Aerobic and anoxic biodegradability of amines applied in CO_2-capture

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ABSTRACT

Integrated and sustainable waste handling is becoming essential in large scale employment of amine-based post combustion CO_2 capture and storage (CCS). We have previously proven the feasibility of biological nitrogen removal of amines in a moving bed biofilm reactor (MBBR) in pre-denitrification mode, thereby serving as a carbon source for denitrification. To evaluate novel solvents, it is essential to test their biodegradability under anoxic conditions. Generally, biodegradability is assessed by standardized aerobic tests, but no equivalent method is available for anoxic degradation. Therefore, a new anoxic batch screening test in syringes was used, measuring the headspace volume expansion due to produced N_2 gas over time. Aerobic biodegradability was measured the conventional way by determining the biological oxygen demand (BOD). Nine different amine samples were tested, including monoethanolamine (MEA) and reclaimer waste. Comparison of biodegradability under aerobic fresh and sea water conditions showed generally improved biodegradation in fresh water. The anoxic screening identified subgroups of amines classified as a) easily degradable, b) slowly degradable and c) undegraded. The results show that BOD alone cannot be relied upon as the only parameter to describe biodegradability. Our anoxic biodegradability test provides essential information on potential carbon sources for denitrification in MBBR and describes the biodegradation kinetics involved.

Amine based processes; Anoxic biodegradability; CCS; Denitrification; Waste generation;

1. Introduction

Since amine based carbon capture and storage (CCS) is moving from the laboratory scale into commercial use, research efforts have now to focus on solvent degradation, emission and waste handling. Aqueous amine solvents are most commonly applied in post-combustion CCS due to their high CO_2 absorption capacity and reaction kinetics. Due to the availability as large scale bulk chemicals, many amines are also relatively low cost (Kumar et al., 2014). Recent works on relevant amine systems include i) acyclic primary amines such as monoethanolamine (MEA) and the sterically hindered 2-amino-2-methyl-1-propanol (AMP), ii) acyclic secondary amines such as diethanolamine (DEA), iii) tertiary amines such as methyldiethanolamine (MDEA) and diethylethanolamine (DEEA), iv) cyclic amines such as piperazine (PZ) and v) its derivatives (Liang et al., 2015). Some other alternative chemical absorbents for CO_2 capture are aqueous alkaline salts of amino acids (Knuutila et al., 2011), phase-change solvents (Pinto et al., 2014), ionic liquids (Kumar et al., 2014) and ammonia (Luis, 2016).

Even though amine based scrubbing is the most widely used technology for post combustion CO_2 capture, many technical solutions have significant potential for improvement. The biggest challenges are the high energy demand of heating the solution for solvent regeneration, followed by solvent loss due to degradation, emissions to air, corrosion, and eco-toxicity (Dutcher et al., 2015; Kumar et al., 2014). Therefore solvent optimization and improvement is at the core of ongoing research (Abu-Zahra et al., 2013).

MEA regarded the benchmark solvent in relation to capture process performance. A recent study has estimated the quantity of generated reclaimer waste for an MEA based process between 1.17 and 3.94 kg/ton CO_2 (Nurrokhmah et al., 2013), whereas an older study from Thitakamol et al. (2007) estimates 4-15 kg of waste per ton of CO_2 captured (Wang et al., 2015). The chemical composition of this waste inevitably depends strongly on the actual amine at use,

as well as flue gas composition and process conditions. In general reclaimer waste will contain water, amine, ammonia, other degradation products, heat stable salts, flue gas impurities and corrosion products.

In a study on key considerations for solvent management, reclaimer waste poses only 7% of the estimated amine loss, whereas water wash makes up 55% of consumed MEA (Reynolds et al., 2012). So far, waste disposal has not received enough attention by the scientific community. Waste management is foreseen to be a topic of increased interest as the amine-based capture technology starts being implemented on large scale. Environmental impacts of carbon capture amines and their degradation products have had much focus over the last years, especially in Europe where environmental law enforcement is strict.

Biological degradation and treatment of amines and amine wastes have been investigated in a multitude of studies, including aerobic biodegradation in seawater and soil, anaerobic detoxification and biogas production, as well as biological nitrogen removal under aerobic and anoxic conditions (Botheju et al., 2010; Brakstad et al., 2012; Eide-Haugmo et al., 2012; Eide-Haugmo et al., 2009; Hauser et al., 2013a; Hauser et al., 2013b; Kim et al., 2010; Mrklas et al., 2004; Ndegwa et al., 2004; Wang et al., 2013a; Wang et al., 2013b). This topic is of great complexity, offering a multitude of options for treating amine waste in an environmentally sustainable manner.

The biodegradability of amines used for CCS is commonly assessed under aerobic conditions. Eide-Haugmo et al. (2012) reported the biodegradability and ecotoxicity of 43 compounds in seawater in an extensive screening study. The biodegradability of the tested amines under these aerobic conditions ranged from <1% to 100%. A follow up study investigated the influence of temperature and concentration, as well as the microbial communities associated with

alkanolamine degradation (Brakstad et al., 2012). Comparative data is lacking for fresh water and other environments, such as anoxic conditions.

Conventionally, biodegradability is assessed by determining the biological oxygen demand (BOD) by a standardized aerobic batch test according to the OECD guideline for testing of chemicals (OECD, 1992). These guidelines include 6 different types of tests, all performed under aerobic conditions. Biodegradation is quantified by measuring the concentration of dissolved oxygen (DO) regularly over 28 days. In general, a substance is readily biodegradable if 60% of the theoretical oxygen demand (ThOD) is reached within 28 days. Furthermore, a test for quantifying biodegradability of chemicals under anaerobic conditions has also been standardized by the OECD (2006). The principle is that anaerobic biodegradability results in production of CO_2 and methane. The increase in headspace pressure reflects the biogas formation and is monitored up to 60 days. However, this test resembles biogas formation in anaerobic digesters and is not necessarily applicable to other anoxic environments (OECD, 2006).

