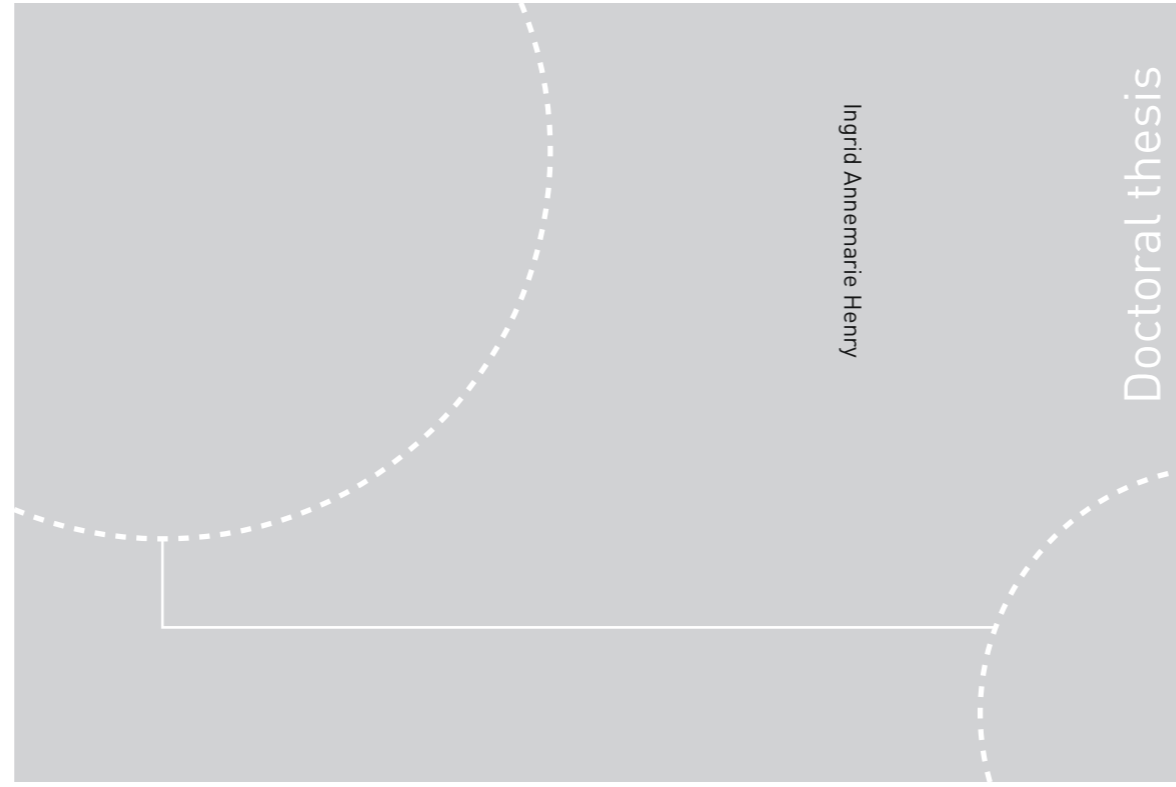


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Ingrid Annemarie Henry

Doctoral thesis

Doctoral theses at NTNU, 2016:359

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Biological Nitrogen Removal of Effluents from Amine-based CO₂ Capture Plants

Screening, Feasibility and Limitations

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Thesis for the Degree of
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Abstract

The increasing large-scale use of amine-based carbon capture and storage (CCS) urges the development of a sustainable waste handling of effluents. However, the development of procedures to improve waste disposal is still in its infancy. In this context, this thesis aimed at testing the feasibility and identifying the essential limiting factors relevant for an up-scaled waste treatment process based on biological nitrogen removal (BNR).

To evaluate the treatment of common solvents, aerobic and anoxic biodegradability of 9 amines were assessed by screening tests. Aerobic biodegradability was estimated by the OECD standard test. Anoxic biodegradability was estimated by a new batch test in syringes with biofilm carriers, measuring volume expansion due to produced N_2 . Results showed striking differences in aerobic (fresh and seawater) and anoxic biodegradability. The anoxic syringe test identified easily, slowly and not biodegradable amines.

Monoethanolamine (MEA) and MEA-based reclaimer (RW) waste were successfully treated in moving bed biofilm reactors (MBBRs). The performance of post- and pre-denitrification was compared, whereas pre-denitrification was identified as the preferred choice of method. Notably, MEA and RW could then serve as a sole carbon source. Essential inhibitory factors were identified by comparing nitrifying activity of MBBRs with or without exposure to organic loading while monitoring the population by pyrosequencing. Organic loading led to heterotrophic enriched and nitrifying MBBRs with 80% efficiency. Nitrification was inhibited at amine concentrations ranging from 9 to 120 mM, while denitrification was stimulated at concentrations up to 100 mM. Heterotrophic enriched nitrifying MBBRs were 5-20 times more sensitive to MEA or organics.

Combining these results, this thesis demonstrates the feasibility of an efficient treatment of waste streams of amine-based CCS based on BNR. Data obtained may form the basis for computerized simulation models needed for up-scaled design and operation.

List of Papers

Paper 1

Hauser, I., Einbu, A., Svendsen, H. F. & Østgaard, K. 2014. Biological treatment of effluents generated by amine based CO₂-capture plants. *Journal of Energy Challenges and Mechanics* 1 (4), 1-5.

Paper 2

Østgaard, K., Shuai, W., Henry, I. A., Sposob, M., Kowarz, V., Haugen, H. H. & Bakke, R. 2016. *Syringe test screening of microbial gas production activity: Cases denitrification and biogas formation*. Submitted to the *Journal of Microbiological Methods*.

Paper 3

Henry, I. A., Kowarz, V. & Østgaard, K. 2016. *Aerobic and anoxic biodegradability of amines applied in CO₂-capture*. Submitted to the *International Journal of Greenhouse Gas Control*.

Paper 4

Hauser, I., Colaço, A., Skjæran, J., Einbu, A., Østgaard, K., Svendsen, H. & Cervantes, F. 2013a. *Biological N Removal from Wastes Generated from Amine-Based CO₂ Capture: Case Monoethanolamine*. *Applied Biochemistry and Biotechnology* 169, 1449-1458.

Paper 5

Hauser, I., Einbu, A., Østgaard, K., Svendsen, H. & Cervantes, F. 2013b. *Biodegradation of amine waste generated from post-combustion CO₂ capture in a moving bed biofilm treatment system*. *Biotechnology Letters* 35, 219-224.

Paper 6

Henry, I. A., Einbu, A., Svendsen, H. F., Bakke, I. & Østgaard, K. 2016. *Inhibition factors in biofilm N removal systems treating wastes generated by amine based CO₂ capture*. *International Journal of Greenhouse Gas Control* 45, 200-206.

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Preface

This thesis is organized around six journal papers, published under Ingrid Hauser (my maiden name) and Ingrid A. Henry. To this date, four have been published and two are submitted. All the papers are enclosed to this thesis as Appendices.

Paper 1 followed an invited lecture and introduces the main aspects and the challenges of this research field. Paper 2 is a methodological study of developing the syringe test screening procedure. The following Papers 3 to 6 are presenting and discussing the different experimental results obtained during this thesis.

Abbreviations

AOB	Ammonia oxidizing bacteria
BNR	Biological nitrogen removal
BOD	Biological oxygen demand
CAS	Chemical abstract service registry numbers
CCS	Carbon capture and storage
COD	Chemical oxygen demand
DO	Dissolved oxygen
EC ₅₀	Effective concentrations where activity reaches 50%
GHG	Greenhouse gas
HRT	Hydraulic retention time
IPCC	Intergovernmental panel on climate change
LC-MS	Liquid chromatography – mass spectroscopy
MAO	Monoamine oxidase
MBBR	Moving bed biofilm reactor
MW _e	Megawatt electrical
NLR	Nitrogen loading rate
NOB	Nitrite oxidizing bacteria
OECD	Organisation for economic co-operation and development
OTU	Operational taxonomic unit
ppm	Parts per million
ThOD	Theoretical oxygen demand

Compound Abbreviations

AMP	2-amino-2-methyl-1-propanol
BHEOX	<i>N,N</i> -Bis(2-hydroxyethyl) oxamide
CH ₄	Methane
CO ₂	Carbon dioxide
DEA	Diethanolamine
DEEA	Diethylaminoethanol
DGA	Diglycolamine
DHU	<i>N,N</i> -di(hydroxyethyl) urea
DIPA	Diisopropanolamine
H ₂ O	Water
HEA	(2-Hydroxyethyl) acetamide
HEEDA	<i>N</i> -(2-hydroxyethyl) ethylenediamine
HEF	2-Hydroxyethyl formamide
HEGly	<i>N</i> -(2-Hydroxyethyl) glycine
HEI	1-(2-Hydroxyethyl) imidazole
HEPO	4-(2-Hydroxyethyl) piperazine-2-one
HSSs	Heat-stable salts
MAPA	3-amino-1-methylaminopropane
MDEA	<i>N</i> -methyldiethanolamine
MEA	2-Aminoethanol
N	Nitrogen
N ₂	Dinitrogen
N ₂ O	Nitrous oxide
NDELA	Nitrosodiethanolamine
NDMA	Nitrosodimethylamine
NHEGly	Nitroso-(2-hydroxyethyl)-glycine

OZD	2-Oxazolidinone
PZ	Piperazine
R	Substituent group
RW	MEA-based reclaimer waste
TEA	Triethanolamine

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1. Introduction

The last two decades have seen increased concerns towards mitigating anthropogenic CO₂ emissions, as they are clearly linked to changes in the climate system. The detrimental effects of climate change can be observed across all continents and oceans in the form of increased atmospheric temperatures, ocean warming, worldwide shrinking of glaciers and ice sheets and rising sea levels according to the Intergovernmental Panel on Climate Change IPCC (2014).

Physical drivers of climate change are anthropogenic greenhouse gas (GHG) emissions, such as carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). Figure 1 depicts the annual amount of emitted CO₂ starting in the year 1850. Prior to the industrial revolution emissions were close to zero, but have increased dramatically, to 34.8 ± 2.9 Giga tons of CO₂ per year in 2011 due to fossil fuel combustion, cement production and flaring (IEA, 2014b; IPCC, 2014). Due to the ever increasing demand of energy, fossil fuels cannot just be replaced by renewable energy sources in the near future (Leung et al., 2014).

Top emitters are China, US, Europe and India, with an estimated contribution of 10.4, 5.2, 3.4 and 2.5 GtCO₂ yr⁻¹ in 2014, respectively (Friedlingstein et al., 2014). These huge discharges have contributed to an atmospheric concentration of 396.0 ± 0.1 ppm CO₂ in 2013, constituting 142% of pre-industrial levels (before 1750) (WMO, 2014). Of this amount, about 50% currently remain in the atmosphere, while the rest is taken up by the ocean and land biosphere (Knutti and Rogelj, 2015). In June 2016 the monthly atmospheric concentration of CO₂ was 406.81 ppm measured in Mauna Loa Observatory, Hawaii (Tans, 2015).

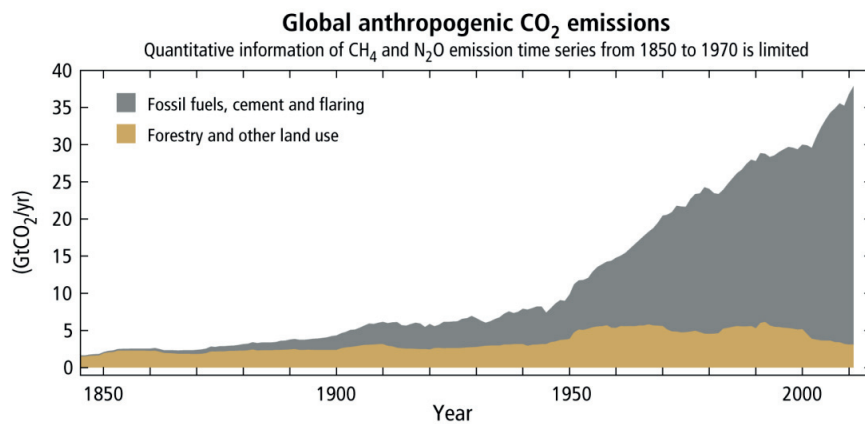


Figure 1 Global anthropogenic CO₂ emissions (IPCC, 2014).

It is recommended by the IPCC that the atmospheric CO₂ level is limited to 450 ppm in order to keep temperature change below 2°C relative to pre-industrial levels, thereby minimizing further negative impact on the climatic system (IPCC, 2014). However, CO₂ is persistent, meaning that even a zero-emission policy will not lead to a full reversal of realized climate changes on human timescales (Knutti and Rogelj, 2015).

The following approaches are considered and adopted by various countries to reduce their CO₂ emissions: improved energy efficiency, and energy conservation, increased usage of low carbon fuels, and renewable energy; applying geoengineering approaches including carbon capture and storage (CCS) (Leung et al., 2014). Thus, future efforts should include research and development of such methods (Heede, 2014; IEA, 2014b; IPCC, 2014).

1.1 Carbon Capture and Storage (CCS)

Carbon capture and storage (CCS) is aimed at reducing emissions at a large scale and has potential applications across several industrial sectors. Apart from fossil fuel power generation, there are also opportunities in heavy industry sectors such as steel and cement that generate GHG emissions as part of the manufacturing process (IEA, 2014a). See also Paper 1.

In CCS, CO₂ is captured, separated, transported and stored for a long term in an underground formation, such as depleted oil and gas reservoirs, unmineable coal beds, saline aquifers or basalts. Currently, there are three main CO₂ capture processes available that can be categorized according to the combustion process, namely, pre-combustion, oxyfuel combustion and post-combustion. In pre-combustion technology, the fossil fuel is gasified in steam to produce syngas (H₂ and CO₂), whereas oxyfuel combustion relies on almost pure oxygen to produce primarily CO₂ and H₂O. In post-combustion, the fossil fuel is conventionally combusted and CO₂ is captured from the resulting gas. Among these technologies, post-combustion capture by amine absorption processes is the most mature technology and can be retrofitted into existing plants (Leung et al., 2014; Dutcher et al., 2015).

A milestone was reached in 2014, when the world's first large scale post-combustion coal-fired CCS project came online at Boundary Dam (Saskatchewan, Canada). The Boundary Dam CCS Project applies amine absorption technology for capturing one million tonnes CO₂ each year (Stéphenne, 2014).

1.2 Amine-based CCS

As illustrated in Figure 2, in an amine-based CO₂ capture process, the flue gas is sent through an absorber column, where it is counter-currently in contact with an aqueous amine solution absorbing the CO₂. A flue gas stream contains typically around 10 vol.% CO₂ and 5 vol.% O₂ (Wang et al., 2015b). The CO₂-

lean flue gas goes through several wash sections and demisters to prevent unwanted emissions of entrained vapours and fine droplets. This scrubbing can be done either with water, acid or proprietary chemicals (Sharma and Azzi, 2014). The rich amine solution is then pumped to a desorber/stripper column where the chemical equilibrium is shifted by heat, typically provided by steam, and the captured CO₂ is released. Temperatures range from 40-50°C in the absorber and 100-150°C in the stripper column (Reynolds et al., 2012). Once separated, the CO₂ is compressed for transport and sequestration. The lean amine solution is then led back to the absorber column, ready for a new capturing cycle as shown in Figure 2.

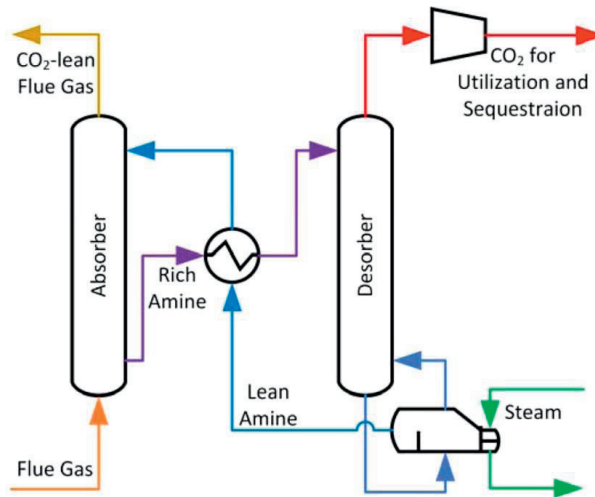
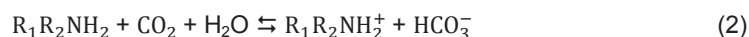
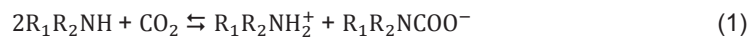


Figure 2 Simplified flow diagram of an amine scrubbing unit (Dutcher et al., 2015).

Amines react with CO₂ via two main chemical reactions - primarily by carbamate formation or bicarbonate formation as given in Equations 1 and 2, respectively. Primary and secondary amines are able to form both, while tertiary amines cannot form carbamate (Dutcher et al., 2015).



Once the amine-CO₂-H₂O system reaches vapour-liquid equilibrium and chemical equilibrium, the amine solution contains cations (protonated amine), free molecules (amine), and anions (carbamate, HCO₃⁻, CO₃²⁻) at the same time. Their concentrations will depend on reaction equilibrium and mass balance (Liang et al., 2015).

1.3 Aqueous Amine Solvents Applied in CCS

Currently, alkanolamines are the most commonly used absorbents due to their low cost and high CO₂ loading (Kumar et al., 2014). The most studied amine has been monoethanolamine (MEA). It has been used for decades in the gas sweetening industry and is often considered the benchmark for amine-based CO₂-absorption. Reducing the energy consumption in the CO₂ capture process has been first priority task in relation with the development of new amine-based processes for CO₂ capture. Also high up on the list, is health and environmental effects of the selected amines, which can represent a show-stopping factor for energetically favourable amines.

Besides MEA, also diethanolamine (DEA), triethanolamine (TEA), methyldiethanolamine (MDEA), 2-amino-2methyl-1-propanol (AMP), diglycolamine (DGA), and diisopropanolamine (DIPA) are of principal commercial interest (Hopkinson et al., 2014). Piperazine (PZ) has also received much attention, but due to its health and environmental effects its application in CCS is still questioned. Recent studies involve blends with other amines such as AMP or 2-methylpiperazine, showing competitive properties when compared to other amine solvent systems (Dutcher et al., 2015; Leung et al., 2014).

Another group of chemical absorbents for CO₂ capture are aqueous alkaline salts of amino acids. Their volatility is very low, resulting in low solvent losses. Due to their natural occurrence, the amino acid salts pose a more environmentally friendly option over alkanolamine solvents. However, they also have their disadvantages, such as precipitating at high concentrations or high

CO₂ loading, thereby lowering mass transfer rates and possibly damaging the process equipment (Liang et al., 2015).

Solvent improvement in terms of factors such as lower overall regeneration energy, high CO₂ absorption capacity, rapid CO₂ absorption rate, low vapour pressure, high degradation resistance, as well as low impact on health and environment is a rapidly developing field with the majority of solvents still being amine-based (Kumar et al., 2014; Hopkinson et al., 2014).

1.4 Amine Solvent Stability

One major challenge associated with amine-based CO₂-capture is, however the inherent loss of amines due to volatility and degradation, leading to reduced process performance over time. Degradation of amine solvents in the CCS system is a complex mixture of reactions. Reactants, intermediates and end degradation products are circulated in the plant and exposed to changing conditions throughout the process cycle. Various degradation and formation mechanisms compete in different units of the plant. Therefore, the composition of the degradation products depends on the combined effects of formation and degradation of compounds within the full process (Einbu et al., 2013).

Degradation mechanisms are either oxidative or thermal, but also influenced by the side-reactions with flue gas components or impurities. The level of impurities in the flue gas depends on its origin, being low in natural gas, increasing in gas-fired flue gas, and being most complex in coal-fired flue gas (Nurrokhmah et al., 2013a; Wang et al., 2015b). Generally, oxidative degradation leads to fragmented amines, resulting in other products such as organic acids, ammonia, and amides. Thermal degradation, occurring mainly at high temperature conditions in the stripping column, forms mostly larger molecules, such as dimers, trimers and amine chains (Dutcher et al., 2015). Carboxylic degradation products can react with amines forming a compound denoted heat stable salt (HSS). HSS anions could include acetate, formate, thiosulfate, sulfate, thiocyanate, oxalate, butyrate and propionates. Water make-

up or flue gas derived HSS anions are chlorides, phosphates, cyanides, and nitrates (Liang et al., 2015).

1.5 Amine Reclaiming in CCS

Due to the degradation mechanisms described above, it is necessary to purify the solvent from time to time. This is essential for maintaining the CO₂ capture capacity, as the accumulation of degradation products and heat stable salts leads to operational problems such as the reduction of CO₂ absorption capacity, corrosion, foaming, fouling, increased viscosity, as well as release of pollutants and toxic degradation products (Wang et al., 2015b).

There are various methods available to keep the contaminant level of the amine solution low, including solution purging, neutralization and amine reclamation. Solution purge involves the removal and replacement of degraded solvent, also called 'bleed and feed'. This technique is not sustainable, as it leads to simultaneous loss of useful solvent. Online neutralization of HSSs is achieved by adding a strong base to the solvent system, rising the pH and thereby releasing the amine trapped by HSSs. The drawback here is that by adding a further impurity, the chemo-physical properties of the solvent system are also worsened. One sustainable and more environmentally sound approach is to reclaim the amine by separating the useful amine from the organic degradation products as well as the HSSs. Reclaiming the amine can be done by thermal reclamation (distillation), ion exchange or electrodialysis (Wang et al., 2015b).

Thermal reclaiming is based on evaporating the useful amine from the degradation products and suspended solids. Therefore, a slip stream is taken from the circulating amine solution and lead to a reclaimer where heat is provided. Initially, the vapour contains mainly water, and then gradually the amine concentration will increase until the vapour composition reaches equilibrium with the circulating solution. In the liquid phase, the high-boiling degradation products and HSSs, as well as some useful amine and water, will accumulate. At the end of the reclaiming cycle, the reclaimer bottom with the

liquid phase is emptied and the generated waste taken out to be handled accordingly (Wang et al., 2015b).

Even though thermal reclamation is one of the oldest techniques used extensively for amine processes in the natural gas sweetening industry, it is to date the only available method to completely remove all degradation products, including heat stable salts, and non-volatile impurities (Liang et al., 2015).

1.6 Waste Management

The arising quantity of waste produced during thermal reclaiming depends on the flue gas composition and operational conditions. Recent studies report 1.17 kg/ton CO₂ to 3.94 kg/ton CO₂ (Nurrokhmah et al., 2013b), while an older study from Thitakamol et al. (2007) estimates 4-15 kg of waste per ton of CO₂ captured (Wang et al., 2015b). The chemical composition of this waste inevitably depends strongly on the actual amine at use, as well as flue gas composition and operational conditions. In general it will contain water, amine, ammonia, other degradation products, heat stable salts, flue gas impurities and corrosion products.

The amount of degraded amine and effluents of solvent and water wash becomes significant, when operating a full scale capture plant removing 1 million tonnes of CO₂ per year, as it is already happening in BD3 SaskPower Canada (Liang et al., 2015). In a study on key considerations for solvent management, reclaimer waste constituted only 7% of the estimated amine loss, whereas water wash made up 55% of consumed MEA (Reynolds et al., 2012). So far waste disposal has not received enough attention by the scientific community, although extended waste monitoring and management forms a very important and necessary part of solvent management.

Sexton et al. (2014) evaluated the reclaimer sludge disposal for MEA, PZ and an MDEA/PZ blend. Solvent loss and degradation were modeled for all three amines. Process modeling and economic analyses were done for two types of flue gas (coal and natural gas), being reclaimed by three different technologies

(thermal reclaiming, ion exchange and electrodialysis). The generated waste could then be classified according to regulatory structures in the United States (US) and European Union (EU). These regulations differ substantially between the US and EU, with the latter being more restrictive. In the US, thermal reclaimer sludge may be classified either non-hazardous or hazardous, depending on the heavy metal content. In the EU, thermal reclaimer sludge may be classified as a hazardous waste due to the significant fraction of the solvent (Sexton et al., 2014). Wang et al. (2015a) classified the thermal reclaimer waste more detailed according to the European List of Wastes as “07 01 08*”, that is “wastes from manufacture, formulation, supply and use of basic organic chemicals”, subgroup “other still bottoms and reaction residues”, according to Directive 91/689/EEC on hazardous waste, indicated by the asterisk *.

For all three solvent systems, the waste streams from ion exchange and electrodialysis contain 95% water and may be classified as non-hazardous in the US, provided no metals are present. Back in the EU, the solvent content of MEA-based waste was not high enough to be classified as hazardous waste. However, the waste of the respiratory sensitizer PZ induces hypersensitivity after inhalation. And thus the MDEA/PZ blend may be classified as hazardous waste in the EU. Also, the authors stress that for their study no real waste was analysed, but assumptions were based on their computer model (Sexton et al., 2014).

In the US, disposal options for hazardous waste are either hazardous waste landfill, fire in a hazardous waste incinerator, fire in a cement kiln licensed to fire hazardous waste, or fire at the power plant. EU regulations limit the options to incineration, due to corrosivity and organic carbon content. Options for non-hazardous waste (only in the US) are landfill, firing in the power plant boiler or firing in a cement kiln. Aqueous waste streams may be treated in wastewater treatment plants, currently not included in US plants, but either on-site or off-site of the CCS plant in Germany. Notably, the amine waste stream has to be treated in an additional unit before discharge (Sexton et al., 2014).

