Capture, Storage and Utilization of Carbon Dioxide by Microalgae and Production of Biomaterials

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Abstract – Carbon dioxide emissions are strongly related to climate change and increase of global temperature. Whilst a complete change in producing materials and energy and in traffic and transportation systems is already in progress and circular economy concepts are on working, Carbon Capture and Storage (CCS) and Carbon Capture and Utilisation (CCU) represent technically practicable operative strategies. Both technologies have main challenges related to high costs, so that further advanced research is required to obtain feasible options. In this article, the focus is mainly on CCU using microalgae that are able to use CO₂ as building block for value-added products such as biofuels, EPS (Extracellular Polymeric Substances), biomaterials and electricity. The results of three strains (UTEX 90, CC 2656, and CC 1010) of the microalgal organism Chlamydomonas reinhardtii are discussed. The results about ideal culture conditions suggest incubation temperature of 30 °C, pH between 6.5 and 7.0, concentrations of acetate between 1.6 and 2.3 g L⁻¹ and of ammonium chloride between 0.1 and 0.5 g L⁻¹, the addition of glucose. This green microalga is a valid model system to optimize the production of biomass, carbohydrates and lipids.

Keywords – Carbon Capture and Storage (CCS); Carbon Capture and Utilisation (CCU); climate change; CO₂; environment

Nomenclature

| Abbreviation | Description |
|--------------|-------------|
| CCS          | Carbon Capture and Storage |
| CCU          | Carbon Capture and Utilisation |
| CCM          | Carbon Concentrating Mechanism |
| CA           | Carbonic Anhydrase |
| CCB          | Calvin-Benson-Bassham cycle |
| rTCA         | Reductive tricarboxylic acid cycle |
| (3HP-4HB)    | 3-hydroxypropionate 4-hydroxybutyrate cycle |
| PBR          | Photobioreactor |
| EPS          | Extracellular Polymeric Substance |
| TAP          | Tris-Acetate-Phosphate |

1. Introduction

Copernicus Climate Change service has indicated 2020 as the globally warmest year in the decade 2011–2020. Europe has experienced in 2020 a 0.4 °C average increase in temperature...
compared to 2019 data. Northern Siberia and parts of the Arctic have registered the highest deviation from 2020 average temperature of 3 °C and even 6 °C in some areas. Atmospheric CO₂ concentrations are on a global increasing trend of ca. 2.3 ppm/year and in May 2020 the concentrations have reached the highest value ever recorded of 413 ppm [1].

Without any actions, theoretical modelling previews that CO₂ could have a 60 % increase by 2100 [2]. To reduce CO₂ concentration levels, three main strategies are suggested:

- to use more wisely natural resources and increase the efficiency of all processes that emit CO₂;
- to use a sustainable energy and sustainable fuels, which are less carbon intensive. The latter are made from renewable and alternative raw materials in replacement of fossil fuels. Biofuels such as biodiesel, biohydrogen and biogas are valid options;
- to capture and remove CO₂ from the atmosphere with technologies like CCS and CCU. CCS consists mainly of three steps: First, carbon dioxide is captured, then CO₂ is concentrated in order to lower transportation costs and then it is stored. There are different techniques to capture CO₂ in CCS technologies, which are mainly physical-chemical or biological and in this paper bio-sequestration is discussed. The storage process consists mainly of geological sequestration (injecting the CO₂ in underground reservoirs) or ocean injection.

These strategies can be combined to have better results. Indeed, CCU consists in both capturing CO₂, thus removing it from the atmosphere, and using it to produce value added products that include biofuels. CCU could be performed by microalgae or bacteria that have CO₂-fixing mechanisms [3].

CCS and CCU both represent promising strategies to tackle CO₂ emissions and hence climate change. The different steps of CCS and CCU technologies and the different organisms that could be used in CCU processes are schematically represented in Fig. 1.

Fig. 1. Scheme of two possible solutions to limit CO₂ increasing levels: CCS and CCU technologies with different processing steps.
2. Technology

2.1. Carbon capture technologies: CCS and CCU

Nowadays, many different methods are operative in carbon capture technologies to capture CO₂. Chemical-physical capture of CO₂ is usually associated with subsequent storage in man-chosen reservoirs while biological capture is usually associated with the increase in biomass of forests, grasslands, and microorganisms. In the following section, these two main groups are presented.

