ENZYME CATALYZED BIOTRANSFORMATION OF CARBON DIOXIDE INTO USEFUL CHEMICALS – A SHORT REVIEW

Kavita Lanjekar[1] and Nikhil Gharat[2]

[1,2]Institute of Chemical Technology, Mumbai, India
Email: kavita.lanjekar@gmail.com

Abstract

Dwindling petroleum feedstocks and increased Carbon dioxide (CO_2) concentrations in the atmosphere currently open the concept of using CO_2 as raw material for the synthesis of well-defined organic compounds. In parallel to recent advances in the chemical CO_2-fixation, enzymatic (biocatalytic) CO_2 conversion is currently being investigated at an increased pace. Enzyme catalytic reactions are found to be environmentally safe catalytic reactions in organic synthesis and, the efficient biocatalytic conversion of Carbon dioxide will help in reducing the greenhouse gas effect and in production of useful chemicals as well. The enzymatic approach for CO_2 conversion has attracted a lot of attention, due to its several advantages compared to conventional processes, such as high yields and selectivity under milder reaction conditions. A detailed search of published reports was done and analyzed in this paper, related to enzyme catalytic conversion of carbon dioxide into useful chemicals.

Keywords: Carbon dioxide, greenhouse gas, enzyme catalysis, FateDH, FaldDH, ADH

I. INTRODUCTION

Enzyme-catalyzed processes have been used industrially for centuries and fermentation processes are well known in history, for example in making wine, beer, bread, and cheese. But, biotransformation differs from fermentation, as it involves the use of particular, often single, enzymes as applied to a particular reactant to give high levels of conversion to a single product [1]. The enzymatic processes can be much faster, cleaner, and easier to operate, as compared to microbial processes. The microbial processes often involve complicated culturing conditions, and in many cases the separation and purification of the products can also be very challenging tasks. [2].

The efficient utilization of carbon dioxide (CO_2) has attracted considerable attention from fundamental research to industrial application in recent years. The efficient utilization of renewable CO_2 will not only help to alleviate greenhouse effect, but also to obtain useful chemicals. Heterogeneous catalysis, electrocatalysis and photocatalysis are presently the three predominant chemical methods for converting CO_2 into some useful chemicals, such as methanol, formic acid and formaldehyde, etc [3-6]. However, these methods suffer from the inherent drawbacks such as, these methods need high temperature and pressure or additional electric or luminous energy and both the selectivity and yields are low. In comparison, the enzymatic approach to convert CO_2 has several advantages such as high yields and selectivity under milder reaction conditions without any significant impact on the environment [7,8].

II. ENZYMATIC CONVERSION OF CO_2

Robyn Obert and Bakul C. Dave (1999) reported a novel and promising approach to convert CO_2 into methanol through consecutive reduction catalyzed by three different dehydrogenases encapsulated in silica gel matrices.

The whole enzymatic reaction consists of three steps:

1. Reduction of CO_2 to formate catalyzed by formate dehydrogenase (FateDH),
2. Reduction of formate to formaldehyde by formaldehyde dehydrogenase (FaldDH), and
3. Reduction of formaldehyde to methanol by alcohol dehydrogenase (ADH).

Reduced nicotinamide adenine dinucleotide (NADH) acts as a terminal electron donor for each dehydrogenase-catalyzed reduction.

$$CO_2 \xrightarrow[NADH]{FateDH} HCOOH \xrightarrow[NADH]{FaldDH} HCHO \xrightarrow[NADH]{ADH} CH_3OH$$