Besides aerobic and anaerobic environments, anoxic ecosystems lie in between these two extremes and play a key role in biodegradation. Under oxygen limiting conditions (ideally < 0.2 mg/L dissolved oxygen) some microorganisms can switch to nitrate respiration, also referred to as denitrification (Lu et al., 2014).

Denitrification is the dissimilatory reduction of nitrate or nitrite to a gaseous N-oxide accompanied by free energy (ΔG^o) transduction (Bueno et al., 2012). The ΔG^o of nitrate respiration is nearly as high as aerobic respiration, making it the next favorable electron acceptor after oxygen (Jørgensen, 2006). The oxidation of organic matter summarized by Jørgensen (2006) is shown in Equation (1) and (2), where organic matter of unspecified composition is symbolized as $[CH_2O]$.

Aerobic respiration, yielding ΔG^{o} -479 kJ/mol:

$$[CH_2O] + O_2\ (g) \rightarrow CO_2\ (g) + H_2O \quad (1)$$

Denitrification, yielding ΔG^{o} -453 kJ/mol:

$$5\ [CH_2O] + 4\ NO_3^- \rightarrow 2\ N_2\ (g) + 4\ HCO_3^- + CO_2\ (g) + 3\ H_2O \quad (2)$$

The dependency of denitrified nitrogen and carbon source is linear, whereas the stoichiometry depends on the type of carbon source (Matějů et al., 1992).

Denitrifying bacteria are mostly facultative aerobes, using either organic (chemoorganoheterotroph) or inorganic (chemolithoautotroph) compounds as electron donors. Heterotrophic denitrifiers have a high physiological and phylogenetic diversity, while the latter autothroph group consists of only a limited number of species. Heterotrophic denitrifiers can be found ubiquitous in soil and aquatic environments. When they grow in biofilms, conditions usually enrich more diverse communities than in activated sludge. This may be due to an increased abundance of concentration gradients of substrates, metabolic intermediates and products allowing bacterial groups with different metabolic properties to coexist. Due to their important role in wastewater treatment, denitrifying bacteria are of particular interest in engineered biological nitrogen removal (BNR) systems (Lu et al., 2014).

In the context of denitrification in BNR, we have previously reported biodegradation of monoethanolamine (MEA) and MEA based reclaimer waste in a moving bed biofilm reactor (MBBR), see Hauser et al. (2013a, 2013b). Furthermore, our study on inhibition factors in N removal systems treating amine waste emphasize the importance of biodegradability under denitrifying conditions, demonstrating that aerobic nitrification was inhibited by all tested amines, whereas anoxic denitrification was stimulated by all compounds at concentrations up to

100 mM (Henry et al., 2016). It is evident that the anoxic environment must be included in the biodegradability assessment of amine solvents.

To date, there is no standardized test protocol for anoxic biodegradability available. Vázquez-Rodríguez et al. (2008) suggested a method for testing anoxic biodegradability under denitrifying conditions based on quantifying the produced CO_2 from sediment extracts. However, for screening novel solvents as potential carbon sources for biological nitrogen removal systems, this procedure may be considered too laborious. Therefore, we propose a method similar to the OECD guidelines for testing biodegradability of chemicals under anaerobic conditions. The principle of our test is to measure the increase in volume in syringes containing MBBR carriers over time. If the tested amine is biodegradable under anoxic conditions, the volume will increase due to formation of gaseous N_2 as an end product of denitrification (Østgaard et al., 2017).

Inoculum quality remains a problem in spite of all international efforts of standardizing such screening tests. As pointed out already by Grady (1984), a negative result does not prove an inherent lack of biodegradability of a compound, but rather that the test conditions were suboptimal. This is not just related to the microbial community and diversity as such, but also to its recent prehistory reflected in current metabolic state, including procedures of enrichment or accommodation commonly applied (OECD 1992). Generally, starvation in the form of limiting access to easily degradable carbon sources will activate alternative inducible metabolic pathways in heterotrophs. By producing wide-spectered hydrolytic enzymes, they will be able to utilize also complex organics such as cell debris and components (proteins, polysaccharides, fatty and nucleic acids) for growth. In short, in the absence of any external carbon source, the inoculum may start to eat itself. Such a background or blank value metabolic activity cannot simply be

148 neglected or subtracted without consideration when evaluating the outcome of screening tests
149 following the guidelines of OECD (1992, 2006).
150 The objective of the present study was to assess the biodegradability under anoxic conditions
151 for 9 amines used for CO_2 capture. This method can be used to identify potential carbon sources
152 for denitrification. Furthermore, these results were compared to results of the standard aerobic
153 biodegradability test in fresh water, as well as to marine biodegradability reported in literature.