2.1.1. Chemical-physical procedures

- Physical absorption. It consists of using solvents, which physically capture CO₂ until the saturation level. Once the solvents are saturated, they are treated to release the CO₂ which can be then stored separately and concentrated. To regenerate the solvent high temperatures are often used [3], [4].
- Adsorption. In this process a specific adsorbent surface is used. It strongly interacts with CO₂ allowing the elimination from the original gas mixture. The adsorbent is then regenerated by either TSA (Temperature Swing Action) or PSA (Pressure Swing Action) inducing CO₂ release [3]–[7].
- Membrane technologies. Selective membranes are used to allow the transfer of CO₂ while other compounds cannot pass through. This technology is very useful in case of high CO₂ partial pressures. To increase the efficiency, many different types of membranes have been developed such as non-facilitated membrane technologies, facilitate transport membrane separation, mixed matrix membrane, gas membrane contractor [3], [4], [8].
- Cryogenic separation. This process is conducted at low temperature conditions and several compressions are used to produce liquid CO₂. The formation of ice and the requirement of low temperatures make this process expensive [3], [4].

2.1.2. Biological procedures

- Forestation. It can be reforestation, afforestation and change in land use. It consists in implementation of both higher plants and lower plants (like mosses in forest floor) using CO₂ to produce solid biomass thanks to photosynthesis.
- Oceanic fertilization. By increasing the concentration of iron and other nutrients in the ocean, the efficiency of the phytoplankton CO₂-uptake increases, so that CO₂ can be removed from the atmosphere [9].
- Microalgae and bacteria. Carbon-fixing microorganisms use CO₂ to increase their biomass. If the microorganisms are able to produce value added compounds then this type of carbon capture can be used for CCU applications [10].

2.2. CCS (Carbon Capture and Storage) technologies

CCS technologies consists in capturing CO₂, concentrating it, transporting it and then storing it in some sort of reservoir that could either be geological or oceanic.

There are different CCS approaches that are used today in energy production facilities:
- Pre-combustion. In this case the separation of CO₂ from the fossil fuel occurs before the burning process. Fuel and O₂ react in a partial oxidation reaction to produce syngas (H₂, CO, CO₂ and lesser amount of other gases), while steam is then added to produce CO₂ and H₂. At this point, CO₂ is captured and compressed whilst H₂ is used as fuel to
power turbines.

- Post-combustion. This is the most used approach in power plants and consists in burning fossil fuels and then adsorbing the CO₂ while is produced during the combustion. The produced steam is used to activate turbines and to produce electricity. If coal is used, then, additionally, toxic gasses are also emitted, like sulphide dioxide (SO₂) and nitrogen monoxide (NO). To avoid polluting effects, the gas mixture needs treatment to be released into the environment.

- Oxy-combustion. This approach uses pure oxygen to burn the fossil fuel determining a flue gas composed only of CO₂ and water vapor. This technique provides an easier capturing and concentration of CO₂ [3], [4].

The first two approaches are the most used and accepted but the development and progress in oxy-combustion approaches is gaining attention.

Transportation can be a very expensive step in CCS strategies. In order to improve the benefit-cost ratio it is important to reduce the distance from capturing to storage sites, to compress the gas, to use pipelines in bulk and to share transportation networks [4].

The choice of the storage location is a crucial step. The site must be porous and permeable in order to guarantee easy injections. Moreover, a rock cap is necessary in order to prevent leaking of CO₂ in the environment. Saline aquifers and depleted basins have been used in the past for their functioning characteristics [4]. Enhanced oil recovery is gaining attention as it can reduce the costs combining fossil fuels extraction with carbon injection. Storing in these underground reservoirs might be a viable option but research on the effects of CO₂ leaking must be performed as well as geological studies to identify proper storage sites.

In conclusion, CCS could be a very important way to reduce carbon emissions, guarantee more work positions and more clean energy in a short period whilst other technologies would be investigated to guarantee more fossil fuel independence. However, the environmental impacts that building the CO₂ transportation infrastructures and possible CO₂ leakage can have on the environment must be considered: Especially on groundwater, soil quality, biodiversity and ecosystems. The cost of the CCS technology is still the main challenge and to overcome it development and research in this field are urgent. For this reason, coupling CCS with CCU could be a viable solution to reduce CO₂ emissions.

