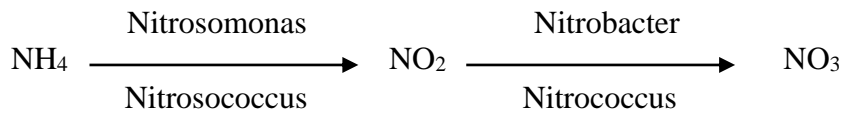


Nitrogen cycle

Nitrification Process :

Nitrification is the biological oxidation, is formally by a two-step process, in the first step oxidation ammonium to nitrite and in the second step the oxidation of the nitrite to nitrate. Nitrification is a second and important step in the nitrogen cycle in soil as convertes of soil ammonia to nitrates, compounds usable by plants.



Nitrification is an aerobic process performed by small groups of autotrophic bacteria different microbes are responsible for each steps . Several bacteria of ammonia-oxidizing bacteria (AOB) , including *Nitrosomonas*, *Nitrosospora*, and *Nitrosococcus*.

In the second step, nitrite is oxidized to nitrate , by groups of nitrite - oxidizing bacteria (NOB), including *Nitrobacter*, *Nitrococcus* *Nitrospira* .

Nitrifying bacteria :

Are chemoautotrophic or chemolithotrophs (family Nitrobacteraceae)depending on the genera (*Nitrosomonas*, *Nitrosococcus*, *Nitrobacter*, *Nitrococcus*) bacteria that grow by consuming inorganic nitrogen compounds. Many species of nitrifying bacteria have complex internal membrane systems that are the location for key enzymes in nitrification: ammonia monooxygenase which oxidizes ammonia to hydroxylamine, and nitrite oxidoreductase, which oxidizes nitrite to nitrate.

Nitrosomonas and *Nitrobacter* are gram negative, mostly rod-shaped, microbes ranging between 0.6-0.4 microns in length. They are obligate aerobes and cannot multiply or convert ammonia or nitrites in the absence of oxygen.

Isolation and detection ammonia oxidizing bacteria:

Procedure :

- 1- Suspend 1 gm of soil sample in 9 ml of Allen I broth (contains $(\text{NH}_4)_2\text{SO}_4$ as ammonia source) .
- 2- Incubate tubes at 28C^0 for a week.
- 3- Mix 1ml microbial suspension with an equal volume of reagent A (salicylic acid ,acetic acid) and reagent B (α-naphtholamine ,acetic acid).
- 4- Let for a few seconds ,formation of red colored deposit illustrate releasing of NO_2 as a result of nitrification process.

Isolation and detection Nitrite oxidizing bacteria:

Procedure :

- 1-Follow previous procedure , but substitute Allen **II** broth instate of Allen **I** broth is contains NaNO_2 as nitrite source for detection of released NO_3 .
- 2- Mix 1ml microbial suspension with drops of nitrate reagent (Diphenylamin DPA) .
- 3- formation of blue colored deposit demonstrate releasing of NO_3 .