Experimental enzyme stock solution was comprised of 10 mg/mL of each enzyme dissolved in 0.1 M phosphate buffer at pH 7. The reaction mixtures

used in the study were prepared by adding 1.0 mL of the enzyme stock solution to 1.0 mL of NADH solution in a polystyrene cuvette such that the final concentration of NADH varied from 0.025 to 0.1 M. The cuvette was covered with Parafilm (the Parafilm cover was used to prevent extensive loss of methanol produced due to evaporation). The gaseous CO_2 was then bubbled through the solution for 3 h using a small nozzle with an approximate outlet diameter of 0.5 mm through a hole made in the Parafilm. The sol-gel encapsulated enzyme samples were prepared using Tetramethoxysilane (TMOS) as precursor for making the silica sol-gel and it was observed that sol-gels prepared without any of the four components fail to show any production of methanol and in order to generate methanol all four species (i.e. FateDH, FaldDH, ADH, and NADH) must be present. The quantitative measurement of methanol was performed by using gas chromatography (GC) and 91.2% yield of methanol (pH 7 and 37°C) was obtained in the study [8].

The subsequent work on enzymatic production of methanol using CO_2 was carried out using the same scheme.

Hong WU et al (2002) demonstrated the comparative yields of methanol by three dehydrogenases in the solution phase (free enzymes) and in silica sol-gel matrix (immobilized enzymes) using different concentrations of enzymes (i.e 7mg/mL of $F_{ate}DH$, 2mg/mL of $F_{ald}DH$ and 2mg/mL of ADH, dissolved in 0.1M phosphate buffer at pH 7). In this study, Tetraethoxysilane (TEOS) instead of TMOS was used as precursor for making silica gel. CO_2 was bubbled through the solution for 8 h and at 37°C for production of methanol. The concentration of methanol was determined by gas chromatography. The results of 100% yield of methanol in solution phase and 91.9% yield in silica sol-gel matrix were achieved [9].

Zhongyi Jiang et al (2003) studied the effect of different pH values, temperature values on methanol yield with the change in the preparative method of silica sol-gel matrix with lower amount of TEOS (1.94 g) as compared to the earlier method (2.6 g). An optimum pH value found out to be pH 7 and optimum temperature 37°C with 92.1% methanol yield was obtained with reaction time of 8 h [10].

The effects of modification of silica gel and ADH on enzyme activity for enzymatic conversion of CO_2 to methanol was studied by Zhongyi Jiang et al (2004). Two approaches were involved: Immobilization of enzyme in modified gels and, Immobilization of modified enzymes in typical gels. Polyethylene glycol (PEG) was used to modify and to adjust the pore size of immobilization carrier silica gel and SC-PEG [Succinimidyl carbonate derivative of mPEG (methoxy polyethylene glycol)] was used to improve the catalytic property of ADH. The results indicated that PEG modification of the silica matrix and/or the ADH significantly increases the enzymatic activities and the storage stability [11].

The efficient conversion of CO_2 catalyzed by three dehydrogenases that are co-encapsulated in an Alginate-Silica (ALG-SiO_2) hybrid gel was demonstrated by Zhongyi Jiang et al (2006). This approach was attempted in order to modify the sol-gel technique, to stabilize the biological activity, and to solve the problems related to encapsulation in silica sol-gel matrix such as:

1. The necessity for the use of co-solvents and catalysts in the sol-gel process due to low water solubility and reactivity of the silica precursor and,
2. The deleterious effect of alcohol liberated from hydrolysis, co-solvents, and catalysts on bioactivity.

The natural biosilicate formation had inspired innovative routes to design carriers for bioencapsulation and the preparation of alginate-silica composites (biosilicate), through the incorporation of silica particles into alginate gel beads, or through the modification of sol-gel silica with alginate was found to be beneficial. In order to co-encapsulate the three dehydrogenases in the ALG-SiO_2 composite, a 1.47 mL aliquot of TMOS solution was vigorously mixed with 4 mL of alginate solution for 5 min, followed by mixing with 1 mL of 0.05 M, pH 7.0 tris-HCl buffer that contained FateDH (4.5 mg), FaldDH (4.5 mg), and ADH (1.0 mg). This mixture was added dropwise into 20 mL of a 0.2 M $CaCl_2$ solution. The biocomposite beads were cured in the $CaCl_2$ solution for 30 min and then were removed for further use. CO_2 was then bubbled with the pressure being maintained at 0.5 MPa and at 37°C. The tubular reactor, with an outer diameter of 2.2 cm, an inner diameter of 1.5 cm, and a height of 15.8 cm