2.3. CCU (Carbon Capture and Utilization) technologies

Carbon Capture and Utilization consists in using the CO₂ for different purposes after that it has been previously captured. Among the wide variety of ways to apply CCU, the use of microorganisms, such as bacteria and microalgae, is very promising. These organisms have evolved efficient carbon fixating mechanisms and they can be used to produce value added products including biofuels that could provide a cleaner way to produce energy.

2.3.1. CO₂ fixation mechanisms

There are mainly six different pathways, which the microorganisms use to fix and utilize CO₂ for metabolic purposes.

1. Calvin-Benson-Bassham cycle (CCB) is the pathway occurring in photosynthetic organisms. It consists of two phases: The first phase is light dependent and NADPH and ATP are produced. The second phase is light independent and carbohydrates and lipids are produced by consuming ATP and NADH.

2. Reductive tricarboxylic acid cycle (rTCA) is typical of organisms, which live in extreme environments characterized by high temperatures, anaerobic and/or acidic conditions;
3. Reductive acetylCoA pathway or Wood-Ljungdahl pathway occurs in anaerobic bacteria and produces methane and acetic acid from CO2;
4. 3-hydroxypropionate 4-hydroxybutyrate cycle (3HP-4HB) uses hydrogen Sulphur (H2S) as energy source;
5. 3-hydroxypropionate bicycle occurs exclusively in some green non-sulphur bacteria living in anaerobic conditions;
6. Decarboxylate 4-hydroxybutyrate cycle: occurs in anaerobic bacteria [2], [3].

These six different pathways can occur in aerobic or anaerobic conditions, as indicated in Table 1 alongside with indications of the enzymes that fix CO2 during these processes.

| Pathway                                      | Conditions                  | CO2-fixing enzyme                                                                 |
|----------------------------------------------|-----------------------------|----------------------------------------------------------------------------------|
| Calvin-Benson-Bassham cycle (CCB)            | Aerobic                     | RuBisCO                                                                          |
| Reductive tricarboxylic acid cycle (rTCA)    | Aerobic/Anaerobic [11]      | 2-Oxoglutarate synthase                                                          |
| Reductive acetylCoA pathway                  | Anaerobic                   | Formate dehydrogenase, CO dehydrogenate/Acetyl-CoA synthase                     |
| 3-hydroxypropionate 4-hydroxybutyrate cycle (3HP-4HB) | Aerobic                     | Acetyl-CoA carboxylase, Propionyl-CoAcarboxylase                                  |
| 3-hydroxypropionate bicycle                  | Anaerobic                   | Acetyl-CoA carboxylase, Propionyl-CoA carboxylase                                 |
| Decarboxylate 4-hydroxybutyrate cycle        | Anaerobic                   | Pyruvate synthase                                                                |

In photosynthetic microorganisms, such as cyanobacteria and microalgae, other than the CCB cycle, another remarkable mechanism is evolved, called Carbon Concentrating Mechanism (CCM). This mechanism has evolved due to the fact that RuBisCO, the key enzyme in photosynthesis, can use as substrate both CO2 and O2. If O2 is used instead of CO2, then the process of photorespiration occurs instead of photosynthesis causing a waste of energy and biological matter. CCMs guarantee high concentrations of CO2 in the proximity of RuBisCO’s active site and favor CO2 fixation through photosynthesis. The most important components in CCM are CAs (Carbonic Anhydrases) and Carboxysomes (in bacteria) or chloroplasts (in eucaryotic cells).

Carboxysomes are bacterial microcompartments that contain RuBisCO. They host the first step of CCB. Being separate compartments, carboxysomes can guarantee high CO2 and low O2 concentrations [2], [3]. Carbonic anhydrase are different enzymes that can serve different purposes if located in different cell compartments. In CCMs, CAs are on the membrane of carboxysomes and are essential because they convert bicarbonate, present in the cytoplasm, into CO2 while transporting it into the carboxysome.

