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About how to capture and exploit the CO₂ surplus that nature, per se, is not capable of fixing

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Summary

Human activity has been altering many ecological cycles for decades, disturbing the natural mechanisms which are responsible for re-establishing the normal environmental balances. Probably, the most disrupted of these cycles is the cycle of carbon. In this context, many technologies have been developed for an efficient CO₂ removal from the atmosphere. Once captured, it could be stored in large geological formations and other reservoirs like oceans. This strategy could present some environmental and economic problems. Alternately, CO₂ can be transformed into carbonates or different added-value products, such as biofuels and bioplastics, recycling CO₂ from fossil fuel. Currently different methods are being studied in this field. We classified them into biological, inorganic and hybrid systems for CO₂ transformation. To be environmentally compatible, they should be powered by renewable energy sources. Although hybrid systems are still incipient technologies, they have made great advances in the recent years. In this scenario, biotechnology is the spearhead of ambitious strategies to capture CO₂ and reduce global warming.

The tremendous impacts of global warming are being felt all over the world due to humans' unsustainable way of life. We have released to the atmosphere more CO₂ than what nature has been capable of fixing. The best strategy requires very deep cuts in emissions, as well as the use of alternatives to fossil fuels around the world. In 2015, the United Nations Conference on Climate Change (COP21), for the first time in more than 20 years of United Nations negotiations, achieved a legally binding climate agreement, with the objective of maintaining global warming below 2°C. Meanwhile, different approaches are being implemented to diminish this environmental problem. In this study, we aimed to update the most promising biotechnological approaches to capture and exploit excess of CO₂ to accomplish human beings quality of life integrated to the sustainability of our planet.

CO₂ capture and storage (CCS): sweeping the dust under the carpet?

One apparently promising technology is referred to as carbon capture and storage (CCS). CCS is a process consisting of the separation of CO₂ from industrial and energy-related sources, transport to a storage location and long-term isolation from the atmosphere (Metz *et al.*, 2005). Because the long-distance transportation of CO₂ to available disposal sites represents a prominent part of the economic and energetic costs of CO₂ capture, it should be preferentially applied to large point sources. This includes large fossil fuel or biomass energy facilities, major CO₂-emitting industries, natural gas production, synthetic fuel plants and fossil fuel-based hydrogen production plants (Aresta and Dibenedetto, 2007). Potential storage methods are geological storage (in geological formations, such as oil and gas fields, coal beds and deep saline formations), ocean storage (direct release into the ocean water column or onto the deep seafloor) and industrial fixation of CO₂ into inorganic carbonates (Metz *et al.*, 2005) (Fig. 1).

In this context, there are several commercially available technologies which can, in principle, be used for the separation of CO₂ from the rest of the flue gases. However, the most employed method is a chemical absorption-desorption process, in which monoethanolamine solution is frequently used to dissolve the CO₂. The almost pure CO₂ is released afterwards from the liquid

