



Need for wastewater treatment

The purpose of wastewater treatment is to remove pollutants that can harm the aquatic environment if they are discharged into it.

- **Suspended Solids** → deposits
- **Oxygen demanding pollutants** (mostly organic matter and ammonia nitrogen) → DO depletion
- **Nutrients** (nitrogen and phosphorus) → eutrophication
- **Toxic pollutants** (mostly organic, thus also DO depletion)

The Biochemical Environment

The most important characteristic of the environment in which micro-organisms grow is the **terminal acceptor** of the electrons they remove as they oxidise chemicals to obtain energy.

Three major types of electron acceptors:

- Oxygen
- Inorganic compounds
- Organic compounds

Aerobic environment

- If dissolved oxygen is present or supplied in sufficient quantity so as to not be rate limiting, the environment is considered to be aerobic.
- Growth is generally most efficient in this environment
- The electrode potential is positive ($> +50$ mV)
- The amount of biomass formed per unit of waste destroyed is high.

Anaerobic environment

- Strictly speaking, any environment which is not aerobic, is anaerobic.
- Within the wastewater treatment field, however, the term anaerobic is normally reserved for the situation in which organic compounds, carbon dioxide and sulphate serve as the major terminal electron acceptor.
- The electrode potential is very negative (< -50 mV)
- Growth is less efficient under anaerobic condition

Anoxic environment

- When nitrate and/or nitrite are present and serve as the primary electron acceptor in the absence of oxygen, the environment is called anoxic.
- The presence of nitrate and/or nitrite causes the electrode potential to be higher than under anaerobic conditions (~ 0 mV).
- The growth is more efficient than under anaerobic conditions, although not as high or as efficient as when oxygen is present.

The biochemical environment has a profound effect on the ecology of the microbial community.

- **Aerobic** operations tend to support complete food chains from bacteria at the bottom to rotifers at the top.
- **Anoxic** environments are more limited.
- **Anaerobic** environments are most limited, being predominantly bacterial.

Nitrogen Removal (Nitrification and Denitrification)

Nitrogen removal is nowadays an important aspect of wastewater treatment processes.

Obstacles in studying the nitrogen conversion processed in activated sludge:

- Large amount of nitrogen compounds involved
- Numerous reactions that can occur
- Slow growth rate of many bacteria involved

Nitrification and Denitrification

(early beginning and state-of-the-art)

- 1890 (Winogradsky) - the first report on microbial oxidation of ammonium
- 1892 (Breal) - the first report on microbial reduction of nitrate to nitrite
- 1926 (Kluyver and Donker) - the basic concepts of nitrification
- 1910 (Beijerinck and Minkman) - isolation of *Paracoccus denitrificans*

- 1990 (Robertson and Keunen) - aerobic denitrification
- 1995 (Muller *et al.*) - heterotrophic nitrification
- 1995 (Mulder *et al.*) - anaerobic ammonium oxidation (ANAMMOX)
- 1995 (Bock *et al.*) - denitrification by autotrophic nitrifying bacteria

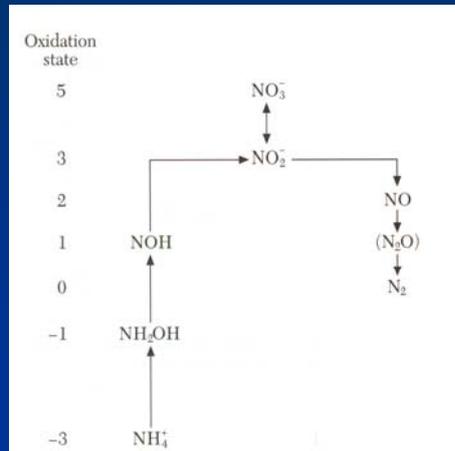
Despite a more than a century long study of the micro-organisms involved, still conflicting and speculative reports on N-conversion appear regularly.

Nitrification

- Nitrification is the conversion of ammonia-N, via nitrite-N, to nitrate-N.
- Can be performed by either heterotrophic or autotrophic bacteria
- In spite the fact that over a 100 heterotrophic species have been cited as forming nitrite from ammonia, significant amounts of nitrate are not thought to be generated heterotrophically in natural systems.
- Nitrification is generally considered being performed by autotrophic bacteria.
- There have been a number of different types of nitrifying bacteria identified, but interestingly, these either oxidise ammonium to nitrite (mostly *Nitrosomonas europaea*) or nitrite to nitrate (mostly *Nitrobacter agilis*).

Nitrification

- For N-removal process it is beneficial if ammonium is only oxidised to nitrite and thereafter denitrified.



