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Nitrification monitoring – Determination of bacterial groups in a biocoenosis with selective inhibition and oxygen uptake rate measurements

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Abstract

In the degradation of ammonia (NH_4^+) to gaseous nitrogen (N_2), the nitrification is one of the two reaction steps. The nitrification itself is divided in two steps and is performed by two different types of bacteria. Current literature has shown that there are types of bacteria, which have the genetic equipment to perform both steps in one bacteria. Nevertheless, in wastewater and landfill leachate treatment, ammonia-oxidizing organisms (AOO) and nitrite-oxidizing organisms (NOO) occur as a symbiosis. The intermediate of the two consecutive reaction steps (NO_2^-, nitrite) is toxic. For this reason, both steps are necessary for the two bacterial groups. To determine the ratio of AOO, NOO and heterotrophic bacteria (which use organic compounds as carbon and energy source) the oxygen uptake rate (OUR) with selective inhibition with N-allylthiourea (ATU) and azide is used. In the inflow of a pilot plant in one street a step by step increased amount of a process water out of a fermentation plant was added to the landfill leachate. For comparison, the other street was supplied only with landfill leachate with the same amount of nitrogen. As a result, comparable values for the different bacterial groups and reproducible results were measured and lead to a better understanding of the analysed nitrification sludge. Deeper understanding of the behavior of the different groups will result in a reduce risk of malfunctions and a more stable operation in the wastewater or landfill leachate treatment plant.

1. Introduction

The nitrogen pathway is very important. Although nitrogen is essential for all organisms, molecular nitrogen is difficult to assimilate. The assimilation is only possible via bacteria, the ions of ammonia (NH_4^+) and nitrate (NO_3^-) or artificial out of N_2 and H_2 via the Haber-Bosch process. Ammonia is a degradation product of wastewater and landfill leachate and leads untreated and without oxygen to eutrophication. The nitrification, the oxidizing of ammonia, can be subclassified in two different steps.

Recent research revealed an identification of organisms, who got the genetic setup to perform both steps alone [1], [2]. Nevertheless, in wastewater and landfill leachate treatment ammonia-oxidizing organisms (AOO) and nitrite-oxidizing organisms (NOO) occur as a symbiosis (Formula 1).

Ammonia oxidizing organisms (AOO)	$NH_4^+ + 1{,}5\ O_2$	→	$NO_2^- + H_2O + 2\ H^+$
Nitrite oxidizing organisms (NOO)	$NO_2^- + 0{,}5\ O_2$	→	NO_3^-
Total reaction/*Comammox*	$\boldsymbol{NH_4^+ + 2\ O_2}$	→	$\boldsymbol{NO_3^- + H_2O + 2\ H^+}$

Formula 1. Reaction steps of the nitrification with AOO, NOO and Comammox.

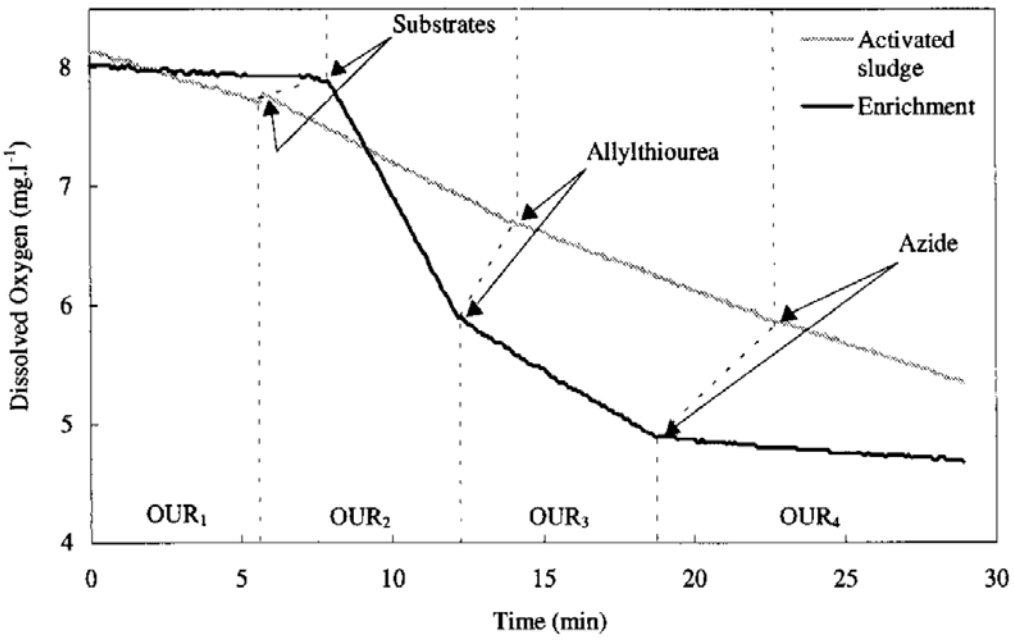

Fig. 1. Different slopes of different OUR with selective inhibition [3]

The treatment of landfill leachate is a continuous operation. The analytics rely on sum parameters such as ion concentration (ammonia, nitrite and nitrate), flow measurement, organic/inorganic dry mass and dissolved oxygen concentration. These parameters are sufficient for a stable operation, but they lack of a deeper understanding of the process. The scope of this research is, to analyse parameters, which are directly connected to the bacterial composition.