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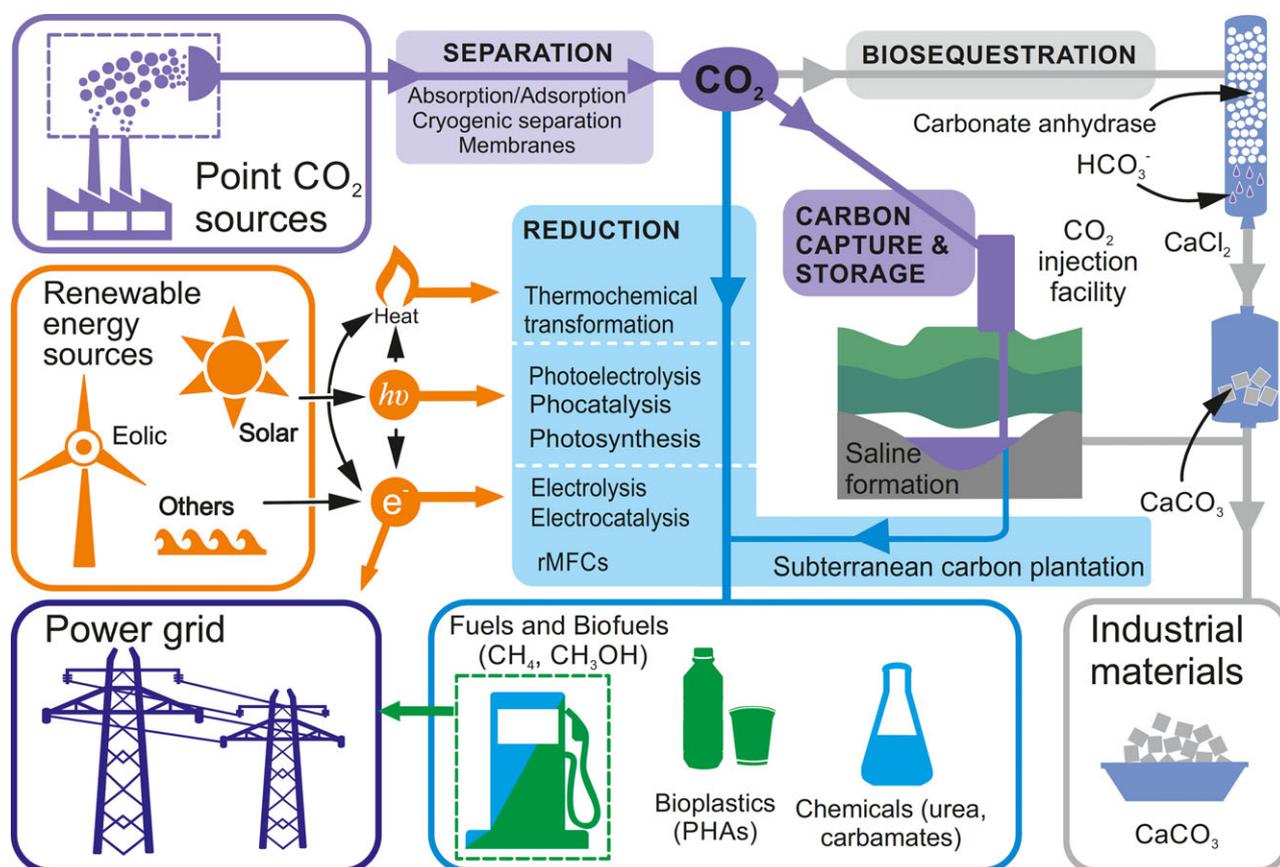


Fig. 1. CO₂ generated in point sources is separated by any of the existing methods (absorption/adsorption, cryogenic or membrane separation). The resulting almost pure CO₂ can be captured and stored (carbon capture and storage – CCS) in geological formations (such as oil and gas fields, and saline formations) or transformed into different products. Carbonate can be produced using carbonate anhydrases from cyanobacteria and CaCl₂ (biosequestration), and the resulting CaCO₃ can be stored or used as precursors for construction materials. CO₂ can also be reduced by different systems (biological, inorganic or hybrid). The energy supplied for the system to be sustainable must come from a renewable power source (wind, sun, etc.). This energy can be provided to the system as electrons (e⁻), photons (hν) or heat. Subterranean carbon plantation is considered an alternative for recycling the CO₂ captured in CCS into methane. The products of CO₂ reduction can be bioplastics (PHAs), bio/fuels like methane or methanol. It can also be transformed into a wide range of chemicals (urea, carbamates, etc.). Fuels produced by CO₂ reduction are a possible solution for the intermittence of renewable energy (power grid), as they can be stored using the existing facilities from fossil fuels.

by heating to 100–150°C. The main drawback of this process is the requirement of intensive energy during regeneration of monoethanolamine solution. Other methods to separate CO₂ include cryogenic fractionation, solid adsorption and membrane separation (Lam *et al.*, 2012; Sreenivasulu *et al.*, 2015).

The European Emissions Trading Scheme and the International Panel on Climate Change (IPCC) recognize that geological storage could be a valid mitigation option (West *et al.*, 2005). In addition, the International Energy Agency (IEA) Blue Map scenario envisages a 19% CO₂ reductions contribution from CCS by 2050 (Scott *et al.*, 2012). However, there are some risks associated with this technology which must be considered. Although extensive physiological research is available, the environmental impacts of elevated CO₂ on terrestrial, sub-surface and marine ecosystems are not fully understood

(West *et al.*, 2005). Uncontrolled leakages could have implications for the environment. In economic terms, leaks into marine and freshwater systems might affect fisheries. For terrestrial systems, leakages might damage crops, groundwater quality and/or human and animal health. Other concerns include acidification, changes in biological diversity and species composition and asphyxiation at high CO₂ concentrations. In addition, biogeochemical processes may be affected as increased CO₂ concentrations could change pH, microbial populations and nutrient supply (Heinrich *et al.*, 2003; Leung *et al.*, 2014).

