

## Harnessing Beneficial Microbes to Mitigate Greenhouse Gas Emissions from Aquaculture

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### Introduction

People around the world are becoming increasingly concerned about global warming. Global warming is mostly triggered by greenhouse gases, that absorb thermal energy emitted by the earth's surface. Aquaculture, like agriculture is an anthropogenic activity, contributes to global warming. The primary greenhouse gases of concern from aquaculture are nitrous oxide ( $N_2O$ ), carbon dioxide ( $CO_2$ ), and methane ( $CH_4$ ). The microbial mitigation of greenhouse gases (GHGs) entails modifying microbial communities to lower emissions of  $N_2O$ ,  $CO_2$  and  $CH_4$  by increasing oxidation, encouraging reduction, and optimizing nutrient cycling. A comprehensive strategy is needed for effective microbial mitigation, balancing nutrient inputs (such as nitrogen), physical pond conditions (such as pH and oxygen), and biological interactions to guide microbial communities toward greater utilization of nutrients and lower GHG emissions, thereby enhancing sustainability. To reduce potential risks, strategies should be developed that enhance the durability of beneficial microorganisms while preventing their dominance over native microbial communities. Using inoculants designed for certain systems or conditions could be one of these tactics, making sure that their effects are confined and do not result in environmental imbalances. Additionally, using precision and scientific management farming could improve aquaculture systems sustainability, preserve microbial diversity, and lower the chance of disruption to the environment.

### Microbial mitigation of nitrous oxide

The goal of microbial mitigation of  $N_2O$  in aquaculture ponds is to control the nitrogen cycle so that certain bacteria are encouraged to transform  $N_2O$  into the innocuous dinitrogen gas  $N_2$ . The main tactics include employing targeted microbial inoculants and modifying the pond habitat to favour  $N_2O$ -reducing bacteria. In aquaculture systems, nitrification (ammonia to nitrate under oxic conditions) and denitrification (nitrate to  $N_2$  under anoxic conditions) are the two primary microbial processes which produce nitrous oxide, a powerful greenhouse gas, as an intermediate by-product. Making sure the last stage of denitrification which makes use of the enzyme nitrous oxide reductase (nosZ gene) is carried out effectively is crucial to mitigation.  $N_2O$  is produced as a byproduct of the nitrification process by ammonia-oxidizing bacteria and

archaea, particularly in low-oxygen environments. A variety of microorganisms can carry out the denitrification process, which converts nitrate to nitrite, nitric oxide,  $N_2O$ , and finally  $N_2$ . When the last stage of conversion to  $N_2$  is impeded, usually because of low C:N ratio or lack of oxygen,  $N_2O$  builds up.

The goal of microbial mitigation techniques is to control the ponds' microbial communities and environmental conditions. Promoting the growth and activity of  $N_2O$ -reducing bacteria with the whole denitrification pathway particularly the nos gene, which is the only biological sink for  $N_2O$  is the main strategy. The capacity of some microbial inoculants like *Cloacibacterium* sp. and several *Bradyrhizobium* sp. to efficiently absorb  $N_2O$  has demonstrated promise in both laboratory and field experiments. One of this century's most important environmental challenges is reducing anthropogenic  $N_2O$  emissions. Given that some bacteria carry out every stage of N transformation in soils, especially during denitrification, manipulating and utilizing soil microorganisms presents a major possibility for further decreasing  $N_2O$  emissions. Denitrification is a microbial process that completes the nitrogen cycle by reducing nitrate ( $NO_3^-$ ) to nitrite ( $NO_2^-$ ) by nitrate reductase (Nar/Nap), nitric oxide (NO) by nitrite reductase (NirS/NirK), nitrous oxide ( $N_2O$ ) by nitric oxide reductase (Nor), and nitrogen gas ( $N_2$ ) by nitrous oxide reductase.

