The adaptation of nitrifying microorganisms to inhibiting substances at meso- and psychrophilic temperature conditions

Conrad Marx, Markus Ahnert, Peter Krebs and Volker Kühn

ABSTRACT

This paper deals with adaption capacity of nitrifiers to allylthiourea (ATU) as a model inhibitor at two temperature levels. Nitrifying communities were developed at 15 (BM15) and 30 °C (BM30). The activity of the nitrifiers was determined by using short-time respiration (STR) tests, oxygen monitoring and *in-situ* measurements. The oxygen monitoring provided information about the temperature-dependent time delay between the dosage of NH_4^+-N or ATU and reaching its characteristic level of effect. The greatly scattered results from the STR tests for BM15 were thus explained by the time delay, which was two to three times higher than for BM30. Furthermore, combining the results of oxygen-monitoring and *in-situ* measurements it can be stated that an adaption to ATU at psychrophilic temperature conditions was not achieved, whereby up to 40% of nitrification was sustained for BM30 at an ATU-concentration over 7 mg/l. The nitrification by BM15 did not start until ATU was degraded to 1–2 mg/l, the typical inhibition concentration for ATU. Hence, the results indicate a population drift to adapted nitrifiers at mesophilic conditions and ATU-degrading microorganisms at psychrophilic temperature conditions, which can have a considerable influence on domestic wastewater treatment in cold climates receiving industrial effluents.

Key words | adaptation, allylthiourea, incubation, inhibition, nitrification, temperature

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NOTATION

$c_{O_2,inh}$	oxygen concentration after addition of ATU [mg/l]
$c_{O_2,nitr}$	oxygen concentration at maximal nitrification [mg/l]
$c_{O_2,sat}$	saturation concentration of dissolved oxygen [mg/l]
$MLSS_E$	mixed liquor suspended solids effluent [g/l]
$MLSS_R$	mixed liquor suspended solids in the reactor [g/l]
SRT_i	sludge retention time [1/d]
V_R	reactor volume [m³]
V_{Sa}	sample volume [m³]
OUR_{max}	oxygen uptake rate after addition of NH_4Cl
OUR_{inh}	oxygen uptake rate after addition of ATU

INTRODUCTION

Fertilizers are applied to cultivated soils in order to perpetually supply crops with sufficient growing media, e.g. nitrogen. Ammonium (NH_4^+) has a good adsorption capability and therefore remains in the upper layer of soil for a long time. The microbial oxidation (nitrification) of NH_4^+ leads to nitrate (NO_3^-), which adsorbs poorly onto soil and easily dislocates vertically. Inhibitors are used to minimize the loss of fertilizer in order to prevent nitrification of reduced N-compounds on the field.

Agricultural fertilizer is passed into surface water bodies due to storm-water induced runoff; fertilizer and inhibitor as one of the main components end up in surface water bodies where they cause, among other things, two negative effects: a rise in ammonium concentration and the inhibition of its natural conversion. The approach of the overarching investigation is a systematic, permanent or temporary discharge of adapted nitrifiers from wastewater treatment plants (WWTPs) to support natural nitrification along with microorganisms that are able to

doi: 10.2166/wst.2013.227

degrade the inhibitor itself. In the case of runoff events going along with the input of fertilizer, adapted microorganisms would already be present in the water body in order to reduce inhibition effects by sustaining nitrification or degrading the substance of interest. The reject water treatment/storage tank at WWTPs hereby constitutes a suitable location for growing nitrifying organisms due to high ammonia concentrations (Salem *et al.* 2004; Krhutkova *et al.* 2006). In practice, different types of bioaugmentation technique have proven successful (BABE® (Salem *et al.* 2003; Henze *et al.* 2008), inNITRI® (Kos 1998; Henze *et al.* 2008), BAR® (Henze *et al.* 2008)) utilizing diverse ratios of return sludge and sludge water to achieve inter alia more favourable growing conditions for nitrifiers by configuring the growing temperature. To investigate the influence of mesophilic and psychrophilic incubation on the adaption of microorganisms to allylthiourea (ATU), biomass of two reactors was acclimated to 15 and 30 °C. ATU was chosen to be the initial substance because of its status as a well-researched nitrification inhibitor (Hall 1984; Iizumi *et al.* 1998; Cui *et al.* 2005). Results may therefore be discussed better than for other less investigated inhibitors. ATU rates among noncompetitive inhibitors and its inhibiting characteristic towards the microbial conversion of ammonium is suggested to derive from its high affinity to metals. Bédard & Knowles (1989) proposed the existence of a binuclear copper site being involved in the catalytic cycle (Figure 1) of ammonia monooxygenase (AMO), which is the responsible enzyme for the oxidation of ammonium to hydroxylamine. The binuclear copper in its reduced state can be bound by ATU and other thioureas, which hinders its original function of providing oxygen for ammonium conversion.

