A Review on the Progress in Chemo-Enzymatic Processes for CO₂ Conversion and Upcycling

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Abstract: The increasing concentration of atmospheric CO₂ due to human activities has resulted in serious environmental issues such as global warming and calls for efficient ways to reduce CO₂ from the environment. The conversion of CO₂ into value-added compounds such as methane, formic acid, and methanol has emerged as a promising strategy for CO₂ utilization. Among the different techniques, the enzymatic approach based on the CO₂ metabolic process in cells presents a powerful and eco-friendly method for effective CO₂ conversion and upcycling. This review discusses the catalytic conversion of CO₂ using single and multienzyme systems, followed by various chemo-enzymatic processes to produce bicarbonates, bulk chemicals, synthetic organic fuel and synthetic polymer. We also highlight the challenges and prospects for future progress in CO₂ conversion via chemo-enzymatic processes for a sustainable solution to reduce the global carbon footprint.

Keywords: chemo-enzymatic; CO₂ conversion; upcycling; carbon footprint; planetary health

1. Introduction

Economic development activities such as industrialization and urbanization have resulted in significant CO₂ production which remains a major factor for global warming, ocean acidification and a dreadful pollutant affecting human health. The predominant source of anthropogenic CO₂ emissions is the combustion of fossil fuels for energy generation in residential, industrial and transportation sectors. For example, burning 1 ton of carbon in fossil fuels produces over 3.5 tons of CO₂ [1]. Atmospheric CO₂ concentration was reported to be 405 parts per million (ppm) in 2017 and is projected to increase to 570 ppm by the end of the century [2]. In light of these circumstances, the International Energy Agency (IEA) has recommended restricting annual CO₂ emissions to <15 gigatonnes to limit the rise in temperature to within 2 °C by 2050 [3,4].

Despite the rising concerns about CO₂ emission, it remains a challenge to propose a truly low-carbon technology to the public market within a short time frame to reduce CO₂ emission to a safer level in the atmosphere [5]. As such, the best approach to address the issue will be capturing and converting CO₂ into useful products or compounds; i.e., CO₂ utilization. Today, various techniques involving chemical, photochemical, electrochemical and enzymatic methods have been reported in the literature to catalyze CO₂...
fixation/conversion reactions to capture and store the increasing concentration of CO$_2$ from the environment as an efficient energy source, or to produce value-added hydrocarbons such as methane, formic acid and methanol [6–9]. Among the aforementioned techniques, chemical, photochemical and electrochemical methods are limited by their low selectivity due to the abundant and stable form of carbon element in CO$_2$ molecules and the challenges to attain a high-performing catalyst [10–12].

On the other hand, an enzymatic method is an eco-friendly approach which has been reported to be efficient for CO$_2$ fixation/conversion reactions owing to their enhanced stereo-specificity and region-chemo selectivity. For instance, the enzymatic technique using three dehydrogenases was first reported by Obert and Dave [13], where the overall process involves the initial reduction of CO$_2$ to formate catalyzed by formate dehydrogenase (FDH); followed by the reduction of formate to formaldehyde by formaldehyde dehydrogenase (F$_a$DH); and finally, the reduction of formaldehyde to methanol by alcohol dehydrogenase (ADH). In the entire process, the reduced nicotinamide adenine dinucleotide (NADH) acts as the terminal donor for all the dehydrogenase-catalyzed reductions. Since methanol emits less CO$_2$ after combustion and has high volumetric and gravimetric energy density, it exhibits significant potential to replace fossil fuels in the future. The enzymatic route for the CO$_2$ to methanol conversion reaction has been reported to be selective and to exhibit higher reaction rates under mild conditions [14,15]. Other studies have reported that issues concerning the stability of the enzyme can be addressed by immobilizing the enzyme in ultrathin, hybrid microcapsules of catechol and gelatin [16,17].

Whilst much of the emphasis has been intensifying on experimental studies of an enzymatic method for efficient CO$_2$ conversion reactions, comprehensive reviews concerning reaction routes and mechanisms for catalytic conversion of CO$_2$ by single and multienzyme systems remain limited. Herein, the authors attempt to gather information from the earliest to the most recent developments regarding the catalytic conversion of CO$_2$ by single and multienzyme systems; and the subsequent upcycling of CO$_2$ via various chemo-enzymatic processes such as the production of bicarbonates, bulk chemicals, synthetic organic fuel and synthetic polymer. Lastly, we highlight the challenges and prospects for future progress in CO$_2$ conversion reactions from the chemo-enzymatic processes.

2. Overview of Chemical Methods for CO$_2$ Conversion

2.1. Electrochemical

The electrochemical method involves using a cathode and an anode, which supply electricity to the electrolyte in a setup illustrated in Figure 1A. An ion exchange membrane separates the electrodes, and the electrode consists of catalyst and substrate layers to facilitate the transfer of CO$_2$ reactant gas and the product. As a result, it is essential to ensure effective mass transfer between the electrodes. In a separate investigation conducted by Yang et al., a novel three-compartment electrochemical cell setup (Figure 1B) was developed, allowing pure formic acid to produce within a concentration range of 5–20 wt% [18]. The improved and consistent electrochemical performance for up to 500 h was attributed to using an electrochemical cell incorporating a Dioxide Materials Sustainion™ anion exchange membrane and a nanoparticle Sn GDE cathode containing an imidazole ionomer.

However, Hori et al. [19] showed the drawbacks of planar electrodes due to mass transfer limitation due to the poor solubility of CO$_2$. It has been reported that using gas diffusion electrodes as a cathode in combination with an improved catalyst deposition layer led to improvements in the electrode performance [20]. Due to their distinctive electrochemical properties, many studies have been carried out by utilizing copper sulfide compounds as a catalyst in the CO$_2$ electrochemical reduction process [21,22]. However, it should be noted that the electrochemical approach requires a high cost of electricity for the reaction.

In this context, current research on lowering overpotentials has been carried out to lower the high electricity cost. For instance, Rosen et al. [23] reported CO$_2$ could be reduced to carbon monoxide (CO) at the applied voltage of 1.5 V to a very low overpotential of
<0.2 V under Pt/Nafion/(Ag, EMIM-BF₄) electrocatalytic system. The system continuously produced CO for >7 h at Faradaic efficiencies >96%. Another example was the electrocatalytic reduction of CO₂ by carbon monoxide dehydrogenase (CODH), a naturally occurring enzyme [24]. Shin et al. [25] achieved a current efficiency of 100% at −0.57 V vs. NHE in a 0.1 M phosphate buffer (pH 6.3) with no overpotential with CODH catalysts. Apart from CO production, methanol can be produced. The electrocatalyst containing histamine generates formic acid, an intermediary compound that subsequently reacts with Pt nanoparticles. This combination enhances the availability of adsorbed hydrogen atoms, producing highly hydrogenated products, such as methanol [26]. Further, the selectivity of the desired product can be increased by using the appropriate electrolyte, catalyst and potential applied to the process.

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**Figure 1.** (A) Basic configuration of electrochemical reduction of CO₂ conversion consisting of cathode and anode through which electricity is applied in the electrolytic solution [27]. Reproduced with permission from Elsevier 2021. (B) Electrochemical conversion of CO₂ to formic acid facilitated by the presence of imidazolium ionomer in the nanoparticle Sn catalyst at GDE cathode [18]. Reproduced with permission from Elsevier 2017.