154 ## 2. Material and methods

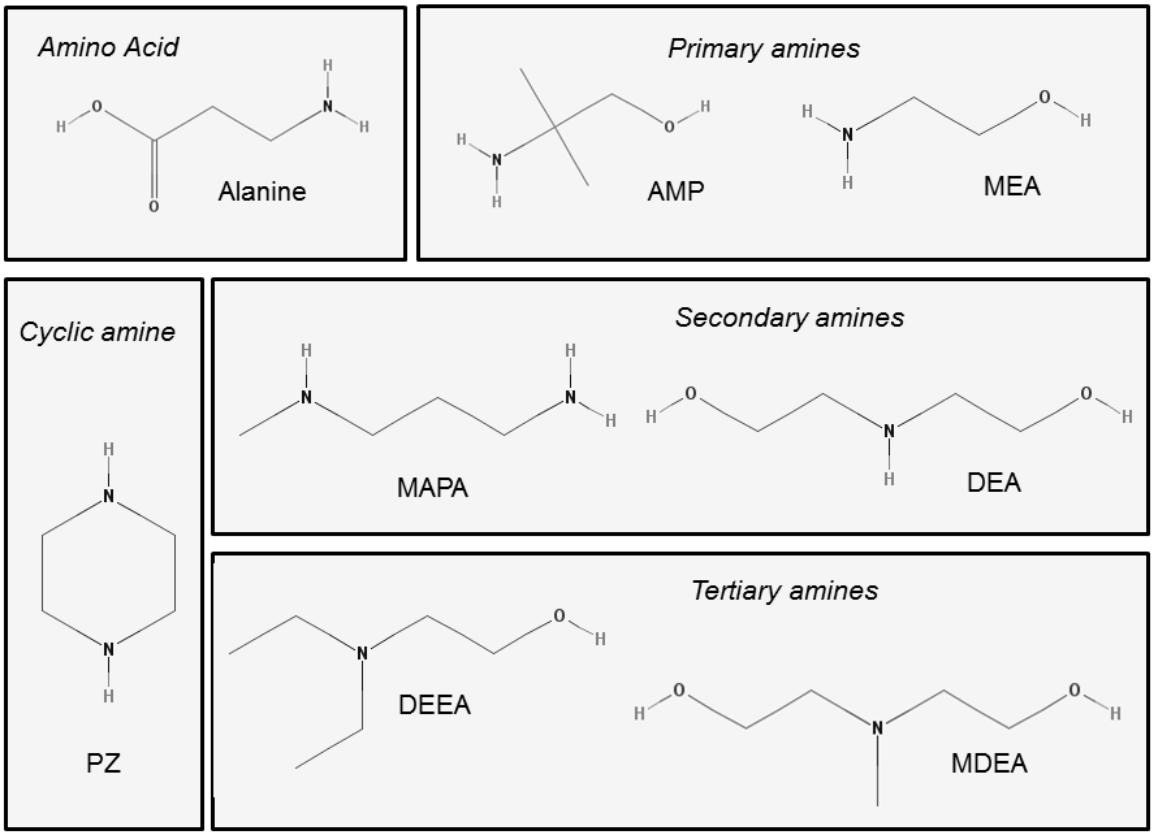
155 ### *2.1. Chemicals*

156 Aerobic and anoxic biodegradability was tested on 9 different compounds with sodium acetate
157 as a positive control. All chemicals are listed in Table 1, including abbreviation, CAS number,
158 formula and theoretical oxygen demand (ThOD). ThOD calculations are based on oxidation of
159 carbon and nitrogen (to nitrate). Chemicals were analytical grade and purchased at Sigma-
160 Aldrich, VWR or Fluka. The test chemicals are sorted according to structure as outlined in
161 Figure 1, in primary, secondary and tertiary amines, cyclic amines, amino acid and reclaimer
162 waste. The chemical composition of the actual MEA based reclaimer waste tested is listed in
163 Table 2, also published previously by Hauser et al. (2013b).
164

165 Table 1 Overview of compounds tested, including abbreviations used, CAS number, formula and
166 theoretical oxygen demand (ThOD). n.a., not applicable

Compound	Abbreviation	CAS	Formula	ThOD[a] (gO2/g)
Positive control				
Sodium acetate	NaAc	127-09-3	$C_2H_3NaO_2$	0.78
Amino acids				
Alanine	Ala	56-41-7	$C_3H_7NO_2$	1.80
Primary amines				
2-amino-2-methylpropanol	AMP	124-68-5	$C_4H_{11}NO$	2.70
2-aminoethanol	MEA	141-43-5	C_2H_7NO	2.36
Secondary amines				
Diethanolamine	DEA	111-42-2	$C_4H_{11}NO_2$	2.13
3-amino-1-methylaminopropane	MAPA	6291-84-5	$C_4H_{12}N_2$	3.45
Tertiary amines				
2-Diethylaminoethanol	DEEA	100-37-8	$C_6H_{15}NO$	2.87
N-methyldiethanolamine	MDEA	105-59-9	$C_5H_{13}NO_2$	2.28
Cyclic amines				
Piperazine	PZ	110-85-0	$C_4H_{10}N_2$	3.35
Reclaimer waste				
MEA based reclaimer waste	RW	n.a.	n.a.	1.42

167 [a] calculations based on carbon and nitrogen oxidation



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169 Figure 1 Structures of the tested amines (Kim et al., 2016), full names are given in Table 1.

170

171 Table 2 Quantification of identified compounds found in the MEA based reclaimer waste tested
172 in this study. See also Hauser et al. (2013b).

Compound	Abbreviation	CAS	Formula	Conc. (g/L)
2-aminoethanol	MEA	141-43-5	C_2H_7NO	586.6
N-(2-Hydroxylethyl)glycine	HEGly	5835-28-9	$C_4H_9NO_3$	42.3
2-Hydroxyethylformamide	HEF	693-06-1	$C_3H_7NO_2$	28.1
4-(2-Hydroxylethyl) piperazine-2-one	HEPO	23936-04-1	$C_6H_{12}N_2O_2$	12.04
1-(2-Hydroxyethyl)imidazole	HEI	1615-14-1	$C_5H_8N_2O$	10.5
Ammonia		7664-41-7	NH_3	8.8
(2-Hydroxyethyl)-acetamide	HEA	142-26-7	$C_4H_9NO_2$	8.2
Nitrate		84145-82-4	NO_3^-	7.5
N-(2-hydroxyethyl)ethylenediamine	HEEDA	111-41-1	$C_4H_{12}N_2O$	4.03
N,N-Bis(2-hydroxylethyl)oxamide	BHEOX	1871-89-2	$C_6H_{12}N_2O_4$	0.06
Nitrite		14797-65-0	NO_2^-	0.046