Inhibition of elimination of nitrite oxidisers can be done by:

- change in pH
- wash-out based on growth rate (SRT, T > 15°C)
- wash-out based on substrate competition (at low DO, → SVI!)

Nitrification



- Most energy is produced and the most of the O₂ is consumed in the first process
- Nitrifying organisms are slow growing and susceptible to various inhibitory effects (growth rate 1 day⁻¹, yield 1 gN → 0.24 g biomass COD, negligible contribution to MLSS, but significant effect on process)
- Under normal conditions the rate of oxidation of nitrite is higher than that of ammonia (no accumulation of nitrite in nitrifying activated sludge systems)
- Protons released during the oxidation of ammonia effect the buffer capacity (if the alkalinity is not at least 1.5-2 mmol/L at the end of nitrification, a significant decrease in pH can take place)
- The specific consumption of O₂ is rather high, which should be reflected in the design of aeration systems (DO ~ 1.5-2.0 mgO₂/L recommended)

Denitrification



- Denitrification is biological reduction of nitrate to nitric oxide, nitrous oxide, and finally, nitrogen gas
- Requires an electron donor (COD) which can be organic material or reduced components such as sulphide or hydrogen. The COD donors are predominantly external RBCOD or organic storage products (PHB). When all these substrates are exhausted, slow endogenous anoxic respiration (denitrification) is also possible. As the rate of hydrolysis under anoxic conditions is rather slow, the availability of particulate substrates for denitrification is rather limited.
- From the stoichiometry, it can be calculated that 1 gNO₃-N equals 2.86 gO₂ in redox reactions. However, the actual consumption of RBCOD for full denitrification of 1 gNO₃-N is ~ 8 gCOD
- Nitrification: generation of protons (H⁺), denitrification: consumption of H⁺ → during denitrification the alkalinity can be partly replenished.

Heterotrophic Nitrification - Aerobic Denitrification

- There is no fundamental argument why denitrification cannot occur under aerobic conditions nor is there a fundamental argument against nitrification by heterotrophic bacteria.
- Nitrification is conventionally measured by the formation of nitrite or nitrate, while denitrification under aerobic conditions is generally not expected. Therefore heterotrophic nitrification - aerobic denitrification is not easily observable.
- Contribution of combined heterotrophic nitrification - aerobic denitrification can be considered negligible in wastewater treatment practice.

Single Reactor High Activity Ammonia Removal over Nitrite - SHARON

- SHARON promotes nitrification to nitrite rather than nitrate. Under normal conditions (5-20°C), growth of *Nitrobacter sp.* is not limiting, so nitrite will not accumulate. However, at high temperatures, the growth rate of *Nitrosomonas sp.* will exceed that of *Nitrobacter sp.* and at a specific retention time it is possible to retain *Nitrosomonas sp.* and wash-out *Nitrobacter sp.* By this up to 25% of oxygen input can be saved.
- Denitrification of nitrite can be achieved, providing sufficient C source is present. With nitrite as electron acceptor, up to 40% of the required amount of C source can be saved in comparison to denitrification with nitrate as electron acceptor.
- SRT=HRT (easy control)
- SRT aerobic = 1 day, SRT anoxic = 0.5 day
- T=25-40°C
- pH= 6.6-7.4
- Heat production!

Single Reactor High Activity Ammonia Removal over Nitrite - SHARON

- Applicable for N-rich wastewater (rejection water from sludge dewatering, leachate from landfill or composting sites, and industrial effluents).
- No sludge retention (SRT) required (due to high operating temperatures and high conversion rates)
- Specific Continuous Stirred Tank Reactor (CSTR) process
- Nitrifiers are slow growing organisms (up to 1 day⁻¹, depending on the temperature), theoretically minimum retention time of ~ 0.7 days can be found, but with reduced efficiency. In case of ammonia pre-treatment with 90% efficiency (1000 mg/L → 100 mg/L) is acceptable (at retention time of 1 day).
- Denitrification is used for pH control
- pH important - NH₃ is substrate!
- CO₂ stripping is essential part of process design.
- Effluent concentration only dependent on growth rate (1/SRT) of bacteria involved, not on influent concentration.

Anaerobic Ammonium Oxidation - ANAMMOX



The process in which under anoxic conditions nitrite is converted to dinitrogen gas with ammonium as electron donor.

- For a long time the oxidation of ammonia was believed to be restricted to aerobic environments.
- Apparently, anaerobic ammonia oxidation is energetically more favourable than 'normal' aerobic nitrification (357 versus 250 kJ/mol)
- The nitrate was found to be preferred electron acceptor (not oxygen)
- Hypothesis: Electron acceptor nitrite is reduced to hydroxylamine which reacts with the electron donor ammonium, producing intermediate hydrazine, and ultimate dinitrogen gas.
- Combination of anaerobic and aerobic ammonia oxidation possible: theoretically, anammox and nitrifiers would be able to coexist under oxygen limiting conditions. The nitrifiers oxidise ammonia to nitrite and keep the oxygen concentration low, while anammox converts toxic nitrite and the remaining ammonium to nitrogen gas.