As a conclusion, organisms that provide molecular tools to fix carbon and/or to produce other biological compounds promise much for the future because they are or can be genetically engineered [12], [13] to produce value added products and higher yields.
3. RESULTS AND DISCUSSION

3.1. Microalgae for CCU

Microalgae are potentially very interesting autotroph organisms that use CCB to produce biomass from CO₂ in the presence of light. These microorganisms have higher carbon uptake rates compared to higher plants (from 10 to 50 folds [10]), they can use nitrogen and sulphur oxides (NOₓ and SOₓ), which are often present in flue gasses, as nutrients, they can grow in non-arable land and also in very differentiated non-favourable climate conditions. Moreover, these organisms naturally produce chemical compounds that can be of economic interests in fields such as dyes, nutraceuticals, cosmetics, biofertilizers, bioactive substances and biomolecules that can be converted into further valuable products [10].

Currently, using microalgae at industrial level to produce specific chemical materials is not an economically viable practice because of the unfavourable benefit-cost ratio. According to an estimation [14], in 2016 the cost of a barrel of algae-based fuel was greater than USD 300, compared with petroleum which was available at 40–60 USD/barrel. Therefore, research are in progress in many scientific laboratories in order to increase the yield and the productivity of these systems. Possible strategies to reduce the costs of these technologies are:

– to combine the use of microalgae with other industrial production lines, for example coupling microalgae cultivation with CO₂ emitting plants: this matching could guarantee both a decrease in CO₂ emission in the atmosphere and high utilization of CO₂ to improve biomass growth or chemicals production (CCU) [4], [15];
– to identify the optimal conditions for growth and value-added chemicals production [15];
– to use different elements of the microalgae culture so that every component could generate profit [3], [15].

3.2. Microalgal culture parameters

Essential parameters to consider to optimize microalgae growth and production are schematically depicted in Fig. 2. Different microalgae species might have different requirements and so it is important to identify the favourable conditions for each species. Moreover, different products can also provide higher yields in different conditions; therefore, it is crucial to identify the ideal parameters for both the microalgal species and the production. In the following are presented the main culture parameters.

– Type of bioreactor. Three types of bioreactors are mainly used in microalgae mass production:

1. Open systems. These are the most commonly used reactors because they are less expensive to install and the upkeep is relatively simple. The tanks are long, not very deep (20–50 cm in depth [16]) and uncovered in most cases. Problems in the culture are various, ranging from risks of contamination to evaporation and scarce mass and CO₂ transfer and light scarcity in some areas of the bioreactor.

2. Photobioreactors (PBRs) or closed systems bioreactors. These are very expensive but guarantee better control of operating conditions, less risk of contamination and provide better yield [17].

3. Hybrid systems. These are composed of two main reactors: The first one is a closed bioreactor and the second one is an open bioreactor. In the PBR, microalgae are grown in optimal conditions until desired density is reached; then the microalgae are transferred into the open bioreactor, in which growth conditions are limiting in order to achieve higher lipids yield (in case these are the desired compounds) [16].
Light and depth. Light is a key element in algal growth because it has to be strong enough to guarantee high photosynthesis rates and has to reach also non-superficial cells. On the other hand, excessive light can damage photosystems decreasing the process yield. Light amount, wavelength, and duration can be modulated to optimize the culture in relation to the specific microalgal organism. Light intensity between 200 and 400 μmol photons m\(^{-2}\) s\(^{-1}\) [15] seems to increase carbohydrates production. Light spectral range can also be modified and for different microalgae species different wavelengths seem to provide best growth rates. Moreover, light/dark photoperiods and pulsed/continuous lighting in different cultures seem to guarantee higher levels in terms of lipid production [15], [16]. Depth is an important parameter due to light penetration. Height of the bioreactors and consequent liquid depth are relevant to avoid self-shading (more superficial microalgae prevent lighting to reach microalgae on the bottom of the bioreactor): a liquid depth between 15 cm and 35 cm is usually selected. Additionally, if CO\(_2\) is sparged in the medium shallow, then bioreactors shorten CO\(_2\) residence time in the medium; as a consequence, growth rate and biomass yield are lower [16].

Nutrients. Essential nutrients should be present in the medium at different concentrations according to the used microalgal strain. Benefit in using flue gas as CO\(_2\) source for algal growth is the presence of NO\(_x\) and SO\(_y\), which can be used as nutrients. However, an excess of SO\(_y\) can acidify the medium damaging the microalgae. It has been reported that often starvation conditions can shift microalgal metabolism and increase lipid production (especially nitrogen starvation) but a correct balance between stress and growth rate must be taken into consideration to effectively increase the process yield [15], [16]. Heavy metals like Fe, Co, Mn, Cu, and Zn are also important nutrients for microalgal growth. Different heavy metals might have different effects on different cultures. In general, high levels of concentration of heavy metals can damage cell growth but, in some cases, proper concentrations (high or very low, depending on the case) can influence positively the biomass production [15], [16].