173

174 ### *2.2. Aerobic Biodegradability test (BOD Test)*

175 A standard fresh water aerobic biodegradation test was performed according to OECD
176 guideline 301 D for testing of chemicals, closed bottle test (OECD, 1992). Surface water was
177 used as microbial inoculum and collected from two unpolluted water sources close to
178 Trondheim, the forest lakes Haukvannet and Theisendammen. Waters were mixed in equal
179 volumes and pre-conditioned by circulation through an aquarium pump for 5-7 days at room
180 temperature in darkness. At the end of the aging period, the enriched water was fortified with

mineral medium and used as inoculum during the biodegradability test according to OECD (1992). The test chemicals were then added to the inoculum. Aged and fortified water without chemicals served as a blank and sodium acetate diluted in aged water served as a positive control. Each test substance was applied to give a final concentration of 2 mg/L in the aged and enriched surface water (OECD, 1992). The solutions were distributed in closed BOD glass bottles (275 mL), and incubated in the dark for 28 days at 20 ± 2˚C. Dissolved oxygen (DO) in the test bottles was measured with an O_2 electrode (Oxi 3315, WTW) in triplicates for test substances, duplicates for blanks and single measurements for the positive control. Measurements were taken at the start of the experiment and after day 5, 7, 14, 21 and 28, and the bottles discharged thereafter (OECD, 1992). Biodegradability was estimated by the biological oxygen demand (BOD), calculated as the difference in DO between the test substance and the blank, and then taken as the percentage relative to the theoretical oxygen demand (ThOD). The ThOD of each test substance is based on the molecular stoichiometric structure, depending on the carbon and nitrogen molecules found in each compound. The total ThOD found in the MEA based reclaimer waste is based on quantification of degradation products in our previous study (Hauser et al., 2013b), whereas their individual contribution to the ThOD is listed in the supplementary information.

Biodegradation rates and half-lives were calculated according to Brakstad et al. (2012), based on first-order rate kinetics by non-linear regression analyses (SigmaPlot 12.5, Systat Software, San Jose, CA, USA, www.sigmaplot.com), given in Equation 3:

$$y = C_0 e^{-kt} \quad (3)$$

where y is amine concentration after time t (days), C_0 is initial concentration and k is the rate constant for the reaction per days of exposure. Half-lives were calculated as ln(2)/k (Brakstad et al., 2012).

2.3. Anoxic Biodegradability (Syringe Test)

The biofilm was grown on polyethylene carriers (Standard AnoxKaldnes K1). Inocula were obtained from a municipal wastewater treatment plant in Trondheim and enriched under denitrifying conditions in steady state conditions as described previously (Hauser et al. 2013a, 2013b).

The inoculum long term stock culture was grown in a denitrification reactor with volume 1.5 l (ht: 15cm, diameter: 20 cm) made of glass, with a water-jacket connected to a VWR water bath set to 22˚C, and operated as a moving bed biofilm reactor (MBBR) run in continuous flow mode. The MBBR reactor was mechanically mixed at a speed of 250 rpm and the influent was fed by using a peristaltic pump, yielding a hydraulic retention time (HRT) of 16 h. The pH was controlled by a Consort Controller R301 and adjusted by automatic addition of 0.3 M HCl or NaOH. To avoid overcompensation, the pH range was set widely, to 6.8 -7.3. The basal medium was prepared according to OECD guideline 301, including 723 mg/L KNO_3 and 400 mg/L sodium acetate, as the electron acceptor and substrate for denitrification, respectively.

For long-term storage, the MBBR carriers were frozen at -20˚C and thawed when needed for the anoxic batch tests. In experiment A, the MBBR carriers were washed and rinsed with basal medium without acetate after each experiment, and kept and reused without any prolonged regeneration phase. In experiment B, the same MBBR carriers were pre-cultured by feeding excess sodium acetate in the continuous flow reactor for 1 week before the syringe test was run.

For the following anoxic syringe test A and B, the same basal medium was used, including nitrate and with different test substances. Test substances were calculated to a final concentration corresponding to a chemical oxygen demand (COD) of 220 mg/L and prepared in basal medium, with pH adjusted to 7.2 - 7.5. NO_3-N was added in excess (110 mg/L), yielding a NO_3-N/COD ratio of 2:1. Sodium acetate served as a positive control, and blanks were basal medium without any carbon source. After preparation, the media were degassed with N_2, determined by an O_2 electrode (Oxi 3315, WTW). Batch experiments were run in 60 ml syringes from BD Plastipak closed air tight with closing cones (Braun). For details, see Østgaard et al. (2017).

For experiment A, each syringe was filled with 5 MBBR carriers and 40 mL test substance and mixed at 20˚C in a horizontal shaker. The gas production in the syringes was read every 1-2 days until day 7, thereafter daily from 12 to 14. To avoid friction derived errors, the piston was first pulled back and released before the value was read. Blanks, positive control and test substances were tested in 5 replicates.

In experiment B, each syringe was filled with 3 MBBR carriers and 40 mL test substance and mixed at 20˚C in a horizontal shaker. The gas production in the syringes was read every 1-3 days until day 21. To avoid friction derived errors, also here the piston was first pulled back and released before the value was read. Blanks and positive control were measured in 5 replicates, test substances in 8 replicates.

3. Results and discussion

3.1. Aerobic Test Stability

The calculations of the aerobic BOD were corrected for blank activity as required by the standard procedure of (OECD, 1992). Our 4 independent experiments were conducted over 9 months and 3 seasons, see Table 3.

Even though the microbial composition of the inoculum must have been changing over time, the oxygen consumption by the blank sample during the test period remained relatively constant, at an average of 2.6 ± 0.3 mg/L DO or 29 ± 3 % of the DO. This is relatively high compared to the positive control sodium acetate, with an average consumption of 3.7 ± 0.5 mg/L, equivalent to 41.6 ± 5.9 % of the DO. The positive control sodium acetate and blank scaled as uncorrected consumed DO are shown separately in Figure 2 A. Please note that the slow but steady oxygen consumption rate of the blank led to an apparent drop in the acetate data after correction as % of ThOD.

Table 3 Additional information for BOD testing - DO consumed at day 28 (mg/L). The initial concentration of DO at day 0 was 8.9 ± 0.1 mg/L for the Blanks.

		Blank	NaAc	MEA	MDEA	DEA	AMP	PZ	RW
Experiment 1	Sep 2014	2.90	4.28	6.55	6.47				
Experiment 2	Mar 2015	2.26	3.93			5.81	6.83		
Experiment 3	Apr 2015	2.42	3.01					6.54	2.65
Experiment 4	May 2015	2.73	3.51					7.61	2.80

3.2. Aerobic Biodegradability

Standardized test results scaled according to OECD (1992) are given in Figure 2 and Table 4. Aerobic biodegradation in fresh water determined by BOD testing at day 28 resulted in biodegradation above 65% for all tested amines, except for the reclaimer waste which remained undegraded under these conditions as shown. This negative result is surprising, since MEA represents approximately 50% of the available carbon in MEA based reclaimer waste, as shown in previous analyses of reclaimer waste (Hauser et al., 2013b). All other chemicals tested may be classified as readily biodegradable (Figure 2 B).