was used. The enzymatic reaction lasted 8 h for the sufficient production of methanol. The methanol yield in ALG-SiO_2 hybrid gel was 98.1%. The significantly improved catalytic properties of the dehydrogenases in the ALG-SiO_2 composite were attributed to less enzyme leakage and creation of the appropriate immobilizing microenvironment such as: High hydrophilicity, moderate rigidity and flexibility, ideal diffusion characteristics and optimized cage confinement effect [12].

Zhongyi Jiang et al (2009) reported the green and efficient conversion of CO_2 to methanol by Biomimetic Coimmobilization of three dehydrogenases in Protamine –Templated Titania. In this study, three dehydrogenases were encapsulated in titania particles through a facile biomimetic titanification. The immobilization process was induced by protamine, and the inorganic phase was formed under mild and ecofriendly conditions. Compared to silica, titania-based materials showed excellent pH stability, superior mechanical strength, and biocompatibility. The biomimetic preparation of titania containing encapsulated dehydrogenases was conducted by the following procedures: the mixture consisting of 0.5 mL of dehydrogenases stock solution (containing FateDH 4.5 mg, FaldDH 4.5 mg, and ADH 1.0 mg), 0.5 mL of protamine solution (A stock solution of protamine 20 mg/mL was prepared in 0.05 M Tris-HCl buffer solution), and 1 mL titanium (IV) bis(ammonium lactato) dihydroxide solution was prepared and agitated for 5 min at room temperature, the resultant enzyme-containing titania particles were recovered by centrifugation for 5 min (3000 r/min) and then washed three times with deionized water. Under optimal conditions (35°C and pH 7.0), the yields of methanol ranged from 35% to 60% in the co-immobilization system with reaction time of 8 h [13].

A novel biopathway to convert CO_2 into formic acid was explored by Zhongyi Jiang et al (2006). Formate dehydrogenase (FateDH) was used as the biocatalyst and reduced nicotinamide adenine dinuncleotide (NADH) as the terminal electron donor.

$$CO_2 + NADH \xrightarrow{\text{Fate DH}} HCOOH + NAD^+$$

To increase the enzyme stability and reusability, to reduce the enzyme cost, and facilitate the sub sequential purification, the enzyme FateDH was encapsulated in a novel alginate–silica (ALG–SiO_2) hybrid gel. This hybrid gel was prepared by in situ hydrolysis and polycondensation of tetramethoxysilane (TMOS) in alginate solution followed by gelation of alginate with Ca^{2+}.

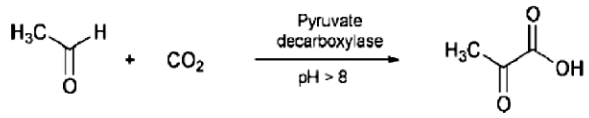
FateDH was immobilized using pure alginate gel beads and hybrid ALG–SiO_2 gel beads, and the results of comparative study showed that, the leakage of the enzyme was significantly reduced by hybridization as compared to pure alginate.

1. The gelation of TMOS was accelerated by alginate and the cross-linking of silica with alginate matrix leads to a compact and porous composite, which had good diffusion characteristics.

2. There was a more homogeneous distribution of silica in alginate matrix and combination of high stability of the mineral with better compatibility of alginate.

3. The enzyme leakage was effectively minimized. The enzyme activity was better retained and enzyme lifetime is increased.