For this type of storage technology to be acceptable, safe practices must be developed, the potential for leaks must be understood, and dangerous situations must be avoided or safely managed (Lee *et al.*, 2010). Other strategies, then, should be elaborated as alternative or, at

least, to complement the geological and ocean storage of CO₂. We will focus on the alternatives arising from microbiological world, especially prokaryotes, and to the contribution they can make to other inorganic technologies.

Transforming greenhouse gases into added-value products

As mentioned, final disposal of CO₂ in different compartments (CCS) is a strategy to diminish its proportion in atmospheric gas composition. However, there are other strategies which are not just aiming at CO₂ capture but also at its transformation and revalorization. A lot of research has been developed into this field. Mikkelsen and coworkers grouped CO₂ transformations into different categories involving different chemical, physical or biological methods (Mikkelsen *et al.*, 2010). To simplify, we will group the systems for CO₂ transformation into three categories: (i) biological (which include living prokaryotes or enzymes); (ii) inorganic transformations (chemical, physical or a combination of both); and a third category (iii) hybrid systems in which the process of CO₂ conversion covers biological and inorganic steps integrated into the same system.

Biological systems for CO₂ transformation

In the field of biological transformations, eukaryotic microalgae and cyanobacteria have been extensively studied for biofuels production and CO₂ sequestration in carbonates via carbonic anhydrases (CAs). These organisms have been proposed for almost 50 years as a source of renewable fuels to reduce global warming (Oswald and Golueke, 1960).

Photosynthetic microorganisms grow 100 times faster than terrestrial plants, and they can double their biomass in <1 day. This is due to their simple cellular structure and large surface to volume ratio that give them the ability to uptake large amount of nutrients from water sources and thus promoting their growth rate. In addition, they can convert solar energy to chemical energy with efficiency of 10–50 times greater than terrestrial plants (Lam *et al.*, 2012). A major appeal of photosynthetic microorganism cultures in greenhouse gas mitigation is that they must use concentrated forms of CO₂, such as provided by power plant flue gases (Benemann, 2003).

Although the potential of microalgae and cyanobacteria to contribute to the world energy and commodities demand is high, there is a large gap between the current available technology and the one needed to supply the potential world demand. It is still necessary to solve a large number of bottlenecks related with biological, engineering and economic aspects (Acién *et al.*, 2012). For

example, photosynthetic microorganisms suffer inefficiencies arising from suboptimal light-harvesting properties including the low energy capture and transfer efficiency of photosynthesis that will not be probably addressed in the near term (Khunjar *et al.*, 2012; Torella *et al.*, 2015). Possible solutions for this issue are discussed in the next sections.

Exploiting CO₂ concentrating mechanism of cyanobacteria

The decline in atmospheric CO₂ levels and rising O₂ were a selective pressure in the past that lead to compensate the inefficient Rubiscos from cyanobacteria, with complementary strategies for CO₂ fixation. They had to develop an effective photosynthetic CO₂ concentrating mechanism for improving carboxylation. This adaptation acts to raise the concentration of CO₂ around Rubisco hence improving the efficiency of CO₂-fixation and providing a survival advantage in limiting CO₂ environments. The main feature of the cyanobacterial CO₂ concentrating mechanism is that cellular Rubisco is partitioned into a protein-bound micro-compartment, called the carboxysome, which allows an internal accumulation of CO₂ with the contribution of carbonic anhydrase (Rae *et al.*, 2011).

Carbonic anhydrase (CA) is an interesting option for CO₂ sequestration coming from the biological world. These are found not only in cyanobacteria but in animals, plants and other microbes. CA catalyses a rapid transformation of CO₂ and water to HCO₃⁻ and protons. As CA has the highest catalytic efficiency for CO₂ hydration ($k_{cat} \sim 106 \text{ s}^{-1}$), it is considered as prominent biocatalytic agent for CO₂ sequestration technology developments. In presence of cations at modest pH *in vitro*, CA converts CO₂ into CaCO₃ (Kanth *et al.*, 2013). CaCO₃ is a common and thermodynamically stable mineral found in rocks worldwide and is the main component of shells of marine organisms, snails and eggs. If the widespread transformation of CO₂ to CaCO₃ is possible, it will represent a stable process for long-term CO₂ storage. The transformation of CO₂ into carbonate compounds using biocatalysts in a biomimetic approach has advantages for thermodynamically stable CO₂ storage as compared to other technologies. This approach does not need a monitoring system for potential leaks, as do CCS, and allows the reuse of carbonate compounds for building or industrial materials (Lee *et al.*, 2010). There are still several problems for practical use of CA such as high cost, limited stability, narrow range of working pH, intoxication due to impurities in flue gas, various issues related to scaling up and operating conditions. So, more stable and high-active CA should be screened or engineered for more practical CA-catalysed CO₂ sequestration system (Kanth *et al.*, 2013).