CO\(_2\) and pH. Carbon dioxide must be at favourable concentration in the medium so that it can guarantee optimal CO\(_2\) fixation. Indeed, too high concentration might inhibit PSII activity and drastically decrease medium pH. Generally, ideal pH levels for microalgal growth are close to neutral or slightly basic (pH ≃ 8) for marine microalgae. Often buffers are used to maintain optimal pH levels, even at high CO\(_2\) concentrations conditions [15], [16].

Mixing. Adequate mixing is essential to guarantee efficient CO\(_2\) transfer rate, homogeneity in the medium and prevent cell-settling. On the other hand, excessive mixing can damage cells and could increase costs. Recently, we have monitored the mixing of an artificial model substrate to analyse the energy consumption [18], [19]. An aqueous cellulose solution was used to emulate the biomass in terms of viscosity and rheological properties [20], [21]. For microalgal cultivation, gas-liquid contacting devices are recommended for heat and mass transfer [16].

Temperature. Different species have different favourable temperature ranges for growth and biomass production. Critical temperatures can affect the metabolism and this is relevant especially in lipids production. At high temperatures lipid production tends to favour saturated fats over unsaturated lipids; this aspect must be taken into consideration when the final aim of microalgal cultivation is the production of biodiesel [16].

Salinity. Salinity might help to shift metabolic pathway. For example, higher salinity conditions increase simple carbohydrates production (in nutritional stressed
conditions) as a response to osmotic stress [15], [16].

3.3. Value-added products

Microalgae represent a very versatile class of microorganisms that can provide several different molecular compounds. Fig. 3 summarizes some possible value-added products, which can be gained from microalgae. Some of these products can be obtained by the same microalgal culture, resulting in lower production costs and making CCU a more economically sustainable option.

Among the value-added compounds, the following prove to be a worthwhile investment.

- PHA (Polyhydroxyalkanoate). This class of compounds might be produced by many different microorganisms, especially in stress nutrition conditions. PHAs can be used as carbon and energy deposit. PHAs are valuable because of the use as bioplastics providing an eco-friendly, biodegradable and biocompatible alternative with similar characteristics to fossil derived plastics [2], [10].

- EPSs (Extracellular Polymeric Substances). These compounds are normally produced by cells and could be interesting building blocks for novel biomaterials [2].

- Bioelectricity. With Microbial Fuel Cells (MFCs) electricity can be produced. O2 is generated by photosynthesis at the cathodic electron acceptor, while CO2 is produced in anodic oxidation as substrate for photosynthesis [22].

- Biogas. Methane, hydrogen and biohythane (a combination of the two gasses) are produced in anaerobic digestion processes during hydrolysis, acidogenesis, acetogenesis and methanogenesis. Biohydrogen can also be produced in biophotolysis or dark fermentation processes [10], [15].

- Bioethanol. Biomass is treated to extract fermentable sugars that are successively converted to produce bioethanol and other bioalcohols. Although microalgae have lower sugar concentrations compared to higher plants, they do not have lignin and therefore sugar extraction is easier to be processed [10], [15].

- Biodiesel: Biodiesel is a biofuel produced from the transesterification of triacylglycerols (TAGs) with an alcohol (usually methanol) in presence of an alkali, acid or enzymatic catalyst to produce FAMEs (Fatty Acid Methyl Esters) [23]. Microalgae represent a valuable biodiesel production system because their lipid
content might reach averagely 60% of their dry weight while nutritional stress conditions and other factors have indicated to increase lipid production up to 90% [10], [15], [16].

- Other compounds. Additional value-added products are pigments used in dyes, food and feed applications, cosmetics and pharmaceuticals but also vitamins and minerals, proteins, carbohydrates and lipids like omega fatty acids [10].

Fig. 3. Scheme of possible value-added compounds that might be produced directly by microalgae or by processing microalgal biomass.

