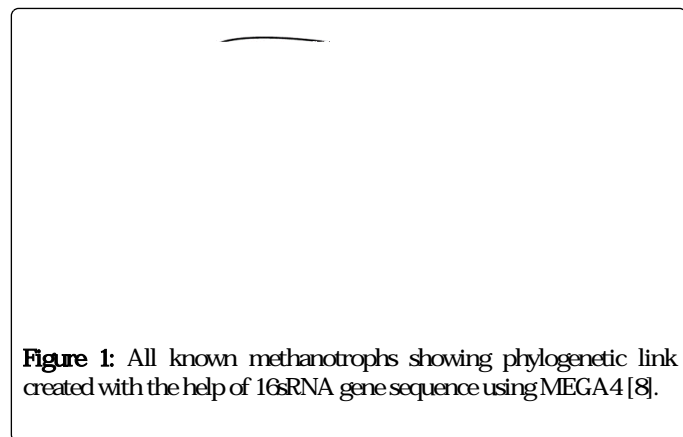




structure of membrane and some other physiological characteristics (Figure 1). The discovery of diverse types helped us to further study of methane-utilizing bacteria and has led to the belief that natural environment is more suitable for them [5,7].

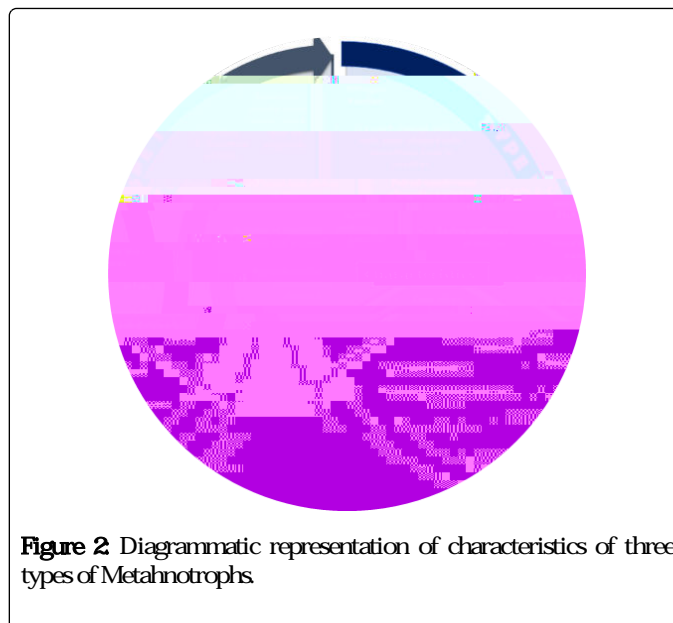


**Figure 1:** All known methanotrophs showing phylogenetic link created with the help of 16sRNA gene sequence using MEGA4 [8].

**Type I:** Type I is the type of methanotrophs that dominates in environments where methane is present in a limited quantity and relatively high levels of combined nitrogen and copper are present. Type II bacteria on the other hand favors environment with high concentration of methane, as well as the environment where dissolved oxygen level is low, and restrictive combined concentration of nitrogen and/or copper. Type I strains can also be characterized on the basis of having other apart from condition characteristics i.e., they have intracytoplasmic membranes throughout the cell in the form vascular disk present in bundles, for carbon absorption they use ribulose monophosphate (RuMP) pathway. They also have phospholipid fatty acids which consist of carbon length of 14 and 16. They can be characterized in a similar comparative way i.e., they too have an intracytoplasmic membranes but unlike Type I it is aligned along the periphery of the cell, with the help of serine pathway they assimilate carbons and they have phospholipid fatty acids with 18 carbon length [5,9].

**Type X: The best of both strains:** Type X strains are a combination of Type I and II strains i.e., they have phospholipid fatty acids of 16 carbon, a RuMP pathway along with possessing ribulose-1,5-bisphosphate, and ability to grow at a temperature which is higher than Type I or II strains [5]. Type X methanotrophs though are quite similar to Type I they are distinguished from type I methanotrophs on the basis that they have enzyme of serine pathway which are lower in levels named as ribulosebisphosphate carboxylase. This enzyme is present in the Calvin Benson cycle. Type X have a DNA, with a distinctive property that it contains G+C content with high moles percentage as compared to type I methanotrophs. Type X have the property that they can grow at higher temperature which isn't present in any other type [10,11].

**Proteobacteria defined types:** The main difference between the types is the pathway they utilize. For the assimilation of carbon type I methanotrophs utilize RuMP pathway, as they are Gammaproteo bacteria (Figure 2). As type II methanotrophs uses Serine pathway for carbon absorption, so they are Alphaproteo bacteria [12].



**Figure 2:** Diagrammatic representation of characteristics of three types of Methanotrophs.

## Ecology

Majority of methane-oxidizing species of bacteria isolated from a wide range of environments suggest that they are mostly aerobic and obligate in nature. However, it cannot be established as a fact due to two main reasons. Firstly, for a fact methane is continuously generated in anaerobic environments, and there is good evidence for its anaerobic oxidation linked which is linked to sulfate reduction by uncharacterized microorganisms present in sediments. Secondly, there is no surety as to if this is the true reflection of relative abundance or just an artifact due to isolation procedures. A thing for ecology for sure is that aerobic environment e.g., soils, surface layers of sediments and natural waters where methane is diffusing have diversity in aerobic methanotrophs population [13,14].

