The Anammox Process for Nitrogen Removal from Waste Water

The Fruitful Collaboration Between Microbiologists and Process Engineers

As a country bordering the Rhine, Switzerland shares in the responsibility to reduce nitrogen export to the North Sea. In wastewater treatment plants, this is currently accomplished through a costly expansion of the main activated sludge system. During the 1990s, however, there were several reports of nitrogen being eliminated rather unexpectedly under certain operating conditions. It was found that this is due to a recently discovered group of bacteria, which were also found in Swiss wastewater treatment plants. Based on this discovery, process engineers have developed a new process that reliably removes nitrogen.

Nitrogen elimination is commonly regarded as the conversion of biologically available nitrogen compounds, such as ammonium (NH_4^+), nitrite (NO_2^-), or nitrate (NO_3^-), to elemental nitrogen (N_2), which is released into the atmosphere as a harmless product. Today, wastewater treatment plants almost always use a biological nitrification/denitrification process for the elimination of nitrogen (see box). Swiss wastewater treatment plants often omit the denitrification step, which means that the discharge water still contains relatively high nitrogen loads, mostly in the form of nitrate. As a country that borders the Rhine, Switzerland has committed itself in the Water Protection Act of 1998 to reduce nitrogen input to the Rhine by 2000 tons by the year 2005. Solutions must, therefore, be found in the very near future. The upgrading of all Swiss wastewater treatment plants with an adequate nitrification/denitrification stage will be expensive. In addition, the operation of this process is energy and resource intensive [1]. Therefore, a new, low-impact process is needed.

Nitrification
= Aerobic oxidation of ammonia by nitrifying bacteria.
Ammonium (NH_4^+) is oxidized by oxygen (O_2) via the intermediate nitrite (NO_2^-) to nitrate (NO_3^-):

$$NH_4^+ + 1.5\ O_2 \Rightarrow NO_2^- + H_2O + 2\ H^+$$
$$NO_2^- + 0.5\ O_2 \Rightarrow NO_3^-$$

Denitrification
= Nitrate respiration by denitrifying bacteria.
Nitrate is reduced under anaerobic conditions to elemental nitrogen (N_2) with the addition of organic carbon (e.g., methanol):

$$NO_3^- + \text{org. carbon} \Rightarrow N_2 + 2\ CO_2$$

Anammox
= Anaerobic oxidation of ammonium by anammox bacteria.
Ammonium is oxidized to elemental nitrogen by nitrite under anaerobic conditions:

$$NH_4^+ + NO_2^- \Rightarrow N_2 + 2\ H_2O$$

Tracking Down Unknown Microorganisms

In the 1980s and 1990s, there were several indications that nitrification/denitrification are not the only processes that can remove ammonium, but that there is an organism that can oxidize ammonium to nitrogen gas using nitrite instead of oxygen as its terminal electron acceptor. Dutch and German scientists first identified these organisms. They belong to the order of Planctomycetales: *Brocadia anammoxidans* and *Kuenenia stuttgartiensis* [2, 3]. The phenomenon of anaerobic ammonium oxidation was also observed in the Swiss wastewater treatment plant of Kölliken. Our investigations revealed that a multilayered system of biofilms had formed in this treatment plant. Such biofilms create pronounced oxygen gradients, where the upper-most layer can be oxygen-rich, while the layer closest to the carrier material can be anaerobic [4]. We assumed that the microorganisms we were looking for were located in the lowest layers of the biofilms. Using specific gene probes and the FISH technique (fluorescence *in situ* hybridization) [5], we were able to confirm the presence of a large population of bacteria belonging to the order Planctomycetes (Fig. 1). Until now, it was not possible to isolate a pure culture of these new bacteria using classic methods of microbiology. We were able, however, to enrich a sample of the biofilm, where approximately 90% of the bacteria were Planctomycetes [6]. The organism present in the Kölliken wastewater treatment plant is *Kuenenia stuttgartiensis.* Using techniques of molecular biology and other physiological experiments, we could show conclusively that *K. stuttgartiensis* oxidizes ammonia to nitrogen under anaerobic conditions [3, 6]; this process is, therefore, called "anaerobic ammonium oxidation" (anammox).

