

Review Article

<https://doi.org/10.20546/ijcmas.2020.905.157>

Utilizing Bacteria to Mitigate Global Climate Change: A Review

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ABSTRACT

At present, the global climate change is one of the biggest challenges in front of human race which results mainly from global warming due to an increased concentration of green house gases in the atmosphere. Among the major green house gases, Carbon dioxide and methane contribute maximum toward the global warming. To mitigate the global climate change the reduction in emission of green house gases is necessary. Microorganisms play a crucial role in the regulation and utilization of these gases. Bacteria can be employed in various ways to address the problem of global climate change through utilization of atmospheric green house gases. This article will present a comprehensive review of the approaches that can present better solutions to reduce the atmospheric green house gases, mainly, CO₂ and methane by using bacteria. Recent advances in biofuel research, nanodevices and creation of engineered carbon eating bacteria are some of the novel approaches which can open the new doors to mitigate global climate change.

Keywords

Utilizing bacteria,
Global climate
change, Green
house

Article Info

Accepted:
10 April 2020
Available Online:
10 May 2020

Introduction

The global climate change is one of the most debated topics over the last few decades from both scientific and political point of view. Global warming is considered as one of the most significant factor leading to climate change. Global warming refers to an increase in average temperature of earth's surface due to an increased concentration of green house gases (GHGs) in the atmosphere

(Venkataramanan, 2011). Green house effect is the heat retention mechanism facilitated by the GHGs which make earth suitable for the survival of living beings. But the gradual increase in the concentration of GHGs leads to an increase in average surface temperature of earth which results in the global climate change (Anderson *et al.*, 2016). Naturally, GHGs are present in the atmosphere in small quantities. Carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O) and ozone (O₃)

are the major GHGs present in atmosphere. Out of these GHGs, CO₂ has been recognized to play most significant role in global warming (Al-Ghussain, 2018). There are broadly two causes of increasing GHGs in the atmosphere: Natural and Anthropogenic causes. Natural causes like volcanic eruptions, oceanic release, respiration, and decomposition causes slow impact on the climate change as compared to anthropogenic causes. Anthropogenic causes, also known as man-made causes are those which involve the human activities leading to release of GHGs into the atmosphere (Kumar *et al.*, 2012). If we look the contribution of various GHGs in global warming, as per United States Environmental protection agency, CO₂ accounts for 81.6 % of all U S green house gases emissions from human activities followed by methane (10.2%), nitrous oxide (5.6%) and fluorinated gases (<2.6%) in year 2017. Among the sources of CO₂ emission transportation contributes maximum (34%) to CO₂ emission followed by electricity (33%) and industry (15%) (EPA, 2017). The emission of methane is largely contributed by natural gas and petroleum industry (31%) and enteric fermentation (27%) by domestic livestock like cattle, swine, sheep, and goats as a part of their normal digestive process (EPA, 2017). It has been observed that there is a significant increase in CO₂ concentration by about 30% since 1950 (Al-Ghussain, 2018). If we look upon our country, in year 2010, India ranked as the third largest GHG emitter and responsible for 5% of total world CO₂ emissions (Chaturvedi *et al.*, 2014; Nejat *et al.*, 2015). According to National Oceanic and Atmospheric Administration (NOAA), USA, global climate summary, 2019, the combined land and ocean temperature has witnessed an average increase of 0.07°C (0.13°F) per decade since 1880. However the average increase after 1881 is 0.18°C (0.32°F) which is more than twice as great (NOAA, 2019). It is, therefore, important to employ

the mitigation strategies which either reduce the emission or utilize the atmospheric GHGs. The first strategy employs the smart ways of energy and waste management, increasing forest cover, adoption of climate smart agriculture and use of green buildings etc. Over the last few decades several strategies has been devised to reduce atmospheric CO₂ emission and synthetic biology is one of them which renders the synthetic life forms to convert CO₂ into fuel, food and useful organic compounds. Among the biological ways of GHG mitigation bacteria are on the forefront. Recent, it has been found that bacteria can be engineered in a manner to generate biomass from the consumed atmospheric CO₂. Some bacteria have their natural capabilities to utilize the gases like methane.