## MMO at Work

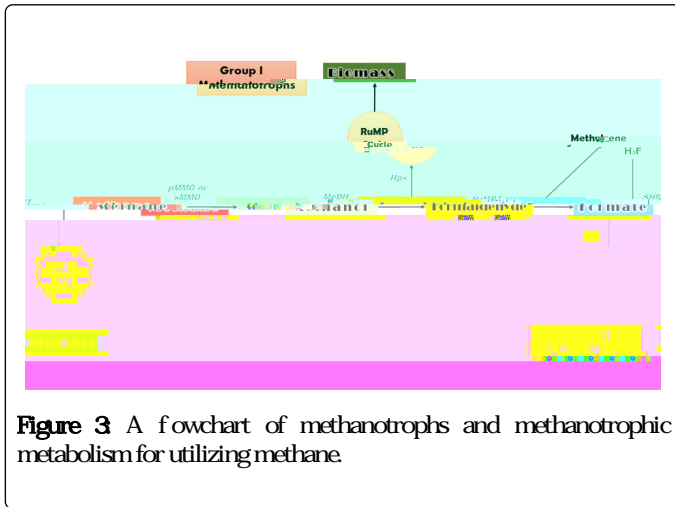
Methane monooxygenase (MMO), all known aerobic methanotrophs the first step in oxidation is the methane conversion into methanol by using MMO [15]. In the second step the methanol further oxidized into formaldehyde, after that it has two options, first is to convert into biomass and other is the further oxidation into formate and then into CO<sub>2</sub>.

Two iso-enzymes of MMO is known: soluble MMO (sMMO), found only as subset of known methanotrophs, and also membrane bound (or particulate) MMO (pMMO). It is located in specialized internal membrane structures, called ICMs [15,16]. sMMO and pMMO both have mixed function of oxidation, that is one atom from O<sub>2</sub> goes to methanol and the other to water, involving the input of 2 electrons and 2 protons. sMMO utilizes NADH, but it is still unknown that what is the physiological electron donor to the MMO [15].

## pMMO vs sMMO

pMMO is found in almost all known methanotrophs, it also shows more affinity towards methane when it is compared to cells which are expressing sMMO (Figure 3). Further studies show that cells shows

higher growth yield which are using pMMO for growth, which signifies that oxidation of methane is more effective by pMMO [17].

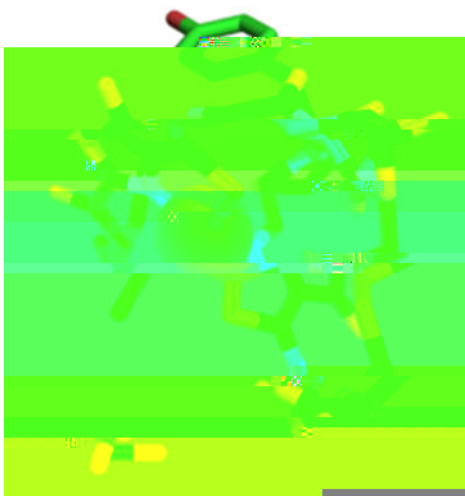


**Figure 3** A flowchart of methanotrophs and methanotrophic metabolism for utilizing methane

### Methanotroph "The Degradation"

In the prevailing twenty five years, study shows that methane-oxidizing bacteria have the ability to degrade wide ranges of halogenated hydrocarbons. Methanotrophic enrichments are capable of degrading priority pollutants e.g., chlorinated hydrocarbons [18], in US and various other countries they are present in aquifers, landfills, wastewaters, and waste disposal sites

methanotrophic degradation of chlorinated hydrocarbons by methanotrophic bacteria is a slow process. Methanotrophic bacteria are able to degrade a wide range of chlorinated hydrocarbons, including polychlorinated biphenyls (PCBs), dieldrin, and dieldrin. Methanotrophic bacteria are able to degrade a wide range of chlorinated hydrocarbons, including polychlorinated biphenyls (PCBs), dieldrin, and dieldrin. Methanotrophic bacteria are able to degrade a wide range of chlorinated hydrocarbons, including polychlorinated biphenyls (PCBs), dieldrin, and dieldrin.



it binds and reduce copper at high affinity. This high binding affinity catch interest of biotechnologists, for using them in biotechnological application including controlling of copper homeostasis in Wilson's disease patient by working as a therapeutic agent [50].

Interestingly, mb can also bind other metals like binding and reduction of both trivalent gold and bivalent mercury ions, their mechanism of binding is very much similar to that of copper ion binding. This can lead to the using of methanotrophs in the field of bioleaching with mining and environmental remediation. Gold nanoparticles and uniform copper can also be produced by Methanobactin [36,49].

## Conclusions and Future Prospects

In this review, we try our best to summarize current knowledge on morphology and application of methanotrophs in environmental bioremediation, mainly for removal of methane from atmosphere. These interesting microorganisms were merely discovered not more than a century ago and attracting great interest. There are plenty more possibilities then discussed in the literature above to make methane-oxidizing bacteria become important and universal microorganism industries. Role of methanotrophs in biogeochemical carbon cycle and also in controlling of global climate change can't be neglected. There is still too much to discover about these organisms like how can unique Methanotrophic structures such as acidophilic, thermoacidophilic, and nitrite-utilizing methanotrophs be best used for the purpose of pollutant degradation? How prevalent are these types of methanotrophs? Can they be with little effort stimulated in situ? Answers to all the above issues will not only guides us to the use of methanotrophs for pollutant degradation but will also help act as a gateway to other interesting issues in methanotrophy. We believe that mighty progress in basic research, together with novel and cutting-edge biotechnological methods eventually will enable the engineering applications of methanotrophs to be realized.

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