However, the efficiency of WWTPs is maintained at higher rates compared to unstressed sludges due to selective and coincidental adaptation of biomass populations (Grunditz *et al.* 1998; Jonsson *et al.* 2001). A large variety of studies regarding the adaptation to inhibitors, e.g. heavy metals (Rusk *et al.* 2004), halogenated hydrocarbons (Nevalainen *et al.* 1993), thiourea (Xiong *et al.* 1998) and salt (Somville 1984) show the possibility of adaptation without purposefully taking the temperature influence into account. Irigoyen *et al.* (2003) found out that temperature is strongly influencing the time of extending nitrogen presence in soils using dicyanidamide and 3,4-dimethylpyrazole phosphate. An increase in temperature from 10 to 30 °C within a short-time range reduced the effect of inhibitory substances by almost 50% and thus needs to be considered in warm climate areas. Due to the growing importance of chemicals being part of wastewater and to extend the view on influencing factors of inhibition, the temperature history of nitrifiers regarding their adaption capacity was investigated.

METHODS

Reactor configuration and operation

Two double-walled glass tanks with a working volume of 10 l each were used for adaptation studies. Each batch reactor was equipped with a stirrer, air diffusers at the bottom, a dosing station for pH-control and a temperature unit (Figure 2). The temperature was set to 30 °C for the reactor containing nitrifying biomass incubated at 30 °C (BM30) and to 15 °C for the reactor containing nitrifying biomass incubated at 15 °C (BM15).

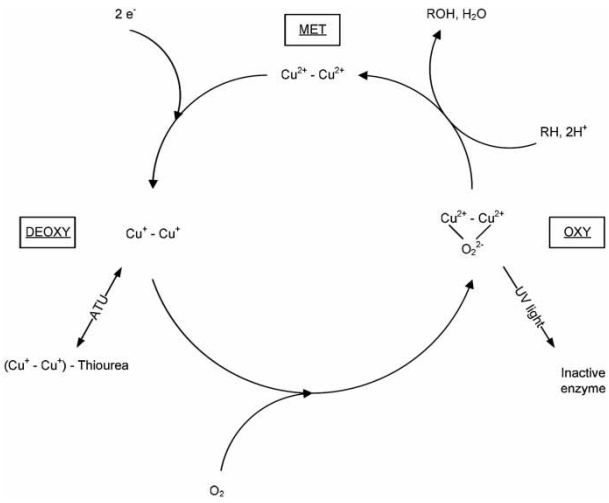

Figure 1 | Catalytic cycle for ammonia monooxygenase (Bédard & Knowles 1989).

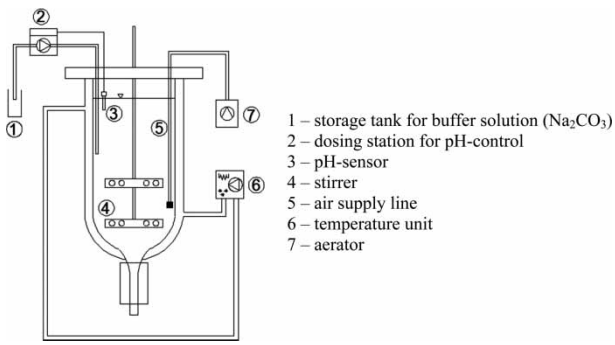

Figure 2 | Reactor configuration.

