Chapter 14
Recent Advances in Enzymatic Conversion of Carbon Dioxide into Value-Added Product

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14.1 Introduction

Nowadays, climate change and global warming are major environmental issue due to continuous increase to atmospheric carbon dioxide concentration and vast growth in industrialization (Yaashikaa et al. 2019). Due to extensive deforestation, agriculture, population growth, rapid industrialization, the current overall concentration of atmospheric CO_2 is 400 ppm as compared to preindustrial level 280 ppm (Giri and Pant 2018) so requires to minimizing of atmospheric CO_2. Carbon dioxide (CO_2) capture, sequestration, and utilization process has been widely recognized effective techniques for reducing CO_2 concentration from the atmosphere. IPCC estimated in 2001 the global average annual mean surface air temperature which is increased between 1.4 and 5.8 °C till 2100. This rise in temperature has been causing global warming, and other climatic changes like in 2006 Australia faced extreme drought of in 1000 years (https://www.theguardian.com/world/2006/nov/08/australia.drought), deadly dust storms in India in May, 2018 (Sarkar et al. 2019), deadliest hurricanes in U.S. (Great Galveston hurricane in 1900, Okeechobee hurricane in 1928, hurricane Katrina in 2005) , forest fire, sea level rise, tsunami, etc., are the main consequences of global climate change (Pant et al. 2017). Currently, different technologies have been used for CO_2 capture and storage (CCS) by carbon capture, transportation, and storage which are main three steps (Huntley and Redalje 2007). The rise in CO_2

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levels in the atmosphere could also minimize by transformation of carbon dioxide into value-added products by different physical, chemical, electrochemical, photochemical, and biological or enzymatic methods. The biological or enzymatic method provides an environmentally friendly and promising way for effective CO_2 conversion or fixation due to high stereo-specificity and chemo-selectivity of enzyme (Shi et al. 2015; Giri et al. 2020; Sharma et al. 2020), while all methods mentioned above have some major drawbacks of these include: large investment and high energy input, high transportation cost, reusability, and environmental toxicity (Irfan et al. 2019). Strategies for CO_2 conversion or fixation by enzymatic method to value-added products not only offer promising new technologies for carbon dioxide reduction but also for an efficient production of value-added products. Enzymes catalyze the biotransformation CO_2 at moderate reaction conditions (temperature, pressure, less require energy) and provide high yield than other transformation methods. This enzymatic transformation of CO_2has attracted increasing international interest for its industrial applications and its capability to turning this greenhouse gas into added value products (Long et al. 2017). The molecule of carbon dioxide shows chemically inert and thermodynamically stable, and some external energy requires an energy input for CO_2 transformation; thus, it would be reasonable to think about suitable enzymatic catalyst as energy sources for CO_2 conversion. The biotransformation of carbon dioxide by enzymatic method is currently under investigation worldwide in various aspects like fuel production of biofuels along with different value-added products and chemicals (methane, organic acids, bicarbonate, glucose, alcohols, etc.) (Yaashikaa et al. 2019).In short, today, it is an urgent need to reduce the rising atmospheric carbon dioxide from the environment and emphasis on green and renewable energy to decrease dependency on conventional fossil fuels. In regards to industrial application, an enzyme system for CO_2 biotransformation simply chooses a potential for carbon dioxide conversion into useful chemicals and fuels for sustainable and clean environment. Finally, the present book chapter mainly focused on enzymatic transformation of CO_2, as well as the future perspectives.

14.2 Enzymatic Transformation of CO_2

Enzymatic CO_2 transformation can be categorized into two types: direct and indirect transformation of carbon dioxide. They are addressed further in the following in more detail.

14.2.1 Indirect Transformation of CO_2

Indirect transformations of CO_2 involve indirectly utilization of carbon dioxide to produce useful chemicals are discussed below. Photosynthesis is one of indirect transformation of CO_2 through microbial transformation or through plant bio-fixation.

