Journal of Bioprocessing & Biotechniques

Jajesniak et al., J Bioproces Biotechniq 2014, 4:3
DOI: 10.4172/2155-9821.1000155

Review Article Open Access

Carbon Dioxide Capture and Utilization using Biological Systems: Opportunities and Challenges

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Abstract

Carbon dioxide capture and utilization (CCU), a concept of turning a greenhouse gas into a useful feedstock, is gaining much attention in recent years. Increasing CO_2 emission into the atmosphere, from fossil fuel combustion and other anthropogenic activities, has forced us to source for more sustainable and economical routes of chemical syntheses. Instead of developing new chemical catalysts and CO_2-based chemistry, we should perhaps learn from Nature. Over the past billions of years, Nature has evolved sophisticated mechanisms for carbon concentration, fixation and utilization, manifested through autotrophy. Many organisms, such as photosynthetic and chemolithoautotrophic organisms, display excellent ability in assimilating CO_2 and converting it into complex molecules. Through the use of enabling technologies (*e.g.*, genetic engineering and protein engineering), the range of CO_2-derived bio-based products is expanding at a rapid pace. Chemicals that can be synthesized biologically include bio-plastics, bio-alcohols, bio-diesel, to name a few. Continued research on multiple fronts and closed collaboration between scientists and engineers are required to further develop biological systems into viable chemical production platforms.

Keywords: Carbon capture and utilization (CCU); Carbon capture and storage/sequestration (CCS); Carbon dioxide (CO_2); Greenhouse gas (GHG); Protein engineering; Synthetic biology

Introduction

Complementing carbon capture and storage/sequestration (CCS) technologies [1], the use of carbon dioxide (CO_2) as a building block for chemical syntheses and as a fluid in a wide range of applications (*e.g.*, extraction, chromatography, food processing, polymer processing, particle engineering and biocatalysis) [2] represents a concrete step toward the goal of a sustainable chemical industry, with concomitant reduction in CO_2 emission into the atmosphere. Driven by green agenda and governments' commitment to reducing carbon footprint (Figure 1), many novel carbon capture and utilization (CCU) technologies to convert CO_2 into fuels or value-added chemicals have been reported [3]. These chemical reactions involving CO_2 are enabled by the use of appropriate catalysts, some of which are inspired by biological systems. The use of physicochemical approaches in CCU has been comprehensively summarized in many excellent reviews [3-9].

Despite the advances made in utilizing CO_2 as a chemical feedstock, large-volume CO_2 conversion is currently limited. Notable examples of industrial use of CO_2 include production of urea (~70 Mt CO_2 per year), inorganic carbonates and pigments (~30 Mt CO_2 per year), methanol (~6 Mt CO_2 per year), salicylic acid (~20 kt CO_2 per year) and propylene carbonate (a few kt CO_2 per year) [4,5,8,10]. On the contrary, photosynthetic organisms convert around 100 Gt of carbon into biomass annually [11]. In other words, Nature has evolved highly sophisticated mechanisms for carbon fixation and utilization; a resource that remains largely untapped and could potentially be a disruptive technology in CCU.

This review is dedicated to exploring both the opportunities and the challenges of applying biological systems in CCU. We first introduce the chemistry of CO_2 and provide an overview of the microorganisms (both eukaryotes and prokaryotes) capable of utilizing CO_2 as a carbon source. We examine prominent biochemical pathways and associated enzymes involved in carbon fixation and metabolism in these microorganisms, leading to bio-based products formation. Subsequently, we discuss enabling technologies such as genetic engineering, metabolic engineering, synthetic biology and protein engineering for improving the performance of biological systems in CO_2 capture or chemical syntheses from CO_2. We also survey recent advances in bioprocess engineering relating to CCU. Finally, a comparison between chemical and biological routes of CO_2 conversion is made and the challenges to the wide application of biological systems in CCU are highlighted.

Chemistry of CO_2 relevant to its utilization

Understanding CO_2 chemistry is of paramount importance to develop new processes based on CO_2 and to comprehend the molecular mechanisms adopted by biological systems. Although containing two polar C=O bonds, CO_2 itself is apolar. CO_2 displays two different reaction sites, namely the electrophilic carbon atom and the nucleophilic oxygen atoms, rendering it a bi-functional molecule. Being the end product of any combustion of materials comprising carbon and hydrogen atoms, CO_2 lies in a potential energy well. The bonding of a third atom to carbon atom (*e.g.*, oxygen or any other) is exothermic. Therefore, CO_2 can theoretically be converted into carboxylates and lactones (RCOOR′), carbamates (RR′NCOOR″), urea (RR′NCONRR′), isocyanates (RNCO) and carbonates [ROC(O)OR′], without any external energetic input [4]. On the other hand, reactions that generate reduced forms of CO_2 require energy, such as formates

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Received February 27, 2014; **Accepted** April 16, 2014; **Published** April 23, 2014

Citation: Jajesniak P, Omar Ali HEM, Wong TS (2014) Carbon Dioxide Capture and Utilization using Biological Systems: Opportunities and Challenges. J Bioprocess Biotech 4: 155 doi: 10.4172/2155-9821.1000155

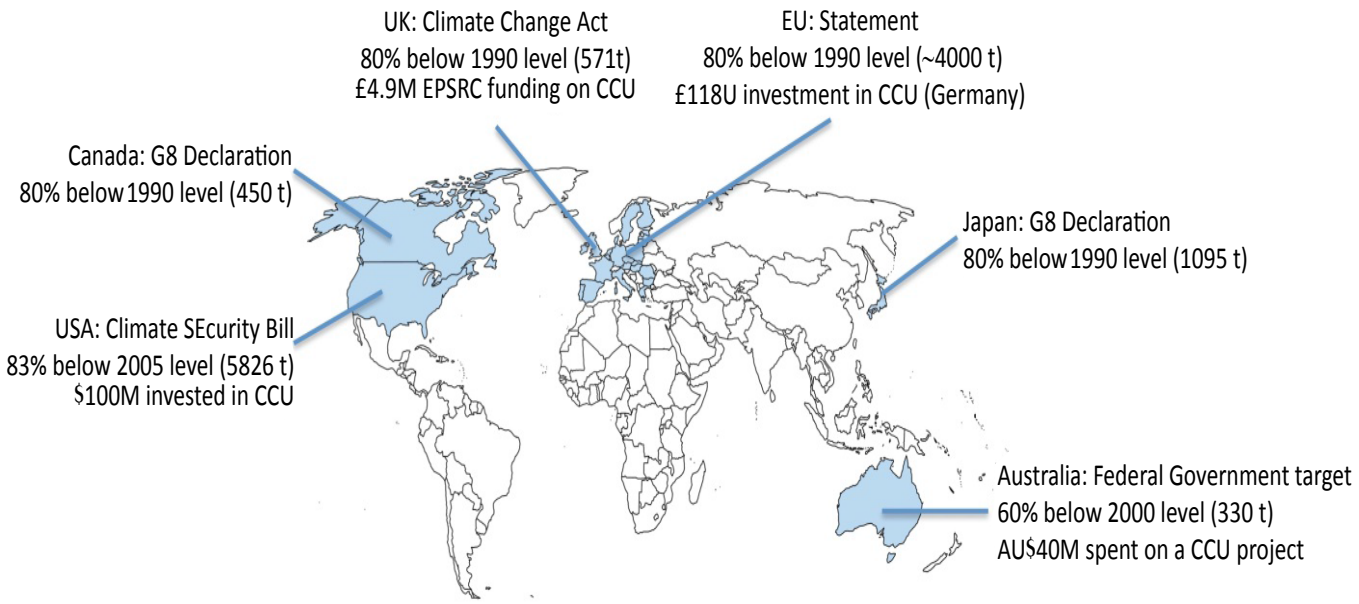

Figure 1: Examples of planned reductions in greenhouse gas emissions by 2050 and recent investments in CCU (based on the information given in [129,130]). Approximate annual emissions of anthropogenic CO_2 are given in brackets [131].