MEA showed the fastest biodegradability of all tested amines, followed by DEA as shown in Figure 2 B. Notably we observed biodegradation even of MDEA and piperazine after a lag time of 7 days, and of AMP after 5 days.

This is in striking contrast to previously reported biodegradability in seawater, where AMP, MDEA and piperazine remained undegraded (Brakstad et al., 2012; Eide-Haugmo et al., 2012). Even at increased temperature, MDEA showed low to negligible ultimate biodegradability in seawater (Brakstad et al., 2012). However, conditions such as aeration or recycling during aging of the water prior to testing (OECD, 1992) might affect the inoculum too.

Generally, conversion was increased and more rapid in fresh water compared to the reported biodegradability of these amines in seawater. For direct comparison of degradation rates in the marine biodegradability test, first-order degradation rates and half-lives were determined and are presented in Table 4.

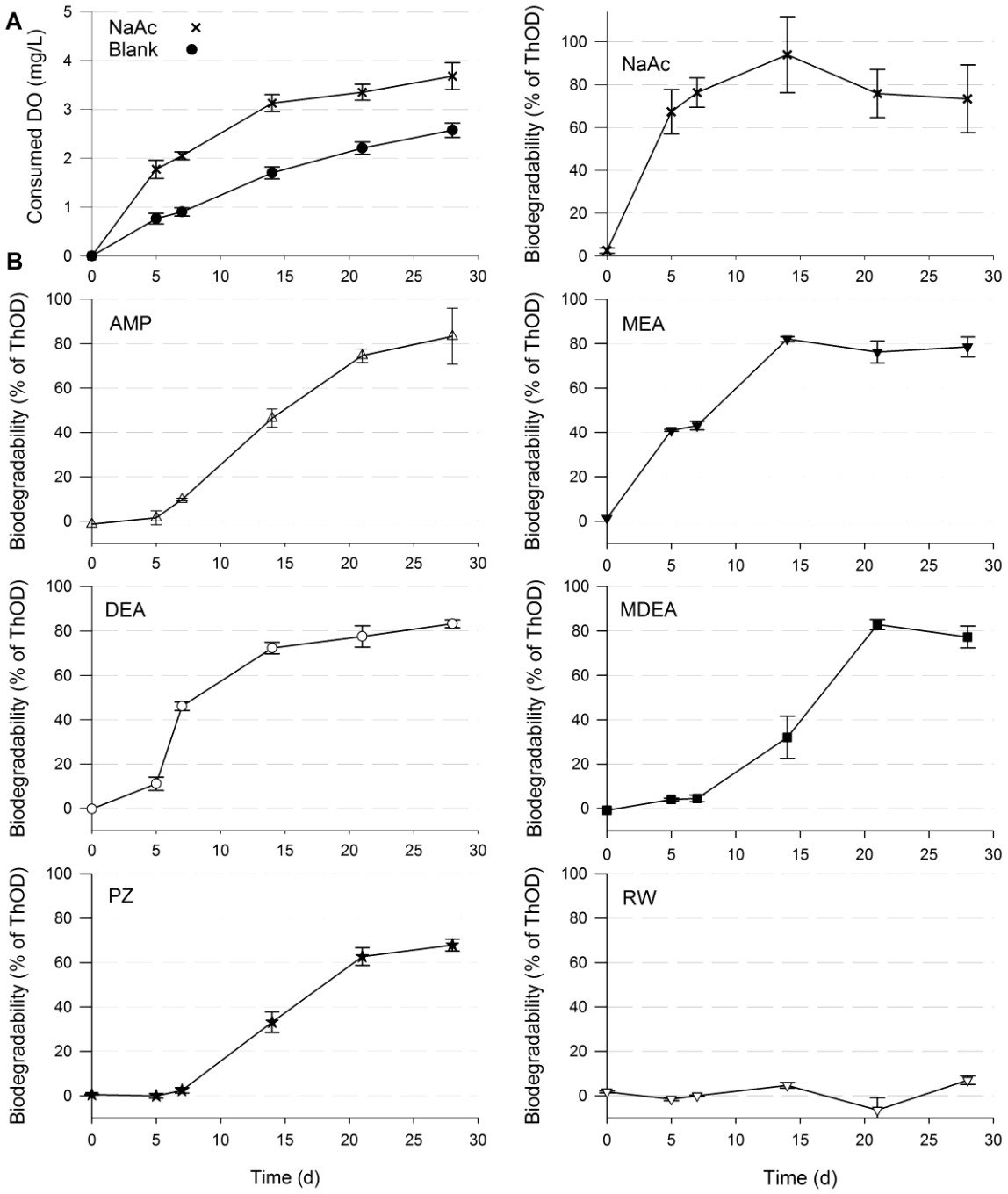


Figure 2 A) Consumed oxygen levels in sodium acetate (×) and blanks (+) during the BOD testing and B) biodegradation of sodium acetate in fresh water given as BOD (% of ThOD) as a function of time. Error bars indicate the SEM of 4 replicates B) Biodegradation of amines in fresh water. The calculated BOD values are corrected for the blank values. AMP (△), MEA

(▼), DEA (○), MDEA (■), piperazine (★) and reclaimer waste (▽). Error bars indicate the SEM of 3 (AMP, DEA, MDEA, MEA) or 6 (Pip, RW) replicates. Note the differences in scaling of graphs in A.

Table 4 Comparing the ultimate biodegradability of amines in fresh water and sea water (Brakstad et al. (2012)[a], Eide-Haugmo et al. (2012)[b]). First-order rate constant (*k*), half-lives in days (d) and ultimate biodegradation (% of ThOD) of 2 mg/L of amines, based on BOD 28 results (OECD, 1992). n.d, not determined.