In order to grow a large population of nitrifiers, reactor operation started with a daily increase of ammonia load up to 1,600 mgNH_4^+/d (160 mg/l). Subsequent to reaching a stable nitrification at this NH_4^+ load, ATU addition started as shown in Figure 3. Due to its lower operation temperature and subsequent minor growth of nitrifiers at 15 °C, BM15 lagged by about 3 months to reach the point of ATU addition. The daily dosage of ATU was increased stepwise (Figure 3), depending on the respective biomass and its ability to fully convert the given ammonia. To be responsive to incomplete NH_4^+-conversion, ATU addition needed to be interrupted temporarily. An average increase of 1.6–1.8 mgATU/(l · month) was achieved in this study without significantly disturbing the nitrification process. Total adaptation time adds up to 7 and 8 months for BM15 and BM30, respectively.

In December 2009, nitrification almost completely failed for BM30 and ATU-, and NH_4^+-addition had to be decreased in the long term. At the point of carrying out the experiments, initial NH_4^+-concentrations were 160 and 120 mg/l for BM15 and BM30. Due to the incident of nitrification failure and the potentially necessary time to restore the point of origin (13 mg/l), the ATU concentration to be investigated for both biomass was set to 7 mgATU/l.

At the time of testing, mixed liquor volatile suspended solids (MLVSS), an indicator of biomass available in the system, was 1.1 and 1.0 g/l for BM15 and BM30, respectively. According to additions of 1.6 and 1.2 gNH_4^+/d, sludge loads result to 0.15 and 0.12 gNH_4^+/(gMLVSS · d). The oxygen concentration of both reactors was adjusted at >4 mg/l. A replacement of the aeration diffusers every 4–8 weeks prevented clogging and ensured sufficient air supply.

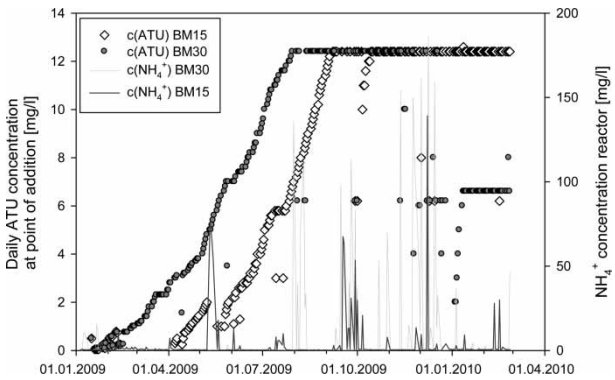

Figure 3 | Stepwise increase of ATU concentration for BM15 and BM30 and their respective ammonia concentration at the end of the day and before the next NH_4^+ addition.

To guarantee a sufficient supply with micro elements, 1 l of supernatant was exchanged weekdays by wastewater withdrawn after primary sedimentation of the WWTP of Dresden-Kaditz, Germany. Samples for influent NH_4^+-N, NO_2^--N and NO_3^--N were taken at least 4 days a week, effluent NH_4^+-N, NO_2^--N, NO_3^--N and ATU concentration were determined daily, dry matter and organic dry matter were analysed 3 days a week.

$$\mathrm{SRT}_i = \frac{V_\mathrm{R}\ \overline{\mathrm{MLSS}_{\mathrm{R}i}}}{\frac{\sum_{i=1}^{28} V_{\mathrm{Sa}_i}}{28}\ \overline{\mathrm{MLSS}_{\mathrm{R}i}} + \frac{5}{7}\ 0.1\ V_\mathrm{R}\ \mathrm{MLSS}_{\mathrm{E}_i}} \quad (1)$$

During the operation, a large number of respiration tests were carried out, which also led to significant losses of biomass. The additional waste of biomass by discharging the supernatant has to be taken into account as well. To include samples and effluent as main losses, Equation (1) was used for calculating the solid retention time. Samples ($V_{\mathrm{Sa},i}$) consist of daily sampling for reactor control (100 ml, weekdays) and irregularly conducted respiration tests. Due to the fact that excess sludge removal was realized through sampling only, a high sludge retention time (SRT) of 60–80 d was reached. A 28 d average of sludge removal (samples) was used for calculation, the mean sludge age for BM15 and BM30 is 69 and 73 d, respectively. Since the study focused on the adaption of nitrifiers in general, high SRT did not play a decisive role as the microbial growth constituted a minor part of interest.