Microbial metabolism and CO₂ utilization

The microbial metabolism plays a critical role in the development and maintenance of earth's biosphere. Unlike eukaryotic organisms, prokaryotes can metabolize a wide range of organic and inorganic matter ranging from complex organic substances like cellulose to inorganic ions like atmospheric gases and metal ions (Oren, 2010). Microbes utilize these substrates to form the useful metabolic products and biomass production. This metabolic diversity of microbes makes them a potential candidate for the reduction of substances which contribute to global warming especially CO₂ and methane. Prokaryotic microorganisms are the main players of biochemical transformation of carbon, nitrogen, sulfur, iron, and other elements (Lamers *et al.*, 2012). Due to this reason they are extensively used in environmental engineering like in the process of reducing environmental pollution known as bioremediation and water purification (Abatenh *et al.*, 2017). Based on the source of carbon for metabolism microbes can be divided into autotrophs and heterotrophs.

Autotrophs utilize CO₂ as a principal carbon source and form their own food whereas the heterotrophs are those organisms which depend on the preformed carbon sources or on other organisms for their food (Hunt *et al.*, 2018). The autotrophs can be further divided into photoautotrophs (photosynthetic) which make use of light energy and chemoautotrophs (chemosynthetic) which utilize chemical energy. In autotrophs most of CO₂ is assimilated through Calvin cycle, with ribulose biphosphate carboxylase (RuBisCO) as main enzyme (Berg *et al.*, 2002). However, heterotrophic bacteria are more abundant in nature but we are more concerned to autotrophic bacteria because they have the more significant role to play in the reduction of green house gases. The autotrophic bacteria can be effectively utilized for the bioconversion of CO₂ into the desired product with higher selectivity.

Methanogenic Bacteria and GHG sequestration

Methane is another most important GHG responsible for global warming. Methane has relatively shorter atmospheric lifetime or short lived than CO₂ but it has a higher global warming potential than CO₂ as it can absorb more energy than CO₂ (Al-Ghussain, 2018). Methane is produced by the decay of organic matter under anaerobic condition. The major natural sources of methane include wetlands, oceans, paddy cultivation and livestock. Among these sources, enteric fermentation in the ruminants contributes around 17% of the global methane production (Knapp *et al.*, 2014). This fermentation is carried out by methanogenic microbes present in the ruminal compartment of Ruminant animals which includes bacteria (archaea), protozoa and fungi. The unique environment of rumen and lower segment of intestine i.e. rapid passage rate, readily available CO₂ and hydrogen (H₂) help the colonization of certain archaea.

These archaea are different from other anaerobic bacteria in their ability to scavenge CO₂ and H₂ generated by the fermentation process to produce methane (Martin *et al.*, 2010). The other substrates for methanogens include formic acid and methyl amines produced by other ruminal microbes. The common rumen methanogenic species includes *Methanobacterium formicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, *Methanoculleus olentangyi* and *Methanosarcina barkeri* (Janssen and Kirs, 2008). These methanogenic organisms produce methane by utilizing CO₂ and hydrogen. The production of methane from ruminants and its impact on global climate change is gaining the concern day by day. Methane also serves as a major source of energy loss in animals and account for about 2-12% loss of gross energy intake (Johnson and Johnson, 1995). So it is desirable to decrease the methane production by livestock without altering animal production to reduce GHG emission and improving feed conversion efficiency of animals as well. There are various strategies or biotechnologies which can be employed to reduce the ruminal methane production targeting ruminal microflora mainly methanogenic bacteria. These techniques includes immunization or biological control of ruminal methanogens, use of probiotics, removal of protozoa or defaunation, mitigation through feed additives, feeding management and genetic manipulations of ruminal microbes for reduced methane production (Martin *et al.*, 2010; Wright *et al.*, 2004; Morgavi *et al.*, 2008). There are certain genes identified in rumen methanogenic bacteria which are involved in the process of methanogenesis viz. *mtr*, *mta*, *mcr*, *hmd* and *fno* (Patra *et al.*, 2017) The variation in expression of these genes alters the ruminal