### 2.2. Thermal

Various catalytic metal particles have been reported for CO₂ conversion. Incorporating silica or alumina support has also been attributed to increased catalytic activity-selectivity performance. Specifically, Cu particles (at variable loadings and particle sizes, including the
nanoscales) supported with ZnO have been extensively explored for methanol production. For instance, Huang et al. [28] modified a range of CuO/ZnO/ZrO$_2$ with ZrO$_2$ support and tested at 240 °C and 3 MPa pressure. Results revealed both CO$_2$ conversion and CH$_3$OH selectivity were appreciably enhanced by the ZrO$_2$ incorporation. Din et al. [29] utilized nano-structured materials developed by supporting the Cu particles with ZrO$_2$. Liang et al. [30] utilized another porous and structured system based on Cu–Zn/Al-foams having monolithic configurations. The catalytic choice with monolithic systems in micro-reactors was shown to be promising in enhancing catalyst stability by hindering coking and elevating reaction activity and thus, the production yield. Other groups of catalysts, such as Pd and Fe, were the alternatives. These findings could be correlated to the works reported by Collins et al. [31] and Bonivardi et al. [32], who employed supported Pd–Ga materials under variable conditions. Their results highlighted that the modification of the catalyst enhanced H$_2$ activation. Another group of catalysts with proficiency is the Fe-based systems. Such work was published by Dorner et al. [33], where CeO$_2$-modified Fe/Mn/Alumina catalysts were developed. Results revealed that CO$_2$ conversion of >14% and CH$_3$OH selectivity >70% could be realized with enhanced stability based on loadings <10 wt%. Over 10 wt% showed the activity/selectivity decayed substantially. Numerous studies have also been reported with other metals, such as La, Mg, Zn, etc., associated with supported Fe particles. Unfortunately, the results were not satisfying (low quality), probably due to poor Fe active site interactions that hindered CO$_2$/H$_2$ adsorption [34]. However, in certain cases, positive effects of sintering inhibition were achieved depending on the support material (i.e., in the order Al$_2$O$_3$ > TiO$_2$ > SiO$_2$) [35,36]. Metal carbides have also been highlighted for CO$_2$ conversion. For instance, Rodriguez et al. [37] showed that TiC could create effective support in situ, especially when loaded with active sites (Au, Ni or Cu). Asara et al. [38] corroborated that Au/TiC materials could yield good reaction properties when the TiC was well structured with improved active surface sites coupled to Au dispersion.

3. In Vitro Conversion of CO$_2$

3.1. CO$_2$ Conversion via Single Enzyme In Vitro

Enzyme-based CO$_2$ conversion has gained significant importance due to its ability to perform complicated reactions and produces a high yield of desired fuels or chemicals. Table 1 shows the enzymes used to produce various products via in vitro CO$_2$ conversion. CO$_2$ conversion in vitro is also an important method for CO$_2$ capture, sequestration and utilization, which can be accomplished by directly adopting a single enzyme as listed below:

- Conversion of CO$_2$ by oxidoreductases;
- Conversion of CO$_2$ by lyases;
- Conversion of CO$_2$ by synthases.

Among the three single enzyme in vitro reactions aforementioned, reactions performed by synthases—such as acetyl-CoA synthase, pyruvate synthase or acetyl-CoA carboxylase, among others—produce products that do not possess any significant importance in our daily life. As such, synthases can be considered unsuitable for in vitro applications. In comparison to other major routes, only oxidoreductases (i.e., formate dehydrogenase (FDH), Carbon dioxide reductase, Carbon monoxide dehydrogenase (CODH), Remodeled nitrogenase) and lyases (i.e., carbonic anhydrase, pyruvate decarboxylase, etc.) have been discovered from certain organisms which assisted in the synthesis of several fuels or chemicals such as formate, CO, methane or bicarbonate, among others. The following section will describe the CO$_2$ conversion routes via a single enzyme in vitro; i.e., the conversion of CO$_2$ by oxidoreductases and the conversion of CO$_2$ by lyases.

3.1.1. Conversion of CO$_2$ by Oxidoreductases

Generally, oxidoreductases are enzymes that catalyze the transfer of electrons from one reducer or electron donor molecule to another oxidant or electron acceptor. NADPH/NADP$^+$ or NADH/NAD$^+$ are usually used as cofactors [39,40]. The conversion of CO$_2$ by oxi-
doreductases produces carbon-based energy sources by reducing the oxidation state of carbon element. Various products can be obtained from the conversion of CO₂ catalyzed by oxoreductases. For example, CO₂ reduction produces formate (an important chemical and fuel) by formate dehydrogenase (FDH), as shown in Equation (1).

\[
\text{CO}_2 + \text{H}^+ + 2e^- \rightleftharpoons \text{HCOO}^- \quad (1)
\]

In the forward reaction, FDH catalyzes the reduction of CO₂ to formate, while in the reverse reaction, it catalyzes the oxidation of formate to CO₂. A reverse reaction is more likely to occur in the presence of a coenzyme (NADH) regeneration system catalyzed by FDH. Although most FDH prefer oxidation reactions, some FDH can reduce CO₂ to formate that can be easily converted to other important chemicals without requiring other organic compounds. FDH can be classified as metal-independent; i.e., metal-free FDH enzymes catalyze reactions from CHOOH to CO₂ using nicotinamide adenine dinucleotide (NAD⁺) as a cofactor, or metal-dependent enzymes based on molybdenum (Mo) or tungsten (W) metals, to reversibly reduce CO₂ to CHOOH by applying a series of enzymes adsorbed from S. fumaroxidans on electrode surface which showed high efficiency in the catalytic reduction of CO₂ to formate (Figure 2A) [41]. The rate of the reaction was highly sensitive to pH, where a six-fold increase was reported from pH 7.5 to 5.5 (Figure 2B).

However, ion-type cofactors such as NADH are costly and not easily available, restricting their application in the catalytic reduction of CO₂ to formate. In view of these limitations, Beller et al. discovered bacterial hydrogen-dependent CO₂ reductase (HDCR) from the acetogenic bacterium Acetobacterium woodii for the conversion of CO₂ to formate [42,43]. The bacterium grows with H₂ and CO₂ at ambient conditions using ancient energy conservation pathways. The HDCR enzyme is composed of four components; i.e., putative formate dehydrogenase (FdhF1/2) and iron-iron hydrogenase (HydA2) as the major subunits surrounded by two small electron transfer subunits (HycB2/3), where FdhF1/2 acts as the catalyst to reduce CO₂ to formate. At the same time, HydA2 activates/oxidizes H₂ into two H⁺ accompanied by acquiring two electrons, while HycB2/3 is responsible for transferring the electrons from HydA2 to FdhF1/2. When utilized for the catalytic conversion of CO₂ and H₂ to formate, a very high reaction rate was reported; i.e., 10 mmol min⁻¹ mg⁻¹. Compared with other chemical catalysts, the hydrogenation of CO₂ was promoted with an initial turnover frequency (TOF) of 70 h⁻¹ [44]. On the other hand, the purified HDCR catalyzed a similar reaction at 30 °C with a TOF of 101 600 h⁻¹, which was approximately 1500 times higher than the chemical catalysis.