Dosages

Adaptation was carried out after having reached steady-state conditions; the nitrifying biomass BM15 and BM30 was tested on their adaption to ATU. Due to operation problems with the reactor of BM30, the daily addition of ATU needed to be dropped from 13 to 7 mg/l. The latter states the concentration to be investigated.

On weekdays, 1 l of supernatant was exchanged with presettled wastewater from the WWTP Dresden-Kaditz. The main characteristics of the feed are to be seen in

Table 1 | Characteristics of the wastewater after primary sedimentation from the WWTP Dresden-Kaditz

Chemical oxygen demand (COD) [mg/l]	Total Kjeldahl nitrogen (TKN) [mg/l]	P_{total} [mg/l]	PO_4-P [mg/l]	pH-value
468.0	57.7	7.4	4.3	7.7

Table 1. After the wastewater had been withdrawn from the fridge it was stored for about 5 h at room temperature before being added to the reactors. That way the impact on the warm and cold reactors was negligibly small, with a drop of only 1 K after addition to the 30 °C reactor and a rise of 0.5 K after addition to the 15 °C reactor. To top up the ammonium concentration, NH_4Cl was used.

For pH-control, sodium carbonate was used. The average value was pH 8.0, which is within the optimum range for nitrifiers, between pH 7.5 and 9.0 (Zhu & Chen 2002). Still, the high temperature and ammonium concentrations of 160 mg/l make the pH value a crucial factor due to ammonia formation. However, a mass balance was carried out and a limitation of nitrification because of ammonia was not observed. The addition of tap water counterbalanced any accruing water losses.

Analytical methods

The chemical analyses of NO_3^--N, TKN, COD, mixed liquor suspended solids (MLSS) and MLVSS were performed according to the national and international standards DIN38405 (part 9), EN25663 and DIN38409 (part 2). NH_4^+-N and NO_2^--N were analysed by using the Merck kits Spectroquant 14752 and Spectroquant 14776, respectively. The analysis of ATU was carried out by a spectrophotometric method described in Richmond *et al.* (1976).

Determination of inhibition and time delay

The inhibition curve of ATU was generated at 20 °C (Figure 4) using short-time respiration (STR) tests. The sludge utilized (MLVSS of 2 g/l, MLSS of 3 g/l) originated from the WWTP of Dresden-Kaditz and was previously aerated for 24 h to attain endogeneous respiration as initial state. The inhibition of BM15 and BM30 determined by STR tests only included measurements of the nitrification rate after NH_4Cl dosage and after the addition of NH_4Cl and ATU ($1\text{-}OUR_{inh}/OUR_{max}$). Endogenous respiration was not considered due to its negligible influence on the maximum rate. STR were started 60 min after adding NH_4Cl or ATU. The duration of STR varied between 5 and 15 min due to different oxygen uptake rates in the range of 10 and 100 $mgO_2/(l \cdot gMLVSS)$ (activated sludge from Dresden-Kaditz and BM15/BM30, respectively) and temperature dependent oxygen saturation concentration between 7 and 10 mg/l. Following interpolation according to methods by Langmuir–Hanes (Langmuir 1918; Hanes 1932) and Michaelis-Menten (Michaelis & Menten 1913) resulted in typical median effective concentrations ($EC_{50} = 50\%$ inhibition) for ATU of 0.94 (L–H) and 1.28 mgATU/l (M–M), respectively (Figure 4). Still, comparing the measuring point for complete inhibition at 5 mgATU/l with the inhibition curves of M–M and L–H a discrepancy up to 20% can be seen. Hence, considering measuring points above 50% an approximation in the form presented in Figure 1 ($R^2 = 0.9857$) is credible here to describe the inhibitory effect more precisely. Calculated EC_{50} values are unaffected by either types of regression curves. EC_{50} values in the literature range from 0.2 to 0.8 mgATU/l (Jonsson *et al.* 2001) and 1.2 mg/l (Konig *et al.* 1998) to 1.3 mg/l (Koenig *et al.* 1999). Determined EC_{50} values are within the range found in the literature and can be regarded as comparable to other investigations.