14.2.1.1 Natural Transformation of CO_2 in Cells

In natural, CO_2 fixation/ transformation into organic materials are important for biological evolution and essential factor for regulating atmospheric CO_2 concentrations (Fuchs 2011; Shi et al. 2015). The carbon dioxide (CO_2) assimilation into organic matter is facilitated by major six CO_2-fixing pathways including the reductive pentose phosphate cycle (Benson–Calvin cycle), the reductive citric acid and acetyl-CoA pathway, 3-hydroxypropinate cycle, 3-hydroxypropinate/4-hydroxypropinate pathway, and the dicarboxylate/4-hydroxybutyrate cycle and converts atmospheric CO_2 to organic compounds (Wolosiuk et al. 1993; Shi et al. 2015). The reductive pentose phosphate cycle or Calvin cycle is one of the most important pathways for photosynthetic organism to incorporate CO_2 into the cell carbon cycle with 7×10^6 g carbon consumption rate on the annual basis (Berg 2011). The key enzymes in Calvin cycle are ribulose-1,5-bisphosphatecarboxylase/oxygenase (RubisCO) and phosphoribulokinase (PRK) which are potential to photosynthetic carbon reduction cycle. Ribulose-1,5-bisphosphatecarboxylase/oxygenase (RubisCO) catalyze the electrophilic addition of carbon dioxide to ribulose-1,5-bisphosphate (5C) compound (Fast and Papoutsakis 2012) and transform to several intermediate products like 3-phosphoglycerate, 1,3-diphosphoglycerate, and 3-phosphate glyceraldehyde, and in the final stage, 3-phosphate glyceraldehyde transform into 5-phosphate ribulose. Furthermore, 3-phosphate glyceraldehyde can be converted into amino acids, sugar, and fatty acids (Shi et al. 2015).

The reductive citric acid cycle or tricarboxylic acid cycle (TCA) converts carbon dioxide and water into carbon compounds. The main following enzymes 2-oxoglutarate ferredoxinoxido reductase, ATP citrate lyase, isocitrate dehydrogenase, pyruvate ferredoxinoxido reductase are involved in the reductive tricarboxylic acid cycle or CO_2 fixation pathways (Hügler et al. 2005). The reductive citric acid cycle involves four steps of carboxylation reactions, in which, succinyl-CoA is reductively carboxylated with carbon dioxide into α-ketoglutarate/2-oxoglutarate in presence of a-ketoglutarate synthase/2-oxoglutarate synthase. Further, α-ketoglutarate/2-oxoglutarate and CO_2 are transformed into verity of compounds like isocitrate, citrate, pyruvate, etc. The pyruvate is then converted into phospho-enolpyruvate (PEP) in presence of pyruvate kinase, and then, oxaloacetate is formed. Finally, oxaloacetate is converted into succinyl-CoA in presence of series of key enzyme (Thauer 2007; Fuchs 2011).

The reductive acetyl-CoA pathway is a major CO_2 fixation mechanism in anaerobic environments to turn CO or CO_2 into carbon cells (Roberts et al. 1994) and mainly found in acetogenic, methanogenic bacteria and Eubacteria. In the reductive acetyl-CoA pathway, CO_2 is converted into formate and carbon monoxide by formate dehydrogenase and CO dehydrogenase (CODH), respectively (Roberts et al. 1994; Berg 2011). Similarly, 3-hydroxypropionate, 3-hydroxypropionate/4-hydroxybutyrate, and dicarboxylate/4-hydroxybutyrate all above six major pathways catalyze the conversion CO_2 or bicarbonate in cells.

14.2.2 Direct Transformation of CO_2

In the direct transformation process, carbon dioxide is converted directly using biocatalysts into useful chemicals. In this method, carbon dioxide is used as substrate and source of hydrogen, and biocatalysts are the additional requirements for conversion. The role of enzyme and products in direct transformation of carbon dioxide is discussed in this section briefly.

14.2.2.1 Transformation of CO_2 to Formate

Demand of future and sustainable energy needs alternatives to current energy technologies mainly based on fossil fuels. Hydrogen has been considered a promising alternative energy feedstock, but hydrogen storage and transportation is a major complexity for its optimum utilization (Schlapbach and Züttel 2011). Schuchmann and Müller (2013) discovered hydrogen-dependent CO_2 reductase from *Acetobacteriumwoodii* bacteria which directly uses dihydrogen and catalyze the hydrogenation of carbon dioxide to formate or formic acid (FA) which is a promising and intermediate reservoir for hydrogen storage and distribution. Formate/formic acid (FA) also used for various chemical intermediates, silage preservation, animal feed additives, and most promising candidate for low-temperature fuel cell (Rees and Compton 2011). NAD-dependent formate dehydrogenase (FDH) from *Candida boidinii* is also used for various CO_2 reductions into formate (Kim et al. 2014) and showed very less CO_2 reducing activity. The formate dehydrogenase (FDH) from *Thiobacillus sp.* (TsFDH) exhibits the high CO_2-reducing activity and showed 5.8 time higher formate production rate as compared to formate dehydrogenase (FDH) from *Candida boidinii* (CbFDH) (Choe et al. 2014).