For a model operating at 900 and 810 MW_e, the annual costs for non-hazardous waste were estimated to range from € 0.16 to €1.2 MM/yr (\$0.21 to \$1.6 MM/yr), while for hazardous waste it would range from €1.9 to €11.4 MM/yr (\$2.5 to \$15.1 MM/yr) (Sexton et al., 2014).

Waste management options for reclaimer waste currently include the reuse for NO_x scrubbing, recycling by co-firing into a coal burner, disposal by incineration, as well as biological treatment. In the case of MEA-based reclaimer waste, secondary biological treatment has emerged as the most economic and environmentally friendly approach (Nurrokhmah et al., 2013a).

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; 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.

1.7 Biodegradation of Amines

1.7.1 General Aspects of Biodegradation

Biodegradation is the process of microbial decomposition of organic substances into simpler substances. Abiotic mechanisms play a role in transformation, but cannot convert organic compounds into inorganic compounds. However, microbial activity may lead to mineralization resulting in CO₂, H₂O and other inorganic compounds (OECD, 1997; Grady, 1984) .

A chemical can be classified as biodegradable, persistent or recalcitrant. The term 'biodegradable' alone does however not imply to which extent the compound is transformed. This can lie anywhere between a primary biodegradation, denoting a single transformation of the substance and total mineralization. Persistence means that a chemical is not degradable under

specific conditions, and recalcitrant is defined as inherent resistance to any degree of biodegradation (Grady, 1984).

From a microbial point of view, biodegradation is limited by physical and biological restrictions (Bressler and Gray, 2003). In order to degrade persistent xenobiotics, there are two major options: i) Gratuitous or fortuitous biodegradation taking advantage of some enzymatic inaccuracy regarding substrate binding, a characteristic of many hydrolytic enzymes. This means that the enzyme can bind analogs of the natural substrate which contain xenobiotic functional groups. ii) Co-metabolism is the transformation of a non-growth substrate in the obligate presence of some other growth substrate or transformable compound (Grady, 1984). In the first case, selective enrichment may be achieved by removing alternative carbon sources, in the latter by adding.

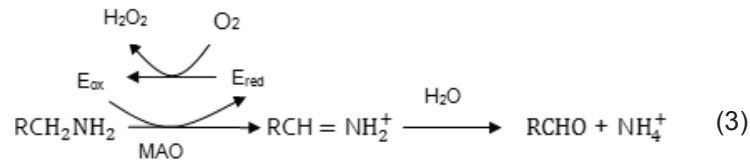
Furthermore, microbial consortia may be able to utilize a sole source of carbon and energy even when a single organism cannot. Typically, the compound may require the sequential metabolism of two or more organisms (Grady, 1984).

1.7.2 Mechanisms of Amine Degradation

Amines can be oxidized abiotically by chemical oxidizing agents, electrochemically and photo chemically (Silverman, 1995). However, interest in microbial oxidative breakdown of amines arose already at the beginning of this century, dating back as far as 1926 (Den Dooren, 1926). The author observed that some organisms are able to utilize amines as a carbon source for growth and some amines can be used as nitrogen source in the presence of glucose by several microorganisms (Gale, 1942).

In biological systems, the oxidation of amines is catalysed by the enzyme monoamine oxidase (MAO, EC 1.4.3.4) (Silverman, 1995). This enzyme is a very promiscuous flavoenzyme, facilitating the oxidation of various primary, secondary, and tertiary alkyl and aryl alkyl amines, whereas primary amines are

the preference (Silverman, 1995). The reaction is a two-step process, as shown in Equation 3.

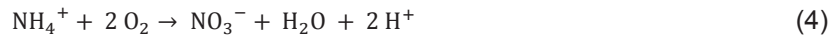


Initially the amine is anaerobically converted by a cysteine residue to the corresponding imine. In the following step the imine is released and hydrolysed to the corresponding aldehyde and ammonia. The second step requires oxygen, as the reduced flavin is inactive and needs to be oxidized back to the active form (Silverman, 1995). The oxidation of amines by flavoproteins was recently reviewed by Fitzpatrick (2010).

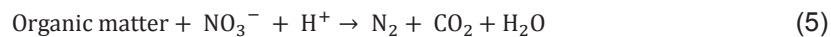
Besides the MAO, the flavin-containing monooxygenase (1.14.13.8) has recently been identified for catalysing the oxidation of secondary and tertiary amines (Chen et al., 2011). Other important enzymes to be mentioned are the ethanolamine oxidase (EC 1.4.3.8), the former copper-containing oxidases, now replaced by primary-amine oxidase (EC 1.4.3.21) and diamine oxidase (EC 1.4.3.22), as well as the ethanolamine ammonia-lyase (EC 4.3.1.7) (Eide-Haugmo, 2011).

1.8 Biological Nitrogen Removal (BNR)

Biological nitrogen removal (BNR) is a well-established technology in the field of waste water treatment, as recently reviewed Zhu et al. (2008). Conventionally, microbial nitrogen removal is achieved by combining autotrophic nitrification with heterotrophic denitrification. In the aerobic step of nitrification, ammonia is sequentially oxidized to nitrite and nitrate by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), respectively, as outlined in detail in Paper 1. Microbial ammonia oxidation is commonly expressed by the total sum given in Equation 4.



The anoxic denitrification step in turn reduces nitrous oxides to inert molecular nitrogen by oxidizing organic matter. This stepwise reduction to molecular dinitrogen gas by denitrifying bacteria is shown in Equation 5.



Contrary to the autotrophic nitrifying bacteria, the denitrifying bacteria are heterotrophic and may grow much faster. The nitrification step may thus be less robust, both because the AOB and NOB are more sensitive towards environmental changes and require longer periods to recover from operational disturbances due to their slow growth rates (Wagner and Loy, 2002).

Notably, the free energy (ΔG°) of nitrate respiration, or denitrification is nearly as high as aerobic respiration, making it the next favourable electron acceptor after oxygen (Jørgensen, 2006). The relationship between denitrified nitrogen and carbon source composition is linear, whereas the stoichiometry also depends on the type of carbon source (Matějů et al., 1992). The content of organic matter in waste water is commonly referred to by its chemical oxygen demand (COD), a collective term for any kind of organic matter scaled according to its degree of oxidation.

There are multiple process combinations available to achieve BNR, whereas just conventional post- and pre-denitrification are considered in this project. In the case of low COD and high ammonia content, post-denitrification may be beneficial. This set-up allows the oxidation of ammonium in the first step, feeding the generated nitrate to the denitrifying reactor reducing it to molecular nitrogen (see Figure 3 A). In this second step, an external carbon source must be added to the denitrifying reactor, since influent organics have already been consumed by heterotrophs in the first aerobic step. The situation may be reversed if both ammonium and organic matter are present in the wastewater. In this case, pre-denitrification may be a better option since additional external carbon source may be avoided (See Figure 3 B). In general, this set-up may

lead to lower operational costs and simultaneous carbon and nitrogen removal. See also Paper 1.

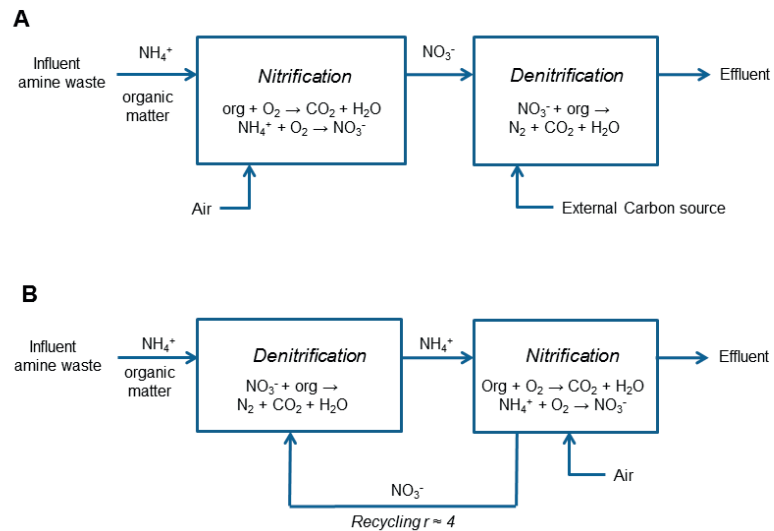


Figure 3 Process flow diagram of a post- and pre-denitrification set-up, A and B, respectively.

However, the performance of a pre-denitrification set-up will strongly depend on the characteristics of the carbon source in the wastewater. In the case of treating wastes from amine-based CCS, it is necessary to verify their biodegradability, possible toxic effects and process kinetics prior to applying BNR.

Biological treatment of effluents generated by CCS was summarized and presented at the first International Symposium on Energy Challenges and Mechanics in Aberdeen 2013 and later published as Paper 1.

1.9 Investigated Amine Solvents and Reclaimer Waste

1.9.1 Amine Solvents

In Table 1 the tested compounds are listed alphabetically, including their full names, abbreviations, CAS numbers and theoretical oxygen demand (ThOD). The corresponding structures are presented in Figure 4. See also Paper 3.

Table 1 Overview of compounds tested, including abbreviations used, CAS number, formula and theoretical oxygen demand (ThOD) (Paper 3).

Compound	Abbreviation	CAS	Formula	ThOD ^a (gO ₂ /g)
<i>Positive control</i>				
Sodium acetate	NaAc	127-09-3	C ₂ H ₃ NaO ₂	0.78
<i>Amino acids</i>				
Alanine	Ala	56-41-7	C ₃ H ₇ NO ₂	1.08
<i>Primary amines</i>				
2-amino-2-methylpropanol	AMP	124-68-5	C ₄ H ₁₁ NO	3.05
2-aminoethanol	MEA	141-43-5	C ₂ H ₇ NO	2.10
<i>Secondary amines</i>				
Diethanolamine	DEA	111-42-2	C ₄ H ₁₁ NO ₂	2.13
3-amino-1-methylaminopropane	MAPA	6291-84-5	C ₄ H ₁₂ N ₂	1.45
<i>Tertiary amines</i>				
2-Diethylaminoethanol	DEEA	100-37-8	C ₆ H ₁₅ NO	2.33
N-methyldiethanolamine	MDEA	105-59-9	C ₅ H ₁₃ NO ₂	2.28
<i>Cyclic amines</i>				
Piperazine	PZ	110-85-0	C ₄ H ₁₀ N ₂	3.35
<i>Reclaimer waste</i>				
MEA-based reclaimer waste	RW	-	-	1.42

^a calculations based on carbon and nitrogen oxidation

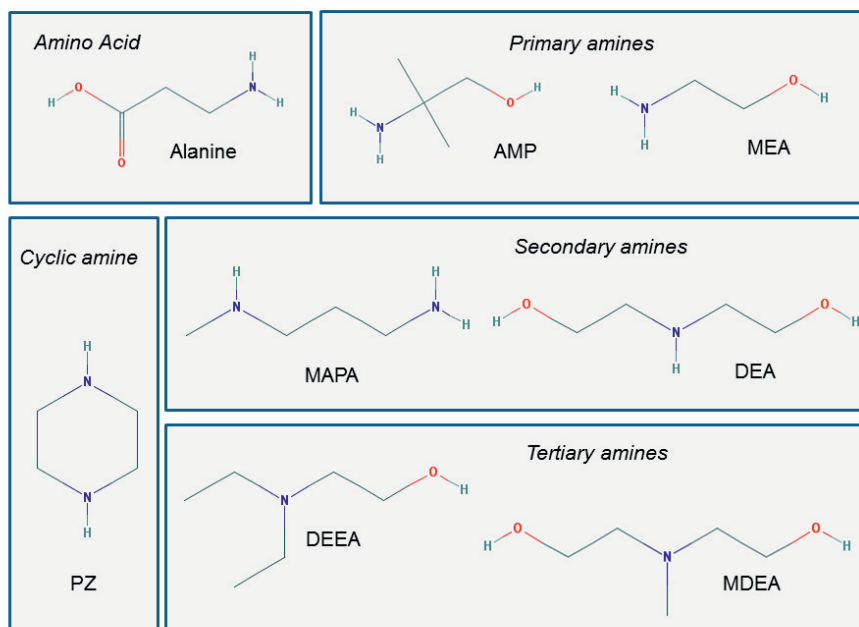


Figure 4 Structures of the investigated amines (Kim et al., 2016), full names are given in Table 1 (Paper 3).

1.9.2 MEA-based Reclaimer Waste

The investigated MEA-based reclaimer waste was generated from a 30 % (w/w) MEA solvent used for capturing CO₂ from coal fired flue gas in 2009. The test rig was run by Aker Clean Carbon's mobile test unit (MTU) capture facility at Longannet in Scotland (UK), 2009.

The main components of the reclaimer waste are listed in Table 2, based on our analysis in Paper 5. By no means does our data include all known process related degradation products of MEA, as this was beyond the scope of this study.

Table 2 Quantification of selected compounds in the MEA-based Reclaimer Waste (Paper 5).

Compound	Abbr.	CAS	Formula	Conc. (g/L)
2-aminoethanol ^a	MEA	141-43-5	C ₂ H ₇ NO	586.6
<i>N</i> -(2-Hydroxyethyl)glycine ^a	HEGly	5835-28-9	C ₄ H ₉ NO ₃	42.3
2-Hydroxyethylformamide ^a	HEF	693-06-1	C ₃ H ₇ NO ₂	28.1
4-(2-Hydroxyethyl) piperazine-2-one ^a	HEPO	23936-04-1	C ₆ H ₁₂ N ₂ O ₂	12.04
1-(2-Hydroxyethyl)imidazole ^a	HEI	1615-14-1	C ₅ H ₈ N ₂ O	10.5
Ammonia ^b		7664-41-7	NH ₃	8.8
(2-Hydroxyethyl)-acetamide ^a	HEA	142-26-7	C ₄ H ₉ NO ₂	8.2
Nitrate ^b		84145-82-4	NO ₃ ⁻	7.5
<i>N</i> -(2-hydroxyethyl)ethylenediamine ^a	HEEDA	111-41-1	C ₄ H ₁₂ N ₂ O	4.03
<i>N,N</i> -Bis(2-hydroxyethyl)oxamide ^a	BHEOX	1871-89-2	C ₆ H ₁₂ N ₂ O ₄	0.06
Nitrite ^b		14797-65-0	NO ₂ ⁻	0.046

^aLC-MS

^b Hach-Lange for water quality

In a recent study by Nurrokhmah et al. (2013a), following MEA-based waste degradation products in a coal-fired CO₂ capture based case, oxidative degradation products were formate, thiocyanate, acetate, thiosulphate, oxalate and sulfate, while thermal degradation products were similar to ours shown in Table 2, being HEEDA, 1-(2-hydroxyethyl) imidazolidone-2 (HEIA) and *N,N*-di(hydroxyethyl) urea (DHU). Besides these well-known degradation products, they also identified minor quantities of nitrosodimethylamine (NDMA) and nitrosodiethanolamine (NDELA). The total amount of nitrogen in their MEA-based reclaimer waste was calculated to be 14% (dry basis) (Nurrokhmah et al., 2013a).

An extensive dataset for oxidative degradation of MEA at increased temperatures and oxygen concentrations was recently published by Vevelstad et al. (2016). The authors monitored the formation of ten primary and seven

secondary degradation products of 30 % (w/w) MEA at lab-scale. Primary products are formed initially by radical and oxidation reactions, including aldehydes, ammonia, alkyl amines (methylamine, dimethylamine, ethylamine and diethyl amine), imine, acids (formic, oxalic, acetic, nitric, nitrous and sulfuric acid), nitrite and nitrate. Secondary degradation products are formed from MEA itself and primary degradation products. The identified compounds were HEGly, OZD, HEPO, HEF, HEA, HEI and BHEOX, whereas HEGly and HEPO are the major degradation products found in pilot samples. Additionally, the nitrosamines NDELA and NHEGly were quantified. In conclusion, they found that HEF, HEI and ammonia contributed most significantly to the nitrogen balance in most of their experiments, while at low oxygen concentration HEGly was the dominant nitrogen containing degradation product. Noteworthy, the total nitrogen balances were closed within 83 – 97% (Vevelstad et al., 2016).

These findings are well in agreement with the quantified compounds of our reclaimer waste, listed in Table 2 and presented in Paper 5. Apart from MEA, we also found that HEGly, HEF, HEPO, HEI and ammonia contributed most to the reclaimer waste composition. Because degradation products are concentrated in the reclaimer waste, our concentrations are higher than those reported by Vevelstad et al. (2016).

1.10 Scope of This Work

The objective of this work has been to determine the feasibility of biological treatment of effluents generated by amine-based CO₂ capture. For CCS to be applied at a large scale, it is essential to include handling of the generated waste in a sustainable manner, as outlined in section 1.6. Therefore, this knowledge has to be integrated when developing efficient and cost-effective CCS for the future.

The main points for reaching this goal were:

1. Screening - Testing the biodegradability of amines under aerobic and anoxic conditions to identify these amines as a suitable carbon source in denitrification is essential for the process. This includes developing a rapid and simple screening test for anoxic conditions.
2. Feasibility - Testing biological nitrogen removal (BNR) of MEA and MEA-based reclaimer waste over a long-term in lab scale bioreactors. This includes comparing the performance of post- and pre-denitrification for MEA and confirming the feasibility of both substances serving as a sole carbon source.
3. Limitations - Identifying the most essential limiting factors relevant for an up-scaled process of BNR on wastes from amine-based CO₂ capture. To operate a high loading, the effect of total organic COD as well as the toxicities of commonly applied amines needs to be determined on nitrifying as well as denitrifying bacterial communities. By pyrosequencing of 16S rDNA amplicons, the relative abundance of nitrifying *versus* heterotrophic bacteria within the nitrifying biofilm can be monitored over time. This enables linking the biofilm community dynamics and composition to the performance.

2. Biodegradability of Amines Applied in CO₂-capture

2.1 Syringe Screening Test for Anoxic Biodegradability

The development of the syringe screening test is presented in Paper 2. 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, the syringe batch test is based on measuring the headspace volume expansion due to produced N₂ gas over time. Thus, syringes were filled with medium and moving bed biofilm reactor carriers (MBBRs) were added as inoculum. The syringes were emptied for air and closed before stacked in an incubator at room temperature. The gas volumes formed were recorded regularly up to 14 days, see Paper 2.

The denitrification test showed saturation response at increasing amounts of inoculum in the form of adapted MBBRs, with well correlated nitrate consumption vs. gas volume formed. 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 $R = 0.9265$ (Paper 2). This is considered sufficient for screening purposes.

The denitrification test efficiently screened different inocula at standardized substrates. Also, different substrates were successfully screened and compared at a standardized inoculum. Inoculum handling and activity may be conveniently standardized by applying biofilm carriers. A robust set of positive as well as negative controls (blanks) should be included to ensure quality of the actual testing, see Paper 2.

With the anoxic syringe test, we present a simple method to predict the biodegradability of amines used in CCS under denitrifying conditions. For future solvent evaluation, this screening method offers a rapid and low cost method, compared to the conventional BOD testing.

2.2 Aerobic Biodegradability of Amines

Aerobic biodegradability in fresh water was determined for AMP, MEA, DEA, MDEA, PZ and MEA-based reclaimer waste according to OECD guidelines (OECD, 1992), see Paper 3.

In accordance with OECD (1992) aerobic biodegradability was estimated by the biological oxygen demand (BOD), calculated as the difference in dissolved oxygen (DO) between the test substance and the blank, and relative to the theoretical oxygen demand (ThOD). The ThOD of each test substance is based on the molecular formula and is dependent 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 on reclaimer waste (see Paper 5). Comparison of our results with previously published results on sea water revealed big differences (Paper 3).

Biodegradability was above 65% for all tested amines, except for the MEA-based reclaimer waste which remained persistent under these conditions (see Figure 5). In general, the conversion was increased and more rapid in fresh water compared to seawater.

Furthermore, we observed biodegradation of MDEA and PZ after a lag time of 7 days, and of AMP after 5 days. This is in contrast to previously reported biodegradability in seawater, where AMP, MDEA and PZ remained undegraded (Brakstad et al., 2012; Eide-Haugmo et al., 2012).

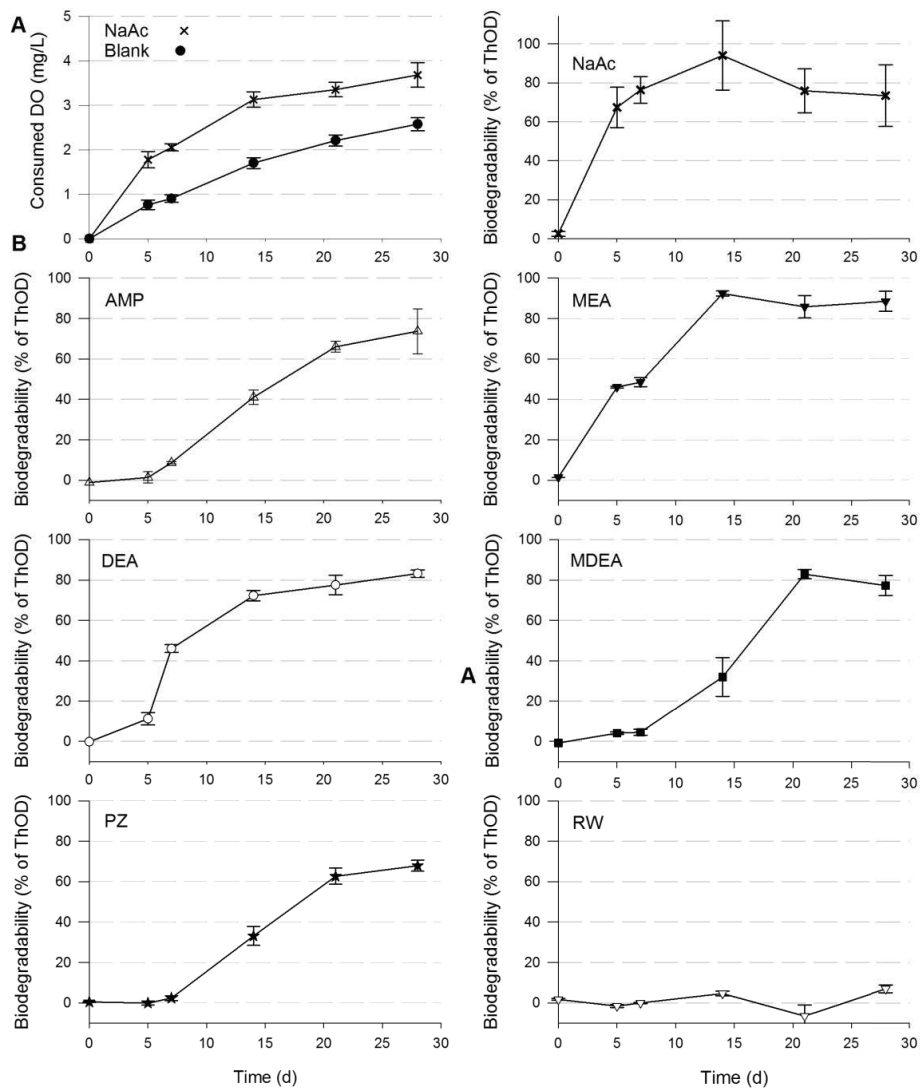


Figure 5 A) Consumed oxygen levels in sodium acetate (x) and blanks (+) during the BOD testing and biodegradation of sodium acetate in fresh water given as BOD (% of ThOD) as a function of time. Error bars indicate the SEM of 4 replicates. B) Biodegradation of amines in fresh water. The calculated BOD values are corrected for the blank values. Error bars indicate the SEM of 3 (AMP (Δ), MEA (▼), DEA (○), MDEA (■), or 6 (PZ (★), RW (▽)) replicates. Note the differences in scaling of graphs in A.

Biodegradation rates and half-lives were calculated for each amine and ranged from 6 to 17 days, whereas for MEA-based reclaimer waste it was longer than 1000 days under these conditions. Those calculated half-lives are within the experimental errors of the measured biodegradation kinetics (see Figure 5B). In the worst cases of AMP and MDEA, apparent differences were 2 to 3 days. Most strikingly, AMP and MDEA showed calculated half-lives significantly shorter than the reported 700 days under marine conditions. DEA and MEA had shorter apparent half-lives in fresh-water with 9 and 6 days, respectively, than in sea water with 24 and 8 days, respectively. One reason for these differences could be the distinct microbial community involved in the two surface waters - β -Proteobacteria pose an important difference between freshwater and marine environment, where they are noticeably absent (Méthé et al., 1998).

In general, ultimate biodegradability, as determined by BOD, may be useful for assessing rapid direct biodegradability of amines in natural ecosystems. However, in an engineered system such as in the case of BNR, these results must be reconsidered. First, the microbial community of surface waters depends on geographical and seasonal variations. This might be directly reflected by the degree of biodegradability. If the substance is not biodegraded, this actually may just indicate the accidental absence of the required bacteria in the chosen inoculum. As pointed out already by Grady (1984), a negative result does not allow to conclude inherent biodegradability of a compound, but just means that the test conditions were simply not satisfying. Furthermore, the author argues that the BOD testing conditions are too stringent for several reasons. Using the compound as a sole carbon and energy source excludes co-metabolism, the small single inoculum limits the genetic capability for degradation, and the relatively short testing time forces acclimation to be the only mechanism. This results in a bias towards only readily biodegradable compounds giving a positive result (Grady, 1984). See also Paper 3.

Secondly, the purely weight based recommended concentration of 2-5 mg/L test substance (OECD, 1992) makes it difficult to compare biodegradability of one

substance with anaerobic respiration based on other electron acceptors. When utilized as a carbon source, the potential chemical energy content quantified as COD would be a better alternative.