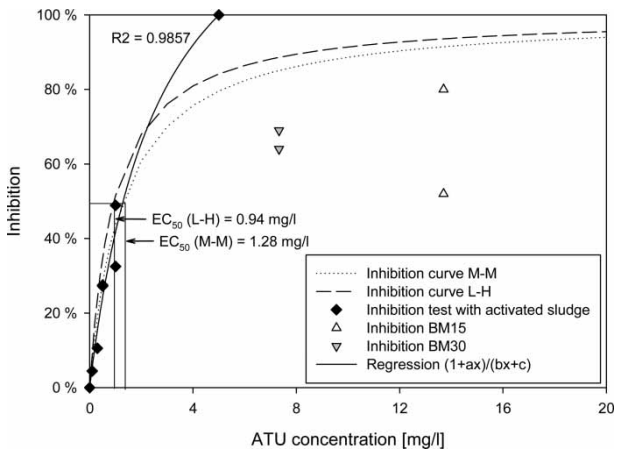

Figure 4 | Inhibition curves of ATU and adaption results of BM15 and BM30.

To investigate the variation of inhibition over time, oxygen-monitoring (Figure 5) and *in-situ* measurements of NH_4^+-N and ATU (Figure 6) were supplementarily carried out. The evaluation of the *in-situ* measurement was carried out using concentrations. There was a need to standardize the experiments with and without the addition of preclarified wastewater to make them comparable. The objective was to observe bacterial responses to the addition of preclarified wastewater, NH_4Cl and ATU, as well as regarding their time delay of oxygen uptake and ammonium/ATU conversion. The time delay was defined as time range between ATU-addition and reaching a maximum of dissolved oxygen (see Figure 5: 3A). The occurrence of the oxygen curve is based on the oxygen-input, influenced by air supply and reactor configurations (k_{La}), and the oxygen-output, by respiration and surface losses. The input value strongly depends on k_{La}-value, thus, due to coincidental

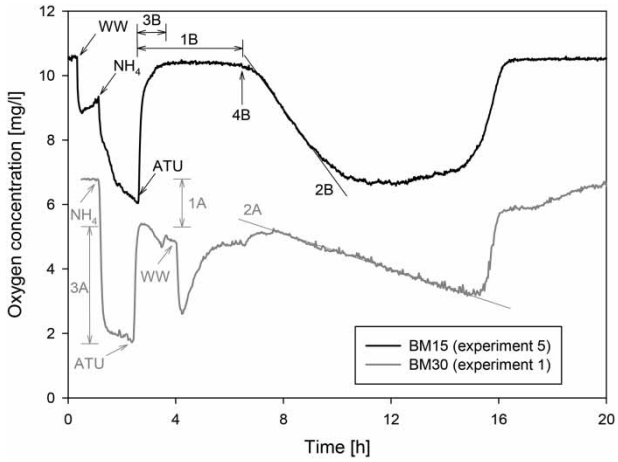

Figure 5 | Oxygen-monitoring of BM15 and BM30. Additions: WW – wastewater; NH_4^+ – ammonium chloride; ATU – Allylthiourea; 1A – oxygen gap between saturation and its respective concentration after ATU- and NH_4^+-addition; 2A – steadily decreasing oxygen level due to increasing activity of nitrifiers (BM30); 3A – difference of oxygen levels before and after ATU-addition; 1B – inhibition plateau at saturation level; 2B – steadily decreasing oxygen level due to increasing activity of nitrifiers (BM15); 3B – time delay for complete formation of inhibition after ATU-addition; 4B – starting point of decreasing oxygen concentration at ATU-concentration of 1–2 mg/l (see Figure 6).

