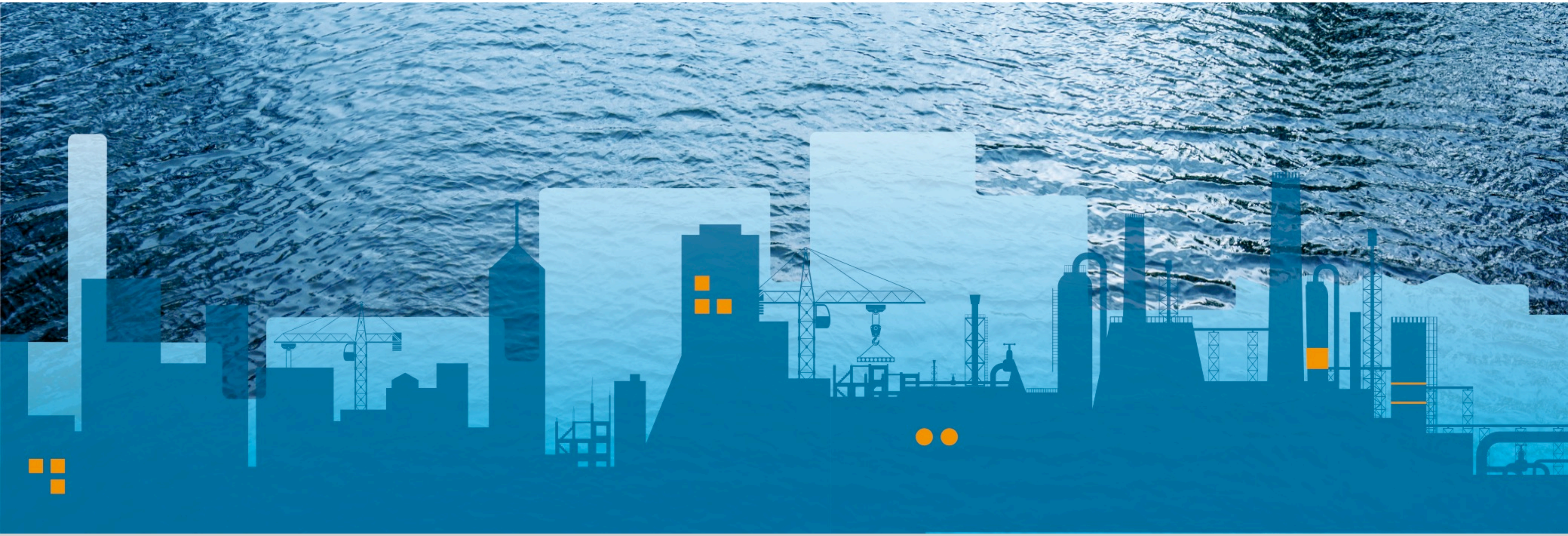
Inhibitors and indicators in biological wastewater treatment processes



Project BEST output (WP2)
Riga Technical University
Dr.Sc.ing. Kristina Tihomirova, Dr..Sc.ing. Kamila Gruškeviča, Dr.sc. Ing. Tālis Juhna

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Introduction

- The biological processes in treatment plants are carried out by very diversified group of organisms (Henze et al., 2000)

- Aerobic microorganisms metabolize the organic matter in the wastewater, multiplying and producing inorganic end-products (principally CO_2, NH_3, and H_2O).

- Biological nitrogen removal is achieved by sequential nitrification under aerobic conditions and denitrification under anoxic conditions.

- Biological nitrogen removal is achieved by phosphorus-accumulating organisms that absorb and accumulate dissolved phosphorus from wastewater, resulting the phosphorus content increase in the sludge.

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Mechanism of biological wastewater treatment

Conventional nitrification-denitrification includes a combination of aerobic nitrification that oxidizes ammonia to nitrite and nitrate in the aeration tank and anoxic denitrification that reduces these nitrification products to nitrogen gas:

$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$ (ammonia oxidizing bacteria (AOB) produce NO_2);

$NO_2^- + 0.5O_2 \rightarrow NO_3^-$ (nitrite-oxidizing bacteria (NOB) produce NO_3);

Heterotrophic denitrification ($NO_3 - N_2$) reaction:

$0.20NO_3^- + 1.20H^+ + e^- \rightarrow 0.10N_2 + 0.60H_2O$

Can be used ANAMMOX ($NO_2 - N_2$) reaction:

- $0.33NO_2^- + 1.33H^+ + e^- \rightarrow 0.17N_2 + 0.67H_2O$

Important for nitrification

Optimal conditions:

- pH 7.8 – 8.0.
- Dissolved oxygen (DO) 2.5 mg/l;
- Long-term aeration;
- Low load on sludge (F/M ratio);
- Sludge retention time (SRT) above 15 d
- Time for AOB un NOB grow – at least 5-6 h
- Sludge density index (SDI) 1.02 – 1.09 g/ml

Process can be affected by:

- High load of NH_4-N
- pH
- Temperature
- Concentration of DO
- High load of COD/BOD
- Heavy metals
- Pharmaceuticals and other toxic compounds

