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Literature Review

Microbial Conversion and Utilization of CO_2

Ge-Ge Wang[1#], Yuan Zhang[2,3#], Xiao-Yan Wang[2,3*] and Gen-Lin Zhang[1]

[1]School of Chemistry and Chemical Engineering, Shihezi University, China

[2]Nutrition & Health Research Institute, China National Cereals, Oils and Foodstuffs Corporation (COFCO), China

[3]Beijing Key Laboratory of Nutrition, Health and Food Safety, China

[#]These authors contributed equally to this work and should be considered co-first authors

Abstract

Rising greenhouse gas emissions have contributed to unprecedented levels of climate change, while microbial conversion and utilization of CO_2 is a practical way to reduce emissions and promote green manufacturing. This article mainly summarizes several natural CO_2 pathways that have been discovered, including the Calvin cycle, the reduced tricarboxylic acid (rTCA) cycle, the Wood–Ljungdahl (WL) pathway, the 3-hydroxypropionate/4-hydroxybutyrate (HP/HB) cycle, the dicarboxylate/4-hydroxybutyrate (DC/HB) cycle, the 3-hydroxypropionate (3HP) cycle, the reductive glycine (rGly) pathway, and artificially designed carbon fixation pathways includes the CETCH cycle, the MOG pathway, the acetyl-CoA bicycle, and the POAP cycle. We also discussed applications of different carbon fixation enzymes, notably ribulose-1, 5-diphosphate carboxylase/oxygenase, pyruvate carboxylase, carbonic anhydrase, as well as formate dehydrogenase. This paper further addressed the development of photosynthetic autotrophs, chemergic autotrophs and model bacteria *Escherichia coli* or yeast produced main products for CO_2 fixation through metabolic engineering, such as alcohols, organic acids, fatty acids and lipids, bioplastics, terpenoids, hydrocarbons, and biomass. Future studies on CO_2 microbial conversion should focus on improving the efficiency of carbon fixation enzymes, metabolic modules of the carbon sequestration pathway, and intracellular energy utilization. Coupled microbial and electrochemical methods for CO_2 fixation, in addition to biological fixation, show considerable promise.

More Information

***Address for correspondence:** Xiao-Yan Wang, Nutrition & Health Research Institute, China National Cereals, Oils and Foodstuffs Corporation (COFCO), Beijing, china,
Email: wangxiaoyan@cofco.com

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Introduction

Global temperatures have reportedly risen by 1 ℃ as a result of human activity and natural events, including ocean sinks, earthquakes, and volcanic activities, since the beginning of the Industrial Revolution. The rapidly increasing CO_2 concentration in the atmosphere has contributed to a number of environmental problems, including global warming, a decline in biodiversity, ocean acidification, and even modifications to industrial production methods and human lives. If growth continues at the current pace, the global temperature will continue to rise, worsening extreme weather, ecological disasters, and other negative effects [1]. In order to alleviate climate stress, 178 countries signed the "Paris Agreement" in 2016. They promise to limit the average global temperature increase this century to 1.5 ℃. By 2030, carbon emissions will need to be reduced globally by 40% in order to achieve that objective.

Carbon capture, utilization and storage (CCUS) technologies use net-carbon or low-carbon energy sources to achieve the purpose of reducing carbon dioxide on a worldwide scale [2,3]. CCUS, which develops an environment that is both sustainable and ecologically friendly, represents a different approach to achieving reduction goals [4]. CCUS technology consists of several steps: CO_2 capture, transportation, utilization, and storage. First CO_2 is absorbed and separated through physical [5] and chemical absorbers [6], metal-organic frameworks (MOFs) [7,8], ionic liquids [9], bioenergy with carbon capture and storage (BECCS) [10], membranes [11], etc. The CO_2 is then transported to a place of storage or a location for carbon use. Lastly, the captured CO_2 is subsequently employed or stored as resources using engineering techniques. The main method that CO_2 is used as a resource includes direct use (such as the production of dry ice, refrigerants, CO_2 microbubble technology [12,13], chemical conversion (such as the production of high-value fuels, chemicals, building materials, and minerals)

[14,15], electrochemical catalytic conversion of CO_2 [16,17], thermal catalysis [18], photocatalysis [19], enzymatic conversion [20], coupling electrochemical CO_2 conversion with CO_2 capture [21], microbial utilization [22] and coupling photo/electrocatalytic with microbial CO_2 utilization [23]. Among these, the technology of microbial CO_2 utilization and conversion is endowed with particular advantages including mild reaction conditions, minimal pollution, low cost, and low energy consumption, which is supposed to be a promising way of reducing carbon emissions [24,25].

In this paper, we will mainly discuss the development of microbial CO_2 utilization and conversion technology. This review concentrates on both natural and artificial CO_2 fixation pathways, as well as the use of key CO_2 fixation enzymes like ribulose-1,5-diphosphate carboxylase/oxygenase (RuBisCo), formate dehydrogenase (FDH), pyruvate carboxylase (PC), and carbonic anhydrase (CA), and the advancement of photosynthetic autotrophs, chemoautotrophs, and model bacteria like *E.coli* and yeast in fixing CO_2 through metabolic engineering, genetic engineering, and synthetic biology. The existing limitations and challenges associated with the biological conversion and utilization of CO_2 are then described.

Carbon dioxide fixing pathways

According to features like ATP usage, carbon fixation reactions, enzymes involved, and carbon species being fixed, the natural carbon fixation pathways have been divided into the Calvin-Benson-Bassham (CBB) cycle, the reductive tricarboxylic acid (rTCA) cycle, the Wood-Ljungdahl (WL) pathway, the 3-hydroxyproppionate (3HP) cycle, the 3-hydroxyproppionate/4-hydroxybutyrate (HP/HB) cycle, the dicarboxylate/4- hydroxybutyrate (DC/HB) cycle and reductive glycine (rGly) pathway. In recent years, scientists have synthesized several in vitro CO_2 fixation routes based on the kinetics of enzyme reactions, including the CETCH cycle [26], the MOG pathway [27], the ACB cycle [28] and the POAP cycle [29] (Table 1) . In the following, we will present these pathways and discuss their reaction and progress.

Natural CO_2 fixation pathway

Calvin-Benson-Bassham cycle: The majority of photosynthetic plants, algae, and proteobacteria use the Calvin-Benson-Bassham cycle (CBB cycle) as their primary route for fixing CO_2 in nature. There are 13 enzymatic processes, of which ribulose-1, 5-diphosphate carboxylase/oxygenase, and phosphoribulokinase are the major enzymes. This cycle can be divided into three stages: (1) carboxylation, also known as CO_2 fixation, a molecule of CO_2 is integrated into a five-carbon compound called 1,5-diphosphate ribulose (RuBP), resulting in an unstable C6 compound that breaks down into two molecules of a three-carbon compound known as 3-phosphoglyceric acid; (2) reduction, NADPH reduces 3-phosphoglyceric acid to Glyceraldehyde 3-phosphate while consuming ATP; (3) regeneration, glyceraldehyde 3-phosphate through a series of reactions to produce ribulose diphosphate [30].

Researchers have successfully introduced the CO_2 fixation process of the Calvin cycle into model organisms like *Escherichia coli* and yeast using genetic engineering techniques. This development overcomes the limits of photoautotrophic carbon sequestration and enables heterotrophic organisms to progress fully into autotrophy utilizing CO_2 as the only carbon source [31]. By integrating the CO_2 fixation pathway with energy consumption pathways in *E. coli*, Gleizer and colleagues [32] created a fully autotrophic *E. coli* strain that can thrive only on CO_2. Gassler and coworkers controlled the endogenous gene expression in *Pichia pastori* to deactivate a portion native methanol metabolism pathway. They then added a heterotrophic CBB cycle for carbon fixation, which transformed *Pichia pastoris* from a heterotrophic to an autotrophic organism capable of utilizing CO_2 for growth [33].