Amine	*K*	Half-life (d)		Ultimate (BOD) (%)	
		Fresh water	Sea water[a]	Fresh water	Sea water[a,b]
AMP	0.0554	**12.5**	>700	**83.3**	<1[b]
MEA	0.0824	**8.4**	8.3	**78.5**	71.2±0.3[a]
					68.0[b]
DEA	0.0752	**9.2**	24.1	**83.2**	66.3±4.0[a]
					62.8[b]
MDEA	0.0514	**13.5**	>700	**77.3**	<1[a,b]
PZ	0.0406	**17.1**	n.d.	**67.9**	3.0[b]
RW	<0.001	**>1000**	n.d.	**3.2**	n.d.

Most strikingly, AMP and MDEA showed half-lives of approximately 14 days compared to more than 700 days under marine conditions. DEA and MEA had also shorter apparent half-lives in freshwater than in sea water. One reason for these differences could be the distinct differences in microbial communities involved in the two cases. ß-Proteobacteria is one important freshwater

297 group that is noticeably absent in marine environments (Methé et al., 1998). In a recent study,
298 high abundance of ß-Proteobacteria has been positively correlated with hydrocarbon degradation
299 in soils (Bell et al., 2013). In marine biodegradation of DEA, phylogenetic analyses indicated
300 that ɤ-Proteobacteria became abundant during the experiment, however, strains growing on DEA
301 or MEA could not be cultivated for gene expression studies during alkanolamine biodegradation
302 (Brakstad et al., 2012).
303 In general, ultimate biodegradability, as determined by BOD may be useful for assessing rapid
304 direct biodegradability of amines in natural ecosystems. However, in an engineered system, such
305 as in the case of biological N removal, these results must be reconsidered.
306 First, the microbial community of surface waters depends strongly on geographical and
307 seasonal variations, as well as the experimental procedure to obtain the inoculum. This might be
308 directly reflected in the apparent degree of biodegradability. If the substance is not biodegraded,
309 this actually just shows the possibly accidental absence of the required bacteria in the chosen
310 inoculum (Grady, 1984). Furthermore, Grady (1984) argues the BOD testing conditions are too
311 stringent for several reasons; using the compound as a sole carbon and energy source excludes
312 co-metabolism, the small single inoculum limits the genetic capability for degradation, and the
313 relatively short testing time forces acclimation to be the only mechanism. This results in a bias
314 towards only readily biodegradable compounds giving a positive result (Grady, 1984).
315 Secondly, the weight based recommended concentration of 2-5 mg/L makes it difficult to
316 compare biodegradability of one substance with anaerobic respiration based on other electron
317 acceptors.

3.3. Anoxic Syringe Test Stability

The relative anoxic biodegradability of amines was assessed in our simple syringe batch test run for 14 and 21 days in experiment A and B, respectively. This method is a modification of the OECD guidelines for quantifying biodegradability of chemicals under anaerobic conditions (OECD, 2006). Instead of measuring the pressure increase in the headspace, we measure the volume increase in syringes as described in the Methods section above. The experimental verification of the syringe test is presented in detail elsewhere (Østgaard et al., 2017). Notably, when testing denitrification in 25 independent samples ranging from 0 to 2.5 mL of volume increase, the correlation coefficient to chemically determined nitrate consumption was R = 0.9265 (Østgaard et al., 2017). This is considered sufficient for screening purposes.

The initial test A showed significant blank activity, *i.e.* gas production as can be seen in Figure 3. This endogenous activity in the absence of external carbon reflects internal turnover of biomass. In this case, biomass was starved prior to the test. Contrary to the OECD guidelines for fermentative anaerobic conditions, starving the biomass prior to the test did not reduce blank activity. Since the positive control sodium acetate showed double activity the test was nevertheless considered just as valid, as in the case of the aerobic tests above.

However, based on these findings the following test B was run with MBBR maintained in a continuous flow reactor fed with excess acetate prior to the syringe test. Noticeably, the blank activity was then recorded as zero in all blank replicates throughout the experiment, see Figure 4.

3.4. Anoxic Biodegradability, Test A:

The average gas production (GP) of amines under anoxic conditions is given in Figure 3. With 5 MBBR carriers, the positive control sodium acetate reached a gas production of 3.5 mL after 7

days and stayed constant until the end of the experiment at day 14. Blanks showed 1.5 mL gas production during the same time period. Alanine showed greater biodegradability than sodium acetate did, which might be due to representing a simpler carbon source. Surprisingly, AMP showed increasing gas production until day 14. This may be addressed to the starvation of the carriers prior to the experiment, possibly inducing the expression of hydrolytic enzymes. MEA was not tested in this particular experiment, but was found generally readily degradable, as illustrated by Østgaard et al. (2017). The gas production of DEA and MAPA followed the same kinetics as for AMP. MDEA, DEEA as well as piperazine showed similar gas production as the blank, indicating they were not inhibiting denitrification at this concentration. Reclaimer waste gave a similar gas production as the positive control did, also with comparable kinetics. In summary, the results of Figure 3 show that starvation of the biofilm might induce the enzymatic machinery for utilizing cell debris as a carbon source.

3.5. Anoxic Biodegradability, Test B:

Testing was then repeated with a biofilm inoculum grown on excess acetate for one week. The average gas production of amines under anoxic conditions is given in Figure 4. With 3 MBBR carriers, the positive control sodium acetate reached a gas production of 1 mL after 3 days and stayed constant until the end of the experiment at day 21. Blanks showed now measurable gas production, reflecting the lack of an available carbon source. Also in this experiment, alanine showed greater gas production than sodium acetate, which might be due to alanine being a simpler carbon source. The primary amines AMP and MEA showed very different behavior: AMP did not give any gas production, but MEA showed increased gas production compared to the positive control sodium acetate. This might be due to the steric hindrance of AMP. Both