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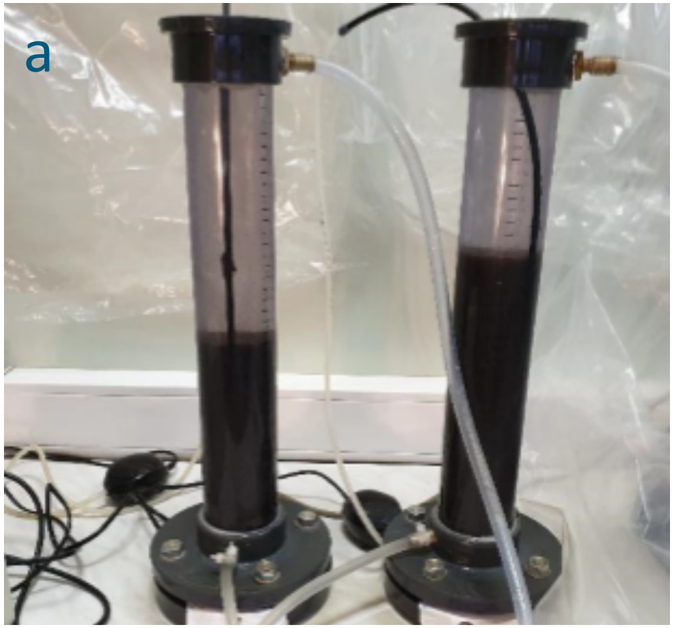
Visual characterisation of problems with activited sludge process



- Sludge **bulking** (a) (risk if SVI_{30} >150 ml/g according to Han et al., 2018);
- Sludge **foaming** (b);
- **Sludge flock degradation**;
- **Floating sludge**.

Inhibitors of process

- **Wastewater process inhibitors are compounds that have a negative impact on WTP's biological treatment system. These substances decrease or diminish efficiency of biological wastewater treatment process.**

- *Food (including milk, fish, and meat processing) industries generate high-strength wastewaters with highly variable concentrations of inhibitors.*

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Inhibitors can be

Heavy metals

Toxic substaces

Overload with typical components

Shock loads of high concentrations of different chemicals used for food processing may disturb biological treatment processes, thus compromise effluent water quality.

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Main indicator of inhibition

Sludge Biotic Index (SBI) has been devised to monitor activated-sludge plant performance (Madoni, 1994, https://doi.org/10.1016/0043-1354(94)90120-1):

Determination of the biological quality of the sludge using numerical values (biotic index) of 0–10 is based on two principles:

1. First, the dominance of the microfauna key groups changes in response to the environmental and operational conditions of the plant.
2. Secondly, the number of morphological species is reduced as the performance of the plant worsens.

This method provides numerical values which enable the operator to monitor the prevalent plant operating conditions on a daily basis.

Main groups of sludge microfauna

- Bacteria (nitrifiers, denitrifiers, organic matter degrading bacteria);
- Protozoa (amoebae, small protozoa (flagellate), infusoria);
- Metazoa (lathes, worms);
- Algae (microalgae);
- Fungi

Sludge microfauna 12 indicative organisms

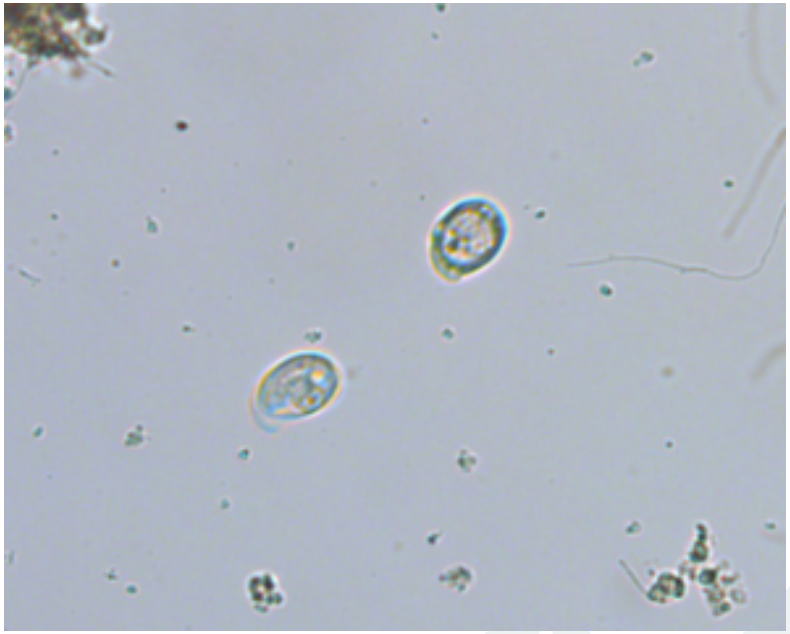
Changes in number and type of microorganisms can: indicate the condition of the treatment process and predict problems!