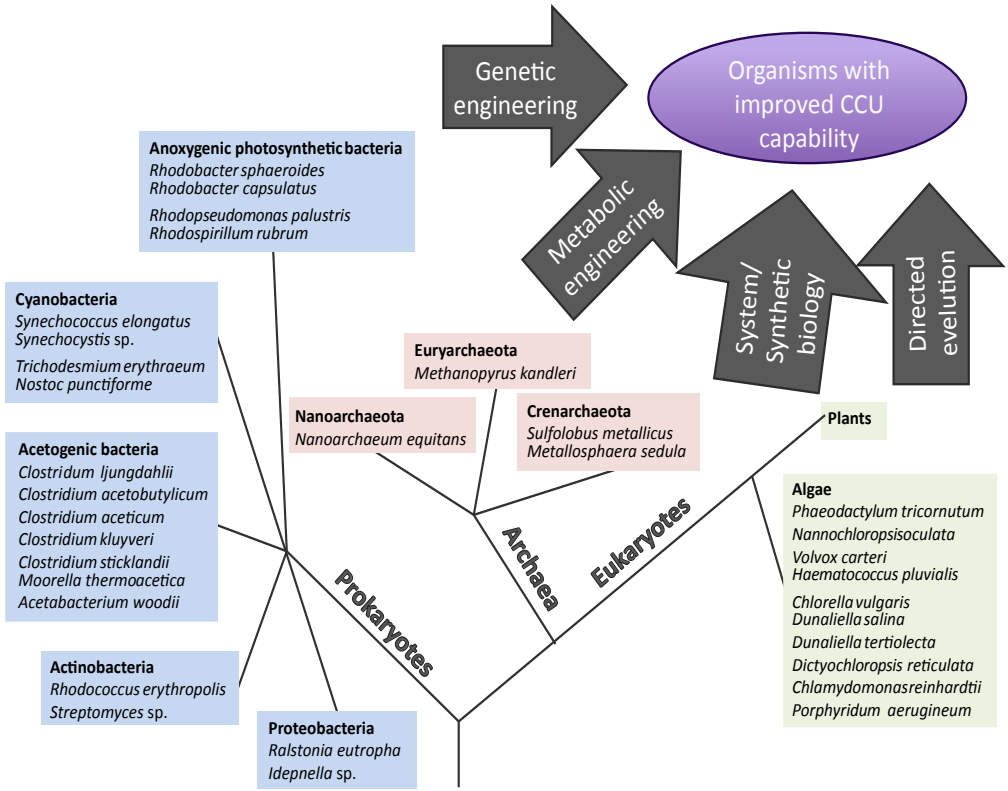

Figure 2: A summary of organisms capable of assimilating CO_2. The scope is further extended, with the advent of enabling technologies such as genetic engineering, metabolic engineering, system and synthetic biology, and protein engineering.

Carbon fixation pathways	Also known as	Organisms	Key CO_2 fixating enzymes	Key CO_2 fixating reaction
Calvin-Benson-Bassham cycle (CBB)	Reductive pentose phosphate cycle	Plants, algae, cyanobacteria, proteobacteria, mycobacteria	RuBisCO	Ribulose-1,5-bisphosphate + CO_2 + H_2O → 2x 3-Phosphoglycerate
Reductive tricarbocylic acid cycle (rTCA)	Reductive citric acid cycle/Reverse Krebs cycle/Arnon-Buchanon cycle	Proteobacteria, green sulfur bacteria, aquificae bacteria	Phosphoenolpyruvate carboxylase 2-Oxogluterate synthase Isocitrate dehydrogenase Pyruvate synthase	Phosphoenolpyruvate + HCO_3^- → Oxaloacetate + P_i Succinyl-CoA + CO2 + Fd_{red}^{2-} + $2H^+$ → 2-Oxoglutarate + CoA + Fd_{ox} 2-Oxogluterate + CO_2 + NAD(P)H + H^+ → Isocitrate + NAD(P)+ Acetyl-CoA + CO_2 + Fd_{red}^{2-} + $2H^+$ → Pyruvate + CoA + Fd_{ox}
Wood-Ljungdahl pathway (W-L)	Reductive acetyl-CoA pathway	Euryarchaeota, proteobacteria, plantomycetes, spirochaetes	Formate dehydrogenase CO dehydrogenase/Acetyl-CoA synthase Formylmethanofuran dehydrogenase*	CO_2 + NAD(P)H + H^+ → Formate + NAD(P)+ CO2 + $2H^+$ + $2e^-$ → CO + H_2O CO + CH_3-CFeSP + HSCoA → CH_3-CO-SCoA + CFeSP + H^+ Methanofuran + CO_2 + $2H^+$ + Fd_{red}^{2-} → Formylmethanofuran + H_2O + Fd_{ox}
3-Hydroxypropionate-4-hydroxybutyrate cycle (3HP-4HB)		Aerobic crenarcheota	Acetyl-CoA/Propionyl-CoA carboxylase Acetyl-CoA/Propionyl-CoA carboxylase	Acetyl-CoA + ATP + HCO_3^- → Malonyl-CoA + ADP + Pi Propionyl-CoA + ATP + HCO_3^- → (*S*)-Methylmalonyl-CoA + ADP + P_i
Dicarboxylate-4-hydroxybutyrate cycle (DC-4HB)		Anaerobic crenarcheota	Pyruvate synthase Phosphoenolpyruvate carboxylase	Acetyl-CoA + CO_2 + Fd_{red}^{2-} → Pyruvate + Fd_{ox} Phosphoenolpyruvate + HCO_3^- → Oxaloacetate + P_i
3-Hydroxypropionate bi-cycle (3-HP)	Fuchs-Holo cycle	Green non-sulfur bacteria	Acetyl-CoA carboxylase Propionyl-CoA carboxylase	Acetyl-CoA + ATP + HCO_3^- → Malonyl-CoA + ADP + P_i Propionyl-CoA + ATP + HCO_3^- → (*S*)-Methylmalonyl-CoA + ADP + P_i

* Found in methanogenic Archaebacteria

Table 1: Reported carbon fixation pathways.

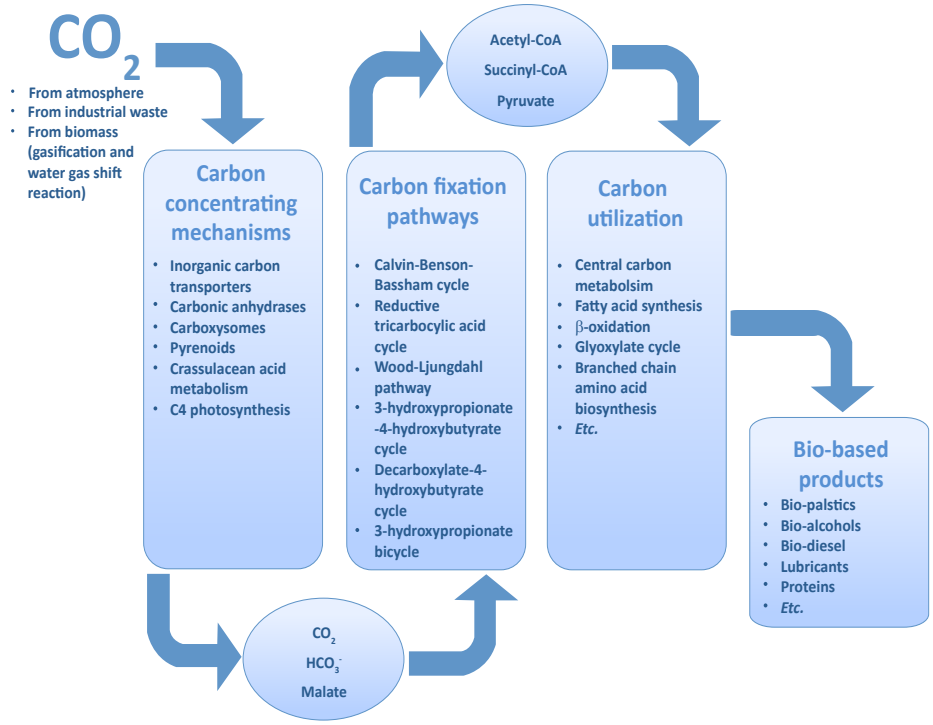

Figure 3: Carbon concentrating mechanisms, carbon fixation pathways and carbon utilization strategies adopted by various autotrophic organisms, leading to bio-based products.