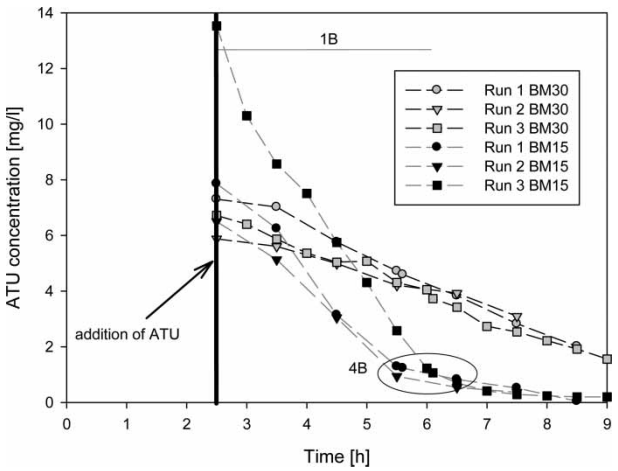

Figure 6 | ATU degradation by BM15 and BM30.

differences between the reactors, a comparison between the curves of BM15 and BM30 is limited. In addition to the inhibition level, the evaluation of the oxygen-monitoring also focused on the increase in oxygen consumption at diminishing inhibition expressed by a negative slope (see Figure 5: 2A and 2B). The inhibition estimated based on oxygen-monitoring was calculated by applying Equation (2).

$$I_{O_2-\text{monitoring}} = \frac{c_{O_2,\text{sat}} - c_{O_2,\text{inh}}}{c_{O_2,\text{sat}} - c_{O_2,\text{nitr}}} \quad (2)$$

Community analysis

The active side of the enzyme ammonia monooxygenase is encoded by the amoA gene, which can be amplified using the polymerase chain reaction (PCR). In addition to ammonia oxidizing bacteria (AOB), the analysis was extended to ammonia oxidizing archaea (AOA), a domain of single-celled microorganisms, which are supposed to hold significant share in natural nitrification (Purkhold 2003). The DNA was extracted using the MO BIO UltraClean DNA Isolation Kit. In order to execute a subsequent terminal restriction fragment length polymorphism (T-RFLP) both labelled and non-labelled primer (Table 2) were used for PCR to amplify the amoA gene of AOB and AOA. Amplification was conducted in 25-µl reactions with the PCR Core Kit (QIAGEN) and the following concentrations:

17.375 µl	Bidestilled water
2.5 µl	Buffer
1.5 µl	MgCl
0.5 µl	dNTP
1.0 µl	amoA 1F-FAM (AOB) or amoA19F-FAM (AOA) (20 pmol)
1.0 µl	amoA 2R (AOB) or amo643R (20 pmol)
0.125 µl	Taq-Polymerase
1.0 µl	Template (dilution: 1:10)
25.0 µl	TOTAL

The PCR was performed under the following settings: initial denaturing step for 5 min at 95 °C followed by 30 cycles of denaturing (1 min at 95 °C), primer annealing (1 min at 53 °C) and amplification (1.5 min at 72 °C) using a PTC-250 thermocycler. Completion lasted 15 min at 72 °C and final cooling was conducted at 8 °C. The PCR products were checked for correct sizes and concentrations on a 1.5% agarose gel and digested overnight at 37 °C with HaeIII (New England Biolabs).

The samples were precipitated and purified with ethanol (1/10 volume of 3 M sodium acetate, pH 5.5 and 2.5 volume of ethanol), spiked and dried with 20 µl Hi-Di (Applied Biosystems) and 0.4 µl ROX as standard (Applied Biosystems). The samples were incubated for 5 min at 95 °C for

Table 2 | Primers used for PCR

	Bacteria	Archaea
Labelled	Bac-amoA-1F-6FAM	Cren-amoA-19F-6FAM
Non-labelled	amoA-2r	Cren-amoA-643R

denaturation and cooled down on ice for 5 min. Subsequently, 2 μl each were loaded onto an ABI PRISM 3100 sequencer (Applied Biosystems) using POP6 as matrix in a 36 cm capillary and an injection time of 15 s. For data processing, GeneMapper (Applied Biosystems) was used. Jaccard similarity coefficients, which interpret the presence/absence data were calculated using the paleoecology statistics freeware package, PAST (Hammer *et al*. 2001).