Only 1h for analysis

Protozoa the naked amoebae (a) and shelled amoebas (b)

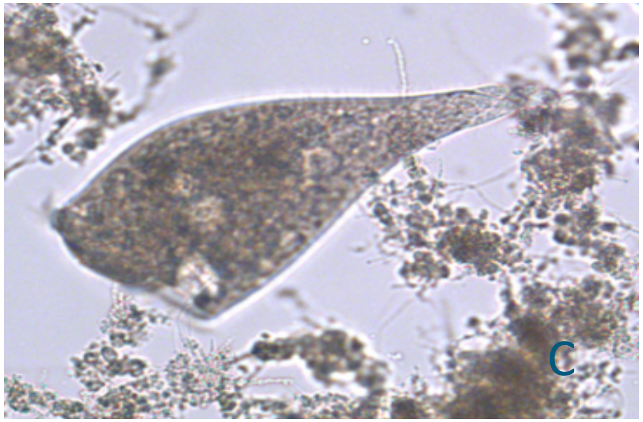
- The naked amoebae (*Limax*, *Mayorella*) is the indicator of low oxygen concentration and toxic compounds in influent;
- The shelled amoebas (*Arcella*) is the indicator of high rate of SRT and well nitrifying sludge.

Protozoa – ciliates



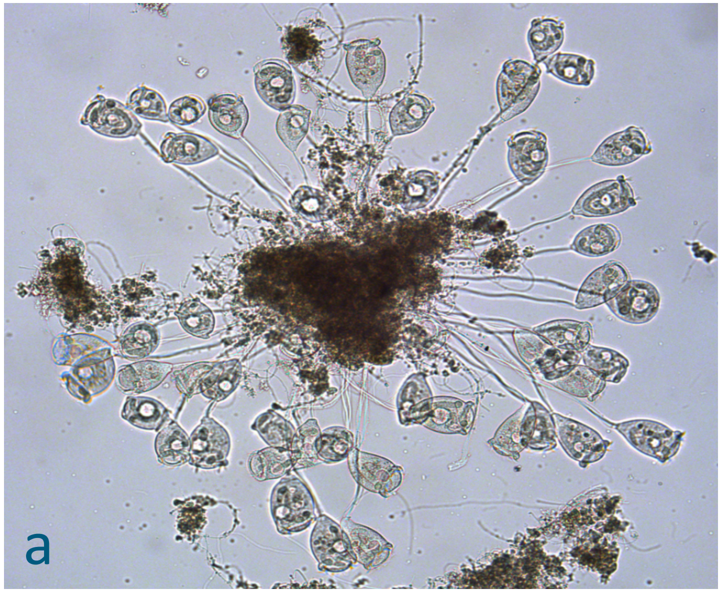
- Ciliates are indicators of effective WWTP.
- *Aspidisca* – stable WWTP conditions, good BOD, COD removal, low DO and NO3 concentration.
- *Chilodonella* – good effluent quality, low DO .

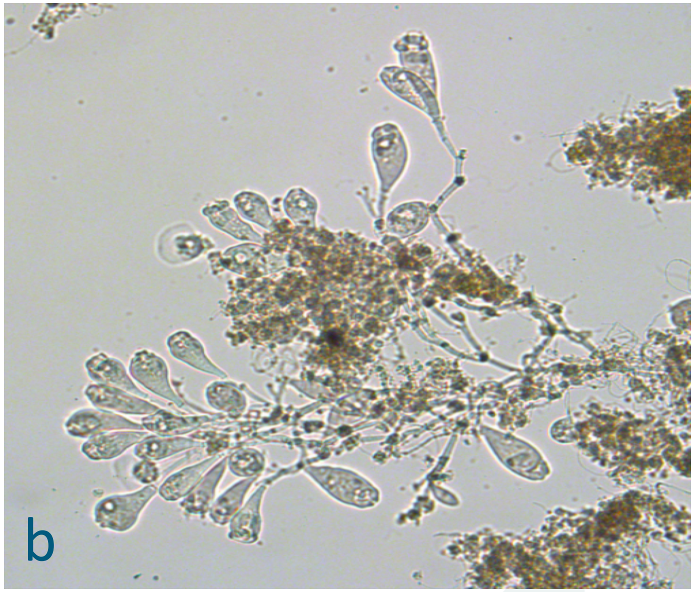
Protozoa – free swimming infusoria



- Free swimming infusoria consume dispersed bacteria;
- *Litonotus* (a)- associated with poor sludge sedimentation capacity;
- *Prorodon* - reduces the number of bacteria;
- *Glaucoma* - insufficient DO, a lot of bacteria, poor effluent quality;
- *Hemiophrys* - a parasite (controls the number of Vorticella), similar to *Litonotus*;
- *Spirostomum (b)* - resistant to low DO, an indicator of nitrogen release.
- *Stentor (c)* - a very good filtering organism, a high quality sludge indicator.