Organisms	Advantages	Disadvantages
Algae	• Wide distribution (particularly in moist environment). • Multiple modes of cultivation available (*e.g.*, open ponds, photobioreactors). • Fast growing. • High cell density. • Fast CO_2 uptake. • High cellular lipid content. • Production of high-value byproducts (*e.g.*, proteins, aquaculture nutrients, fertilizers). • Genetic modification tools available.	• Light requirement (*e.g.*, intensity, wavelength). • Water requirement. • Large amount of phosphorous required as a fertilizer. • Biodiesel fuels derived from algae have poor cold flow properties and low oxidative stability.
Cyanobacteria	• Grow easily with basic nutritional requirements. • Cultivation is simple and inexpensive. • Higher photosynthetic levels and growth rates compared to algae and higher plants. • Contain considerable amount of lipids (mainly in the thylakoid membranes). • Wide range of fuels potentially obtained from cyanobacterial biomass (*e.g.*, H_2, ethanol, photanol, diesel, methane). • Genetic modification tools available.	• Temperature (optimum growth temperature 20-30°C),pH (optimum pH 7-9) and light intensity affect productivity. • After carbon, nitrogen (*e.g.*, nitrate, urea, ammonia) is the most limiting nutrient for biomass production. • Agitation (*e.g.*, aeration, pumping and mechanical stirring) is necessary, increasing the operating cost of cell cultivation.
β-Proteobacteria	• Aerobic microorganisms and easier cultivation compared to clostridia. • Diverse carbon sources and carbon utilization pathways. • Natural ability to store carbon intracellularly as PHA. • Genetic modification tools available.	• Gas fermentation is still under development.
Clostridia	• Diverse range of carbon substrates (*e.g.*, simple or complex carbohydrates, CO_2/H_2 and CO). • Diverse pathways for production of useful metabolites/industrial products (*e.g.*, ethanol, acetate, acetone, lactate, butanol, 2,3-butanediol, valeroate, caproate, carpylate and Closthioamide). • Tolerance to toxic metabolites and substrates. • Genetic modification tools available.	• Anaerobic cultivation could be expensive. • Gas fermentation is still under development.
Archaea	• Natural ability to produce CH_4 (methanogenesis) by methanogens. • Natural ability to accumulate PHA in some archaeal species. • Excellent sources of thermostable enzymes (*e.g.*, carbonic anhydrases).	• Gas fermentation is still under development. • Difficult to emulate growth conditions. • Genetic modification tools unavailable for most archaeal species.

Table 2: Advantages and disadvantages of applying CO_2-utilizing microorganisms for industrial-scale chemical production.

($HCOO^-$), oxalates ($[C(O)O]_2^{2-}$), formaldehyde (H_2CO), carbon monoxide (CO), methanol (CH_3OH) and methane (CH_4) [4]. Most of the current industrial uses of CO_2 are highly energy-intensive processes. CO_2 is a relatively inert molecule; its reactivity is greatly enhanced by the judicious choice of catalysts. In this regard, metal complexes are prime candidates as catalysts owing to the ability of CO_2 to interact with metal centres [4,10]. This property of CO_2 has fuelled the rapid development of new chemistry based on CO_2. To summarize, major scientific and technological challenges of using CO_2 as a C-1 building block include: 1) identifying pathways and products, 2) addressing energetic constraints and 3) understanding and developing new catalysts [7]. Not surprisingly, Nature has provided clues to addressing some, if not all, of these challenges.

Diverse microorganisms capable of utilizing CO_2

Organisms capable of CO_2 assimilation are not restricted to photosynthetic organisms (*e.g.*, plants, algae, cyanobacteria) only. Research endeavour on autotrophic bacteria has started to gain its momentum. In the section below, we summarize CO_2-utilizing microorganisms (Figure 2) that are most extensively studied and could potentially be further developed into industrial-scale bioprocesses. For each of these microorganisms, we provide a brief introduction, discuss their carbon fixation pathways (Table 1, Figure 3), and consider their advantages/disadvantages for exploitation in biological CCU (Table 2). Obviously, these microorganisms have not been evolved by Nature for industrial-scale production of desired chemicals; many of their inherent properties (*e.g.*, growth characteristics, types of metabolites produced, thermo stability, and tolerance to inhibitors) are not fit for purpose. The application of genetic engineering has made it possible to improve their phenotypes and to expand their repertoire of chemical syntheses. Therefore, we also highlight recent developments in engineering these microorganisms for CCU. Worthy of note, this list of microorganisms is by no means exhaustive.

Algae

When thinking of photosynthetic and carbon fixating microorganisms of significant industrial relevance, alga is perhaps the most likely one that stands out. Diverse and found widely throughout the biosphere, algae exist in many forms including the larger sized macro-algae and the more commonly studied and smaller sized micro-algae. Micro-algal species can further be subdivided in accordance to their habitats and structures into classes including green algae, diatoms, red algae, yellow-green algae, golden algae, brown algae and euglenoids [12]. One other subdivision of micro-algae, cyanobacteria, will be discussed in the following section due to their unique characteristics, distinctive from all other micro-algae classes.

As eukaryotes, being either unicellular or more complex multicellular organisms, algae have typical organelles (*i.e.,* chloroplasts) characteristic of most photosynthetic organisms [13]. Algae are able to utilize CO_2 through the Calvin-Benson-Bassham (CBB) cycle (Table 1), converting the inorganic carbon into complex organic compounds. The key enzyme in CBB cycle is ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which catalyzes the carboxylation of ribulose 1,5-bisphosphate to give two molecules of 3-phosphoglycerate (3PG) [14]. One molecule of 3PG is channelled into central metabolic pathways, while the other is utilized in the continuation of the cycle. RuBisCO is, however, a notoriously inefficient carboxylase. In addition to its affinity to CO_2, it also binds O_2 (i.e., oxygenase activity) leading to photorespiration and unwanted products [15]. Such inefficiency of RuBisCO is problematic due to the high O_2 and low CO_2 concentrations present in our atmosphere. To compensate for the imbalance between the high demand of inorganic carbon and low ambient CO_2 concentration,

algae have evolved carbon dioxide concentrating mechanisms (CCMs). There are three major constituents of CCM: 1) active bicarbonate (HCO_3-) uptake transporters, 2) a suite of carbonic anhydrases (CAs) localized strategically within the cells, and 3) a subcellular micro-compartment within which most RuBisCO is located (*i.e.*, the pyrenoids within the chloroplasts) [16]. Inorganic carbon is taken up via either CO_2 diffusion across cellular membranes or the energy-driven accumulation of HCO_3- through the use of membrane pumps [17,18]. This increases the cytosolic inorganic carbon pool, which is unable to diffuse back through the membrane due to the negative charge HCO_3- carries. HCO_3- then enters pyrenoids and the fast acting CA (widely acknowledged as the fastest enzyme discovered in Nature) converts HCO_3- to CO_2 for subsequent utilization by RuBisCO [17,19]. Tight packaging of RuBisCO and high CO_2 concentration within pyrenoids increase RuBisCO's catalytic efficiency, while isolating carboxylation reaction from other competing reactions.

Both macro- and micro-algae are researched and exploited for their ability to fix inorganic carbon. Their potential is attributed to their wide distribution (particularly in moist environments), high biomass capability, fast CO_2 uptake and utilization, and finally and importantly their ability to produce secondary products from the biomass that are of high commercial value [12,20,21]. In algae, the most industrially-relevant component of biomass is lipid, a source for the production of secondary products such as biofuels and lubricants. Therefore, selecting high lipid-producing strains and optimizing the conditions of culture, such as light, temperature and pH, to increase the yield of lipid is vital to maximize the benefit of algal carbon capture and utilization. Biomass production and CO_2 fixation parameters of different micro-algal species and cultivation conditions have been reviewed elsewhere [21].

In recent years, substantial progress has been made in the area of algal transgenics [22,23]. The most advanced genetic tools available have been developed for model organisms such as the green algae *Chlamydomonas reinhardtii* and *Volvox carteri* and the diatom *Phaeodactylum tricornutum*. Nuclear transformation has been achieved for many types of algae, including the industrially-relevant species such as the green micro-algae *Dunaliella salina* and *Haematococcus pluvalis* [24]. Different strategies for modifying green algae have been developed. RNA silencing was used to down-regulate the entire gene family encoding for light-harvesting antenna complexes of *C. reinhardtii* [25]. The engineered alga exhibited an increased efficiency of cell cultivation under elevated light conditions. Random mutagenesis via ^{60}Co γ irradiation was applied to improve the productivity of green alga Chlorella pyrenoidosa in the presence of flue gas containing 15% v/v CO_2 [26]. The biomass yield was increased by 50% and CO_2 fixation efficiency reached 37%, under optimized conditions, corresponding to a CO_2 fixation rate of 1.54 g CO_2/L/day. It is worth noting that algal transgenics has opened up an exciting possibility of using eukaryotic algae for recombinant protein production [27]. Green alga *C. reinhardtii* was demonstrated to express complex mammalian proteins in its chloroplasts, including a full-length IgG1 human monoclonal antibody [28]. Specht *et al.* comprehensively reviewed the current state of this field of research and its potential future applications [29].