For the nitrification, oxygen is crucial. A measurement of the oxygen uptake rate characterizes the process of nitrification. In addition, with selective inhibitors, an estimation of the shares of the different bacterial groups AOO, NOO and heterotrophs is possible. In figure 1, the resulting different slopes of the OURs with inhibition are shown.

The scientific question of the experiment in general was, if the combined treatment of landfill leachate with process water out of a fermentation plant has a short-term and long-term influence on the nitrification. With the pilot landfill leachate treatment plant, a direct comparison between the two streets is possible [4].

2. Materials and Methods

For the experiments with the selective OUR, the Cenotox 2.0 of Dimatec was used. The analytical device does not use model sludge like other devices; the sludge is taken directly out of the process. With the device, an online and atline analysis is possible.

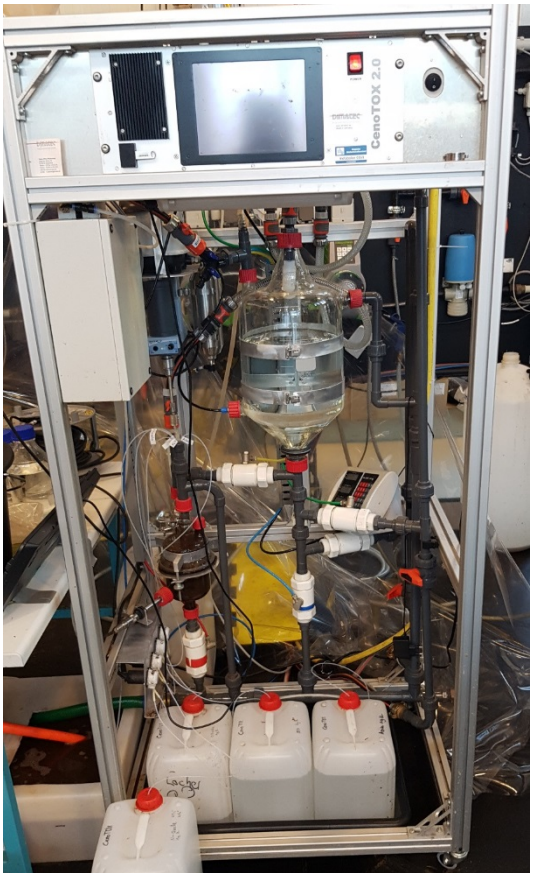

Fig. 2. Picture of the Dimatec Cenotox 2.0

Together with the company Dimatec, the device was improved with the collaboration of TH Cologne. Designed for big scale plants some changes were necessary. In the first method the measurement of all three OURs (simultaneously) lead to loss of a high amount of sludge. Hence, the analytical method was changed from online to atline. In addition, a new software is developed to enable the online measurement.

In figure 2, the Cenotox is shown. The activated sludge is aerated in the big reactor on the top of the picture. Afterwards, the aerated sludge is transferred to the measurement reactor at the bottom. The carbon source, the nitrogen source and the inhibitors are placed on the ground of the device.

According to literature [3] N-Allylthiourea and Azide were used for inhibition. In own experiments we got better results with a concentration of 120 µM ATH and Azide. In contrast, the concentrations of ATH (86 µM, [3]) and Azide (24 µM, [3]) were different in literature. The monitoring was started at day 280 of the experiment. Before and during the whole experiment, the ratio of process water was increased step by step. At day 480 of the experiment, the activated sludge of the control street was disposed and activated sludge from the other lane was transferred in the emptied street.

3. Results & Discussion

The online setup of the Dimatec Cenotox 2.0 is shown in Fig. 3. The red arrows indicate the bioreactor 1-1 (left side) and 2-1 (right side). With blue arrows and lines, the sampling location of the bioreactors and the piping is shown. On comparison with atline measurement, online measurements required 4 times more sludge. Therefore, the measurements were performed in atline mode instead of online mode.