3.4. Chlamydomonas reinhardtii green alga

*Chlamydomonas reinhardtii* is a unicellular green alga, which is widely used as model system for microalgal organisms [24]. It offers a simple life cycle, easy isolation of mutants and several possible techniques for molecular engineering studies. Nowadays, it is the most well characterized eukaryotic freshwater algae. This microalgal species seems to be very promising for lipid generation and thus for potential biodiesel production. Different parameters can be varied in order to identify the most convenient conditions in *C. reinhardtii* cultures for biomass, carbohydrate and lipid productions. In particular, pH, temperature, acetate concentration and ammonium chloride concentration are the parameters showing to be dominant. The effects of the different parameters on performance and productivity have been considered in different strains. Three strains seem particularly interesting and the main results are summarized in the tables: UTEX 90 (Table 2) [24], [25], CC 2656 (Table 3) [24], [25], and CC 1010 (Table 4) [24], [25].

For applications in CCU, both acetate and CO₂ can be used as carbon source, creating mixotrophic conditions which favour productivity of both biomass and carbohydrates [26]. Experiments on acetate as carbon source for microalgal growth indicate that *C. reinhardtii* prefers this source because it increases the expression of specific periplasmatic proteins [24]. The UTEX 90 and CC 2656 strains show different ideal values for the parameters, especially regarding acetate and ammonium chloride concentrations for biomass and carbohydrates-lipids production. The results in terms of productivity at the different culture conditions used in the two strains are shown in Table 2 and Table 3 [24], [25].

Using an airlift photobioreactor it was reported that cultivations with CO₂-air mixture at 7% (v/v) and under optimized conditions guarantee higher productivity in terms of biomass, carbohydrates and lipids in comparison with productivity of cultures without CO₂ injections.
The value of 7% (v/v) was specifically selected because for higher CO₂-to-air percentage the biomass and carbohydrate productivity decreases [25] due to altered pH levels and consequent increased expression of CA [27]. On the other hand, low CO₂ concentrations favor photorespiration over photosynthesis due to the fact that RuBisCO has affinity for both CO₂ and O₂ in term of dependence on the CO₂/O₂ ratio.

### TABLE 2. BIOMASS, CARBOHYDRATE AND LIPID PRODUCTION FROM STRAIN UTEX 90 UNDER IDEAL CULTURE CONDITIONS [24], [25]

| Working parameter | Ideal conditions for UTEX 90 | Biomass (mg L⁻¹ d⁻¹) | Carbohydrate (mg L⁻¹ d⁻¹) | Lipid (mg L⁻¹ d⁻¹) |
|-------------------|-------------------------------|----------------------|---------------------------|-------------------|
| pH                | 7                             | 983                  | 30.86                     | 25.29             |
| Acetate (g L⁻¹)   | 2.3 (for biomass)              | 235.7                | 80.79                     | 39.36             |
|                   | 1.56 (for carbohydrate/lipid) |                      |                           |                   |
| Ammonium Chloride (g L⁻¹) | 0.50 (for biomass) | 1.5                  | 88.72                     | 42.43             |
|                   | 0.10 (for carbohydrate/lipid) |                      |                           |                   |
| Incubation temperature (°C) | 30                          | 147.15               | 36.79                     | 36.79             |

Tris-Acetate-Phosphate (TAP) is a standard maintenance medium often used for *C. reinhardtii*: Ammonium NH₄⁺ serves as primary nitrogen source and Tris buffers the pH. Effects on microalgal growth and lipid production have been studied using the CC 1010 strain of *C. reinhardtii*. Among the parameters, the nitrogen and phosphorus starvation (TAP &N and TAP &P) and the glucose and vitamin B₁₂ (Cyanocobalamin) concentration were considered in relation to biomass concentration, lipid content and lipid productivity [28]. Some results are summarized in Table 4. Maximal lipid productivity (43 g L⁻¹ day⁻¹) was obtained under N starvation conditions with the addition of 0.1% glucose. Indeed, heterotrophic cells direct excess carbon towards lipid biosynthesis so that the presence of glucose in the medium improves lipid production. Similar positive effects of the nutrients on the lipid production have been observed also in other green microalgae [29]. The addition of glucose and glycerol provide higher lipid production also in *Chlorella vulgaris* [30] and *Tetraselmis suecica* [31]. On the other side, the addition of vitamin B₁₂, which is essential for some microalgal strains, does not seem to affect lipid production in CC 1010. Studies suggest that *Chlamydomonas* microalgae are able to produce cobalamin so that exogenous vitamin present in the medium is generally stored in the cell [32].