Microbial inoculants can either favourably alter microbial ecosystems or directly increase these microbes. *Bradyrhizobium japonicum*, which is mainly employed to fix nitrogen in soybeans, can also function as a denitrifier. Depending on its capacity to convert  $N_2O$  to  $N_2$ , it can act as a source or sink for  $N_2O$ . Even if they are not nosZ+ by nature, some microbial inoculants can nonetheless affect community composition to increase the abundance of nosZ genes and lower  $N_2O$  emissions. For instance, applying the fungus *Trichoderma viride* to water spinach greatly decreased the production of  $N_2O$  by increasing the relative abundance of nirK, nirS, and nosZ genes. In a similar way, *Bacillus amyloliquefaciens* increased nosZ genes, resulting in a 50% decrease in  $N_2O$  emissions. Furthermore, it has been demonstrated that *Bacillus*-based consortia, which are frequently employed to encourage root development, reduce  $N_2O$  emissions in soil microcosms.

### Microbial mitigation of methane

Among the greenhouse gases, methane is around 23 times more powerful than carbon dioxide. Up to one-third of the present global warming is caused by anthropogenic sources that either directly or indirectly release methane into the atmosphere. Before methane is released into the atmosphere, methanotrophic bacteria or methanotrophs can operate as a biofilter and use methane as a source of energy. They are the only known significant biological sink for atmospheric methane and are crucial in lowering the methane load by up to 15% of the total amount of methane destroyed worldwide. Methanotrophs can be found in a variety of environments and in a broad range of pH, oxygen, temperature, and salinity due to their physiologically adaptive nature. Both aerobic and anaerobic microbes utilize CH<sub>4</sub>. Group I (Gammaproteobacteria), Group II (Alphaproteobacteria), and Group III (Verrucomicrobia) are the three primary groups of aerobic methanotrophs. They are found in a variety of settings and are important for the natural oxidation of methane. Methane monooxygenase (MMO), by oxidation convert methane into methanol, is used by all known aerobic methanotrophs. After being oxidized to formaldehyde, methanol can either be further oxidized to CO<sub>2</sub> or converted to biomass. There are two known MMO iso-enzymes: membrane bound (or particulate) MMO (pMMO), which is observed in methanotrophs, and cytoplasmic soluble MMO (sMMO), which is noticed in the subdivision of methanotrophs. Methanotrophs are highly concerning for their industrial utilization due to their unique microbiological and metabolic traits.

### Microbial mitigation of carbondioxide

In aquaculture, microbes use photosynthesis (algae/cyanobacteria) to convert CO<sub>2</sub> to renewable energy, while advantageous bacteria help control pH and organic

matter, lowering overall emissions. Certain microbes, such as those with carbonic anhydrase, effectively convert CO<sub>2</sub> to bicarbonate, sustaining carbon, and lowering methane/nitrous oxide along with CO<sub>2</sub>. The main CO<sub>2</sub> consumers are photosynthetic microbes (Phytoplankton, Microalgae, Cyanobacteria), which use sunlight to transform dissolved CO<sub>2</sub> into organic matter, so serving as carbon sinks throughout the day. Bacteria like *Bacillus pumulis*, *B. licheniformis* that produce carbonic anhydrase, quickly transforms CO<sub>2</sub> into bicarbonate, which helps control carbon and neutralize pond pH. Sulfate reducing bacteria can indirectly affect the carbon cycle by reducing methane and nitrous oxide under specific circumstances, such as when calcium sulfate is supplied.

In our case study, we have isolated the beneficial bacteria *Bacillus subtilis*, *B. licheniformis*, *B. megaterium*, *Methylobacillus flagellates*, *Methylophaga thiooxydans* and *Methyloversatilis discipulorum* from the brackishwater aquaculture systems which have the potential for greenhouse gas mitigation in aquaculture ponds.

### Conclusion

Although the microbial solutions show promise in the mitigation of GHGs, more investigations are required to identify the powerful microbial strains, especially *in situ* microbiome consortia. The microbial approach to mitigating greenhouse gas emissions focuses on improving beneficial bacterial populations by addressing the complexity of native microbial ecosystems, refining microbial-based strategies for broad applicability across diverse farming systems and environments, and integrating microbial inoculants with organic amendments, precision aquaculture, and best management practices. Such approaches ensure that microbial solutions are adaptable to potential climate change scenarios, economical, and environmentally responsible.

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