Protozoa – attached infusoria

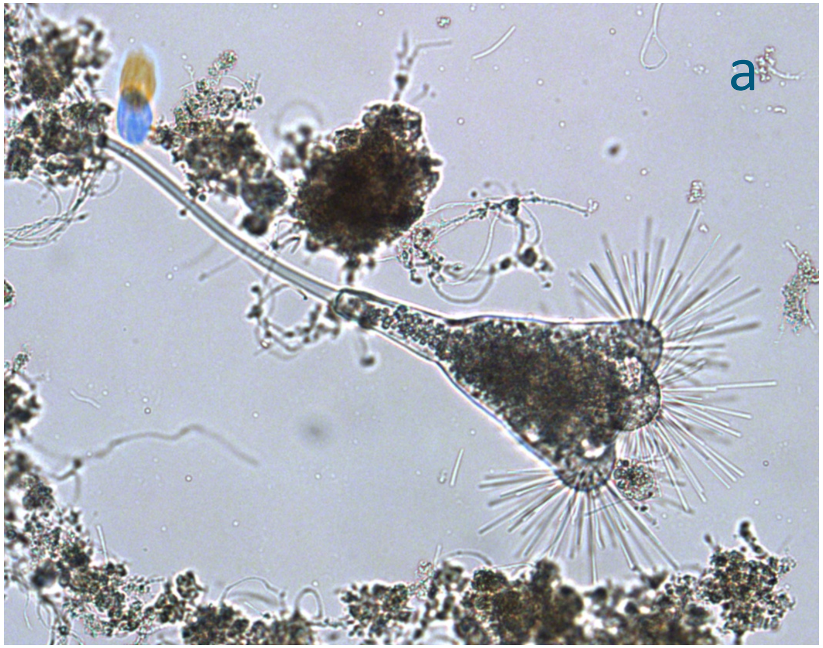




- *Vorticella microstoma* (a) - an indicator of high organic load, show good acclimatization properties.
- *Vorticella convallaria* lives in sludges with normal loads and in plants that deal well with the biodegradation of pollutants.
- *Epistylis* (b) and *Carchesium* are indicators of well nitrifying sludge and high degree of purification.

Protozoa – suckers infuzoria

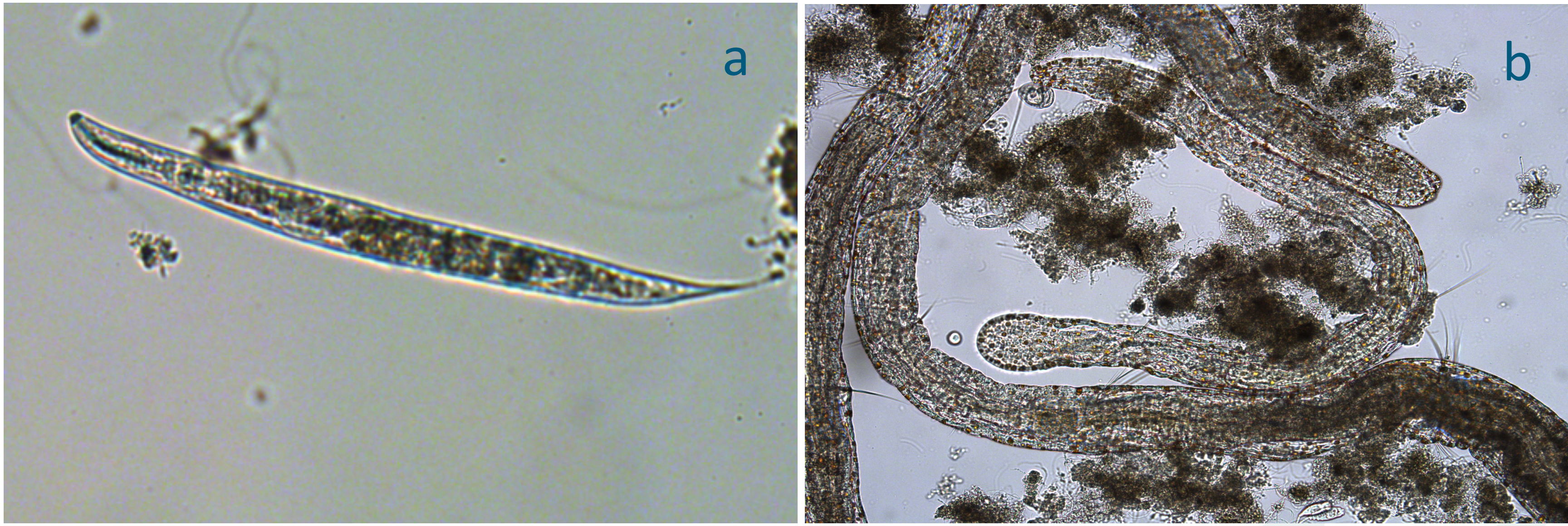
- Appears in sludge with low load, then also becomes as parasite in infusion colonies.
- *Tokophrya (a), Podophrya (b, with stalked ciliates colony), Acineta* are suckers that grow when toxic pollutants are completely degraded and indicate high quality purification.