Cyanobacteria

Photosynthetic prokaryotes span across five phyla: cyanobacteria, proteobacteria, chlorobi, chloroflexi and firmicutes. As mentioned earlier, cyanobacteria are often described as micro-algal species. However unlike algae, they are prokaryotic, with their photosynthetic pigment present within their cytoplasm rather than specialized organelles as in eukaryotic plants and algae. These organisms are able to fix nitrogen from the atmosphere through nitrogenase as well as inorganic carbon. Like algae, cyanobacteria are diverse and widely distributed, existing either in biofilms [30] or as individual planktonic cells. Cyanobacteria are believed to have played a key role in the early atmosphere formation lowering the CO_2 concentration and increasing the oxygen (O_2) concentration [31]. Nowadays, cyanobacteria are still considered a key player among photosynthetic organisms, accounting for 20–30% of Earth's primary photosynthetic activity [32].

RuBisCO is responsible for the carbon utilization in cyanobacteria, catalyzing the same reaction as in algae in the CBB cycle. Cyanobacteria rely on carboxysomes to achieve CCM. Analogous to algal pyrenoids described above, carboxysomes are extraordinarily efficient proteinaceous micro-compartments that encapsulate RuBisCO [33]. Even though cyanobacterial RuBisCOs typically display low affinity for CO_2, they are capable of high growth level at low CO_2 concentration due to the action of carbonic anhydrase (CA) in concentrating intracellular CO_2 level [34].

Cyanobacteria are more efficient at the carbon fixation from the atmosphere than algae owing to their simpler structure [35]. However, they are not able to yield the same biomass level [36]. That being said, cyanobacteria are still sought after for inorganic carbon fixation as they possess a simpler genetic make-up that can easily be engineered for improved biomass yield [37], increased RuBisCO's CO_2 affinity [34,38] and production of useful chemicals [35].

Advances in the metabolic engineering of cyanobacteria, including production of ethanol, isobutanol and isoprene, were comprehensively reviewed [39]. Since then, the number of potential products that can be produced by cyanobacteria from CO_2 has greatly expanded. By transferring a modified CoA-dependent 1-butanol production pathway to cyanobacterium *Synechococcus elongates,* autotrophic production of 1-butanol was demonstrated [40]. The same species of cyanobacterium was also metabolically engineered to achieve isopropanol production from CO_2 and light [41]. The developed system produced 26.5 mg/L of isopropanol after 9 days of cultivation. In comparison, *Clostridium beijerinckii* can achieve an isopropanol titre exceeding 1.5 g/L [42]. *S. elongates* was also reported to be engineered to produce improved yields of 2,3-butanediol (2.38 g/L) [43]. Further, autotrophic 1,2-propanediol production was achieved in *S. elongates,* with a reported titre of 150 mg/L under optimized conditions [44]. The production of terpenoids, a diverse group of organic compounds with significant industrial applications, in cyanobacteria has been described [45]. Heterologous genomic integration of the mevalonic acid pathway genes from *Enterococcus faecalis and Streptococcus pneumoniae* in a cyanobacterium of *Synechocystis* genus, already expressing heterologous isoprene synthase, led to a 2.5-fold increase in isoprene production [46]. By heterologous expression of appropriate synthases in *Synechocystis* sp., production of sesquiterpene β-caryophyllene [47] and monoterpene β-phellandrene [48] were also demonstrated.

β-Proteobacteria

Belonging to the class of β-proteobacterium, *Ralstonia eutropha* (also known as *Cupriavidus necator*) is a Gram-negative bacterium found in soil environment. This bacterium can afford autotrophic, heterotrophic and mixotrophic growth, depending on the types of carbon source available.

In the absence of organic substances, *R. eutropha* thrives on

hydrogen (H_2) as sole energy source, fixing CO_2 via the CBB cycle [49]. Besides RuBisCO, CA is of great importance for fine-tuning CO_2 concentration in autotrophic metabolism. Analysis of the *R. eutropha* H16 genome sequence revealed the presence of four CA genes: can, *can2, caa* and *cag* [50].

R. eutropha is a typical representative of the aerobic H2 oxidizer [51], making it an easier microbe to handle compared to obligate anaerobe. What is most desired from *R. eutropha* is its ability to capture CO_2 and utilize it to produce chemicals of commercial value [52]. The biggest appeal of working with *R. eutropha* is perhaps its ability to store carbon within its cytoplasm in the form of polyhydroxyalkanoates (PHAs), or more generally known as bio-plastics. PHA granules usually consist of short chains of poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). However, polymers of varying lengths can also be produced through genetic manipulation. These bio-plastics are highly sought after commercially for their green credentials, being a source of carbon sink and biodegradable while also sharing desired properties of the traditionally produced plastics. Other than its ability to produce polymers of varying characteristics, *R. eutropha* is also desired for its diverse carbon utilization pathways and its bio-compatibility for production of medicinal compounds [53].

Reported genetic modifications of *R. eutropha* concentrate mainly on expanding the repertoire of useful compounds produced by this species and increasing its production yield [54]. A lot of research has been done on using genetic engineering to obtain a variety of different PHA copolymers that exhibit better mechanical properties than PHB using diverse carbon sources such as plant oils or fructose [54-56]. Large quantity production (up to 40% cellular dry weight) of cyanophycin, a useful protein-like polymer, was shown [57]. Other useful compounds that can be produced in significant quantities from genetically modified *R. eutropha* include ferulic acid (a precursor to vanillin biotransformation) [58] and 2-methylcitric acid [59]. Despite the significant expansion in the number of potential biochemicals produced by *R. eutropha*, most of the discussed processes utilize organic compounds as a carbon source instead of CO_2. The absence of versatile genetic tools specifically for *R. eutropha* has certainly contributed to this. Recently, Bi *et al.* reported the development of a genetic toolbox for the metabolic engineering of this species, which consists of a set of plasmids bearing a variety of origins of replication, promoters, ribosomal binding sites and 5'-mRNA stem-loop structures [60]. All these genetic elements were analyzed and characterized in details. The usefulness of this toolbox was further demonstrated by performing an optimization of hydrocarbon production in *R. eutropha* resulting in a 6-fold titre improvement. Beller and co-workers engineered *R. eutropha* for the production of fatty acid-derived, diesel-range methyl ketones. They achieved a productivity of 50–180 mg/L under chemolithoautotrophic growth conditions, using CO_2 and H_2 as the sole carbon source and electron donor, respectively [61]. Also relevant to CCU is the production of isobutanol in *R. eutropha.* Li *et al.* reported production of isobutanol and 3-methyl-1-butanol from CO_2 using an integrated electromicrobial process [62]. This system involved electrochemical generation of formate from CO_2, followed by conversion to isobutanol and 3-methyl-1-butanol using genetically modified *R. eutropha.* The final titre was reported to exceed 1.4 g/L (approx. 850 mg/L isobutanol and 570 mg/L 3-methyl-1-butanol).

Ideonella sp., like *R. eutropha*, is capable of storing biological polymers intracellularly from captured inorganic carbon [63]. An advantage of this species over *R. eutropha* is its higher tolerance to carbon monoxide (CO) and H_2 gases, suggesting its potential use with industrial exhaust gases as feed for fermentation.

Clostridia

Clostridia are anaerobic, Gram-positive bacteria. They are of major significance to: 1) human and animal health and physiology, 2) anaerobic degradation of simple and complex carbohydrates, 3) the carbon-cycle and acidogenesis, and 4) degradation/bioremediation of complex organic chemicals [64].

Many strains in the *Clostridium genus* are able to fix CO_2 (using H_2 as an electron donor) or CO alone, and also use other C1 compounds, such as formate ($HCOO^-$) and methanol, as sole carbon sources. They accomplish this using the Wood–Ljungdahl (WL) pathway (Table 1), in which two molecules of CO_2 are reduced to produce one molecule of acetyl-CoA with CO or H_2 being used to generate reducing equivalents.