RESULTS AND DISCUSSION

At the investigated ATU concentrations of 13 and 7 mg/l, nitrification was identified by STR tests (see Figure 4) which indicate successful adaptation of the nitrifying biomass BM15 and BM30 at 15 and 30 °C, respectively.

Evaluating the curves for BM15 and BM30 in Figure 5 shows a different picture and requires the distinction between two main characteristics. Firstly, after ATU-addition the curve of BM15 returns to the saturation concentration and remains there for 3–4 h (see Figure 5: 1B) while BM30 maintained a certain part of its previous oxygen consumption rate (see Figure 5: 1A). Secondly, for BM15 the decrease of oxygen level, which in turn means an increase of activity of nitrifiers, proceeds at a higher rate than for BM30 (see Figure 5: slopes 2A and 2B). Table 3 summarizes the information provided by the oxygen-monitoring; inhibition values of BM15 and BM30 were calculated using Equation (2), exemplified with

$c_{O_2,sa} - c_{O_2,inh} = 1A$ and

$c_{O2,sat} - c_{O2,nitr} = 1A + 3A$ for BM30.

Except for one measurement, the obtained data are close together, between 96–97 and 71–79% inhibition for BM15 and BM30, respectively. The within-series values of absolute inhibition scatter significantly, while the percentages of experiments 1, 2, 4 and 5, 6, 7 vary only slightly. This discrepancy was observed despite the fact that reactor conditions and measuring method, which have influence on, for example, the oxygen supply, were not changed during the experiments. Further, experiment 4 exhibits a significant difference in time delay and the widely diverse slopes of declining curves (see Table 3) throughout experiments 1–4 which suggest a strong influence of the 'form on the day' of participating microorganisms. The slopes and time delays of BM15 do not show such a variation, which might be explained according to the lower incubation temperature and thereby to a greater continuity of the microorganisms in terms of ATU degradation.

The test results for inhibition of BM30 using STR tests and oxygen-monitoring differ only by about 10%. The significant deviation for BM15 (>40%) can be explained by the fact that characteristic inhibition requires at least 80–90 min to take shape. Diversified sample taking within this time frame leads to enormous variation in oxygen consumption and hence inhibition calculation (Figure 4). In case of BM30, time delay did not cause this error because characteristic inhibition, in the majority of cases, had already been developed before collecting the samples. This observation suggests that the use of ATU in connection with the determination of carbon respiration via STR tests is not as trivial at low temperatures.

During the first 3–4 h of sampling (Figure 6: 1B) ATU conversion rate was three times higher for BM15 than for BM30 (Figure 6). The subsequent drop of ATU-degradation by 90% occurred after the ATU concentration fell below 1–2 mg/l (Figure 6: 4B). ATU removal by BM30 remained constant and exceeded the sampling time by about 2 h.

Table 3 | Results of the oxygen-monitoring

Experiment	ATU-concentration [mg/l]	Inhibition [mg O_2/l]	Inhibition [%]	Time delay ATU [min]	Slope of declining curve [mg O_2/(l · h)]
BM30		**(e.g. 3A in Figure 5)**			**(e.g. 2A in Figure 5)**
1	7.3	3.57	72	22	– 0.290
2	7.3	4.18	71	24	– 0.466
3	7.3	2.25	38	26	– 0.614
4	7.3	2.75	78	38	– 0.381
BM15				**(e.g. 3B in Figure 5)**	**(e.g. 2B in Figure 5)**
5	12.8	4.28	96	84	– 1.098
6	12.8	3.25	97	86	– 1.169
7	12.8	2.54	96	82	– 1.098

For both cultures, BM15 and BM30 NH_4^+-conversion accelerated after 3–4 and 2 h, respectively, regardless of whether an addition of preclarified wastewater took place or not. For comparison, five out of six experiments started off with an approximate concentration of about 7 mgATU/l. One experiment using BM15 was carried out applying an initial ATU-concentration of about 13 mg/l. The deployed concentration corresponded to the daily dosage at this time (Figure 3) and represents the actual state of adaptation of BM15 (see Reactor configuration and operation). The degradation speed was slightly higher than in the other experiments using BM15 and dropped after 3.5 h when a concentration of about 1 mgATU/l was reached. The higher daily dose of ATU given to BM15 might be the reason for its higher degradation potential. Induced growth and hence higher abundance of bacteria being able to use ATU as substrate led to enhanced detoxification and allowed nitrification to occur at 'normal' EC_{50}.