Carbon monoxide (CO) is another important product that can be produced by reducing CO₂ using carbon monoxide dehydrogenase (CODH). CO exhibits significant fuel value, which can be readily converted to methanol as liquid fuel and is an essential feedstock in various synthetic processes such as Fisher-Tropsch, Monsanto and Cativa processes as shown in Equation (2).

\[
\text{CO} + \text{H}_2\text{O} \rightleftharpoons \text{CO}_2 + 2\text{H}^+ + 2e^- \quad (2)
\]

The reduction of CO₂ to CO can be achieved with the aid of two types of CO dehydrogenase catalyzing the reaction. Firstly, CO dehydrogenases containing Fe₃S₄Ni in the active center of oxygen-sensitive obligatory anaerobes such as Moorella thermoacetica, which was first reported by Shin and co-workers [25]. A significant advancement in their study was the combination of electrocatalysis/photocatalysis with the enzymatic conversion of CO₂ to CO. These enzymes can oxidize CO at a rate of 40,000/s using methyl viologen cation as oxidant at 70 °C, and can reduce CO₂ at a rate of 45/s while also exhibiting close thermodynamic potential for the CO₂/CO pair. However, the electrocatalysis/photocatalysis conversion of CO₂ requires significant overpotential, which consumes a lot of energy and might generate a mixture of products such as CO, methane and methanol. In order to address this concern, Armstrong et al. conducted multiple studies wherein immobilized NiFe CODH was evaluated for enzyme activity while rotating at high speed in an anaerobic sealed cell, using pyrolytic graphite as a substrate [45,46]. Authors reported that the
standard reduction potential for interconversion between CO₂ and CO was much higher (E = −0.46 V) than without a catalyst (E = −1.9 V), which ascertains the lower overpotential requirements while achieving high selectivity for yielding 100% CO. The second class of CODH contains air-stable MoSCu, commonly found in aerobes such as *Oligotropha carboxidovorans*, in the active center. Some studies have reported that glutamate (Glu 763) and phenylamine (Phe 390) are important for binding ligaments of Mo and Cu to perform their activity. In contrast, other studies have reported that these enzymes achieve a lower catalytic conversion rate; i.e., 100/s for CO oxidation [47,48].

Methane is another product that can be produced by converting CO₂ using remodeled nitrogenase as described in Equations (3) and (4). In earlier studies by Yang et al., it was reported that nitrogenase was not capable of triggering the reaction to catalyze eight electron reduction of CO₂ to methane since no methane was detected even after 20 min of reaction [49]. However, when several amino acids (i.e., α-195, α-70 or α-191) that approach FeMo-cofactor active sites (Figure 2C) that control substrate binding and reduction reaction were substituted in appropriate molecules, the catalytic behavior was altered (Figure 2D). The remodeling of nitrogenase by replacing α-195 with Gln and α-70 with Ala successfully catalyzed the formation of methane from CO₂ where 16 nmol (methane) and nmol⁻¹ (MoFe protein) were obtained in the 20 min of reaction.

\[
    \text{N}_2 + 8\text{H}^+ + 16\text{ATP} + 8\text{e}^- \rightarrow 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{Pi} \tag{3}
\]

\[
    \text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \tag{4}
\]

Figure 2. (A) Schematic of catalytic interconversion between CO₂ and formate. (B) Kinetics of catalytic reaction by tungsten containing FDH as a function of pH value [41]. Copyright (2012) Yang et al. (C) FeMo cofactor with some key amino acid residues [49]. (D) CH₄ formation as a function of time for different MoFe proteins [49]. Copyright (2008) National Academy of Sciences, USA.
**Table 1.** Enzymes used for production of various products via in vitro CO$_2$ conversion (modified from [48]).

<table>
<thead>
<tr>
<th>Product</th>
<th>Enzyme</th>
<th>Advantages</th>
<th>Limitations</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate (HCO$_3^-$)</td>
<td>Carbonic anhydrase</td>
<td>Rapid cycle</td>
<td>• Unstable, cannot be reused.</td>
<td>[50–52]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Cannot obtain value-added products.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Poor activity in harsh conditions.</td>
<td></td>
</tr>
<tr>
<td>Formate (HCOO$^-$)</td>
<td>Formate dehydrogenase</td>
<td>Obtaining value-added products</td>
<td>• Requires modification for electrocatalytic/photocatalytic application.</td>
<td>[53–55]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Favors forward reaction.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Expensive cofactor.</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Requires improvement for long-term covalent immobilization.</td>
<td></td>
</tr>
<tr>
<td>Carbon dioxide reductase</td>
<td></td>
<td></td>
<td>• Doesn’t require cofactor</td>
<td>[42,43,56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Reaction direction easily controlled by substrate concentration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• CO can easily inhibit reactions in cascade system</td>
<td></td>
</tr>
<tr>
<td>Methanol (CH$_3$OH)</td>
<td>Multiple dehydrogenases (FDH + FaDH + ADH)</td>
<td>Value added products.</td>
<td>• Not stable for long-term activity</td>
<td>[13,57,58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Can be processed at moderate conditions</td>
<td>• Requires immobilization for industrial application.</td>
<td></td>
</tr>
<tr>
<td>Methane (CH$_4$)</td>
<td>Remodeled Nitrogenase</td>
<td>Not inhibited by H$_2$</td>
<td>• O$_2$ sensitive enzymes</td>
<td>[59,60]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Requires modification to improve turnover number and frequencies</td>
<td></td>
</tr>
<tr>
<td>Carbon Monoxide (CO)</td>
<td>Carbon monoxide dehydrogenase</td>
<td></td>
<td>• Air stable,</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Product reaction not easily convertible to value-added products</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Low stability</td>
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</tbody>
</table>

3.1.2. Conversion of CO$_2$ by Lyases

Lyase is an enzyme that facilitates the cleavage of various chemical bonds using techniques that do not involve hydrolysis or oxidation but rather substitution reactions. This enzymatic activity often results in the creation of a new double bond or ring structure. Conversely, the reverse reaction, known as the Michael reaction, allows for the carboxylation of unprocessed molecules with carbon dioxide to produce valuable products. For example, carbonic anhydrase (CA) can convert CO$_2$ to bicarbonate, as shown in Equation (5).

$$\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \quad (5)$$

CA commonly exist in mammals, plants, algae or bacteria and plays the primary role in the interconversion between CO$_2$ and bicarbonate to maintain acid-base balance in the blood and other tissues. Based on the origin of enzymes and their structural fold, several classes of CA are available, such as $\alpha$, $\beta$, $\gamma$, $\delta$ and $\zeta$, all of which are composed of a tetrahedral active center with a metal ion such as divalent zinc or related metal ion [61,62]. For the reaction mechanism of CA, a hydroxide atom in the active site attacks a CO$_2$ molecule and then reacts with the water molecule from the reaction environment to release protons.
Higher CO$_2$ concentration directs to bicarbonate production. To date, CA catalytic conversion has been used for absorption, membrane separation, mineralization/precipitation for CO$_2$ capture, sequestration and utilization. For example, the CA catalytic conversion of CO$_2$ by chemical absorption using regenerable alkaline solvents increases the CO$_2$ absorption rate in the alkaline aqueous solvents with lower heat and energy input for desorption [64]. Despite the advantages, the harsh conditions involved in the absorption process, such as high temperature (50–125 $^\circ$C), high concentration of organic amines and trace contaminants, causes enzyme denaturation, which leads to reduced enzyme activity and stability [27].

Several modifications have been developed to overcome the aforementioned limitations, such as sourcing CA from thermophilic organisms, generating thermo-resistant CA via protein engineering techniques, immobilizing CA on suitable supports or modifying the absorption process. For instance, in a study by Zhang et al., CA was immobilized on nonporous silica-based nanoparticles in a potassium carbonate-based absorption process to improve enzyme stability and chemical resistance [65]. Authors reported that immobilized CA retained 56–88% of its original activity for a 60-day period at 50 $^\circ$C, in comparison to free CA, which retained only 30% of its original activity. Another study by Hwang et al. revealed using a CA-assisted method to create crystalline CaCO$_3$ composites together with in situ CA entrapment [66]. This study largely used free CA as a catalyst to speed up the precipitation and mineralization processes. Authors reported that CA immobilized in CaCO$_3$ composites retained 43% catalytic activity. Additional investigations have noted other immobilization carriers since CaCO$_3$ is pH and temperature sensitive, which may prevent the application of CA in particular regions. For example, Forsyth et al. reported the green approach for the recyclable conversion of CO$_2$ to CaCO$_3$ catalyzed by CA encapsulated bio-inspired silica [67]. Authors highlighted that immobilized CA exhibited little enzyme leaching with excellent recycling, storage and thermal stabilities.