ability to produce methane. Certain substances like rice straw extract has been shown to alter the expression level of genes involved in the process of methanogenesis (Faseleh *et al.*, 2013). Another livestock derived source of methane is the ruminant excreta e.g. Cow dung which can be effectively converted to biogas that primarily consist of methane and CO₂. It does not pose severe threat to the climate change as it is consumed as fuel for the household and other purposes. Apart from animal sources, the other sources like wetlands, oceans, rice fields and landfills cause a huge and serious atmospheric emission of methane produced by methanogens which are not much characterized yet (Pandey *et al.*, 2015). So, it is clear that identification, characterization and manipulation of methanogens using biotechnological approaches is necessary to reduce the atmospheric emission of methane.

Role of Soil and Ocean microbes in reducing GHGs emission

Soil microbes play a crucial role to establish ecological equilibrium that sustains the life processes. The important roles that soil microflora play includes decomposition of organic matter, recycling of nutrients, production of trace gases and biotransformation of metals and elements (Gupta *et al.*, 2014). Through these processes soil microbes play a vital role in the production and consumption of GHGs. To utilize the soil microbes in mitigation of GHG production, it is prerequisite to understand their role as contributors and as reactive components of global climate change. The gases produced by human activities can be effectively utilized by soil microbes and neutralize their effect on climate change. Soil serves as a good habitat of vast varieties of microbes which comprise of decomposers, nitrogen fixing and denitrifying bacteria, methanogens, photosynthetic cyanobacteria,

and chemolithoautotrophs etc. (Oertel *et al.*, 2016). Decomposers play an important role in the regulation of global environmental temperature. The process of decomposition largely depends on the atmospheric temperature. As temperature increases and reach to its optimum value, bacteria become more active and perform the decomposition faster releasing more CO₂ into the environment. It has been observed that targeting all GHGs simultaneously for mitigation would be more effective and advantageous than targeting CO₂ alone (Weyant *et al.*, 2006; Aldy *et al.*, 2010). Biological nitrogen fixation (BNF) is an important phenomenon discovered by Beijerinck in 1901, through which molecular nitrogen present in the air is converted into nitrogenous compounds like ammonia (NH₃) in the soil by a group of microorganisms called nitrogen fixing bacteria. These bacteria may be free living or found in association with certain plants like leguminous plants in a symbiotic manner (Kumar and Rao, 2012). Another group of soil microbes called denitrifying bacteria convert a potential GHG, nitrous oxide into nitrogen gas which is harmless. It has been found that this ability is also present in other microorganisms which can be utilized to mitigate the emission of N₂O in the atmosphere. Cyanobacteria are photosynthetic bacteria which play a vital role to reduce the GHG emission mainly from the oceans. *Prochlorococcus* and *Synechococcus* are abundantly found single cell cyanobacteria in marine ecosystem and are reported to remove about 10 billion tons of carbon from the air each year which is about two-third of the total carbon fixation that occurs in the oceans (Willey *et al.*, 2009; Gupta *et al.*, 2014). So an important fact emerged through this finding is that, the inherent or natural capabilities of these microorganisms can be effectively harnessed through the use of biotechnological methods for the reduction of GHGs.