Table 1: Characteristics of pathways for CO_2 fixation.

Pathway	Key enzymes	Carbon source	Product	ATP	NAD(P)H	Ref
CBB cycle	Ribulose-1,5-diphosphate carboxylase/oxygenase Phosphoribulokinase	3 mol CO_2	GA-3P	9 mol	6 mol	[30]
rTCA cycle	ATP-citrate lyase 2-ketoglutarate synthase	2 mol CO_2	Acetyl-CoA	2 mol	4 mol	[36]
W-L pathway	Formate dehydrogenase CO dehydrogenase Formylmethanofuran dehydrogenase	2 mol CO_2	Acetyl-CoA	1 mol	4 mol	[39]
DC/HB cycle	4-Hydroxy butyryl-CoA dehydratase	1 mol CO_2 + 1 mol HCO_3^-	Acetyl-CoA	3 mol	4 mol	[45]
HP/HB cycle	4-Hydroxy butyryl-CoA dehydratase	2 mol HCO_3^-	Acetyl-CoA	4 mol	4 mol	[42]
3HP cycle	Malonyl-CoA reductase Malyl-CoA lyase	3 mol HCO_3^-	Pyruvate	5 mol	5 mol	[47]
rGly cycle	Reductive glycine cleavage complex	1 mol CO_2	Pyruvate	2 mol	3 mol	[48]
CETCH cycle	Crotonyl-CoA carboxylase/reductase	1 mol CO_2	Glyoxylate	1 mol	7 mol	[26]
MOG pathway	Phosphoenolpyruvate carboxylase	CO_2	Glyoxylate	8-12 mol	6 mol	[27]
ACB cycle	Ferredoxin oxidoreductase	2 mol CO_2	Acetyl-CoA	5 mol	5 mol	[28]
POAP cycle	Pyruvate carboxylase Acetate-CoA ligase Oxaloacetate acetylhydrolase Pyruvate:ferredoxin oxidoreductase	2 mol CO_2	Oxalate	2 mol	1 mol	[29]

Methanol is a C1 substrate that methylobacterium uses as a source of biomass and energy. *Methylobacterium extorquens* AM1 was engineered by Borzyskowski and group to carry out both energy metabolism and biomass synthesis, with energy coming from methanol metabolism and biomass synthesis deriving from CO_2 metabolism via the Calvin cycle [34].

Through the CBB cycle, photosynthetic autotrophs naturally fix about 300 billion tons of carbon dioxide each year. The rate of carbon fixation is a key factor limiting plant development in environments under conditions of abundant water and light. To increase the metabolic flow of the CBB cycle, CO_2-fixing enzyme efficiency must be enhanced [35].

Reductive tricarboxylic acid cycle

The reductive tricarboxylic acid (rTCA) cycle has since been found in *Thiobacillus viridis*, bacteria, and archaea. In this cycle (Figure 1) , the enzyme ATP-citrate lyase breaks down citrate into acetyl-CoA and oxaloacetic acid. Malate dehydrogenase then catalyzes the enzymatic reduction of oxaloacetic acid to malic acid, which generates fumaric acid through fumaric hydration. Fumaric acid is further transformed into succinic acid through the enzyme reductase. Finally, succinic acid generates citrate by redox [36].

Malubhoy and coworkers engineered yeast strains to produce succinic acid (SA) from pyruvate by rTCA cycle, the greatest yield of SA was 0.23 Cmol/Cmol glycerol [37]. Kang and colleagues overexpressed pyruvate carboxylase, malate carboxylase, and malate transporter simultaneously adding xylose metabolic pathways and knocking out the natural yeast pathways that create ethanol and glycerol. Through the rTCA cycle, this allowed engineered yeast to produce malic acid [38].

WL pathway

The WL pathway, also known as the reductive acetyl-CoA

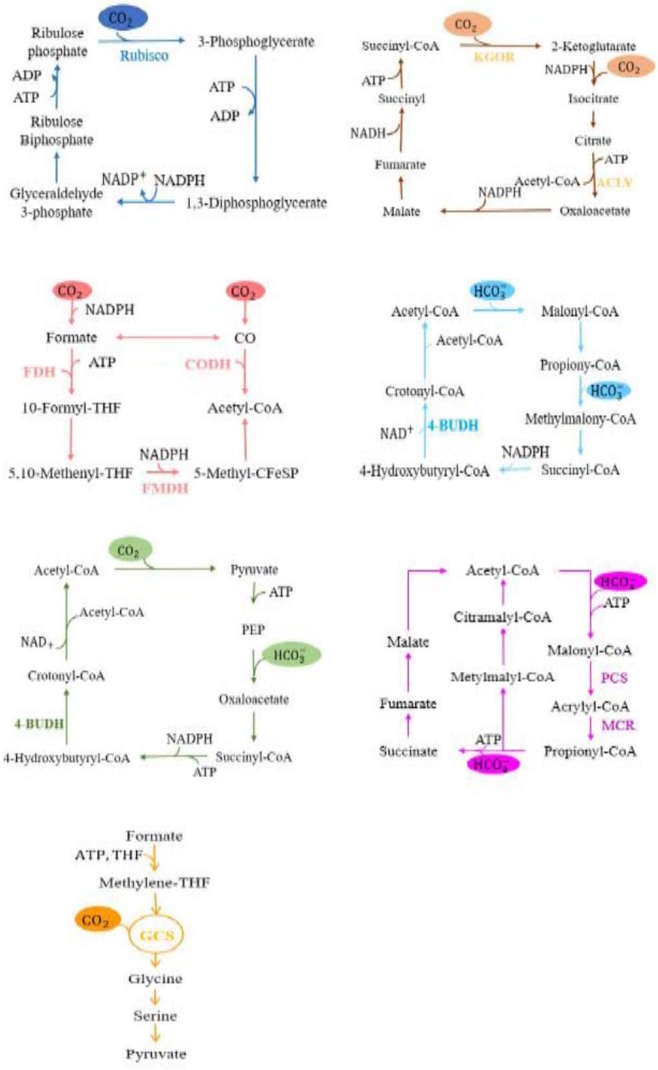

Figure 1: Natural CO_2 fixation pathways. the CBB cycle (in blue) [30]; the reductive TCA cycle (in brown) [36]; the Wood–Ljungdahl pathway (in pink) [39]; the HP/HB cycle (in cyan) [42]; the DC/HB cycle (in green) [44]; the 3-HP cycle (in purple) [47]; the reductive glycine pathway (in orange) [48]. RuBisCo: Ribulose-1, 5-diphosphate Carboxylase/oxygenase; ACLY: ATP-Citrate Lyase; KGOR: 2-Ketoglutarate ferrioxoreductase; FDH: Formate Dehydrogenase; CODH: CO Dehydrogenase; FMDH: Formylmethanofuran Dehydrogenase; 4-BUDH: 4-Hydroxy butyryl-CoA dehydratase; PCS: Propionyl CoA synthase; MCR: Malonyl-CoA Reductase; GCS: the Glycine Cleavage System.

pathway, was discovered by Wood, Ljung-dahl, and others, and exists in acetonogenes, acetogenes, and some fungi. During one of the two branches, one CO_2 molecule is reduced to a single methyl group, while in the other branch, one molecule of CO_2 is reduced to CO, which then cooperates with the methyl group and CoA to generate acetyl-CoA. Acetyl-CoA can be further converted into biomass or acetyl phosphate. Subsequently, the acetyl combines with ADP to produce ATP and acetate [39]. CO dehydrogenase (CODH), formate dehydrogenase, and formylmethanofuran dehydrogenase are the main enzymes in this pathway (Figure 1).

