1 **Biotransformation in water and soil of nitrosamines and nitramines**
2 **potentially generated from amine-based CO_2 capture technology**

3

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ABSTRACT

12 Nitrosamines (NSAs) and nitramines (NAs) are identified as possible degradation products
13 from amine-based post-combustion CO_2-capture (PCCC). Selected NSAs and NAs were
14 subjected to aerobic and anaerobic biodegradation studies. In a screening study with 20 µg/L
15 NSAs and NAs at 20°C, only NSAs and NAs containing hydroxyl groups (alkanol compounds)
16 exhibited aerobic biotransformation > 10% after incubation in 28 days. Extending the
17 biodegradation period to 56 days resulted in ≥ 80% biotransformation of examined alkanol
18 NSAs and NAs at 20°C. Biotransformation (20°C; 56 days) of the NSA NDELA at different
19 concentrations (1-100 µg/L) did not differ significantly, but both water sources and
20 temperatures affected biotransformation of tested the compounds. Anaerobic biotransformation
21 occurred rapidly (56 d) with alkanol NSAs and NAs, but not with alkyl compounds.
22 Interestingly, 1st order rate coefficients and half-lives indicated comparable or even faster
23 anaerobic than aerobic biotransformation at the same temperature. Predictions of
24 biotransformation pathways suggested that the -OH substituent of alkanol NSAs and NAs was
25 more susceptible to degradation than nitroso- and nitro-substituents.

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28 Key words: Nitrosamines, nitramines, CO2-capture, biodegradation, adsorption, water, soil

1. Introduction

Amine-based solvents are used as chemical absorbents in post-combustion CO_2 capture (PCCC) processes. The PCCC processes may result in the formation of a number of degradation products, of which the potential generations of nitrosamines (NSAs) and nitramines (NAs) have been of concern, since these compounds are considered to be carcinogenic compounds (da Silva and Booth, 2013; Wagner et al., 2014). Most NSAs tested so far have been highly toxic and carcinogenic in mammalian studies (Bogovski et al., 1972; Låg et al., 2009). Less is known about the NAs, but these tend be mutagenic and carcinogenic at a less potent level than for NSAs (Khudoley et al., 1981; Mirvish et al., 1980; Selin, 2011). NSAs and NAs can be formed in PCCC process when NOx reacts with amines and are emitted to the air with the CO_2-depleted flue gas. Secondary amines form stable NSAs and NAs, while primary amines form stable NAs, but unstable NSAs. Tertiary amines are dealkylated to secondary amines before nitrosation or nitration (Dai et al., 2012). The risk of NSA generation is decreasing in the order secondary amines > tertiary amines > primary amines (Tønnesen, 2011). Another important source of NSA and NA formation is through atmospheric photooxidation of the amine solvent present in the emissions from PCCC processes (da Silva, 2013; Nielsen et al., 2012). Although these products may be generated in very small concentrations in the PCCC process, they can be emitted to the air together with the CO_2-depleted flue gas. The carcinogenic characteristics of NSAs have led to suggested low acceptance levels in drinking water, e.g. 4 ng/L in Norway for both NSAs and NAs (Låg et al., 2011).

In the environment, NSAs and NAs may be subject to several degradation processes, including hydrolysis, photolytic degradation and biodegradation under oxic or anoxic conditions. Whilst NSAs exhibit a strong absorbance peak at ~340 nm wavelength in the solar spectrum, NAs do not absorb in the natural sunlight range (Sørensen et al., 2015). NSAs and NAS are highly water-soluble with preferential partition to water in soil adsorption studies

55 (Gunnison et al., 2000; Sørensen et al., 2013), and they will therefore quickly partition into
56 water and undergo wet deposition to aquatic and terrestrial environments. Furthermore, NSAs
57 and NAs in aquatic and soil environments were mainly reported to be resistant to hydrolysis
58 under acidic and basic conditions (Ho et al., 1996; Saunders and Mosier, 1980; Sørensen et al.,
59 2015). In aquatic systems, rapid photodegradation of NSAs has been reported, with half-lives
60 of 10-35 minutes and 60-220 minutes reported for summer and winter conditions, respectively
61 (Plumlee and Reinhard, 2007; Sørensen et al., 2015). Aqueous photolysis appears to be
62 significantly influenced by pH and oxygen levels, and generates a broad range of degradation
63 products (Lee et al., 2005).

64 During conditions of limited or negligible radiation (e.g. in soil and sediments, in water
65 below photo-zone and under ice-coverage, during night-time and in winter season in northern
66 and southern temperate regions), NSA biodegradation becomes an important depletion process.
67 Due to the persistence of NAs to photolysis, biodegradation of these compounds as a depletion
68 process is important at all light conditions, although limited biodegradation data have been
69 reported for PCCC-related NAs (Gundersen et al., 2014; Sørensen et al., 2013). NSA
70 biodegradation studies in aquatic and soil systems have been performed primarily with *N*-
71 nitrosodimethylamine (NDMA) and *N*-nitrosodiethanolamine (NDELA). While NDMA
72 biotransformation in freshwater was reported to be 91% after 15 days (Aubert et al., 1978),
73 mineralization half-lives in lake waters were reported to be 10 and 40 days (Gunnison et al.,
74 2000; Kaplan and Kaplan, 1985; Oliver et al., 1979). In a study of NDELA biodegradation in
75 lake water and soil reported biotransformation, but no mineralization after 90 days (Yordy and
76 Alexander, 1980). Biodegradation studies of NDMA showed mineralization to be related to
77 initial NSA concentrations, with increased mineralization when the NDMA concentrations
78 were reduced (Kaplan and Kaplan, 1985). Under anaerobic conditions in soil, biodegradation
79 half-lives of NDMA and nitrosomorpholine varied from 70 days to more than 100 days

80 (Patterson et al., 2010; Zhou et al., 2009). Aerobic biodegradation of NDMA was suggested to
81 result in conversion of the NSA to nitromethylamine or methylamine by bacterial strains
82 possessing toluene monooxygenases or propane monooxygenases, respectively (Fournier et al.,
83 2009; Fournier et al., 2006). Anaerobically, NDMA was proposed to be degraded to
84 dimethylamine under methanogenic conditions (Tezel et al., 2011).

85 The objective of the current study was to generate aquatic biodegradation data for NSAs
86 and NAs identified as likely amine degradation products present in PCCC emissions, with
87 relevance to precipitation to soil and water. NSA and NA concentration, together with
88 environmental parameters as temperature and oxygen were investigated for their influence on
89 biodegradation rates. As they may be precipitated in catchment areas and end up in drinking
90 water and agricultural soil, these data are of importance for prediction of the environmental fate
91 of these potential degradation products from amine-based PCCC plants.

92

93 **2. Materials and Methods**

94 *2.1. Chemicals*

95 NSAs and NAs (>99% purity) were supplied by Chiron, AS, Trondheim, Norway. The
96 chemicals used in the present study are described in Table 1. The structural characteristics of
97 the chemicals are shown in Table S1 (Supplementary Data; SD). NSA and NA stock solutions
98 to be used in the experiments were stored in Milli-Q water at 4°C.

99

100 *2.2. Water sources*

101 Natural fresh water was collected from a river (Nedre Leirfoss) and a lake (Haukvatnet)
102 close to Trondheim, Norway (63°26'N, 10°23'E) and used as the microbial sources in most
103 experiments. The waters from the two sources were mixed in equal volumes prior to use (termed
104 "mixed water"). Some studies were conducted with lake waters in the catchment area of the

105 pilot-scale PCCC test facility of the Technology Centre at Mongstad (TCM) (60°48'N, 5°1'E),
106 north of Bergen, Norway. These water sources included the lakes Rotavatnet, Storavatnet,
107 Torsteinsvatnet and Steinsvatnet, which were recently described in a TCM baseline study
108 (Grung et al., 2012). The Mongstad waters were shipped to SINTF's laboratories in Trondheim
109 and used as sources for biotransformation studies. All water sources were low in nutrient
110 contents (e.g. tot-P < 12 μg/L), indicating oligotrophic conditions (Grung et al., 2012;
111 Trondheim kommune, 2013).

112

113 *2.3. Aerobic biodegradation*

114 Aerobic biotransformation studies were performed at different temperatures (5, 10 and
115 20°C) for periods of up to 56 days. Water was acclimated/aged at temperatures to be used in
116 each experiment 5-6 days prior to start. During aging periods, some of the water (10 L) was
117 filtered through submerged aquarium pumps with a filter wool insert at each temperature (5, 10
118 and 20°C) for microbial enrichments. At the end of the acclimation period, the filter wool
119 materials were stirred well in 1 L of the water that was circulated through its aquarium pump.
120 This "enriched" water was then mixed with the rest of the water acclimated at the same
121 temperature, which was then used in each experiment. Before start of the tests, this water was
122 aerated for 20 minutes (bubbling of sterile-filtered air), and amended with inorganic nutrients
123 (N-, P-, Ca-, Mg-, and Fe-sources) (OECD Guideline 301D, 1992).

124 Aerobic biotransformation was performed in the aged and amended waters with NSA or
125 NA concentrations of 1.0 – 200 μg/L, with 1 L capped flasks (SCHOTT), with 800 mL solutions
126 in each flask (triplicate samples). Similarly treated water without amine compounds were
127 distributed as blank solutions, while 100 mg/L $HgCl_2$ was added to flasks with NSA or NA as
128 sterilized solutions. The flasks were incubated at the three temperatures in complete darkness
129 for 56 days with constant stirring. Samples from each flask were collected for chemical analyses

130 (5-6 mL aliquots) after 0, 7, 14, 21, 28, 42 and 56 days of incubation. All samples were stored
131 at -20°C prior to analysis.

132 Biochemical oxygen demand (BOD) of NSAs was determined after 28 days of incubation
133 at 20°C, according to the OECD Guideline 301D screening test (OECD Guideline 301D.,
134 1992), using final substance concentrations of 2.0 mg/L.

135

136 *2.4. Anaerobic biotransformation*

137 Anaerobic biotransformation studies were performed with fat clay collected from a
138 terrestrial local source at Eberg, Trondheim, Norway (63°25'N, 10°25'E), which served as
139 bacterial inoculum. The clay had grain sizes < 5 µm, a water porosity of 30%, and an organic
140 carbon content of 4.5% (dry weight). The clay was mixed with sterilized lake water
141 (Haukvatnet; autoclaved at 121°C for 15 min), and acclimated/aged at 20°C for 7 days in the
142 dark. The anaerobic biotransformation tests were performed as described in OECD Guideline
143 308 (OECD Guideline 308, 2002), in sediment/water amended with inorganic nutrients (OECD
144 Guideline 311, 2006). A reducing agent (0.055 g/L Na_2S x yH_2O) was supplied and anoxic
145 conditions controlled by a redox indicator (0.001 g/L resazurine).