Methylophilum infernorum, an extremely acidophilic methanotrophic aerobic bacteria first isolated and described in 2007, act as a biofilter to reduce methane emissions to atmosphere, and therefore serve as a potential target in strategies to combat global climate change (Dunfield *et al.*, 2007). The bacteria belongs to Verrucomicrobia phylum and was first isolated from Hell's Gate (Tikitere), a methane-emitting geothermal field in the North of New Zealand and known to have a diverse habitat ranging from many terrestrial and aquatic habitats. The complete genome of *Methylophilum infernorum* has been sequenced which can give some useful insights about its role in methane mitigation (Hou *et al.*, 2008). *Methylobacillus* is another genus of anaerobic bacteria found largely in marine and fresh water ecosystems and act as one of the most important carbon recycler as they recycle carbon compounds as methane, methanol and methylated amines (Hanson & Hanson, 1996). A major group of photosynthetic microorganisms generally referred as aerobic anoxygenic phototrophic bacteria (AAPB) found mainly in aquatic ecosystem and act as sink for CO₂ sequestered from the atmosphere (Li *et al.*, 2017; Jiao *et al.*, 2003). Biomineralization is the widespread natural process of chemical alteration by microbes results in the precipitation of several minerals (Anbu *et al.*, 2016). This process serves as an effective mean of CO₂ sequestration. Microbially induced calcite precipitation (MICP) is an example of biomineralization which causes the removal of CO₂ from environment by converting CO₂ into carbonate minerals that can form different crystals such as calcite, aragonite, dolomite and magnesite using microbes (Ferris *et al.*, 1994; Mitchell *et al.*, 2010). It has been seen that, cement and concrete industry is responsible for 5 % of the total industrial energy consumption and 5 % of anthropogenic CO₂ emissions (Worrel *et al.*, 2001). Therefore, the natural properties of

biomineralizing microbes can be effective utilized in Bioconcrete and Biocement formation which is energy efficient and ecofriendly way of cement formation (De Muynck *et al.*, 2010; Anbu *et al.*, 2016). It has been proposed by many scientists that use of Carbonic anhydrase enzyme to reduce atmospheric CO₂ can be an alternate method of biological sequestration of CO₂ (Ramanan *et al.*, 2009; Dhami *et al.*, 2014). Carbonic anhydrase is a zinc containing metallo-enzyme which is ubiquitous in prokaryotes and eukaryotes and involved in numerous physiochemical processes like photosynthesis, respiration, CO₂ and ion transport and maintenance of acid base balance.

Biofuel production and CO₂ utilization

The increase in global population demands more energy supply for improving the life quality. Among the energy sources, fossil fuels are used as a major energy source for past many years (Razzak *et al.*, 2013). The consumption of fossil fuel is a potential cause of climate change as its combustion leads to massive emission of CO₂. Due to this reason, the research encompassing the generation of ecofriendly alternative to fossil fuels has gained the importance from last some years and Biofuel is on the forefront among these. The use of biofuels can reduce fossil fuel consumption and hence reduce the emission of CO₂ into atmosphere. The CO₂ emitted through Biofuel burning are easily utilized by plants from atmosphere by the process of photosynthesis which makes biofuels carbon neutral (Demirbas, 2009). Biodiesel, bioethanol, biobutanol and biohydrogen are some of the examples of biofuels. Several approaches of biofuel production utilizing microbes have been well recognized and well established (Zhu, 2019). Biofuels are broadly classified into two categories: Primary and secondary biofuels (Rodionova *et al.*, 2017). Primary biofuels comprise of unprocessed

biofuel plant woods used for cooking. The secondary biofuels are processed liquid fuels produced from plant biomass utilizing algae and microbes. Plant biomass is considered as one of the best raw material for the production of biodiesel, bioalcohol and fermentation driven biohydrogen. The secondary biofuels are further categorized as first, second and third generation biofuels (Dragone *et al.*, 2010; Rodionova *et al.*, 2017). First generation biofuels comprise of the production of bioethanol or butanol and biodiesel from conventional starch and sugar rich plants like corn, barley, wheat, sugarcane and potato etc. The secondary biofuels are produced from novel starch, oil and sugar crops such as *Jatropha*, cassava or *Miscanthus*. The tertiary biofuels are produced by microalgae or seaweeds (Heiman, 2016). The genetic engineering tools utilizing *E. coli* and *Bacillus subtilis* can be employed to produce bioalcohol, isoprenoids and fatty acids derivatives in higher amounts (Dragone *et al.*, 2010). *Clostridium acetobutylicum* and *Clostridium beijerinckii* has been reported to utilize acetone-butanol-ethanol fermentation for the production of biofuels (Gronenberg *et al.*, 2013). Molecular hydrogen is one of the most promising sources of renewable energy produced by photosynthetic microbes including green alga (Martinez-Perez *et al.*, 2007). Some bacteria are capable of producing molecular hydrogen anaerobically using carbohydrate rich biomass without light by the process known as dark fermentation (Hay *et al.*, 2013).