2.3 Anoxic Biodegradability of Amines

The relative anoxic biodegradability of alanine, AMP, MEA, DEA, MAPA, MDEA, DEEA, MDEA, PZ and MEA-based reclaimer waste was assessed in a simple batch test run for 21 days (see Paper 3). The experimental verification of the syringe test is presented in detail elsewhere (Paper 2).

The initial anoxic experiment A showed significant blank activity, recorded as gas production (see Paper 3). This endogenous activity in the absence of external carbon reflects internal turnover of biomass. In this case, biomass was starved prior to inoculation. Contrary to the OECD guidelines for fermentative anaerobic conditions (OECD, 2006), starving the biomass prior to the test did not reduce blank activity. Instead, pre-starvation of the biofilm inoculum might induce the enzymatic machinery for utilizing cell debris as a carbon source. See Paper 3. Since the positive control sodium acetate showed doubled 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 recorded as zero in all blank replicates throughout the experiment.

As illustrated in Figure 6, the positive control sodium acetate rapidly reached maximum gas production after 3 days and stayed constant until the end of the experiment at day 21. Blanks showed now measurable gas production during the entire incubation period, reflecting the lack of a suitable carbon source. Alanine followed the same rapid biodegradation as the control and gave even

greater gas production, possibly due to being a simpler carbon source. The primary amines AMP and MEA showed very different behaviour: AMP did not give any gas production, while MEA was rapidly utilized just as rapid as the positive control. Both secondary amines, DEA and MAPA had a prolonged lag phase of approximately 10 days, but thereafter, the gas production increased steadily in most replicates. The tertiary amines MDEA and DEEA, as well as the cyclic amine PZ did not show any measurable gas production during the entire experiment. MEA-based reclaimer waste followed the same rapid utilization as MEA did (Paper 3).

With the anoxic syringe test we showed that alanine, MEA, and MEA-based reclaimer waste were suitable carbon sources for the denitrification process under anoxic conditions (Paper 3). The secondary amines DEA and MAPA required a lag phase before they could be utilized as a carbon source. Selective enrichment could then probably be applied to improve degradation. This does not directly apply for AMP, DEEA, MDEA and PZ, as they could not be utilized at all under current experimental anoxic conditions, not even after an extended incubation period of 21 days (Paper 3).

In general, the actual microbial consortia determine the observed biodegradability of amines. If biological nitrogen removal is the main goal, BOD values do not predict the biodegradability under denitrifying conditions. For example, MEA-based reclaimer waste remained persistent under aerobic conditions, but was rapidly degraded in the syringe test under denitrifying conditions (see Paper 3).

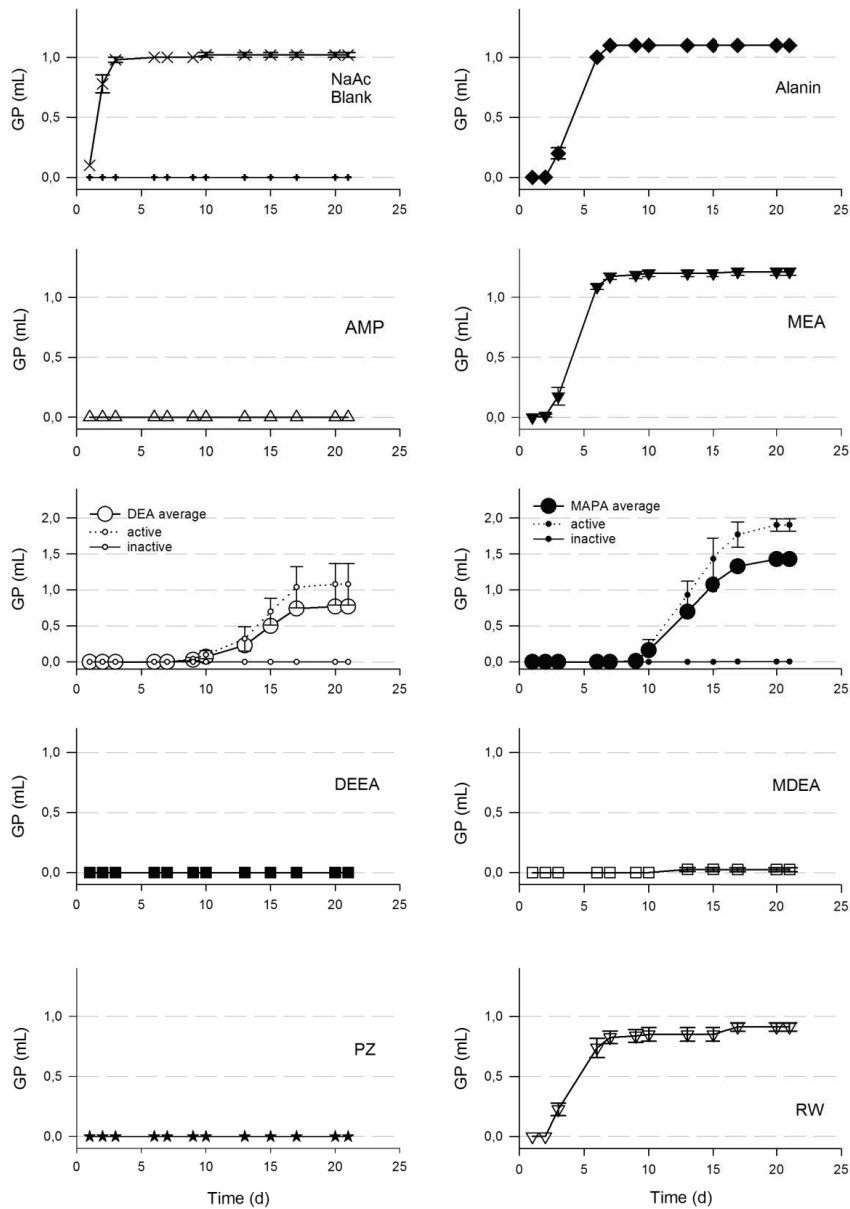


Figure 6 Anoxic biodegradability of amines given as average gas production (GP) in mL as a function of time. Error bars indicate the SEM of 5 (Positive control sodium acetate (×), Blank (+), DEA (○), MAPA (●)) or 8 (alanine (◆), AMP (△), MEA (▼), MDEA (■), DEEA (□), PZ (★)) replicates. DEA and MAPA had 2 and 3 inactive syringes respectively, Therefore, active and inactive data were treated separately (see Paper 3).

3. Biological Nitrogen Removal

3.1 BNR of MEA in Post- and Pre-denitrification

This chapter describes how biological nitrogen removal can be achieved in wastewater containing MEA in post- as well as pre-denitrification treatment systems (see Paper 4).

Although several reports have documented the aerobic and anoxic biodegradation of MEA, no scientific reports are available comparing the efficiency of post-denitrification and pre-denitrification treatment systems. This information is essential to develop efficient and cost effective MEA removal from industrial wastewaters. For this purpose, nitrifying and denitrifying biofilms were used in moving-bed biofilm reactors (MBBRs), set up in post- and pre-denitrification processes (see Figure 3 A and B). The principles of BNR are described in section 1.7 and summarized in Paper 1, while BNR of MEA can be found in detail in Paper 4. It should be noted that the actual degree of filling was 36% for the nitrifying and 21% for the denitrifying MBBR, which is below 70%, as recommended by Ødegaard (1999) for a full scale plant. At moderate filling, the activity can be assumed to be proportional to the carrier surface area as long as transport is not affected.

The post-denitrification set-up (Figure 3 A) was operated in continuous flow for 42 days with medium containing ammonium and MEA, whereas ethanol was added to the denitrifying reactor as a carbon source. Biodegradation of MEA readily occurred by the nitrifying biofilm. See Figure 7. At the same time, the ammonium concentration increased drastically. This increased concentration corresponds to the expected equimolar release of ammonium from MEA hydrolysis. As most MEA was removed in the nitrification step, negligible levels reached the denitrification reactor. Even though nitrite was gradually accumulating in the nitrification reactor, both nitrate and nitrite were reduced in the following denitrification step. The process performance of the system is given in Figure 7 and Table 3.

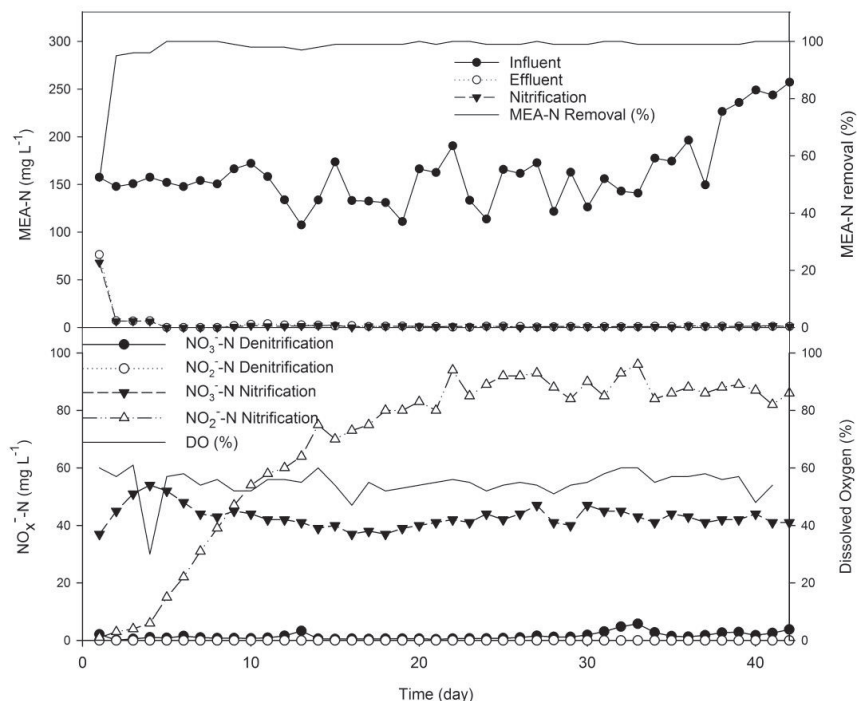


Figure 7 Performance of the post-denitrification BNR system treating MEA (see Paper 4).

The pre-denitrification system (Figure 3 B) was operated directly after the post-denitrification system in continuous flow for further 138 days with medium containing ammonium for the first 19 days. To ensure denitrifying activity, ethanol was added for the first week. Thereafter, ethanol was omitted, and MEA was added to the medium as a sole carbon source. See Figure 8.

MEA was instantly degraded in the anoxic denitrifying reactor constituting the first step of the treatment system. At the same time, ammonium concentration increased. It should be noted that, denitrifying activity was maintained with MEA as a sole carbon source, although denitrification of high nitrate levels required time for adaption of the culture. The following nitrifying reactor received only small amounts of MEA (<10 mg/L), because most MEA ($86 \pm 10 \%$) was already

removed in the preliminary denitrifying reactor. As a consequence, the ammonium concentration in the effluent was also accordingly low, approaching zero when no more ammonium was fed. The overall process performance is summarized in Figure 8 and Table 3.

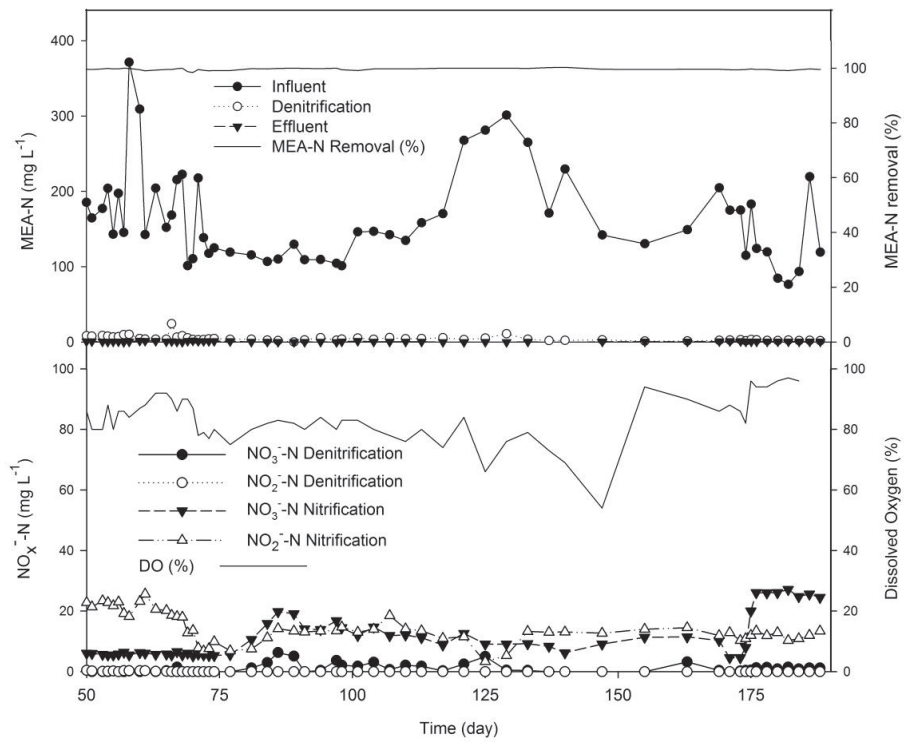


Figure 8 Performance of the pre-denitrification BNR system treating MEA (see Paper 4).

Table 3 Process performance of post- and pre-denitrification systems during the degradation of MEA. ^aCOD balance includes the supplied ethanol, indicating large excess of available carbon.

	Post-denitrification	Pre-Denitrification
Operational time (days)	42	138
Influent		
MEA-N (mg/L)	162	164
NH ₄ ⁺ -N (mg/L)	54	15
Total N (mg/L)	216	179
COD (mg/L)	2848	752
Removal Efficiency (%)		
MEA-N	99	99
Total Nitrogen	77	77
COD removal ^a	61	90

This study clearly demonstrates the advantage of pre-denitrification over post-denitrification for achieving biological nitrogen removal from MEA contaminated effluents. Besides the removal of MEA, also the removal efficiency of total nitrogen, as well as organic matter was high without any additional carbon source.

Most importantly, we i) verified that MEA could successfully be utilized as a sole carbon source in the denitrification process and ii) identified pre-denitrification as the choice of method over post-denitrification. These findings provide valuable insight for further development of efficient treatment systems for effluents containing MEA, such as MEA-based reclaimer waste.

3.2 BNR of Reclaimer Waste in Pre-denitrification

After verifying that MEA could serve as a sole carbon source for pre-denitrification, the next step was to successfully treat MEA-based reclaimer waste in the same manner. See Paper 5. The aim of this study was to evaluate the capacity of a pre-denitrification system to achieve nitrogen removal and biodegradation of reclaimer waste without external carbon source supply.

The identified components of the reclaimer waste are listed in Table 2 (section 1.9.2.). MEA accounts for close to 50 % of the total COD measured in the reclaimer waste ($1,575 \pm 40$ COD g/L). This large fraction of MEA is consistent with findings of Botheju et al. (2012) and Strazisar et al. (2003). The listed components are selected and do not represent a full analysis of the reclaimer waste, as this is not in the scope of this study.

The MBBR pre-denitrification system was operated with a filling degree of 21 % in the denitrifying and 36 % in the nitrifying MBBR. This is lower than 70 %, as recommended by Ødegaard (1999) for a full scale plant. The MEA-based reclaimer waste was diluted 1:1000 in basal medium and fed continuously throughout the study. Also here, MEA-based reclaimer waste was instantly utilized as a carbon source for denitrification. As the biodegradation of MEA is initiated by a hydrolytic reaction releasing ammonium and acetaldehyde (Ndegwa et al., 2004), the observed increase of ammonium concentration is a direct consequence of the biodegradation of MEA in the denitrifying reactor. By stopping the external supply of $\text{NH}_4^+\text{-N}$ in the medium, nitrite was no longer accumulating and the oxidation of ammonium to nitrate increased in the nitrifying reactor. The reactor performance over time is illustrated in Figure 9.

The COD removal remained constant at 71 ± 1 % even during addition of external nitrate as an electron acceptor. The fact that nitrate concentration increased during this period of external addition suggests that the remaining organic fraction was not biodegradable under denitrifying conditions. This apparently persistent organic fraction was constant at 378 ± 36 COD mg/L

(28%) throughout the experiment. This non-biodegradable fraction was also noted under anaerobic co-digestion with readily biodegradable acidic substrates by Botheju et al. (2011), suggesting this might represent unidentified MEA degradation products.

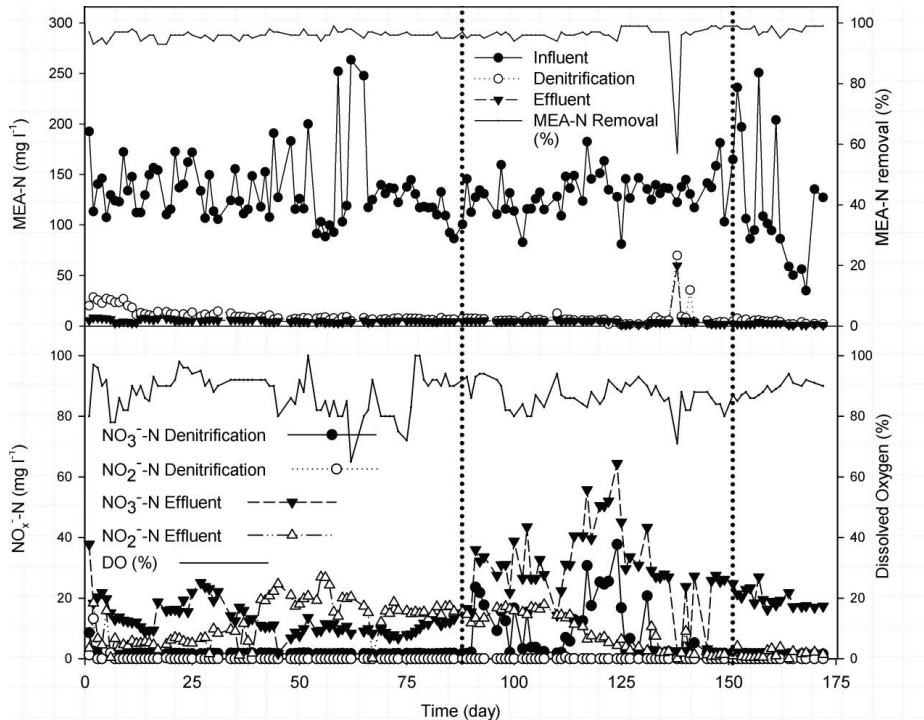


Figure 9 Performance of the pre-denitrification BNR system for treating MEA-based reclaimer waste (see Paper 5).

At day 97 samples from the nitrifying and denitrifying reactor were analysed for selected known degradation products and quantified by LC-MS (Paper 5). HEI and both substituted amides, HEF and HEA, as well as the amino acid HEGly, could be largely degraded in the denitrifying reactor, while the nitrifying reactor utilized the leftovers of HEF and HEA almost completely and HEI and HEGly to a lesser extent. HEPO and BHEOX could not be further degraded in the nitrifying reactor, as the concentration received from the denitrifying reactor remained unaffected.

The BNR of MEA-based reclaimer waste achieved 98 ± 1 % removal of MEA and 72 ± 16 % removal of total nitrogen in pre-denitrification mode. Notably, 93 ± 7 % of MEA was already removed in the anoxic denitrifying reactor and the following nitrifying reactor received only small amounts of MEA (<10 mg/L).

Throughout the experiment, the removal efficiency of MEA, total nitrogen and COD was stable. Additional ammonium or nitrate did not affect the system efficiency. This study shows that those experimental denitrifying conditions appear suitable for treating MEA-based reclaimer waste in a pre-denitrification system.

4. Process Considerations of BNR

4.1 Inhibition Factors

For an up-scaled process, the most essential inhibitory factors need to be identified, particularly those restricting efficiency at high loading. While previous reports have documented biodegradation and biological nitrogen removal of various amines under different environmental conditions, essential process parameters such as the inhibition potential of amines on microbiological processes are still lacking. See Paper 6.

Nitrifying biofilm tends to be stratified, with the NOB located in the inner part of the biofilm and the AOB closely associated with heterotrophic bacteria throughout the biofilm (Okabe et al., 1999). This is advantageous for the nitrifying bacteria in terms of shear stress protection, but on the other hand this stratification can lead to suffocation of the inner located bacteria due to oxygen limitation. This competition for space in the oxygen gradient between heterotrophic and nitrifying autotrophic bacteria of biofilms has been well documented (Nogueira et al., 2002; Vogelsang et al., 2002).

In particular, in the presence of an external carbon source, the fast growing heterotrophic bacteria of the outer layer may outcompete the slow growing

autotrophs for oxygen and space. This factor must be considered when evaluating the treatment of amines and amine-based reclaimer waste by biofilm reactors. As the amine represents an available carbon source for heterotrophs, it might significantly change the stratification and composition of the biofilm, depending on availability and toxicity of the amine.

To identify the most essential inhibitory factors, three MBBRs (100mL carriers) were operated with carriers for 48 days in continuous mode as described in Paper 6. MBBR 1 was run as a control to confirm process stability, while chronic exposure to organic loading (NaAc) was tested in duplicate in MBBR 2 and 3 in order to verify reproducibility, as illustrated in Figure 10. To study population dynamics during inhibition and quantify the relative abundance of nitrifying bacteria over time, the bacterial communities of all 3 MBBRs were characterized by pyrosequencing of 16S rDNA amplicons. These sequence data were used to determine the relative abundance of AOB + NOB. Furthermore, toxicity tests of selected amines and organic loading were performed in batch tests to determine the EC₅₀ for the rate-limiting nitrifying step, as well as the denitrifying process. Additionally, the effect of increased heterotrophic bacteria within the nitrifying biofilm was documented by re-testing the toxicity of sodium acetate and MEA after long-term exposure to organic loading (Paper 6). The experimental timeline is shown in Figure 10.

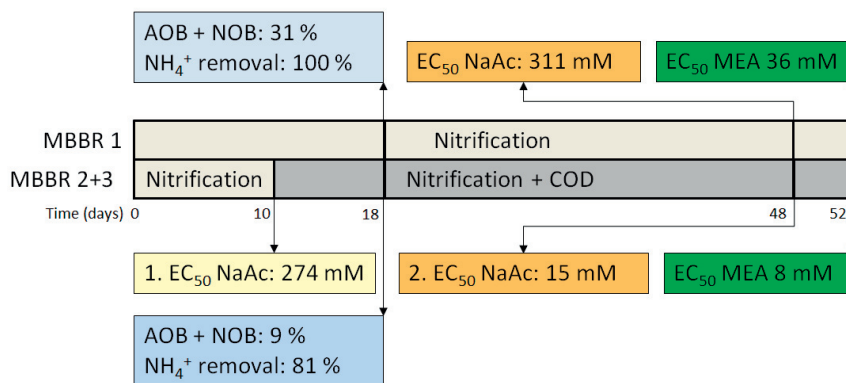


Figure 10 Experimental timeline of the 3 nitrifying MBBRs presented in Paper 6.

4.1.1 Nitrification

After 10 days of continuous nitrification, MBBR 2 and 3 additionally received organic loading in form of sodium acetate (NaAc). After 1 week (at day 18) the ammonia removal efficiency dropped by 20 % in MBBR 2 and 3, compared to control reactor MBBR 1 running on pure nitrification, as shown in Paper 6. At the same time, there were also significantly less nitrifying bacteria in both the treated nitrifying MBBR compared to the control reactor receiving no organic loading. The relative abundance of AOB and NOB during the entire experiment is shown in Figure 11, together reaching a maximum of 65 % in the control reactor MBBR 1.

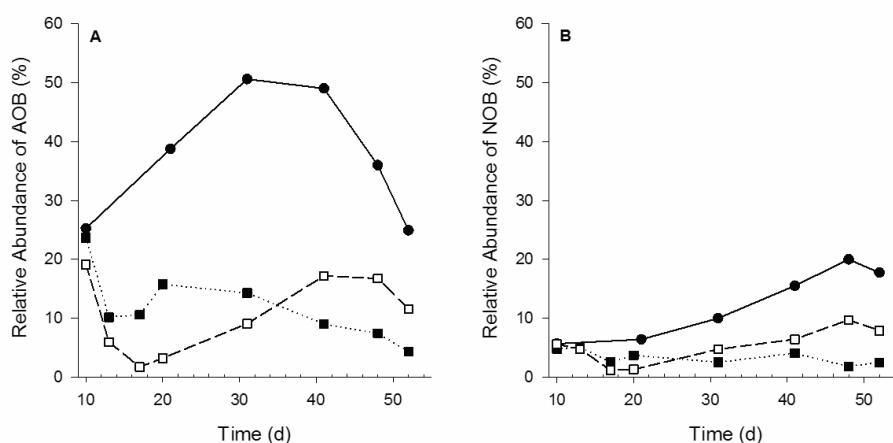


Figure 11 Relative quantities (%) of AOB (A) and NOB (B) as identified by 454-pyrosequencing. Closed circles (●) represent MBBR 1 without organic loading, squares show data from MBBR 2 (■) and 3 (□) receiving 300mg/L COD in the feed from day 11 onwards (Paper 6).

To determine changes in acute sensitivity towards chronic exposure to organic loading, EC_{50} values were determined both before and after chronic exposure to sodium acetate at day 10 and day 48, respectively. For comparison, the control reactor MBBR1 was also tested at day 48 for acute toxicity of sodium acetate to document long-term stability towards organic loading. See also Figure 10 and

Paper 6. During this inhibition test, the biofilm (100mL) is exposed to increasing concentrations of the test substance and each concentration monitored for 3 h. Results show decreasing nitrate production with increasing concentration of the test substance. The slope of each concentration is used for generating a dose response curve by setting the initial slope (0mM) as the reference value of 100% (Paper 6), see Figure 12.