Carboxysomes are not just interesting for microbiologists. Growth and productivity in important crop plants is limited by the inefficiencies of the C3 photosynthetic pathway. Introducing into C3 plants CO₂-concentrating mechanisms, such as carboxysomes, could overcome these limitations and lead to increased yields (Rae *et al.*, 2017). Recently, different groups described the recombinant expression of synthetic carboxysome shells in *Escherichia coli* (Cai *et al.*, 2016) and *Corynebacterium glutamicum* (Baumgart *et al.*, 2017) as intermediate step before jumping onto plants for improved CO₂ fixation and concomitant increased biomass production.

Rational consortia between photoautotrophs and heterotrophs

As CO₂ fixation tools, cyanobacteria can be co-cultivated with other heterotrophic microorganisms providing them of reduced compounds from CO₂. In this strategy, photosynthesis would provide organic carbon to an optimized heterotrophic organism (such as *E. coli*) which in turn would transform it into an added-value compound. For example, the capability of sucrose-secreting cyanobacteria to act as a platform for the construction of a light-driven consortia was evaluated (Hays *et al.*, 2017). In this work, the cyanobacteria *Synechococcus elongatus* PCC 7942 was paired with three disparate heterotrophs: *Bacillus subtilis*, *E. coli* or *Saccharomyces cerevisiae*. These synthetic consortia could be stabilized over the long term (weeks to months) and persisted in the face of selected perturbations (dilution, periods of darkness and phase changes in growth media). It could also be programmed for photoproduction of target compounds and proteins, such as alpha-amylase and the bioplastic polyhydroxybutyrate (produced by co-culturing cyanobacteria with recombinant *B. subtilis* or *E. coli* respectively). The principal advantage of this system is its modularity, and the wide range of products that could be synthesized by means of genetically flexible organisms associated with cyanobacteria.

Natural and engineered CO₂ fixation pathways

Today, six autotrophic CO₂ fixation mechanisms are known: (i) the Calvin–Benson reductive pentose phosphate cycle; (ii) the reductive citric acid cycle (Arnon–Buchanan cycle); (iii) the reductive acetyl-coA (Wood–Ljungdahl) pathway; (iv) the hydroxypropionate (Fuchs–Holo) bicycle (3-hydroxypropionate cycle); (v) the 3-hydroxypropionate/4-hydroxybutyrate and (vi) the dicarboxylate/4-hydroxybutyrate cycles (Berg, 2011). Many heterotrophs have been engineered so far with recombinant CO₂ fixation enzymes and pathways (Parikh *et al.*, 2006; Mueller-Cajar and Whitney, 2008; Guadalupe-Medina *et al.*, 2013; Antonovsky *et al.*, 2016). However, they still

grow as mixotrophs, requiring an organic carbon source for the production of the starting substrates of linear CO₂ fixation pathways and/or for the regeneration of ATP and/or electron donors. Converting these engineered heterotrophs into true autotrophs would require the functional transplantation of complete CO₂ fixation cycles and the transplantation of, and integration with, energy-harvesting systems (Claassens *et al.*, 2016; Claassens, 2017).

To overcome limitations of the natural carbon fixation pathways, an extensive *in silico* study identified alternative pathways that combine existing metabolic building blocks from various organisms. This work suggested that some of the proposed synthetic pathways could have significant quantitative advantages over their natural counterparts, such as the overall kinetic rate (Bar-Even *et al.*, 2010). In another attempt to improve CO₂ fixation, Schwander *et al.* published the construction of a non-natural optimized CO₂ fixation cycle called the ‘crotonyl–coenzyme A (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA’ (CETCH) cycle (Schwander *et al.*, 2016). The 12 enzymes involved in the CETCH cycle come from six organisms across all three domains of life: one from a plant (*Arabidopsis thaliana*), one from humans (*Homo sapiens*) and the other 10 enzymes from microbes. Although the CETCH cycle has the fewest reactions and the lowest requirement for ATP and NADPH among the aerobic CO₂ fixation pathways, it produces glyoxylate, a less reduced metabolic intermediate. As CETCH cycle functionality has only been demonstrated *in vitro*, it should be transplanted into a selected chassis organism to develop a whole-cell biocatalyst (Gong and Li, 2016; Schwander *et al.*, 2016).