Calculation of inhibition using Equation (2) at time points of measured ATU concentration leads to respective inhibition curves representing the actual state of adaption of BM15 and BM30 (see Figure 7). As can be seen, data obtained from oxygen monitoring are quite suitable to describe inhibition behaviour of biomass in batch systems. Values from oxygen-monitoring and STR agree well for BM30, whereas results for BM15 differ significantly due to the time delay after adding ATU. Compared to EC_{50} calculated by M–M and L–H with unadapted sludge from the WWTP Dresden-Kaditz, it can be assumed that affinity to ATU for both biomasses (EC_{50}(BM15) and EC_{50}(BM30)) significantly rose during the adaptation process.

The results of T-RFLP archaea analysis did not show any signals for BM15. For BM30, only one operational taxonomic unit of archaea was found at 555 base pairs (bp). AOB were present in different abundances and diversity in both cultures, which suggests that bacteria are mainly responsible for ammonia conversion in an ATU-influenced environment. The predominant peaks in the T-RFLP profile of BM15 were 60 and 115 bp and those of BM30 were 65, 455, 480 and 490 bp. Even though the larger number of predominant peaks was found for BM30, BM15 shows the higher diversity due to many subdominant short-chained signals. All signals above 450 bp disappeared in the T-RFLP profile of BM15, which leads to the assumption of an incapability of these strains to grow under psychrophilic incubation conditions. Jacaard similarity coefficients for AOB showed values below 0.15, which underlines the diversity difference between the cultures.

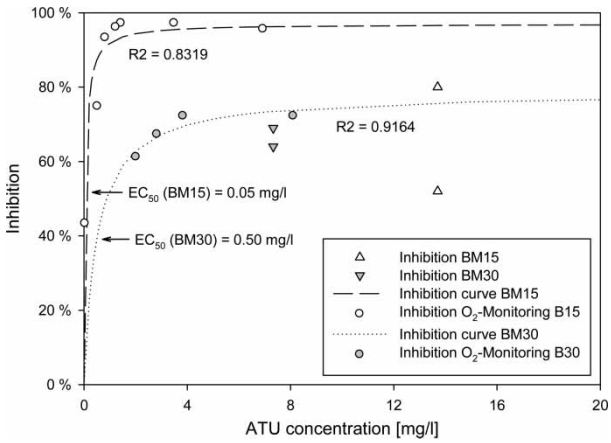

Figure 7 | ATU-inhibition curves of BM15 and BM30.

CONCLUSIONS

The results showed only a minor adaptation to ATU for nitrifiers acclimated to 15 °C. Only very limited nitrification took place until ATU concentration was degraded below 1–2 mg/l, which is close to its typical inhibition concentration. The adaptation rather drifted for the benefit of ATU-degrading microorganisms instead of nitrifiers. BM15 developed a significantly higher degradation potential for ATU, which was the key process for carrying out time-delayed nitrification. An evaluation by means of to what extent the higher ATU concentration added to BM15 was responsible for better degradation results was not possible. In contrast, the nitrifiers incubated at 30 °C still kept about 20–40% of their activity at a concentration near 7 mg ATU/l.

The conducted experiments have shown that the incubation temperature at which mixed cultures develop in some way also seem to predetermine the ability or disability to adapt to certain substances. Through conducting further experiments investigating the adaptation to nitrification inhibitors it might be possible to group certain strains by their adaptability and optimal environmental conditions in order to facilitate the prognosis of whether a wastewater treatment strategy can be promising in certain process conditions, e.g. specific climates.

ACKNOWLEDGEMENTS

This work as part of the research project 'Control of wastewater treatment processes to support ecotechnology

measures in rivers' was financed by the Deutsche Forschungsgemeinschaft DFG (German Research Foundation). The authors thank A. Marquardt for her support at the centre for environmental research (UFZ) and her contributions to this research.

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First received 6 September 2012; accepted in revised form 26 February 2013