14.2.2.2 Transformation of CO_2 to Carbon Monoxide

Carbon monoxide dehydrogenase (CODH) is a type of dehydrogenese enzyme that can convert carbon dioxide into carbon monoxide in the presence of the required electron donor like NADH, NADPH, MV^{+2}, etc. by following equation (Sultana

et al. 2016). The reduction of carbon dioxide into carbon monoxide possesses fuel value that offers a feasible new alternative energy sources (Olah 2005).

$$CO_2 + 2H^+ + 2e^- \longleftrightarrow CO + H_2O$$

The reduction of carbon dioxide into carbon monoxide is primarily catalyzed by Ni and Fe containing CO dehydrogenases ([NiFe] CODH) through reversible redox reaction and the synthesis of acetyl-COA (Jeoung and Dobbek 2007). The active site of ([NiFe] CODH) contains Ni and Fe centers bridged bound to three sulfur ligands (3Fe-4S) which is coordinated with His, Cys amino acid residue, sulfido, and a H2O/OH-ligand. In the catalyzing reaction of CO_2 reduction, two-electron process occurs during the reduction process and form reduce Ni center followed by carbon dioxide is bound to the Ni center with Ni-C bound and stabilized by Fe center. Simultaneously, the hydrogen bond is formed between carboxylate oxygen atoms (O_2) and protonated histidine residue (His93). The Fe_1 loss water molecule and formed a CO_2 complex, and another oxygen molecule (O_1) of CO_2 complex is bound to Fe_1 and formed hydrogen bound with a protonated lysine residue (Shi et al. 2015). First time in 2003 (Shin et al. 2003) implement the reduction of CO_2 to CO in vitro by utilizing ([NiFe] CODH) from *Moorella thermoacetica* which showed efficient combination of enzyme conversion of CO_2 to CO. The expression of Fe proteins (vnfH- and nifH)-encoded of Mo- and V-nitrogenases in *Azotobacter vinelandii* strains commonly the reduction of nitrogen (N_2) to ammonia (NH_3) also capable to catalyze CO_2 to CO in vitro and vivo (Seefeldt et al. 1995; Rebelein et al. 2016).

14.2.2.3 Transformation of CO_2 to Methanol

The enzymatic conversion of carbon dioxide into methanol is a promising new recycling technique not only for green house management but also for efficient fuel production (Obert and Dave 1999). The use of enzyme for transformation of CO_2 to methanol provides a facile low-temperature route and higher energy capacity for direct fuel generation from carbon dioxide. In 1994, Kuwabata et al. used two-enzyme system for conversion of carbon dioxide into methanol using FateDH and methanol dehydrogenase (MDH) as catalyst and pyrroloquinolinequinone (PQQ) as an electron mediator. The enzyme FateDH reduces CO_2 to formate, and the enzyme, methanol dehydrogenase (MDH), reduces formate into formaldehyde and then methanol.

The conversion of CO_2 to methanol is catalyzed by three oxido-reductases: formate dehydrogenase (FateDH), formaldehyde dehydrogenase (FaldDH), and alcohol (ADH) dehydrogenases like methanol dehydrogenase (MDH) which are dependent on the reduced form of cofactor β-Nicotinamide adenine dinucleotide (NADH) (Amado 2013). The following enzymes are able to catalyze the sequential reduction of CO_2 to value added products like formate, formaldehyde, and methanol, presented in Fig. 14.1.

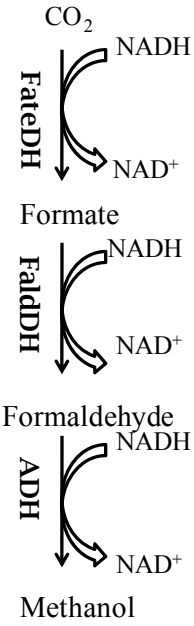

Fig. 14.1 Enzymatic transformation of CO_2 to methanol promoted by FateDH, FaldDH, and ADH

However, this method still has several problems like deleterious of enzyme by cosolvents and catalyst in sol–gel process needs some modification in process to improve the stability the biological activity (Xu et al. 2006). The immobilization of enzyme and hybrid enzymetic/photocatalytic approach for high enzymatic activity improved stability for efficient CO_2 transformation into methanol which was recently proposed in many studies (Aresta et al. 2014; Luo et al. 2015). The photocatalytic/enzymatic integrated approach combining by heterogeneous photocatalysts in the process of NADH regeneration from NAD^+ represents an important step toward the potential application in hybrid CO_2 reduction technology to methanol.