3.2. CO$_2$ Conversion via Multienzyme In Vitro

In living cells, enzymes catalyze a wide variety of metabolic processes that involve multiple reaction steps. The formation of macromolecular enzyme complexes efficiently transfers the intermediates from one catalytic site to the other [68]. A multienzyme-based approach for CO$_2$ conversion utilizing naturally occurring reactions may prove efficient and cost-effective. To date, at least six CO$_2$ conversion pathways have been validated: the Calvin cycle, reductive tricarboxylic acid (TCA) cycle, reductive acetyl-CoA pathway, 3-hydroxypropionate bicycle, dicarboxylate/4-hydroxybutyrate cycle, and reductive glycine pathway; in a variety of living cells [69,70]. As a result, this process, known as metabolic channeling, has motivated researchers to artificially combine multienzyme biocatalysts to achieve the desired goal of creating bio-based compounds. Among them, using cell-free enzymes or immobilized multiple enzymes cascade systems for CO$_2$ fixation is an up-and-coming area. These types of cascades have several benefits over cellular conversion. In such multienzyme cascade systems, the enzyme rate of reaction can be enhanced, and a large concentration of CO$_2$ can be processed while recycling enzymes for several cycles. Reaction rates are efficiently optimized without using toxic substrates or reaction intermediates, thus facilitating metabolic pathways by regulating the activities of competing pathways [71]. Pyruvate dehydrogenase (PDH) complex, tryptophan synthase channel, fatty acid oxidation pathway and citric acid cycle, are metabolic channeling examples that mimic natural enzyme complexes artificially [72]. For instance, direct product transfer as a substrate from one enzyme to another nearby enzyme with the bulk phase without equilibration [71], as illustrated in Figure 3. Such a scenario happens when the distance between two active sites is short, and thus the overall reaction rate can be enhanced efficiently [73]. In this regard, different active sites can be closer spatially and assembled as a complex to provide efficient reaction control with multifunctional enzymes (multienzyme cascade systems). Moreover, these systems offer ease in separating and purifying the end product. This section reviews
various CO$_2$ conversion via multienzymes in vitro strategies which have been exploited so far.

![Figure 3](image.png)

**Figure 3.** Schematic illustration of the substrate channeling effect between (A) two free-floating enzymes (E1 and E2) and (B) two proximate enzymes (E1/E2). Diffusion of the substrates (S1 and S2) and enzymes (E1 and E2) is assumed to be directional. Reproduced with permission from Hwang and Lee [71]. Copyright 2019 American Chemical Society.

### 3.2.1. Conversion of CO$_2$ to Methanol by Multiple Dehydrogenases

One of the important products of CO$_2$ conversion is methanol, for the following reasons: (i) the recycling of “greenhouse” gas, and (ii) the efficient production of sustainable/renewable fuel alternatives. In comparison to the fuels (CO, methane, etc.) produced from the single-enzyme route, the liquid methanol produced from multienzyme reactions has much higher energy capacity and is easier to transport. In early 2007, Bilal El-Zahab et al. demonstrated methanol production from CO$_2$ by applying a group of enzymes, i.e., FDH, FaDH, and ADH. The reaction catalyzed by these enzymes was an NADH dependent reaction [74]. To carry out the in vitro reaction, they immobilized all the enzymes and the NADH covalently on the polystyrene microparticle (~500 nm) (Figure 4A). The reaction was conducted by bubbling the CO$_2$ gas in the reaction mixture; and a promising improvement in cofactor utilization was observed during the production of methanol from CO$_2$. On the other hand, another approach for methanol production was investigated by constructing an immobilized multienzyme cascade. For instance, the support matrix for the enzyme entrapment was fabricated as a hybrid microcapsule from catechol modified gelatin (GelC) [57]. Enzyme-GelC cascade was synthesized by systematic stepwise entrapment of three enzymes, FAD, FaDH, and alcohol dehydrogenase, via physical and covalent attachment by forming silica nanoparticle layer facilitating enzyme-GelC-Si cascade (Figure 4B).
This microreactor showed an enhanced methanol yield of up to 71%, along with a high recyclability rate, activity, and stability strength. Similarly, another study was investigated by carrying out the co-immobilization of FDH, FaDH, and alcohol dehydrogenase (ADH) on the commercially available ultrafiltration (UF) flat-sheet polymeric membrane by employing a simple pressure filtration method [17]. Two distinct approaches were used; i.e., co-immobilization and sequential immobilization. For co-immobilization, a simple method of UF membrane fouling was used under pressure until the membrane was saturated with enzymes (Figure 4C). In sequential immobilization, three different UF membranes were saturated with enzymes sequentially and stacked onto each other.

In the past, enzymatic reductions for CO\textsubscript{2} were limited because a sacrificial coenzyme, such as nicotinamide adenine dinucleotide (NADH), was required to provide electrons and the hydrogen equivalent. However, a new bio-electrocatalytic reduction method for converting CO\textsubscript{2} to methanol was studied, which involves injecting electrons directly from electrodes into immobilized enzymes, thus eliminating the need for the coenzyme NADH [75]. To achieve this, a carbon-felt electrode was created by depositing FDH, FaDH, and ADH within an alginate-silicate hybrid gel (Figure 4D). Using this technique, the researchers were able to produce almost 0.15 ppm of methanol. This method of immobilizing enzymes on electrodes presents an opportunity for electrochemical applications in various reactions where an alternative to the costly sacrificial coenzyme NADH is desired [76].

A magnetic nanoparticle multienzyme cascade was developed by immobilizing ADH, FaDH, and FDH powered by NAD+/NADH and glutamate dehydrogenase (GDH) as the coenzyme regenerating system [77] (Figure 4E). The stepwise reaction scheme led to only a 2.3% yield of methanol per NADH. However, in a batch system under CO\textsubscript{2} pressure, combining the four immobilized enzymes increased the methanol yield by 64-fold. Therefore, this study indicated a successful regeneration of NADH in situ, envisaging a real possibility of using immobilized enzymes to perform the cascade CO\textsubscript{2}-methanol reaction.

### 3.2.2. Conversion of CO\textsubscript{2} to Other Fuels/Chemicals/Materials by Use of a Multienzyme System

Developing novel reaction routes for the direct production of advanced fuels/chemicals/materials (with higher number of carbon) would also be highly competitive. Recently, a relatively simpler multienzyme route for converting CO\textsubscript{2} and ethanol into L-lactic acid noted a novel and sustainable alternative to synthesizing building blocks for biodegradable polymers [78] (Figure 5A). The catalytic process is composed of three single-enzyme routes: (1) the oxidation of ethanol to acetaldehyde by ADH in the presence of NAD+, (2) the synthesis of pyruvate from CO\textsubscript{2} and acetaldehyde catalyzed by pyruvate decarboxylase, and (3) the reduction of pyruvate to L-lactic acid by lactate dehydrogenase (LDH) in the presence of NADH. It is worth noting that the cycling of NAD+/NADH could be achieved via the first and third single-enzyme routes. Another encouraging advance in technology was reported on the photosynthetic recycling of CO\textsubscript{2} into isobutyraldehyde [79] (Figure 5B). Herein, genetically engineered *Synechococcus elongatus* PCC7942 underwent expression with several key enzymes; i.e., Rubisco, acetolactate synthase (AlsS), aceto-hydroxy acid isomeroreductase (IlvC), dihydroxy-acid dehydratase (IlvD) and 2-ketoacid decarboxylase (Kdc). Since Rubisco was the bottleneck in carbon fixation in Cyanobacteria, overexpression of this enzyme could increase the reaction rate of CO\textsubscript{2} and, thus, the productivity rate of isobutyraldehyde.
Figure 4. (A) Chemical route for the attachment of enzymes and cofactor onto polystyrene particles. Reproduced with permission from El-Zahab et al. [74] Copyright 2008 Wiley. (B) GelCSi microcapsules with enzymes. Reproduced with permission from Wang et al. [57] Copyright 2014 American Chemical Society. (C) Co-immobilization and sequential immobilization of enzymes in membranes for methanol production from CO₂. Reproduced with permission from Luo et al. [17] Copyright 2015 Elsevier. (D) Electrochemical CO₂ reduction using enzymes. Reproduced with permission from Schlager et al. [75] Copyright 2016 Wiley. (E) CO₂-methanol cycle using immobilized FDH, FaDH, and ADH. Originally published in Marques Netto et al. [77] under Creative Commons Attribution 4.0 International License.