EC₅₀ values of sodium acetate were an average of 274 mM before and 15 mM after long-term organic exposure in MBBR 2 and 3. The EC₅₀ value of sodium acetate was estimated to be 311 mM in the control MBBR 1 at day 48, which is close to the other reactors prior to chronic exposure. See the dose responds curve in Figure 12 and results in Figure 10.

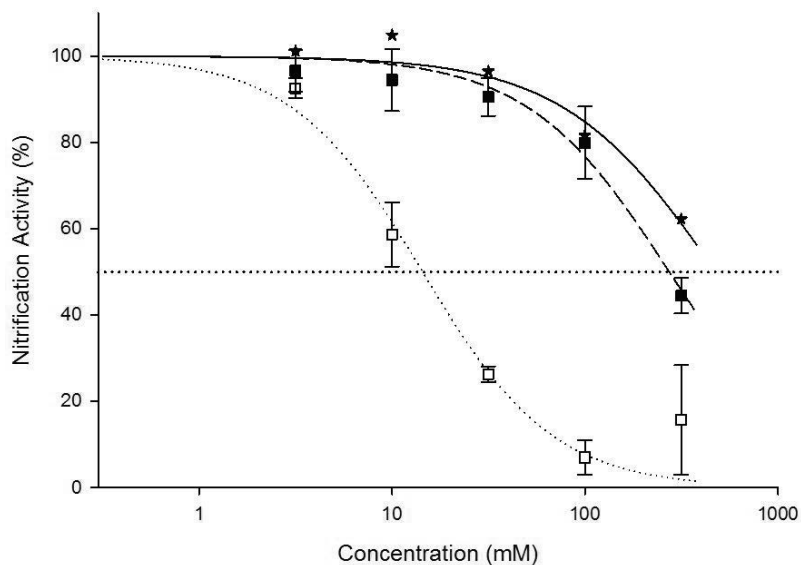


Figure 12 Dose response curve of nitrifying biofilm exposed to sodium acetate. Squares represent average data from MBBR 2 and 3 - Closed squares (■) from the 1. EC₅₀ NaAc: 274 mM prior and open squares (□) from the 2. EC₅₀ NaAc: 15 mM after chronic exposure to organic loading. Stars (★) show data from the control reactor MBBR 1 - EC₅₀ NaAc: 311 mM. Error bars indicate standard deviation of 2 reactors (Paper 6).

These results clearly illustrate the negative impact of heterotrophic bacteria on the nitrifiers of the biofilm, resulting in an approximately 20-fold higher sensitivity towards acute organic shock after long-term moderate exposure to organic loading. The EC₅₀ of MEA was then determined only 96 hours after the acute toxicity test of organic loading. The highly enriched nitrifying control biofilm in MBBR 1 showed a much higher tolerance towards acute exposure to MEA (EC₅₀ 36 mM) than both of the heterotrophic enriched MBBR 2 and 3 did (EC₅₀ 7 and 8 mM). This clearly illustrates that the biofilm composition is a crucial factor in the inhibition potential of amines.

MEA, MEA-based reclaimer waste (RW), as well as AMP, DEA, MDEA and PZ were tested for their inhibition potential on nitrifying biofilms prior to the above described chronic exposure experiment. The EC₅₀ values ranged from 9 to 118 mM, as shown in Figure 13.

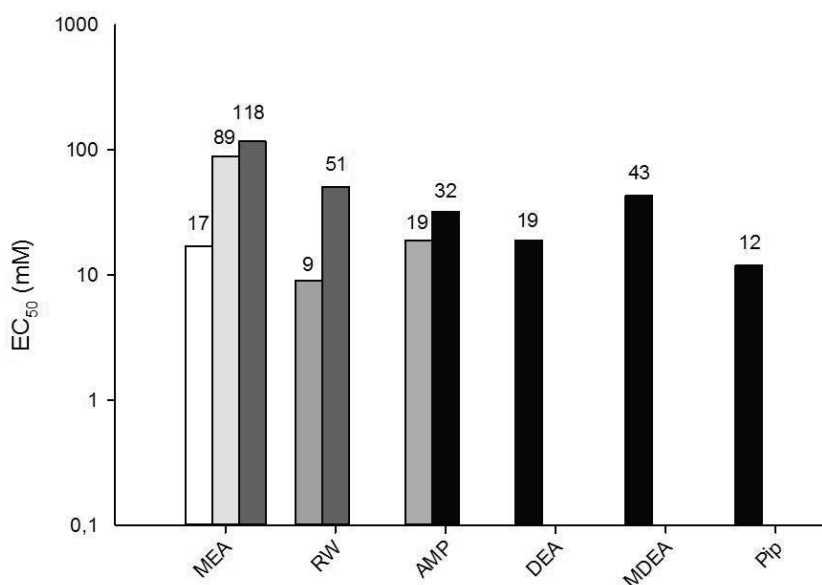


Figure 13 EC₅₀ values of the nitrifying biofilm for MEA, MEA-based reclaimer waste, AMP, DEA, MDEA and piperazine (Pip). Independent replicate tests are shown as bars, whereas the degree of shading indicates the chronological order of testing, with white as the first and black as the last experiment (Paper 6).

The illustrated changes in acute toxic response in different repeats can largely be related to differences in the biofilm composition. The first toxicity test of MEA (EC_{50} 17 mM) was conducted right after treating MEA in a post-denitrification set-up, where organics in the form of MEA entered the nitrifying reactor directly for 42 days (Paper 4). The consecutive tests were repeated while the nitrifying biofilm was operated under pure nitrification conditions with insignificant loadings of organic matter (Paper 4 and 5). As expected, the inhibitory effect was reduced as the heterotrophic content in the biofilm diminished over time, in the case of MEA the EC_{50} values ranged from 17 to 118 mM in these experiments.

Thus, it was confirmed that the increased inhibition of amines on the nitrifying biofilm activity was linked to the biofilm composition, as shown with sodium acetate as well as MEA as organic loading tested consecutively on the very same biofilm under defined operating conditions (Paper 6). When treating waste streams of these amines by biological nitrogen removal, the pre-denitrification configuration can prevent an organic overload of the autotrophic nitrification step, as long as the denitrification unit initially consumes most of the organics.

4.1.2 Denitrification

As described in detail in Paper 6, AMP, DEA, MDEA, MEA, MEA-based reclaimer waste (RW), and PZ were also tested for their inhibition potential on the denitrifying biofilm. As expected, this heterotrophic biofilm was more resistant to high loadings of amine waste and amines. The denitrification activity, assessed by nitrate consumption over time, in fact increased at low concentrations up to 100 mM of all tested substances, most probably stimulated by the additional carbon source. The heterotrophic consortia of denitrifying bacteria seem to cope very well even with high loadings of amines, as well as with MEA-based reclaimer waste.

Only at the highest test concentration of 316 mM, AMP and DEA showed some inhibition, at 30 % and 19 %, respectively (Paper 6). Due to the apparently stimulating effect of all tested amines, any EC₅₀ values could not be determined. The increased denitrification activity was most pronounced with MEA-based reclaimer waste, where the denitrifying biofilm reached almost 250% of the initial activity. This might be due to the small organic acids and other compounds found in the reclaimer waste (Nurrokhmah et al., 2013a), representing a readily available carbon source.

5. Conclusions & Outlook

One of the main outcomes of this thesis is the development of a rapid screening method for anoxic biodegradability of amine solvents. The aerobic and anoxic biodegradability of amines used for CO₂-capture was assessed by screening tests (see Paper 3), using the conventional BOD test for testing aerobic biodegradability, according to the OECD guidelines (OECD, 1992). However, the corresponding anoxic biodegradability was estimated by an in-house developed method, a simple batch test identifying potential carbon sources for denitrification at a high through-put rate at low cost (see Paper 2). With the anoxic syringe test, we present a simple method to predict the biodegradability of amines used in CCS under denitrifying conditions. For future solvent evaluation, this innovative screening method offers a rapid and low-cost method, compared to the conventional BOD testing. This thesis highlights that screening for biodegradability should generally cover a broader spectrum than aerobic conditions.

When testing biodegradability under aerobic conditions in freshwater, DEA and MEA were rapidly degraded. AMP, MDEA and PZ were degraded after one week incubation, while MEA-based reclaimer waste was not degraded under our aerobic conditions. These results show that biodegradability in freshwater is

more favourable compared to seawater, especially for AMP, MDEA and PZ which were persistent under marine conditions (Paper 3).

With the anoxic screening batch test, we identified three possible categories of biodegradability under denitrification conditions. (i) Easily biodegradable, such as alanine, MEA and MEA-based reclaimer waste. (ii) Slowly biodegradable after a lag phase, such as DEA and MAPA; and (iii) not degraded under current conditions, such as AMP, DEEA, MDEA, and PZ. The differences in degradation kinetics are visualized in Figure 14 (Paper 3).

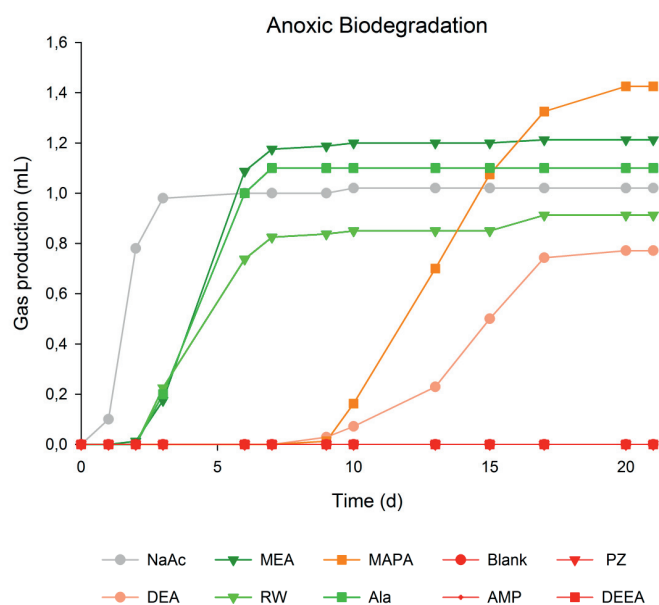


Figure 14 Anoxic biodegradation of amines (Paper 3).

The rapid anoxic biodegradability of MEA and MEA-based reclaimer waste was also confirmed by each of them serving as a sole carbon source for BNR in a pre-denitrification system (Papers 3, 4, and 5). The group of slowly biodegradable amines, such as DEA or MAPA, may require more time for being utilized as a sole carbon source, as shown in the screening test of Paper 3 and

depicted in Figure 14. Therefore, longer start-up times of maybe 2 weeks and/or long-term pre-selective enrichment of the inoculum should be considered. Also negative results of persistent amines may be retested in a continuous flow system, by testing with enrichment at low amine concentrations in the absence of alternative carbon sources (Nörtemann and Hempel, 1991).

As shown with MEA-based reclaimer waste, the ultimate aerobic BOD value of 3 % would seem to exclude any attempt of BNR, but under denitrifying conditions, MEA-based reclaimer waste was rapidly degraded (Paper 3 and 5). In reverse, AMP was rapidly degraded under aerobic conditions, but could not be utilized at all under denitrifying conditions in the syringe test. This is in agreement with our pilot studies of AMP in a pre-denitrification system (results not included). Our findings highlight the importance of considering the appropriate inoculum for assessing the biodegradability of amines in engineered ecosystems. Thus, the anoxic screening test is a first important step for estimating the feasibility of applying BNR on the respective amine and its waste.

The work carried out within this thesis confirms the suitability to treat MEA and real MEA-based reclaimer waste efficiently with BNR. The performance of a post- and pre-denitrification system for BNR from MEA contaminated effluents was compared as shown in Paper 4. MEA was rapidly degraded in both configurations, regardless of whether the first treatment unit was aerobic or anoxic. Pre-denitrification was then chosen as the preferred method, based on two reasons, i) the removal of MEA and total nitrogen was the same in both systems, whereas ii) MEA served as a sole carbon source in pre-denitrification and thus no additional carbon source was required. Besides MEA and nitrogen, organic matter was also efficiently removed under pre-denitrification conditions. Essentially, we also proved the feasibility of treating real MEA-based RW with BNR (Paper 5). Both nitrogen and organic matter were rapidly removed in the pre-denitrification system. Also in this case MEA-based RW served as a sole carbon source, making BNR in pre-denitrification mode a cost-effective treatment option. However, we observed a persistent fraction of COD that was

not degraded under denitrifying conditions. This was also observed during anaerobic co-digestion by Botheju et al. (2011). This fraction should be identified in further studies.

Even though we proved pre-denitrification to be highly efficient for treating MEA and MEA-based reclaimer waste (Paper 4 and 5), this might not apply for other amines or their waste products. In some cases, additional long-term selective enrichment attempts will be necessary to develop an inoculum with significant capacity in anoxic biodegradation of the actual compound. Post-denitrification might then be a useful approach when the amine remains persistent under anoxic conditions, but is biodegradable under aerobic conditions. This implies that an extra carbon source must be added to the denitrifying reactor, allowing aerobic biodegradation and/or co-metabolism. Based on the results of this thesis, some treatment options are indicated in Table 4.

Table 4 Summarized results of Paper 3 to 6. Treatment options for BNR in post- or pre-denitrification (Post-D and Pre-D) configuration are indicated with a +. EC₅₀ Nit... Nitrification inhibited by 50%; EC₅₀ Denit... Denitrification inhibited by 50%;

	Biodegradability		Inhibition		BNR	
	Aerobic	Anoxic	EC ₅₀ Nit	EC ₅₀ Denit	Post - D	Pre - D
Ala		Readily				+
AMP	Readily	<i>Persistent</i>	19 - 32mM	> 316 mM	+	-
MEA	Readily	Readily	17-118mM	> 316 mM	99 %	99 %
DEA	Readily	Slowly	19mM	> 316 mM	+	+
MAPA		Slowly				+
DEEA		<i>Persistent</i>				-
MDEA	Readily	<i>Persistent</i>	43mM	> 316 mM	+	-
PZ	Readily	<i>Persistent</i>	12mM	> 316 mM	+	-
RW	<i>Persistent</i>	Readily	9-51mM	> 316 mM	-	98 %

Furthermore, this thesis identified nitrification as the limiting process of BNR, supporting the application of pre-denitrification for following two reasons: Firstly, nitrification was inhibited at low organic loading and/or amine concentrations, while denitrification was stimulated at the same concentrations. The initial denitrifying step appears stable up to high amine concentrations (see Paper 6). Secondly, the purer the autotrophic nitrifying biofilm was, the less sensitivity was shown towards inhibition by amines. Thus, we proved that the acute inhibition of nitrification activity is linked to the biofilm composition. This means that the more efficient the denitrification unit works, the less heterotrophic growth will occur in the aerobic step, and the more stable the post-nitrification treatment will operate.

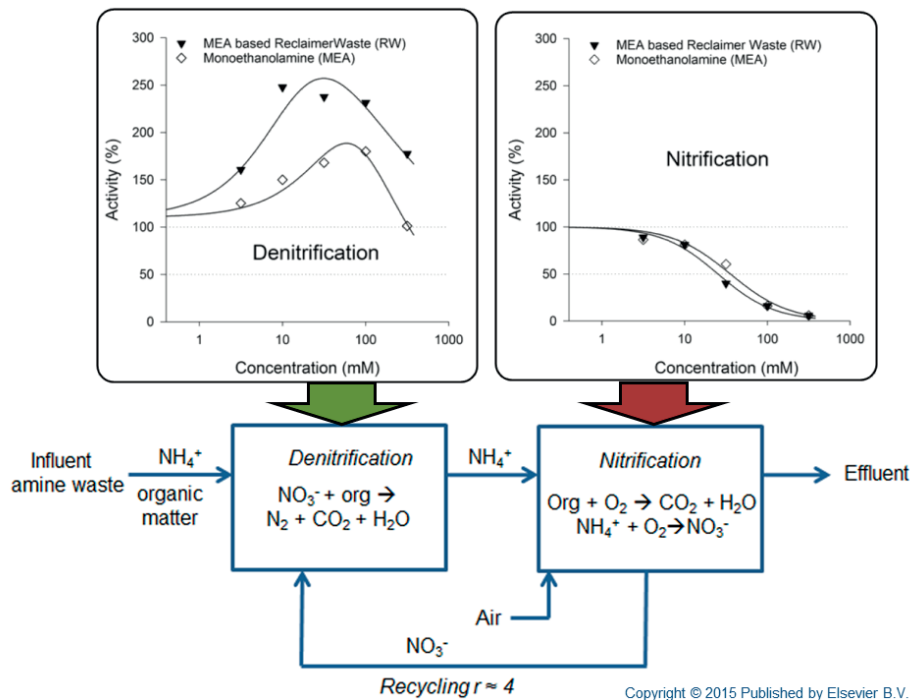


Figure 15 Inhibition within the pre-denitrification system for BNR (see Paper 6).

The contrasting effect of MEA and MEA-based RW on the nitrifying and denitrifying biofilms is highlighted in Figure 15. This implies that BNR may become unstable at high loadings where higher levels of amines can leak through to the nitrification reactor and inhibit nitrate production. The lack of recycled nitrate in turn, will limit anoxic respiration in the denitrification reactor even further. Therefore, operational conditions need to be monitored and controlled. This can be achieved by restricted feeding or addition of external electron acceptor, or even by adding an aerobic heterotrophic step prior to nitrification. Inevitably, these process control options need to be tested by simulation, followed by experimental verification at a lab scale before scaling up to pilot testing.

This study demonstrates the feasibility of BNR to treat amine waste, but does not include the design of a treatment system for reclaimer waste. As the experiments were conducted at a lab scale, this down-scaled process cannot directly reflect the process at a larger scale. Inevitably, intermediate steps should be tested before a full treatment system can be designed. Therefore, future works on designing an up-scaled process should include the evaluation of the process performance at a pilot scale. Depending on the process chosen, typical design values (Ødegaard, 1999; Ødegaard et al., 2009) may be useful for developing an up-scaled process. However, proper simulation models would be a very strong tool in optimizing the design.

In fact, a dynamic computer model could be a most helpful tool in developing both design and operation of such systems. To operate at high loading, the identified inhibition factors should also be included. In this context, future efforts should focus on extending the biokinetic model of Activated Sludge No 1 (ASM1) to include a proper 2-step description of nitrification, *i.e.* nitrite formation, followed by nitrate formation. This allows identifying transient nitrite accumulation during dynamic operation. Even more important, we now have proper data from our lab-scale experiments to include a quantitative description

of inhibition by specific amines, as well as of organic stimulation of heterotrophs interfering with nitrification in the aerated zone (Paper 6).

Finally, the methods and knowledge developed during this thesis work may set the framework for designing an up-scaled process of BNR of amine waste. To make CCS a sustainable process, this cost-effective waste treatment system should be integrated in the design and operation of any amine-based CO₂ capture plant.

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Paper I



Biological treatment of effluents generated by amine based CO₂-capture plants

生物处理由胺二氧化碳捕获设备所产生的污水

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Abstract - Carbon capture and storage (CCS) is a currently developed technology to fight climate change by reducing greenhouse gas emissions from large point sources. There are various capturing principles, whereas to date most of the pilot plants are based on amine absorption. One commonly used primary amine is monoethanolamine (MEA).

During the process of carbon capture, the solvent is subject to various degradation mechanisms due to oxidation, thermal strain, and unwanted side reactions within the system. After reclaiming the usable solvent for recycling, these degradation products accumulate as waste and need to be treated accordingly. Depending on the solvent, different degradation products may be found in this so called reclaimer waste, with ammonia as a dominant end product. Volatile products may also be emitted through the exhaust gas. Estimates from a full scale amine-based capture plant predicts approximately 0.2 ppm amine and 20 ppm ammonia in the emissions. For a full scale capture plant removing 1 million tons CO₂ annually, these concentrations implicate emissions of significant environmental impact.

Effluents from those various sources within the capture plant can be treated biologically to obtain nitrogen removal as well as general detoxification. Our studies have shown that MEA, as well as MEA-based reclaimer waste, can be treated with biological nitrogen removal, which is a well-established method within the field of wastewater treatment. Most important, by applying a recycled pre-denitrification reactor configuration, we have shown that the amine and its organic degradation products will serve efficiently as the carbon source needed for the denitrification step. Future development has to take these findings into consideration.

Keywords – Biodegradation; Carbon capture and storage; Monoethanolamine; Nitrification

I. INTRODUCTION

Carbon capture and storage (CCS) has received much attention in the recent years. It is thought that with CCS, the global CO₂ emission can be reduced until society is technologically as well as morally ready to shift from fossil fuel to alternative energy sources. In fact, the public acceptance of CCS depends on a variety of factors, such as the type of information, religious faith and others [1, 2]. However, to date many knowledge gaps of the technological side need to be filled before CCS can be employed at a large scale.

CCS relies on various capture mechanisms, whereas amine based post combustion CO₂-capture has been tested on more than 25 pilot plants, approaching full scale application on coal-fired plants [3]. One reason why carbon capturing from large point sources is not yet commercially viable is that such a large scale application of solvent has to carefully consider essential environmental aspects such as solvent emissions or spillage, as well as waste handling [4-9]. It is not only the solvent itself, but also various degradation products that have to be included in the assessment. The solvents used in the CCS process need to meet many criteria. For example, the solvent needs to have good thermodynamic and mass transfer properties and be stable at process conditions, while at the same time being easily degradable in the environment [10]. Monoethanolamine (MEA) is an example of a well-studied alkanolamine, as it has been applied for decades in the gas sweetening industry and also found application in CCS [11].

II. SOURCES OF WASTE IN CCS

During the process of carbon capture, the solvent reacts with components of the flue gas. In general, the solvent is also

subject to oxidative as well as thermal degradation. In flue gas coming from a fossil fuel-fired boiler there are CO₂, O₂, CO, SO_x, NO_x, fly ash, and other impurities, which make it very complex to predict all side reactions [12]. Therefore, waste effluents of CCS may include compounds in the liquid as well as in the gas phase. During CCS operation, a slip stream from the stripper column is taken and the degraded solution containing high molecular weight compounds and heat stable salts is separated via distillation from the useful amine [13], see Fig. 1. This so called 'reclaimer waste' contains mainly amine, ammonia, heat stable salts and other degradation products. The actual composition will depend on the type of solvent, process conditions, and flue gas quality. According to recent literature the generated amount of this type of waste ranges from 1.17kg/ton CO₂ to 3.94kg/ton CO₂ depending on flue gas composition and operational conditions [9].

Another source of waste produced in CCS is the exhaust gas. To avoid unwanted emissions of volatile amines or degradation products to the environment, the exhaust gas goes through multiple water washes. These water wash sections will remove ammonia from the gas, but over time they become saturated. Therefore, the circulating water needs to be exchanged and treated.

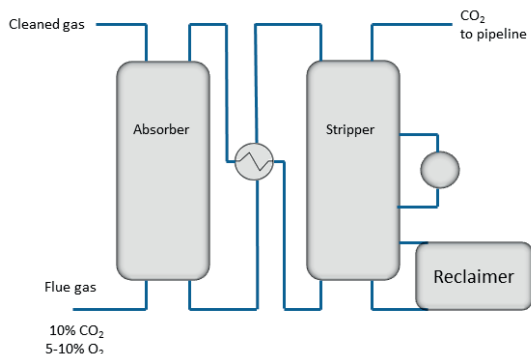
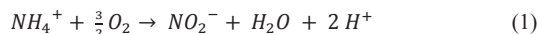


Fig. 1 Process flow diagram of typical post-combustion CO₂ capture by amine absorption, adapted from [13].

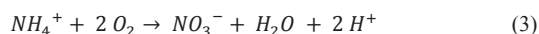
Maximum atmospheric emissions from simulations of a coal fired MEA based capture plant are reported with 5.5 and 1.14 mg/Nm³ (dry CO₂ lean Flue gas) for MEA and NH₃, respectively [14]. Other reported volatile compounds are diethanolamine (DEA), formaldehyde, acetaldehyde, acetone, methylamine and acetamide [14].

III. BIOLOGICAL NITROGEN REMOVAL

Biological nitrogen removal is based on the sequential reduction of ammonia to inert nitrogen. It is a key process in the natural nitrogen cycle and has been applied for wastewater treatment during the last century. This two-step process can be divided into nitrification and denitrification. The first step comprises the oxidation of ammonia to nitrate via nitrite and is facilitated by two groups of bacteria, namely the ammonia oxidizing bacteria (AOB) and the nitrite oxidizing bacteria (NOB), in two steps (Eqs. (1) and (2)):

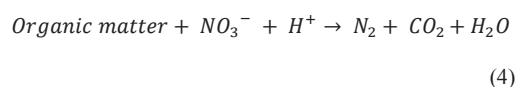


Commonly expressed by the total sum (Eq. (3)):



Both of these phylogenetically unrelated bacteria groups are chemolithoautotrophic, meaning they use inorganic compounds such as NH₄⁺ and nitrite as energy source while utilizing carbon dioxide as the carbon source. Due to the low energy yield, they grow very slow compared to the denitrifying bacteria in the following step.

In the next step, the generated nitrate is stepwise reduced to molecular nitrogen by denitrifying bacteria, as shown in Eq. (4).