14.2.2.4 Transformation CO_2 to Methane

Recently, researchers found remodeled nitrogenase of *Rhodopseudomonas palustris* is able to take CO_2 from the air and turn it into methane. Purified remodeled nitrogenases of Rhodopseudomonas palustris containing two amino acids substitutions (α-195 by Gln and α-70 by Ala) near the site of its FeMo cofactor are able to CO_2 transformation into methane (Shi et al. 2015; Fixen et al. 2016). The bacterial Mo-dependent nitrogenase enzyme catalyzes the dinitrogen (N_2) to two ammonia molecule and evolution of H2 by following equation.

$$N_2 + 8H^+ + 16ATP + 8e^- \longrightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

Remodeled nitrogenases reduce CO_2 to methane by eight electrons and make it unique among all known catalyzed enzyme reactions. Furthermore, the reduced CO_2

is also able to react with acetylene to form propylene by following reactions (Omae 2012; Yang et al. 2012).

$$CO_2 + 8H^+ + 8e^- \longrightarrow CH_4 + 2H_2O$$

$$CO_2 + HC = CH + 8H^+ + 8e^- \longrightarrow H_2C = CH - CH_3 + 2H_2O$$

14.2.3 Transformation of CO_2 to Bicarbonate

Another strategy of enzymatic CO_2 conversion into value product is CO_2 mineralization in bicarbonate by enzyme carbonic anhydrase (CA) with high turnover rate. Recently, CA mediated conversion of CO_2 has attracted much attention toward green house management. Carbonic anhydrase is zinc containing metalloenzyme comprises three histidine residues, and a hydroxide ion or water molecule catalyzes the carbon dioxide by following equation (Giri and Pant 2019a).

$$CO_2 + H_2O \longrightarrow HCO_3^- + H^+$$

The CO_2 sequestrating by carbonic anhydrase for precipitating carbonate minerals are being actively investigated (Prabhu et al. 2011; Giri et al. 2018; Giri and Pant 2019a). CA can categorized into five distinct classes of α, β, γ, δ, ζ with few structural similarities, but all CAs similar active site of a divalent zinc ion or associated metal ion. The proposed mechanism of CO_2 hydration and CO_2 mineralization by CA could be summaries as follows: Nucleophilic attack on C atoms of CO_2 by zinc (Zn^{2+}) bound –OH to yield bicarbonate then displaced by molecule of water (Giri and Pant 2019a) (Fig. 14.2).

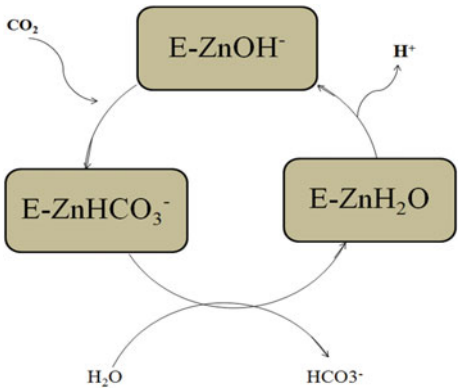

Fig. 14.2 Schematic representation of CA reaction mechanism to accelerate CO_2 uptake to facilitate carbonate precipitation

The first CA was discovered in 1933 by Meldrum and Roughton from erythrocytes in the role of transition of the bicarbonate anions (Meldrum and Roughton 1933) and in 1940 Neish identified the first plant originated CA and its different characteristics from previously known erythrocytes CA (Neish 1940). The prokaryotic CA was discovered by Veitch and Blankenship in 1963 and extracted from *Neisseria Sicca* in 1972. The active center of α CA contained by Zn atom and tetrahedral coordinated with three histidine residues and single water molecule, predominate in mammals (Domsic and McKenna 2010; Giri and Pant 2019a). However, the β CA shows oligomeric quaternary structure and Zn atom tetrahedral coordinated with two molecule of cysteines, and single molecules of histidine and aspartate. In the γ class of CA, the Zn atom is coordinated in a penta mode to three molecules of histidines and two water molecules (Alber and Ferry 1994). The β CA predominate in eukaryotes and γ CA were mostly present in Archaea. The δ class of CA found in *Thalassiosira weissflogii* showed a different amino acid sequence, compared to other α, β, and γ CAs (Tripp et al. 2001). Several research group purified carbonic anhydrase from organisms that thrive in extreme environments utilize to formation of calcium carbonate (Capasso et al. 2012). The converting CO_2 into bicarbonate by using enzyme CA in biomimetic approaches is thermodynamically favorable compared to other CO_2 mitigation techniques and carbonate minerals further used for building and industrial applications (Giri et al. 2018; Sharma and Kumar 2020).