146 At the start of the experiment 35 g clay (porosity approximately 30%) and 65 mL
147 freshwater (water:clay ratio of 4:1) from lake Haukvatnet was added to 100 mL serum flaks
148 with butyl rubber septa. The flasks were capped, flushed with N_2, and then incubated at 20°C
149 for up to 26 days to ensure anoxic conditions. Control flasks were sacrificed for measurements
150 of redox potential and pH until stable conditions. Test chemicals from stock solutions (200
151 mg/L) were then gently mixed into the top layer of the water above the sediment at final
152 concentration of 100 µg/L. Controls included blank samples without chemicals and sterilized
153 samples (autoclaved and poised with 100 mg/L $HgCl_2$). The flasks were gently mixed without
154 disturbing the sediment and incubated at 20°C for up to 56 days. Samples (triplicate) of each

155 test chemical were sacrificed for chemical analyses after 0, 7, 14, 21, 28 and 56 days of
156 incubation.
157
158 *2.5. Chemical and microbial analyses*
159 NSAs and NAs in biotransformation and soil adsorption studies were quantified by direct
160 injection on an Agilent 1290 LC coupled with an Agilent 6490 QqQ MS system, mainly as
161 described previously (Sørensen et al., 2015). The analytes were separated by reverse phase
162 chromatography on various columns and mobile phases (See Table S2 in SD). Analyte retention
163 times were within the range of 1 to 10 min, and the limits of quantification (LOQ) were within
164 the range of 0.1 to 1 ng/mL. Where possible, deuterated calibration homologues of the test
165 NSAs and NAs were employed (see Table 1), but when these were not available, the closest
166 deuterated analogue was used. The deuterated NSA calibration standards (Table 1) were stored
167 in Milli-Q water at -20°C, except NPz and DNPz which were stored in Milli-Q water at 4°C.
168 Deuterated NA standards were stored in Milli-Q water at 4°C, except Pz-NO2 which were
169 stored in methanol at 4°C. Blank samples never exhibited NSA or NA concentrations above the
170 lower limit of quantification (LOQ). The precision (repeatability) of analysis was better than
171 5% relative standard deviation for all analytes, based on replicate analyses.
172 Total microbial cell concentrations were determined in the different lake waters after
173 biodegradation periods of 28 and 56 days, using epifluorescence microscopy (1250x
174 magnification) with 4'6-diamidino-2-phenylindole (DAPI) as fluorescent nucleic acid stain.
175 Inhibition of bacterial growth by nitrosamines was assessed by preparing dilution series of each
176 nitrosamine from the 100 mg/L stock solutions in ranges of 2 mg/L to 1 µg/L.
177 Inhibition of bacterial growth by NSAs were tested in Nutrient Broth (Fluka BioChemika)
178 added with final concentrations of 2000, 1000, 100, 10, and 1 µg/L nitrosamine.
179 Acclimated/aged samples of mixed water (1 mL; 20°C) as inocula were incubated with the

180 different NSA concentrations at 20°C for 4 days with continuous agitation, and bacterial growth
181 measured daily spectrophotometrically (OD_{600}).
182 For PCR-analyses, water samples (300 ml) were filtered (Sterivex filter cartridges, 0.2
183 µm pore exclusion limit; Millipore, Bedford, Ma), nucleic acids extracted from the cartridges,
184 and bacterial 16S rRNA genes amplified by PCR as previously described (Brakstad and
185 Lødeng, 2005). PCR products were analyzed by denaturing gradient gel electrophoresis
186 (DGGE), using 20–70% of denaturing agent (Teske et al., 1996).
187
188 *2.6. Calculations*
189 Non-linear regression, linear regression, paired *t*-test and Anova analyses were performed
190 in GraphPad Prism vs. 6.0 software (GraphPad Software Inc., La Jolla, CA, USA). First-order
191 rate coefficients (k1) were determined by non-linear regression analyses with determination of
192 lag-phases included using the option "plateau followed by one-phase decay" in the software.
193 The rate coefficients were determined for the decay-period, the plateau period defined the lag-
194 phase, and half-lives were determined from rate coefficients and plateau periods (t1/2 = plateau
195 period + 0.693/k1). Q_{10} was determined by linear regression analyses of rate coefficients (k1)
196 determined at different temperatures, based on Arrhenius plots (Bagi et al., 2013). Paired t-test
197 and Anova analyses were used to determine significance ($P<0.05$) between two or multiple data
198 sets, respectively.
199

201 **3. Results and Discussions**

202 *3.1. Screening of biodegradation*

203 Screening of biotransformation was performed with 10 NSAs and 6 NAs in the mixed
204 water, at initial concentrations (20 µg/L) and high incubation temperature (20°C). These were
205 selected as potential degradation products from relevant amine candidates for carbon capture
206 processes (da Silva and Booth, 2013; Gjernes et al., 2013). The results (Fig. 1) showed that only
207 one NSA and two NAs showed biotransformation > 10% after 28 days of incubation; NDELA
208 (24±1% biotransformation), MEA-NO_2 (27±2% biotransformation) and AMP-NO_2 (27±8%
209 biotransformation). The biotransformation results of the NSAs were confirmed by BOD
210 analyses (Table S3 in SD), which showed that none of the NSAs were considered as ready
211 biodegradable after 28 days of incubation at 20°C (BOD ≥ 60% of ThOD) (OECD Guideline
212 301D, 1992). The low NSA biodegradability determined in the current study was in contrast to
213 previous respirometric studies in water or soil (Gunnison et al., 2000; Kaplan and Kaplan,
214 1985). Testing of inocula from the mixed water with the NSAs NDELA, NDMA and NPz (1
215 µg/L to 2000 µg/L) in Nutrient Broth did not show inhibition of growth curves for any of the
216 NSAs with increasing concentrations (Fig. S1 in SD), and one-way Anova analyses did not
217 show significant differences between concentrations (P>0.05). This indicated that lack of
218 NDMA and NPz biodegradation was not the result of bacterial inhibition (results not shown).

219 Biotransformation of NSAs and NAs was determined in the mixed water at 20°C for an
220 extended period of 56 days. The compounds selected for the analyses represented both NSAs
221 and NAs of alkanol, alkyl, and cyclic structures. Since concentrations in aquatic environments
222 are very low, we made efforts to use as low concentrations as we considered possible, based on
223 their LOQs (Table S2). Initial nominal concentrations of 5-20 µg/L were therefore used for the
224 NSAs and NAs, except DMNA and MNA, in which nominal concentrations of 50 µg/L were
225 selected. Only NDELA, MEA-NO_2 and AMP-NO_2 showed further degradation as a result of

226 the extended incubation period, with 80±9% (NDELA), 99% (MEA-NO2) and 90±4% (AMP-
227 NO_2) biotransformation at the end of the experiment (Fig. S2 in SD). Non-responsive periods
228 (lag-phases), first-order rate coefficients and half-lives were determined for the biodegradable
229 NSA and NAs (Table 2). The determination of lag-periods and 1st order rate coefficients
230 resulted in overall half-lives (sums of lag-periods and half-lives determined from rate
231 coefficients [k1]) for NDELA, MEA-NO_2 and AMP-NO_2 ranging from 28.2 - 35.1 days (Table
232 2).

233 The results from this experiment showed that only NSA and NA compounds containing
234 hydroxyl (-OH) groups (alkanol compounds) were susceptible to significant biotransformation
235 under the selected conditions, while no biotransformation was detected for any of the alkyl or
236 cyclic NSA or NA compounds included in this study. Although lag-periods of 9-19 days were
237 shown in this experiment, these non-responsive periods may be lower in environments
238 previously exposed to these compounds, and with microbial communities adapted to
239 degradation of NSAs and NAs. Interestingly, the lack of NDMA biotransformation in our
240 studies differed from previous mineralization studies with lake water or soil showing increased
241 degradation of the nitrosamine with decreased initial concentrations (Gunnison et al., 2000;
242 Kaplan and Kaplan, 1985; Yang et al., 2005). However, no alkanol compounds were
243 investigated in these studies, and possible effect of hydroxyl groups on biodegradation were
244 therefore not considered. However, the fact that alkyl NSA are biodegradable in aquatic
245 environments, show that the nitroso-group of the NSA, and possibly the nitro-group of the NA,
246 may be attacked by the microbes at optimal environmental conditions.
247

248 *3.2. Effects of compound concentrations on aerobic biotransformation*

249 Aquatic biodegradability may be affected by initial concentrations of the compounds. For
250 instance, high concentrations of NDMA (> 15 µg/L) was shown to reduce biodegradation of

251 NDMA (Kaplan and Kaplan, 1985). In our study, the importance of initial concentration on
252 biotransformation was studied with NDELA at 20°C for 56 days in the mixed water, and with
253 initial concentrations of 100, 10 and 1 µg/L. The biotransformation of NDELA at the three
254 concentrations was comparable (Fig. 2; Fig. S3, SD), reaching 87±11% (100 µg/L), 80±9% (10
255 µg/L) and 75±1 % (1 µg/L) after 54 days. Transformation did not differ significantly between
256 the concentrations used in the study (P>0.05; two-way Anova test) and did not show the same
257 concentration-dependent trends between low concentrations (1.5-150 µg/L) as observed for
258 NDMA (Kaplan and Kaplan, 1985). Our data are partly in agreement with results from a
259 biodegradation study of 0.05-1 µg/mL [U-^{14}C]NDELA in water from different lakes performed
260 at 22°C, which resulted in more than 90% depletion in most samples after 20 days. However,
261 results differed considerably between water sources and water sampling season, and
262 mineralization was negligible (Yordy and Alexander, 1980).
263
264 *3.3. Different water sources*
265 Water source may affect NSA/NA biodegradation (Yordy and Alexander, 1980), and the
266 biotransformation capacity of water collected from different lakes was compared using an
267 alkanol NA (AMP-NO_2). The water sources included the mixed water, and four lakes in the
268 catchment area of the TCM test center at Mongstad (Torsteinsvatnet, Rotavatnet, Storavatnet,
269 and Steinsvatnet). Biotransformation of AMP-NO_2 (20 µg/L) was determined at 20°C after 28
270 and 56 days (Fig. 3). The biotransformation ranged from 9±6% to 66±21% after 28 days and
271 from 33±16% to 97±2% after 56 days. As expected from the low initial concentration of AMP-
272 NO_2, microbial analyses (epifluorescence microscopy and PCR-DGGE) did not show specific
273 stimulation of bacterial concentrations or communities in any of the waters (Fig. S4 and Fig.
274 S5 in SD). The degradation in Torsteinsvatnet was significantly lower than in the other lake
275 waters (P< 0.05; two-way Anova analyses), while none of the other water sources resulted in

276 significant differences (two-way Anova). Microbial concentrations, determined by
277 fluorescence microscopy, were initially lower in Torsteinsvatnet than in the other water sources,
278 but reached similar concentrations after 28 and 56 days (Fig. S4 in SD). An aquatic baseline
279 survey of the lakes in the TCM catchment area showed that Torsteinsvatnet had lower pH
280 (4.85), lower concentrations of NH_4-N, NO_3-N and total P, and higher background
281 concentrations of alkylamines than the other lakes (Grung et al., 2012). Water quality
282 parameters may therefore have impacts on the potential for biodegradation of AMP-NO_2 and
283 other alkanol-compounds. Furthermore, suppression of AMP-NO_2 degradation caused by
284 existing alkylamine concentrations may also be occurring in this water. These varying
285 environmental parameters in natural waters may also explain the differences in
286 biotransformation observed for NDMA in the present study compared to the data reported in
287 other studies (Gunnison et al., 2000; Kaplan and Kaplan, 1985; Yang et al., 2005).
288
289 *3.4. Temperature-related aerobic biotransformation*
290 Biotransformation of NDELA, MEA-NO_2 and AMP-NO_2 were compared in the mixed
291 water at three water temperatures, 20°C, 10°C, and 5°C, representing non-freezing conditions
292 in temperate climates. These temperatures are relevant for seasonal variations in temperate
293 regions like the western coast of Norway. First-order biotransformation rate data were
294 determined after lag-periods (Table S4 in SD), showing temperature-dependent rate coefficients
295 (k1) and half-lives for all three compounds. The influence of temperature on biodegradation of
296 organic pollutants has often been explained by the Q_{10}-approach, which describes the increases
297 in rates of enzymatic reactions at a rise in temperature of 10°C, based on Arrhenius plots (Bagi
298 et al., 2013). Comparison of overall rate coefficients at 20°C and 10°C (Fig. 4) resulted in Q_{10}-
299 values from Arrhenius plots of 1.5±0.2 (NDELA), 2.3±1.1 (MEA-NO_2) and 3.9±0.9 (AMP-
300 NO_2), indicating a larger influence of water temperature on the studied NAs than the NSA. In

301 a previous study Q_{10} of 1.67 was determined for monoethanolamine in soil, with temperatures
302 of 6, 14 and 25°C (Sorensen et al., 1997).
303
304 *3.5. Anaerobic biotransformation*
305 Initial studies in our laboratory showed that NSAs (NDMA and NDELA) and NAs
306 (DMNA and MEA-NO_2) exhibited <10% soil adsorption to different clay types (results not
307 shown), in agreement with other studies showing negligible adsorption of NSAs to soil particles
308 (Gunnison et al., 2000; Kaplan and Kaplan, 1985; Oliver et al., 1979; Yang et al., 2005).
309 Biodegradation of NSAs and NAs in soil systems will therefore appear in the mobile pore water
310 phase in the soil, or eventually ending up in groundwater or surface water.
311 During transport through soil or sediments oxygen may be limited, and a biodegradation
312 experiment under anoxic conditions was therefore conducted with two NSAs (NDMA and
313 NDELA) and two NAs (DMNA and MEA-NO_2). The study was performed at 20°C over a
314 period of 56 days, in anoxic and pre-sterilized lake water (Haukvatnet), and with clay sediment
315 as microbial inoculum (ISO Standard 11734, 1995; OECD Guideline 308, 2002). The anaerobic
316 test showed biotransformation of the alkanol compounds (NDELA and MEA-NO_2), but not the
317 alkyl compounds (NDMA, MDNA), as evidenced by the degradation curves (Fig. S6 in SD).
318 These data are in agreement with the results from the aerobic experiments, where only the
319 alkanol compounds exhibited significant biotransformation over a period of 28-56 days.
320 Interestingly, determination of 1st order rate coefficients and half-lives (Table 3) showed even
321 faster degradation than in the aerobic experiments at the same temperature (Table 2), although
322 rate coefficients and half-lives did not differ significantly between aerobic and anaerobic
323 biotransformation tests ($P > 0.05$) when compared by paired *t*-test. These degradation tests may
324 have been affected by the bacterial concentrations, since temperate lake sediments may contain
325 up to 10^3 times higher bacterial concentrations than lake water (Pace et al., 1990; Duhamel and

Jacquet, 2006). Also other studies have shown fast NSA biotransformation under anoxic conditions, including a study of 7 NSAs in soil-river water columns, resulting in half-lives of 1.3-7.1 days (Drewes et al., 2006). Experiments with NDMA showed faster mineralization in soil/water systems than in lake water at low concentrations (10-15 µg/L), although oxygen content was assumed higher in the lake water than in the soil/water system (Kaplan and Kaplan, 1985).