362 secondary amines, DEA and MAPA had a lag phase of approximately 10 days, but thereafter, the
363 gas production increased steadily. As could be expected for such labile systems, out of 8
364 replicates, we observed 2 and 3 completely inactive syringes respectively. To give a better
365 picture of the results, we calculated the average of active and inactive syringes separately, as
366 illustrated in Figure 4. The tertiary amines MDEA and DEEA, as well as the cyclic amine
367 piperazine did not show any measurable gas production. Reclaimer waste gave less gas
368 production as MEA did, which might be due to the lower concentration of MEA in the reclaimer
369 waste. See Figure 4. These findings highlight three possible categories of biodegradability under
370 denitrification conditions. (i) Easily biodegradable, such as alanine, MEA and MEA based
371 reclaimer waste. (ii) Slowly biodegradable after a lag phase, such as DEA and MAPA; and (iii)
372 difficult, such as AMP, DEEA, MDEA, and piperazine.
373 We have already verified the rapid biodegradation under denitrifying conditions for MEA and
374 MEA based reclaimer waste, serving as a sole carbon source for biological nitrogen removal in a
375 pre-denitrification system. Future works should include the verification of the biodegradability
376 after a lag phase for DEA and MAPA. Furthermore, to quantify the actual degree of degradation,
377 a reference or positive control should be included suitable for independent chemical analysis.
378 These values may then be applied to calibrate the whole test on a COD scale.
379
380

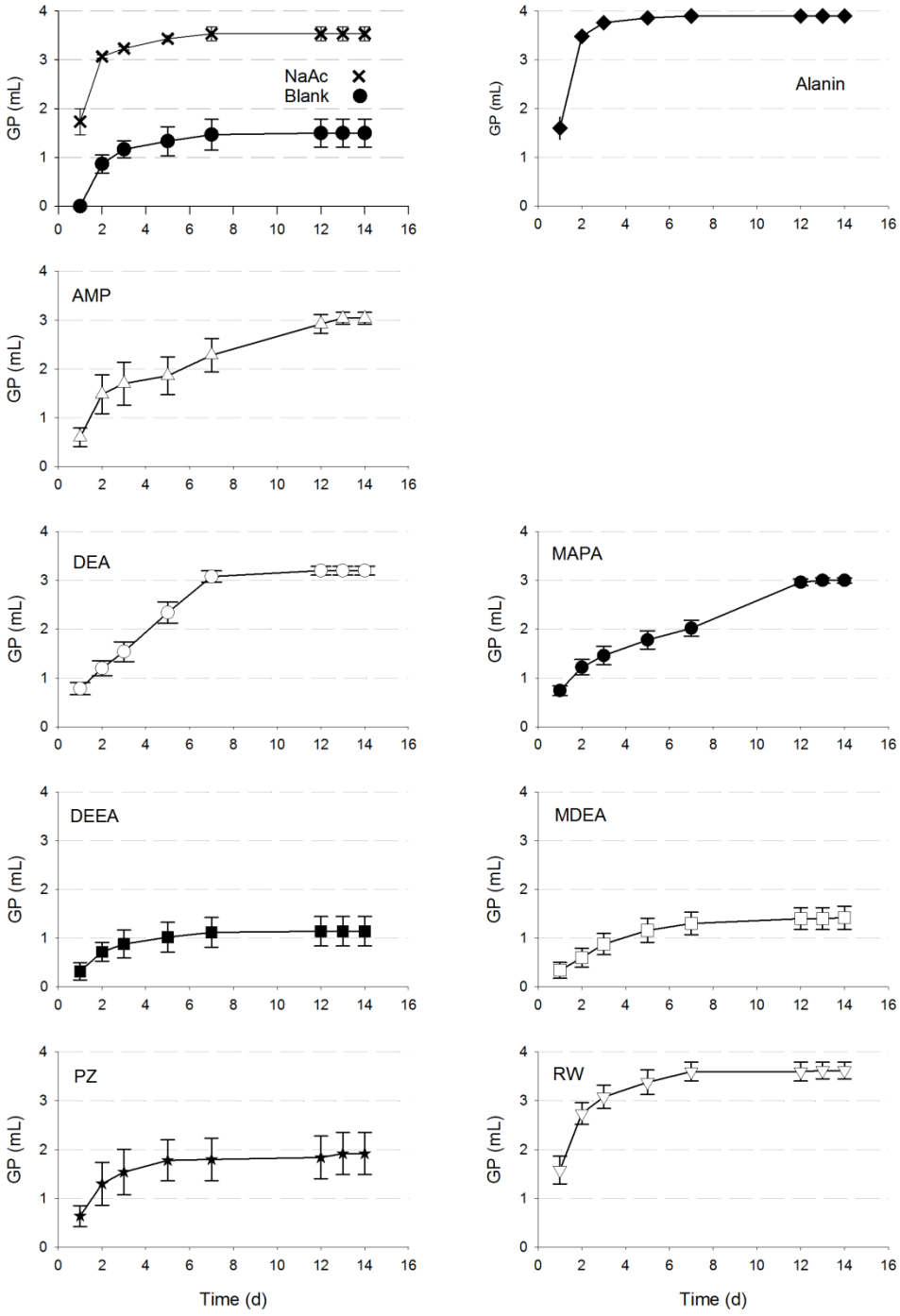
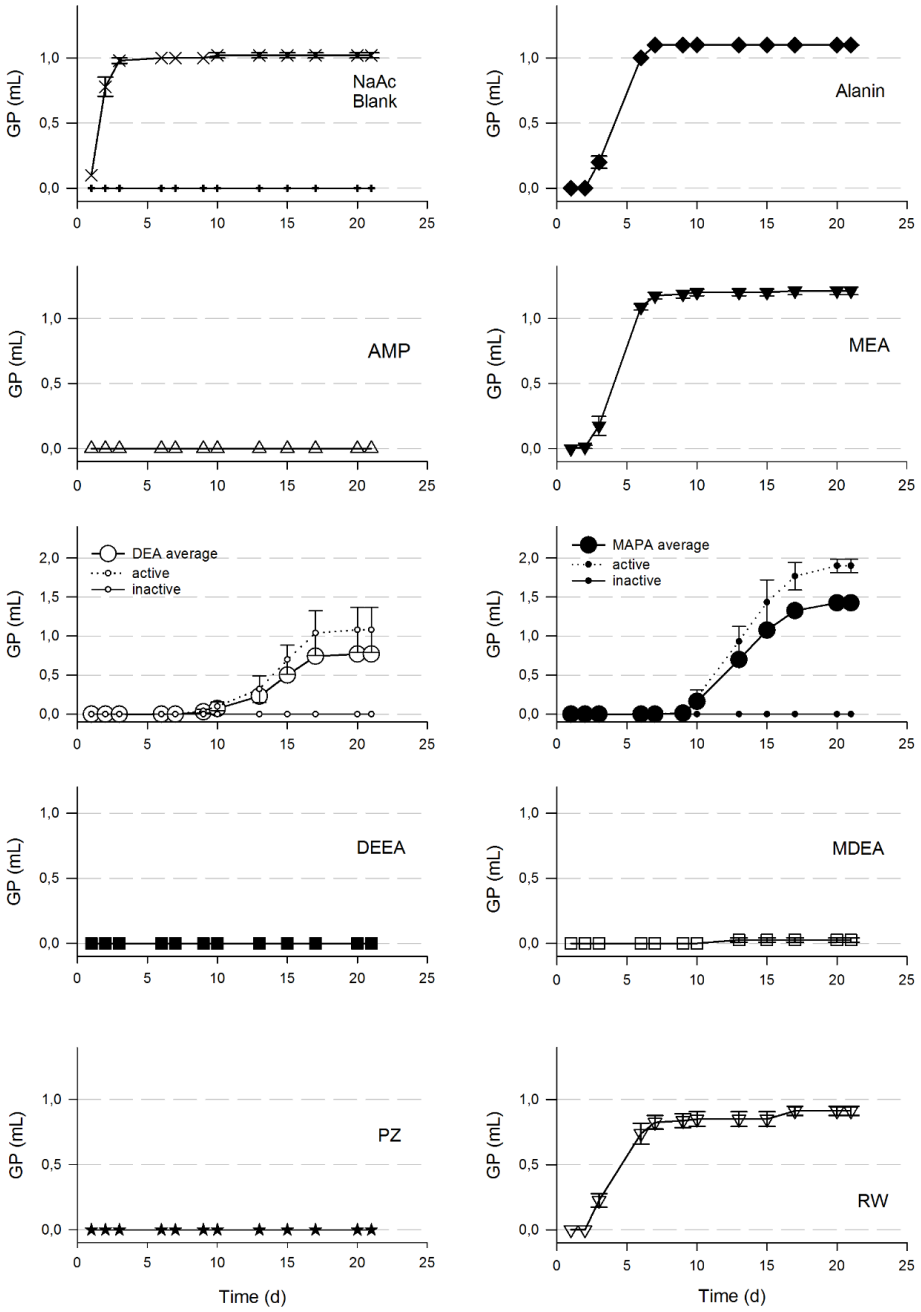


