

Applicability of carboxydotrophic bacterial carbon monoxide dehydrogenase (CODH) in carbon sequestration and bioenergy generation

Ashima Anand and T Satyanarayana*

Department of Microbiology, University of Delhi, South Campus, New Delhi 110 021

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This review presents applicability of carboxydotrophic bacterial carbon monoxide dehydrogenase (CODH) in carbon sequestration and bioenergy generation. CODH catalyzes reversible conversion of CO₂ to CO. These bacteria use CO and CO₂ as source of energy and carbon for growth, and therefore, play a vital role in maintaining very low CO level in the environment. The enzyme finds application in detection and mitigation of CO levels, and also to produce bioenergy from CO₂.

Keywords: Bioenergy, Carbon dioxide (CO₂), Carbon monoxide (CO), Carboxydotrophic bacteria, Carbon monoxide dehydrogenase (CODH)

Introduction

Carbon monoxide (CO) is naturally produced by human body as a signalling molecule, and thus may play a role as a neurotransmitter or a blood vessel relaxant¹. High levels of CO are poisonous and toxicity arises due to its affinity towards transition metals such as iron (Fe) present in the heme and cytochrome oxidase. CO is attracted to haemoglobin about 200 times stronger than oxygen [CO + Hb → HbCO (carboxyhemoglobin)], and thus, unable to deliver oxygen to the tissues; this condition is known as toxaemia. CO levels²⁻⁶ in different locations varies considerably (Table 1). Exposure to CO (> 100 ppm) can be dangerous to human health, because it readily binds to haemoglobin in red blood cells and can cause rapid and lethal toxaemia, that may result in seizures, coma and, if untreated, can be fatal. Workers in enclosed conditions in coal mines and in transport and sewage treatment can suffer increased exposure to CO. Use of tobacco is the major source of CO in a closed area. Presence of a special class of microorganisms (carboxydotrophs) in environment is responsible for CO to be present in trace amounts (0.06-0.15 ppm) in atmosphere. Several bacteria in environment possess an enzyme carbon monoxide dehydrogenase (CODH) that plays a role in utilizing CO and CO₂ as source of energy

and carbon for growth. This enzyme can be used for converting highly toxic CO to innocuous CO₂, and in the conversion of CO₂ to CO. Possibility of converting CO to hydrocarbons (ethane, propane and butane) has been shown using vanadium nitrogenase from *Azotobacter vinelandii*². This review presents role of carboxydotrophic bacteria in nature, and potential applications of CODH.

Carboxydotrophic Bacteria

Carboxydotrophic bacteria are capable of oxidizing CO to CO₂. This interconversion between CO and CO₂ is catalyzed by the presence of enzyme CODH (CO + H₂O ↔ CO₂ + 2H⁺ + 2e⁻). Carboxydotrophs can grow at elevated levels of CO (> 100 ppm)⁷. Two major groups of microbes (aerobes and anaerobes) are present; hence there is a difference in CODH enzyme. Bacterial strains devoid of CODH cannot grow with CO or CO₂ and H₂.

Aerobic Carboxydotrophs

Aerobic CO users are able to oxidize CO to CO₂ using CODH enzyme and present in diverse environments. Majority of CO oxidizers are thermophiles, capable of survival and growth at high temperatures (50-122°C). In hot climates, mainly volcanic eruptions, CO levels are high (0.6-5540 ppm)⁸. Among thermophilic and aerobic carboxydotrophs, *Bacillus schlegelli* grows on CO very fast with a doubling time of 3 h, optimally at 65°C, while no growth below 50°C, and CODH belongs

*Author for correspondence
E-mail: tsnarayana@gmail.com

Table 1—CO levels in different locations and its effect on human body system

Location	CO conc., ppm	Effect of CO poisoning on body system ⁶
Atmospheric level	0.1 ³	Neurologic headache, dizziness (mild), seizures, coma, CNS depression (severe)
Gas stoves, vehicle emission, solid waste disposal	5-15 ⁴	Cardiac decreases myocardial function and chest pain
Automobiles exhaust	100-200 ⁵	Metabolic-acidosis
Wood fire exhaust	5000 ⁴	Pulmonary-edema in 10-30% cases
Car exhaust without catalytic convertors	7000 ⁴	Multiple organ failure due to high exposure of CO.