The urease is also a metalloenzyme containing nickel belonging to the group of hydrolases that play an important role in nitrogen requirement in plants and microorganism. The function of urease is to catalyze hydrolysis of urea into ammonia and carbamate. The carbamate further hydrolyzed into the additional mole of ammonium and carbonic acid. The shifting of bicarbonate toward carbonate ion increases the influx of calcium ions and obligates the bacterial export of calcium ion outside the bacterial cell. The availability of calcium and dissolve inorganic carbon in the microenvironment precipitated calcium carbonate outside the cell by following equation (Castro et al. 2016) (Fig. 14.3; Table 14.1).

$$CO(NH_2) + H_2 \longrightarrow NH_2COOH + NH_3$$

$$NH_2COOH + H_2O \longrightarrow NH_3 + H_2CO_3$$

$$H_2CO_3 \longrightarrow HCO_3^- + H^+$$

$$2NH_3 + 2H_2O \longrightarrow 2NH_4^+ + 2OH^-$$

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \longrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$$

$$Ca^{2+} + \text{Cell} \longrightarrow \text{Cell} - Ca^{2+}$$

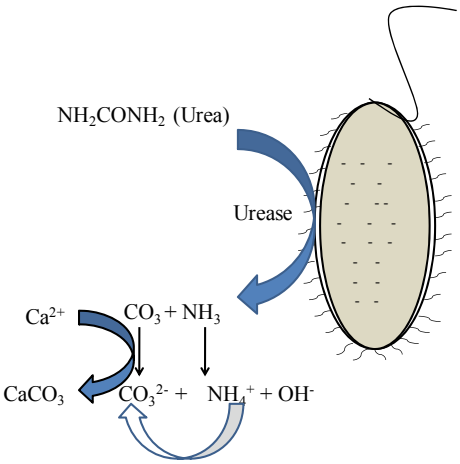

Fig. 14.3 Schematic diagram of carbonate formation by urease activity

Table 14.1 Bacterial carbonic anhydrase and role in bicarbonate precipitation

CA source	Isolation site	Calcite precipitation	References
Bacillus sp.CR2	Mine tailing soil	2.32 mg/cell mass (mg)	Achal and Pan (2011)
Lysinibacillus sp. strain LHXY2	Karst cave	980 mg/100 ml	Lü et al. (2019)
B. pasteurii NCIM 2477	Culture	–	Sarada et al. (2009)
Bacillus and *Virgibacillus*	Desalination plant	–	Silva-Castro et al. (2015)
B.megateriumSS3	Calcareous soil	187 mg/100 ml	Dhami et al. (2013)
B.turiniensis	Calcareous soil	167 mg/100 ml	Dhami et al. (2013)
Bacillus sp. strain (5C-1)	Karst cave	–	Wang et al. (2010)
Citrobacter freundii	Himalayan rocks	230 mg CaCO3/mg of purified CA	Giri et al. (2018)
Pseudomonas spp.	Himalayan rocks	–	Giri and Pant (2019b)

$$\text{Cell} - \text{Ca}^{2+} + \text{CO}_3^{2-} \longrightarrow \text{Cell CaCO}_3$$

In general, enzyme carbonic anhydrase and urease play an important role in production in carbonate by biomineralization. Biocementation and biodeposition are also new emerging technologies which offer a cost effective, environmentally sound, and appropriate alternative on the conventional construction industry. Furthermore, the use of these technologies promises to reduce CO_2 emissions and mitigate the effects of climate change.