Figure 3 Anoxic Biodegradability, Experiment A. Average gas production (GP) in mL of amines under denitrifying conditions as a function of time. Positive control sodium acetate (×), Blank

384 (+), alanine (◆), AMP (△), MEA (not done), DEA (○), MAPA (●), MDEA (■), DEEA (□),

385 piperazine (★) and reclaimer waste (▽). Error bars indicate the SEM of 5 replicates.



386

387 Figure 4 Anoxic Biodegradability, Experiment B. Average gas production (GP) in mL of amines

388 under denitrifying conditions as a function of time. Positive control sodium acetate (×), Blank

(+), alanine (◆), AMP (△), MEA (▼), DEA (○), MAPA (●), MDEA (■), DEEA (□), piperazine (★) and reclaimer waste (▽). Error bars indicate the SEM of 5 (sodium acetate, DEA, MAPA) or 8 (alanine, AMP, MEA, MDEA, DEEA, piperazine) replicates. DEA and MAPA had 2 and 3 inactive syringes respectively, Therefore, active and inactive data were treated separately (see text).

4. Conclusion

This study presents the biodegradability of selected amines tested under aerobic and anoxic conditions, based on two different types of biodegradability tests.

Under aerobic conditions in fresh water, DEA and MEA were rapidly degraded. AMP, MDEA and piperazine were degraded after one week incubation, while MEA based reclaimer waste was not degraded at all under those aerobic conditions. These results showed improved biodegradability compared to seawater, especially for AMP and MDEA which have been reported persistent under marine conditions.

Under anoxic conditions, our results show that alanine, MEA, and reclaimer waste were suitable carbon sources for denitrification. The secondary amines DEA and MAPA required a lag phase of approximately 10 days before they could be utilized as a carbon source. This does not apply for AMP, DEEA, MDEA and piperazine, as they could not be utilized at all under anoxic conditions in our tests, even after an extended incubation period of 21 days. In this context, it should be mentioned that the concentration of these tested chemicals was well below the observed inhibitory concentration reported previously (Henry et al., 2016).

In general, the microbial consortia play a major role in the biodegradability of amines. If biological nitrogen removal is the main goal, aerobic BOD values do not predict the biodegradability under denitrifying conditions. As shown with MEA based reclaimer waste, the

412 ultimate BOD value of 3 % would exclude any attempt of biological nitrogen removal, but under
413 denitrifying conditions, MEA based reclaimer waste was rapidly degraded in the syringe test, as
414 well as in continuous pre-denitrification systems (Hauser et al., 2013b). Oppositely, AMP was
415 rapidly degraded under aerobic conditions, but could not be utilized as carbon source under
416 denitrifying conditions in the syringe test. This is in agreement with preliminary pilot studies of
417 AMP in a continuous pre-denitrification reactor system (results not included). Our findings
418 highlight the importance of considering the appropriate inoculum before assessing the
419 biodegradability of amines in engineered ecosystems.
420 With the anoxic syringe test, we present a simple method to predict the biodegradability of
421 amines used in CCS under denitrifying conditions. For future solvent evaluation, this screening
422 method offers a rapid and low cost method, compared to the conventional BOD testing.

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431 ## Appendix A. Supplementary data

432 Supplementary data related to this article can be found in a separate file and consists of 1 Table
433 with a total of 3 pages.

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