Metazoan– lathes

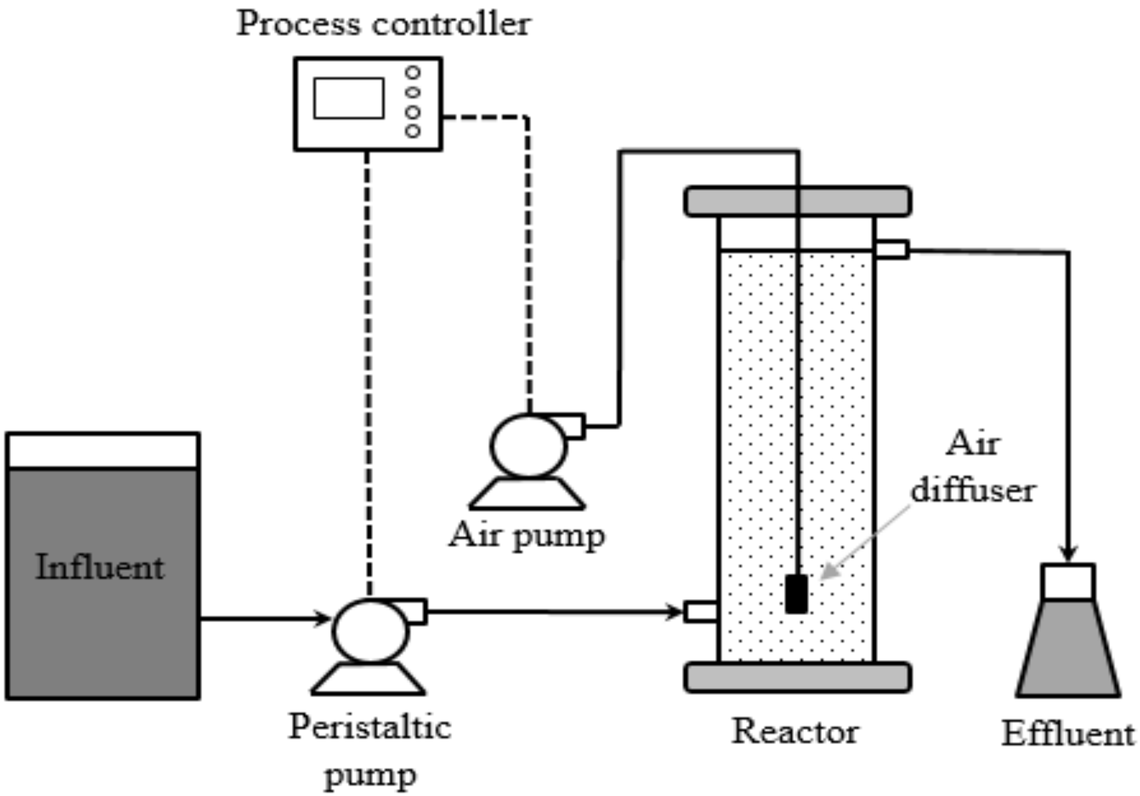
- Rotaria (a), Cephalodella (b), Nommata, Philodina) - large, more complex than protozoa;
- Feed on bacteria, suspended solids and protozoa;
- Are sensitive to changes in the environment, a large number of lathes indicates good operation of the wastewater purification process;
- Are also a good indicator of well nitrification.

Metazoan – worms



- The *Nematode* (a) lives in bioreactors with poor mixing and low oxygen concentration;
- *Aeolosomes* (b) form large colonies in well-nitrifying sludges low-load biorectors; they are associated with well-precipitated sludge and good release of suspended solids

Lab-scale tests for inhibition research used in BEST project



Sequencing batch reactors (SBR) cycle:

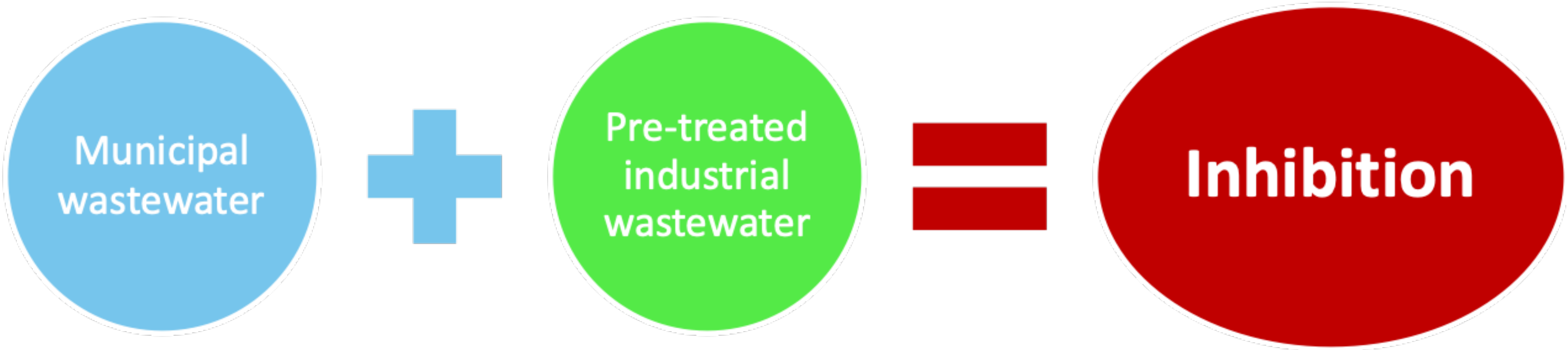
- 25 min pump in/out;
- 215 min aeration;
- 30 min sedimentation.

Sampling:

after 1 cycle when inhibitor added or 0 h contact time with sludge;
After 5 cycles or 24 h;
After 10 cycles – 48 h;
After 15 cycles– 72 h.

Denisova et al. (2018) Granular Sludge Conference, Netherlands, Delft.

Ammonia shock load



30-100 mg/l **Up to 1000 mg/l**

Municipal wastewater nitrogen concentration varies from 30 mg/l up to 100 mg/l (Henze et al., 2008).
Pre-treated industrial wastewater nitrogen concentrations can reach up to 1000 mg/l (Adam et al., 2019).