Clostridia, particularly *Clostridium* spp., display many desirable traits for biotechnological applications: 1) the ability to utilize a broad spectrum of carbon substrates (*e.g.*, simple and complex carbohydrates, CO_2/H2 and CO), 2) diverse pathways for production of useful metabolites/industrial products (*e.g.*, ethanol, acetate, acetone, lactate, butanol, 2,3-butanediol, valeroate, caproate, carpylate and Closthioamide), and 3) tolerance to toxic metabolites and substrates [64]. Being an obligate anaerobe, however, makes the cultivation of *Clostridium* more difficult. Normal atmospheric condition is usually fatal to most *Clostridium* species.

Early work with *Clostridium* focused mainly on the production of acetic acid and related products [65-68]. Research on CCU using genetically modified *Clostridium* species suffers a relatively slow kick off, owing to the lack of relevant genetic tools, as in the case of *R. eutropha.* Nonetheless, the repertoire of genetic tools applicable to clostridia has significantly expanded following the development of plasmid DNA technologies and chromosomal manipulation technologies [64]. The use of the mobile group II introns for targeted gene disruption (Targetron or ClosTron) is popular for genetic engineering of *Clostridium* [69-71]. Application of ClosTron was demonstrated through engineering *Clostridium acetobutylicum* for acetone-butanol-ethanol fermentation [72] and for expression of cellulosome [73]. Papoutsakis group developed a resolvase overexpression approach for enhancing native homologous recombination in clostridia [74]. Similarly, Leang et al. utilized double-crossover homologous recombination and an improved electroporation protocol to conduct a proof-of concept gene deletion study on *Clostridium ljungdahlii* [75].

Archaea

The domain of Archaea encompasses microorganisms that are encountered in exceptional ecological niches, such as high (thermophiles and hyperthermophiles) or low (psychrophiles) temperatures, acidic environment (acidophiles), high salinity (halophiles) and anaerobic atmosphere (methanogens). CO_2-type hydrogenotrophic methanogens *(e.g., Methanothermobacter thermautotrophicus, Methanothermobacter marburgensis, Methanobrevibacter aboriphilus, Methanocaldococcus jannaschii, Methanosarcina barkeri)* are capable of methane production (CH_4; biological methanogenesis) using H_2 as energy source and CO_2 (or CO and $HCOO^-$) as carbon source [76]. This intriguing group of microorganisms presents us with an opportunity of alternative energy production. Further, strains of several haloarchaeal genera, including *Haloarcula, Haloferax, Halobiforma* and *Haloquadratum*, have been found to accumulate PHA [77].

Members of autotrophic archaea, such as *Metallosphaera, Sulfolobus, Archaeoglobus,* and *Cenarchaeum species*, capture CO_2 via

the 3-hydroxypropionate-4-hydroxybutyrate (3HP-4HB) cycle (Table 1) [78]. The 3HP-4HB cycle utilizes inorganic carbon in the form of HCO_3-. In this cycle, a single acetyl-CoA molecule and two HCO_3- molecules are used to form a succinyl-CoA, which in turn is converted via 4-HB into two acetyl-CoA molecules.

As many archaea are extremophiles, these organisms represent excellent sources of thermostable CA enzymes suitable for industrial CO_2 capture [34] and platforms for gas fermentation [79]. The identification of new archaeal isolates and the development of genetic tools for engineering archaea would certainly expand the product range of gas fermentation.

Genetic engineering of archaea has recently picked up its pace. Keller *et al.* reported the heterologous expression of five genes from the carbon fixation pathway of archaeon *Metallosphaera sedula* in a hyperthermophilic archaeon *Pyrococcus furiosus,* which grows optimally on carbohydrates at 100°C [80]. Engineered *P. furiosus* was demonstrated to successfully incorporate CO_2 into 3HP, an important chemical building block, at 70˚C that is the optimal growth temperature of *M. sedula*. At this temperature, metabolism of the engineered *P. furiosus* is substantially limited, significantly reducing metabolic burden during 3HP production. This temperature-dependent strategy for production of biochemicals, based on the work of Basen *et al.* [81], provides a potential approach to manipulating carbon metabolism.

Synthetic biology: A powerful tool for advancing biological CCU

Direct application of naturally occurring organisms is frequently hindered by their undesirable properties (*e.g.*, insufficient product yield, demanding cultivation conditions, and inadequate growth rate). Synthetic biology has emerged as a powerful tool for engineering living organisms that allows for alteration of existing biochemical pathways and introduction of heterologous pathways into organisms with favorable traits or features [82]. Considering recent advances in enabling technologies such as protein engineering, metabolic engineering and system biology [83], synthetic biology appears to be a perfect tool for enhancing biological processes and making them feasible and more economically viable. It is not surprising that, in recent years, synthetic biology has also become central to biological CCU. In this section, special attention is given to *Escherichia coli* and *Saccharomyces cerevisiae.*

Genetically modified *Escherichia coli*

Owing to its simple cultivation, relatively high growth rate and an extensive repertoire of genetic tools available, Gram-negative bacterium *Escherichia coli* remains the frontrunner in the development of new technologies for CCU, despite its heterotrophic nature.

Heterologous expression of RuBisCO and phosphoribulokinase (PRK) in *E. coli* under different fermentation conditions was attempted by Zhuang *et al.* [84]. When grown in media supplemented with L-arabinose, a significant CO_2 fixation rate was observed [67 mg CO_2/mole arabinose/L/h] resulting in 15% reduction in CO_2 emission during fermentation. Heterologous expression of CA in E. coli is widely reported. Expression of CA from *Methanobacterium thermoautotrophicum* and *Methanosarcina thermophile* in the periplasm of *E. coli* resulted in whole-cell biocatalysts for CO_2 hydration [85]. The developed systems successfully hydrated CO_2, with catalytic efficiencies and turnover numbers only an order of magnitude lower than those of free enzymes, plausibly due to mass transport limitations. When calcium chloride ($CaCl_2$) and calcium carbonate ($CaCO_3$) were added to the solution, 50–70% increase in $CaCO_3$ precipitation was observed, in comparison to uncatalyzed reaction. Despite lower efficiency, whole-cell biocatalysts provide two significant advantages over the use of dissolved CA for CO_2, namely higher stability (100% activity retention after 24 h of use) and bypassing the need for protein purification (whole cells can be separated and recycled). Similar strategy was used in periplasmic expression of CA from *Neisseria gonorrhoeae* in *E. coli* [86].

Recent advances in CCU using *E. coli* are not limited to expression of individual enzymes. Functional carboxysomes from Halothiobacillus neapolitanus, encoded by 10 genes, were successfully expressed in *E. coli*. These bacterial micro-compartments are capable of in vivo self-assembly and carbon fixation [87]. Expressed carboxysomes were purified using sucrose gradient centrifugation and reported to fix CO_2 *in vitro* as well.

Genetic engineering of *E. coli* has also made it possible to efficiently convert photosynthetic organisms into valuable chemicals. Brown algae, for example, are considered promising feedstock for carbon-neutral biofuels production. Their economic viability is hindered however by inability of industrial microorganisms to metabolize alginate, one of their most abundant polysaccharides. Wargacki *et al.* reported the discovery of a DNA fragment from *Vibrio splendidus* encoding for enzymes responsible for alginate transport and metabolism [88]. Its expression in *E. coli* resulted in creation of microbial platform capable of metabolizing alginate, with a yield of 0.281 weight ethanol/weight dry macroalgae (about 80% of maximum theoretical yield based on the total polysaccharide composition).

Genetically modified *Saccharomyces cerevisiae*

In comparison to *E. coli,* model eukaryotic microorganism, *Saccharomyces cerevisiae,* has attracted less attention as a potential solution to anthropogenic CO_2 emission. Heterologous expression of prokaryotic RuBisCO from *Thiobacillus denitrificans* and PRK from *Spinacia oleracea* was demonstrated in *S. cerevisiae* [89]. To obtain a significant number of functional RuBisCO molecules, co-expression of *E. coli* protein-folding chaperones GroEL and GroES was necessary. The developed system was characterised by a 90% reduction in the by-product glycerol formation and a 10% increase in ethanol production, for a sugar-limited culture.

Another relevant example is improved production of malic acid in *S. cerevisiae* by engineering a CO_2 fixating pathway that proceeds via the carboxylation of pyruvate [90,91]. Engineered *S. cerevisiae* strain produced malate at a titre of up to 59 g/L, with a malate yield of 0.42 mole/mole glucose.