In the absence of oxygen, some bacteria may use nitrate as the terminal electron acceptor for respiration instead of oxygen. Most denitrifying bacteria are facultative, meaning they can switch their respiration from oxygen to nitrate. Denitrification occurs then only under severe oxygen limiting conditions, because oxygen is energetically more favorable than nitrate [15].

Denitrifying bacteria are heterotroph, meaning they need organic carbon for energy metabolism, as well as for growth. The denitrifying bacteria belong taxonomically to various subclasses of the *Proteobacteria*. However, the ability to denitrify can also be found among archaea and core enzymes have even been found in fungi [15].

IV. BIODEGRADATION

Many natural occurring compounds have a functional role in at least one or more microbial metabolic pathways. This means that bacteria utilize them as a carbon source, or in their energy metabolism. Xenobiotics are man-made compounds, which do not occur naturally. However, many of these compounds may also be utilized by microbes if they can be made available to the bacteria and the conditions are right.

The persistency of a compound will depend on the chemical structure, the concentration and the environmental conditions for degradation. Monoethanolamine (MEA) is an easily degradable alkanolamine, but at high concentrations it was shown to persist for decades in soil [16]. Another aspect to consider when treating undefined mixed waste is that certain compounds might act synergistic or antagonistic in combination [17].

In general, biodegradation depends on chemical reactions catalyzed by extra- and intra-cellular enzymes. Larger molecules are hydrolyzed to smaller compounds before cellular uptake and the final oxidation to carbon dioxide. In the absence of an external electron acceptor, reduced products such as methane will accumulate. Thus, MEA based reclaimer

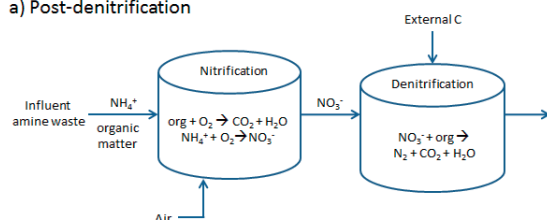
waste has been successfully degraded even under anaerobic conditions for biogas production [18-21].

A common measure for the biodegradability of a compound is the so called biological oxygen demand (BOD). During this test the microbial aerobic degradation of a compound is determined by measuring the oxygen consumption during degradation over a set time frame, such as 7 days in the standardized OECD Test No. 301 [22]. For solvents used in CCS, a lot of work has been invested to test the biodegradability of amines in seawater [23], whereas data on freshwater is yet scarce. We are currently testing amine biodegradability in freshwater with both oxygen and nitrate as alternative electron acceptors for oxidation.

V. PROCESS CONSIDERATIONS

For biological nitrogen removal there are multiple alternative process solutions available. The post- and pre-denitrification set-up illustrated in Fig. 2 have recently been tested for treatment of MEA [24]. A post-denitrification set-up is beneficial if the influent contains ammonia and only low amounts of organic matter. The aerated nitrifying reactor will convert ammonia to nitrate that serves as an electron acceptor in the second denitrifying step. Amine waste contains substantial amounts of ammonia, but also vast amounts of organic matter.

a) Post-denitrification



b) Pre-denitrification

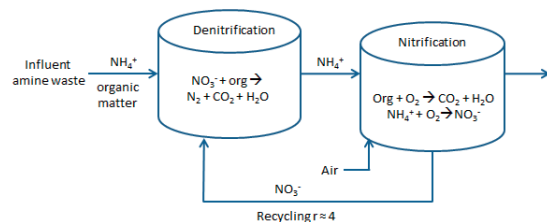


Fig. 2 Alternative process configurations.

This may be utilized aerobic by heterotrophic competing with the nitrifiers for oxygen, thus decreasing the nitrification efficiency. In an open system, autotrophic nitrifying bacteria are always accompanied by heterotrophic bacteria, and their competition for space and oxygen is a well-known phenomenon, particularly in biofilm systems [25]. For the nitrogen removal efficiency this does not have to play a major role, but in terms of economy, the additional feed of organic

matter in the following denitrifying step may be of significance.

The obvious solution is to feed the heterotrophic denitrifying bacteria with the available organic matter in the amine waste. This can be achieved with the pre-denitrification set-up where the amine waste reaches the anoxic denitrification reactor first, see Fig. 2b. Here the amine can be biodegraded, resulting in ammonia and other organic compounds. The organic matter represented by the amine itself serves as a carbon source, while the bulk fraction of ammonia continues into the aerated nitrifying reactor (Fig. 2b). Ammonia will there be biologically oxidized to nitrate, which then has to be recycled to the denitrifying bacteria, to serve as the electron acceptor for their respiration. One disadvantage of this set-up may be that there will always be some nitrate lost in the effluent. However, the cost saving advantage of not needing any additional carbon source is the most important factor. We have recently successfully treated MEA as well as real reclaiming waste from an amine based CO₂-capture plant with this set-up [24, 26].

Another crucial factor in bioprocess engineering is the retention of the biocatalyst in a continuous flow. Losses have to be minimized so that bacterial growth can compensate to maintain a steady state activity. This is of particular importance for the slow-growing nitrifiers, with a doubling time of 1 day or more. We have applied so called moving bed biofilm carriers to achieve successful retention and activities [24, 26].

The corresponding diffusion transport dominated micro-environment of the biofilm has been found to determine the actual organic loading capacity as well as toxic inhibition of such as system, work is in progress to understand and model those essential process parameters in detail.

VI. NEW SOLVENTS

Developing new capture solvents is currently an ongoing research topic [27]. As mentioned above, the solvent must show stability during process conditions, as well as be easily degradable in the environment. According to Hoff et al. [10] most of the first generation post combustion solvents belong to one of the following groups: Two- component buffer plus promoter systems (activated AMP or MDEA), single component amine system with high molecular efficiency (MEA, Piperazine), amino acid systems using strong base or amine as neutralizing agent (KOH + Glycine), or promoted carbonates (K₂CO₃ + activator) [10]. All of these solvent groups show more or less chemical degradation during process condition. Volatility is another important aspect. Amino acids show low volatility [10], and in terms of biodegradability in marine environment, amino acids show low toxicity and high biodegradability. However, tertiary amines, compounds containing quaternary carbons and some solvents (such as AMP and MDEA) did not degrade easily in sea water [23]. Researchers are currently working on third generation solvents, aiming on improving their energy efficiency.

VII. CONCLUSION

Biodegradability tests of solvents need to be applied before large scale utilization in CCS can be done. For many solvents data exist, but not for all relevant environments. A limitation of the BOD testing is that just the aerobic degradation is determined and not the anoxic degradability which is crucial in biological waste treatment as illustrated.

Efficient waste and effluent treatment must be integrated in the evaluation and choice of future solvent systems. We have shown how this can be tested in lab scale to develop suitable compact bioprocess plants for this purpose [24, 26].

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Paper II

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Syringe test screening of microbial gas production activity: Cases denitrification and biogas formation

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Abstract

Mass produced plastic syringes may be applied as vessels for cheap, simple and large scale batch culture testing. As illustrated for the cases of denitrification and of biogas formation, metabolic activity was monitored by direct reading of the piston movement due to the gas volume formed. Pressure buildup due to friction was shown to be moderate. A piston pull and slide back routine should always be applied before recording gas volume to minimize experimental errors. As illustrated, inoculum handling and activity may be conveniently standardized by applying biofilm carriers. A robust set of positive as well as negative controls ("blanks") should be included to ensure quality of the actual testing.

The denitrification test showed saturation response at increasing amounts of inoculum in the form of adapted moving bed biofilm reactor (MBBR) carriers, with well correlated nitrate consumption vs. gas volume formed. As shown, the denitrification test efficiently screened different inocula at standardized substrates. Also, different substrates were successfully screened and compared at a standardized inoculum.

The biogas potential test showed efficient screening of different types of food waste substrates (carbohydrate, protein, fat). In a second case, reclaimer waste from a monoethanolamine (MEA) based CO₂ capture facility was monitored for a period of 4 weeks, showing successful use of co-feeding to support waste treatment.

In total, syringe test screening of microbial gas production seems highly efficient at a low cost when properly applied.

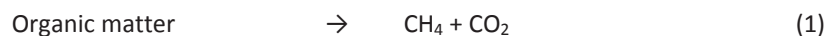
Keywords: Biodegradation, screening test, syringe test, denitrification, biogas formation.

Introduction

In a time of increasingly complex and sophisticated methodology, it may be revealing to find fields of science where simplicity may still be relevant. Mass screening of microbial activity in open system batch cultures is one case still of significance. In principle, this is either applied to compare different substrates at a hopefully standardized inoculum, or to compare different inocula at some standardized substrate.

This is applied both in routine surveillance as well as in research, as in the OECD / ISO standardized tests for ecotoxicology and biodegradation (OECD 1981, OECD 1992, OECD 2004, OECD 2006). Either, some substrate consumption or some product formation may be recorded. Substrates in the widest sense could be some organic or inorganic electron donor, just as well as some acceptor as in the case of the respiratory BOD test (Clescerl *et al.* 1999). Product formation is only applicable for activity measurements in cases of product accumulation, mainly in cases of anaerobic or fermentative conditions.

In particular cases, microbes may create products forming a stable and separate gas phase, such as in the case of biogas production, given in Equation 1:



The exact product balance depends on the redox state of the substrate and the products' water solubility.

Another case is the type of anaerobic respiration denoted anoxic or denitrifying reaction, with NO_3^- as the electron acceptor, given in Equation 2:



The accumulation of inert dinitrogen gas will directly reflect the respiration rate. Due to its low water solubility, it will readily separate out as a gas phase.

The simplest way of measuring such a gas phase accumulation, is putting the culture into a syringe and record the gas production as the volume expansion reflected by the movement of the piston. But does it really work? If so, it is so trivial that somebody must have thought of it before. To our best knowledge and googling, we have been able to trace an origin back to studies of livestock digestion physiology, particularly the rumen fluid (Menke *et al.* 1979, Menke and Steingass 1988, Duan *et al.* 2006). In those early days, only glass syringes were available and the field of application very restricted. We do not claim originality for the idea, but today, disposable syringes in suitable sizes are cheap and readily available, and the multitude of possible applications much wider.

There is a growing demand for reliable and low cost methods to investigate bio-reactions, especially in developing countries where research is poorly funded while there is a great demand for efficient environmental biotechnology. We have observed the usefulness of the

presented protocol through research and education development aid projects. A purpose of publishing this method is to boost data generation regarding feed sources for biogas generation and pollution mitigation processes.

The purpose of this current work is to present recommended procedures for widely different illustrative examples of syringe test applications, as well as some guidelines to avoid the major potential pitfalls of this approach.

Materials and Methods

a) General aspects

Disposable plastic medical syringes (BD Plastipak, Franklin Lakes, NJ USA) were applied with volumes 100 mL or 60 mL. After filling, syringes were closed air tight either by adding a needle and silicone rubber stopper, or directly by plastic closing cones (Braun, Melsungen, Germany). Stoppers with syringe valves (Mininert™ from VICI AG Int., Schenk, Switzerland) were applied for sampling by suction to a connected syringe for later analysis of gas composition.

A pressure friction test was run by filling 60 ml syringes with 40 ml of water, then carefully adding pressurized air until a volume expansion of 3 mL was achieved. The equilibrium pressure before and after expansion was recorded by an on line Beamex multifunction calibrator MC5 (Pietarsaari, Finland). This procedure was repeated in triplicate for each syringe examined.

Inocula were based on samples from natural sediments, from wastewater treatment plant, from biogas facilities at pilot or lab scale, as well as from long term adapted lab cultures. Types of inocula included suspended cultures, granules or moving bed biofilm carriers. See details below.

Incubations were performed at room temperature as well as in incubators (in house and Infors HT Minitron 22 C, Bottmingen, Switzerland) set at temperatures of 22, 25 or 35 °C. Stirring was obtained by a variety of laboratory shakers, including rotating (in Minitron at 110 rpm), linear (in house at 140 rpm) or tilting (Nutating Mixer, VWR, Radnor, PA USA), preferably with the syringes stacked in horizontal position.

A piston slide test was included in one initial experiment on denitrification (see below) , where after 12 d of incubation, gas volumes denoted “before” were recorded directly, then the pistons were pulled and let slide back to a new stable position before re-reading the volumes denoted “after”. Later on, the latter procedure was adapted as standard.

b) Denitrification

Suspended inocula were obtained from a local river sediment (Nidelva, Trondheim) and from activated sludge of the wastewater treatment plant at Statoil’s liquefied natural gas facility Hammerfest LNG, Melkøya. Biofilm cultures were obtained by long term lab enrichment cultures originating from local domestic wastewater, grown on moving bed biofilm reactor (MBBR) polyethylene carriers type Standard AnoxKaldnes K1 with 10 mm outer diameter (Veolia Water Technol. – AnoxKaldnes, Lund, Sweden).

The basal medium was prepared according to OECD guideline 301 on testing biodegradability (OECD 1992) with nitrate added corresponding to 100 mg/L of NO_3^- -N. Substrates tested included sodium acetate and a variety of amines, such as monoethanolamine (MEA), diethanolamine (DEA), methyldiethanolamine (MDEA), 2-amino-2-methylpropanol (AMP) and piperazine. See also Henry *et al.* (2016a).

Syringes of 60 mL were filled with 40 mL of medium plus inoculum, emptied for air and closed before stacked in an incubator at 21 °C. Gas volumes formed were recorded daily unless otherwise stated.

At end of incubation, nitrate concentrations left were determined by Hach –Lange assays for water quality (Hach-Lange, Düsseldorf, Germany) according to manufacturer’s instructions.

c) Biogas

The suspended anaerobic sludge inoculum was obtained from a long term UASB lab enrichment as described by Wang *et al.* (2013a; b; c), based on cultures originating from livestock manure, local river sediment, a domestic wastewater treatment plant sludge digester in Porsgrunn, Norway and granules from a UASB methane reactor treating wastewater from the pulp and paper industry at Norske Skog Saugbrugs AS, Halden, Norway.

In comparative tests of different mixed substrates, different organic mixtures resembling food wastes were made by mixing 1) apple juice as a source for carbohydrate, 2) yeast extract as a protein source and 3) cream as a source of fat, as shown in Table 1:

Table 1. Composition of mixtures tested for biogas potential.

Sample name	Mixture of substrate	Volume ratio	COD ratio
A	apple juice (carbohydrate) and yeast extract (protein)	45:55	~50:50
B	apple juice (carbohydrate) and cream (fat)	95.5:4.5	~70:30
C	yeast extract (protein) and cream (fat)	97.8:2.2	~80:20
D	apple juice (carbohydrate), yeast extract (protein) and cream (fat)	44.6:53.2:2.2	~40:40:20

Amine absorption is a technique commonly applied in carbon capture and storage (CCS). In another experiment, reclaimers waste was collected at a MEA based CO_2 capture facility of a

coal fired power plant. In general, such wastes do contain undegraded amine as well as degradation products thereof, ammonia being the dominant one in addition to organic intermediates (Strazisar *et al.* 2003, Hauser *et al.* 2013). Test concentration was 20 g/L. A mixture of glucose (1.7 g/L), peptone (3.0 g/L) and yeast extract (3.6 g/L) was applied as a separate co-substrate feed.

The biogas potential standardized test was performed in 100 mL syringes filled with a 30 mL volume of mixed substrate and inoculum. Any air entered during filling was pushed out through the needle before closing with a stopper.

The syringes were then stacked horizontally in a fume hood at room temperature (22 ± 3 °C) when comparing different inocula, at 35 °C in the experiments comparing different substrate mixes. A tilting shaker (Rocking platform shaker, VWR Collection) was applied in those particular cases.

Gas volume was recorded 1 – 2 times per day, depending on activity. After prolonged gas accumulation, produced biogas was removed to avoid overfilling the reactors. Produced biogas was just emptied in the hood, or collected in a sample container by pushing through interconnective valves into a smaller syringe for analysis of gas composition.

At end of incubation, gas volumes formed were sampled and gas composition determined by gas chromatography (HP 5890 A, Hewlett-Packard, Palo Alto, CA USA). In a two-column system, the Molsieve 5A column was used to separate H₂, O₂, N₂, CH₄, CO, and the Porabond Q Tandem column to separate air, CH₄, and CO₂. Argon was used as the carrier gas. Nitrogen was applied as the valve gas. The injection temperature was 120 °C, and the detector temperature was 150 °C. The oven was kept constant at 40 °C.

The liquid content of volatile fatty acids VFA was also determined by gas chromatography, using a HP 6890 serial C (Hewlett-Packard) with a flame ionization detector and a capillary column (DB-FFAP 30 m long and 0.25 mm ID, 0.25 µm film). Helium was used as the carrier gas, at flow velocity 24 mL/min. The detector gases were hydrogen and air. The injector and the detector temperatures were set to be 200 °C and 250 °C, respectively. The oven was programmed to start to start at 80 °C and hold for one minute, then to 180 °C at a rate of 30 °C/min, then to 230 °C at a rate of 100 °C/min.

Results and discussion

a) Sources of error

The pressure friction test described above gave data summarized in Figure 1 for 10 different syringes tested in triplicate. Error bars show standard deviations based on those sequential measurements. Repeatability was generally good.

The average pressure increase was found to be 21.9 mbar, that is + 2.1 % of the current atmospheric pressure, that is rather moderate.

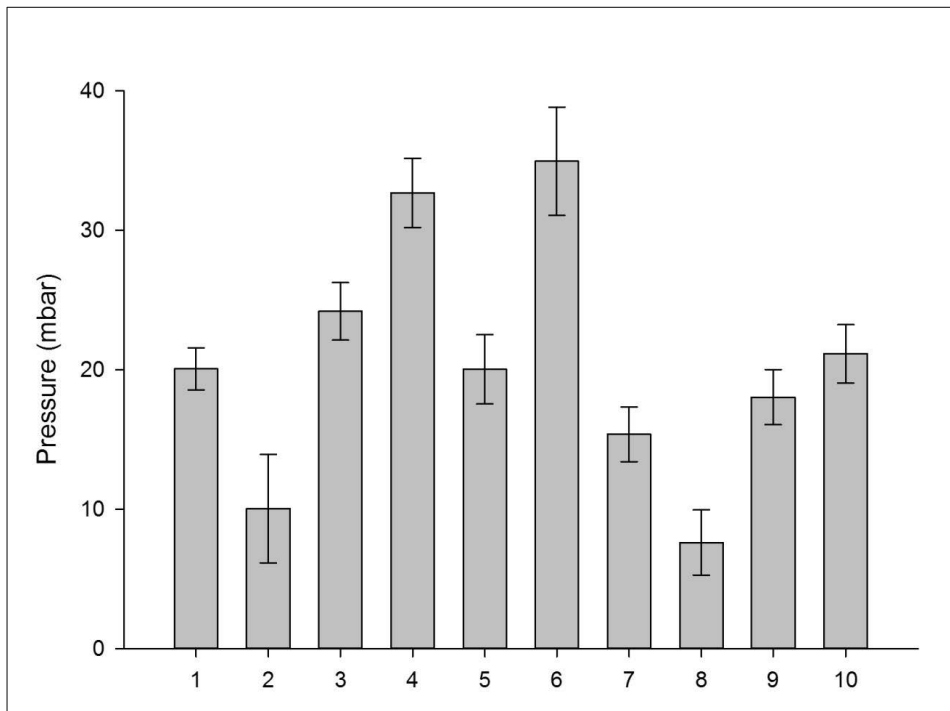


Figure 1 Recorded pressure increase after expanding volume of 40 mL liquid with 3 mL air for 10 different syringes selected at random. Error bars show standard deviations based on 3 sequential measurements.

Individual recordings were within $2.1 \pm 0.9\%$ (s.d.). According to Student's *t*-test, the three highest values were significantly higher than three lowest. In conclusion: Standard mass produced syringes may have systematic and different friction errors. When all 30 recorded values were pooled and plotted as a histogram (not included), a reasonably symmetrical distribution verified that a normal distribution may be assumed to be valid also in this case, with a mean value of 21.9 and ± 4.4 in s.d. for triplicates, ± 9.9 in single measurements.

As illustrated, those mechanical friction errors may be moderate but should be taken into consideration when planning statistical testing. In addition, also the biological variation has to be included when planning the number of replicates needed to obtain a certain level of significance in comparative testing.

The piston slide test described above was included to reveal how such frictional mechanical errors might affect real recordings. In an experimental set of 5 replicates of 5 different carbon sources for denitrification, that is a total of 25 independent reactors, volumes were recorded both before and after the piston was forcibly pulled to let slide back to a stable position again. There were clearly large differences between syringes, with volume changes ranging between the extreme values of 0 and 0.9 ml, on the average 0.30 ± 0.05 (s.e.m.) ml.

When data were sorted according to the expected volume increase due to N_2 formation, experimentally determined by the corresponding nitrate consumption, linear regression analysis gave correlations as shown in Figure 2. The volume differences recorded for each culture increased at larger volumes, at a reasonably constant relative value.

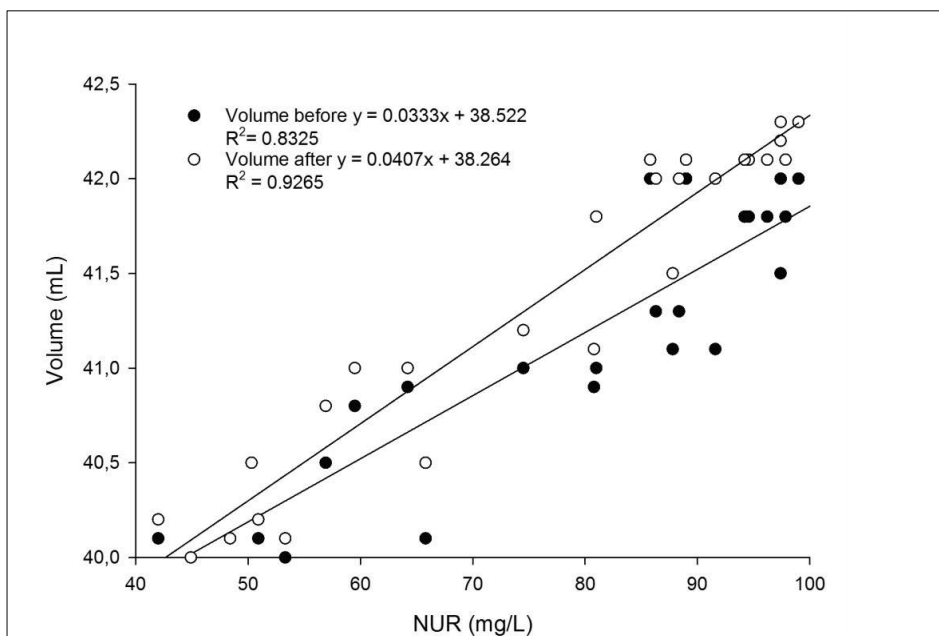


Figure 2 Linear correlations between recorded volume expansions and corresponding nitrate utilization rate (NUR) for 25 denitrifying cultures, with volumes recorded before and then after a pull and piston slideback operation.

It may be noted that there was an apparent threshold, with a nitrate consumption above 40 mg/L of NO_3^- -N of even at zero gas volume, corresponding to 40 mL of liquid and total volume in the graph (Fig. 2). This is surprising, compared to tabulated values of 20 mg/L for N_2 solubility in freshwater at room temperature.

Most important, the correlation coefficient was clearly improved by the piston pullback procedure, to a value of $R^2 = 0.93$ illustrated in the upper graph. This procedure was therefore applied in all later recordings.

Concerning additional biological variations, in this case operating with 5 replicates gave a typical experimental error in recorded volume increase for each of the 5 compounds tested of $\pm 22\%$ (s.e.m.). This is considered acceptable for most practical applications.

Other factors to consider are, based on general observations (results not included): A shaker should preferably be used if mass transfer limitations are expected, but manual handling twice a day when reading gas production has been found to be sufficient in all cases tested. Stacking the syringes horizontally in a tray to contain spills in the incubator is preferred. Keeping the syringes in vertical position on racks has also been practiced.

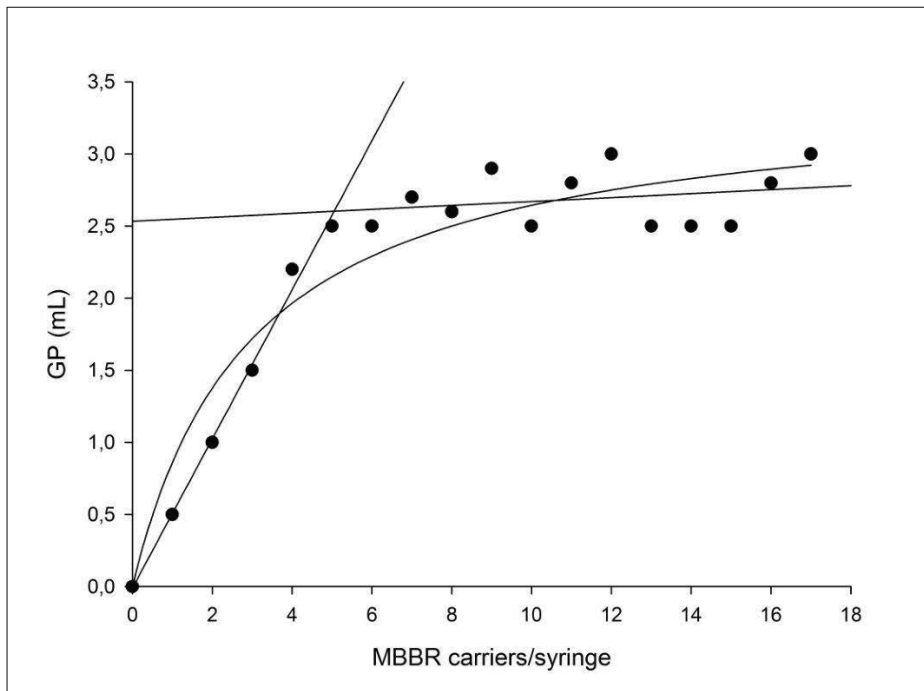


Figure 3 Gas production recorded after 1 d of incubation, starting with 400 mg/L acetate (equivalent to 286 mg/L COD) and 100 mg/L of $\text{NO}_3\text{-N}$, shown as a function of amount of inoculum in the form of biofilm carriers. Curved line shows one site saturation kinetics $f = B_{max} * (x) / (Kd + (x))$ for total data set ($R^2 = 0,9101$), straight lines linear regression of different subsets 0-5 and 6-17 carriers.

b) The denitrification test

As already illustrated in Figure 2, volume recordings based on the piston pullback procedure showed a good correlation to the denitrification activity determined by direct chemical analysis, with a correlation coefficient of $R = 0.93$. This is considered adequate for most practical applications.