Nanotechnological approaches utilizing bacteria

Nanotechnology has emerged as one of the most fascinating research areas over the last some years and can be utilized to address a variety of issues including utilization of atmospheric GHGs to reduce its effect on

global climate change (Stander and Theodore, 2011; Khan *et al.*, 2012). Nanotechnology basically deals with the manipulation of matter at nanoscale i.e. the dimensions of sizes in the range of one-billionth of a meter to generate useful products. Nanotechnology can enable the development of nanodevices which perform artificial photosynthesis where biocatalysts are used along with the semiconductor based light absorbers. This device can effectively mimic the natural process of photosynthesis. Few bacterial species have also been reported to be used as biocatalytic agents like *Sporomusa ovata* and genetically engineered *E.coli* (Liu *et al.*, 2015). The development of nanowire-bacteria hybrids is a novel technology which possesses a high reaction rate of CO₂ reduction and can be effectively utilized for unassisted solar CO₂ fixation to value-added chemicals (Liu *et al.*, 2015). As discussed earlier, ubiquitously present Carbonic anhydrase enzyme can play an important role in atmospheric CO₂ utilization. Single enzyme nanoparticle (SEN) based biosensor utilizing carbonic anhydrase has also been reported for gaseous CO₂ sequestration (Yadav *et al.*, 2011).

Carbon eating bacteria: A novel discovery

With the advent of genetic engineering and synthetic biology techniques, it is no more a big challenge to attain the targeted transformations in the natural biochemical pathways of microbes. A recent research claims the conversion of obligate heterotroph to full autotrophy over laboratory timescales, where a group of researchers from Israel have developed a carbon eating *E.coli*. The bacterium is engineered in such a way that it can generate biomass carbon from CO₂ by utilizing a non native Calvin cycle (Gleizer *et al.*, 2019). Generally autotrophs dominate over the heterotrophs on the earth in terms of utilization and sequestration of atmospheric

CO₂ and supply of biomass (Bar-On *et al.*, 2018). The better understanding of their metabolic pathways enables scientists to construct the synthetic autotrophic *E.coli* with modified metabolic pathways (Smith and Stitt, 2007; Ort *et al.*, 2015). Under the normal circumstances *E.coli* produces formate hydrogenlyase (FHL) enzyme which oxidizes formic acid to carbon dioxide and couples that reaction directly to the reduction of protons to molecular hydrogen (McDowall *et al.*, 2014). The researchers unlocked the reverse reaction of FHL where pressurized CO₂ and H₂ allow FHL to function as a hydrogen dependent CO₂ reductase and generate formate which accumulates outside the cell (Roger *et al.*, 2018). The reducing power generated from this single carbon molecule formate (HCOO-) can be used for carbon fixation using Calvin-cycle for harvesting energy (Gleizer *et al.*, 2019). The target has been achieved by employing different strategies which includes the incorporation of mutations in genes encoding for various enzymes to modify the metabolic pathways. The Chemostat cell culture based directed evolution led to complete trophic mode change in around 200 days (Gleizer *et al.*, 2019). Undoubtedly, this study will provide a new and effective way of reducing atmospheric CO₂ and serve as a stepping stone to future efforts for seeking new solutions toward the problem of global climate change.

Conclusion of the study is as follows:

It is quite clear that bacteria can be utilized in multiple ways to address the problem of global climate change by reducing emission and utilization of atmospheric GHGs. Looking toward the extend of the problem it is necessary to explore more than one option by utilizing the advanced technologies with special reference to microorganisms. There are several bacterial species which are still uncharacterized and can be used for the

mitigation of GHGs. Advances in nanotechnology, genetic engineering, genomics, bioinformatics, synthetic biology and artificial intelligence can be explored for the context of global climate change.

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How to cite this article:

Shaurya Dumka, Chinmay Gupta and Aman Kamboj. 2020. Utilizing Bacteria to Mitigate Global Climate Change: A Review. *Int.J.Curr.Microbiol.App.Sci*. 9(05): 1385-1394. doi: <https://doi.org/10.20546/ijemas.2020.905.157>