Inorganic transformations

The strategies for CO₂ transformations can be grouped on the basis of the type of energy supplied to the CO₂ for coping with its thermodynamic and kinetic stability (ΔG°_f 396 kJ mol⁻¹). They can be classified as (i) chemical and thermochemical or as (ii) photochemical and electrochemical. They will be summarized briefly here, but for more detailed reviews see references (Aresta and Dibenedetto, 2007; Mikkelsen *et al.*, 2010).

Chemical and thermochemical transformations

The chemical transformations include all those strategies getting the leverage over the little reactivity window offered by the CO₂ structure. This transformation method is based on the electrophilicity of the central carbon and the electron-rich behaviour of the two oxygen atoms hosted in the molecular structure. For this reason, the CO₂ chemical reactions are usually associated with, for example, low-valent metal complexes (e.g. Ni, Pd) (Sakakura *et al.*, 2007). In addition, when the catalytic

reactions are not able to promote the CO₂ activation, the reactions are usually coupled with high temperature and pressure conditions (thermochemical conversion) and/or with high-energy compounds like hydrogen (Mikkelsen *et al.*, 2010).

The range of products reached by chemical conversion technologies is wide, encompassing both chemical carbon-based compounds with high oxidation state (e.g. carbamates, urea and polymeric materials), and energy-rich, and consequently, more reduced molecules, such as formic acid, formaldehyde, methanol, methane and other hydrocarbons (Mikkelsen *et al.*, 2010; Liu *et al.*, 2015b). However, despite this vast product scenario, currently the application of CO₂ as a feedstock for industrial production is limited to the production of urea, cyclic carbonates, polymers and carbamates (Mikkelsen *et al.*, 2010). When a catalyst or a chemical activator is not available, the CO₂ can be converted under extremely high temperature and pressure conditions (Lou *et al.*, 2003; Treacy and Ross, 2004).

Photochemical and electrochemical

Leaf-mimic system and artificial photosynthesis are alternative strategies for converting the CO₂. These carbon fixation systems offer a way to avoid the high-energy cost characterizing thermochemical conversions. They try to mimic the structural design of carbon catalyst centres or to reproduce the equivalent electron paths discovered in natural CO₂ conversion systems (Berardi *et al.*, 2014; Banerjee *et al.*, 2016). Photochemical conversion methods are based on the exploitation of the sun-light as energy source, a feature that renders this strategy sustainable for a further applications (Blankenship *et al.*, 2011; Herron *et al.*, 2015).

The artificial photosynthesis works towards a direct CO₂ conversion for the synthesis of hydrocarbons or oxygenated products (Chang *et al.*, 2016). The devices adopted for these methodologies are composed of the minimum components of natural photosynthetic systems, such as a light-capturing antenna, a catalyst and an electron sacrificial donor compound. In this kind of devices, light promotes the electron transfer from the antenna system to the CO₂, the catalyst favours a photo-assisted multi-electron transfer, and eventually, a sacrificial electron donor closes the electron cycle. The CO₂ fixation devices are classified as photocatalytic system, if both reduction and oxidation occur in a same location, or as photo-electrochemical cells if the redox reactions are spatially separated (Chang *et al.*, 2016). In the latter, the redox reactions are carried out on the surface of two electrodes, which can be physically separated by a membrane (generally nafion) (Yim *et al.*, 2015). The main products are generally represented by CO, formate and oxalate, even if

also the synthesis of hydrocarbons, as methane or ethane, is attested (Jhong *et al.*, 2013).

For electrochemical CO₂ conversion, transition metals are the most used catalysts (Jhong *et al.*, 2013). Among them, copper has the best selectivity to produce hydrocarbons and formic acid. It also offers the potentiality to catalyse the synthesis of C1-, C2-, C3-based compounds and hydrocarbons with a faradic efficiency higher than 50% (Peterson and Nørskov, 2012). However, materials used for catalysing the reactions are not able to promote high values of reaction rate together with high selectivity, energetic efficiency and current density so far (Kuhl *et al.*, 2012)(Montoya *et al.*, 2017). Therefore, new cathode materials or modification of the catalyst surface is under investigation (Peterson and Nørskov, 2012).