The three fundamental parameters to consider in testing will be the inoculum, the organic substrate and the electron donor. The activity rate may obviously be speeded up by increasing the biomass of the inoculum. When using biofilm grown on carriers such as the moving bed reactor type K1, this is achieved by simply adding more carriers per syringe, as shown in Figure 3. A rapid response could be observed within one day. However, at higher values of inoculum, there appears to be some bi-phasic or saturation effect. Standardized testing was therefore restricted to 5 carriers or less per syringe.

Concerning the balance of the electron donor organic substrate and the electron acceptor nitrate, it may be noted that according to basic stoichiometry, it would take 2.86 g of COD to fully reduce 1 g of $\text{NO}_3\text{-N}$ to dinitrogen gas. Consequently, if testing at or standard initial 100 mg/L $\text{NO}_3\text{-N}$, nitrate depletion may limit the degradation if the test substrate exceeds 286 mg/mL. It should also be evident that some fixed-time test, as in the case of the standard BOD_7 , should not be applied unless conditions are kept within the proper concentration ranges for that incubation period. Below, we illustrate some cases of time-graph kinetics:

To illustrate the screening of inocula, four widely different sources of denitrifying cultures listed in the Methods section were compared by testing their ability for anaerobic degradation of acetate. Adapted biofilm carriers were sampled from continuous lab reactors exposed to monoethanolamine (MEA) for more than 5 months, while non-adapted were sampled from long term lab reactors exposed to domestic sewage only. In an attempt to quantify active biomass, COD was determined for each suspended culture as well as for maximal scrape-off per biofilm carrier. For the sediment sample, inoculating 6 mL of sediment at 2800 mg/L COD gave 16.8 mg COD per syringe, while for the Melkøya sludge, 2.5 mL of 4920 mg/L COD gave 12.3 mg COD per syringe. The scrapeoff material per carrier corresponded to 2.2 mg of COD, adding 5 carriers then gave 11.0 mg COD per syringe for the adapted biofilm. The non-adapted carriers were not quantified but can assumed to be of the same magnitude. It should be stressed that biofilm activity is determined by the three-dimensional surface area rather than volume or biomass. In conclusion, within a factor of 2 the inocula can be considered to represent similar amounts of organics.

Figure 4 A shows the corresponding gas volumes produced over a period of up to 10 days. Clearly, pre-adapted biofilms responded faster than non-adapted. However, maximal conversion was reached within 3-4 days in both cases. The Melkøya sample was less active, but also showed quite high denitrifying capacity without any significant lag. It is striking that even our river sediment sample showed significant activity. Although sediment conditions

may easily go anaerobic, nitrate is difficult to identify there as a dominant electron acceptor for anaerobic respiration. The blank sample shown represents surface water only.

It should be noted that when complete inocula in this case were incubated with just pure water as substrate, recorded background volumes never exceeded 0.1 mL (results not included), in spite of the significant organic loading represented by the biomass of the inoculum itself. Later testing has revealed that this might not always be the case (Henry *et al.* 2016b), it seems that prolonged starvation of the inoculum culture may induce hydrolytic enzymes stimulating the ability to use the biomass itself as carbon source. If so, this will lead to enhanced blank values even in the absence of other external substrates. Since such enzymes might be quite unspecific, simply subtracting high background values could be highly misleading. Instead, the quality as well as the quantity of inoculum should be reassessed if high control values are observed.

To illustrate the screening of different substrates, data for the amine MEA, commonly applied in carbon capture and storage (Hauser *et al.* 2014), are shown for the same inocula in Figure 4 B. Clearly, this is a much less favourable substrate, with a much slower metabolic response. However, also in this case all inocula were directly able to degrade also this chemical by anoxic respiration. Again, the river sediment showed the slowest response, with the biofilm carriers as the apparent winners. Such amines should evidently not be considered or treated as xenobiotic in this context, see also Brakstad *et al.* (2012), Eide-Haugmo *et al.* (2012).

A thorough screening of anoxic vs. aerobic degradation of a multitude of relevant amines is presented in detail by Henry *et al.* (2016b).

Clearly, the kinetics observed in Figure 4 A and B reflect adaptive responses as well as selective growth within the microbial communities involved. This should always be taken into consideration when trying to standardize some inoculum for mass screening.

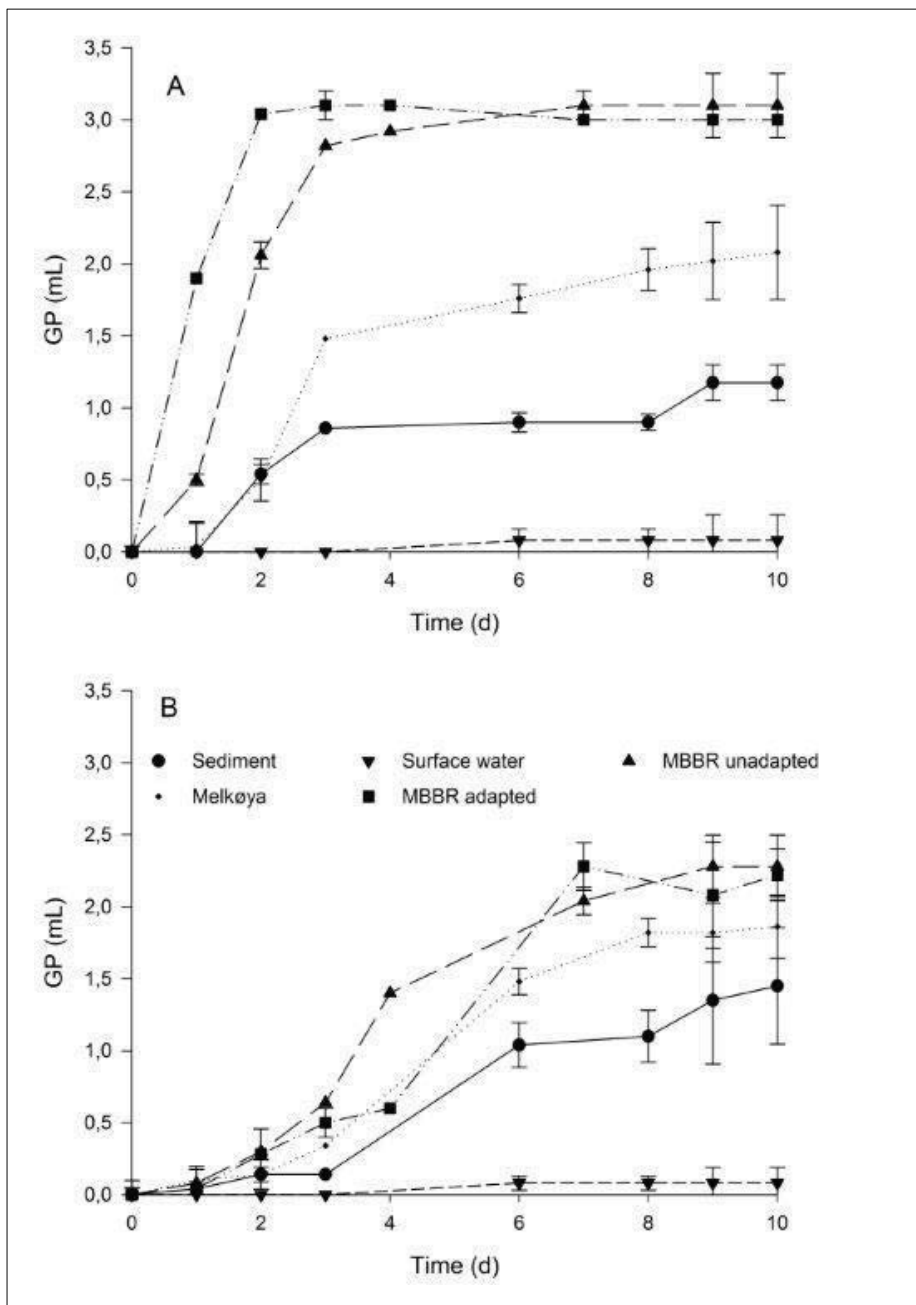


Figure 4 Gas production recorded as a function of time with different inocula as indicated, starting with 286 mg/L of substrate COD and 100 mg/L of $\text{NO}_3\text{-N}$. **A:** With acetate. **B:** With monoethanolamine MEA. Error bars indicate the standard error of the mean (SEM) of 5 replicates.

c) Biogas potential test

Also in this case, different inocula may be screened and compared at some standardized substrate (results not included).

Instead, different substrates may be compared for the same type of inoculum, as illustrated in the case of the mixes resembling food waste listed in Table 1. See Figure 5.

As shown in Figure 5 A, biogas production started rapidly, reaching high levels within the first recordings at 18 h of incubation. As expected, the samples A and B dominated by carbohydrates responded most rapidly.

As shown in fig. 5 B, after 184 h of incubation, the same feeds were added a second time. The response was as immediate and striking as for the first. It may be noted that sample E, representing the blank now feed the same protein + fat mix as C in the first feeding, showed a similar response. Over time, though, sample D, containing all three major food groups, gradually took the lead. At end of experiment at day 28, gas production had ceased in all reactors, at a total volume of ranging from D at 408 mL to B at 261 mL for those fed twice. Ranked from high to low production, the observed order was D>A>C>B.

Gas flow analysis showed corresponding results, with highest levels the first days after feeding (results not included). VFA, pH and gas composition were also determined after 506 h of digestion. At the time, sample B had gone sour at a pH of 3.9, with a corresponding gas composition of 8 % CH₄, 60 % CO₂ and 25 % N₂. For the others, pH stayed within pH 6.8 – 7.8, with a healthy biogas composition of 59 - 64 % CH₄, 20 - 40 % CO₂ and 0 % N₂. A total VFA of 0.1 – 8.6 g/L had compositions dominated by 0 – 3.7 g/L acetate, 0 – 1.6 g/L propionate, 0 – 0.6 g/L isobutyrate, 0 – 3.0 mg/L butyrate and 0.1 – 1.1 mg/L isovalerate, with others all below 0.05 mg/L.

In conclusion, the biogas syringe test as performed provided a simple and useful method to test the potential of different wastes for biogas production. In this case, substrate mixes of carbohydrate + protein + fat close to real food waste obtained the greatest production. High lipid content seemed to slow down production at room temperature.

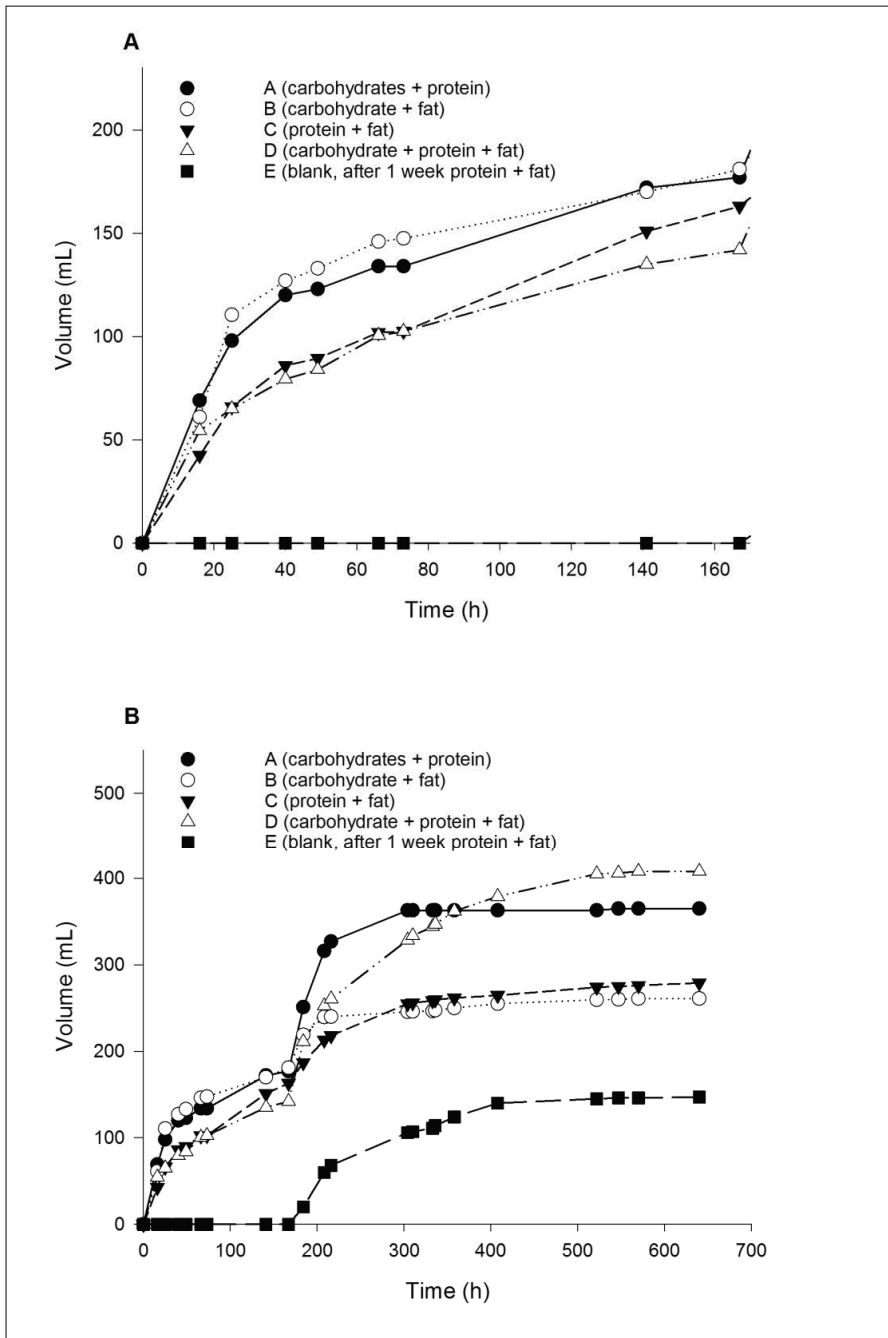


Figure 5 Biogas production recorded as a function of time with different substrates as indicated. **A:** Initial period of one week. **B:** With re-feeding after one week. Note different time scales.

Reclaimer waste generated by amine based CCS is a dark and smelly mixture, generally not particularly suitable for bacterial survival and proliferation. Figure 6 shows the anaerobic treatment of such waste at our test concentration of 20 g/L when added co-substrate feed to stimulate bacterial growth.

As shown in Figure 6 A, the accumulated biogas generation at 25 °C ceased after 2 weeks when only fed the easily degradable co-feed. Addition of the MEA-containing reclaimer waste did not speed up biogas production rate. However, prolonged gas production continued for an additional period of more than a week, with a final increase of 11.5 mL compared to that of c-substrate only.

As shown in Figure 6 B, increasing the temperature to 35 °C clearly speeded up the process, with the co-substrate feed alone reaching its plateau at 8 days, at a level 35 % above that of 25 °C. With reclaimer waste present, biogas production continued until day 23, reaching a final level 28.5 % above that of lower temperature. It should be noted that both temperatures are well within the mesophilic range. Generally, biocatalyzed processes from enzyme to cellular level tend to have temperature coefficients corresponding to a doubling of activity per 10 °C, well in accordance with the pattern presented in Figure 6.

Previous semi-continuous feed tests have shown accumulated biogas containing between 75 to 84 % methane (Wang et al. 2013a), giving an average of 80 % applied in mass balance calculations (not shown). Then, according to total and effluent recordings of COD, the COD recovery as methane at 25 °C was estimated to be 66 % for co-substrate only and 56 % for reclaimer waste + co-substrate, with corresponding values at 35 °C of 89 % and 70 %, respectively (Wang 2014).

In conclusion, Figure 6 illustrates how prolonged gas accumulation can be recorded efficiently for long periods just by removing produced biogas after recordings to avoid overfilling the syringes. In this way, both biogas production rates and total conversions can be precisely determined for a multitude of substrate combinations in a single experiment.

Finally, instead of just one extra feed as illustrated in Figure 5, the basic batch test described above can also be extended into a more systematic sequential batch test by removing a predetermined liquid volume (*e.g.* 1 mL) to be replaced by fresh feed at regular intervals.

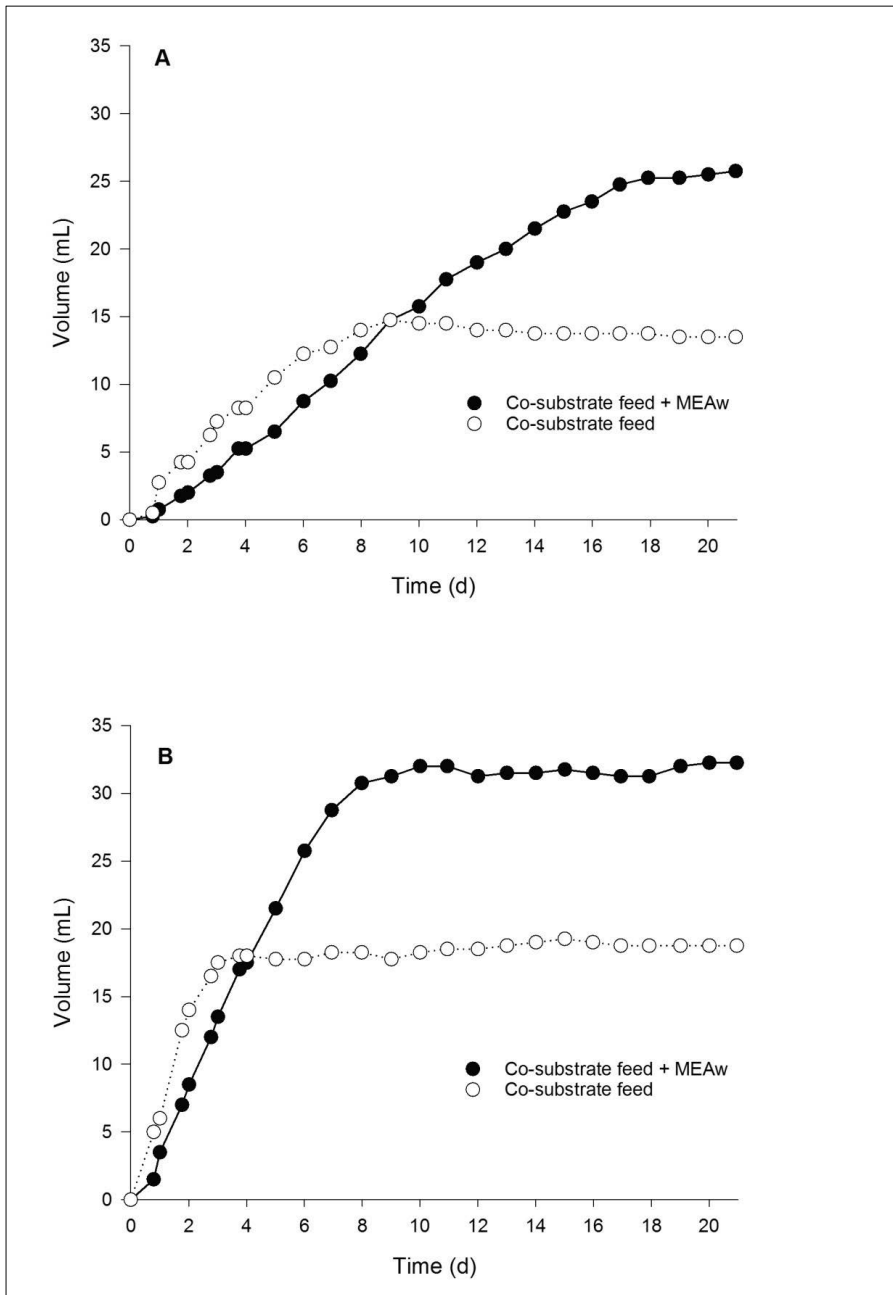


Figure 6 Biogas production extended by sequential batch regime recorded as a function of time with co-substrate feed alone or combined with 20 g/L reclaimer waste as indicated. **A:** At 25 °C. **B:** At 35 °C.

Conclusions

The pressure friction test revealed 2 % pressure buildup and significant differences between syringes. This is considered to be a moderate source of error. However, wear should be minimized by restricting reuse.

The piston slide test revealed that piston pull and slideback should always be applied before recording gas volume to minimize experimental errors.

Inoculum handling and activity may be conveniently standardized by applying MBBR biofilm carriers as illustrated here, or aerobic or anaerobic UASB biogas granules (Nordgård *et al.* 2015), or gel entrapped cultures even including frozen storage (Vogelsang *et al.* 1999).

As always, a robust set of positive as well as negative controls (“blanks”) should be included to ensure the quality of the actual testing. For heterotrophic growth, glucose or acetate (as illustrated here) may be recommended as a positive reference. Most important, the internal metabolic activity of the inoculum alone should be assessed and restricted if necessary.

The denitrification test showed saturation response at increasing amounts of inoculum in the form of adapted MB biofilm carriers, with well correlated nitrate consumption vs. gas volume.

The denitrification test efficiently screened different inocula at standardized substrates. Also, the denitrification test efficiently screened different substrates at standardized inoculum.

The biogas potential test showed efficient screening of different types of food waste substrates (carbohydrate, protein, fat).

The biogas potential test was also successfully extended by running it as a long-term sequenced batch reactor for a period of 4 weeks. Experiments thereby documented successful use of co-feeding to degrade MEA based reclaimer waste from amine based carbon capture.

In total, syringe test screening of microbial gas production seems highly efficient when properly applied. The versatility of this approach has been illustrated above.

As a cheap and simple screening method, it should be helpful in expanding the field of applied environmental biotechnology also in developing countries.

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Paper III

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Aerobic and anoxic biodegradability of amines applied in CO₂-capture

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ABSTRACT

Integrated and sustainable waste handling is becoming essential in large scale employment of amine-based post combustion CO₂ 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 N₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ (Nurrokhmah et al., 2013), whereas an older study from Thitakamol et al. (2007) estimates 4-15 kg of waste per ton of CO₂ 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 CO₂ 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).

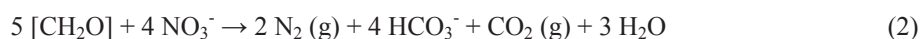
Besides aerobic and anaerobic environments, anoxic ecosystems lie in between these two extremes and play a key role in biodegradation. Under oxygen limiting conditions (ideally < 0.2 mg/L dissolved oxygen) some microorganisms can switch to nitrate respiration, also referred to as denitrification (Lu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

Denitrification is the dissimilatory reduction of nitrate or nitrite to a gaseous N-oxide accompanied by free energy (ΔG°) transduction (Bueno et al., 2012). The ΔG° 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 [CH₂O].

Aerobic respiration, yielding ΔG° -479 kJ/mol:



Denitrification, yielding ΔG° -453 kJ/mol:



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 autotroph 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 CO₂ 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 N₂ as an end product of denitrification (Østgaard et al. 2016).

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

neglected or subtracted without consideration when evaluating the outcome of screening tests following the guidelines of OECD (1992, 2006).

The objective of the present study was to assess the biodegradability under anoxic conditions for 9 amines used for CO₂ capture. This method can be used to identify potential carbon sources for denitrification. Furthermore, these results were compared to results of the standard aerobic biodegradability test in fresh water, as well as to marine biodegradability reported in literature.