Protein engineering for enhanced biological CCU

Protein engineering is equally important as the genetic engineering approach described in the previous section and contributes to the advancement of biological CCU. Many naturally occurring enzymes/proteins have not been evolved or designed for industrial applications [92]. Their properties, however, can be tailored via state-of-the-art protein engineering approaches such as rational design and directed evolution [93]. Enzymatic activity, specificity, selectivity, thermostability, tolerance to organic solvents and inhibitors are only a few examples of protein properties that can be improved using protein engineering. In this section, we discuss selected examples of protein

engineering in CCU.

RuBisCO and RuBisCO activase

Engineering RuBisCO, particularly to improve its selectivity, is not trivial [94]. Site-directed mutagenesis study on RuBisCO from green alga *C. reinhardtii* resulted in proteins variants with decreased CO_2 selectivity and lower CO_2 utilization efficiency [95]. This study echoed the generally accepted hypothesis that selectivity of RuBisCO is determined by complex interactions between amino acids residues distant from the active site. Therefore, research effort is channeled toward applying directed evolution to improve the catalytic efficiency of RuBisCO [96].

Parikh *et al.* introduced a clever strategy for high-throughput selection of functional RuBisCO variants [97]. The CBB cycle was partially reconstructed in *E. coli* and the genetically modified bacteria were grown on minimal media supplemented with pentose. The growth of this engineered *E. coli* was dependent on the presence of a functional RuBisCO, therefore creating a novel platform for directed evolution of this carboxylase. The use of this selection system was demonstrated by evolving cyanobacterial RuBisCO. Selected protein variant displayed 5-fold increase in specific activity compared to that of wild-type enzyme. The observed increase in the specific activity was attributed to the 4–9-fold increase in the yield of properly folded L subunits of RuBisCO in *E. coli* [98]. Nonetheless, a detailed investigation of the enzymatic activity of RuBisCO variant showed a 12% increase in carboxylation turnover rate and a 15% improvement in its K_M for CO_2, with no observed change in CO_2/O_2 selectivity.

RuBisCO activase is the catalytic chaperone of RuBisCO, promoting dissociation of sugar from RuBisCO's active site [99]. Gene shuffling was applied to engineer thermostability of *Arabidopsis thaliana* RuBisCO activase isoform RCA1 [100]. After two rounds of DNA shuffling and screening, the best variant exhibited nearly 2-fold increase in specific activity after 15 min of incubation at 45˚C. At 40˚C, 98% of the RuBisCO retained its activation state in the presence of activase mutant. In the case of wildtype activase, only 70% of the RuBisCO was active. The thermostable variant was also expressed in RCA1 deletion mutant of *A. Thaliana*. The transgenic plant, when grown at constant 26˚C or exposed daily to 30˚C for 4 hours, demonstrated higher growth rate and higher photosynthetic rate.

Carbonic anhydrase

One of the most popular options for carbon capture is chemical absorption of CO_2 using alkaline solvents [*e.g.*, aqueous monoethanolamine (MEA) or methyldiethanolamine (MDEA)] [101]. The disadvantage of this method is significant heat loss during the desorption process. CA has been reported to facilitate the use of solvents with lower heat of desorption and, consequently, to reduce the total energy demand of the whole process [104]. The biggest obstacle to using CA in this process is the harsh operating conditions, such as high temperature and high concentration of organic amine. Potential solutions to this problem include enzyme immobilization, utilization of CA from thermophilic species and protein engineering. Codexis utilized directed evolution to increase the activity and stability of CA under the described conditions [102,103]. The evolved enzyme showed a significantly higher stability at elevated temperatures and high concentration of alkaline solvent. The half-life of CA was increased by approximately five orders of magnitude (to 20 hours) in 4.2 M MDEA and at 75˚C [102]. For a long time, chemical modifications have played an essential role in modifying protein properties [104,105]. By exposing CA to cross-linking agents, Codexis was able to further improve its stability and specific activity at high concentration of alkaline solvents. The chemically modified polypeptides were reported to have up to five times higher specific activity in 4.2 M MDEA and at 50˚C than the unmodified protein [106].

Other (de)carboxylases

In addition to those carboxylating enzymes found in the carbon fixation pathways (Table 1), other (de)carboxylases that exist in the Nature also have huge biotechnological potential. Salicylic acid (de) carboxylase (Sdc) that catalyzes reversible carboxylation of phenol to salicylic acid (Kolbe–Schmitt reaction) has been isolated from a salicylic acid-degrading yeast *Trichosporon moniliiforme* WU-0401 [107]. Recombinant *E. coli* expressing Sdc converted phenol to salicylic acid with a 27% (mole/mole) yield, corresponding to a titre of 10.6 mM, after 9 h cultivation at 30°C. The isolated enzyme also catalyzes the conversion of m-aminophenol to *p*-aminosalicylic acid (PAS), an important bioactive compound widely used as an anti-tuberculosis agent. Directed evolution was used to improve the catalytic properties of this enzyme [108,109]. The evolved protein variant, Y64T-F195Y-Sdc, showed a 12-fold higher specific carboxylation activity toward *m*-aminophenol than the wild-type enzyme [109]. Recombinant *E. coli* expressing Y64T-F195Y-Sdc produced 140 mM PAS from 200 mM *m*-aminophenol within 9 h [109].

Designed/artificial peptides/proteins for carbon capture

Short peptides constitute another class of biological molecules whose properties could potentially have an impact in the field of CCU.

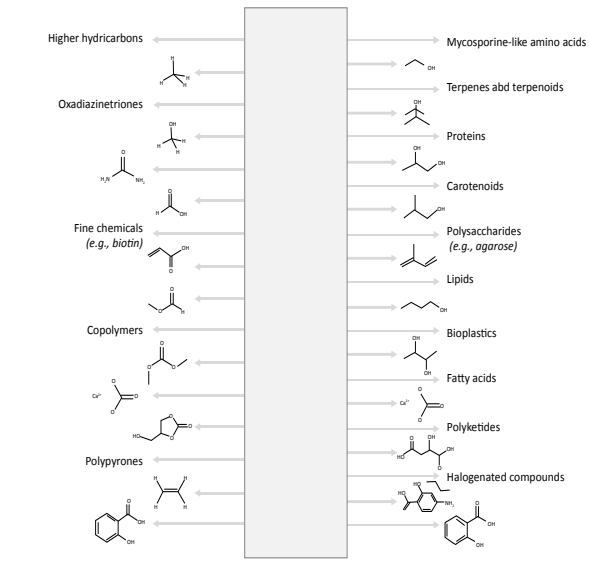

Figure 4: A comparison of CO_2-derived chemicals via chemical [3,7,9,127,128] and biological routes. Three observations are derived from this comparison: 1) some chemicals (*e.g.*, salicylic acid, methane) can be produced via either a chemical or a biological route, 2) biological routes complement chemical approaches and expand the range of chemicals that can be derived from CO_2, and 3) complex molecules (*e.g.*, protein) can only be produced via a biological route because a microorganism can be regarded as an integrated chemical plant encompassing a multitude of biocatalysts.