Papoutsakis and colleagues expressed 11 enzymes and auxiliary core protein genes from Clostridium in *Clostridium acetobutylicum* demonstrating that the two branches can work independently of one another. They discovered that acetyl-CoA synthase (ACS) catalyzes the condensation of CO with methyl for the synthesis of acetyl-CoA, whereas CODH catalyzes the reduction of CO_2 to CO. They also learned that CODH/ACS forms complexes that connect two branches of the WL route [40]. By introducing ACS and ACDH into *E. coli*, Hu, and colleagues from Jiangnan University created a novel CO_2 fixation metabolic route (HWLS). They combined the fixation and utilization of carbon dioxide in two modules, producing butyrate and malic acid with yields of 1.48 mol/mol glucose and 0.79 mol/mol glucose, respectively [41].

HP/HB cycle

The autotrophic thermococcus utilizes acetyl-CoA/propionyl-CoA carboxylase as the primary carboxylase for CO_2 fixation, according to research by Berg and colleagues [42]. Through the reduction of 3-hydroxypropionic acid, one molecule of acetyl-CoA and two molecules of bicarbonate are transformed into succinyl-CoA in this system. Succinyl-CoA is then reduced to 4-hydroxybutyric acid, which is subsequently converted into two molecules of acetyl-CoA by 4-hydroxybutyryl CoA dehydratase (Figure 1). Recycling bicarbonate within cells represents a more feasible evolutionary pathway than CO_2. In *Pyrococcus furiosus*, Keller and team effectively produced 3-hydroxypropionic acid by overexpressing five HP/HB cycle genes derived from *Metallosphaera sedula* [43].

DC/HB cycle

The dicarboxylic acid/4-hydroxybutyric acid (DC/HB) cycle exists in anaerobes and facultative aerobes, such as Thermoproteales and *Pyrolobus fumarii* [44]. One molecule of CO_2 and one molecule of bicarbonate are fixed by pyruvate synthase and phosphoenolpyruvate (PEP) carboxylase in this pathway, respectively, to produce succinyl-CoA [45]. The DC/HB cycle has not been successfully expressed heterologously because it requires unique iron, sulphur, and thioester proteins, and other things.

3HP cycle

Through the acetyl-CoA/propargyl-CoA carboxylase, the 3-hydroxypropionate (3HP) cycle effectively integrates two moles of bicarbonate [46]. The four steps in this metabolic process are as follows: (1)propionyl CoA synthesis from acetyl-CoA; (2) propionyl CoA conversion to succinate; (3) glyoxylate production; and (4) glyoxalate and propionyl CoA assimilation reactions to generate pyruvate followed with acetyl CoA regeneration. Through acetyl-CoA/propargyl-CoA carboxylase, the 3-HP cycle assimilates 2 moles of bicarbonate [47]. The enzyme of this cycle has noteworthy characteristics such as effective CO2 fixation, oxygen insensitivity, abundant intermediates, and the capacity to regenerate glycolic acid and glyoxylic acid. In *E. coli* K12, Mattozzi and colleagues expressed genes in an operon linked to the 3-HP pathway from various species. They successfully set up a heterologous 3-HP metabolic pathway and evaluated its functionality via a growth curve. The findings show that the four subpathways are all capable of functional heterologous expression [47].

Reductive glycine pathway

Anaerobic bacteria use the reductive glycine (rGly) route to assimilate formate. Tetrahydrofolate, a cofactor in this pathway, activates formate into formyl-tetrahydrofolate while expending one molecule of ATP in the process. Then, methylene tetrahydrofolate is produced by further reducing formyl-tetrahydrofolate. After the condensation and reduction of methylene tetrahydrofolate by CO_2, NH3, and NADH, glycine is produced. The reversible glycine cleavage system (rGCS), which consists of the aminomethyltransferase T protein, glycine decarboxylase P protein, dihydrolipoamide dehydrogenase L protein, and the aminomethyl carrier H protein, is the main component of rGlyP [48,49].

Bang and colleagues expressed tetrahydrofolate (THF) cycle and formate dehydrogenase in *E. coli* to create a formic acid utilization route, which allowed the engineered strain to grow without the need for additional glucose supplementation [50,51]. Cruz and coworkers overexpressed the enzyme MIS1 together with other enzymes involved in the glycine cleavage/synthesis system in yeast, establishing a glycine synthesis route that allows yeast to generate glycine from formate and CO_2 [52]. The reductive glycine pathway was modularized by Kim and colleagues into four parts (C1, C2, C3, and energy), and these modules as well as methanol dehydrogenase were introduced into *E. coli*. As a result, C1 substrates such as methanol, formic acid, and CO_2 were effectively used by modified bacteria for growth [53].

The rGly pathway, along with the WL pathway and TCA cycle, is one of the most efficient ATP pathways among the verified CO_2 fixation pathways. As a result, under anaerobic industrial circumstances, the reductive glycine pathway has become an incredibly attractive production method. However, insufficient ATP biosynthesis in anaerobic conditions prevents the production of a variety of compounds via these pathways [48]. In order to produce ATP efficiently and use a variety of

single-carbon substrates for sustainable biosynthesis, future research should focus on investigating and improving the glycine synthase system.

Artificial CO_2 fixation pathways

In addition to the natural pathways for CO_2 fixation mentioned above, researchers have also created a number of artificial pathways for CO_2 fixation depending on features like topology, ATP efficiency, and thermodynamics (Figure 2) .

CETCH cycle

The CETCH cycle, which consists of 17 enzymes obtained from nine distinct species including animals, plants, and microbes, indicates the synthetic CO_2 fixation route in vitro. The CETCH cycle compared to the naturally occurring CO_2 fixation pathway, demonstrates higher kinetic and thermodynamic favorability after multiple optimization through enzyme engineering and metabolic engineering. This cycle overcomes the carboxylation bottleneck in natural carbon fixation pathways by employing highly carboxylated enoyl-CoA carboxylases/reductases (ECRs) as a major enzyme for converting CO_2 into organic molecules at a rate of 5 nanomoles per minute per milligram of protein. ECRs exhibit catalytic efficiency 2-4 times better than RuBisCo, the primary carbon fixation enzyme of the CBB cycle. It exists in secondary metabolism, utilizing molecular oxygen as a substrate and not participating in autotrophic carbon fixation pathways. Notably, this new carbon fixation route uses less energy and calls for fewer reaction steps [26].

MOG pathway

The MOG pathway was synthesized by Bar-Even and coworkers by combining the metabolic modules of many organisms. They compared the specific activity and affinities of phosphoenolpyruvate carboxylase, pyruvate carboxylase, acetyl-CoA and propionyl-CoA carboxylase and other carboxylases towards CO_2 or HCO_3^- and metabolic pathways, revealing that these metabolic pathways generate glyoxylate via the same metabolic pathway. Consequently, these pathways were termed the MOG (malonyl-coenzyme A-oxaloacetate glyoxylate) pathways. Notably, the MOG pathways exhibit significant quantitative advantages, such as the overall kinetic rate, cycle rates, and carboxylation efficiency [27].

Acetyl-CoA bicycle

Acetonogenic bacteria metabolize C1 substrates into C2 metabolites, such as acetyl-CoA. Wu, et al. sequentially connected three functional modules—carbon fixation, gluconeogenesis, and non-oxidizing glycolysis, establishing the reductive acetyl-CoA bicycle (ACB) process, a novel CO_2 fixation method, by following this metabolic pathway [28]. In the ACB pathway, two molecules of the C1 substrate (CO_2 or formic acid) and two molecules of acetyl-CoA are catalyzed by the enzyme pyruvate:ferredoxin oxidoreductase (Pfor), resulting in two molecules of pyruvate. Then, through gluconeogenesis, these pyruvate molecules are converted into one molecule of hexose. Through glycolysis, the hexose is further broken down into three molecules of acetyl-CoA. One of the acetyl-CoA molecules generates a C2 product, the other two molecules reenter the cycle. After one full cycle, two molecules of a C1 chemical generate one molecule of acetyl-CoA. In natural syngas fermentation strain *Clostridium ljungdahlii* DSM 13528 coexpressing phosphoketolase and the ACB pathway, the engineered bacteria growth rate and carbon fixation efficiency increased under three different culture conditions: gas only, sugar only, and gas-sugar mixture [54].