to molybdenum (Mo) hydroxylase family, as growth is Mo dependent⁹. *Streptomyces thermoautotrophicus* (mycelial carboxydrotroph) is isolated from compost, and capable of growth on CO, and inactivation of CODH enzyme by methanol suggested that it also belongs to Mo hydroxylase family¹⁰. *Pseudomonas carboxyhydrogena*¹¹, *P. thermocarboxydovorans*¹¹, and *Oligotropha carboxydovorans*¹² belong to the class of proteobacteria, isolated from sewage (wastewater) and compost. Several species of *Mycobacterium* possess CODH activity. *Mycobacterium* is the causative agent of serious diseases in human beings and mammals such as *M. tuberculosis* causes TB (tuberculosis), *M. leprae* causes leprosy, *M. bovis* causes bovine TB, and *M. marinum* causes opportunistic infections. Most of the *Mycobacterium* spp. can grow and utilize CO and methanol as a source of carbon and energy, and in contrast, *M. tuberculosis* does not use methanol. Also, enzymes for RuBisCO cycle and C1 assimilation pathway are present¹³. Humans contain CO (1-5 ppm) in respiratory gases that prove to be beneficial for the growth of *M. tuberculosis* to survive as tubercle bacilli inside the host. *Mycobacterium* spp. also possesses CODH along with NODH (nitric oxide dehydrogenase) activity¹⁴.

Several microorganisms are inhabitants of marine environment (oceans and other brackish water bodies). CO oxidizers are also present in marine habitats. Concentration of CO is highest in surface water but decreases with the fading of light in water. Among marine carboxydrotrophic microbes, *Silicibacter pomeroyi*¹⁵ is aerobic and gram negative, uses CO as an energy source, and CO oxidation continues in marine waters. *Stappia* spp. (*Rhodobacteraceae* family) is aerobe, gram negative and capable of utilizing CO. All *Stappia* spp. oxidize CO at <1000 ppm (0.1%)¹⁶.

Plant symbiotic bacteria (*Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*) and many other bacterial species, belonging to Rhizobiales, have ability to oxidize CO. *Bradyrhizobium*, a gram negative soil bacterium, maintains symbiotic association with soybean. *Rhizobia* fix nitrogen during symbiosis with legumes. CO is produced by plants in roots and nodules, and is utilized by bacteria with the help of CODH. CO presence can, however, inhibit nitrogenase activity as CO binds to leghaemoglobin (leg Hb), thereby preventing binding of oxygen to legHb, and thus the presence of oxygen will interfere with nitrogenase activity¹⁷. Soil CO oxidizers, a large group of aerobic carboxydrotrophs, belong to this category. *Arthrobacter 11/X*¹¹, *Nocardia salmonicolor*¹⁸ and *Pseudonocardia carboxydivorans*¹⁹ strain Y8^T and gram positive actinobacteria isolated from soil contain Mo – CODH.

Anaerobic Carboxydrotrophs

Obligate anaerobes (methanogens) use CODH for reduction of CO₂ to CO in CO₂ fixation. Thermophiles (*Carboxydotherrmus hydrogenoformans*), isolated from hot swamp mud of Kunashir islands in Russia, optimum growth at 68°C²⁰, grows well on CO. This bacterium contains CODH along with acetyl CoA synthetase (ACS)⁸. *C. hydrogenoformans*, a strict anaerobic thermophile, utilizes CO and converts it into CO₂ and H₂, while methanogens (Archaea) produce CO under certain conditions. Thus transfer of CO among species is an example of mutualistic association²¹. Various other carboxydrotrophic microorganisms isolated from thermal vents include *Thermosinus carboxydivorans*, a hyperthermophile, which exhibits high growth yields on CO; *Carboxydocella thermoautotrophica*²² and *Carboxydothrachium pacificum*²⁴ have also been isolated from thermal deep vents. Methanogens that are

capable of CO utilization include *Methanopyrus kandleri*, a hyperthermophile, isolated from black smoker of the Gulf of California, grows at 84-110°C, produces methane from CO₂ and H₂²³; *Methanothermobacter thermoautotrophicus*, isolated from sewage sludge, capable of growth on CO, converting it to methane. *Methanosarcina*, *Methanobacterium* and *Methanobrevibacter* can also grow on CO⁸.

Anaerobes such as acetogenic bacteria (*Morella thermoacetica*) possess CODH and use the enzyme in Wood – Ljungdahl pathway for acetate synthesis. A population of bacteria survives in the hindgut of lower termites exhibiting mutualism²⁴. Acetate generated is utilized by the insects and lower termites. CODH plays an important role in maintaining a mutualistic association between bacteria and their hosts as in *Treponema*, a spirochaete. While feeding on lignocellulosic material, termites and insects generate CO₂ and H₂, which are utilized by microbes (gut microflora) with the help of CODH, thereby converting it into acetate, the major source of energy for termites.