The recycling stability of immobilized FateDH both in pure and hybrid alginate gels were tested for 10 successive batch reactions at 37°C and the activities of the first batch were taken to be 100%. It was observed that FateDH immobilized in hybrid ALG–SiO_2 gels retained about 69% of its activity after 10 cycles, while that in alginate gel decreased almost to zero and it was mainly due to the lower leakage of enzyme in early stage of the activity assay and well-retained conformational feasibility of enzyme. The optimum reaction condition was found to be at pH 7.0 and 37°C, under these conditions, the highest yield of formic acid catalyzed by the immobilized FateDH was up to 95.6%, only a little lower than that of the free form enzymatic reaction (98.8%) with reaction time of 8 h [14].



Hideaki Maeda et al (2001) demonstrated the usefulness of the reverse reaction of pyruvate decarboxylase in the production of pyruvic acid from acetaldehyde and carbon dioxide.

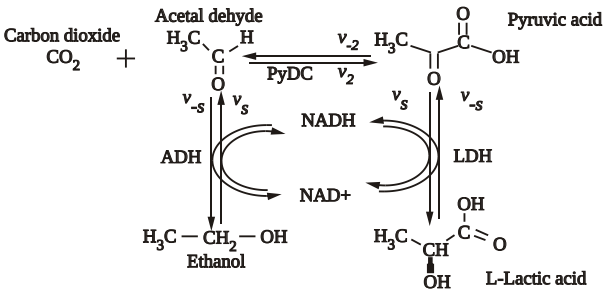
Pyruvate decarboxylase is known as a catalyst of the decarboxylation reaction of pyruvic acid, to produce acetaldehyde and this enzyme requires thiamin pyrophosphate as a coenzyme for catalytic activity. The reverse reaction of this enzyme is of interest as a catalytic procedure for carboxylation. In this study, the carboxylation of acetaldehyde using the reverse reaction of pyruvate decarboxylase, without the use of organic solvents, was studied in detail.

Brewer's yeast pyruvate decarboxylase (1 unit) and thiamin pyrophosphate were added at 4°C to a solution of acetaldehyde (100 mM) in sodium bicarbonate buffer (1 ml) in a 1.5 ml microcentrifuge tube. The reaction mixture was warmed to 25°*C* quickly, and then shaken on a vortex mixer at room temperature. After 1 h, the reaction mixture was chilled on ice, and then subjected to HPLC analysis immediately. The amount of pyruvic acid was calculated from the peak area of HPLC analysis calibrated with commercially available pyruvic acid standards. The yield was estimated based on acetaldehyde.

The effects of concentration of bicarbonate buffer and, effect of pH on the reaction were evaluated. The maximum yield of the reaction, at 500 mM $NaHCO_3$–$Na2CO_3$ buffer and pH 11 was 81% [15].

A novel multienzyme reaction system, with a designed internal cofactor regeneration loop for production of L-lactic acid (an important building block for synthesis biodegradable polymers) from carbon dioxide and ethanol was reported by Xiaodong Tong et al (2011). A triad catalytic system was investigated in this work for conversion of carbon dioxide and ethanol with cofactor regeneration achieved within the synthetic loop, without requiring additional chemical for cofactor regeneration. The process consists of

1. The synthesis of pyruvate from carbon dioxide and acetaldehyde catalyzed by pyruvate decarboxylase (PyDC) and,
2. Subsequent reduction of pyruvate to lactate by lactate dehydrogenase (LDH).
3. The regeneration of the cofactor NADH needed for reduction of pyruvate was achieved through the oxidation of ethanol catalyzed by alcohol dehydrogenase (ADH).

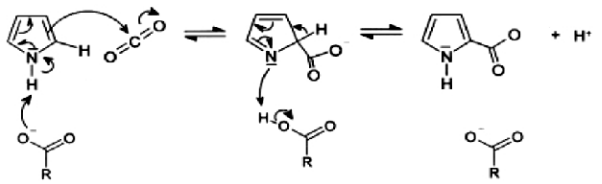


Reactions were conducted by purging CO_2 into a buffer solution (pH of 9.5, Sodium carbonate and sodium bicarbonate) containing ADH, PyDC, and LDH (with a concentration of 0.25 mg/mL for each enzyme). It was found that the concentrations of the lactate product kept increasing during the 12 h reaction time, with the same amount of ethanol reduced concurrently and, the two reaction intermediates, acetaldehyde and pyruvate, maintained relatively stable throughout the course of reaction. However, the concentration of pyruvate was maintained at a much lower level than acetaldehyde, indicating the rate of production of pyruvate was a limiting step in the reaction system.