POAP cycle

The POAP cycle consists of a four-step reaction: (1) pyruvate:ferredoxin oxidoreductase (Pfor) carboxylates acetyl-CoA to pyruvate, which contrary to the natural metabolic pathway, is the most difficult step in the cycle;

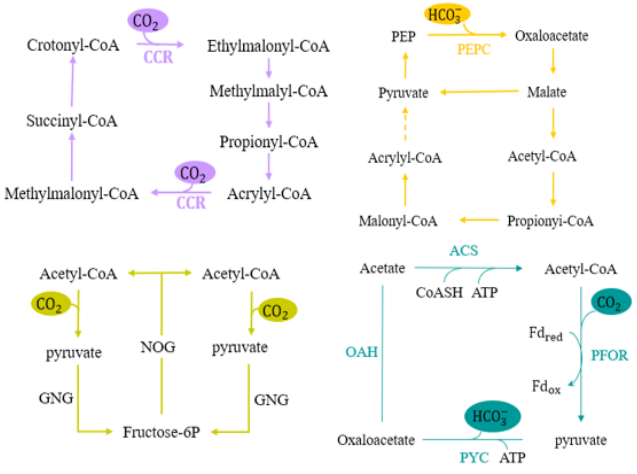

Figure 2: Synthetic CO_2 fixation pathways. The CETCH pathway (in purple) [26]; The MOG pathway (in yellow) [27]; the ACB cycle (in green) [28]; and the POAP cycle (cyan) [29]. CCR: Crotonyl-CoA carboxylase/reductase; PEPC: Phosphoenolpyruvate carboxylase; GNG: Gluconeogenesis; NOG: Non-Oxidative Glycolysis; PFOR: Pyruvate Ferredoxin Oxidoreductase; PYC: Pyruvate Carboxylase; OAH: Oxaloacetate Acetylhydrolase; ACS: Acetate-CoA Ligase.

(2) pyruvate carboxylase (Pyc) then converts pyruvate into oxaloacetic acid through carboxylation; (3) oxaloacetic acid is hydrolyzed by the enzyme oxaloacetate acetylhydrolase (Oah), which releases acetic acid and oxalic acid; (4) acetic acid is converted into acetyl-CoA by the enzyme acetyl-CoA ligase (Acs) [29].

The POAP pathway completes one cycle, the conversion of two molecules of CO_2 into one molecule of oxalic acid at the expense of two molecules of ATP, and one molecule of NAD(P)H. This cycle involves the fewest steps among artificial carbon fixation cycles and enables fixing CO_2 under anaerobic and higher temperature conditions.

Despite having an advantage over thermodynamics, dynamics, and energy utilization effectiveness, the artificial CO_2 fixation pathway still has challenges, such as the complexity of a multi-enzyme system and a reliance on exogenous energy sources, which fall below the level of the standards of industrial biological manufacturing.

The key enzymes for carbon dioxide fixation: Ribulose-1, 5-diphosphate carboxylase/oxygenase

In the CBB cycle, 1, 5-diphosphate carboxylase/oxygenase (RuBisCo) catalyzes the carboxylation of ribulose 1, 5-diphosphate (RuBP) with CO_2 to form 3-phosphoglyceric acid (3-PG). RuBisCo also exhibits high sensitivity towards oxygen and can oxidize ribulose 1, 5-diphosphate to generate 2-phosphoglycolic acid (2-PG) [55]. Based on its structural variations, RuBisCo can be classified into four forms, forms I, II, III, and IV. Form I RuBisCo consists of eight large subunits and eight small subunits. The presence of small subunits enhances CO_2 concentration within the environment and large subunit facilitates carboxylation. This structure is termed as L_8S_8 which is commonly found in eukaryotic and prokaryotic photosynthetic organisms. Form II RuBisCo comprises eight large subunits known as L_8 structures primarily observed in spirorubidiums, dinoflagellates, and purple non-sulfur photosynthetic bacteria. Form III RuBisCo enzymes are composed of 2-10 large subunits predominantly existing in archaea or a few bacteria. While form IV, also called the Rubisco-like protein (RLP), does not catalyze either of these reactions. There appear to be six different clades of RLP, mainly present in Bacillus, chlorothiobacillus, and Archaeococcus [56]. Pang, et al. expressed forms I and II of the Rubisco enzyme in *E. coli* to study how the two forms of the enzyme affect CO_2 fixation. They found that the activity of these two enzymes for fixing CO_2 was comparable [57].

Fujihashi, et al. designed the mutant SP8-T289D by contrast sequencing RuBisCo from a variety of organisms and introduced it into the mesophilic, photosynthetic bacterium *Rhodopseudomonas palustris*. As a result, the engineered bacterium displayed an approximately two-fold increase in specific growth rate in comparison to the control bacterium [58]. Aigner, et al. coexpressed the chaperone proteins Cpn60/Cpn20, Raf1/Raf2, RbcX, and bundle-sheath defective-2 (BSD2) with *Arabidopsis thaliana* RuBisCo in *E. coli*. They found that BSD2 was crucial for stabilizing the assembly of plant RuBisCo large subunit until the small subunits were available [59].

The ability to fix CO_2 has been demonstrated by heterologous expression of the bacterial RuBisCo in yeast, *E. coli*, and other microbial hosts [60-63]. However, the Rubisco only catalyzes a limited number of molecules per minute, low protein assembly efficiency, energy consumption, carboxylation efficiency, and specific CO_2 binding affinity lead to a low carbon fixation efficiency through heterologous expression [64]. Meanwhile, the difficult process of expressing RuBisCo from plants in microbes frequently leads to insufficient carboxylation functionality. In order to increase the effectiveness of heterologous expression in microorganisms, it is vital to explore the carboxylation active site of the RuBisCo and to optimize both carboxylation efficiency and specificity.

Pyruvate carboxylase

Pyruvate carboxylase (PC) catalyzes the carboxylation of pyruvate and HCO_3^- to form oxaloacetic acid and phosphoenolpyruvate carboxylase (PEPC) catalyzes the carboxylation of phosphoenolpyruvate (PEP) with HCO_3^- to form oxaloacetic acid and inorganic phosphate. Oxaloacetic acid, a significant TCA cycle intermediate, is used in the biosynthesis of several amino acids, including lysine, threonine, and aspartate. Therefore, the fixation of CO_2 by pyruvate carboxylase has great significance for amino acid production [65].

Zelle, et al. expressed pyruvate carboxylase PYC2, malate dehydrogenase (MDH), and malic acid transporters SpMAE1, and the malic acid production in *Saccharomyces cerevisiae* reached 59 g/L [66]. To further increase fumaric acid production, Xu, et al. knocked down gene fum1 encoding fumarase and overexpressed the fumaric acid transporter and pyruvate carboxylase [67]. Xiberras, et al. constructed the yeast succinic acid biosynthesis pathway and replaced the NAD-dependent dihydroxyacetone pathway with the native glycerol metabolism pathway. The yield of succinic acid produced by modified bacteria under batch culture conditions in glycerol was 10.7 g/L [68].