Hybrid systems: Integrating different technologies for the design of more efficient CO₂ transforming systems

Hybrid systems for conversion of CO₂ could be the solution for two different problems emerging from the battle against climate change; on one side, photosynthetic organisms suffer inefficiencies arising from non-optimal light-harvesting properties (Khunjar *et al.*, 2012; Torella *et al.*, 2015). In a typical fermentation process of sugar cane to ethanol, the final product contains only almost 0.2% of the available solar energy. This low efficiency is mainly due to ineffective plant photosynthesis, but also to energy losses in the subsequent processing of biomass and microbial fermentation. In comparison with biological photosynthesis, the efficiency of photovoltaic solar panels is very high; solar panels that are currently available have solar to electricity efficiencies around 18%, and new innovations may enable efficiencies of more than 40% (Claasens *et al.*, 2016). By the other side, renewable energy sources have to deal with the problem of intermittence generation of electric-power. In particular, the amount of solar radiation incident on the earth's surface depends upon many factors such as location (latitude), time of the day, inclination of the surface, declination and weather. Storing solar energy is critical for continuous processing during these fluctuations (Herron *et al.*, 2015). As solar and wind penetration increases in our systems, the intermittency of these two energy sources seriously compromises the stability and quality of grid power (Tuller, 2017).

One option of energy storing could come for hydrogen technology. Water splitting powered by renewable energy sources can lead to the production of hydrogen as a fuel. Hydrogen has many attractive attributes – it is clean burning and can be efficiently converted back to electricity via fuel cells. Hydrogen lacks volumetric energy density, this is the amount of energy stored per

unit volume, and cannot be easily stored and distributed like hydrocarbon fuels. Its utility is much greater as an onsite fuel for converting CO₂ to CH₄ or for generating heat, electricity or syngas (Tuller, 2017). Liquid fuels are more appealing as a solar storage medium because of their attractive energy density and existing sophisticated distribution and storage infrastructures. However, attempts to produce liquid fuel via inorganic CO₂ reduction have generally poor specificity and energy efficiency (Torella *et al.*, 2015).

Thus, the problem of energy intermittence and low light-harvesting properties of photosynthesis could be solved by integrating into a hybrid configuration the energy coming from renewable sources with biological systems as producers of storable fuels such as methane, alcohols, alkanes and other chemicals. This innovative technology is carried out in reverse microbial fuel cells and takes advantage of a higher CO₂ reduction turnover from the electrochemical side and more interesting carbon products from the bio-side. In a standard microbial fuel cell, organisms oxidize organic fuels and transfer electrons into an electrochemical system so that fuels are converted to electrical energy. In a reverse microbial fuel cells, this process is reversed so that electrical energy is used by bacterial cells to drive CO₂ fixation to high-energy organic compounds (Khunjar *et al.*, 2012). The origin of these electrons may be any renewable energy source (sun, wind, etc.). The process of delivering these electrons to the cell is called extracellular electron transfer (EET). This transfer can be done in an indirect or direct fashion, depending whether they

require or not the diffusion of a mobile component for electron transport (Rabaey and Rozendal, 2010) (Fig. 2).

Indirect EET

In a reverse microbial fuel cells, the indirect method for EET involves the production or use of so-called electron shuttles (or electron mediators), which transport the electrons from the electrode to the cell. The use of a mediator enables the utilization of planktonic cells in the bioreactor and can also enable separate-stage designs that afford spatial and temporal decoupling of energy capture and bioproduction. This allows both processes to be operated and optimized separately. In the mediated approach, electrons are first transferred from the electrode to a soluble mediator, and then, the mediator is oxidized by the cell. Inorganic compounds that are linked with chemoautotrophy and can be electrolytically regenerated (such as NH₃, H₂, NO₂⁻, Fe²⁺ and H₂S), are an attractive options for this platform as they can facilitate the construction of multicarbon organics from CO₂ using natural carbon fixation pathways (Khunjar *et al.*, 2012).

Ammonia can be used as an indirect EET taking advantage of its low cost, abundance, safety and solubility. For example, *Nitrosomonas europaea*, a chemolithoautotroph, was used as the biocatalyst due to its inherent capability to utilize ammonia as its sole energy source for growth. Calculations indicated that overall production efficiency could approach approximately 2.7% under optimal electrolysis conditions. Considering a conversion efficiency of 10% from solar energy, the biomass production efficiency of this system