2. Material and methods

2.1. Chemicals

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

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

Compound	Abbreviation	CAS	Formula	ThOD ^a (gO ₂ /g)
<i>Positive control</i>				
Sodium acetate	NaAc	127-09-3	C ₂ H ₃ NaO ₂	0.78
<i>Amino acids</i>				
Alanine	Ala	56-41-7	C ₃ H ₇ NO ₂	1.08
<i>Primary amines</i>				
2-amino-2-methylpropanol	AMP	124-68-5	C ₄ H ₁₁ NO	3.05
2-aminoethanol	MEA	141-43-5	C ₂ H ₇ NO	2.10
<i>Secondary amines</i>				
Diethanolamine	DEA	111-42-2	C ₄ H ₁₁ NO ₂	2.13
3-amino-1-methylaminopropane	MAPA	6291-84-5	C ₄ H ₁₂ N ₂	1.45
<i>Tertiary amines</i>				
2-Diethylaminoethanol	DEEA	100-37-8	C ₆ H ₁₅ NO	2.33
N-methyldiethanolamine	MDEA	105-59-9	C ₅ H ₁₃ NO ₂	2.28
<i>Cyclic amines</i>				
Piperazine	PZ	110-85-0	C ₄ H ₁₀ N ₂	3.35
<i>Reclaimer waste</i>				
MEA based reclaimer waste	RW	n.a.	n.a.	1.42

^a calculations based on carbon and nitrogen oxidation

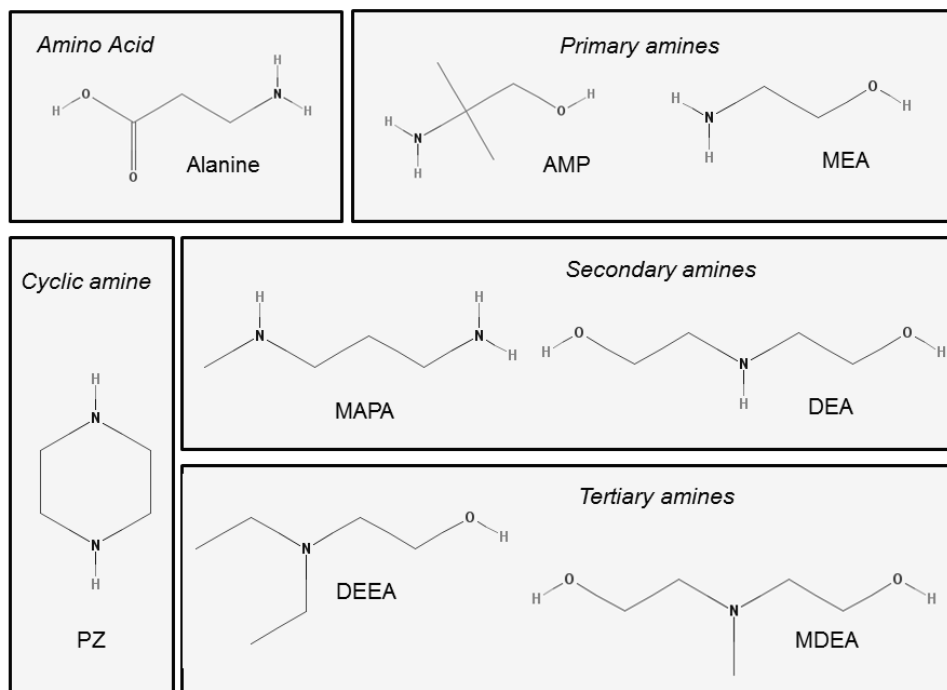


Figure 1 Structures of the tested amines (Kim et al., 2016), full names are given in Table 1.

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

Compound	Abbreviation	CAS	Formula	Conc. (g/L)
2-aminoethanol	MEA	141-43-5	C ₂ H ₇ NO	586.6
<i>N</i> -(2-Hydroxyethyl)glycine	HEGly	5835-28-9	C ₄ H ₉ NO ₃	42.3
2-Hydroxyethylformamide	HEF	693-06-1	C ₃ H ₇ NO ₂	28.1
4-(2-Hydroxyethyl) piperazine-2-one	HEPO	23936-04-1	C ₆ H ₁₂ N ₂ O ₂	12.04
1-(2-Hydroxyethyl)imidazole	HEI	1615-14-1	C ₅ H ₈ N ₂ O	10.5
Ammonia		7664-41-7	NH ₃	8.8
(2-Hydroxyethyl)-acetamide	HEA	142-26-7	C ₄ H ₉ NO ₂	8.2
Nitrate		84145-82-4	NO ₃ ⁻	7.5
<i>N</i> -(2-hydroxyethyl)ethylenediamine	HEEDA	111-41-1	C ₄ H ₁₂ N ₂ O	4.03
<i>N,N</i> -Bis(2-hydroxyethyl)oxamide	BHEOX	1871-89-2	C ₆ H ₁₂ N ₂ O ₄	0.06
Nitrite		14797-65-0	NO ₂ ⁻	0.046

2.2. Aerobic Biodegradability test (BOD Test)

A standard fresh water aerobic biodegradation test was performed according to OECD guideline 301 D for testing of chemicals, closed bottle test (OECD, 1992). Surface water was used as microbial inoculum and collected from two unpolluted water sources close to Trondheim, the forest lakes Haukvannet and Theisendammen. Waters were mixed in equal volumes and matured by circulation through an aquarium pump for 5-7 days at room 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). Essential nutrients for the bacteria as well as 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 $20 \pm 2^\circ\text{C}$. 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-adapted 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. NO₃-N was added in excess (110 mg/L), yielding a NO₃-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 N₂, determined by an O₂ 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. (2016).

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.

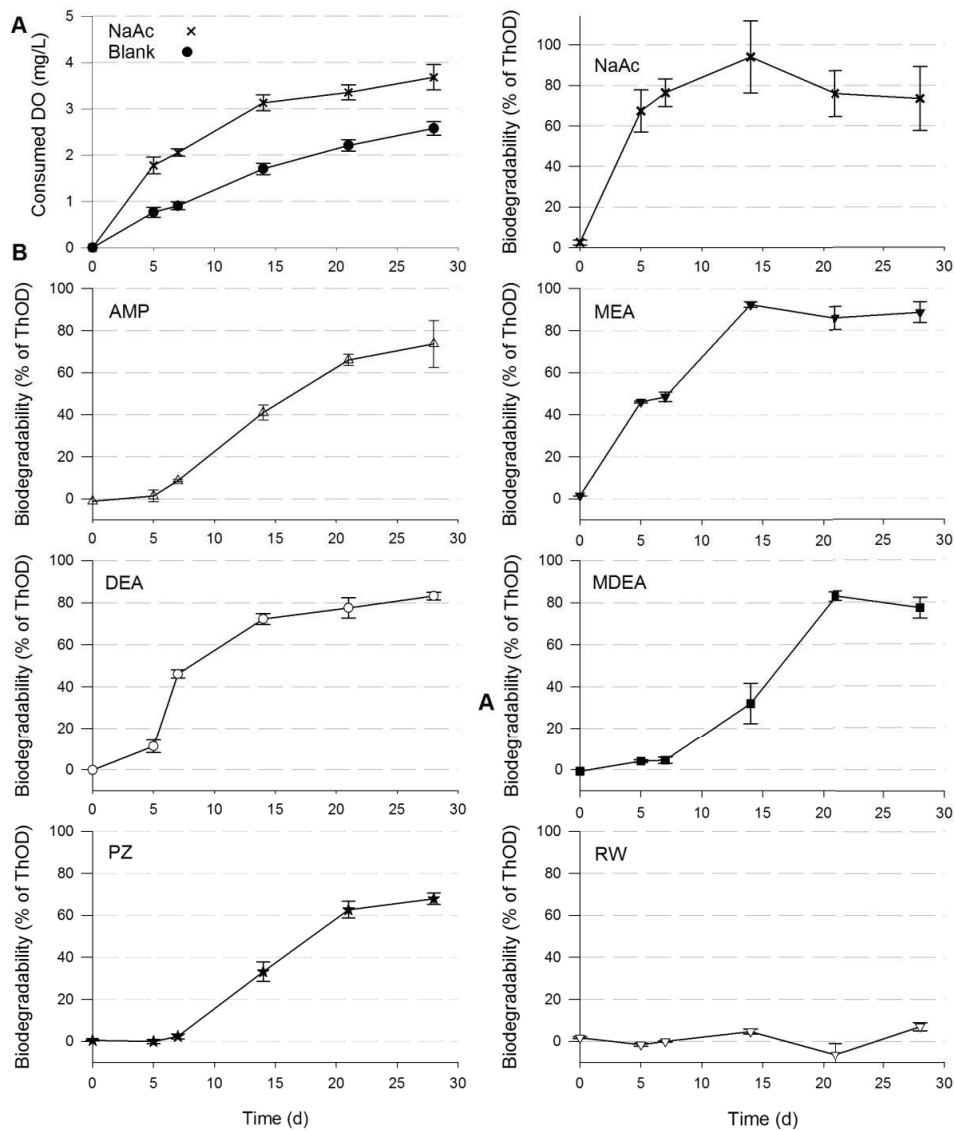


Figure 2 A) Consumed oxygen levels in sodium acetate (x) and blanks (+) during the BOD testing and B) biodegradation of sodium acetate in fresh water given as BOD (% of ThOD) as a function of time. Error bars indicate the SEM of 4 replicates B) Biodegradation of amines in fresh water. The calculated BOD values are corrected for the blank values. AMP (Δ), 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	<i>K</i>	Half-life (d)		Ultimate (BOD) (%)	
		Fresh water	Sea water ^a	Fresh water	Sea water ^{a,b}
AMP	0.0468	14.8	>700	96.5	<1 ^b
MEA	0.114	6.1	8,3	88.4	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. β -Proteobacteria is one important freshwater

group that is noticeably absent in marine environments (Méthé et al., 1998). In a recent study, high abundance of β -Proteobacteria has been positively correlated with hydrocarbon degradation in soils (Bell et al., 2013). In marine biodegradation of DEA, phylogenetic analyses indicated that γ -Proteobacteria became abundant during the experiment, however, strains growing on DEA or MEA could not be cultivated for gene expression studies during alkanolamine biodegradation (Brakstad et al., 2012).

In general, ultimate biodegradability, as determined by BOD may be useful for assessing rapid direct biodegradability of amines in natural ecosystems. However, in an engineered system, such as in the case of biological N removal, these results must be reconsidered.

First, the microbial community of surface waters depends strongly on geographical and seasonal variations, as well as the experimental procedure to obtain the inoculum. This might be directly reflected in the apparent degree of biodegradability. If the substance is not biodegraded, this actually just shows the possibly accidental absence of the required bacteria in the chosen inoculum (Grady, 1984). Furthermore, Grady (1984) argues the BOD testing conditions are too stringent for several reasons; using the compound as a sole carbon and energy source excludes co-metabolism, the small single inoculum limits the genetic capability for degradation, and the relatively short testing time forces acclimation to be the only mechanism. This results in a bias towards only readily biodegradable compounds giving a positive result (Grady, 1984).

Secondly, the weight based recommended concentration of 2-5 mg/L makes it difficult to compare biodegradability of one substance with anaerobic respiration based on other electron 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., 2016). 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., 2016). 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. (2016). 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

secondary amines, DEA and MAPA had a lag phase of approximately 10 days, but thereafter, the gas production increased steadily. As could be expected for such labile systems, out of 8 replicates, we observed 2 and 3 completely inactive syringes respectively. To give a better picture of the results, we calculated the average of active and inactive syringes separately, as illustrated in Figure 4. The tertiary amines MDEA and DEEA, as well as the cyclic amine piperazine did not show any measurable gas production. Reclaimer waste gave less gas production as MEA did, which might be due to the lower concentration of MEA in the reclaimer waste. See Figure 4. These findings highlight three possible categories of biodegradability under denitrification conditions. (i) Easily biodegradable, such as alanine, MEA and MEA based reclaimer waste. (ii) Slowly biodegradable after a lag phase, such as DEA and MAPA; and (iii) difficult, such as AMP, DEEA, MDEA, and piperazine.

We have already verified the rapid biodegradation under denitrifying conditions for MEA and MEA based reclaimer waste, serving as a sole carbon source for biological nitrogen removal in a pre-denitrification system. Future works should include the verification of the biodegradability after a lag phase for DEA and MAPA.

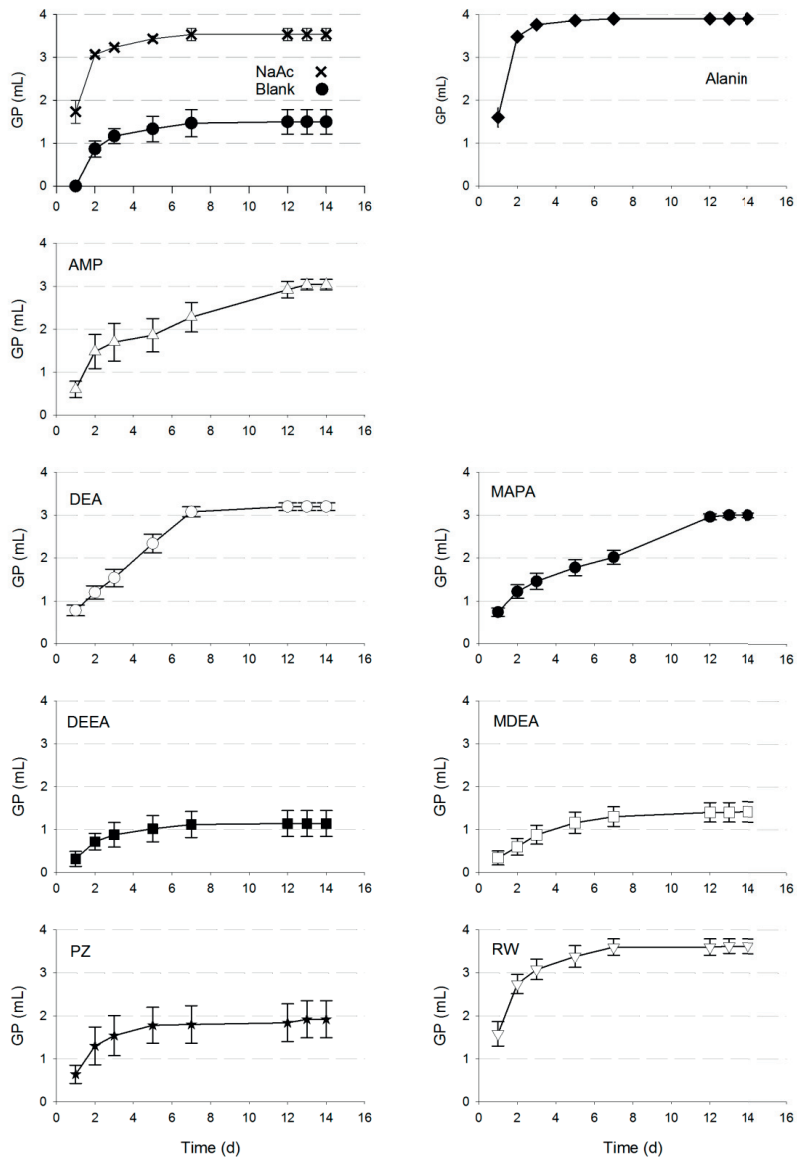


Figure 3 Anoxic Biodegradability, Experiment A. Average gas production (GP) in mL of amines under denitrifying conditions as a function of time. Positive control sodium acetate (x), Blank

(+), alanine (◆), AMP (△), MEA (not done), DEA (○), MAPA (●), MDEA (■), DEEA (□), piperazine (★) and reclaimer waste (▽). Error bars indicate the SEM of 5 replicates.

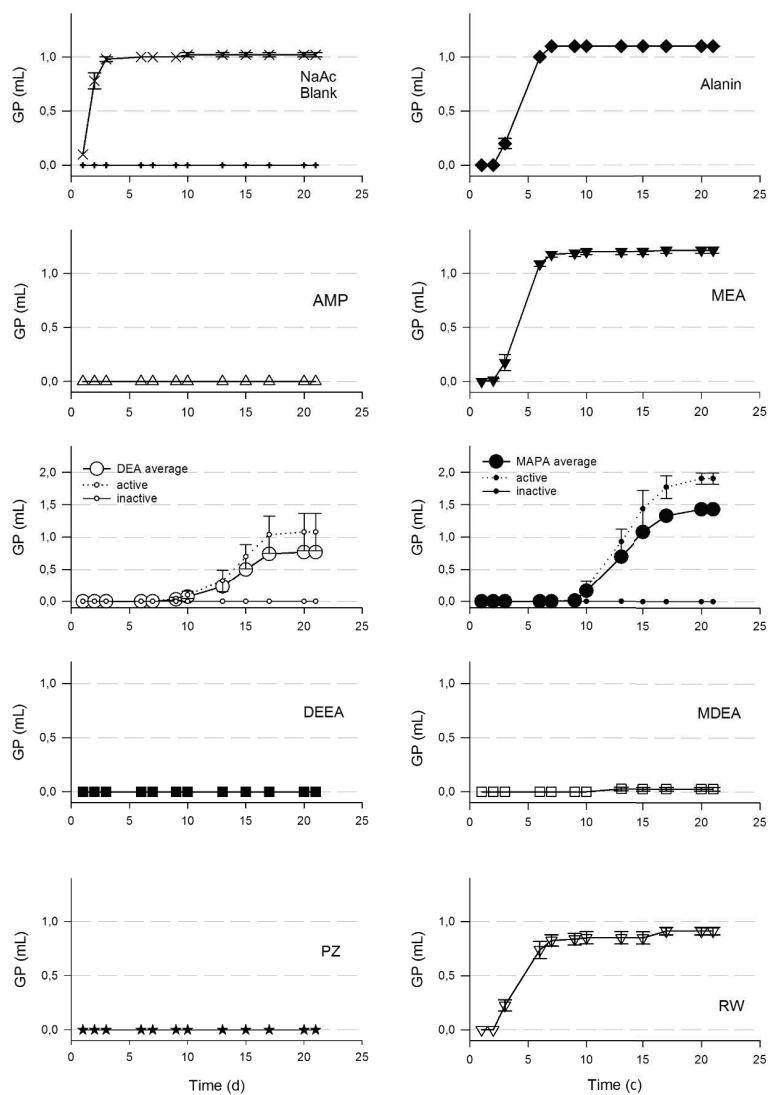


Figure 4 Anoxic Biodegradability, Experiment B. Average gas production (GP) in mL of amines under denitrifying conditions as a function of time. Positive control sodium acetate (×), Blank

(+), 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.

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 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 ultimate BOD value of 3 % would exclude any attempt of biological nitrogen removal, but under denitrifying conditions, MEA based reclaimer waste was rapidly degraded in the syringe test, as

well as in continuous pre-denitrification systems (Hauser et al., 2013b). Oppositely, AMP was rapidly degraded under aerobic conditions, but could not be utilized as carbon source under denitrifying conditions in the syringe test. This is in agreement with preliminary pilot studies of AMP in a continuous pre-denitrification reactor system (results not included). Our findings highlight the importance of considering the appropriate inoculum before assessing the biodegradability of amines in engineered ecosystems.

With the anoxic syringe test, we present a simple method to predict the biodegradability of amines used in CCS under denitrifying conditions. For future solvent evaluation, this screening method offers a rapid and low cost method, compared to the conventional BOD testing.

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Appendix A. Supplementary data

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

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Paper IV

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Paper V

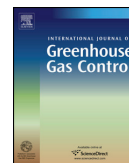
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Paper VI



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Inhibition factors in biofilm N removal systems treating wastes generated by amine based CO₂ capture



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ABSTRACT

We have previously shown that biological nitrogen removal by pre-denitrification as illustrated may be a feasible approach for treating wastes generated by amine based CO₂ capture. In order to identify limiting factors for successful up-scaling, we first compared the nitrifying activity of moving bed biofilm reactors (MBBR) with or without chronic exposure to organic loading in the form of acetate while monitoring population dynamics in the biofilms by pyro-sequencing. Our results show that the long-term abundance of heterotrophic bacteria is an essential factor in inhibition of nitrification efficiency. Secondly, the inhibition potential of the commonly applied amines monoethanolamine (MEA), 2-amino-2-methyl-1-propanol (AMP), diethanolamine (DEA), methyl-diethanolamine (MDEA), piperazine (Pip), as well as MEA based reclaimer waste (RW) were tested on separate nitrifying and denitrifying MBBRs. Results show that nitrification was inhibited by 50% at EC₅₀ concentrations from 9 to 120 mM, whereas denitrification was stimulated by all compounds at concentrations up to 100 mM. Nitrifying biofilms long-term adapted to organic loadings were 5–20 times more sensitive towards inhibition than those maintained without organic feeding, by both MEA and by organic loading. The crucial factor for the total process is therefore maintaining nitrification by avoiding overloading of amines or other organics in the second reactor.

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1. Introduction

Carbon capture and storage (CCS) is increasingly receiving attention as a measure to mitigate greenhouse gas emissions. In post combustion CO₂ capture, the most commonly applied scrubbing solvent is still monoethanolamine (MEA), but recently a full scale plant was set in operation at Boundary Dam, Canada, based on a mixed diamine system (Stéphenne, 2014). Many different blended systems have also been suggested, among them phase change systems (Pinto et al., 2014). For medium to high pressure applications promoted methyl-diethanolamine (MDEA) and sterically hindered and cyclic amines such as 2-amino-2-methyl-1-propanol (AMP), often mixed with piperazine (Pip) are applied for CO₂ capture.

In such amine based CO₂ capture plants, the aqueous amine solution is subject to oxidative and thermal degradation, carbamate polymerization, as well as side reactions with flue gas impurities

(Gouedard et al., 2012; Bello and Idem, 2005; Goff and Rochelle, 2004; Lepaumier et al., 2011a,b; Supap et al., 2011; Davis and Rochelle, 2009; Vevelstad et al., 2011, 2013, 2014; Fredriksen and Jens, 2013). In order to keep the capture capacity up, high molecular weight degradation products and heat stable salts are separated from the intact solvent in a reclaimer unit and removed as so called 'reclaimer waste' (Goff and Rochelle, 2004; Wang et al., 2015). The resulting degradation products vary among the amines in quality, quantity and toxicity (Fredriksen and Jens, 2013; Eide-Haugmo et al., 2009; Rohr et al., 2013; McDonald et al., 2014). MEA-based waste composition and treatment options were recently reviewed by Nurrokhmah et al. (2013a) and Nurrokhmah et al. (2013b), concluding secondary biological treatment to be the most economical option (Nurrokhmah et al., 2013a). Despite the fact that various amines applied in CCS have their distinct properties, they all end up as waste containing ammonia as well as more or less toxic nitrogenous organic degradation products.

Biological nitrogen removal is based on the sequential aerobic microbial oxidation of ammonia to nitrate, followed by anoxic reduction of nitrate to inert molecular nitrogen, denoted nitrification and denitrification, respectively (Zhu et al., 2008). Both microbial consortia have widely different substrate requirements as well as growth kinetics. Nitrification is facilitated by two groups

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of chemolithoautotrophic bacteria – the ammonia oxidizing bacteria (AOB) and the nitrite oxidizing bacteria (NOB). Contrary to these bacteria, the denitrifying bacteria are heterotrophic and may grow much faster. The nitrification step may thus be less robust, both because the nitrifiers are more sensitive towards environmental changes and require longer periods to recover from operational disturbances due to their slow growth rates (Wagner and Loy, 2002).

Our recent studies on MEA (Hauser et al., 2013a) and MEA based reclaimer waste (Hauser et al., 2013b) have proven the feasibility of applying biological nitrogen removal (BNR) on such waste streams at a lab-scale (Hauser et al., 2014). In our applied pre-denitrification set-up, the waste is first treated in the anoxic denitrification reactor and then enters the aerobic nitrification reactor (see TOC graphic). All organic matter which is biodegradable under denitrification conditions may be oxidized in the first step, and most of MEA is hydrolyzed to ammonia with acetaldehyde as the first organic intermediate (Ndegwa et al., 2004). This effluent is then led to the aerobic nitrification reactor, where the influent ammonia is oxidized via nitrite to nitrate. MEA and other organics still present may be oxidized by oxygen and further degraded, stimulating heterotrophs also in this reactor step. The average MEA and chemical oxygen demand (COD) removal achieved were 98 ± 1 and $71 \pm 1\%$, respectively (Hauser et al., 2013b).

In moving bed biofilm reactors (MBBR), the bacteria grow as a biofilm on polyethylene carriers (Rusten et al., 2006). It has been shown that the nitrifying biofilm is stratified, whereas the NOB are located in the inner part of the biofilm and the AOB are closely associated with heterotrophic bacteria throughout the biofilm (Okabe et al., 1999). This is advantageous for the nitrifying bacteria in terms of shear stress protection, but on the other hand this stratification can lead to suffocation of the inner located bacteria due to oxygen limitation. This competition for space between heterotrophic and nitrifying autotrophic bacteria in the diffusive gradient of biofilms has been well documented (Nogueira et al., 2002; Vogelsang et al., 2002). Especially, in presence of an external carbon source, the fast growing heterotrophic bacteria on the surface may outcompete the slow growing autotrophic for oxygen and space. This factor must be considered when evaluating the treatment of amines and amine based reclaimer waste by biofilm reactors. As the amine represents an available carbon source for heterotrophs, it might significantly change the composition of the biofilm, depending on availability and toxicity of the amine.

Although several studies on amines used in CCS address the biodegradability (Eide-Haugmo et al., 2009) and potential toxic effect on humans (McDonald et al., 2014; Rohr et al., 2013), none of them assess the inhibiting effect of amines on bacteria, as involved in nitrification or denitrification. This information is essential to model process parameters in detail and consequently develop cost efficient waste treatment systems with high operational stability.

The main scope of this study is to identify the most essential inhibitory factors relevant for an up-scaled process. Therefore, three moving bed biofilm reactors (MBBR) were operated for 48 days in continuous mode. Reactor 1 was run as a control to confirm process stability, while chronic exposure to organic loading was tested in duplicate in order to verify reproducibility. To study inhibition and the effect on the relative abundance of nitrifying bacteria within the nitrifying biofilm over time, the bacterial communities were characterized by pyrosequencing of 16S rDNA amplicons. The sequence data were used for determining the relative abundance of ammonia and nitrite oxidizing bacteria.

Furthermore, inhibition tests of selected amines and organic loading (sodium acetate) were performed in batch tests to determine the EC_{50} (effect concentration where the activity reaches 50%) on the rate-limiting nitrifying step, as well as on the denitrifying biofilm. Our choice of amines represents a primary (MEA), secondary (DEA), tertiary (MDEA), sterically hindered (AMP)

and heterocyclic amine (piperazine). Additionally, the effect of increased heterotrophic bacteria within the nitrifying biofilm was verified by re-testing MEA toxicity after long-term exposure to sodium acetate.