Biodegradation of precipitated NSAs and NAS in soil and sediment systems will mainly occur in the pore water and groundwater, often under oxygen limitations, and with generation of CH_4 as the result of the mineralization process (Bradley et al., 2005; Sharp et al., 2005). Previous studies have reported variable NSA degradation rates in soil under anaerobic conditions. Several NSAs were shown to biodegrade under methanogenic conditions (Sharp et al., 2005; Tezel et al., 2011), and it has been suggested that the NDMA biotransformation pathway under anaerobic conditions may involve the reduction of the nitroso group and subsequent N-N cleavage (Padhye et al., 2009). When ^{14}C-labelled NDMA was mixed with the sandy soil (50 µg/l soil slurry) half-lives for mineralization ranged from 11-35 days under aerobic conditions and from 26-39 days under anaerobic conditions (Gunnison et al., 2000). An *in situ* groundwater biodegradation study of NDMA showed an estimated 80% biotransformation after 626 days, with a calculated half-life of 70 days (Zhou et al., 2009), while a 12-month anaerobic study with sediment columns showed NDMA and nitrosomorpholine half-lives of > 100 days (Drewes et al., 2006; Patterson et al., 2010).

3.6. Biodegradation pathways

To our knowledge biodegradation pathways of NSAs have only been reported for the alkyl NDMA (Fournier et al., 2009; Fournier et al., 2006; Tezel et al., 2011), while no such information exists for relevant NAs. By using the Pathways Prediction System of the

351 Biocatalysis/Biodegradation Database (http://eawag-bbd.ethz.ch/) the alcohol substituents of
352 NDELA, MEA-NO_2 and AMP-NO_2 were predicted to be more susceptible to degradation than
353 the nitroso- and nitro-substituents. This was also in agreement with our results showing that the
354 alkanol NSAs and NAs were faster biotransformed than the alkyl NSAs and NAs (Fig. 1).
355 According to the Biocatalysis/Biodegradation Database the alcohol groups are expected to be
356 transformed to carboxylates (via aldehydes), which may be subject to decarboxylation
357 (http://eawag-bbd.ethz.ch/). Alkanol compounds like NDELA may then be degraded to alkyl
358 compounds as NDMA (Fig. S7 in SD). Further degradation of alkyl compounds like NDMA
359 may involve conversion of the nitroso group into a nitro group (Fournier et al., 2009; Fournier
360 et al., 2006). The NSA biodegradation pathways contrast the proposed photodegradation
361 mechanisms, suggesting radical formation of the nitroso-group and transformation to
362 alkylamines (Sørensen et al., 2015).
363
364 **4. Conclusions**
365 In this study, it was shown that none of the tested NSAs and NAs were readily
366 biodegradable. However, alkanol NSAs and NAs were biotransformed in aquatic environments,
367 both at aerobic and anaerobic conditions at low concentrations. Aerobic biotransformation rates
368 were related to temperature, but seemed to be mainly similar at different low-range
369 concentrations. While NSAs will photodegrade rapidly, this study has shown that
370 biotransformation of alkanol NSAs may be an important process under the absence of light,
371 both in water and sediments. Biodegradation also represents an important environmental
372 degradation pathway for alkanol NAs that are not susceptible to photodegradation (Sørensen et
373 al., 2015). Since the NSAs and NAs with the fastest biotransformation all contained hydroxyl-
374 groups, this lead us to suspect that biotransformation is associated with microbial attacks on the
375 hydroxyl- rather than the nitroso- or nitro-groups. Further studies are needed to investigate NSA

376 and NA biodegradation pathways experimentally, in order to determine potential
377 biotransformation products of NSAs and NAs from PCCC-related sources and to investigate if
378 potential carcinogenicity will persist in light-protected environments like soil and sediments.
379
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389
390 **Supplementary data**

391 Supplementary data associated with article is presented in the online version, at.....
392
393

394 ## References

395 Aubert, J., Petit, L., Puel, D., 1978. Etude de la degradabilite des nitrosamines en milieu marin.
396 Rev. Internat. d'Océanograph. Médic. LI-LII, 45-53.

397 Bagi, A., Pampanin, D.M., Brakstad, O.G., Kommedal, R., 2013. Estimation of hydrocarbon
398 biodegradation rates in marine environments: A critical review of the Q(10) approach. Mar.
399 Environ. Res. 89, 83-90.

400 Bogovski, P., Preussman, R., Walker, E.A., 1972. N-Nitroso Compounds, Analysis and
401 Formation: Proceedings of a Working Conference held at the Deutsches
402 Krebsforschungszentrum, Heidelberg, Federal Republic of Germany, 13-15 October 1971,
403 IARC Scientific Publications World Health Organisation, Lyon.

404 Bradley, P.M., Carr, S.A., Baird, R.B., Chapelle, F.H., 2005. Biodegradation of N-
405 nitrosodimethylamine in Soil from a Water Reclamation Facility. Bioremed. J. 9, 115-120.

406 Brakstad, O., Lødeng, A., 2005. Microbial diversity during biodegradation of crude oil in
407 seawater from the North Sea. Microb. Ecol. 49, 94-103.

408 Dai, N., Shah, A. D., Hu, L., Plewa, M. J., McKague, B., Mitch, W. A., 2012. Measurement of
409 nitrosamine and nitramine formation from NOx reactions with amines during amine-based
410 carbon dioxide capture for postcombustion carbon sequestration. Environ. Sci. Technol. 46,
411 9793-9801.

412 da Silva, E.F., Booth, A.M., 2013. Emissions from Postcombustion CO2 Capture Plants.
413 Environ. Sci. Technol. 47, 659-660.

414 Duhamel, S., Jacquet, S., 2006. Flow cytometric analysis of bacteria- and virus-like particles in
415 lake sediments. J. Microbiol. Meth. 64, 316-332.

416 Drewes, J., Joerg, E., Hoppe, C., Jennings, T., 2006. Fate and Transport of N-Nitrosamines
417 Under Conditions Simulating Full-Scale Groundwater Recharge Operations. Water Environ.
418 Res. 78, 2466-2473.

419 Fournier, D., Hawari, J., Halasz, A., Streger, S.H., McClay, K.R., Masuda, H., Hatzinger, P.B.,
420 2009. Aerobic Biodegradation of N-Nitrosodimethylamine by the Propanotroph *Rhodococcus*
421 *ruber* ENV425. Appl. Environ. Microb. 75, 5088-5093.

422 Fournier, D., Hawari, J., Streger, S.H., McClay, K., Hatzinger, P.B., 2006. Biotransformation
423 of N-nitrosodimethylamine by *Pseudomonas mendocina* KR1. Appl. Environ. Microb. 72,
424 6693-6698.

425 Gjernes, E., Helgesen, L. I., Maree, Y., 2013. Health and environmental impact of amine based
426 post combustion CO_2 capture. Energy Procedia, 37, 735-742.

427 Grung, M., Ranneklev, S., Garmo, Ø., Wright, R.F., Myking, T., Heegaard, E., Øyen, B.-H.,
428 Schei, F.H., Blom, H.H., 2012. Terrestrial and aquatic baseline study and monitoring program
429 for CO2 Technology Centre Mongstad. NIVA, p. 98.

430 Gundersen, C.B., Andersen, T., Lindahl, S., Linke, D., Vogt, R.D., 2014. Bacterial Response
431 from Exposure to Selected Aliphatic Nitramines. Energy Procedia 63, 791-800.

432 Gunnison, D., Zappi, M.E., Teeter, C., Pennington, J.C., Bajpai, R., 2000. Attenuation
433 mechanisms of N-nitrosodimethylamine at an operating intercept and treat groundwater
434 remediation system. J. Hazard. Mat. 73, 179-197.

435 Ho, T.-F.L., Bolton, J.R., Lipczynska-Kochany, E., 1996. Quantum yields for the
436 photodegradation of pollutants in dilute aqueous solution: Phenol, 4-chlorophenol andN-
437 nitrosodimethylamine. J. Adv. Oxid. Technol. 1, 170-178.

438 ISO Standard 11734., 1995. Water Quality – Evaluation of the "Ultimate" Anaerobic
439 Biodegradability of Organic Compounds in Digested Sludge – Method by Measurement of the
440 Biogas Production. International Organisation for Standardisation.

441 Kaplan, D.L., Kaplan, A.M., 1985. Biodegradation of N-Nitrosodimethylamine in Aqueous and
442 Soil Systems. Appl. Environ. Microbiol. 50, 1077-1086.

443 Khudoley, V., Malaveille, C., Bartsch, H., 1981. Mutagenicity Studies in Salmonella
444 typhimurium on Some Carcinogenic N-Nitramines in Vitro and in the Host-mediated Assay in
445 Rats. Cancer Res. 41, 3205-3210.

446 Lee, C., Choi, W., Yoon, J., 2005. UV Photolytic Mechanism of N-Nitrosodimethylamine in
447 Water: Roles of Dissolved Oxygen and Solution pH. Environ. Sci. Technol. 39, 9702-9709.

448 Låg, M., Andreassen, Å., Instanes, C., Lindeman, B., 2009. Health effects of different amines
449 and possible degradation products relevant for CO2 capture. The Norwegian Institute of Public
450 Health, Oslo, Norway, p. 30.

451 Låg, M., Lindeman, B., Instanes, C., Brunborg, G., Schwarze, P., 2011. Health effects of amines
452 and derivatives associated with CO2 capture. The Norwegian Institute of Public Health, p. 45.

453 Mirvish, S.S., Bulay, O., Runge, R.G., Patil, K., 1980. Study of the carcinogenicity of large
454 doses of dimethylnitramine, N-nitroso-L-proline, and sodium nitrite administered in drinking
455 water to rats. J. Nat. Cancer Inst. 64, 1435-1442.

456 Nielsen, C. J., Herrmann, H., Weller, C., 2012. Atmospheric chemistry and environmental
457 impact of the use of amines in carbon capture and storage (CCS). Chem. Soc. Rev. 41, 6684-
458 6704.

459 OECD Guideline 301D, 1992. Ready Biodegradability. Closed Bottle Test., OECD Guideline
460 for the Testing of Chemicals. Organisation for Economic Cooperation and Development
461 (OECD), Paris.

462 OECD Guideline 308, 2002. Aerobic and Anaerobic Transformation in Aquatic Sediment
463 Systems, OECD Guideline for the Testing of Chemicals. Organisation for Economic
464 Cooperation and Development (OECD), Paris.

465 OECD Guideline 311, 2006. Anaerobic Biodegradability of Organic Compounds in Digested
466 Sludge: By Measurement of Gas Production, OECD Guideline for the Testing of Chemicals.
467 Organisation for Economic Cooperation and Development (OECD), Paris.

468 Oliver, J.E., Kearney, P.C., Kontson, A., 1979. Degradation of herbicide-related nitrosamines
469 in aerobic soils. J. Agric. Food Chem. 27, 887-891.

470 Pace, M. L., McManus, G. B., & Findlay, S. E., 1990. Planktonic community structure
471 determines the fate of bacterial production in a temperate lake. Limnol. Oceanograph. 35, 795-
472 808.

473 Padhye, L., Tezel, U., Mitch, W.A., Pavlostathis, S.G., Huang, C.-H., 2009. Occurrence and
474 Fate of Nitrosamines and Their Precursors in Municipal Sludge and Anaerobic Digestion
475 Systems. Environ. Sci. Technol. 43, 3087-3093.