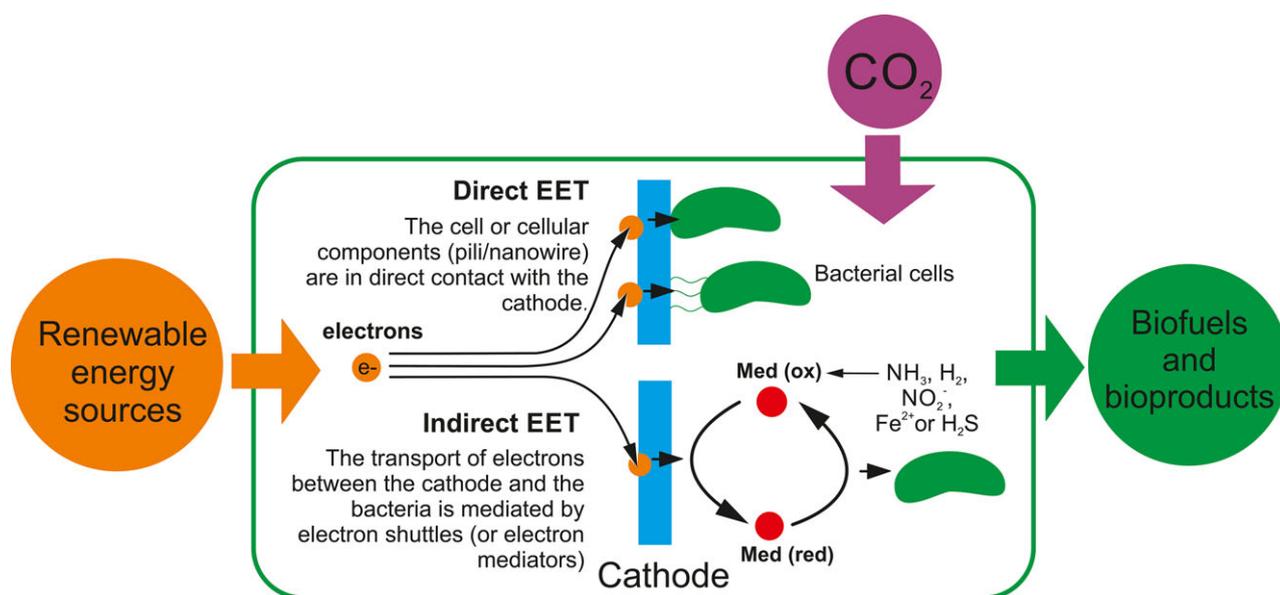


Fig. 2. Schematic representation of CO₂ reduction and extracellular electron transfer (EET) in hybrid systems. They are based on reverse microbial fuel cells and take advantage of the high CO₂ reduction turnover of electrolysis and the versatility of microbial metabolism.

was $0.27 \pm 0.02\%$. This level of efficiency is comparable to photon to biomass conversion efficiencies observed for photosynthetic systems (around 1%) (Khunjar *et al.*, 2012), so further efforts are required to improve these results, by considering more efficient conversions from solar energy, or modifying the genetic backgrounds of the strain.

Ralstonia eutropha, as a model chemolithoautotroph species, is a promising tool for the development of hybrid systems. This bacterium is able to grow using electrochemically generated H_2 as energy source. Engineered strain of *R. eutropha* H16 was studied for the production of isobutanol and 3-methyl-1-butanol as the target fuels. Growth of *R. eutropha* was inhibited by reactive oxygen and nitrogen species when electric current was introduced. To circumvent this problem, a porous ceramic cup was used to shield the anode. This shield provided a tortuous diffusion path for chemicals. Therefore, the reactive compounds produced by the anode could be quenched before reaching the cells growing outside the cup. Using this approach, healthy growth of *Ralstonia* strain LH74D and production of over 140 mg l^{-1} biofuels were achieved with the electricity and CO_2 as the sole source of energy and carbon respectively (Li *et al.*, 2012). Based on electrochemically generated H_2 as energy source, *R. eutropha* was also engineered for the production of fatty acid-derived, diesel-range methyl ketones. These modifications included overexpression of a cytoplasmic version of the TesA thioesterase, the deletion of two putative β -oxidation operons and heterologous expression of three genes (the acyl coenzyme A oxidase gene from *Micrococcus luteus* and *fadB* and *fadM* from *E. coli*). These genetic modifications led to the production of 180 mg l^{-1} under chemolithoautotrophic growth conditions (Muller *et al.*, 2013).

There are other ongoing projects using hybrid systems for CO_2 capture. These projects aim to bridge a cost-effective CO_2 capture and purification, with electrochemical conversion of CO_2 , followed by the fermentation of the CO_2 -reduction intermediates (such syngas and C1 water-soluble molecules). By selecting the appropriate microorganism, a wide range of valuable products can be synthesized, such as PHAs, isoprene, lactic acid and methane (www.celbicon.org). For example, *Rhodospirillum rubrum*, a purple non-sulfur bacterium, can produce PHAs from CO as carbon and energy source (Revelles *et al.*, 2016). This feature and its metabolic versatility make this species interesting as biological tool for CO_2 fixation.