Carbonic anhydrase

The carbonic anhydrase (CA) is the primary enzyme responsible for the hydration of atmospheric CO_2, which has a catalytic conversion frequency of 10^6 s^{-1}. In addition to CO_2 capture, CA also can be used for multi-enzyme-catalyzed conversions, chemical-enzyme-catalyzed conversions, and biological conversions [69] . Interaction with RuBisCo, phosphoribulokinase (PRK), and other enzymes involved in natural CO_2 fixation pathways is necessary to enable the CA

function in biosynthesis. Introducing CA into *E. coli*, the flux of the CO_2-fixing bypass pathway increased from 13% to 17% [70]. Gleizer et al., coexpressed CA, RuBisCo, PRK, and FDH in *E. coli* to transform heterotrophic organisms into complete autotrophic organisms through laboratory evolution techniques [32]. Effendi et al., expressed human carbonic anhydrase in *E. coli* MG1655 using a dual promoter σ70 and heat shock protein (HSP70A) instead of inducers to enhance its activity under high-temperature conditions. Cadaverine was successfully produced by the modified bacteria with a yield of 36.7 g/L using CO_2 as a substrate [71].

Formate dehydrogenase

The formate dehydrogenase (FDH), commonly known as CO_2 reductase enzyme, catalyzes the transformation of CO_2 into formic acid [72,73]. Du, et al. overexpressed yeast formate dehydrogenase in *S. cerevisiae*, which led to modified yeast that consumed 30% more glucose and produced 13% more ethanol [74]. Wang, et al. combined CO_2 fixation and formate consumption in yeast. The modified bacteria formate utilization rate was continuously improved to 0.48 g/L/h, and the FFA titer reached 10.1 g/L under glucose-feeding conditions [75]. Under anaerobic conditions, *E. coli* produces formate hydrolyzase (FHL), which oxidizes formic acid into CO_2 and H_2. By raising the pressure of CO_2 and H_2 gases inside the reactor, Roger, et al. discovered that FHL efficiently converted CO_2 and H_2 to formate and formate extracellular concentrations accumulated to above 500 mM [76]. Based on FDH's extraordinary efficiency at lowering CO_2, it has the potential to directly air capture and use carbon. Formic acid, a byproduct of FHL metabolism, can either be used as an energy source or transformed into other useful substances by chemical or biological processes [77,78].

CO_2-fixing microorganisms: CO_2-fixing Autotrophs

Autotrophs use CO_2 as their main or only source of carbon by photosynthesis or chemosynthesis. It can be achieved to reduce CO_2 emissions by modifying autotrophic microorganisms to produce bioproducts while also fixing carbon dioxide.

Photoautotroph

Photosynthetic autotrophic microalgae are able to fix CO_2 through their metabolic pathways by using CO_2 fixation enzymes like RuBisCo and CA. Microalgae are an excellent resource of biobased feedstock for the generation of biofuels because they have better photosynthesis and a greater CO_2 fixation efficiency than terrestrial plants [79]. Microalgae also have advantages in cultivation, growth rate, and oil content. Microalgae are therefore essential for the production of biomass and the fixation of carbon [80,81]. Wei, et al. overexpressed the RuBisCo activator enzyme (nRCA) from *Nannochloropsis oceanica* in *Nannochloropsis spp.*, which increased the biomass output by 46%, the large subunit protein expression level by 45%, the growth rate by 32%, and the productivity of the lipids by 41% [82]. Wang and Shin, et al. modified the light-trapping antenna protein to increase microalgae photosynthetic system efficiency for solar energy utilization and carbon fixation rate [83,84]. Using 15% CO_2 (v/v) as a screening stress and a spotting plate method, Jin, et al. determined that *Heynigia riparia* SX01 had the highest biomass productivity (0.39 g/L/day) and CO_2 fixation rate (0.71 g/L/day) [79]. This study offers insightful information on employing microalgae to convert CO_2 from flue gas into biomass feedstock.

Cyanobacteria are another photoautotrophic bacterium that can be easily designed and has low food requirements, similar to microalgae. Researchers have successfully manipulated cyanobacteria to produce alcohols, alkenes, terpenes, and organic acids [85-89]. However, due to their need for light and gas supply, photosynthetic bacteria are only able to produce a limited amount of light-sensitive, volatile, and intracellularly unstable chemicals. Li, et al. proposed an integrated strategy (iPRCC) that combines a carbon sequestration module and a resting cell catalysis module. *E. coli* was manipulated genetically to transform intermediates into light-sensitive products and intracellularly unstable molecules after modified cyanobacteria were used to drive metabolism toward stable substrates. This study expands the possible use of carbon-negative biosynthesis technology while using CO_2 biosynthesis for high-value compounds like vanillin [90].

Along with microalgae and cyanobacteria, purple non-sulfur bacteria like *Rhodospiralis* and *Rhodobacter spheroides*, as well as anaerobic photosynthetic purple sulfur bacteria, can utilize carbon dioxide to produce chemicals [91]. Fixen, et al. used a transcription factor NifA mutant to activate nitrozyme expression, regulate intracellular metabolism of electrons and energy, and drive *Rhodopseudomonas palustris* to convert CO_2 into methane [92].

Chemolithoautotrophic

Chemoautotrophs obtain energy by oxidation of environmental electron sources such as ammonia, hydrogen, carbon compounds, and sulfur. The hydrogen-oxidizing bacterium *Ralstonia eutropha* (also known as *Cupriavidus necator*) exhibits a wide range of metabolic processes, employing CO_2 as the sole carbon source and H_2 and O_2 gases as substrates [93]. *R. eutropha* efficiently produces PHB and directs the carbon flux towards biofuels and other high-density carbon chemicals like isobutanol, methyl ketone, isoprene, sucrose, modified PHB, and plant growth accelerator via metabolic engineering techniques to optimize their metabolic pathway [93].

Wang, et al. successfully constructed a glucose metabolic pathway in *C. necator* H16, by blocking the ED and PHB synthesis routes, enabling hydrogen-oxidizing bacteria to effectively utilise glucose, glycerol, and CO_2 for inositol

synthesis [94]. Joshua, et al. successfully developed a heterologous (R)-1, 3-butanediol biosynthesis route in *C. necator* H16 by implementing (R)-3-hydroxybutyraldehyde CoA and pyruvate-dependent pathways, knocking out competing pathways, and increasing butanediol synthesis gene expression levels [95]. Liu, et al. created a novel water-splitting biosynthetic system that enables *R. eutropha* to synthesize chemicals using hydrogen produced during the water decomposition process under low CO_2 concentration. With up to 50% energy efficiency during CO_2 reduction, this artificial photosynthetic system demonstrates amazing potential and offers a platform for microorganisms to make use of light energy to fix CO_2 [96].

Through the enzyme action of formate dehydrogenase, *C. necator* is able to convert formates into CO_2 and then participate in the Calvin cycle. However, the high ATP need of this process restricts the amount of biomass production. Claassens, et al. established a reductive glycine route in *C. necator*, achieving similar growth rates compared to the wild type, after short-term evolution, which offers the potential for biologically converting formic acid [97]. Even though *R. eutropha* has a highly developed genetic system for the bioconversion of carbon dioxide and the capacity to manufacture a variety of chemical products, more research is required to improve its genetic tools and synthetic biology techniques compared to other model microorganisms [98].

Common chemoautotroph Clostridium mainly fixes carbon via the reductive CoA process. Cheng et al., overexpressed *aor*, *adhE2*, and *fnr* in *Clostridium carboxidivorans*, which increased butanol and ethanol yields by 18% and 22%, respectively [99]. Huang, et al. utilized a phage serine integrase-mediated site-specific genome engineering technique introducing heterologous phage attachment/integration (Att/Int) systems in *Clostridium ljungdahlii*, the modified strain produced a butyric acid yield of 1.01 g/L [100]. *Clostridium ljungdahlii* is an anaerobic, non-photosynthetic mixotrophic bacteria, that uses both organic and inorganic compounds, such as sugar and CO_2 and H_2. Jones et al., genetically modified *C. ljungdahlii* and the engineered strain acetone output had reached 138% of the theoretical maximum [101].