14.2.3.1 Conversion of CO_2 to Other Chemicals

Beside CA, there are several other types of carboxylases in cells and have also been explored to catalyze the carboxylation of raw materials and have potential to other biocatalytic applications including: carboxylation of epoxide, aromatic compounds, hetro-arometic compounds, and aliphatic substrate (Glueck et al. 2010). The enzymatic carboxylation reactions derived mainly from catabolic pathways with potential for biocatalytic applications given. The reactions are categorized into four classes, by according to substrate type:

Biocarboxylation of Epoxides

A novel enzymatic reaction has been investigated involving the metabolism of aliphatic epoxides by *Xanthobacter* strain Py2 (Allen and Ensign 1996). The pathways of epoxy degradation seem to be regulated by the presence or absence of CO_2. In the absence of CO_2, propene-grown *Xanthobacter* Py2 catalyze the isomerization of aliphatic epoxides. On the other hand in the presence of CO_2, the carboxylation reaction of epoxide formed acetoacetate and beta-hydroxybutyrate (Glueck et al. 2010; Shi et al. 2015). Both CO_2 dependent and no-dependent pathways were reported to be dependent on NAD^+. A novel type NADPH-dependent pyridine nucleotide-disulfide oxidoreductase is very essential protein for epoxide degradation (Swaving et al. 1996).

Biocarboxylation of Aromatic (Phenolic) Compounds

The second route of carboxylation reaction is also called aromatic carboxylation. Initially, partially purified phenylphosphate enzymes from *Thauera aromatic* were used in the carboxylation of phenol in presence of CO_2 to synthesize p-hydroxybenzoic acid under ambient conditions (Aresta Dibenedetto 2002). The metabolism of aromatic (phenolic) compounds in aerobic bacteria is commonly proceeding via oxidation using molecular oxygen as a co-substrate. The anaerobic carboxylation in *Thauera aromatic* proceeds via involving two enzyme system (i) phenylphosphate synthase (ATP-dependent activation of phenol to phenylphosphate) and (ii) regioselective (para-)carboxylation of the activated intermediate to p-hydroxybenzoic acid by metal (Mg^{2+}, Mn^{2+} and K^+)-dependent phenylphosphate carboxylase. The divalent metal ion in enzyme acts as a Lewis acid by increasing the electrophilic nature of CO_2. Acetyl-CoA is the final product of aromatic (phenolic) degradation by oxygen-sensitive phenylphosphate synthase (Glueck et al. 2010). The first example of biotechnological application of a carboxylase was phenol to p-hydroxybenzoic acid at ambient temperature and pressure (Aresta et al. 1998). 4-hydroxybenzoate decarboxylases purified from *Enterobacte spp.*, *Clostridium hydroxybenzoicum,* and *Chlamydophila pneumonia* can also catalyze phenol in the presence of CO_2 to yield 4-hydroxybenzoate. Similarly, 3,4-dihydroxybenzoate

decarboxylase from *Clostridium hydroxybenzoicum* can catalyze phenol and catechol in the presence of bicarbonate into 3,4-dihydroxybenzoate (Miyazaki et al. 2001).

Biocarboxylation of Hetero-aromatic Compounds

Pyrrole-2-carboxylate decarboxylase is an important enzyme for hetero-aromatic compounds synthesis. The Pyrrole-2-carboxylate decarboxylase from Bacillus megaterium PYR2910 can be used for formation of Pyrrole-2-carboxylate, which is a potential herbicide used in agricultural purposes (Omura et al.1998).

14.2.3.2 Biocarboxylation of Aliphatic Substrates

Pyruvate decarboxylase can be used for enzymatic synthesis of pyruvic acid from acetaldehyde and CO_2, which again converted into lactic acids by using multi-enzyme systems. Lactic acids can also be formed from ethanol and CO_2 which can be used in food, cosmetic, pharmaceutical, and chemical industry (Miyazaki et al. 2001; Tong et al. 2011).

14.3 Future Perspectives and Conclusion

Biocatalysts are generally expensive, stability issue, activity, and reusability, which restrict their optimum use in industrial purposes. Several enzymatic engineering such as chemical modification, genetic engineering, and immobilization need further optimum use of enzyme with economic viable, improving enzymatic activity and stability and reusability (Giri and Pant 2019b). Biocatalysts are needed faster CO_2 transformation for industrial and environmental purposes. Thus, considerable research is required toward the CO_2 management into valuable products by discovery of novel enzyme system as well as enzyme engineering system.

Although, a considerable research should also been focus in novel enzymatic and multi enzymatic technologies for CO_2 conversion into different products. Scientific and technical advances are still needed for link between fundamental and industrial research in CO_2 transformation and mitigation.

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