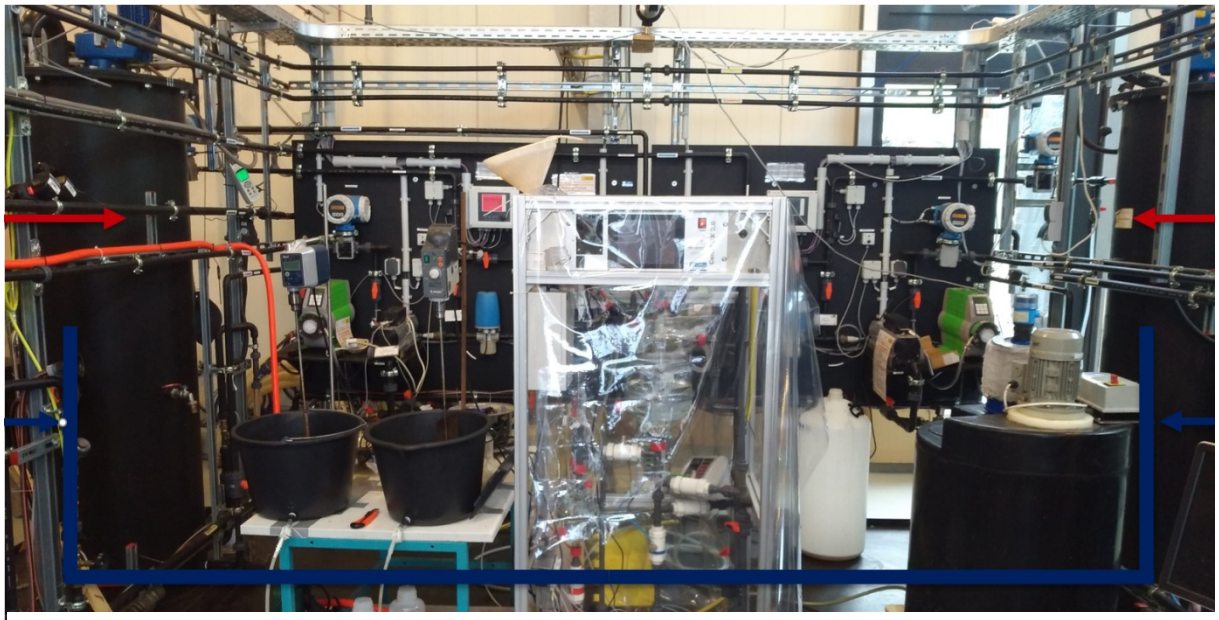

Fig. 3. Dimatec Cenotox 2.0 implemented in the semi-technical scale

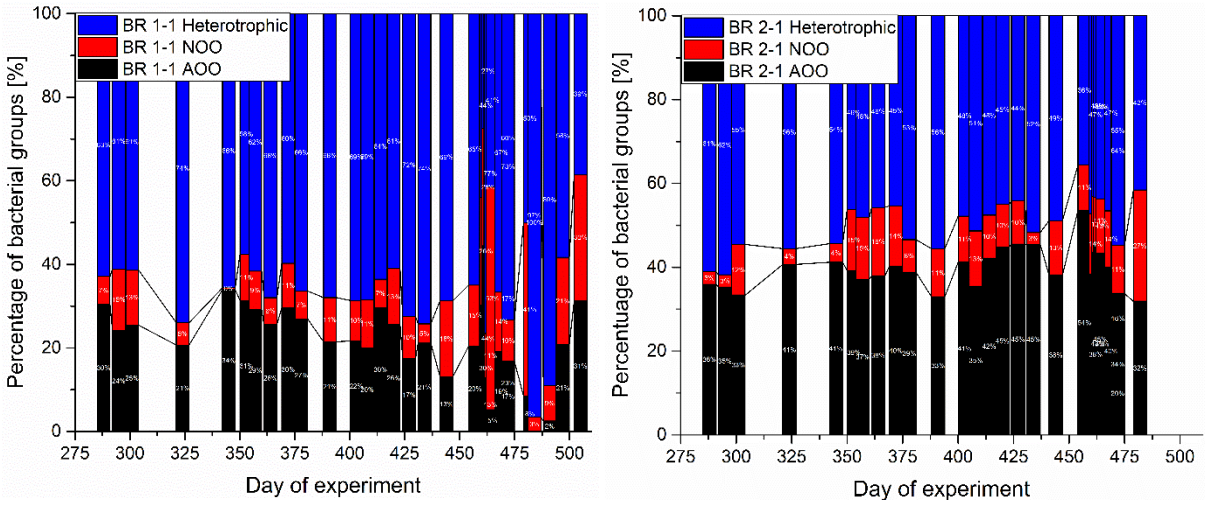

Fig. 4. Results of AOO, NOO and Heterotrophic bacteria in BR 1-1 (left) and BR 2-1 (right)

In Fig. 4 the results of the measured OURs are shown. Over the whole experiment, on average the share of AOO and NOO is reduced in the street with process water. Nevertheless, the measured values are stable within a range of ± 10 %. This result is, due to

the increased ratio of process water during the investigated period, remarkable. As a first result, the used concentrations of process water is not toxic for the nitrification and a stable operation of the nitrification step is possible. In addition, the transfer of the sludge from street one in street two and the stress for the biocoenosis is in comparison with the measured values comprehensible. For this reason, as a second result of the experiments, interruptions and malfunctions of the continuous process are reflected in the measured OURs.

Conclusions

The selective OUR is a suitable tool to determine the activity of the bacterial groups for nitrification. The Cenotox 2.0 was successfully customized for the pilot plant. The nitrification was monitored over 250 days and the control of a stable nitrification. No short-term or long-term toxic effect on the biocoenosis was observed. In addition, mechanical stress for the activated sludge could be comprehended in the measured values of the OUR.

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