Additionally, via either the Calvin cycle or the CoA pathway, electroautotrophs can directly or indirectly use electricity as a source of energy for fixing carbon dioxide.

CO_2-fixing heterotroph

Natural C1 metabolizing microorganisms can convert CO_2 into biofuels and chemicals. However, due to ineffective carbon fixation capacity, which results in significant carbon loss, their bioproduction efficiency is far less than heterotrophs. Industrial model microorganisms have become an attractive host for the construction of third-generation biorefineries in contrast to naturally CO_2-fixing microorganisms because of their advanced molecular and synthetic biology tools and well-established fermentation processes [63].

Yeast

Yeast performs exceptionally well in industrial settings, and creating CO_2-fixing yeast strains presents an attractive choice for developing carbon-neutral industrial processes. Anaerobic fermentation byproducts like glycerol can be produced due to redox cofactor imbalances. It can increase both carbon use and biological production, and decrease byproduct production by using CO_2 produced during fermentation as an electron acceptor for NADH oxidation in microorganisms.

Xia, et al. overexpressed the reductive pentose phosphate pathway in *S. cerevisiae* SR8 as well as the CO_2 fixation enzyme of the CBB cycle to improve xylose fermentation [62]. Prk, RuBisCo, and GroESL were expressed in Saccharomyces IMU032 by Guadalupe, et al. Engineered strain produced 90% less glycerol and 10% more ethanol under conditions of a sugar-limited medium with glucose and galactose fermentation [63]. Li, et al. built the CBB pathway in *S. cerevisiae* and fermented it in a YP medium supplemented with 70 g/L maltose and 40 g/L xylose, the modified yeast CO_2 fixation rate was 336.6–436.3 mg/CO2/L/h [64]. Compared to the metabolism of glucose, the sorbitol metabolism by glycolysis produces one more molecule of NADH. By introducing the sorbitol metabolic pathway into yeast, Van Aalst, et al. increased the efficiency of biological production by providing additional electrons for carbon fixation pathways [102]. Gassler, et al. changed the *Pichia pastoris* endogenous methanol absorption system into a CO_2 fixation pathway through chromosome integration technology. Completely autotrophy strain using CO_2 as the only carbon source was successfully obtained after laboratory evolution [33]. Both metabolic engineering and protein engineering should be taken into consideration to optimize Rubisco-PRK pathways and increase protein expression levels in order to achieve functional expression of RuBisCo in yeast; establishing natural mechanisms for intracellular CO_2 concentration is also essential for effective carbon sequestration [65].

Yeast fixes carbon dioxide using also the rTCA cycle. Xiberras, et al. integrated expression cassettes for three enzymes that convert oxaloacetate to SA in the cytosol ("SA module") into the yeast genome to produce succinic acid, achieving a maximum yield of 0.22 g/g glycerol [67]. Xu, et al. increased fumaric acid production to 1.6 g/L by metabolic engineering with *S. cerevisiae* EN.PK2-1C as host [68]. Recently, researchers coupled electrocatalysis and biosynthetic techniques to produce glucose and free fatty acids from CO_2 and water, yielding 2.2 g/L of glucose and 448.5 mg/L of free fatty acids [103]. Additionally, expressing pyruvate carboxylase expression in yeast can also synthesize amino acids, ethanol, and other compounds.

Escherichia coli

E. coli is a potential heterotrophic organism to metabolize CO_2 due to rapid growth, and abundant genetic tools. Zhuang,

et al. successfully expressed RuBisCo and PrkA in *E. coli*, the engineered strain CO_2 emission decreased by 15% compared to the wild strain (JB), while the CO_2 fixation rate remained constant at 67 mg CO_2/mol arabinose/L/h, similar to microalgae and cyanobacteria [61]. Gong et al., introduced CA into *E. coli* to enhance intracellular CO_2 concentration [70]. Gleizer, et al. coexpressed RuBisCo, Prk, and FDH in modified *E. coli* to convert heterotrophic bacteria to total autotrophic utilizing CO_2 as the only carbon source [32]. When RuBisCo is in the activated state, it catalyzes the conversion of the substrate RuBP and CO_2 into two molecules of 3-phosphoglyceric acid (PGA). Pang, et al. coexpressed RuBisCo and Rca in *E. coli* to study the effect of the activating enzyme on carboxylation activity; which improved host overall metabolism by reducing intracellular RuBP inhibition on the expression of RuBisCo [57]. Hu, et al. overexpressed pyruvate carboxylase in *E. coli* and the engineered bacteria malic acid production rose by 110% [104]. Bang, et al. constructed recombinant THF and reduced glycine pathways in *E. coli*, this increased the flux of formic acid and CO_2 assimilation towards pyruvate synthesis from 4.5% to 14.9% [50]. Heterologous expression of the carbon fixation pathway allows host assimilation of C1 substrates, but one or multi-step reactions are insufficient for effective CO_2 fixation. The efficiency of carbon fixation enzymes may be significantly increased by creating an environment where autotrophs assimilate CO_2 in the heterologous host. *Prochlorococcus marinus* MED4 carboxylase structural protein, stabilizing factor, molecular chaperone, and auxiliary module were coexpressed in *E. coli* to successfully engineer the synthesis of carboxysomes (CBs), which led to a notable improvement in CO_2 assimilation capability [105]. Although the construction of carboxysomes is essential for effective CO_2 assimilation, due to the large number of related genes and complicated assembly process, it imposes development expenses. Accelerating CO_2 fixation using carboxylases in heterotrophic bacteria requires more research.

Although the conversion of heterotrophic *E. coli* strains to autotrophic has been accomplished, more study is still required to improve metabolic flux, enhance C1 capture and utilization efficiency, and accelerate the growth of "synthetic autotrophic microorganisms" [106].

Microbial CO_2 fixation by metabolic engineering

The bioconversion of CO_2 exhibits low energy consumption, less pollution, a wide range of products, and high conversion efficiency. Utilizing microbial metabolism to convert CO_2 into biobased chemicals is a fundamental strategy in addressing the challenge of increasing global atmospheric CO_2 concentration [1]. There are two main ways for microorganisms to metabolize carbon dioxide and produce value-added compounds: one involves building biosynthetic production pathways in naturally carbon fixation organisms, and the other involves converting heterotrophic production strains into "synthetic autotrophic strains" through the use of carbon fixation pathways. This section examines developments in the metabolic engineering of microbial conversion of carbon dioxide into biomass, biofuels, and other important biobased compounds (Figure 3).

Alcohols

Synechococcus and engineered cyanobacteria use carbon dioxide and solar energy to produce alternative fuels or chemicals, which has the potential to greatly reduce reliance on fossil fuels and minimize carbon emissions. Kusakabe et al., successfully engineered a pathway in *Synechococcus elongatus* PCC7942 for isopropanol synthesis, after optimizing production conditions, the engineered cyanobacteria produced 26.5 mg/L of isopropanol [85]. By deleting the regulatory gene *cp12* and overexpressing key enzymes of the oxidative pentose phosphate pathway in *S. elongatus* PCC7942, Kanno, et al. created engineered bacteria that can produce 12.6 g/L of 2,3-butanediol in both dark and light environments [86]. Shen, et al. modified the *S. elongatus* PCC7942 by introducing ketoacid decarboxylase, alcohol dehydrogenase, and citramalate pathways to improve the biosynthesis of isoleucine, a precursor to 2-ketobutyrate. The ultimate 2-methyl-1-butanol concentration of the modified bacterium was 200 mg/L, highlighting the first time 2MB was produced through photosynthetic [87]. In *Synechocystis sp. PCC* 6803, Yao, et al. expressed the fatty acyl-CoA reductase gene *maqu_2220* and knocked off competing pathways, which resulted in directed carbon flux towards fatty alcohol synthesis and a final titer reached 2.87 mg/g dry weight [107]. Li, et al. used *R. eutropha* H16 as the host bacteria and introduced isobutanol and 3-methyl-1-butanol (3MB) synthesis pathways. The modified strain LH74D used formate produced by CO_2 electrochemistry as a carbon source with a yield of 0.57 g/liter for 3MB production. This study showed the viability of employing carbon dioxide as a feedstock and electricity as an energy source to drive the biological conversion of carbon dioxide into different compounds [108].