Figure 5. (A) The multienzyme route for the synthesis of isobutyraldehyde from CO₂. AlsS: acetolactate synthase; IlvC: acetohydroxy acid isomeroreductase; IlvD: dihydroxy-acid dehydratase; Kdc: 2-ketoacid decarboxylase, Reproduced with permission from Tong et al. [78] Copyright 2011 Wiley. (B) The multienzyme route for the synthesis of L-lactic acid from CO₂ and ethanol. Originally published in Atsumi et al. [79] under Creative Commons Attribution 4.0 International License.
4. Applications of Chemo-Enzymatic Approaches for CO₂ Upcycling

Elevated average worldwide temperatures, also known as “global warming” and “climate change,” have sparked a global movement to cut the quantity of carbon dioxide (CO₂) in the atmosphere. Popular strategies that have been considered and used to reduce CO₂ emissions include CO₂ capture and storage (CCS) and turning CO₂ into fuels and chemicals. The CO₂ Utilization Program of the US Department of Energy (DOE) principally emphasizes the mineralization and polymerization processes, CO₂ improved retrieval of hydrocarbons, and production of chemicals [80]. These industries have a significant chance of converting high CO₂ capabilities into cost-effective products. Despite the many products that can be made from CO₂, there is a significant difference between the amount of CO₂ produced and the amount of CO₂ transformed. Using CO₂ to generate fuels and chemicals is a win-win method for reducing atmospheric CO₂ and effectively utilizing carbon resources [10], as illustrated in Figure 6. Many scientists are eager to investigate and create new catalytic methods that will allow CO₂ to be converted into platform chemicals that may be used to create bio-based products. This section will explore various applications of the upcycling of CO₂ using a chemo-enzymatic approach.

The technique, known as chemo-enzymatic, integrates biocatalytic reaction with the production of bulk chemicals. The enzymatic approach offers various benefits, including greater selectivity, yield, and the lack of changeable reaction conditions. The production of such chemicals with excellent stereoselectivity is crucial in the current environment [81]. Diverse methods or strategies have been documented for the systematic synthesis of enzyme-based catalysts, for optimizing reaction processes, and for the clarification of reaction mechanisms in order to improve the effectiveness of the enzyme catalytic process. Additionally, carbon capture and storage (CCS) technology has gained widespread adoption to remove and recycle CO₂. One notable advancement in CCS technology is carbon capture, utilization, and storage (CCUS), which has the potential to reduce CO₂ emissions substantially. The captured CO₂ can be directed towards a novel output process, where it undergoes transformation and is utilized in a variety of ways [82]. This procedure efficiently accomplishes the upcycling and reusing of CO₂ and goes beyond simple storage. Table 2 summarizes the products formed via the chemo-enzymatic approach of utilizing CO₂. The advantages and disadvantages of CO₂ conversion via chemical (electrochemical and catalytic thermal) and chemo-enzymatic approaches are summarized in Table 3.

Table 2. List of products formed via chemo-enzymatic approach of CO₂ conversion.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Products</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carboxydothermus hydrogenoformans CO Dehydrogenase I[Ch Ni-CODH I]</td>
<td>Carbon monoxide (CO)</td>
<td>[24]</td>
</tr>
<tr>
<td>Dehydrogenases in an ALG – SiO₂</td>
<td>Methanol</td>
<td>[83]</td>
</tr>
<tr>
<td>Formate Dehydrogenase</td>
<td>Formate</td>
<td>[41]</td>
</tr>
<tr>
<td>Nitrogenase</td>
<td>Methane</td>
<td>[84]</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>Bicarbonate (HCO₃⁻)</td>
<td>[85]</td>
</tr>
<tr>
<td>Nanocatalyst (Na–Fe₃O₄/HZSM-5)</td>
<td>Gasoline</td>
<td>[86]</td>
</tr>
<tr>
<td>Nanoparticles (PdZn/TiO₂)</td>
<td>Dimethyl Ether</td>
<td>[87]</td>
</tr>
<tr>
<td>Inorganic catalyst ZnO-ZrO</td>
<td>Starch</td>
<td>[88]</td>
</tr>
<tr>
<td>Formolase(FLS) and D-fructose-6-phosphate aldolase (FSA)</td>
<td>Carbohydrate (l-Erythrulose)</td>
<td>[89]</td>
</tr>
<tr>
<td>Hybrid nanocatalyst (C@Fe–Al₂O₃)</td>
<td>Salicylic acid</td>
<td>[90]</td>
</tr>
<tr>
<td>PdCu alloy nanoparticles</td>
<td>Urea</td>
<td>[91]</td>
</tr>
<tr>
<td>Metallic heterogenous catalyst</td>
<td>Cyclo carbonates</td>
<td>[92]</td>
</tr>
<tr>
<td>Cu–Ru–Metallic Organic Framework (MOF)</td>
<td>Ethanol</td>
<td>[92]</td>
</tr>
<tr>
<td>Platinum/cobalt catalysts</td>
<td>Butanol-based fuel</td>
<td>[93]</td>
</tr>
</tbody>
</table>
Table 3. Advantages and disadvantages of various CO$_2$ conversion approaches.

<table>
<thead>
<tr>
<th>Approaches</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrochemical</td>
<td>• Recyclable electrolytes</td>
<td>• Low catalyst lifespan</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>• High selectivity</td>
<td>• Economically not feasible</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Long term stable operation</td>
<td>• Require more electrical energy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Easy to scale-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermal</td>
<td>• High yield production</td>
<td>• Require high temperature</td>
<td>[34,95]</td>
</tr>
<tr>
<td></td>
<td>• Low catalyst stability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemo-enzymatic</td>
<td>• Bulk chemicals production</td>
<td>• Require high temperature</td>
<td>[27,85]</td>
</tr>
<tr>
<td></td>
<td>• High selectivity and yield</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Reaction requires less variable conditions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6. Sustainable CO$_2$ conversion and utilization.

4.1. Conversion into Minerals

In order to speed up the hydration and dehydration of carbon dioxide, carbonic anhydrase (CA) is a crucial metalloenzyme in biological systems. CA is mainly responsible for the (inter)conversion between CO$_2$ and bicarbonate. It is known that the enzyme carbonic anhydrase increases CO$_2$ absorption rates. A divalent zinc ion in the enzyme’s active site catalyzes the process. With reaction speeds of up to $10^8$ s$^{-1}$, CA is one of the fastest recorded enzymatic reactions [96]. CA-catalyzed processes have been shown effective in capturing gaseous carbon dioxide from industrial emissions in aqueous solutions. The three most widely used techniques for CA-catalytic conversion of CO$_2$, including capture, sequestration, and utilization, are absorption, membrane separation, and precipitation/mineralization. These methods offer promising solutions for reducing carbon emissions in industrial settings [64]. The chemical absorption method, which uses CA-catalytic CO$_2$ conversion with regenerable alkaline aqueous solvents, is one of the most prominent applications of the chemo-enzymatic process of CO$_2$ conversion.