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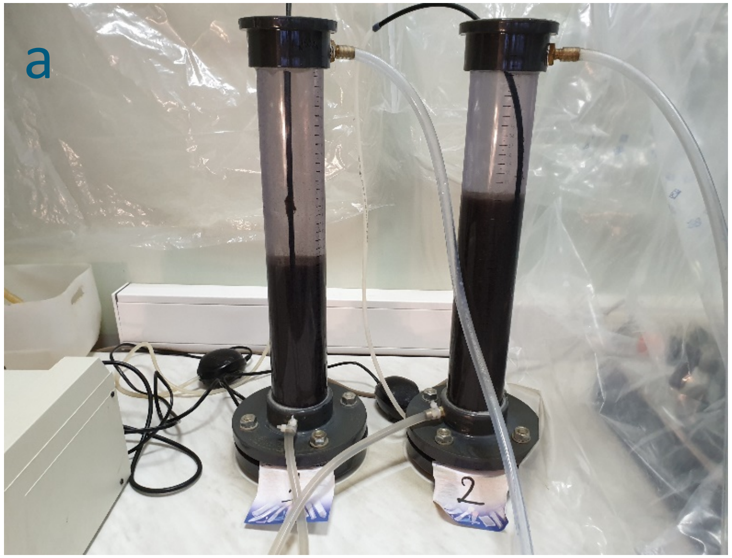
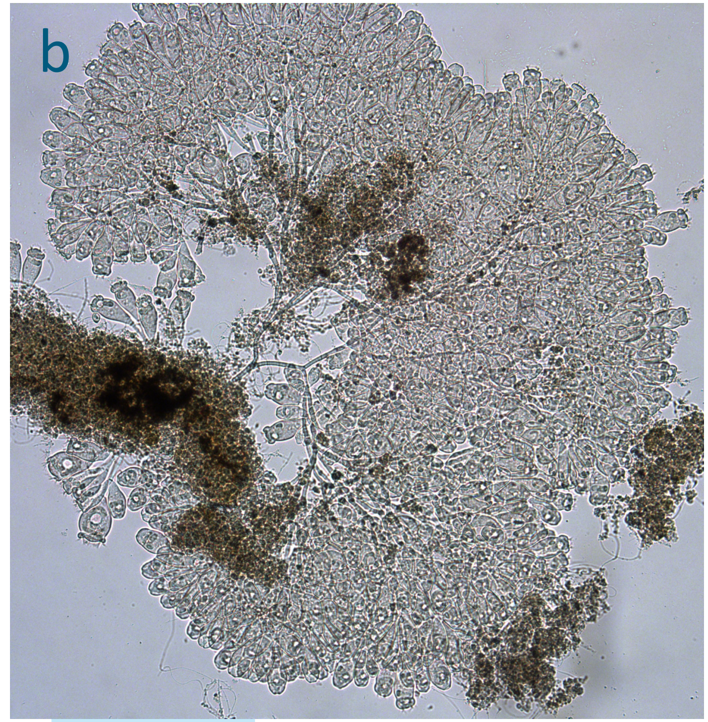
Indicators of ammonia shock load





- Inhibition of AOB process;
- decrease in inorganic carbon concentration
- decrease pH in effluent;
- increase EC in effluent;
- Increase SVI_5 (a) and *Epistylis sp*. (b) concentration in activated sludge

Take home messages:

- Regeneration of the nitrification process after the shock organic compound load – after 10-24 hours (Hamoda and Al-Sharekh, 1999).

- At the same time, if ammonia concentration increases gradually, then after 48 hours ASP is capable of resisting up to 3 times higher ammonia concentrations than it was initially recorded (Puigagut et al., 2005).

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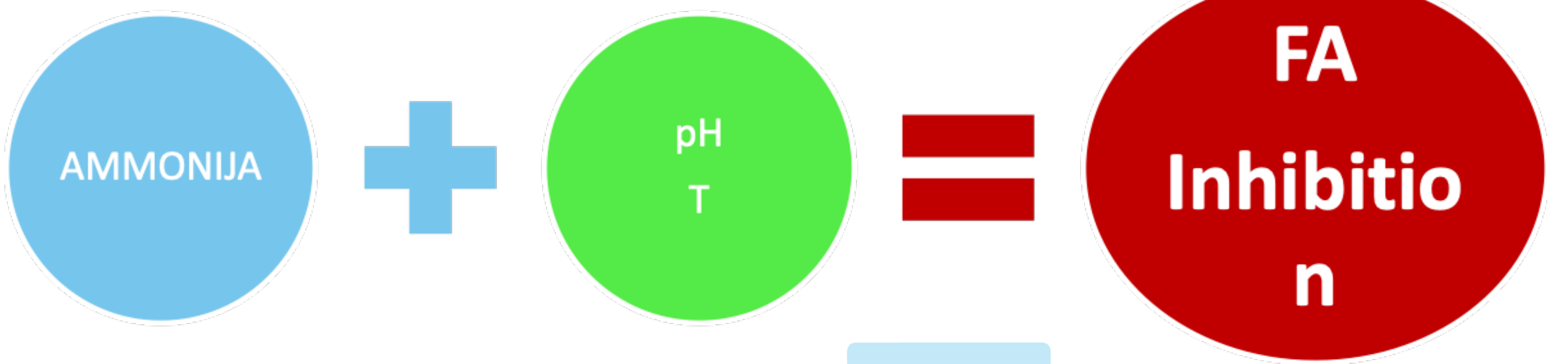
pH and Free Ammonia (FA)

Ionized (NH_4^+) and unionized (or free ammonia NH_3 – FA) ammonia equilibrium is influenced by pH level: increase of pH leads to increase of FA concentration and in alkaline conditions (pH > 7) influence is higher (Liu et al., 2019).