476 Patterson, B.M., Shackleton, M., Furness, A.J., Pearce, J., Descourvieres, C., Linge, K.L.,
477 Busetti, F., Spadek, T., 2010. Fate of nine recycled water trace organic contaminants and
478 metal(loid)s during managed aquifer recharge into a anaerobic aquifer: Column studies. Water
479 Res. 44, 1471-1481.

480 Plumlee, M.H., Reinhard, M., 2007. Photochemical Attenuation of N-Nitrosodimethylamine
481 (NDMA) and other Nitrosamines in Surface Water. Environ. Sci. Technol. 41, 6170-6176.

482 Saunders, D.G., Mosier, J.W., 1980. Photolysis of N-nitrosodi-n-propylamine in water. J.
483 Agric. Food Chem. 28, 315-319.

484 Selin, N.E., 2011. Environmental Guidelines and Regulations for Nitramines: A Policy
485 Summary (Revision 2). Massachusetts Institute of Technology (MIT), p. 21.

486 Sharp, J.O., Wood, T.K., Alvarez-Cohen, L., 2005. Aerobic biodegradation of N-
487 nitrosodimethylamine (NDMA) by axenic bacterial strains. Biotechnol. Bioengineer. 89, 608-
488 618.

489 Sorensen, J., Hawthorne, S., Gallagher, J., Thompson, J., Harju, J., Evans, J., Chollak, D., 1997.
490 Assessment of the subsurface environmental fate of amines used by the gas industry, SPE/EPA
491 Exploration and Production Environmental Conference. Society of Petroleum Engineers.

492 Sørensen, L., Silva, E.F.d., Brakstad, O.G., Zahlsen, K., Booth, A., 2013. Preliminary Studies
493 into the Environmental Fate of Nitrosamine and Nitramine Compounds in Aquatic Systems.
494 Energy Procedia 37, 683-690.

495 Sørensen, L., Zahlsen, K., Hyldbakk, A., Silva, E.F.d., Booth, A.M., 2015. Photodegradation
496 in natural waters of nitrosamines and nitramines derived from CO2 capture plant operation. Int.
497 J. Greenh. Gas Contr. 32, 106-114.

498 Teske, A., Wawer, C., Muyzer, G., Ramsing, N.B., 1996. Distribution of sulfate-reducing
499 bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable-number
500 counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA
501 fragments. Appl. Environ. Microb. 62, 1405-1415.

502 Tezel, U., Padhye, L.P., Huang, C.-H., Pavlostathis, S.G., 2011. Biotransformation of
503 Nitrosamines and Precursor Secondary Amines under Methanogenic Conditions. Environ. Sci.
504 Technol. 45, 8290-8297.

505 Trondheim kommune, 2013. Forslag til tiltaksanalyse for Trondheim kommune. Omfatter
506 vannforekomster i vannområdene Nidelva, Nea og Gaula. Trondheim kommune, Miljøenheten,
507 Kommunalteknikk (in Norwegian).

508 Tønnesen, D., 2011. Update and Improvement of Dispersion Calculations for Emissions to Air
509 from TCM's Amine Plant: Part l-Worst case Nitrosamines and Nitramines. Norwegian Institute
510 for Atmospheric Research (NILU), p. 48.

511 Wagner, E. D., Osiol, J., Mitch, W. A., Plewa, M. J., 2014. Comparative in vitro toxicity of
512 nitrosamines and nitramines associated with amine-based carbon capture and storage. Environ.
513 Sci. Technol. 48, 8203-8211.

514 Yang, W.C., Gan, J., Liu, W.P., Green, R., 2005. Degradation of N-nitrosodimethylamine
515 (NDMA) in landscape soils. J. Environ. Qual. 34, 336-341.

516 Yordy, J.R., Alexander, M., 1980. Microbial metabolism of N-nitrosodiethanolamine in lake
517 water and sewage. Appl. Environ. Microbiol. 39, 559-565.

518 Zhou, Q., McCraven, S., Garcia, J., Gasca, M., Johnson, T.A., Motzer, W.E., 2009. Field
519 evidence of biodegradation of N-Nitrosodimethylamine (NDMA) in groundwater with
520 incidental and active recycled water recharge. Water Res. 43, 793-805.

521

522

Tables and Figures

523 **Table 1.** Summary of nitrosamines and nitramines used in this study. The compounds were provided
524 as neat products or dissolved in dichloromethane (DCM) or acetonitrile.

525

Chemical	Abbreviation	Solvent	CAS no.	Deuterated analogue for quantification
Nitrosamines				
N-nitrosodiethanolamine	NDELA	Neat	1116-54-7	NDELA-d8
Nitrosopiperidine	NPIP	DCM	100-75-4	NPIP-d10
Nitrosodiethylamine	NDEA	Neat	55-18-5	NDEA-d10
Nitrosodimethylamine	NDMA	Neat	62-75-9	NDMA-d6
Nitroso-N-methylethylamine	NMEA	DCM	10595-95-6	NMEA-d3
Nitrosomorpholine	NMOR	Acetonitrile	59-89-2	NMOR-d8
Nitroso-N-propylamine	NDPA	Neat	621-64-7	NDPA-d14
Nitrosopyrrolidine	NPYR	DCM	930-55-2	NPYR-d8
Nitrosopiperazine	NPz	Acetonitrile	5632-47-3	NPz-d4
Dinitrosopiperazine	DNPz	Acetonitrile	140-79-4	DNPz-d8
Nitramines				
Dimethylnitramine	DMNA	Neat	4164-28-7	DMNA-d6
Ethanolnitramine	MEA-NO_2	Neat	74386-82-6	MEA-NO_2-d4
Methylnitramine	MNA	Neat	598-57-2	MNA-d3
N-nitropiperazine	Pz-NO_2	Neat	42499-41-2	Pz-NO_2-d6
1-methyl-2-(nitroamino)-1-propanol	AMP-NO_2	Neat	1239666-60-4	-
Diethylnitramine	DENA	Neat	7119-92-8	-

Table 2. Aerobic biotransformation rates in mixed water of NSAs and NAs at 20°C determined by 1st order rate coefficients with standard deviations (k1±SD) after a non-responsive lag-period. Half-lives were determined from average k1-values. The sum of the lag-period and half-lives (∑lag+half-life) are also shown.

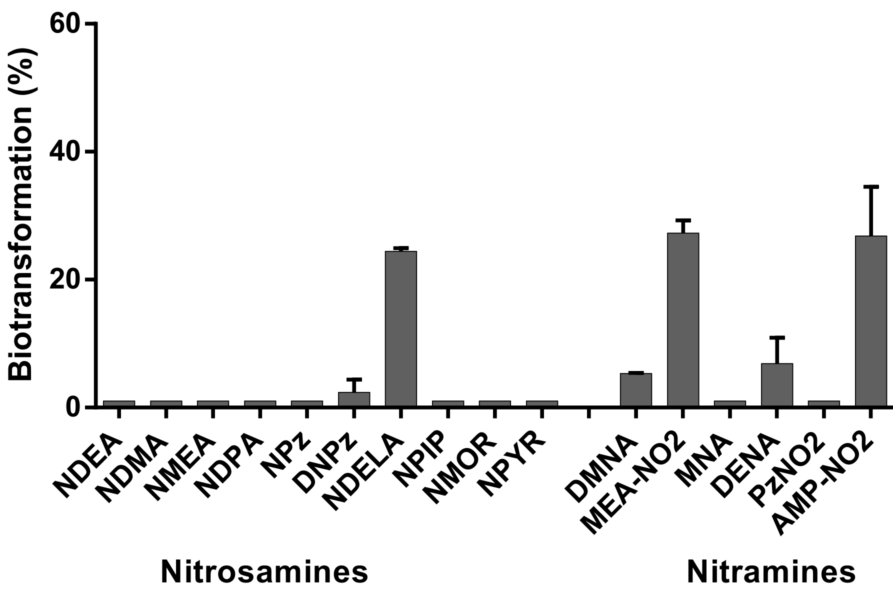
Compound	Lag-period (days)	k1±SD	Half-life (days)	∑lag+half-life (days)	R^2
Nitrosamines (NSAs)					
NDELA	9.0	0.0313±0.0023	22.2	32.2	0.9595
NDMA	ND[A)]	< 0.001	>500	ND[A)]	ND[A)]
NPz	ND[A)]	<0.001	>500	ND[A)]	ND[A)]
Nitramines (NAs)					
DMNA	ND[A)]	<0.001	>500	ND[A)]	ND[A)]
MEA-NO_2	18.8	0.0741±0.0115	9.4	28.2	0.9297
MNA	ND[A)]	<0.001	>500	ND[A)]	ND[A)]
DENA	ND[A)]	<0.001	>500	ND[A)]	ND[A)]
Pz-NO_2	ND[A)]	<0.001	>500	ND[A)]	ND[A)]
AMP-NO_2	19.3	0.0440±0.0038	15.8	35.1	0.9429

[A)] Not determined

Table 3. Anaerobic biotransformation rates of NSAs and NAs at 20°C determined by 1st order rate coefficients with standard deviations (k1±SD) after a non-responsive lag-period. Half-lives were determined from average k1-values. The sum of the lag-period and half-lives (∑lag+half-life) are also shown.

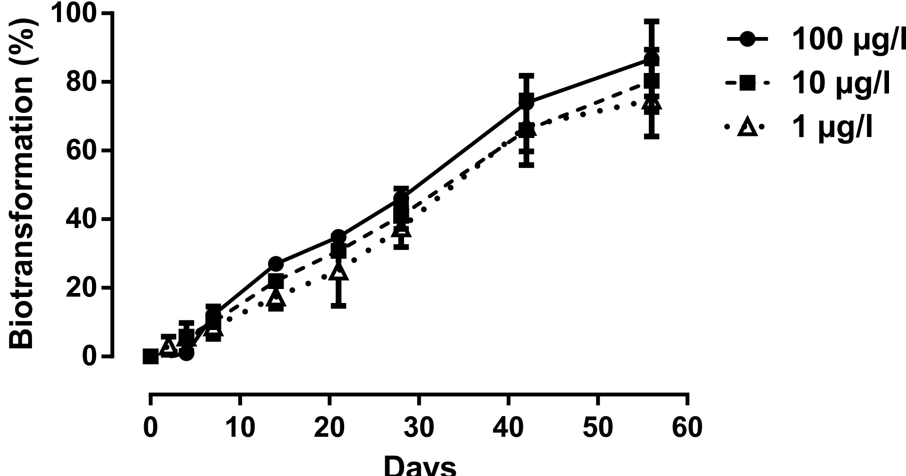
Compound	Lag-period (days)	k1	Half-life (days)	Σ lag + half-life (days)	R^2
Nitrosamines (NSAs)					
NDELA	6.9	0.0383±0.0025	18.1	25.0	0.9934
NDMA	ND[A)]	< 0.001	>500	ND[A)]	ND[A)]
Nitramines (NAs)					
DMNA	1.0	0.0039±0.0005	180	181	0.9560
$MEA-NO_2$	7.0	0.0980±0.0009	7.1	14.1	0.9999

[A)] Not determined



526

527 **Fig. 1**. Biotransformation of NSAs and NAs in mixed water after 28 days of incubation at 20°C.
528 Biotransformation was determined as percentage reduction in water samples compared to sterilized
529 controls. Error bars show SD of replicates.



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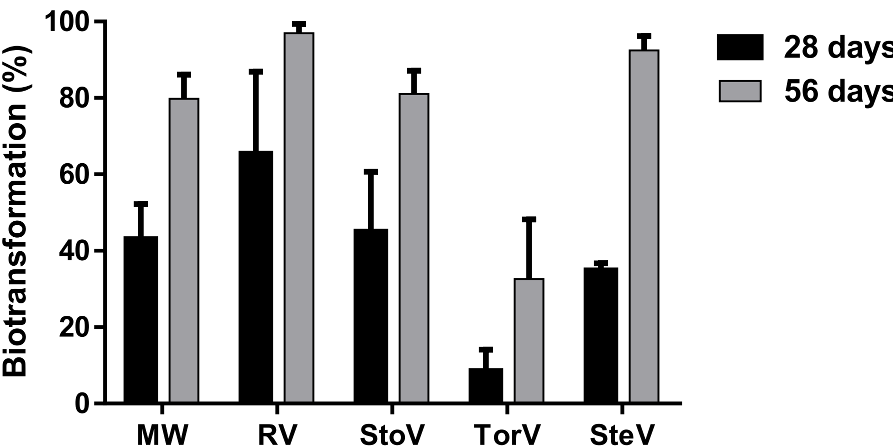
531 **Fig. 2.** Biotransformation at 20°C of NDELA in mixed water at three initial concentrations (1, 10 and
532 100 µg/L) determined as % depletion in normal water compared to sterilized water (control) at each
533 sampling. Error bars describe SD of triplicate samples.