Bio-based products	Organisms	Species	Chemicals	Productivity	References
Bio-plastics	Proteobacteria	*Ralstonia eutropha*	Poly-3-hydroxybutyric acid	Under growth with H_2/CO_2, 61 g/L of PHB was formed in 40 h, representing ~70% of total cell weight.	[132]
	Proteobacteria	*Ideonella* sp. O-1	Poly-3-hydroxybutyric acid	The content of PHB in the cells reached ~80% (w/w).	[63]
	Algae	*Phaeodactylum tricornutum*	Poly-3-hydroxybutyric acid	PHB accumulated to 10% of algal dry weight.	[133]
Bio-alcohols	Proteobacteria	*Rhodobacter sphaeroides Rhodobacter capsulatus*	Ethanol	~800 mg/L ethanol from CO_2 and non-water hydrogen source.	[134]
	Cyanobacteria	*Synechococcus elongatus* PCC 7942	Ethanol	Ethanol production rate of 0.18 μg/L/hr from CO_2 and water.	[135]
	Cyanobacteria	*Synechococcus elongatus* PCC 7942	Isopropanol	26.5 mg/L of isopropanol after 9 days.	[41]
	Cyanobacteria	*Synechococcus elongatus* PCC 7942	Isobutyraldehyde, isobutanol	Productivity of isobutyraldehyde of 6230 μg/L/hr was achieved, and 450 mg/L of isobutanol was produced in 6 days.	[136]
	Cyanobacteria	*Synechococcus elongatus* PCC 7942	*n*-Butanol	*n*-Butanol accumulation reached 14.5 mg/L in 7 days.	[40]
Bio-diesel	Algae	*Nannochloropsis oculata*	Lipids	The maximal biomass and lipid productivity in a semi-continuous system were 0.480 and 0.142 g/L/d with 2% CO_2 aeration.	[137]
	Algae	*Chlorella vulgaris*	Lipids	The maximal biomass and lipid productivity were 3.83 g/L and 0.157 g/L/d with CO_2 aeration rate of 0.5 vvm.	[138]
Other chemicals	Algae	*Porphyridium aerugineum*	Polysaccharide	~2.5 mg/mL in 20 days.	[139]
	Proteobacteria	*Ralstonia eutropha*	Methyl ketone	50-180 mg/L under chemolithoautotrophic growth conditions.	[63]
	Cyanobacteria	*Synechocystis* sp. PCC 6803	Isoprene	Accumulation of ~50 μg isoprene/g of dry cell weight/day.	[140]
	Cyanobacteria	*Synechocystis* sp. PCC 6803	Sesquiterpene β-caryophyllene	3.7 μg of β-caryophyllene/g of dry cell weight/week.	[47]
	Yeast	*Trichosporon moniliiforme*	Salicylic acid	Phenol was converted to salicylic acid with a 27% (mol/mol) yield at 30°C for 9 h.	[107]

Table 3: Representative bio-based products derived from CO_2.

Hydrophobic dipeptides (*e.g.*, VA, AI, AV, VV, IV, IA, VI and LS) have been reported to undergo self-assembly forming low-density crystal structures containing nanochannels [110]. This type of material has been shown to selectively absorb CO_2 over nitrogen (N_2) and methane (CH_4) providing a promising alternative to current CO_2 separation methods [111]. Similar properties have been observed in hexapeptide-based amyloid fibres (VQIVYK), which have been demonstrated to bind CO_2 via carbamate formation [112]. Binding was undiminished in the presence of water and heating the material to 100°C resulted in its regeneration. Short peptides have also been displayed on the surface of CA-overexpressing *S. cerevisiae* cells to increase the mineralization rate of $CaCO_3$ [113]. For a concentration of $2x10^8$ cells per millilitre, the display of peptides derived from glycophorin A (PEVPEGAFDTAI) on the cell surface led to a nearly 50% increase in the mineralization rate. This improvement was attributed to favourable electrostatic interactions between the acidic peptides and calcium ions. We strongly believe that designed or artificial peptides and proteins would be the next wave of biological materials for CCU applications.

Bio-based products from CO_2

The stunning array of CO_2-utilizing microorganisms available (section 3), the ability to genetically modify microorganisms (section 4) and the use of protein engineering to tailor enzymatic/protein properties (section 5) have vastly expanded the repertoire of bio-based products that can be synthesized directly from CO_2 (Figure 4, Table 3). Representative bio-based products are discussed herein. Biological CCU is no doubt a desirable alternative to biorefining that relies on biomass feedstock.

Bio-plastics

In *R. eutropha* and *Ideonella* sp., CO_2 is reduced to form PHB, typically under the conditions of nutrient limitation but with an excess of a carbon source. Purified PHB is a biodegradable and biocompatible thermoplastic, exhibiting thermal and mechanical properties similar to those of petrochemically-derived polypropylene. This has induced a considerable interest in the commercial production of this polymer. PHB is well suited for use as food packaging material in view of its resistance to water and UV radiation and its impermeability to O_2. It has also been applied in surgical sutures. Importantly, PHB can be processed using pre-existing technologies and used in combination with other synthetic polymers. A few platform chemicals that can be derived from PHB, chemically or enzymatically, include (*R*)-3-hydroxybutyric acid (3HB), methyl ester of 3HB, crotonic acid, acetoacetic acid and 1,3-butanediol *etc* [114].

Bio-alcohols

Early efforts in direct incorporation of CO_2 to produce bio-alcohols have focused mainly on ethanol production in *Rhodobacter* and *Synechococcus* [115]. The use of ethanol as a replacement for conventional gasoline is challenged by the facts that ethanol has low

energy density (70% that of gasoline) and is corrosive to current engine technology and fuel infrastructure [116]. Further, it readily absorbs water leading to separation and dilution in the storage tank [116]. Biological production of isopropanol is feasible. Isopropanol can be used to partially replace gasoline. It is also applied, instead of methanol, to esterify fat and oil for biodiesel production, which reduces its tendency to crystallize at low temperatures. Isopropanol can further be dehydrated to yield propylene for making polypropylene [117]. In recent years, we observe a shift in research focus toward the biological productions of more advanced biofuels, such as isobutanol and *n*-butanol. Butanol is essentially noncorrosive and immiscible with water. Its energy density (~82-90%) is closer to gasoline [116]. Butanol can be used as a solvent or a gasoline blendstock. Isobutanol is readily converted through known processes to a variety of hydrocarbons (*e.g.*, isobutylene and paraxylene). *n*-Butanol, on the other hand, is used as production intermediate for butyl acrylate, butyl acetate and dibutyl phthalate. Equally attractive, butanol can be efficiently and effectively used in existing production, distribution, marketing and end-user assets [118].

Bio-diesel

Due to their high lipid content and ease of cultivation, algae are ideal starting materials for the production of bio-diesel that may be used to replace non-renewable petroleum-based transport fuels. Lipids, in the form of triacylglycerides (TAGs), typically provide a storage function in the cell that enables algae to endure adverse environment conditions. Once extracted, lipid can be converted into fatty acid methyl esters (FAME) or biodiesel via transesterification reaction (*e.g.*, with the use of methanol) [119]. The physical characteristics of FAME are similar to those of fossil diesel fuels. Importantly, it is non-toxic and biodegradable. The use of bio-diesel as a low-blend component in the transport fuel (*i.e.,* up to 7%) does not require any changes in the distribution system, therefore avoiding expensive infrastructure changes.

Other chemicals

With the advent of genetic engineering and protein engineering (sections 3-5), bio-based products from CO_2 extend further to include chemicals such as acetone, isoprene, isobutyraldehyde, malic acid, salicylic acid and terpenoids *etc* (Figure 4, Table 3). It is worth emphasizing that complex biopolymer (*e.g.*, protein) synthesis from CO_2 using photosynthetic organism (*e.g.*, *C. reinhardtii*) is also feasible [29]. This synthesis would not have been possible with a chemical route.

Bioprocess engineering

Industrial-scale implementation of any biochemical process for chemical production, including biological CCU discussed above, requires that we establish its sustainability and economic viability. To this end, a close collaboration between scientists and engineers is necessary. Input of engineering expertise is critical to address process design and/or integration, which usually take place after pilot study or laboratory-scale proof-of-concept. Production of biofuels from algae is by far the most studied and promising method of biological CCU. A considerable amount of engineering effort has gone into addressing the inherent challenges of large-scale algal cultivation. Some of these challenges also apply to other autotrophic organisms. Therefore, we use algal cultivation as an example.

Challenges

On an industrial scale, algae are usually grown in either open ponds, including raceway ponds, or photobioreactors [120]. Open ponds are simpler to design and construct, however, they are limited by large surface area requirement, high cultivation costs, low biomass productivity and contamination [121]. To overcome these problems, the use of closed system cultivation such as photobioreactors has been proposed.

Kumar *et al.* provided a detailed review on photobioreactor design and challenges of industrial-scale algal cultivation [122]. Flue gas constitutes one of the main sources of CO_2 for algal cultivation but its high temperature, varying composition and the presence of sulfur and nitrogen oxides pose major problem for efficient culture. Such operating conditions also necessitate careful selection of a suitable algal strain. Light intensity is another major consideration as insufficient amount of light results in poor growth while excessive light intensity is likely to induce photoinhibition. Even when the intensity of incident light is fully optimized, individual cells are exposed to varying amounts of light unless adequate mixing is provided. The mixing of cell culture requires a considerable energy input and, consequently, constitutes a significant portion of the total operating cost. Oxygen accumulation can be problematic as trapped oxygen is likely to reduce photosynthetic efficiency. These are only a few examples of the challenges that we face in order to improve the economic viability of the bioprocess [120,122-124]. Fortunately, many advances have been made to address the discussed issues.