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$



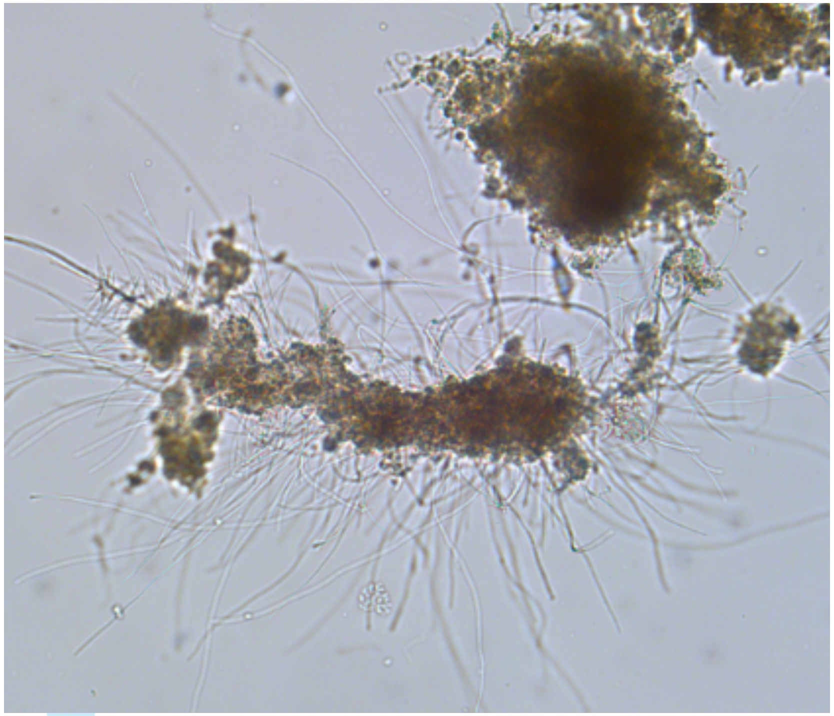
The one of the reasons of WWTP influent pH drops can be salt-sand mixtures applied to roads in winter, that contributes acidic runoff from roads.

High hydraulic rate of acidic natrium chloride runoff influences the total pH of the WWTP influent, if road runoff permeates in the sewage systems through sewage manholes (Henrikson & Brodin, 2012).

Impact of pH and Free Ammonia (FA)

- AOB (ammonia oxidizing bacteria) inhibition by FA concentration 10 – 150 mg/l (Anthonisen et al., 1976).
- NOB (nitrite oxidizing bacteria) inhibition by FA concentration 0.1 – 1.0 mg/l (Anthonisen et al., 1976).
- The range that inhibits NOB, but do not affect AOB and also provides nitrites accumulation in sludge can be 5 – 10 mg FA/l (Chung et al., 2006).
- Inorganic carbon (IC) source limitation for 4 days can be enhancing factor of inhibition for AOB according to Tora et al. (2010)

Indicators of process inhibited by high FA and high pH



(a) Filamentous sludge floccule

- Inhibition of NOB;
- Decrease concentration of NO3-N in effluent;
- High inorganic carbon concentration in the influent and effluent of reactors;
- Increase of SVI5 un SVI30 after 48h;
- Filamentous sludge bulking (a).

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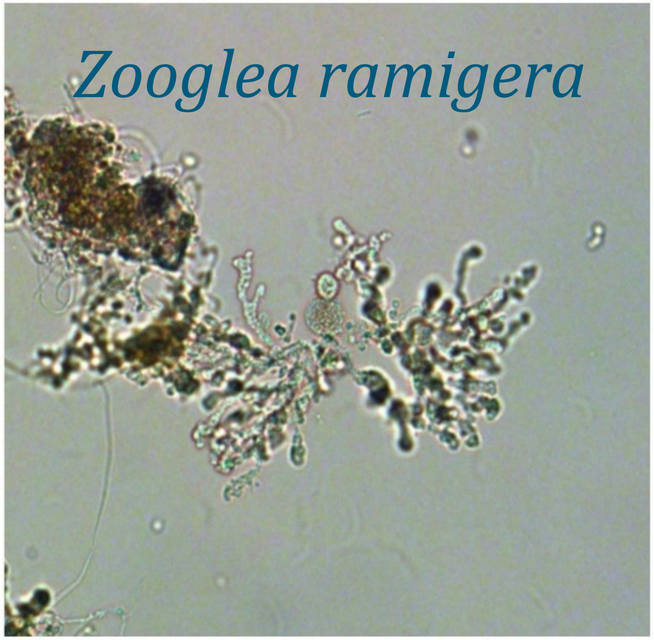
Impact of low FA and low pH factors

- **Inhibition of AOB** un **NOB;**
- NO_2-N accumulation in sludge;
- Decrease of NO_3-N concentration;
- Low inorganic carbon concentration in the influent and effluent of reactors;
- **Increase of SVI$_5$** un **SVI$_{30}$ after 48h**;
- Filamentous sludge bulking, sludge leaching, foaming, flake degradation;
- ***Zooglea*** concentration increase.