534



535

536 **Fig. 3**. Percentages biotransformation of AMP-NO_2 in normal water relative to sterilized water from
537 different lake water sources after incubation at 20°C in 28 and 56 days. The lakes compared were
538 mixed water (MW), Rotavatnet (RV), Storavatnet (StoV), Torsteinsvatnet (TorV), and Steinsvatnet
539 (SteV).

540

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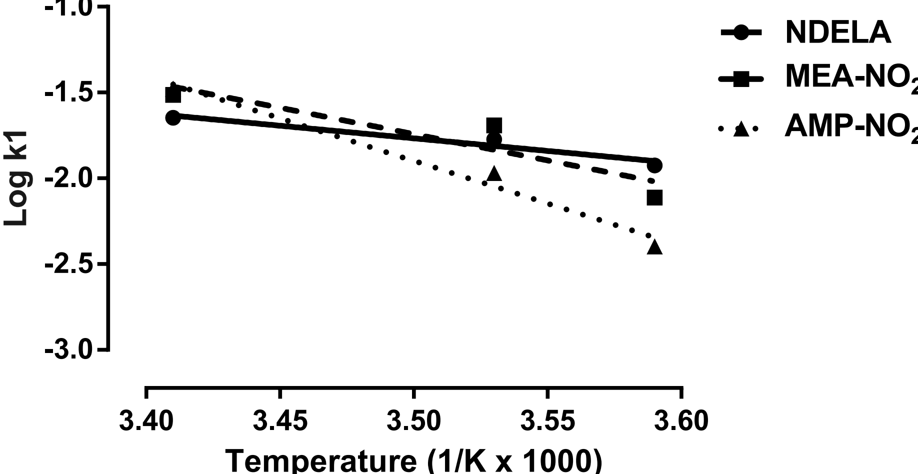
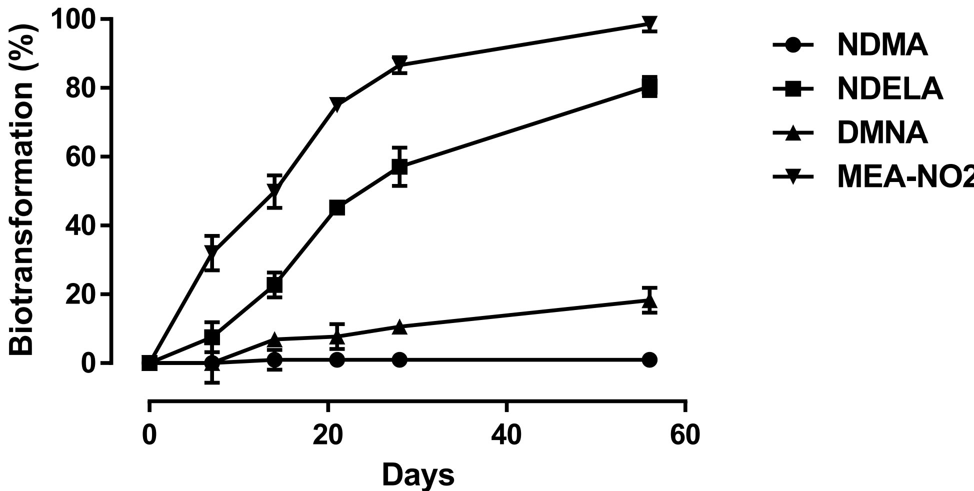


542

543 **Fig. 4.** Temperature-dependence of overall rate coefficients (Logk1) for NDELA, MEA-NO_2 and
544 AMP-NO_2 in mixed water. The overall rate coefficients were calculated from the sums of lag-phases
545 and half-lives (see Table S5 in SD).

546



547

548 **Fig. 5**. Anaerobic biodegradation of NDMA, NDELA, DMNA and MEA-NO_2 in a water-
549 sediment system. The results are shown as % biotransformation in normal water compared to
550 sterilized water (% of control) at each sampling.

Supplementary Data

Table S1a. Chemical structures and selected environmental data of the nitrosamines used in this study estimated from the structure-activity relationship program EPIWEB 4.1.

Chemical	Structures	Soil adsorption (Koc)	[A)]Bioaccumulation (log Kow)	Atmospheric oxidation (half-life; hrs)
NDELA		0.2242	-1.28	4.3
NPIP		12.04	0.7223	5.0
NDEA		14.03	0.48	7.2
NDMA		3.683	- 0.57	50.7
NMEA		8.01	0.04	12.7
NMOR		3.528	-0.44	1.7
NDPA		43.03	1.36	5.3
NPYR		5.976	-0.19	8.3
NPz		2.332	-1.49	1.2
DNPz		2.332	-0.85	3.0

[A)] Compounds with log Kow ≥ 3 are considered to be bioaccumulating
[B)] Based on overall OH-rate constant

Table S1b. Chemical structures and selected environmental data of the nitramines used in this study estimated from the structure-activity relationship program EPIWEB 4.1.

Chemical	Structures	Soil adsorption (Koc)	[A]Bioaccumulation (log Kow)	[B]Atmospheric oxidation (half-life; hrs)
DMNA		6.50	-0.52	33.5
MEA-NO_2		0.09	-2.74	1.6
MNA		1.94	-1.51	82.3
DENA		22.65	0.46	6.7
Pz-NO_2		0.43	-2.18	1.2
AMP-NO_2		0.29	-1.87	1.9

[A] Compounds with log Kow ≥ 3 are considered to be bioaccumulating
[B] Based on overall OH-rate constant

Table S2. Summary of the analytical methods and limits of quantification used to analyse and quantify nitrosamines and nitramines for this study.

<table>
<tr><th>Analyte</th><th>Column</th><th>Mobile Phase</th><th>Ion Source</th><th>LOQ (ng/mL)</th></tr>
<tr><td>NDELA</td><td rowspan="3">Ascentis Express RP-Amide
(15cm x 4.6 mm; 2.7µm particle size)</td><td rowspan="3">Formic Acid/MeOH</td><td rowspan="3">APCI/ES
I</td><td rowspan="3">1</td></tr>
<tr><td>NPIP</td></tr>
<tr><td>NDEA</td></tr>
<tr><td>NDMA</td><td rowspan="2">Ascentis Express RP-Amide
(15cm x 4.6 mm; 2.7µm particle size)</td><td rowspan="2">Formic Acid/MeOH</td><td rowspan="2">APCI</td><td rowspan="2">1</td></tr>
<tr><td>NMEA</td></tr>
<tr><td>NMOR</td><td rowspan="3">Ascentis Express RP-Amide
(15cm x 4.6 mm; 2.7µm particle size)</td><td rowspan="3">Formic Acid/MeOH</td><td rowspan="3">APCI</td><td rowspan="3">0.1</td></tr>
<tr><td>NDPA</td></tr>
<tr><td>NPYR</td></tr>
<tr><td>NPz</td><td>Discovery HS F5
(15cm x 4.6 mm; 3µm particle size)</td><td>0,1wt% Ammonium
Acetate/MeOH,</td><td>APCI</td><td>1</td></tr>
<tr><td>DNPz</td><td>Ascentis Express RP-Amide
(15cm x 2.1 mm; 2.7µm particle size)</td><td>Formic Acid/ACN</td><td>ESI</td><td>10</td></tr>
<tr><td>DMNA</td><td>Zorbax eclipse plus c18 RRHD
(5cm x 2.1 mm; 1.8µm particle size)</td><td>25mM Formic Acid,
isocratic</td><td>ESI</td><td>1</td></tr>
<tr><td>MEA-NO_2</td><td>Thermo Scientific Hypercarb
(15cm x 4.6 mm; 3µm particle size)</td><td>0.1wt% Ammonium
Acetate/MeOH,</td><td>APCI</td><td>0.1</td></tr>
<tr><td>MNA[a]</td><td>Zorbax eclipse plus c18 RRHD
(5cm x 2.1 mm; 1.8µm particle size)</td><td>25mM Formic Acid/ACN</td><td>ESI</td><td>1</td></tr>
<tr><td>PZ-NO_2</td><td rowspan="3">Discovery HS F5
(15cm x 2.1 mm; 3µm particle size)</td><td rowspan="3">25mM Formic Acid/ACN</td><td rowspan="3">ESI</td><td rowspan="3">1</td></tr>
<tr><td>AMP-NO_2</td></tr>
<tr><td>DENA</td></tr>
</table>

[a] Required derivatisation with dibutylamine before analysis

Table S3 Biodegradability in mixed water of NSAs measured respirometrically and calculated as % BOD of ThOD).

Name	Abbreviation	Biodegradability - BOD (% BOD of ThOD) ± SD			
		7 days	14 days	21 days	28 days
Nitrosamines					
Nitrosodiethylamine	NDEA	< 1	< 1	< 1	< 1
Nitrosodimethylamine	NDMA	< 1	< 1	< 1	< 1
Nitroso-N-methylethylamine	NMEA	< 1	< 1	< 1	< 1
Nitroso-N-propylamine	NPDA	< 1	< 1	< 1	2.4±0.6
Nitrosopiperazine	NPz	< 1	< 1	< 1	< 1
Dinitrosopiperazine	DNPz	< 1	< 1	< 1	< 1
Nitrosodiethanolamine	NDELA	2.6±0.2	1.3±1.5	4.0	10.2±5.5
Nitrosopiperidine	NPIP	< 1	< 1	< 1	< 1
Nitrosomorpholine	NMOR	< 1	< 1	< 1	< 1
Nitrosopyrrolidine	NPYR	< 1	< 1	< 1	< 1

Table S4. Biotransformation rates of NDELA, MEA-NO_2 and AMP-NO_2 in mixed water at three temperatures, 20°C, 10°C and 5°C, determined by 1st order rate coefficients with standard deviations (k1±SD) after a non-responsive lag-period. Half-lives were determined from average k1-values. The sum of the lag-period and half-lives (∑lag+half-life) are also shown.

Compound and temperature	Lag-period (days)	k1[B)]	Half-life (days)	Σlag+half-life (days)	R^2
NDELA					
20°C	10.0	0.03340±0.0038	20.8	30.8	0.9277
10°C	0.0	0.0169±0.0021	41.1	41.1	0.7776
5°C	0.0	0.0119±0.0022	58.3	58.3	0.5778
MEA-NO_2					
20°C	12.4	0.0679±0.0158	10.2	22.6	0.9458
10°C	18.6	0.0447±0.0064	15.5	34.1	0.9088
5°C	7.0	0.0084±0.0016	82.9	89.9	0.6678
AMP-NO_2					
20°C	0.6	0.0343±0.0027	20.2	20.8	0.9911
10°C	1.3	0.0110±0.0009	63.2	64.5	0.9316
5°C	0.0	0.0040±0.0008	172.9	172.9	0.6612

Table S5. Overall aerobic biotransformation rate coefficients (k1) in mixed water of NDELA, MEA-NO_2 and AMP-NO_2 at three temperatures, 20°C, 10°C and 5°C. The rate coefficients were determined from the sum of the lag-period and half-lives (∑lag+half-life), as described in Table S4.

