Nitrification is a **two-step biological process** by which aerobic bacteria oxidize ammonium to nitrate. Bacteria oxidize ammonium ions (NH$_4^+$) to nitrite (NO$_2^-$) in the first step and then oxidize nitrite to nitrate (NO$_3^-$) in the second step.

Ammonium ions are typically generated from the hydrolysis of urea and degradation of organic nitrogen compounds in municipal wastewater. High concentrations of ammonium ions are often found in food processing wastewaters rich in protein. Ammonium ions found in refinery wastewater are often a result of the hydrolysis of amines used for corrosion inhibition in crude.
The release of nitrogen can have negative impacts on the aquatic life and health of the receiving stream. Several nitrogen species, specifically nitrite and ammonia, are toxic to fish. Ammonium and nitrite can deplete oxygen from a receiving stream from the resulting microbial activity. Since nitrogen is a nutrient, it can promote algae growth and eutrophication.

Nitrification is a sensitive process and is more easily interrupted than other biological wastewater treatment processes due to the relatively slow growth rate of nitrifying bacteria. This also means recovery from nitrification upsets can be slow. Frequent sources of nitrification problems include environmental factors, toxicity, solids washout, and loading variation.
What’s the difference between ammonium and ammonia?

In most wastewater circles, people talk about the “ammonia” concentration, but it’s technically inaccurate to describe nitrification as the oxidation of ammonia. Ammonium (NH$_4^+$) is ionized and ammonia (NH$_3$) is not, and it is actually the ionized form, ammonium, that is oxidized by nitrifying bacteria. The major factor that determines the proportion of ammonia and ammonium is pH of the water, although temperature also plays a role to a lesser extent (Figure 1). The chemical equation that drives the relationship between ammonium and ammonia is: $\text{NH}_3 + \text{H}_2\text{O} \leftrightarrow \text{NH}_4^+ + \text{OH}^-$

The likely reason for mixing up the two comes from the analytical test method. Most methods measure the value as ammonium-N plus ammonia-N and a table, similar to one that was used to create Figure 1, must be used to determine the proportion based on pH and temperature. Since ammonia is toxic to aquatic life at very low concentrations, it is of greater concern and therefore usually the species of interest when running the test. And, because the laboratory analysis measures the sum of ammonia and ammonium, that sum is often discussed (inaccurately) as “ammonia”.

![Graph showing the relationship between pH and the proportion of ammonia and ammonium at different temperatures.](image-url)
What microorganisms are responsible for nitrification?

This series of pictures was taken of the same floc particle using molecular probes and various microscopy techniques. (A) is a floc particle from a nitrifying biomass stained green for all bacterial types. (B) is the same floc particle with AOB stained red. (C) is an overlay of the two pictures showing the spatial distribution of AOB within a well-formed floc particle.

The organisms responsible for nitrification can be described in different ways. Most broadly, they are autotrophs. They require oxygen and obtain carbon from CO$_2$ and HCO$_3$. They can also be described by which step of the process they drive: ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). *Nitrosomonas* and *Nitrobacter* are the organisms most commonly associated with ammonia and nitrite oxidation, respectively, but with the influx of new tools from molecular biology, it is understood that there are other AOB including *Nitrosospira* and NOB including *Nitrospira* that can play important roles in the nitrification process.

\[
2\text{NH}_4^+ + 3\text{O}_2 \rightarrow 2\text{NO}_2^- + 2\text{H}_2\text{O} + 4\text{H}^+
\]

Oxygen required: 3.43 lb / lb N

\[
2\text{NO}_2^- + \text{O}_2 \rightarrow 2\text{NO}_3^-
\]

Oxygen required: 1.14 lb / lb N

Total oxygen required: 4.57 lbs/lb N
What is the optimum pH range for nitrification?

The optimal pH for nitrification is between 7.0 and 8.5. Nitrification can occur outside this range, but plants should adjust pH to the optimal range for recovery and start-ups until nitrification is well established.

How does temperature impact nitrification?

Nitrification readily occurs between 15 – 30°C. As temperatures decrease below 10°C or above 35°C nitrification rates slow down and it can become more difficult to maintain.
How much alkalinity is required for nitrification?

The reaction that converts nitrate to nitrite creates nitrous acid (NHO₂) which consumes alkalinity and can lower pH. The theoretical demand for alkalinity is 7.14 lbs (as CaCO₃) per lb of ammonium converted to nitrate. Without enough alkalinity, the pH of the system could crash.

\[
\text{NH}_4^+ + 1.83 \text{ O}_2 + 1.98 \text{ HCO}_3^- \rightarrow \\
0.98 \text{ NO}_3^- + 0.021 \text{ C}_5\text{H}_7\text{NO}_2 + 1.88 \text{ H}_2\text{CO}_3 + 1.04 \text{ H}_2\text{O}
\]

What are typical DO ranges required for nitrification?

The theoretical oxygen demand for complete nitrification is 4.57 lbs per lb nitrogen. Typically, nitrifying plants will have a dissolved oxygen concentration above 2.0 mg/L, but may fully nitrify well below that. For a recovery or start-ups, a DO > 2.0 mg/L is recommended until nitrification is well established.
What compounds are toxic to nitrifiers?

Rapid inhibition can be caused by exposure to certain organic and inorganic compounds. Even intermittent exposure can cause nitrification disruptions.

Sometimes inhibition is caused by the accumulation of compounds on floc particles where nitrifiers are colonized. The long term exposure to these compounds can cause a steady decline in nitrification performance.

Compounds which cause acute toxicity:
- Cyanide
- Phenol
- Chlorinated hydrocarbons
- Metals
- Amines
- Spent caustic waste

Compounds which cause chronic toxicity:
- Long chain fatty acids
- Fluorides
- Surfactants
- Metals
- Oils

Can all types of wastewater systems nitrify?

Activated sludge systems are generally well suited for nitrification if the conditions are favorable. Trickling filters and rotating biological contactors (RBCs) also are good for nitrification. Nitrification in lagoons and aerated stabilization basins (ASBs) can be challenging due to the low concentration of biological solids.

Several higher life forms are indicators of low ammonia, including rotifers, crawler ciliates, and suctoria.
Novozymes offers microorganisms specially selected for the ability to thrive at low temperatures.

Low temperatures can have dramatic effects on a microbial community. When this happens it is important to have a microbial community that consists of microbes that are effective at removing organics at lower temperatures.

Many biological wastewater treatment plants experience a reduction in activity at lower operating temperatures. Typically, lower temperatures result in reduced BOD and COD degradation. Novozymes’ cold weather products exhibit growth and activity below 55º F (13º C) and improve treatment at cold temperatures.

**Toler-X 5100 performs well in extreme conditions**

Toler-X 5100 helps plants to comply with their permits and improves plant efficiency at low temperatures. It contains a blend of psychrophilic (cold-loving) microorganisms and is active all the way down to 2 ºC (36 ºF). The strains in Toler-X 5100 have a wide range of organic degradation abilities and can be used at a wide range of industrial facilities.

Combined with appropriate operational adjustments, bioaugmentation can also offset polymer and other chemical costs this winter.

**The key benefits include the following:**

- Improves COD reduction at low temperatures
- Improves system stability at low temperatures

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**BONUS QUESTION:**

How can Novozymes help you nitrify?
Thanks!

We appreciate you taking the time to read Novozymes’ Nitrification Q&A!

Have a question?

Have a question about nitrification that wasn’t included in this document? Send it on to wastewater@novozymes.com.