Anaerobic Ammonium Oxidation – a Sustainable Process for Nitrogen Removal

Process engineers were able to build on this knowledge. Wastewater treatment plants with anaerobic sludge digestion produce a particularly ammonium-rich effluent, which is nowadays recombined with the influent to the treatment plant. Consequently the nitrogen load to be treated is increased by approximately 15–20%. Instead of "recy-

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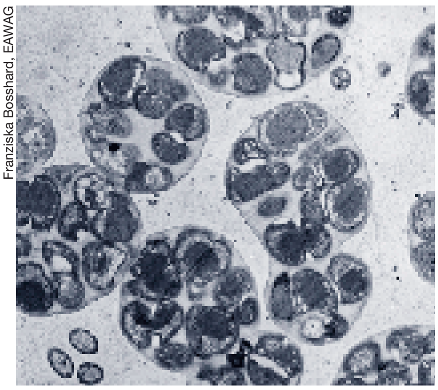

Fig. 1: Anammox bacteria form dense, globular clusters. Individual bacteria are visible as small, open circles within the clusters. The cell clusters have a diameter of approximately 15 µm. Magnification: 1000x.

cling” this effluent, it could be treated separately using the new anammox process, requiring minimal input of energy and other resources. What is required, however, is nitrite, which is not present in the effluent but is formed as an intermediate product in the nitrification process (see box). The following two-step process (Fig. 2) is a possible solution: in a first, aerated reactor, ammonium is partially oxidized to nitrite *(partial nitritation),* which is then reduced to elemental nitrogen in a second oxygen-free reactor using the remaining ammonium *(anammox).* The overall process is, therefore, called “partial nitritation/anammox”, or simply the anammox process. So far, it can only be used for effluents rich in ammonium. Thanks to genetic methods, anammox bacteria can be detected easily and rapidly, which is important during the startup phase and in determining process failure. Compared to traditional nitrification/denitrification, the anammox process has several advantages (Fig. 3):

- Oxygen additions can be reduced by 60 %. This also reduces the energy required to introduce the oxygen.
- Anammox bacteria do not require organic carbon. By contrast, organic carbon, such as methanol, has to be added in the nitrification/denitrification process.
- Anammox bacteria produce very little biomass, reducing the amount of sludge that has to be disposed of.

The new anammox process uses not only less energy and fewer resources, but it is also less expensive than conventional nitrification/denitrification.

Testing the Anammox Process

In order to test the feasibility of this two-stage process in practice, EAWAG has built and operated a pilot plant (4 m^3) in cooperation with the wastewater treatment plant Werdhölzli (Zurich) and other partners [7]. This pilot project has yielded new insights into the process and has confirmed its applicability. In addition, the pilot plant has been used to develop guidelines for the scale-up and operation of full-scale plants. Unfortunately, the new process is still met with caution and hesitation, mostly because of the slow growth rates of the anammox bacteria and the lack of experience in operating the process. Due to the many advantages of the process, however, we anticipate the construction of the first large-scale anammox reactors over the next few years.

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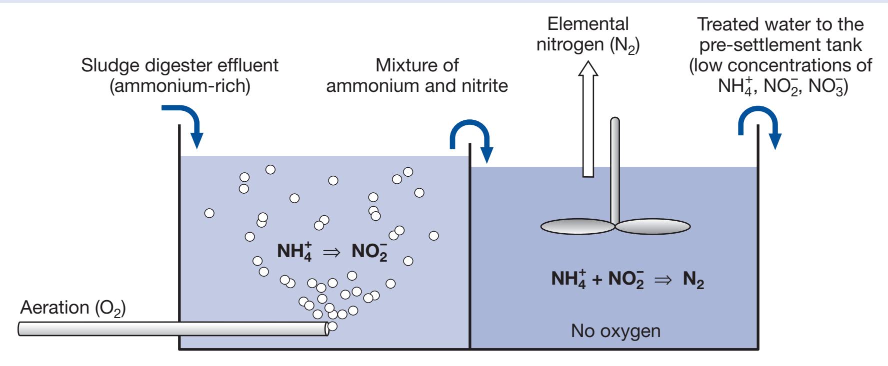

Fig. 2: The anammox process.

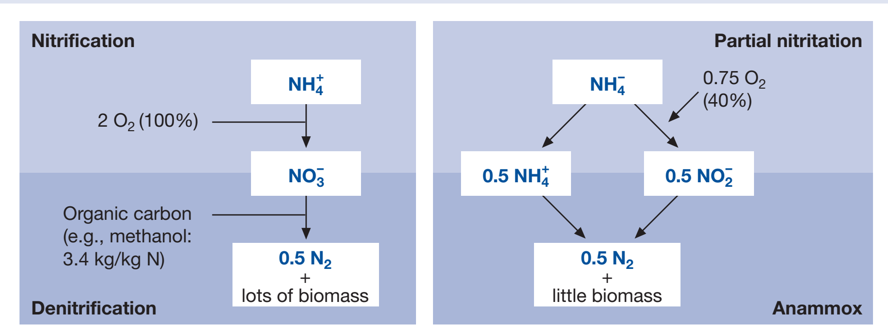

Fig. 3: Comparison between the anammox process and conventional nitrogen removal by the nitrification/denitrification process.

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