### TABLE 3. BIOMASS, CARBOHYDRATE AND LIPID PRODUCTION FROM STRAIN CC 2656 UNDER IDEAL CULTURE CONDITIONS [24], [25]

| Working parameter | Ideal conditions for CC 2656 | Biomass (mg L⁻¹ d⁻¹) | Carbohydrate (mg L⁻¹ d⁻¹) | Lipid (mg L⁻¹ d⁻¹) |
|-------------------|-------------------------------|----------------------|---------------------------|-------------------|
| pH                | 6.5                           | 780                  | 19                        | 12.85             |
| Acetate (g L⁻¹)   | 2.1 (for biomass)              | 171.43               | 46                        | 23.01             |
|                   | 1.56 (for carbohydrate/lipid) |                      |                           |                   |
| Ammonium Chloride (g L⁻¹) | 0.50 (for biomass) | 1.15                 | 51.6                      | 24                |
|                   | 0.20 (for carbohydrate/lipid) |                      |                           |                   |
| Incubation temperature (°C) | 30                          | 125.72               | 23.89                     | 16.35             |
Finally, the results of the three strains of *C. reinhardtii* are compared in Table 5. The setting of the optimal conditions refers to the lipid production. For CC 1010 it does not correspond to an increase of biomass. It is estimated that the cells of this strain act to accumulate lipids instead of growing by successive cell divisions.

**TABLE 4. BIOMASS CONCENTRATION, LIPID CONTENT AND LIPID PRODUCTIVITY IN STRAIN CC 1010 OF *C. reinhardtii* [28]**

| Media conditions | TAP & N | TAP-N +0.1 % Glucose | TAP-N +0.2 % Glucose | 0.05 % Glucose | 0.2 % Glucose | 0.001 % vitamin | 0.003 % vitamin |
|------------------|---------|----------------------|----------------------|----------------|--------------|----------------|----------------|
| Biomass conc. (g L⁻¹) | 0.77 ± 0.04 | 0.37 ± 0.04 | 0.40 ± 0.04 | 0.80 ± 0.05 | 0.85 ± 0.02 | 0.8 ± 0.03 | 0.76 ± 0.04 |
| Lipid content (%) | 24 ± 2 | 59 ± 6 | 47 ± 5 | 28 ± 0.6 | 22 ± 2 | 24 ± 1 | 23 ± 2 |
| Lipid productivity (g L⁻¹) | 15.2 ± 0.4 | 43 ± 5 | 34 ± 3 | 15 ± 0.3 | 15 ± 0.8 | 15 ± 0.3 | 15 ± 0.6 |

**TABLE 5. BIOMASS, CARBOHYDRATE AND LIPID PRODUCTIVITY IN STRAINS UTEX 90, CC 2656 AND CC 1010 OF *C. reinhardtii* [25], [28]**

| Productivity (mg L⁻¹ d⁻¹) | UTEX 90 | CC 2656 | CC 1010 | Initial | Optimal | Initial | Optimal | Initial | Optimal |
|---------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| Biomass                   | 119 ± 6 | 510 ± 30| 106 ± 6 | 360 ± 20| 770 ± 40*| 370 ± 30*|
| Carbohydrate              | 26 ± 1 | 270 ± 10| 19 ± 1 | 170 ± 10| –**     | –**     |
| Lipid                     | 21 ± 1 | 113 ± 6 | 11 ± 1 | 66 ± 3 | 15.2 ± 0.4| 43 ± 5  |

*Refers to mg L⁻¹, ** data not available

4. CONCLUSIONS

CCS and CCU are valid approaches to reach the zero-emission target. Further improvements are necessary to have fully economically and sustainable integrations. Microalgae are very promising in CCU because they have higher CO₂ uptake rates, they are generally easier to cultivate than higher plants, they naturally produce interesting compounds and they might be easily genetically engineered [33] to produce value added products. The identification of the optimal culture conditions to produce lipids is a crucial step to make biodiesel more competitive on the market. There are already many biobased products produced from microalgal biomass and further research and studies could indicate and identify more economically sustainable ways to include CCU in modern circular economy.

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