In this study, a kinetic model was developed based on reaction kinetic parameters determined separately for each reaction step and it predicted well the reaction rates, and yields of the multienzyme reaction system. Lactate was successfully synthesized with 41% of ethanol converted in a batch reaction, while a turnover number of 2.2 day^{-1} was reached for cofactor regeneration in a reaction with continuous feeding of ethanol [2].

Toru Nagasawa et al (2001) demonstrated the synthesis of Pyrrole-2-carboxylate from carbon dioxide and pyrrole using reverse reaction of Pyrrole-2-carboxylate decarboxylase enzyme. Pyrrole-2-carboxylate decarboxylase was purified from *B. megaterium* PYR2910 and the reverse reaction was performed at 20°C in a tightly closed reaction vessel containing potassium phosphate buffer (pH 5.5), ammonium acetate, pyrrole, dithiothreitol, enzyme and $KHCO_3$. A pH 5.5 buffer was used to counteract the pH increase by the HCO_3^- addition and during

purification and storage of the enzyme, dithiothreitol was found to stabilize the enzyme. The optimum temperature and pH for CO_2 fixation were 45°C and pH 7.0, respectively.



Due to an equilibrium constant of 0.3–0.4 M, the decarboxylase enzyme also catalyzes the reverse carboxylation of pyrrole after the addition of bicarbonate and for the synthesis of pyrrole-2-carboxylate, the reverse reaction was optimized and the equilibrium shifted towards the carboxylate. For highest carboxylation yields, saturating amounts of 3 M $KHCO_3$ were used leading to a shift of the reaction equilibrium towards the carboxylate. HCO_3^- addition was accompanied by CO_2 gas evolution resulting in an increased pressure in the tightly closed reaction vessel of 1.38 atmospheres which supported the reverse reaction productivity 2.5-fold compared to atmospheric pressure. As biocatalyst, either concentrated cells with an optical density at 610 nm of 40, previously grown under inducing conditions, or the purified enzyme, both in a concentration corresponding to 100 units enzyme activity/ml, were employed. Additionally, acetate as enzyme cofactor and L-ascorbate as anti-oxidizing, enzyme protecting agent was added to the reaction mixture. For maximal CO_2 fixation rates, 300 mM pyrrole was optimal. Higher pyrrole concentrations inhibited the enzyme. The product yield was 230 mM (25.5 g/l) pyrrole-2-carboxylate from 300 mM pyrrole in a batch reaction and 325 mM (36.1 g/l) from 400 mM pyrrole in a fed batch reaction. The yield after bioconversion was 80%, limited by the equilibrium and the overall yield after isolation was 52% [16].

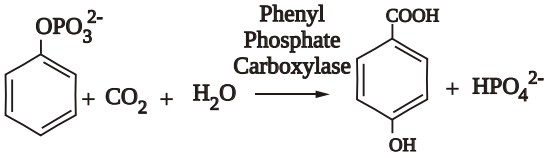
Michele Aresta et al (2006) reported a new biotechnological synthetic approach for production of 4-hydroxybenzoic acid using phenylphosphate carboxylase enzyme, extracted from Thaurea aromatica, that selectively carboxylates phenylphosphate in the para position, without any formation of the ortho isomer. They showed that the enzyme also works in supercritical carbon dioxide.

The carboxylation of phenol in the strict anaerobe Thauera aromatica proceeds via a two-enzyme step involving the

1. ATP-dependent activation of phenol to phenylphosphate mediated by phenylphosphate synthase, followed by
2. regioselective (para-) carboxylation of the activated intermediate to p-hydroxybenzoic acid by phenylphosphate carboxylase.