Anaerobic Ammonium Oxidation - ANAMMOX

- Anammox have extremely low growth rate (0.1- 0.05 d⁻¹)
- Unlikely they will be present in normal heterotrophic denitrifying wastewater processes. In autotrophic denitrifying systems accumulation will take many months.
- In the process, bacteria are enriched on a mineral medium containing ammonia, nitrite and bicarbonate as the only carbon source.
- Relatively high conversion rate (up to 4 kg N/m³.d), sufficient for full-scale application.
- Autotrophic systems with very long SRT or biofilm systems.
- The process allows to denitrify with ammonium as electron donor - no organic substrate (COD) is needed in N-removal process.

SHARON-ANAMMOX

- Partial nitrification of ammonia to nitrite by fast growing nitrifiers, and denitrification of nitrite to dinitrogen gas using ammonia as electron donor.
- Partial conversion of ammonia to nitrite (~50%) to obtain 1:1 ammonia-nitrite mixture (effluent) in SHARON process. The effluent of SHARON was used as influent for ANAMMOX.
- Oxygen requirement for N-removal reduced by 60%.
- No COD is needed.
- Sludge production is minimised.
- Net CO₂ emissions strongly decreased.
- Combined systems for COD and N removal nowadays can be operated independently.
- This makes it possible to optimise COD and N removal processes separately.

Comparative stoichiometry of N-removal processes

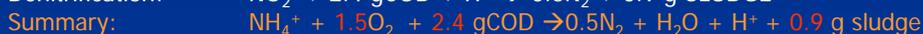
Conventional N-removal



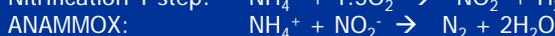
ANAMMOX



SHARON



SHARON/ANAMMOX



Phosphorus Removal

- Biological phosphorus removal (BPR) has become a well established process and is applied in many full-scale WWTPs.
- The organisms involved have a complex physiology in which formation and consumption of storage polymers poly-phosphate, poly-hydroxy-alkanoates and glycogen play a dominant role.
- BPR discovered by accident in full-scale WWTP in India in 1959 by Srinath (aerobic P-uptake).
- The first full-scale processes were designed in 1970's.
- In 1980's more interdisciplinary research including microbiology and process engineering to better understand the basic phenomena of BPR.

Phosphorus Removal

- Levin and Shapiro (1965), conducted the first structural investigation on BPR (aerobic P-uptake and non-aerobic P-release).
- Levin (1966) Patent for the Phostrip process. Developed without proper understanding of the mechanisms of BPR, but still applied in WWT (the concept accepted in 80's only, when the basics became clear).
- Fuhs and Chen (1975) *Acinetobacter sp.* responsible for BPR, however these organisms are not involved in the actual process (Bond *et al*, 1995, etc.).
- BPR Biochemical concepts were developed by engineers !!! However all had contacts with microbiologists or knowledge in microbiology.

Phosphorus Removal

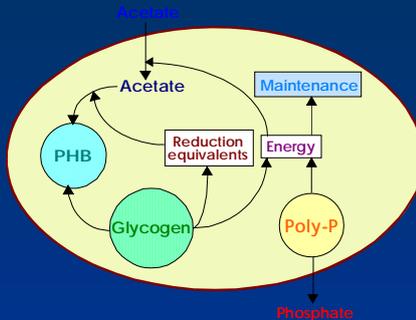
- No pure culture isolated for BPR yet!
- PAOs are heterotrophs.
- PAOs, bio-P bacteria, P-removing micro-organisms etc.
- Traditional method: chemical P removal by addition of salts to wastewater (increased salinity, accumulation of chemical precipitate in the sludge, extra costs for treatment of additional sludge, larger tanks to keep the same amount of biological sludge, low costs of chemical addition, simple and stable process, easier to understand and operate in comparison to BPR)

Phosphorus Removal

- Efficiency of the process is directly related to the formation of PAOs.
- Need for truly anaerobic conditions (no O_2 , no NO_3)
- VFA present in the wastewater or fermentation product
- Design heavily dependent on wastewater characteristics
- Two basic process configuration:
 1. Full biological process and
 2. Combined biological-chemical process