Compound and temperature	Σlag+half-life (days)	Overall k1
NDELA		
20°C	30.8	0.0225
10°C	41.1	0.0169
5°C	58.3	0.0119
MEA-NO_2		
20°C	22.6	0.0306
10°C	34.1	0.0203
5°C	89.9	0.0077
AMP-NO_2		
20°C	20.8	0.0333
10°C	64.5	0.0175
5°C	172.9	0.0040

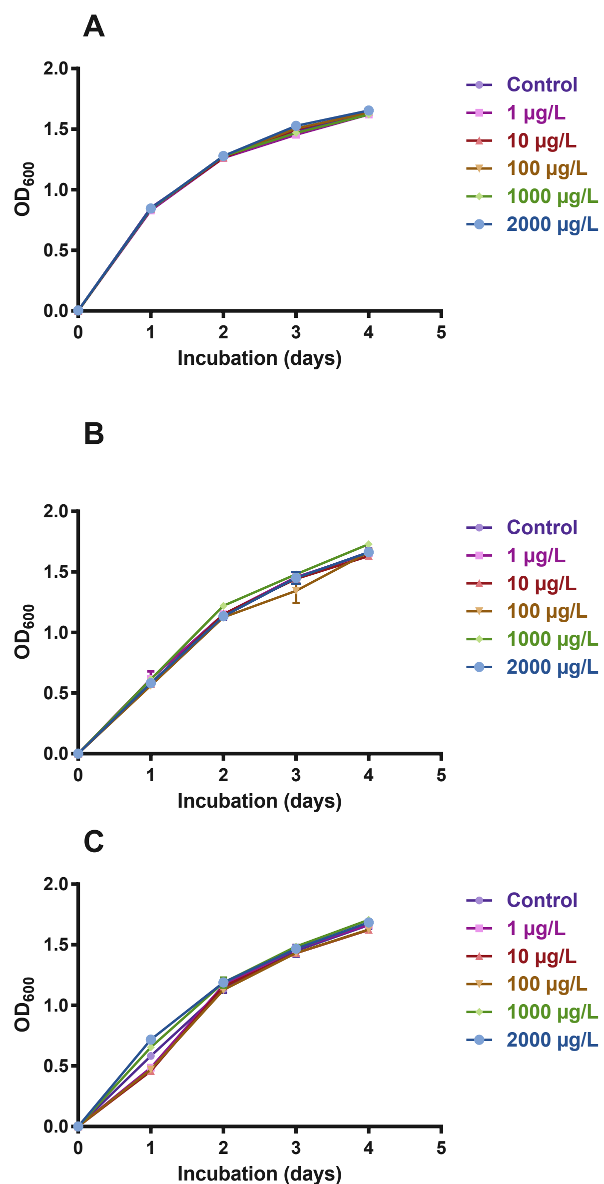


Fig. S1. Growth curves of bacteria from mixed water in Nutrient Broth with different concentrations of the NSAs NDEAL (A), NDMA (B) and NPz (C). The controls represent Nutrient Broth without NSAs. Error bars represent SD of three replicates.

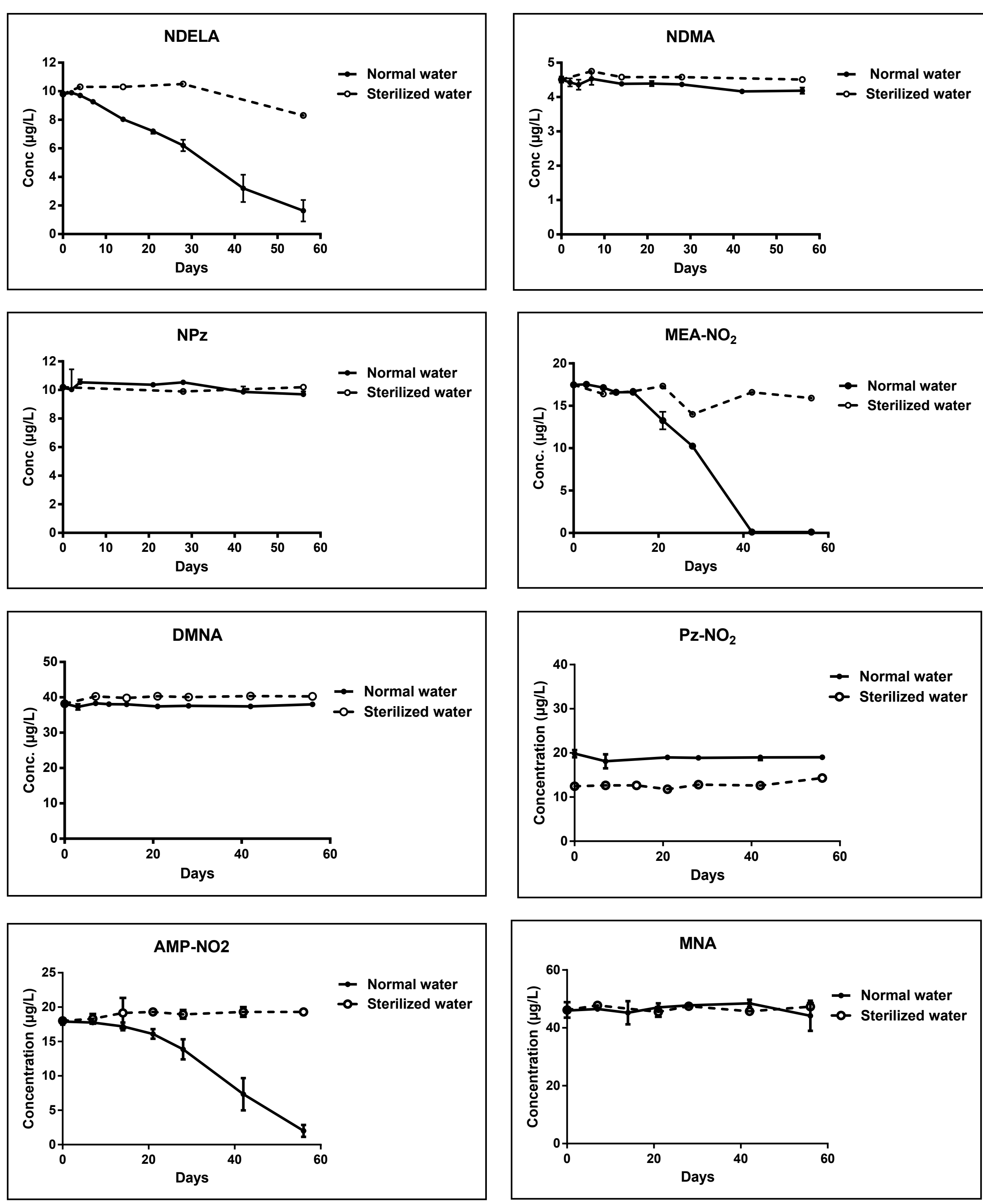
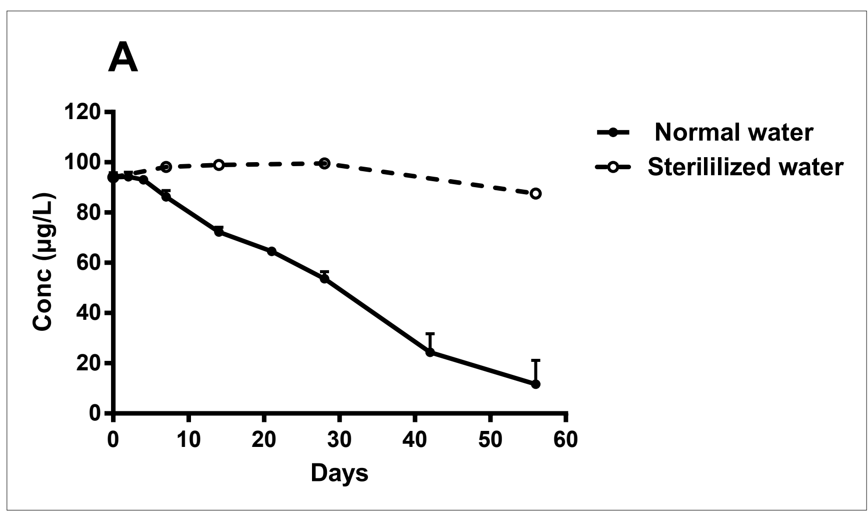
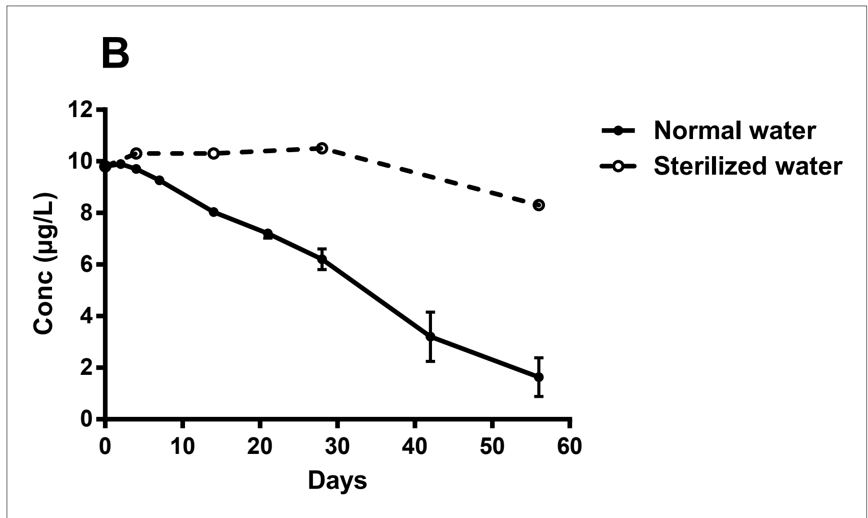


Fig. S2. Depletion of nitrosamines (NDELA, NDMA, NPz) and nitramines (MEA-NO_2, DMNA, Pz-NO_2, AMP-NO_2, MNA) in mixed water at low initial nominal concentrations (5-40 µg/L) and incubation temperature of 20°C. The compounds represented alkanol-compounds (NDELA, MEA-NO_2, AMP-NO_2), alkyl-compounds (NDMA, DMNA, MNA) and cyclic compounds (NPz, Pz-NO_2). Error bars represent SD of three replicates.





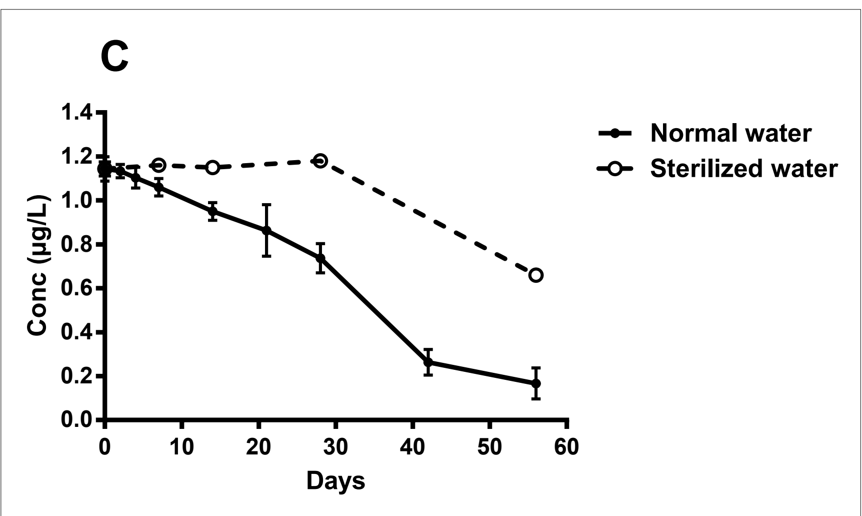


Fig. S3. Depletion of NDELA in mixed water at biotic and normal and sterilized conditions at initial nominal concentrations of 100 µg/L (A), 10 µg/L (B) and 1 µg/L (C).

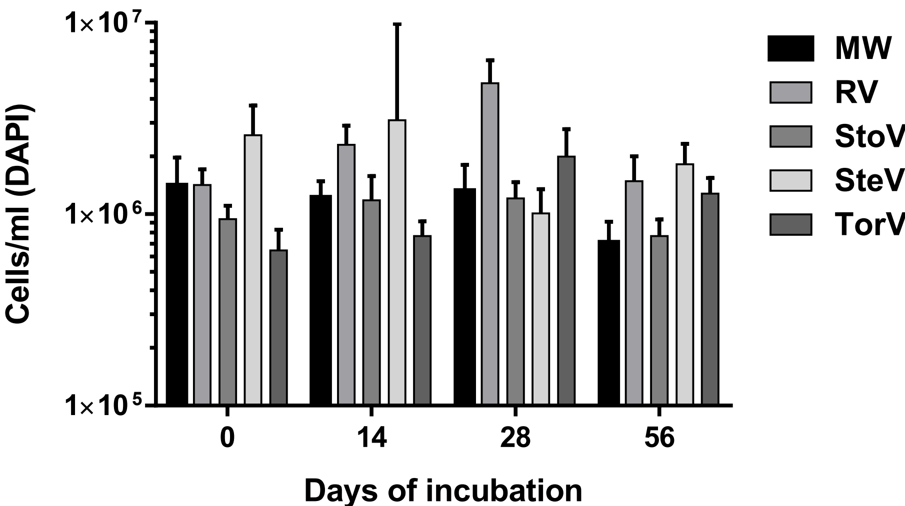


Fig. S4. Total concentrations of microbes in different lake water sources during biodegradation of AMP-NO2 at 20°C. Error bars show SD. The lakes compared were the mixed water (MW), Rotavatnet (RV), Storavatnet (StoV), Torsteinsvatnet (TorV), and Steinsvatnet (SteV).