The biogenic precipitation of calcium carbonate, an important natural process, is facilitated by CA. As a result, biomimetic techniques have great potential for CO$_2$ capture, storage, and utilization. The first step in CO$_2$ sequestration is separating CO$_2$ from other gaseous components in flue gas produced during fuel combustion. A common chemical
absorption method is utilized, whereby CO₂ is removed from flue gas in an absorber column and desorbed in a heated stripper column to yield relatively pure CO₂ (Figure 7A). By utilizing immobilized CA as a packed column membrane contractor, these scrubbers can emit CO₂ gas and precipitate CaCO₃ (Figure 7B). Furthermore, coupling a CA-catalyzed conversion process with a chemical absorption process may increase CO₂ absorption rates in alkaline aqueous solvents while also enabling the use of aqueous solvents with reduced heat/energy input for desorption.

**Figure 7.** Schematic representation of CO₂ capture, storage and utilization process. (A) Represents the CO₂ sequestration process that involves the removal of CO₂ from the flue gas that is in the absorber column and then desorbed in a heated stripper column to attain relatively pure CO₂. (B) Depicts the process of bicarbonate formation. The immobilized CA (scrubber column) absorbed the released CO₂, and the precipitation column’s carbonate ions were transformed into the compound CaCO₃ in the presence of a CaCl₂ solution. Reproduced with permission from Kanth et al. [97] Copyright 2013 Wiley.

CO₂ Solution Inc. has developed a commercialized technology for biomimetic CO₂ capture and storage. It employs a patented biotechnology infrastructure that can be used in the coal-fired power generating industry, the oil sands industry, as well as other CO₂-intensive industries, using CA as a biocatalyst. Cement plants release CO₂, which CO₂ Solution Inc. collects and transforms into bicarbonate ions, which are then transformed into limestone and utilized as raw goods for cement production [97], as shown in Figure 7B. Calcite is the main byproduct of CaCO₃ catalyzed by CA. The calcite CaCO₃ minerals are currently employed in numerous industrial processes. These consist of a medicinal source in antacids and calcium supplements, cement constituent, paper filler and coating, white coloring in paints, and granulating excipient. Calcite is also a component of limestone, marble, and chalk [98].

Despite the numerous advantages of the chemical absorption process, it has been observed that harsh conditions, including high temperatures, high levels of organic amines, and trace pollutants, can result in enzyme denaturation. This is a major limitation in the industrial process. Various methods have been employed to overcome this limitation in investigating enzyme-enhanced CO₂ sequestration. These methods include the purification and analysis of CA from different sources [99,100], as well as the immobilization of CA to enhance its resistance to abrasive environments [101]. Many studies have been done
exploring the immobilization technique to improve the CA for high CO₂ sequestration, which includes using materials such as mesoporous silica [102], nanofibers [103], nanoparticles [104], and membranes [105]. By co-precipitating enzymes and inorganic components, several advanced methods for enzyme immobilization have recently been devised. Recently, novel CA from alkalophilic cyanobacterium showed improved thermal stability and outstanding CO₂ collection capabilities [106]. Another recent discovery in improving CO₂ sequestration technology and linking it to additional reactions for upcycling is the acceleration of CO₂ sequestration via pressure. This is done by introducing carbonic anhydrase into a high-pressure reactor to catalyze the hydration of CO₂ and produces precipitated CaCO₃ [107]. All these efforts are made to enhance the sequestration, upcycling and recycling of CO₂.

Recent advancements in metabolic and protein engineering have paved the way for using synthetic biology as a powerful tool for enhancing biological function and commercial viability. An example of this can be seen in the successful expression of Methanobacterium thermoautotrophicum in the periplasm of *E. coli*, creating a whole-cell biocatalyst for CO₂ hydration. These processes have been shown to effectively hydrate CO₂ with catalytic yield and efficacy comparable to free enzymes. However, a lower yield disparity may result from mass transportation restrictions. In comparing the operation with and without calcium components (carbonate and chloride), it has been observed that the presence of CaCl₂ and CaCO₃ results in a 50–70% increase in carbon precipitation [108].

The latest development in 3D printing has enabled the creation of a novel apparatus consisting of buoyant and biocatalytic constituents, which has enhanced interfacial CA catalysis to utilize and convert CO₂ into CaCO₃ effectively. This innovation has simplified the alignment and positioning of immobilized CA at the interfacial region. The resulting bioreactor has demonstrated the ability to expedite CO₂ hydration at gas-liquid interfaces, thereby expediting the conversion of CO₂ [109].

4.2. Conversion into Chemicals

The economic worth of CO₂ can be increased by using it to synthesize bulk compounds. The most prominent byproducts for CO₂ utilization in conventional chemical industry operations are urea and salicylic acid, and urea generation is the most significant chemical route for CO₂ utilization [110]. The interaction of ammonia with CO₂ is the primary industrial technique for producing urea. The production of methylammonium using supercritical NH₃ and CO₂ is the first reaction in the urea manufacturing process. The second reaction is the dehydration of methylammonium to produce urea. An efficient catalyst for the concurrent electrocatalytic coupling of N₂ and CO₂ to accomplish sustainable urea production was discovered to be PdCu alloy nanoparticles on TiO₂ nanosheets [91]. In order to accelerate the synthesis of urea, “N—N” spontaneously established a C-N bond with the CO created by reducing CO₂. Due to its superior stereo-specificity and region/chemo-selectivity, the enzymatic method—which was inspired by the CO₂ metabolic process in cells—offers a green and effective substitute for effective CO₂ conversion. The decarboxylase enzyme is one of the popular enzymes used to convert CO₂ into biodegradable chemicals.

An example of this is using pyruvate decarboxylase in biodegradable chemical conversion, which offers the advantage of an environmentally safe technique [48]. These decarboxylases have drawn a lot of interest recently because they can also chemically catalyze the carboxylation of the substrate with CO₂. Four distinct enzymatic carboxylation reactions obtained from catabolic pathways have been created in vitro, as stated by Faber and co-workers: (1) the carboxylation of epoxides, (2) the carboxylation of aromatics, (3) the carboxylation of hetero-aromatics, and (4) the carboxylation of aliphatics [64]. CO₂ with phenol can produce salicylic acid under high pressure and temperature. A study by Ijima discovered that supercritical CO₂ and phenol would successfully synthesize salicylic acid at room temperature in the presence of a Lewis acid catalyst. Under specific circumstances, the phenol was transformed into salicylic acid by adding 10 mmol of aluminium bromide [111]. In an alternative investigation, the catalytic activities of
various alkali metals were evaluated. The findings indicate that potassium carbonate positively affected the production of salicylic acid from supercritical CO$_2$ and phenol. The results demonstrated that potassium carbonate was the most efficient catalyst among all the base catalysts, with a 99% selectivity for salicylic acid carboxylation. The key impact was achieved by optimizing the reaction parameters to maintain reaction efficiency. Under specific conditions, the conversion rate of phenol was 69.3%, while salicylic acid’s production and selectivity reached 69.3% and 99%, respectively. These results suggest that potassium carbonate has great potential as a catalyst for the carboxylation of phenol to produce salicylic acid [112].

4.3. Conversion into Organic Fuels

Catalytic hydrogenation can be utilized to convert CO$_2$ into organic compounds such as methane, methanol, formic acid, and dimethyl ether. This process produces “green fuel” that, unlike traditional fossil fuels, does not generate additional CO$_2$ emissions upon consumption.

4.3.1. Conversion into Methane and Methanol

Methane, the principal constituent of natural gas, is a critical energy source for residential and industrial use. The methanation process, which involves converting a mixture of CO$_2$ and H$_2$ gases with an H$_2$ to CO$_2$ ratio of 4, is responsible for the production of CH$_4$. This exothermic reaction requires a catalyst and heating for efficient conversion. Extensive research has identified Co, Ni, Ru, and other active elements as effective catalysts for this reaction [113]. Martins et al. developed a novel adsorption-conversion cycle technology focused on CO$_2$ hydrogenation that allowed for the simultaneous isolation and use of CO$_2$ [114]. In this method, a concurrent adsorption reactor isolates the biogas well (a CO$_2$/CH$_4$ combination), and it also produces methane by reacting the separated CO$_2$ with the entering H$_2$. This is a very promising approach for capturing and using CO$_2$, but the advancement of this study field depends on improving process operating parameters.