Numerous research have suggested that yeast can make ethanol expressing the heterologous carbon fixation pathway.

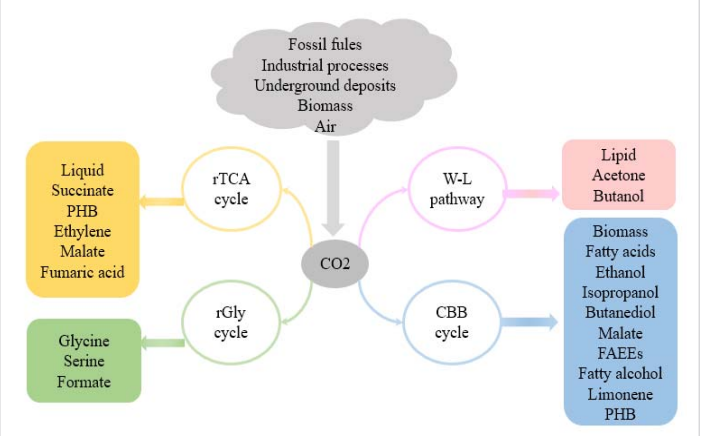

Figure 3: Various commodity chemicals and bioproducts derived from CO_2 by microorganisms.

This strategy reduces the generation of glycerol and increases the output of ethanol by using CO_2 as an electron acceptor for NADH reoxidation [109].

Organic acids

Hu, et al. coupled carboxylation reactions that produce ATP in the main metabolic pathway with consume ATP in the natural carbon fixation pathway in *E. coli* to improve the yield of malic acid [104]. Yu, et al. improved gene expression levels by combining promoters (P4, P17, and P19), CO_2 transport genes (sbtA or bicA), and fixed genes (ppc and pck) in *E. coli*. the modified strain AFP111 compared to the control strain, succinic acid yield increased by 37.5%, reaching 89.4g/L [110]. Kang, et al. overexpressed pyruvate carboxylase, malate carboxylase, and malate transporter in yeast and introduced the xylose fermentation pathway, the resulting strain produced 61.2 g/L of malic acid in fed-batch culture [38]. Wang, et al. successfully enhanced 3-HP biosynthesis in the cyanobacterium *Synechocystis sp*. PCC 6803 by enhancing the expression levels of essential enzymes, improving precursor supplies of malonyl-CoA and NADPH, and suppressing competing routes. The 3-HP yield of the modified strain reached 837.18 mg/L [111]. D-lactate is essential to produce polylactic acid. The methylglyoxal synthase gene from *E. coli* was inserted into the cyanobacterium *S. elongatus* PCC7942 to directly manufacture lactic acid from carbon dioxide through methylglyoxal utilizing dihydroxyacetone phosphate (DHAP). The maximum lactate titer obtained was 13.7 mM (1.23 g/l) [112]. Besides organic acids, microorganisms can also use CO_2 to synthesize inorganic acids such as acetate and butyric acid [113].

Fatty acids and lipids

Fuel made from fatty acids is an important biofuel. Microalgae are examples of photosynthetic microorganisms that can use fatty acid synthase to turn CO_2 into malonyl-CoA, synthesize fatty acids, and extend the carbon chain. These compounds, such as triglycerides and polyphosphate triglycerides, can then be hydrolyzed for use in the chemical, food, and energy industries [114,115]. Wang, et al. designed a C1 substrate assimilation platform in *S. cerevisiae* to synthesize free fatty acids (FFAs) from CO_2 and formic acid. The formic acid utilization rate increased 21.8 times in the modified strain KW301, while the fatty acid output increased 33.7 times, reaching 10.1 g/L [75]. Li, et al. controlled the metabolic pathway of *R. eutropha* H16 to synthesize fatty acids using H_2, CO_2, and O_2 as substrates, in a gas autotrophic fermentation system. The engineered bacteria B2 generated free fatty acids and reached 60.64 mg/g in less than 48 hours [116]. Hu, et al. modified *Clostridium acetogenes* to produce biodiesel from synthetic gas in an integrated continuous reactor system with an output of 18 g/L of C16-C18 triacylglycerides [117].

Bioplastics

The production and consumption of non-biodegradable plastics have been steadily increasing over the last few decades, causing a significant environmental load on the environment. The replacement of petroleum-based plastics, on the other hand, offers promising prospects owing to the easy degradability and sustainability of bioplastics. Polyhydroxybutyrate (PHB), is a biodegradable polymer synthesized through microbial fermentation [118]. Proteobacteria, cyanobacteria, purple non-sulfur bacteria, and other microbes produce PHB by a process that is catalyzed by the three enzymes PhaA, PhaB, and PhaC. Mozumder, et al. utilized *C. necator* in syngas (10% CO_2, 75% H_2, and 15% O_2) to create PHB, with a yield of 42.9 g/L [119]. Karmann et al., designed *R. rubrum* for synthesizing PHB by utilizing syngas consisting of CO and CO_2 as carbon sources and energy sources [120]. Weiss, et al. developed a synthetic symbiotic system where the *S. elongatus* PCC 7942 CscB secretes sucrose to support *Halomonas boliviensis* to produce PHB, resulting in a yield of 28.3mg PHB/L/d [121]. Chen et al., constructed a microbial electrosynthesis system (MES) for the CO_2-driven synthesis of PHB by *R. eutropha* [122]. Costa, et al. used a two-stage fermentation with *Clostridium autoethanogenum* as the host to create PHA [123]. Even though the production of PHB by microorganisms on a large scale has been accomplished using CO_2, further research discovered that utilizing sugar as the substrate rather than CO_2 as the substrate produced more PHB. Therefore, the ability of microbes to capture and use CO_2 still needs significant improvement.

Terpenoids

Terpenoids, referred to as isoprenoids or terpenes, are a broad and diverse class of chemical molecules with several industrial uses, such as in the food, cosmetics, and pharmaceutical industries. Microorganisms produce isoprenoids through the mevalonate pathway (MEV) and methylerythritol 4-phosphate (MEP) pathway [124]. By expressing menthollimonene synthase and abies α-red myrrh synthase, Fiona, et al. effectively engineered *Synechococcus sp.* PCC7002 to generate limonene and α-red myrrh in polychlorella, with yields of 4 mg/L and 0.6 mg/L, respectively. This achievement offers a promising platform for the production of terpene compounds using algae [89]. The engineered *Anabaena sp.* PCC7120 was constructed with coexpressed limonene synthase gene (*lims*) as well as a DXP operon. Under higher light intensity, the limonene yield and productivity increased 6.8 and 8.8 times more than the control strain, respectively [125]. Gao, et al. modified *S. elongates* to use the methylerythritol phosphate route to produce isoprene, resulting in the designed strain isoprene production reaching 1.26 g/l from CO_2 [126].

Hydrocarbons

Hydrocarbons, which are made of carbon and hydrogen, are crucial parts of petroleum. Alkanes and alkenes can be produced by cyanobacteria using a natural carbon fixation route. By optimizing the expression level of the *Pseudomonas*

syringae ethylene forming enzyme (*efe* code), as well as adjusting light intensity and nutritional conditions, Justin, et al. increased ethylene synthesis in *Synechocystis sp.* PCC6803 and achieved a maximum ethylene yield of 171 mg/L/d [127].In order to produce ethylene utilizing 2% CO_2 as a carbon source, Tomas, et al. overexpressed the *Sy-efe* gene in *Synechocystis sp.* PCC 6803 [128].