Carbon monoxide Dehydrogenase (CODH)

CODH is classified into two groups (aerobic and anaerobic); aerobic CODH is oxygen stable, contains Flavin and Mo, inhibited by methanol, and K_m value is less (53 µM) in *Pseudomonas carboxydovorans*, whereas anaerobic CODH is oxygen labile, contains Ni (nickel), inhibited by potassium cyanide (KCN), and K_m value is high (5 mM) in *Methanosarcina barkerii*. Aerobic CODH belongs to Mo hydroxylase family, and consist of a dimer of heterotrimer. CODH is made up of 3 subunits⁷ [small {Cox S: Fe-S (sulphur) protein}, medium (M: FAD binding protein) and large {L: catalytic Mo cytosine dinucleotide (MCD) binding protein}]. CODH of *Pseudomonas carboxydovorans* is reported to be attached to the inner cytoplasmic membrane, thus enzyme is soluble²⁵. Based on sequence difference, genome organization and catalytic properties, there are two forms of aerobic CODH². Form I (OMP: *Oligotropha*, *Mycobacterium* and *Pseudomonas*) has been characterized for its ability to oxidize CO. Form II (BMS: *Bradyrhizobium*, *Mesorhizobium* and *Sinorhizobium*) is much similar to form I. Cox M and S of form II are entirely same as that of form I but Cox L differs in the active site.

In aerobic CO oxidizers, CODH enzyme carries the conversion of CO to CO₂, which undergoes carbon fixation via Calvin Benson Cycle. Carboxydotrophs

contain genes for RuBisCO enzyme (cbbL). This leads to the fixation of CO₂ into cell biomass. While in anaerobes, CODH is oxygen labile, heat stable and Ni is present at catalytic centre. In Nickel limited cultures, induction of CO leads to accumulation of apo protein (an inactive CODH). Addition of Ni leads to activation of CODH, thus Ni is either involved in catalysis or required for the formation of CODH²⁶. Major role played by anaerobic CODH is synthesis of acetyl CoA from CO using Wood Ljungdahl pathway. In acetogenic bacteria (*Moorella thermoacetica*), acetate is produced while growing on CO as a sole energy source⁸. While in methanogens, methane is produced from CO. *Methanobacterium thermoautotrophicum* and *Methanosarcina barkerii* convert CO ($4\text{CO} + 2\text{H}_2\text{O} = \text{CH}_4 + 3\text{CO}_2$ ²⁷). Anaerobic CODH is of two types²⁷: i) unifunctional CODH with no ACS function, bacteria (*Rhodospirillum rubrum* and *Carboxydotherrmus hydrogenofomans*) utilize CO as source of carbon and energy ($\text{CO} + \text{H}_2\text{O} = \text{CO}_2 + \text{H}_2$); and ii) bifunctional CODH carries out dual role (CODH/ACS function). *Moorella thermoacetica*, an acetogenic bacterium, catalyses synthesis of acetyl CoA via reductive acetyl CoA pathway using Wood-Ljungdahl pathway, thereby synthesizing acetate from acetyl CoA. Attempts have been made to clone and express CODH encoding genes²⁸. Genes for CODH have been cloned and characterised well from gram-negative bacteria (*Pseudomonas thermocarboxydovorans*, *Oligotropha carboxydovorans* and *Hydrogenophaga pseudoflava*). CODH gene has also been cloned from gram-positive *Mycobacterium* sp JC1.

Applications of CO Oxidizing Organisms and CODH

CODH activity has been utilized in biological filters²⁹, based on the principle that CO has a very high affinity for haeme molecule. Haemoglobin can be used to soak up the toxic gas, and thereby, continuously removing CO. These CO filters can be employed in cigarette filters to remove CO by converting it into CO₂. Both CO oxidizing microbes and enzymes can be used. In biofuel cells, an electrical circuit is set up and at anode CODH catalyzing reaction takes place as $\text{CO} \rightarrow \text{CO}_2 + 2\text{H}^+ + 2\text{e}^-$. Protons are transferred to cathode, where it combines with oxygen to form water ($\text{O}_2 + 4\text{H}^+ \rightarrow 2\text{H}_2\text{O}$), while electrons generated are used for producing electrical power. In biosensors, CO based probes are being designed, which can help in CO detection. CO based detectors are important as CO is tasteless and odourless gas, and thus

can go undetected. Syngas (a mixture containing varying amounts of CO and H₂) is used as a fuel in internal combustion engines. *Azotobacter vinelandii*, an important N fixer, contains vanadium nitrogenase, which has been shown to reduce CO to ethylene, ethane, propane and butane².

Conclusions

CODH of carboxydophilic bacteria plays an important role in maintaining atmospheric CO concentration at a safe level. Both aerobic and anaerobic carboxydophilic bacteria have been isolated from soil, volcanic areas and oceans, and represent the major sinks of CO. Majority of CO oxidizers belong to varied physiological metabolic groups (homoacetogens, methanogens and photoautotrophs). Potential use of CODH is in mitigating CO in atmosphere and conversion of CO₂ to syngas and alkanes.

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