The conversion of carbon dioxide (CO$_2$) to methanol through a multienzyme process has gained widespread attention as a promising pathway due to recycling the “greenhouse” gas and creating sustainable biofuels. Although methanol can be synthesized via the catalytic hydrogenation of CO$_2$, the occurrence of the reverse water gas shift reaction (RWGS) can limit its production. This reaction consumes a portion of the hydrogen (H$_2$), decreasing methanol yield. Simultaneously water production can also negatively impact the catalyst’s catalytic activity, compounding the reduction in methanol production [115]. Therefore, the choice of catalyst is crucial to the hydrogenation of CO$_2$ to methanol. Li et al. investigated the Cu/ZrO$_2$ catalyst’s catalytic mechanism for CO$_2$ hydrogenation to methanol [116]. Because of the distinctive crystalline structure of ZrO$_2$, it was discovered that it could react with Cu. This enhanced Cu’s dispersion stabilized Cu nanoparticles during catalytic CO$_2$ hydrogenation, successfully prevented the RWGS reaction, and encouraged methanol production.

The multienzyme reaction creates liquid methanol, which is simpler to ship and has much greater energy capabilities. For the first time, Yoneyama et al. reported the successful electrochemical reduction of CO$_2$ to methanol using pyrroloquinolinequinone (PQQ) as a coenzyme and FateDH and methanol dehydrogenase (MDH) as catalysts. This method provided a simple means of producing methanol straight from CO$_2$ under benign circumstances [64]. Dave and co-workers reported a strategy involving the sequential reduction of CO$_2$ to methanol, with multiple dehydrogenases as the catalysts and NADH as a cofactor, to benefit the essence that dehydrogenases can efficiently catalyze the reduction of CO$_2$ in the existence of an appropriate cofactor [13].

4.3.2. Conversion into Ethanol

The potential of microbe-based ethanol generation has garnered significant attention in recent years. Conventionally, biofuels are produced through the solventogenic conversion
of biomass obtained from photosynthetic microbes. However, this technique has been demonstrated to be ineffective owing to its lower energy conversion efficiency [117]. As a result, current research is focused on the direct conversion of CO$_2$ into biofuels by photosynthetic bacteria. While only a limited number of cyanobacterial species are capable of producing ethanol as a byproduct during biological fermentation at lower concentrations, efforts are underway to increase the generation levels to make the process more cost-effective. By inserting the pyruvate decarboxylase (pdc) and alcohol dehydrogenase II (adh) genes from *Z. mobilis* into the chromosomes of *Synechocystis* sp. PCC6803, Dexter and Fu (2009) attempted to produce ethanol and successfully produced 550 mg/L [118].

4.3.3. Conversion into Formic Acid

Formic acid is a crucial industrial compound with various applications in animal feed preservation, textile tanning, and chemical synthesis. The current global annual production of formic acid stands at 800,000 T, primarily generated by reacting methanol and carbon monoxide with a robust base [119]. Numerous studies on CO$_2$ hydrogenation have explored various reaction media, including organic solvents, supercritical CO$_2$, aqueous solutions, and the addition of organic or inorganic bases or task-specific ionic liquids [120]. The most extensively studied catalysts for CO$_2$ hydrogenation to formic acid conversion are homogeneous catalysts, which offer milder reaction conditions and improved catalytic efficiency compared to heterogeneous catalysts. Catalysts that dissolve in water are often employed in CO$_2$-to-formic acid conversion systems. According to Zhao et al., RhCl(mtppms)$_3$ was utilized as a catalyst to hydrogenate CO$_2$ into formic acid in a sodium formate solution [120]. In a recent study, researchers have conducted the biocatalytic conversion of CO$_2$ into formic acid using a biocatalytic micromixer consisting of two enzymes, carbonic anhydrase (CA) and formate dehydrogenase (FDH). These two enzymes were biomineralized in a zeolitic imidazolate framework-8 composite thin film and modified with polydopamine/polyethyleneimine [121].

4.3.4. Conversion into Dimethyl Ether (DME)

The potential utilization of dimethyl ether (DME) as a substitute fuel or precursor for creating various value-added products, such as gasoline, aromatics, and olefins, has led to significant interest in converting CO$_2$ to DME. While DME has primarily been used as a replacement for ozone-depleting chlorofluorocarbon (CFC) chemicals, such as aerosol propellant in spray bottles, there has been a growing focus on its potential as an environmentally friendly alternative fuel [122]. DME can be generated directly by CO$_2$ hydrogenation or by dehydrating methanol after CO$_2$ hydrogenation. Since methanol dehydration can produce DME, the catalyst that converts CO$_2$ into dimethyl ether is typically a mixed catalyst made of catalysts for methanol synthesis and dehydration, such as a molecular sieve [123]. Many studies have recently been done to create a CO$_2$ hydrogenation catalyst that is extremely effective [124,125]. Several approaches have been put forth to develop a catalyst with good CO$_2$ conversion, DME selectivity, and stability properties that can make DME through one-pot hydrogenation of CO$_2$ [126]. A more appealing substitute for the two-step process has recently been suggested: direct synthesis in a single step. The one-step procedure combines the two reactions of methanol synthesis (via CO hydrogenation) and methanol dehydration to DME in the same reactor while utilizing hybrid bifunctional catalysts in environments that encourage methanol synthesis. The hybrid catalysts required for direct DME production need to combine acid sites for subsequent methanol dehydration and metal sites for selective hydrogenation of CO to methanol [127]. Some elements of the bifunctional catalyst, the Cu-based catalyst, are anticipated to continue being the most effective catalyst for the CO$_2$-to-methanol reaction step.
4.3.5. Conversion into Olefins

Light olefins (C2=C4=), which are required to manufacture high value-added substances and plastics, are in great supply on a global scale. Processing hydrocarbons, the direct/indirect conversion of synthesis gas (CO + H\textsubscript{2}), and the hydrogenation of CO\textsubscript{2} can all be used to create light olefins. The most recent technique to be researched among these is the catalytic hydrogenation of CO\textsubscript{2}, which may help reduce atmospheric CO\textsubscript{2} emissions.

The production of olefins from CO\textsubscript{2} can take place in many distinct ways (Figure 8), based on the catalyst being utilized: (1) the CO-mediated altered Fischer-Tropsch to olefins (FTO) route; (2) the methanol to olefins (MTO) route [27]; and (3) the direct CO\textsubscript{2} hydrogenation to olefins utilizing promoted and multifunctional catalysts (Equation (6)).

\[
n\text{CO}_2 + 3n\text{H}_2 \rightarrow C_n\text{H}_{2n} + 2n\text{H}_2\text{O} \tag{6}
\]

![Figure 8](image_url)

**Figure 8.** Schematic diagram illustrating the three major pathways for producing olefins from CO\textsubscript{2}: (1) modified Fischer-Tropsch to olefins (FTO) route; (2) methanol to olefins (MTO) route; and (3) direct olefin formation over multifunctional, promoted catalysts. RWGS—reverse water-gas shift. Adapted from [128].

It was reported that the FTO route (1) is much more effective than the MTO technique in the context of hydrocarbon generation [128]. Therefore, to optimize the production of olefins through the methanol-to-olefin (MTO) route, minimizing the formation of byproducts such as CO, CH\textsubscript{4}, C\textsubscript{2}-C\textsubscript{4} alkanes and C\textsubscript{5+}, hydrocarbons is crucial. Significant CO\textsubscript{2} conversions can encourage the production of such byproducts, underscoring the need to mitigate their generation. Conventionally, the MTO process involves two separate reactors: the first for methanol production and the second for olefin production over zeolite catalysts. However, a more efficient approach involves employing a bifunctional catalyst with metallic and acidic properties within a single vessel for the catalyzed reaction [129].