Biomass

Heterotrophs exclusively consume organic chemicals, while autotrophs possess the ability to utilize CO_2 for the synthesis of valuable compounds. The primary objective in synthetic biology is to engineer heterotrophic organisms capable of harnessing CO_2 as a carbon source for biomass production. In an electrochemical-biological system designed by Zheng, et al. [103], yeast with a deactivated glucose metabolic pathway produced long carbon chain molecules like glucose and fatty acids from acetic acid and acetate created by electrocatalyzed CO_2. This process yielded 2.2 g/L of glucose. Wang et al., genetically modified *C.necator* to fix CO_2 and produce glucose with a yield of 253.3 mg/L, providing a viable approach for microorganisms to generate glucose from CO_2 [129].

The bioconversion of CO_2 has many advantages, such as low energy consumption, a wide range of products, higher conversion rates in large-scale production, and no competition for food and land resources (Table 2). Around 11.5 million tons of CO_2 are annually converted through biotechnology into a variety of goods worldwide, but this number is considerably

Table 2: Some value-added bioproducts derived from CO_2

Pathway	Organism	Carbon Source	Key genes	Product	Ref
CBB	*E. coli*	L-arabinose, xylose	*cbbM, PrkA*	Ethanol, acetate	[61]
CBB	*Saccharomyces cerevisiae*	maltose, xylose	*XR, XDH, XKS, sPRK, cbbM*	Ethanol	[64]
CBB	*S. cerevisiae*	glucose, galactose	*GroEL, GroES, cbbM, PRK*	Ethanol	[63]
CBB	*S. cerevisiae*	glucose, formate	*cbbM, prk, fdh*	Free fatty acids	[75]
CBB	*S. elongatus* PCC7942	CO_2	*thl, atoAD, adc, adh*	Isopropanol	[85]
CBB glycolytic	*S. elongatus* PCC7942	CO_2, glucose	*galp, zwf, gnd, rbcLXS*	Butanediol	[86]
CBB	*S. elongatus* PCC7942	CO_2	*Kivd, YqhD*	2-Methyl butanol	[87]
CBB	*S. elongatus* PCC7942	CO_2	*MsLS*	Limonene	[88]
CBB	*S. elongatus* PCC7942	CO_2	*pdc, adh, atfA, xpkA, pta*	Fatty acid ethyl esters	[89]
CBB	*S. elongatus* PCC7942	CO_2	*pal*	Olefins, Cinnamaldehyde, Curcumin	[90]
CBB glycolytic	*E. coli*	CO_2, glucose	*pck, mdh*	Malate	[104]
CBB	*M. extorquens AM1*	CO_2	*Prk, cbbM*	Cell growth	[34]
CBB	*C. necator*	CO_2	*HAD1, cbbY2*	Glucose	[129]
CBB	*Synechocystis* sp. PCC6803	CO_2	*maqu_2220*	Fatty alcohol	[107]
CBB	*R.eutropha* H16	CO_2	*AlsS, ilvCD, kivd & yqhD*	Isobutanol, methyl-butanol	[108]
CBB	*S. cerevisiae*	glucose	*Rubisco, GroEL/GroES, PRK*, non-ox PPP	Ethanol	[109]
CBB	*Synechocystis* sp. PCC6803	CO_2	*mcr, accB, accC, accA, accD, birA, pntA, pntB*	3-HP	[111]
CBB	*S. elongatus* PCC7942	CO_2	*mgsA*	Lactate	[112]
CBB rTCA	*R. eutropha* H16	$CO_2/O_2/H_2$	*acc, Ltes, Fas, acpS*	Fatty acids	[116]
CBB	*C. necator*	$CO_2/O_2/H_2$		Lipid	[119]
CBB	*S. elongatus* PCC7942, *H. boliviensis*	CO_2	*CscB*	Ethanol or hydrocarbon fuels	[121]
CBB TCA	*C.necator* H16	Glucose, Glycerol, CO_2	*ScIPS, EcIMP*	myo-inositol	[94]
CBB	*C. necator H16*	CO_2	*bld, adhE, dra, s-adh, PDC*	(R)-1,3-butanediol	[95]
CBB TCA EM-CoA	*R. rubrum*	CO_2, acetate		PHB	[120]
TCA	*S. cescerevisiae*	xylose	*PYC1, PYC2, MDH3, SpMAE1*	Malate	[38]
TCA	*S. cescerevisiae*	glucose	*RoPYC, SFC1*	fumaric acid	[68]
TCA	*S. cescerevisiae*	Glycerol	*PYC2, MDH3-R, fumR, FRDg-R*	Succinic acid	[67]
TCA	*Synechocystis* 6803	CO_2	*efe*	Ethylene	[127]
TCA	*E. coli*	CO_2, glucose	*sbtA, bicA, ppc, pck*	Succinate	[110]
rGly		Methanol, CO_2		Glycine, Serine, Pyruvate	[49]
rGly	*E. coli*	CO_2, FA	reconstructed THF cycle, *gcvTHP, lpd*	Glycine, serine	[50]
rGly	*C. necator*	Formate	*ftl, fch, mtdA,gcvT, gcvH, gcvP*	Biomass	[97]
W-L	*C. carboxidivorans*	$CO/CO_2/H_2$	*aor, adhE2, fnr*	Ethanol, butanol	[99]
W-L	*C. ljungdahlii*	CO/CO_2	Att/Int system	Butyric acid	[100]
W-L	*C. ljungdahlii*	$H_2/CO/CO_2$	*thl, ctfAB, adc*	acetone	[101]
W-L	*M. thermoacetica, Yarrowia lipolytica*	H_2/CO_2		Lipid	[117]
W-L	*Clostridium autoethanogenum*	$CO/CO_2/H_2/N_2$		PHA, bioethanol	[123]
W-L	*Clostridium acetobutylicum*	CO_2		Acetone, butanol	[133]
Electrosynthesis	*Clostridium autoethanogenum*	CO_2		Butyrate	[113]
Biosystem-electro	*S. cerevisiae*	CO_2	*yihx, agpP*	Long-chain compounds	[103]

insufficient in comparison with the annual CO_2 emissions (24 billion tons). The application of microorganisms, particularly heterotrophs, to convert CO_2 and use it to synthesize bioproducts still needs further research.

Discussion

Through synthetic biology and metabolic engineering, the researchers have synthesized carbon sequestration pathways with lower energy consumption and fewer reaction steps, screened enzymes for carbon sequestration with higher carboxylation efficiency, and improved the CO_2 assimilation ability of natural CO_2-fixing microorganisms, these advancements have further facilitated the achievement of carbon sequestration and emission reduction targets. However, microbial CO_2 fixation research is still in the early stages of development, there remain several drawbacks, such as low carbon sequestration efficiency and energy capture efficiency, as well as huge disparities between goal product production and industrial requirements. Therefore, more research is needed to explore in terms of CO_2 fixation efficiency, and utilization of energy [130-132].

Conclusion

Thoroughly investigated and optimized natural carbon sequestration pathway and directed evolution of carbon fixation enzymes by rational and semi-rational design enable increase in the efficiency of key enzymes and metabolic modules of the carbon sequestration pathway. In order to efficiently use energy, realize optical drive, and electric drive biocatalysis, it is necessary to optimize the energy utilization systems of electric autotrophs and chemoautotrophs and develop new materials for capturing light energy, generating electricity. Furthermore, by enhancing the electron transfer mechanism and coupling electro-biological CO_2 fixation, this will have the potential to use microorganisms on a broad scale to produce added value products from CO_2, while simultaneously decreasing the cost of microbial conversion technology.

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