Direct EET

Direct transfer typically involves at least a series of periplasmic and outer membrane complexes. In recent

years, the involvement of pili or pilus-like appendages (called nanowires in this context) was established. It has been suggested that nanowires also establish electron transport between different microorganisms in a community (Rabaey and Rozendal, 2010). Nevin proposed the term 'microbial electrosynthesis' for the reduction of CO_2 to multicarbon compounds with electrons donated from an electrode as the electron donor (Nevin *et al.*, 2010).

The bioelectrochemical reduction of CO_2 has been also postulated as a promising process to obtain methane. The term electromethanogenesis has been applied to the process of producing methane using CO_2 as the sole carbon source, using electroactive microbes in an engineered system (biocathode) powered with electric current. CO_2 can be fixed either by direct EET or indirect EET; however, we will mention examples of the former here. Electromethanogenesis-based technologies have a great potential for storing renewable energy in the form of methane, improving waste treatment processes or upgrading gas streams containing CO_2 . In all cases, future studies must focus on further up-scaling, increase process efficiencies and reduce operation costs to reach coexistence with well-established technologies, or even a hypothetical overtaking (Blasco-Gómez *et al.*, 2017).

Electromethanogenesis can also add value to CO_2 stored in geological formations by means of subterranean carbon plantation. This concept proposed by Sato and coworkers, is based on CCS technologies, *in situ* biological conversion of the stored CO_2 to methane and harvest of the biogenic methane as a recycled energy source (Sato *et al.*, 2013). When supplied with CO_2 through CCS operations, such reservoirs could function as natural bioreactors that prompt methanogens to convert CO_2 to methane. Intermittent electrical energy provided by, for instance, wind turbines and photovoltaic cells can be stored in a stable form as methane. The current limitation of the system is the relatively slow rate of electromethanogenesis, and further studies are required on electrochemical reduction of CO_2 under more realistic conditions: at higher (hydrostatic) pressures, in presence of solid (rock) surfaces and in microbial symbioses (Kuramochi *et al.*, 2013; Sato *et al.*, 2013).

Methane may not be the only product of electrosynthesis. Using consortia of cathodophilic microorganisms from brewery wastewater, it is possible to obtain a mixture of products (acetate, methane and hydrogen) through electrosynthesis in the same process, with CO_2 as the only carbon source. The electrochemical evidence suggests that the electron transfer between the electrode and microbes in a biofilm operates in the absence of soluble redox active components in the medium (Marshall *et al.*, 2012). Liu and coworkers created a two-step strategy that mimics natural photosynthesis, where light capture by a biocompatible nanowire array interfaced and

directly provided reducing equivalents to living organisms. The high-surface-area silicon nanowire array harvests light energy to provide reducing equivalents to the anaerobic bacterium, *Sporomusa ovata*, for the photoelectrochemical production of acetic acid under aerobic conditions (21% O₂). The resulting acetate (~6 g l⁻¹) fed genetically engineered *E. coli* to produce a variety of value-added chemicals. The yield of target molecules was as high as 26% for n-butanol, 25% for one of the isoprenoid compounds (amorphadiene) and up to 52% for PHAs, comparable with literature values (Liu *et al.*, 2015a).

Concluding remarks

Global warming is an ongoing threat for the maintenance of ecological systems, for economy and human life quality. Solving the problem implies the convergence of different approaches to achieve a net CO₂ removal from the atmosphere. CCS may account for part of the solution, but they must be complemented with other green technologies with lower environmental risks and higher economical sustainability. Microbial biotechnology can lend a great hand to this aim by providing recombinant microorganisms able to transform the low reactive molecule of CO₂ into a wide variety of compounds, such as biofuels, bioplastics and chemicals. New hybrid technologies should also be explored to make the whole process more efficient. Electrochemical cells, as inorganic systems for CO₂ fixation, have the potentiality to sequester high amounts of CO₂, but their low selectivity and capacity for generating added-value products are limitations in a commercial scenario. In contrast, biological systems are much more versatile for the production of C-based molecules than electrochemical cells, but they are quite inefficient in harnessing solar energy. A promising strategy for sequester and fix CO₂ is definitively based on the combination of bio and electrochemical systems.

Conflict of interest

None declared.

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