Recent advances

Research on bioprocess engineering of algae has expanded greatly in recent years, both in scope and diversity. Advances made span across all stages of large-scale algal cultivation, from CO_2 supply to product extraction (Table 4). Worthy of note, life cycle analysis of large-scale bio-diesel production from algae was also reported using commercial data available [125]. The technological diversity that we see in Table 4 suggests that any economically viable CCU using biological system can only be a result of interdisciplinary collaboration.

Future prospects and conclusion

The principles of green chemistry have become firmly entrenched in academia as well as industry. They serve as a blueprint, guiding the design and development of environmentally benign products and processes. Poliakoff and co-workers proposed a mnemonic "PRODUCTIVELY" (Table 5), which captures the spirits of green chemistry [126]. Our analyses show that the idea of applying biological systems to achieve CCU aligns with the philosophy of green chemistry (Table 5). Importantly, the range of CO_2-derived chemicals using biological systems is expanding rapidly and will soon catch up with CO_2 transformation via chemical routes (Figure 4, Table 3) [3,7,9,127,128].

A possible biological route for chemical production does not always guarantee its eventual translation into a viable industrial process. If we consider the principles of green engineering (Table 6; again summarized perfectly using a mnemonic "IMPROVEMENTS" [126]), there are challenges that need to be addressed according to our assessment, should we adopt a biological route for CCU. Key bioprocess design and engineering issues include process safety (if H_2 is utilized as an energy source), directing carbon source into bio-product formation, control of cultivation conditions (*e.g.*, temperature, pH, light, O_2, nutrients) to maximize cell density and product yield,

Production stage	Recent advances	References
CO_2 supply	• Optimization of flue gas settings via computer simulations to promote microalgae growth.	• [141]
Light supply	• Solar spectrum conversion using a photoluminescent material.	• [142]
Water supply	• Construction of algal-bacterial microcosm capable of processing organic waste.	• [143]
Algal strain selection	• High throughput screening method for rapid identification of microalgae with high CO_2 affinity.	• [144]
Reactor design	• Design of an airlift-driven raceway reactor.	• [121]
	• Design of a photobioreactor utilizing Taylor vortex flow.	• [145]
Reactor settings optimization	• pH-based method for determining CO_2 fixation capacity of microalgae under given conditions.	• [146]
Cell harvesting	• New method of algae flocculation based on decreasing pH of culture media.	• [147]
Product separation	• Analysis of different methods of cell disruption for efficient lipid extraction.	• [148]
Life cycle analysis	• Life cycle analysis (LCA) of large-scale algal bio-diesel production using commercial data.	• [125]

Table 4: Selected examples of advances in various stages of large-scale algal cultivation.

Principles of green chemistry*		Biological CCU
P	Prevent wastes.	Recyclable bio-wastes.
R	Renewable materials.	Light/H_2 as energy source and CO_2/flue gas as carbon source.
O	Omit derivatization steps.	CO_2 converted via integrated biochemical pathways.
D	Degradable chemical products.	Biodegradable products.
U	Use safe synthetic methods.	Use of non-pathogenic organisms.
C	Catalytic reagents.	Specialised enzymes, micro-compartments or organelles.
T	Temperature, pressure ambient.	Mild cultivation conditions.
I	In-process monitoring.	Process control for bioreactors or fermenters is available.
V	Very few auxiliary substances.	Other non-carbon nutrients derived from biomass.
E	E-factor, maximize feed in product.	Yield optimization via strain selection, genetic engineering and synthetic biology.
L	Low toxicity of chemical products.	Biocompatible products.
Y	Yes it's safe.	Generally safe. Cautions in large-scale H_2/O_2/syngas utilization.

* Adapted from [126]

Table 5: Alignment of biological CCU with the principles of green chemistry.

Principles of green engineering*		Challenges of applying biological CCU
I	Inherently non-hazardous and safe.	The use H_2/O_2/syngas presents explosion safety challenges to large-scale production.
M	Minimize material diversity.	Less of a biological problem.
P	Prevention instead of treatment.	Bio-wastes are inevitable in fermentation.
R	Renewable material and energy inputs.	Concentration, composition, temperature and pressure of CO_2 source have direct impact on organismal growth and productivity. The same applies to energy source (*e.g.*, light intensity and wavelength *etc*).
O	Output-led design.	Design of biological system is not trivial and requires sound knowledge at both molecular and system level. Robust genetic tool is lacking for modification of some organisms.
V	Very simple.	Biological system is inherently complex, highly integrated and regulated.
E	Efficient use of mass, energy, space & time.	Energy and carbon source are channelled into cell growth and biomass accumulation, instead of chemical production. Low productivity is an issue. Biological membrane could be a barrier to mass/energy transfer. Some enzymes display promiscuous activities (moonlighting). Maintaining strict anoxia for anaerobic cultivation, sparging, and cell stirring can be costly and energy intensive.
M	Meet the need.	Less of a biological problem.
E	Easy to separate by design.	Most organisms or enzymes are not tolerant to solvents used in product separation.
N	Networks for exchange of local mass & energy.	Less of a biological problem.
T	Test the life cycle of the design.	Less of a biological problem.
S	Sustainability throughout product life cycle.	Less of a biological problem.

*Adapted from [126]

Table 6: Challenges of applying biological CCU, identified through principles of green engineering.

product extraction/purification and techno-economics *etc.*

In term of technology readiness level, algae and cyanobacteria are frontrunners. β-Proteobacteria (*e.g., Ralstonia* spp.), clostridia (*e.g.*, *Clostridium* spp.) and archaea (*e.g.,* methanogens) could eventually overtake photosynthetic microorganisms, considering rigorous research activities in these areas. Regardless the type of microorganism used, we need to pay attention to essential prerequisites for a successful bioprocess development: 1) appropriate strain selection (*e.g.*, cell density, growth characteristics, CO_2 fixation rate, product type and yield), 2) media demand in addition to CO_2, 3) physiological parameters (*e.g.*, gas concentration), 4) bioprocess mode (*e.g.*, batch, continuous), and 5) bioreactor design (*e.g.,* continuous stirred tank reactor, fixed-bed reactor, membrane reactor) [129-131].

Biological CCU is likely not a stand-alone technology and could potentially be coupled to other well-established chemical processes such as gasification and water gas shift (WGS) reaction. Biomass sources like wood and straw contain large proportion of materials that cannot be degraded and fermented easily by microorganisms. A good alternative would be biomass gasification, a partial oxidation of carbonaceous compounds into a mixture of CO, CO_2 and H_2 (*i.e.,* syngas). More CO_2

and H_2 can be produced via subsequent WGS reaction ($CO + H_2O \rightarrow CO_2 + H_2$), which in turn are utilized by CO_2/H_2-type microorganisms (*e.g.*, *Ralstonia* spp., methanogens).

In addition to technology aspects described in this review, the advancement of biological CCU is highly dependent on other crucial factors such as R&D funding (Figure 1), commitment to reducing carbon footprint (Figure 1), governmental policies (*e.g.*, carbon tax, cap-and-trade system) and incentives for CCU (*e.g.,* tax credit for renewable energy and for developing/deploying energy-efficient equipments/technologies). Often, these factors trigger much discussion and debate, at both national and international levels.

In view of the ample opportunity available within our biosphere and the maturation of enabling technologies (both computational and experimental), we believe research effort will continue to be invested on biological CCU. In the near future, we foresee growth in the following areas: 1) isolating useful enzymes or strains from extreme environments, 2) accumulation of genomic, transcriptomic, metabolomic and proteomic data of CO_2-utilizing organisms, 3) development of robust transformation and genetic tools for CO_2-utilizing organisms, 4) application of synthetic biology and protein engineering to enhance biological CCU, and 5) further development of gas fermentation technology (*e.g.*, fermenter design, gas sparging, gas mixing, gas/liquid mass transfer). Considering the amount of financial and research investment that have already gone into CCS, we predict that future climate change mitigation will rely on a synergistic combination of both CCS and CCU technologies, with biological systems playing a significant role.

As a concluding remark, industrial-scale implementation of biological CCU can only be realized via close collaboration between scientists and engineers.

Acknowledgement

Pawel Jajesniak and Hossam Eldin Mohamed Omar Ali have contributed equally. We thank ChELSI and EPSRC (EP/E036252/1) for financial support. Omar Ali HEM is supported by Sheffield–A*STAR scholarship.

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