algae cultivation still requires arable land and are capable of surviving even in places that other crops plants cannot inhabit, difficult to meet in industrial zones. To ensure all economic and other potential constraints are avoided, industrial waste gas removal from industrial zones needs to be practically demonstrated. However, interdisciplinary methodologies encompassing system engineering, chemical engineering, biotechnology, materials and manufacturing engineering are critical to sufficiently tackle this problem (Li and Kang, 2011). In a similar vein, increasing the added value of microalgae products and the overall economic process will require applying these products in medicine and food. However, it is vital to evaluate and verify the quality of algal products cultured by industrial waste gas (Li and Kang, 2011; Zhou and Ruan, 2014; Roger et al., 2018).
- 6 There is a risk of parasitism of microalgae used in carbon capture and storage by some bacterial and fungal species. These can have a devastating effect on the commercial cultivation of microalgae, directly attacking the microalgae, resulting in death or actively competing for nutrients within the growth environment. When these interactions are not effectively managed, the productivity of the system falls dramatically (Yao et al., 2019). In the past few years, some important pathogens of microalgae used in commercial cultivation systems have been identified. These include fungi such as Chytridiomycota (Kagami et al. 2012), Aphelids (Karpov, 2013), and other parasites such as Amoebozoa, which are invasive dinoflagellates (Chambouvet et al., 2011; Chambouvet et al., 2008).

7.1. Improving bio-capture of CO₂ through genetic engineering and metabolic modifications

Genetic engineering and metabolic modifications are global strategies extensively studied for improving bio-capture of CO₂ using microalgae (Ruiz-Ruiz, 2020; Goma et al., 2016). One of these main strategies is the modification of the Ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCO) system, which is employed as the primary carboxylase system in the carbon fixation of atmospheric carbon dioxide. This approach is aimed at improving the selectivity, velocity and productivity of microalgae cells. Unfortunately, this approach has produced little success because the simultaneous enhancement of RuBisCO's selectivity and velocity could not be achieved (Eva et al., 2019; Zhu et al., 2010). Interestingly, it has been suggested that modifying microalgae's catalytic rates or its relative affinity toward CO₂ may improve RuBisCO (Ruiz-Ruiz, 2020). Through these two approaches, which also involves overexpressing genes encoding for RuBisCO's subunits or through the overexpression of some natural variants of this enzyme, RuBisCO's activity has shown significant improvements. The emerging picture suggests that this resulted in a four-fold increase in the microalgae cells' activities and an increase in growth and biomass productivity (Cheah et al., 2015; Atsumi et al., 2009; Iwaki et al., 2006; Liu et al., 2010; Chen et al., 2012).

Generally, increasing catalytic velocity and selectivity through RuBisCO modification is a preferable approach; however, overcoming the selectivity problem could also be overcome with a bioreactor designed to contain a high concentration of CO₂ (Ng et al., 2017; Kamennaya et al., 2015). Ironically, RuBisCO is not the only enzyme capable of enhancing carbon fixation. Various high-profile enzymes involved in the Calvin-Benson cycle, such as sedoheptulose-1, 7-bisphosphatase, transketolase and aldolase, have also been engineered to enhance carbon fixation

(Ng et al., 2017; Kamennaya et al., 2015; Zhou et al., 2016). However, abiotic factors such as excess light, which can induce photo inhibition, have been shown to affect the efficiency of carbon fixation. These factors can result in inefficient utilization of light and decreased photosynthetic competence of microalgae cell (Ng et al., 2017; Kamennaya et al., 2015; Zhou et al., 2016).

Several genetic and molecular modification approaches to improve microalgae's photosynthetic efficiency have been attempted (Blankenship and Chen, 2013; Ort et al., 2015; Seth and Wangikar, 2015; Wobbe et al., 2016). An interesting approach is the molecular modification of the truncated light-harvesting antenna (TLA). This concept seeks to increase light penetration into the microalgae cells by reducing the antenna size in microalgae, resulting in higher biomass productivity (Beckmann et al., 2009; Masuda et al., 2003; Mussnug et al., 2003; Cazzaniga et al., 2014). Other reported benefits of this approach are: i) the prevention of over-absorption of photons by individual cells, ii) enabling deeper sunlight penetration into the culture, and iii) allowing more cells to be productive (Kirst and Melis, 2014). In recent times, novel genome-editing tools have been used in the gene modification of microalgae cells. Some of these tools are CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats – associated protein 9), Zinc-Finger Nucleases (ZFN), and Transcription Activator-Like (TAL) Effector Nucleases, TALEN (Shin et al. 2016; Spicer and Purton, 2016). These tools are used to insert genes into specific locations with the microalgae's genome (Huang et al., 2016; Yao et al., 2016). Furthermore, an integrative technology known as omics is another sustainable approach for enhancing carbon capture using microalgae (Banerjee et al. 2016). Research and development activities and interests in this area are gaining immense attention lately. Supposing that all approaches are developed and applied successfully in microalgae, this will enhance the production of natural bio-products and improve the functionalities of microalgae cells used for the bio-capture of carbon (Huang et al., 2016).

7.2. Environmental impacts

Since fossil fuels are responsible for greenhouse gas emissions, it is rational to shift from conventional fuels to microalgae biofuels, which has shown tremendous environmental benefits. Algae can utilize the excess CO₂ in the atmosphere for growth and biofuel production. However, extensive research to understand the potential disadvantages of these biofuels are still limited (Allah et al., 2016). Nevertheless, as demonstrated so far in this review, bio-carbon capture can contribute immensely to neutralizing the levels of CO₂ emissions coming from anthropogenic activities. Regardless of the challenges associated with algae value-added products and biofuel, promising and sustainable results have been reported by Usher et al. (2014) and Slade et al. (2013). For instance, the utilization of raw algal oil in an internal combustion engine approach showed lower NOx emissions (Tsaousis et al., 2014). Furthermore, an emulsified biodiesel blended with microalgae, yeast or bacteria biodiesels showed lower NOx emissions for microalgae biodiesel blends (Wahlen et al., 2013). These exciting findings suggest that microalgae's capture and utilization of CO₂ to produce valuable products and biofuel offer economic opportunities over a range of timescales and positive environmental benefits. However, algal blooms due to over cultivation with algae could result in dissolved oxygen and BOD deficiency in ponds, which could have potentially dangerous implications for the pond's biodiversity. This environmental effect could be resolved through controlled growth and harvesting.

8.0. Conclusion

Biological CO₂ capture through fast-growing microalgae from point sources is one of the critical aspects that can ultimately help decarbonise and, hence, ameliorate global warming. Significant carbon dioxide emissions emanate from power generation, combustion of fuels and process

industries. As an antidote, we can leverage microalgae's ability to capture CO₂ and lock them into their organelles. Unlike the conventional carbon capture techniques mostly applicable to power plants, this approach is suitable for carbon emission from the transportation sector and, at the same time, a source of biofuel for the sector resulting in carbon neutrality. Furthermore, the highly developed carbon concentrating mechanisms (CCM) comprising carbonic anhydrase (CA) can significantly capture atmospheric CO₂ and convert the captured CO₂ into biomass. Although numerous articles reporting on the bio-capture of CO₂ by microalgae have been published, more research is needed to mitigate global warming. Nonetheless, we envisage that this promising technique will contribute immensely to reducing atmospheric CO₂ to combat global warming in the near future. Hence, it can be concluded that before bio-capture of CO₂ through microalgae can become a reality, it is imperative to continue investing extensively in the research and development of technology and technical expertise in this area towards a sustainable green environment.

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