A recent study conducted by Guo et al. developed a fully integrated catalyst with an enzyme-like structure using calcined CC ash, iron carbides, and alkali boosters to convert CO\textsubscript{2} hydrogenation directly into linear α-olefins (LAO), with selectivity in hydrocarbons of at least 40% and a total olefin selectivity in hydrocarbons of 72%. The output specificity in this “CO\textsubscript{2}-LAO” process came close to Fischer-Tropsch synthesis (FTS). It is shown that the bio-promoter reconfigured iron catalysts have a synergistic impact on enhancing CO\textsubscript{2} hydrogenation efficiency in comparison to unpromoted and chemically supported iron-based catalysts [130].
4.4. CO₂ Utilization in Polymer Synthesis

In the area of CO₂ chemical upcycling, creating CO₂ polymers with a commercial benefit from CO₂ is a major field of study. The two most viable ways of CO₂ upcycling for the creation of synthetic polymers are the generation of polycarbonate (PC) and polyurethane (PU) from CO₂ [131].

4.4.1. Chemo-Enzymatic Synthesis of Polycarbonates

The thermoplastic polymer containing carbonate units, commonly known as polycarbonate (PC), has attracted significant attention owing to its potential for recyclability. Notably, PC is biodegradable and can be synthesized using CO₂ as a comonomer. The current state-of-the-art approach for producing aliphatic polycarbonate (PPC) involves copolymerizing CO₂ with epoxide. Extensive research has focused on developing new catalysts and catalytic mechanisms to enhance PPC production. This innovation is crucial for meeting the increasing demand for sustainable and environmentally friendly plastics.

Liu has recently reported the discovery of a novel bimetallic nickel compound, which exhibited exceptional catalytic activity in accelerating the copolymerization of CO₂ and epoxides. This finding has led to the successful synthesis of CO₂-based poly(cyclohexene carbonate)s (PCHCs) with carbonate linkages exceeding 99%, via the copolymerization of CO₂ and cyclohexene oxide, and under the catalytic influence of the bimetallic nickel compound. This breakthrough finding has significant implications for developing sustainable and environmentally-friendly polymer materials [132].

4.4.2. Chemo-Enzymatic Synthesis of Polyurethanes

Polyurethane (PU) is a commonly used polymer in manufacturing various polymeric materials and plastic sponges. Due to its remarkable properties, it has found widespread use across numerous industries following years of scientific research and development. Nonetheless, the conventional synthesis methods of PU involve the utilization of isocyanates, which pose significant safety risks [133]. A new development on the path to the production of PU is the substitution of isocyanate with CO₂. This will not only reduce the issue of CO₂ emissions but also realize the resource exploitation of CO₂. Several recent advancements in the production of PU include employing CO₂ as a carbonyl source to swap out isocyanate and polysiloxane with amino modifications [134], synthesizing polyurethane thermoplastics via polycondensation of CO₂ with mixed binary diamines [135], and the recent disclosure of a procedure for the indirect production of polyurethane from CO₂ that is solvent- and catalyst-free [136].

5. Significance of Chemo-Enzymatic Methods in Comparison

Chemo-enzymatic describes a synthetic strategy that integrates biocatalytic reactions for large-scale chemical synthesis. The enzymatic approach offers multiple benefits, including enhanced selectivity, yield, and tolerance towards diverse reaction conditions [27]. In the current situation, producing such chemicals with high stereoselectivity is important [81]. A schematic representation of the chemo-enzymatic approach to CO₂ conversion is presented in Figure 9.

The chemo-enzymatic route has demonstrated the ability to convert CO₂ into bulk chemicals. The process involves the conversion of CO₂ into CO by [NiFe] carbon monoxide dehydrogenase, which can then be further transformed into various value-added products, such as hydrocarbons, methanol, and acetic acid [24]. Previous studies have extensively investigated the production of formate, formic acid, and methanol via the enzymatic reduction of CO₂ [137]. Additionally, combining electrochemical and chemo-enzymatic approaches has resulted in a Faradic efficiency of 10% [75]. CO₂ can also be converted into methane via the enzyme nitrogenase. Yang et al. [84] studied nitrogenase for methane production, where 1 nmol of nitrogenase produced 21 nmol of methane at optimized conditions. Carbonic anhydrase (CA) is another enzyme with a higher reaction rate for CO₂ conversion when compared with other enzymes [27]. CA is also involved in the
process of CO$_2$ capture and CO$_2$ sequestration. Although this reaction requires high temperature, which might affect the enzyme activity, this setback can be overcome by enzyme immobilization onto beads/matrix materials. In this regard, this process further assists CO$_2$ capture and conversion in producing valuable products [138]. One notable chemo-enzymatic mechanism is the Michael addition, where the enzyme lyase serves as the catalyst. The carboxylation of molecules, involving the cleavage of chemical bonds and leading to the formation of valuable chemical compounds, can be accomplished by utilizing this enzyme [64].

![Chemo-enzymatic CO$_2$ utilization and conversion](image)

**Figure 9.** Chemo-enzymatic CO$_2$ utilization and conversion [27]. Reproduced with permission from Elsevier 2021.

The chemo-enzymatic approach towards CO$_2$ conversion is a promising avenue for achieving high selectivity and product yield. However, there are some potential drawbacks associated with this approach, including high cost; and efforts have been made to increase its activity and stability. It is crucial to conduct scale-up pilot research studies in industrial settings to assess the viability of this approach for large-scale applications. Moreover, computational methods can be employed to design novel enzymes that can enhance the efficiency of CO$_2$ conversion. These advances in enzyme design hold significant potential for achieving higher yields and reducing the overall cost of the process. The development of new inventions and research in the field of CO$_2$ capture, utilization, and sequestration is gaining momentum worldwide. The goal is to move towards sustainable solutions that can mitigate the impact of CO$_2$ emissions on the environment. Researchers, governments, and people are working together to promote this vital research, which is expected to play a crucial role in achieving global sustainability targets.

While turning waste CO$_2$ into high-value products is a promising complementary strategy for improving the carbon balance and financial prospects. Bioenergy transformation to carbon capture and storage (BECCS) is also gaining considerable attention as a potential carbon-negative technology for the future. Combining chemo- and bio-catalysis can produce beneficial synergies in the chemical value chain when one of the two catalytic fields is insufficient. The long-standing research goal of combining these two catalytic fields represents an economically and environmentally appealing concept, as it can potentially avoid time- and capacity-consuming work-up stages of intermediates.
6. Conclusions

The increasing atmospheric CO\(_2\) concentration is a significant environmental issue, leading to global warming. In response, efforts are being made to collect and transform CO\(_2\) into value-added goods. CO\(_2\) utilization can be directly used or transformed into compounds with added value. Enzymatic technology is a promising approach, utilizing CO\(_2\) metabolic processes in cells and providing an environmentally friendly way to upcycle CO\(_2\). Many methods for rational enzyme-based catalyst synthesis, reaction process refinement, and reaction mechanism clarification have been documented, contributing to improved chemo-enzyme catalytic activity. Despite recent advancements, more research is necessary for large-scale utilization, cheaper and environmentally stable catalysts, and the industrialization of developed technologies. Finding low-energy pathways for CO\(_2\) conversion through efficient enzyme-based technology requires further exploration. The significant scientific and technical advances in CO\(_2\) conversion and upcycling presented in this review provide a basis for future research to address the urgent need for effective CO\(_2\) utilization and the reduction of carbon emissions.


**Funding:** The authors thank Sunway University for the International Research Networks Grant Scheme 2.0 (STR-IRNGS-SET-GAMRG-01-2022) for this work.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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