In previous study, Michele Aresta et al (1998) discovered that 4-OH benzoic acid can be synthesized from phenol and CO_2 at room temperature and, sub-atmospheric pressure of CO_2 with 100 % selectivity, using a Carboxylase enzyme in water medium [7]. But, the use of aqueous media resulted in complex recovery of the product of reaction. In order to have a simpler system, Michele Aresta et al (2005) tried the use of an organic solvent, such as acetonitrile, but the activity of the enzyme was very low because of de-activation. Therefore, sc-CO_2 was used as solvent and reagent, and it has been found that, the enzyme performs the same function encountered in the water system and showed the activity comparable to an aqueous medium.

It was the first report on a carboxylase enzyme functioning in a supercritical fluid to give a C–C bond formation and using directly carbon dioxide as reagent.



Bacteria of Thauera aromatica were grown in anaerobic conditions on phenol as substrate to prepare the cellular extract and, the semipurified cellular extract was then used in the carboxylation assays. The activity of the enzyme depends on K^+ and Mn^{2+} hence, $MnCl_2$, KCl, and imidazole-HCl (pH 7) were used along with the enzyme. The divalent metal ion was supposed to act as a Lewis acid by increasing the electrophilic character of CO_2, whereas K^+ was assumed to support the regioselectivity in close analogy to the chemical process.

The reaction was carried under CO_2 pressure (25.0 MPa) at 308 K for 4 h and a 4.2% conversion

of Phenylphosphate into 4-hydroxybenzoic acid (compared to 7% conversion in water medium) was obtained. The formation of 4-hydroxybenzoate was determined spectrophotometrically, followed by HPLC analysis at 254 nm using reverse phase C-18 column [17].

Scott A. Ensign et al (1997) purified and characterized Acetone carboxylase enzyme from an aerobic bacterium *Xanthobacter* strain Py2. This study reveals the molecular properties of the first bacterial acetone-metabolizing enzyme to be isolated and suggested a novel mechanism of acetone carboxylation to form acetoacetate, coupled with ATP hydrolysis and, AMP and inorganic phosphate formation.

$$\text{acetone} + CO_2 \rightarrow \text{acetoacetate} + \text{AMP} + 2P_i$$

The physiological function of acetone carboxylase is to convert acetone, a toxic and recalcitrant organic molecule, to acetoacetate, which is a central metabolite. Acetone carboxylase exhibited an obligate requirement for ATP as a cofactor. Acetone carboxylation is a thermodynamically unfavourable process and the hydrolysis of ATP to ADP would provide sufficient energy to drive the carboxylation reaction. During the course of acetone carboxylation, AMP and inorganic phosphate form as the products of ATP hydrolysis. Acetone carboxylation presumably involves nucleophilic attack of the carbanion of acetone on CO_2 (or bicarbonate). The carbanion might be formed by general base abstraction of a proton, but would be hard to generate and highly unstable due to the high pKa of the methyl group. The carbanion could be stabilized by keto to enol tautomerization, and the enol tautomer could be further stabilized by the transfer of a phosphoryl (or other) group from ATP to the oxygen atom of the enolate. Nucleophilic attack of the enol on CO_2 (or bicarbonate) with concomitant hydrolysis of the oxygen–phosphate bond would result in the formation of acetoacetate.

It was found that Acetone carboxylase was comprised of three polypeptides with molecular weights of 85,300, 78,300, and 19,600 arranged in an $\alpha_2 \beta_2 \gamma_2$ quaternary structure, and Acetone carboxylase exhibited a *V*max for acetone carboxylation of 0.225 %mol acetoacetate formed $min^{-1}mg^{-1}$ at 30°*C* and pH 7.6 and apparent *K*m values of 7.80 μM (acetone), 122 μM (ATP), and 4.17 mM (CO_2 plus bicarbonate) [18]. For an industrial application, Acetoacetate produced using Acetone carboxylase enzyme, can be converted to 3-hydroxybutaoate (a building block for formation of biodegradable plastic) by using 3-hydroxybutyrate dehydrogenase enzyme.