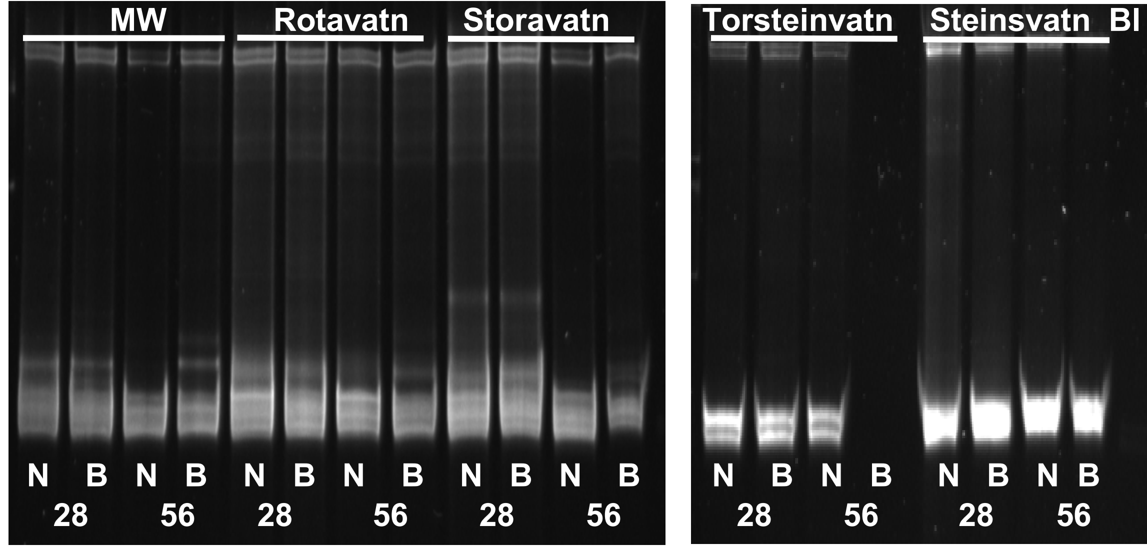


Fig. S5. Denaturing gradient gel electrophoresis (DGGE) of bacterial PCR products from water samples after 28 or 56 days of biodegradation of AMP-NO_2. The results are shown for mixed water (MW), Rotavatn, Storavatn, Torsteinvatn and Steinsvatn. DGGE results are shown for samples with nitramine (N) and from blank water samples without nitramine (B). A sterilized negative control (Bl) was also included.

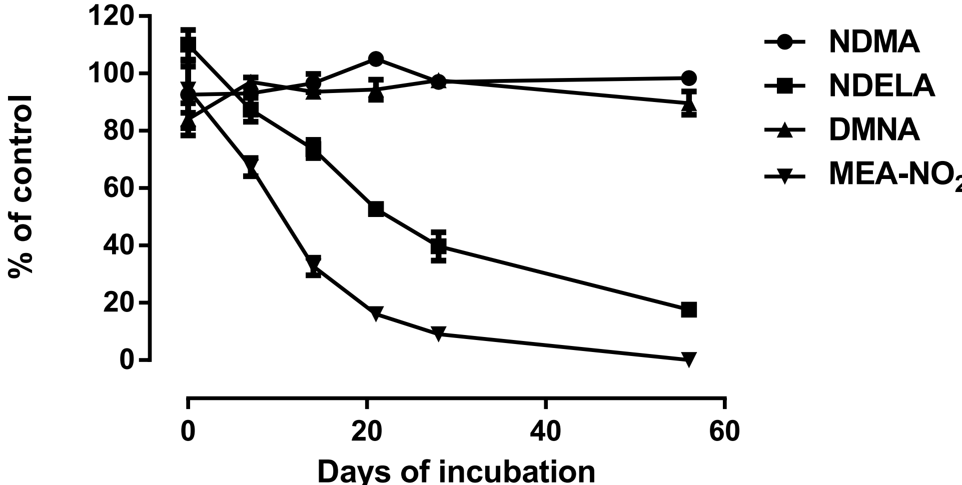
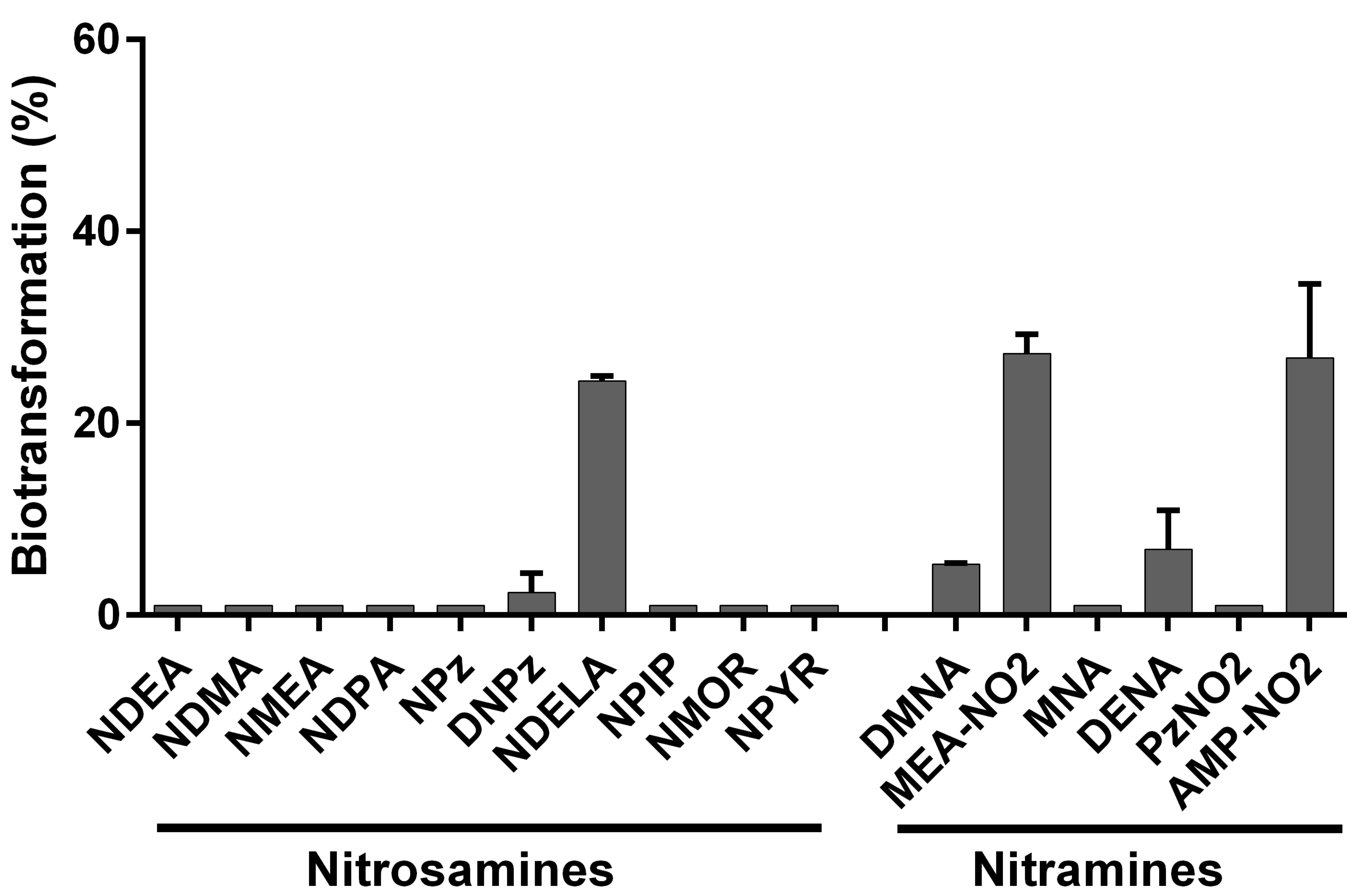
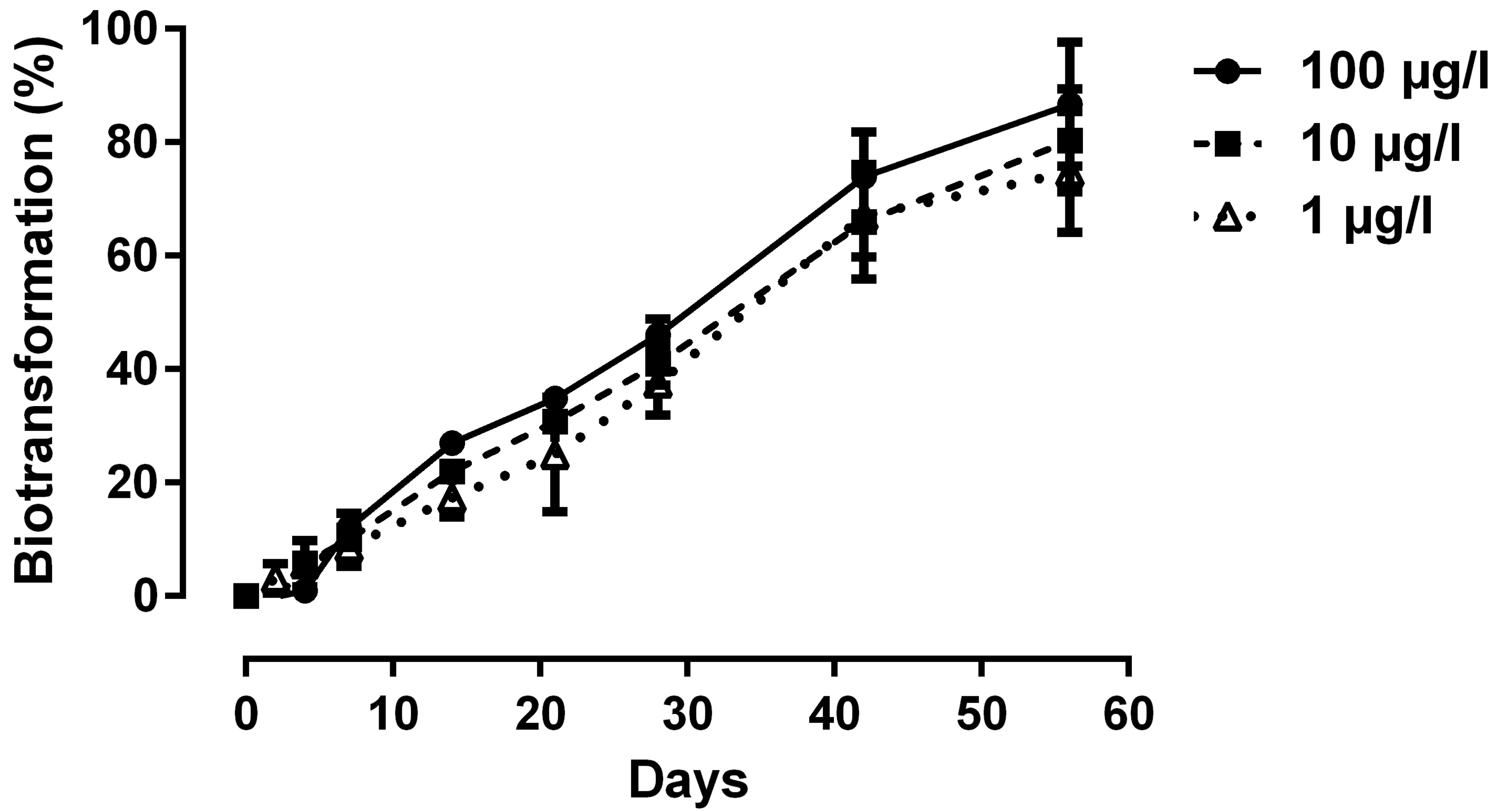