Low FA and low pH indicators



Pin-point sludge flocs condition → appearance refers to an environmental shock, that would enhance sludge microorganisms not to agglomerate and form a settleable flocs (Stypka, 1998).
Reactor had white billowy foam → foam occurs to sludge with high or too low organic loading, toxic conditions, excessive wasting, *zoogloea* high abundance (Gerardi, 2002).
Zoogloea ramigera and *Zoogloea uva* had considerable increase

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Take home messages:

- Low FA (approximately 0.1 mg/l) under pH 6.5 is more destructive for the ASP-process than high FA under pH 8.5 (approximately 10 mg/l).

- Low FA reactor sludge microfauna had no population decrease comparing with high FA reactor. Although, low FA under pH 6.5 lead to zoogloea, gymnamoebae and *Chilodonella* sp. development.

- Zoogloea high abundance influenced sludge settleability, that led to sludge wash out from low FA reactor with following organic load increased and sludge foaming.

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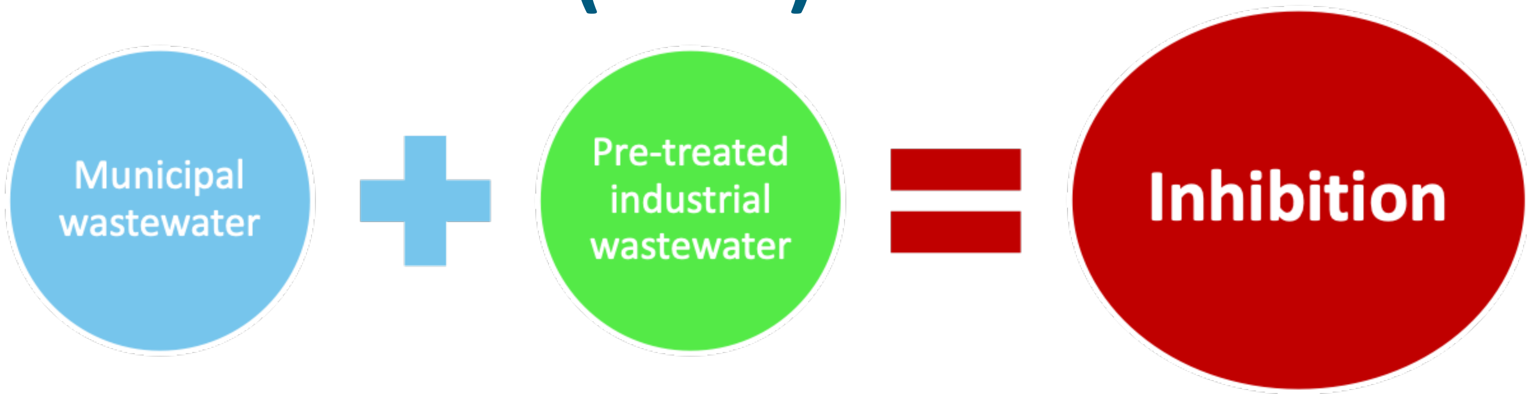
Sodium chloride (NaCl)



High concentration of NaCl is mainly caused by shortcut discard of the industrial wastewater into the municipal sewage system – for example, dairy product wastewater with NaCl 15-20% solution.

The second reason of the high NaCl concentration is salt-sand mixtures from the roads infiltrated into the sewage system. In this case, sand-salt mixture road wastewaters are acidic (Henrikson & Brodin, 2012).

High concentration of the NaCl can be distinguished by high wastewater electrical conductivity (EC). Usually, EC of the municipal and pre-treated industrial wastewater at the influent of WWTP is approximately 700 – 3000 µS/cm (Rodriguez-Sanchez et al., 2017).

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Effect: Microfauna osmotic shock

Cephalodella (before NaCl addition)

Cephalodella (after NaCl addition)

Shortcut discard of the sodium chloride (3.3 g/l one SBR-cycle) lead to AOB and NOB bacteria inhibition for 24 h.

Effluent carbon concentration (TOC and IC) and COD increases right after NaCl addition and keeps at high values for 24 h.

The most valuable indicator of the NaCl inhibitor influence is electrical conductivity in the effluent, as it increases after NaCl addition and shows sludge accumulation ability of salts for 24 h.

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Take home messages:

- AOB and NOB inhibition can be described by sludge leaching with following COD increase and degradation of microfauna
- Sludge microfauna have osmotic shock and dewatering after NaCl addition
- The most NaCl resistant microfauna genus/species are: testate amoebae *Arcella*, crawling ciliate *Aspidisca*, stalked ciliate *Epistylis*.

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