Another important work on Acetone Carboxylase is done by Stephen J. Birks and David J. Kelly (1997). They described an assay for acetone carboxylation, in which fixation of radiolabelled carbon dioxide from $NaH^{14}C0_3$ is measured in the presence of acetone, ATP, magnesium ions and acetyl-CoA. Acetone carboxylase activity was specifically induced by growth of Rhodobacter *capsulatus* on acetone or butanone and was associated with a high-molecular-mass protein complex containing two major polypeptides, of 70 and 85 kDa. Partial purification of the activity was achieved by FPLC ion-exchange chromatography, which confirmed that the 70 and 85 kDa proteins were subunits of the enzyme and suggested that at least one additional protein (60 kDa) may be associated with carboxylase activity. Acetone carboxylase activity was also demonstrated in cell-free extracts of acetone grown *Rhodomicrobium vannielii* and the denitrifying bacterium *Thiosphaera pantotropha* [19].

III. CONCLUSION

CO_2 issue is truly global and thus people and governments worldwide should seriously consider supporting research and industrial commitments on CO_2 conversion and utilization, as well as international collaborative research involving developing countries. The enzyme catalytic utilization of Carbon dioxide provides an eco-friendly way for production of carbon-based chemical products. However, the literature survey indicates that most of the experimental works were carried out on laboratory scale. There are several advantages of enzymatic conversion such as high selectivity, high yield, less amount of waste, milder reaction conditions. But, they do possess some drawbacks such as high cost of enzymes and cofactors, longer reaction times as compared to conventional methods etc. It would be interesting and also of great importance to discover the new enzymatic reaction pathways using different enzymes for Carbon dioxide utilization and new ideas to overcome the drawbacks related to enzyme catalysis.

REFERENCES

[1] J.T. Sime and G.B. Kauffman, 1999, Applications of Biocatalysis to Industrial Processes, Journal of Chemical Education, Vol. 76, No. 12, 1658.

[2] X. Tong, B. Zahab, X. Zhao, Y. Liu, P. Wang, 2011, Enzymatic Synthesis of L-Lactic Acid From Carbon Dioxide and Ethanol with an Inherent Cofactor Regeneration Cycle, Biotechnology and Bioengineering, Vol. 108, No. 2, 465-469.

[3] S.W. Park, O.S. Joo, K.D. Jung, H. Kim, S.H. Han, 2001, Development of ZnO/Al_2O_3 catalyst for reverse-water-gas-shift reaction of CAMERE (carbon dioxide hydrogenation to form methanol via a reverse-water-gas-shift reaction) process, Applied Catalysis A: General 211, 81.

[4] M. Azuma, K. Hashimoto, M. Hiromoto, 1990, Electrochemical Reduction of Carbon Dioxide on Various Metal Electrodes in Low-Temperature Aqueous $KHCO_3$ Media, Journal of The Electrochemical Society 137, 1772.

[5] M. Subrahmanyam, S. Kaneco and N. Alonso-Vante, 1999, A screening for the photo reduction of carbon dioxide supported on metal oxide catalysts for C_1–C_3 selectivity, Applied Catalysis B: Environmental 23, Issue 2-3, 169-174.

[6] S. Kuwabata, K. Nishida, R. Tsuda, H. Inoue, H. Yoneyama, 1994, Photochemical Reduction of Carbon Dioxide to Methanol Using ZnS Microcrystallite as a Photocatalyst in the Presence of Methanol Dehydrogenase, Journal of The Electrochemical Society vol 141, issue 6, 1498-1503.