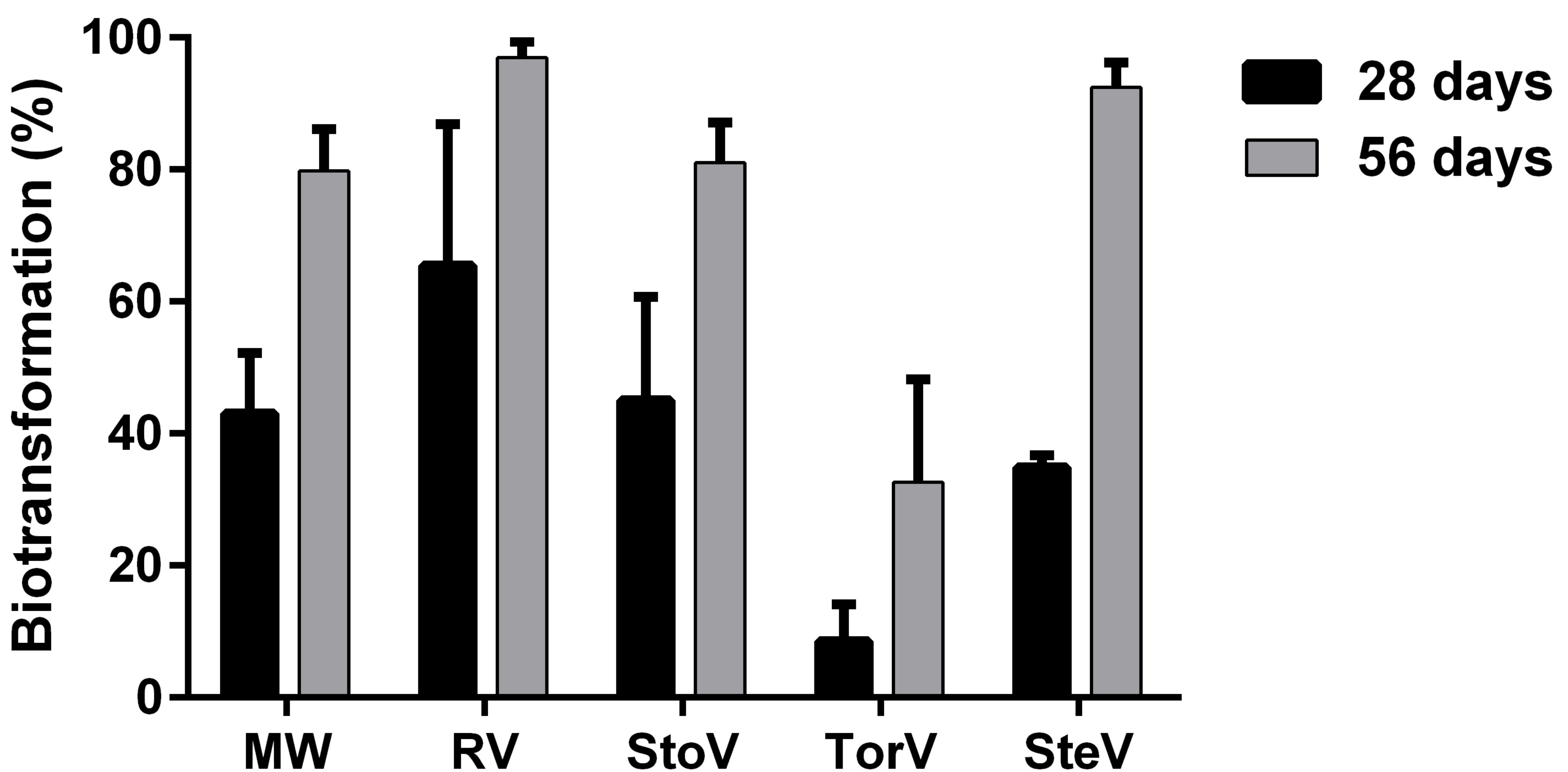
Fig. S6. Anaerobic biodegradation of NDMA, NDELA, DMNA and MEA-NO_2 in a system of clay and sterilized mixed water. The results are shown as % concentration of compounds in normal water compared to sterilized water (% of control) at each sampling.

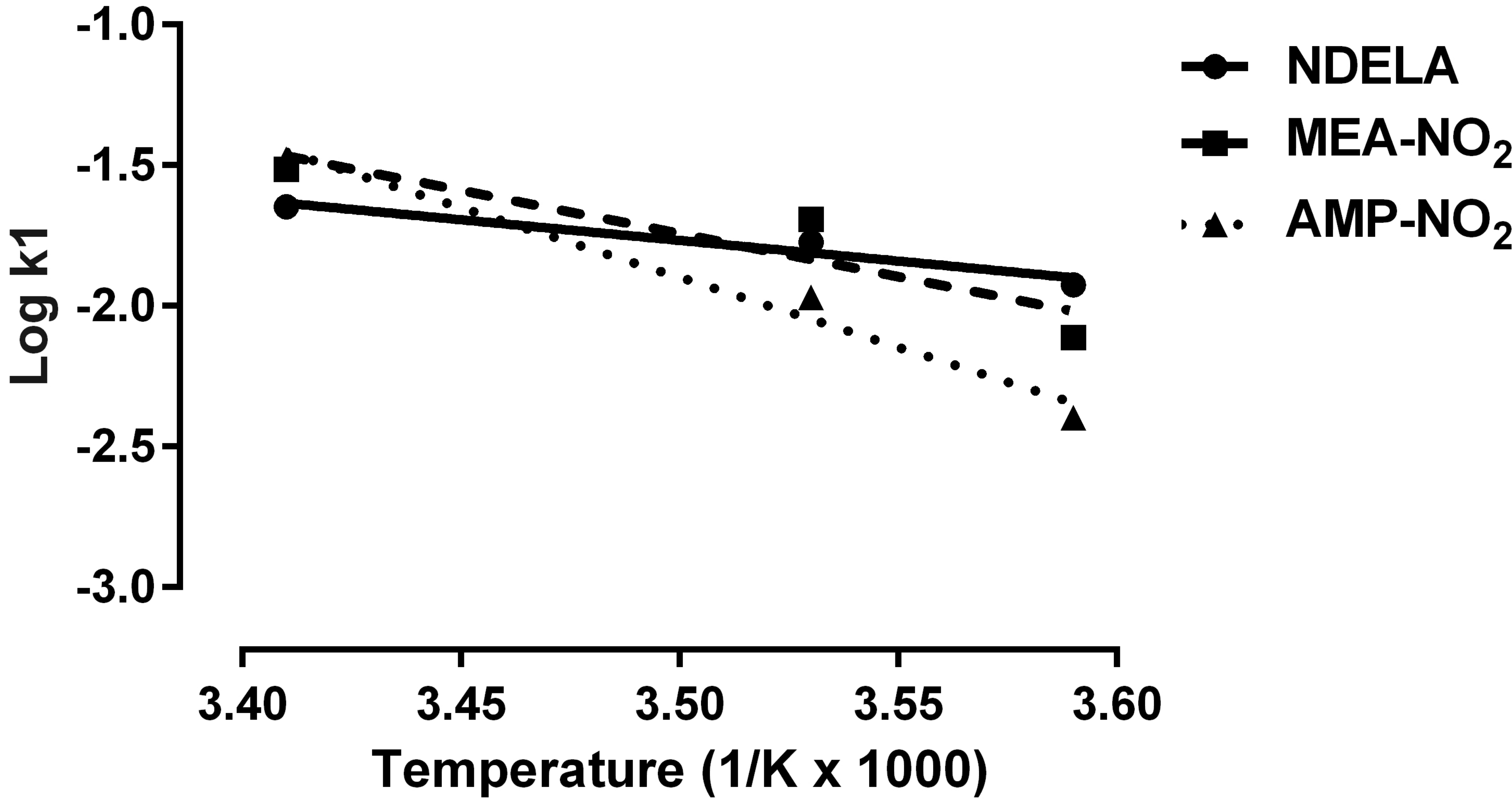
Fig. S7. Possible biodegradation pathways of NDELA to NDMA, based on rules in the Pathways Prediction System of the Biocatalysis/Biodegradation Database (http://eawag-bbd.ethz.ch/).



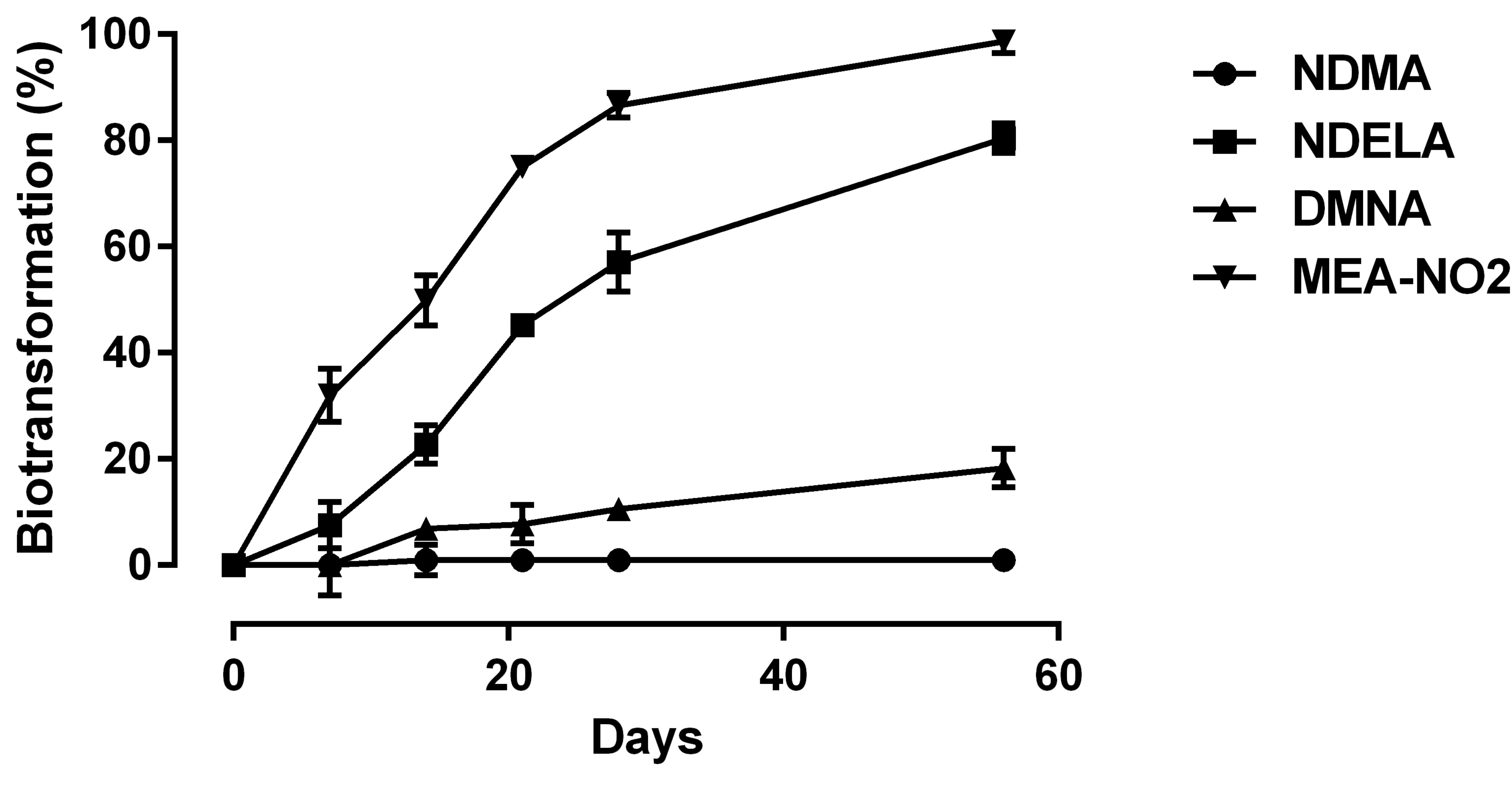
Biotransformation (%)
60
40
20
0
NDEA
NDMA
NMEA
NDPA
NPz
DNPz
NDELA
NPIP
NMOR
NPYR
DMNA
MEA-NO2
MNA
DENA
PzNO2
AMP-NO2
Nitrosamines
Nitramines


Biotransformation (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
Days
100 µg/l
10 µg/l
1 µg/l


Biotransformation (%)
100
80
60
40
20
0
MW
RV
StoV
TorV
SteV
28 days
56 days



Log k1
-1.0
-1.5
-2.0
-2.5
-3.0
3.40
3.45
3.50
3.55
3.60
Temperature (1/K x 1000)
NDELA
MEA-NO2
AMP-NO2


Biotransformation (%)
100
80
60
40
20
0
0
20
40
60
Days
NDMA
NDELA
DMNA
MEA-NO2