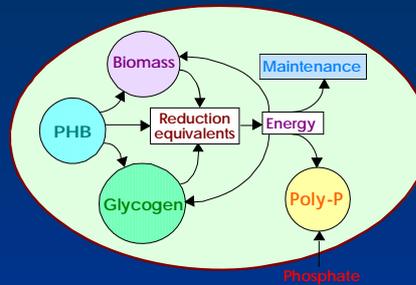
Fully biological P-removal - METABOLISM

Anaerobic conditions



Acetate uptake
 PHB formation
 Glycogen utilization
 Maintenance
 P-release

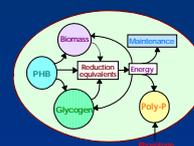
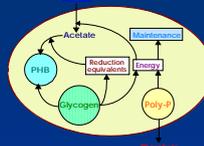
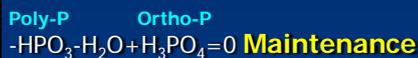
Aerobic conditions



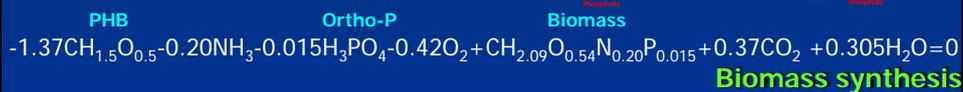
PHB utilization formation
 Glycogen formation
 Maintenance
 Biomass formation
 P-uptake

Fully biological P-removal - STOICHIOMETRY

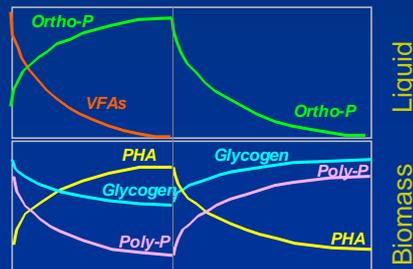
Anaerobic conditions



Aerobic conditions



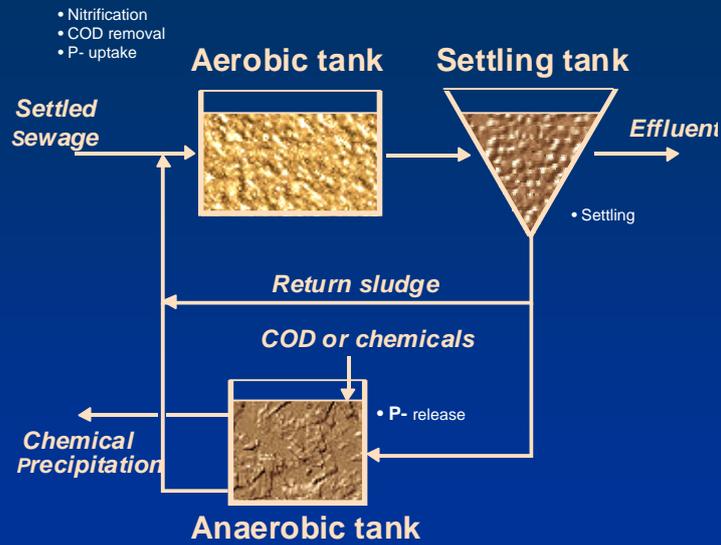
Fully biological P-removal process



Combined biological and chemical P-removal

- PAOs can accumulate up to 12% P on dry weight basis, normal bacteria 1-3% only.
- BPR needs to be combined with CPR in cases of:
 - COD/P ratio can be low, production of PAOs insufficient
 - Lowered biomass production by prolonging sludge age for nitrification
- Careful dosing of chemicals needed to avoid that all P is chemically fixed - no P for poly-P - no PAOs!
- BPR only: 20gCOD/gP
- BPR/CPR: 2 gCOD/gP
- In line addition of chemicals - accumulation of chemical sludge in the system - impact on nitrification.
- As alternative to in-line treatment, off-stream Phostrip process can be used.

Combined biological and chemical P-removal Phostrip process



Combined biological N and P-removal

UCT Process



- Prevention on NO_3^- to enter anaerobic tank - negative impact on P release by direct substrate competition between heterotrophic denitrifiers and PAOs.

Combined biological N and P-removal

- Prevention on NO_3 to enter anaerobic tank - negative impact on P release by direct substrate competition between heterotrophic denitrifiers and PAOs. Denitrifiers win!
- This observation and the fact that *Acinetobacter* cannot denitrify, induced belief that P and N removal are competing processes.
- However, recently it has been shown that denitrifying bacteria can also be involved in BPR (anoxic P-uptake).
- Often significant contribution to BPR of DPAOs!
- Use of DPAOs in BPR means less COD needed for BPR!
- New processes in which the slow-growing nitrifiers and fast growing heterotrophs are separated. After anaerobic reactor, the sludge/water mixture is separated: the water containing ammonium is nitrified separately and returned with sludge in the anoxic reactor.