[7] M. Aresta, E. Quaranta, R. Liberio, C. Dileo and I. Tommasi, 1998, Enzymatic synthesis of 4-OH-benzoic acid from phenol and CO_2: the first example of a biotechnological application of a Carboxylase enzyme, Tetrahedron 54, issue 30, 8841-8846.

[8] B.C. Dave and R. Obert, 1999, Enzymatic Conversion of Carbon Dioxide to Methanol: Enhanced Methanol Production in Silica Sol-Gel Matrices, Journal of the American Chemical Society, 121, 12192.

[9] Z. JIANG, Hong WU, Songwei XU, Shufang HUANG, 2002, Enzymatic Conversion of Carbon dioxide to Methanol by Dehydrogenases encapsulated in Sol-gel Matrix, Fuel Chemistry Division Preprints, 47(1), 306.

[10] Hong WU, Z. JIANG, Song Wei XU, Shu Fang HUANG, 2003, A New Biochemical Way for Conversion of CO_2 to Methanol via Dehydrogenases Encapsulated in SiO_2 Matrix, Chinese Chemical Letters Vol. 14, No. 4, 423 – 425.

[11] Hong Wu, Shufang Huang, Zhongyi Jiang, 2004, Effects of modification of silica gel and ADH on enzyme activity for enzymatic conversion of CO_2 to methanol, Catalysis Today 98, 545–552.

[12] Song-wei Xu, Yang Lu, Jian Li, Zhong-yi Jiang and Hong Wu, 2006, Efficient Conversion of CO_2 to Methanol Catalyzed by Three Dehydrogenases Co-encapsulated in an Alginate-Silica (ALG-SiO2) Hybrid Gel, Industrial & Engineering Chemistry Research, 45, 4567–4573.

[13] Qianyun Sun, Yanjun Jiang, Zhongyi Jiang, Lei Zhang, Xiaohui Sun and Jian Li, 2009, Green and Efficient Conversion of CO_2 to Methanol by Biomimetic Coimmobilization of Three Dehydrogenases in Protamine-Templated Titania, Industrial & Engineering Chemistry Research, 48 (9), 4210–4215.

[14] Yang Lu, Zhong-yi Jiang, Song-wei Xu and Hong Wu, 2006, Efficient conversion of CO_2 to formic acid by formate dehydrogenase immobilized in a novel alginate–silica hybrid gel, Catalysis Today 115, 263–268.

[15] Masaya Miyazaki, Mitsukuni Shibue, Kazuya Ogino, Hiroyuki Nakamura and Hideaki Maeda, 2001, Enzymatic synthesis of pyruvic acid from acetaldehyde and carbon dioxide, Chemical Communications, 1800–1801.

[16] Marco Wieser, Toyokazu Yoshida, Toru Nagasawa, 2001, Carbon dioxide fixation by reversible pyrrole-2-carboxylate decarboxylase and its application, Journal of Molecular Catalysis B: Enzymatic 11, 179–184.

[17] Angela Dibenedetto, Rosa Lo Noce, Carlo Pastore, Michele Aresta and Carlo Fragale, 2006, First in vitro use of the phenylphosphate carboxylase enzyme in supercritical CO_2 for the selective carboxylation of phenol to 4-hydroxybenzoic acid, Environmental Chemistry Letters 3: 145–148.

[18] Miriam K. Sluis and Scott A. Ensign, 1997, Purification and characterization of acetone carboxylase from *Xanthobacter* strain Py 2, Proceedings of the National Academy of Sciences USA, Vol. 94, 8456–8461.

[19] Stephen J. Birks and David J. Kelly, 1997, Assay and properties of acetone carboxylase, a novel enzyme involved in acetone-dependent growth and CO_2 fixation in *Rhodobacter capsulatus* and other photosynthetic and denitrifying bacteria, Microbiology, 143, 755-766.

Ms. Kavita Lanjekar Junior Research Fellow (JRF) M.Tech Green Technology, Institute of Chemical Technology (I.C.T.), Mumbai, India.