2. Material and methods

2.1. Experimental set-up

3 1 L (ht: 15 cm, diameter: 9 cm) moving bed reactors made of glass, with water-jackets (connected to a Cole-Parmer Polystat water bath set to 25 °C) were set-up in continuous flow. All reactors were mechanically mixed at a speed of 250 rpm. The influent was fed with a peristaltic pump at a hydraulic retention time (HRT) of 6 h. Air was supplied through a sparger and an O₂ electrode (Oxi 3315, WTW) was used to measure the dissolved O₂ level at every sampling. The pH was controlled by a Consort Controller R301 (reactors 1 and 2) or (Ingold pH amplifier) (reactor 3) and adjusted by automatic addition of 0.5 M and 0.3 M HCl or NaOH, respectively. To avoid high salinity, pH ranges were set to 7.3–7.8 for the nitrification and 6.8–7.3 for the denitrifying reactor.

2.2. Inocula and media

The biofilm was grown on polyethylene carriers (Standard AnoxKaldnes K1; Rusten et al., 2006). Inoculum for the nitrifying biofilm originated from a municipal wastewater treatment plant in Trondheim and enriched under nitrifying conditions until steady state. The denitrifying biofilm was inoculated with sludge from the same facility without a previous enrichment process. Both biofilms had previously been exposed to MEA (Hauser et al., 2013a) and MEA based reclaimer waste (Hauser et al., 2013b). For the chronic exposure to organic loading, reactors 1 and 2 were inoculated with biofilm used to treat MEA based reclaimer waste (Hauser et al., 2013b) and reactor 3 to biofilm which had been short-term exposed to MEA. The basal medium composition for nitrification was as follows (g/L): (NH₄)₂SO₄, 0.236; K₂HPO₄, 0.4; NaHCO₃, 1.0; 10 mL/L trace metal solution containing (g/L): MgSO₄·7H₂O, 2.5; CaCl₂·2H₂O, 1.5; FeCl₂·4H₂O, 0.2; MnCl₂·2H₂O, 0.55; ZnCl₂, 0.068; CoCl₂·6H₂O, 0.12; NiCl₂·6H₂O, 0.12; and EDTA, 2.8; 0.384 g/L sodium acetate anhydrous, equivalent to 300 mg/L COD was added to reactors 2 and 3 from day 11 onwards. Reactor 1 was used as a control receiving no organic loading. For the inhibition tests under denitrifying conditions, the basal medium was prepared in deaerated water, containing (g/L): K₂HPO₄, 0.533; NH₄Cl, 0.253; KNO₃, 4.0; yeast extract, 0.05; ethanol, 1.0; 10 mL/L trace metal solution as described above.

2.3. Chemical analyses

Samples were taken every 2–3 days with syringes (BD Plastipak) and filtered with 0.45 μm filters (Sarstedt) to remove suspended biomass. All concentrations of NH₄⁺-N, NO₃⁻-N, NO₂⁻-N and chemical O₂ demand (COD) were determined with assays from Hach-Lange for water quality (Hach Lange, Germany), procedures were carried out according to manufacturers' instructions.

2.4. Analyses of microbial community

Standardized biofilm samples were collected by picking one MBBR carrier with forceps from each of the 3 reactors every 3–5 days, and the samples immediately frozen at -20 °C without any buffer or pretreatment. DNA was extracted by using a Power Soil DNA Isolation Kit (MO BIO Laboratories, California), and the 16S v4 region was amplified by a semi-nested PCR protocol for pyrosequencing (Vik et al., 2013).

The primers 329F (5'-ACKGBCCARACWCCTACG-3') and 802R (5'-TACCRGGGTATCTAAKCCYGT-3') were used for the external PCR. For the internal PCR cycles, fusion primers were used. They included the 16S targeting sequences (518F: 5'-CAGCAGCCGGTAAKAC-3' and 802R as the forward and reverse primer, respectively) for amplifying the v4 region and the adaptors A and B for the 454 pyrosequencing (forward and reverse primer, respectively). Both the forward and reverse PCR primers included one of the Multiplex Identifier (MID) sequences MID15 to MID 38 (Roche, TCB No. 005-2009). For both external and internal amplification, the reactions were run for 22 cycles (98 °C 15 s, 50/53 °C (for external and internal PCR, respectively) 20 s, 72 °C 20 s) with 0.6 μM of each primer, 200 μM of each dNTP, 2 mM MgCl₂, Phusion Hot Start II High-Fidelity DNA polymerase and reaction buffer from Thermo Scientific Finnzymes. PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega). An equimolar amplicon library was generated using the SequalPrep Normalization Plates (96) (Invitrogen). Finally, the pooled amplicon sample was concentrated using Amicon Ultra 0.5 mL 30 K spin columns (Millipore) and sequenced on 1/4 of a 454 plate with a GS FLX instrument at the Norwegian Sequencing Centre (<http://www.sequencing.uio.no>). The resulting pyrosequencing data were deposited at the European Nucleotide Archive (study accession number PRJEB11630).

2.5. DNA sequence data processing and statistical analysis

The sequence data were processed using the QIIME pipeline version 1.8.0 (Caporaso et al., 2010). Low quality reads were removed in an initial quality filtering, so that the remaining sequences had a length of at least 200bp, a minimum quality score of 25, and no ambiguous bases in the primer sequence. The sequences were de-noised using the Denoiser.py (Reeder and Knight, 2010) with default parameters. UCHIME was used for identifying chimeric sequences (Edgar, 2010). After quality trimming and chimera removal, the sequence reads were clustered to generate operational taxonomic units (OTUs) at 97% sequence similarity. The open-reference OTU picking workflow in Qiime was employed with the Greengenes core data set gg.13.5.fasta for OTU picking and taxonomic assignments. The data set was examined for known AOB and NOB taxa: Nitrosomonadaceae, Nitrospira, Nitrobacter, Nitrotoga, Nitrococcus, and Nitrospina (Isobe and Ohte, 2014; Monteiro et al., 2014). Relative abundances of AOB and NOB were determined as % of the total reads. Statistical analyses were done with SigmaPlot 12.5 (Systat Software, San Jose, CA, USA, www.sigmaplot.com).

2.6. Acute inhibition test

In this batch test, 100 mL of well developed biofilm carriers of either nitrifying or denitrifying type were exposed to increasing concentrations ranging from 0 to 316 mM of the test substance to determine the EC₅₀ value. The nitrifying or denitrifying reactors were filled with basal medium containing either 50 mg/L NH₄⁺-N or 533 mg/L NO₃-N, respectively. Samples were taken every 30 min over a time period of 3 h, filtered with 0.45 μm filters and analyzed for NO₃-N. After 3 h, the reactors were drained and the next test concentration was added. Following this procedure, the biofilm was exposed to a series of 0, 3.16, 10, 31.6, 100 and 316 mM of test substance. The reclaim water was obtained from the Aker Clean Carbon's mobile test capture facility in Longannet in Scotland (UK), while performing tests on a coal-fired power plant during a campaign with MEA in 2009. All other tested chemicals were analytical grade. All test solutions were prepared in the respective basal medium for nitrification or denitrification, and pH adjusted to 7.5 with 6 M HCl. After the last and highest concentration, the biofilm was rinsed with tap water and transferred into basal medium

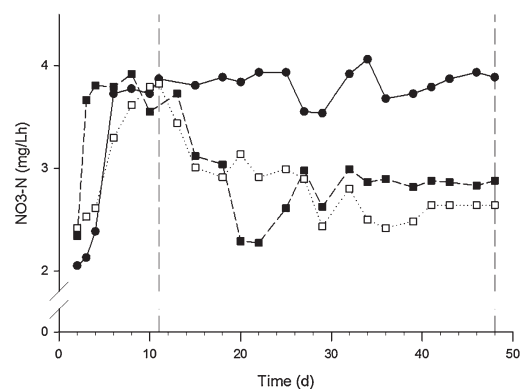


Fig. 1. Nitrification activity given as NO₃-N production per hour. Closed circles (●) represent reactor 1 without organic loading, squares show data from reactor 2 (■) and 3 (□) receiving 300 mg/L COD in the feed from day 11 onwards. Inhibition tests of sodium acetate were performed on day 11 and day 48, indicated by dashed lines.

(reactor 1) or basal medium with sodium acetate (reactors 2 and 3). EC₅₀ values were estimated with 'REGTOX', a Microsoft Excel macro for non-linear regression analysis (Vindimian et al., 1983).

3. Results and discussion

3.1. Impact of chronic organic loading on nitrification

All 3 nitrifying reactors were started as batch reactors and switched to continuous operation after day 1 (HRT = 6 h), as complete conversion from ammonia to nitrate occurred with only minor accumulation of nitrite (max. 9.7 mg/L). The initial nitrification activity was similar in all 3 reactors; detailed data are given in Fig. 1 and Table S1 in Supplementary material.

After 11 days continuous nitrification, organic loading to reactors 2 and 3 started, corresponding to a C/N-ratio of 2.2. The ammonia removal dropped in both reactors, as illustrated in Fig. 1. However, reactor 1 remained stable while running on pure nitrification. After day 18, all reactors gradually stabilized, at average removal efficiencies of 83 ± 4% and 79 ± 6% of the ammonia loading for reactors 2 and 3, respectively.

Our observed inhibition of nitrification by organic loading at 20% is similar to the 10% loss of ammonium removal by Nogueira et al. (2002) or 50% by Ballinger et al. (2002). It is evident that this inhibition effect will be concentration dependent. In artificial biofilms consisting of gel entrapped nitrifiers, we observed complete inhibition when exposed to high loadings of sodium acetate (data not shown). However, this inhibition was clearly reversible, and 25–50% of the original nitrification activity was recovered within a week after the organic shock loading.

The quantitative inhibition depends on many factors, such as the loading rate, oxygen level, the pre-history of the biofilm, as well as its structure and composition. Furthermore, it has previously been shown that the initial microbial community as well as the type of carbon source influence the population dynamics of autotrophic and heterotrophic bacteria in a biofilm, thereby affecting the nitrification performance (Okabe et al., 1996). The dissolved oxygen level remained relatively constant in all reactors, ranging from 5.9 to 6.7 mg/L DO, indicating sufficient excess of oxygen in the bulk medium. However, in a biofilm oxygen limitation may be caused by the diffusion resistance of the outer boundary layer as well as the internal biofilm diffusion (Chen et al., 2006). Micro-electrode recordings have shown that in a nitrifying biofilm, the oxygen concentration within the biofilm fell rapidly below 2 mg/L,

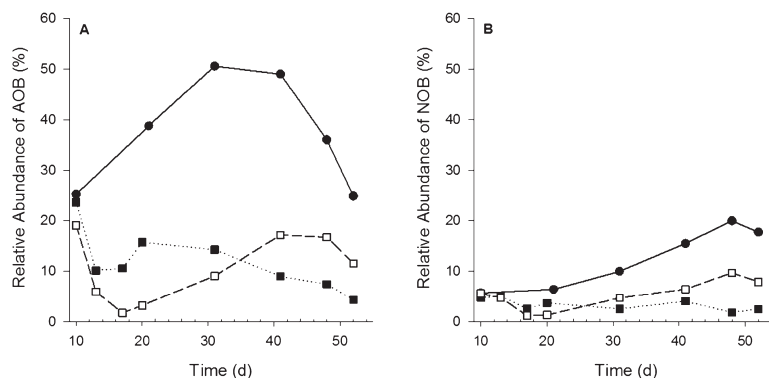


Fig. 2. Relative abundance (%) of sequence reads representing AOB (A) and NOB (B) as identified by 454-pyrosequencing. Closed circles (●) represent reactor 1 without organic loading, squares show data from reactor 2 (■) and 3 (□) receiving 300 mg/L COD in the feed from day 11 onwards.

the general half saturation constant of nitrifiers, even though the bulk solution had a high DO level (Chen et al., 2006). Therefore, even if the bulk solution has a high DO level, oxygen can become limiting for nitrification within the biofilm.

3.2. Relative abundance of nitrifiers during chronic organic loading

Bacterial communities were characterized for all 3 nitrifying MBBRs during chronic exposure of reactors 2 and 3 to organic loading, starting at day 11. After denoising and chimera removal, a total of 32673, 33171, and 34242 sequence reads were obtained for the R1, R2, and R3 reactor samples, respectively, corresponding to an average of 4539 ± 1082 sequence reads per sample. In total 29 OTUs were classified as Nitrosomonadaceae (AOB). These AOB reads constituted up to 50% of the total reads for the control reactor samples (Fig. 2A). Of these, only two were classified at the genus level: one as *Nitrosomonas*, and one as *Nitrososphaera*. For NOBs, 6 OTUs were classified as *Nitrospira*, but generally only comprised minor parts of the total community (Fig. 2B). No *Nitrobacter* OTUs were identified. However, 19 OTUs, not classified at the genus level, were assigned to the family Bradyrhizobiaceae, which includes *Nitrobacter*. We therefore included the Bradyrhizobiaceae reads when determining the ratios of NOB in the sampled communities as a rough but achievable estimate to illustrate relative community changes over time.

Fig. 2 shows the relative abundance of sequence reads representing nitrifying bacteria (AOB and NOB) from day 11 until day 52 during the experiment. After 1 week exposure to organic loading (day 18), there appeared to be significantly less nitrifying bacteria in both the treated nitrifying MBBR ($P < 0.001$, One Way ANOVA) compared to the control reactor receiving no organic loading.

3.3. Acute inhibition of organic loading on nitrification

To determine changes in acute sensitivity towards chronic exposure to organic loading on the nitrification activity, EC_{50} values were determined prior to day 11 and after chronic exposure to sodium acetate (day 48) on the nitrifying reactors described above. For comparison, the control reactor was tested at day 48 for acute inhibition of sodium acetate to document long-term stability towards organic loading.

During this test, the biofilm was exposed to increasing concentrations of the test substance and each concentration monitored for 3 h. One set of typical results is illustrated in Fig. 3, showing the

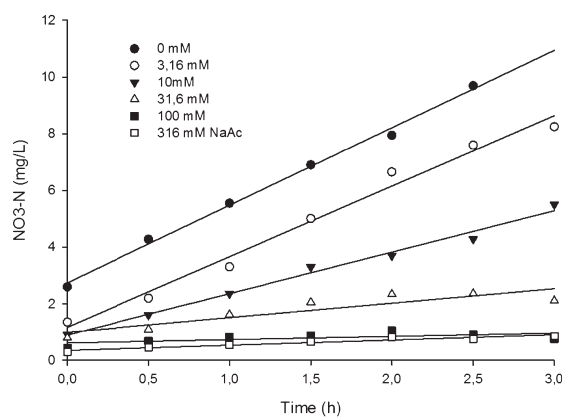


Fig. 3. Example of the inhibition test, case showing the effect of sodium acetate on the nitrifying biofilm after chronic exposure to organic loading at day 48. Please note that linearity was documented for 3 h as illustrated in each case before transfer to the next and higher dose of the test series.

decreasing nitrate production with increasing concentration of test substance. The slope of each concentration was used for generating the dose response curve; whereas the initial slope (0 mM) is set as the reference value of 100% (Fig. 4).

The EC_{50} value of sodium acetate for the control, highly enriched nitrification at day 48 was estimated to be 311 mM, which is close to the other reactors prior to chronic exposure, with an average EC_{50} value of 274 mM. After chronic organic exposure, however, the EC_{50} dropped to 15 mM sodium acetate (see Fig. 4). These results emphasize the negative impact of heterotrophic bacteria on the nitrifiers of the biofilm, resulting in an approximately 20-fold higher sensitivity towards acute organic shock after long-term moderate exposure to organic loading. Over time, we have found this type of biofilm pre-history to be the dominant factor in inducing apparent lack of reproducibility in acute toxic impact on biofilm activity.

3.4. Acute inhibition of amines on nitrification

To determine the acute inhibition of monoethanolamine (MEA) on the nitrification activity, EC_{50} values were determined just 96 h after the last acute inhibition test of organic loading on the

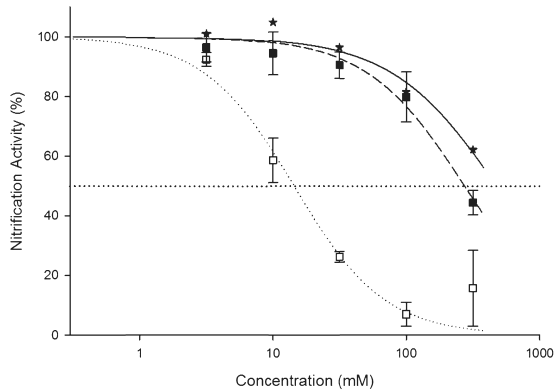


Fig. 4. Dose response curve of nitrifying biofilm exposed to sodium acetate. Squares represent average data from reactors 2 and 3 – closed squares (■) from the first inhibition test at day 11 prior to chronic exposure to organic loading (EC_{50} 274 mM), open squares (□) from the second inhibition test at day 48 after chronic exposure (EC_{50} 15 mM). Error bars indicate standard deviation of 2 reactors. Stars show data from the control reactor at day 48 (EC_{50} 311 mM).

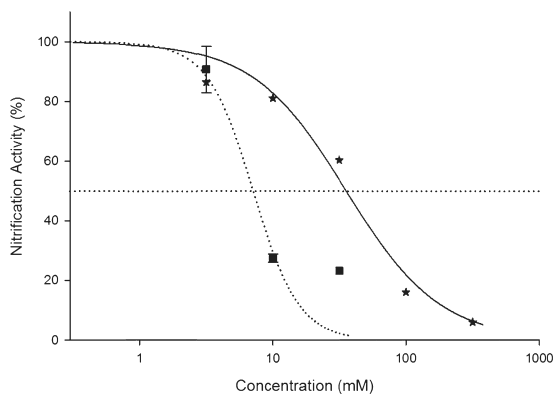


Fig. 5. Dose response curve of nitrifying biofilm exposed to monoethanolamine (MEA). Squares (■) represent average data from reactors chronically exposed to organic loading at day 52 after chronic exposure (EC_{50} 7 mM). Error bars indicate standard deviation of 2 reactors. Stars show data from the control reactor under pure nitrifying conditions at day 52 (EC_{50} 36 mM).

nitrifying reactors described above. In the time between the tests, reactors were operated in continuous mode as before, which is with and without organic loading. Thus, consecutive testing of MEA was performed on biofilms with bacterial composition as determined by sequencing. The highly enriched nitrifying control biofilm showed a much higher tolerance towards acute exposure to MEA (EC_{50} 36 mM) than both of the heterotrophic enriched biofilms did (EC_{50} 7 and 8 mM), see Fig. 5. This clearly illustrates that the biofilm composition is a crucial factor in the inhibition potential of amines. Due to the extreme organic shock loading ahead of testing, these data of MEA were not included in the following comparison of different amines, although the values fell within the expected range.

MEA, MEA based reclaimers (RW), as well as AMP, DEA, MDEA and piperazine were tested for their inhibition potential on nitrifying biofilms prior to the chronic exposure experiment. The EC_{50} values ranged from 9 to 118 mM (see Fig. 6). The corresponding dose response curves are given in the Supplementary material Figs. S1–S4. The variable response to acute inhibition in the replicates can largely be an effect of differences in the biofilm composition.

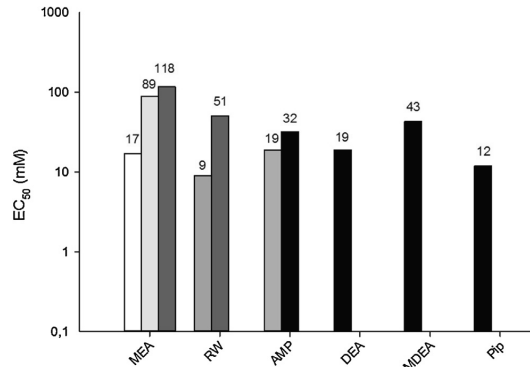


Fig. 6. EC_{50} values of the nitrifying biofilm for monoethanolamine (MEA), MEA based reclaimers (RW), and 2-amino-2-methyl-1-propanol (AMP), diethanolamine (DEA), methyldiethanolamine (MDEA) and piperazine (Pip). Independent replicate tests performed at intervals of 6–18 months are shown as bars, whereas the degree of shading indicates the chronological order of testing, with white as the first and black as the last experiment.

The first toxicity test of MEA (EC_{50} 17 mM) was conducted right after treating MEA in a post-denitrification set-up, where organics in the form of MEA entered the nitrifying reactor directly for 42 days (Hauser et al., 2013a). The consecutive tests were repeated while the nitrifying biofilm was operated under pure nitrification conditions with insignificant loadings of organic matter (Hauser et al., 2013a,b). As expected, the inhibitory effect was reduced as the heterotrophic content in the biofilm diminished over time, in the case of MEA the EC_{50} values ranged from 17 to 118 mM in these experiments, see Fig. 6.

Thus, it was confirmed that the increased inhibition of amines on the nitrifying biofilm activity was linked to the biofilm composition, as shown with sodium acetate as well as MEA as organic loading tested consecutively on the very same biofilm under defined operating conditions. When treating waste streams of these amines by biological nitrogen removal, the pre-denitrification configuration can prevent an organic overload of the autotrophic nitrification step, as long as the denitrification unit consumes most of the organics. We have previously reported that the amount of amine reaching the nitrification unit under steady state operations was generally low, with less than 14% for MEA (Hauser et al., 2013a) and 7% for the MEA based reclaimers (Hauser et al., 2013b).

3.5. Acute inhibition of amines on denitrification

DEA, MEA, MEA based reclaimers (RW), MDEA, piperazine and AMP (COD values of 1218 < 1309 < 1575 < 1746 < 1857 < 1975 mg/L, respectively), were tested for their inhibition potential on the denitrifying biofilm. As expected, this heterotrophic biofilm was more resistant to high loadings of amine waste and amines. The denitrification activity, assessed by nitrate consumption over time, in fact increased at low concentrations up to 100 mM of all tested substances, probably stimulated by the additional carbon source, see Fig. 7.

Only at the highest test concentration of 316 mM, AMP and DEA showed some inhibition, at 30% and 19%, respectively. Due to the stimulating effect of all tested amines, any EC_{50} values could not be determined. The increased denitrification activity was most pronounced with MEA based reclaimers, where the denitrifying biofilm reached almost 250% of the initial activity. This might be due to the small organic acids and other compounds found in the reclaimers (Nurrokhmah et al., 2013a), which present a

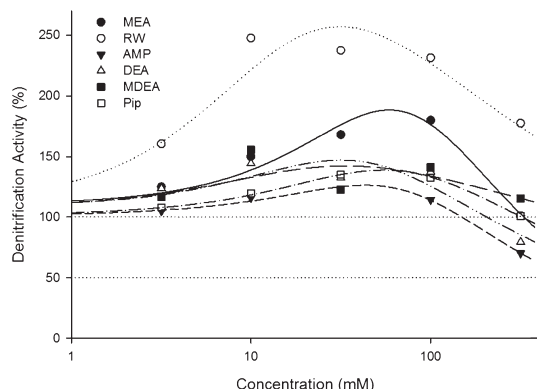


Fig. 7. Dose response curve of denitrifying biofilm exposed to monoethanolamine (MEA), MEA based reclaimer waste (RW), and 2-amino-2-methyl-1-propanol (AMP), diethanolamine (DEA), methyldiethanolamine (MDEA) and piperazine (Pip).

readily available carbon source. Biodegradation under anoxic conditions has also previously been reported for DEA, as well as for MDEA (Brakstad et al., 2012).

3.6. General aspects

Our results show that pre-denitrification should be the favourable method to apply biological nitrogen removal on amine based CCS waste for two reasons: First, MEA, MEA based reclaimer waste, MDEA and piperazine showed no denitrifying inhibition up to 316 mM in the initial denitrification step, whereas AMP and DEA gave just a slight inhibition at 316 mM. Secondly, the nitrification activity showed less sensitivity towards inhibition by amines the purer the autotrophic nitrifying biofilm was. This means that the more efficient the denitrification unit works, the more stable the post-nitrification treatment will operate. The contrasting effect of MEA and MEA based reclaimer waste on the nitrifying and denitrifying biofilms are summarized in Fig. 8. The similarities in graph A and B are striking, probably reflecting that undegraded MEA still was the dominant contaminant of this reclaimer waste (Hauser et al., 2013b).

However, operational disturbances may create conditions of accidentally increased organic loading reaching the aerobic post-nitrification step, leading to an immediate reduction in nitrate production for recycling. As long as this electron acceptor remains the limiting factor of the pre-denitrification, this type of negative feedback may ruin operational stability unless conditions are closely monitored and controlled by restricted feeding or addition of external electron acceptor. A computer model is under development to simulate such an operational regulation.

Our study presents the first attempt to elucidate the potential limiting factors for scaling up biological nitrogen removal of wastes generated by amine based CCS. By determining the EC_{50} values for both nitrifying and denitrifying biofilms, we could identify possible bottlenecks of the process. The heterotrophic consortia of denitrifying bacteria seem to cope very well even with high loadings of amines, as well as with MEA based reclaimer waste. However, our experiments do not allow us to fully deduce the actual biodegradability of the tested amines. For MEA and MEA based reclaimer waste, multiple corresponding studies have addressed the biodegradability under aerobic, anoxic and anaerobic conditions (Hauser et al., 2013a,b; Kim et al., 2010; Ndegwa et al., 2004; Ohtaguchi and Yokoyama, 1997; Wang et al., 2013). However, for AMP, DEA, MDEA and piperazine, data are scarce and biological

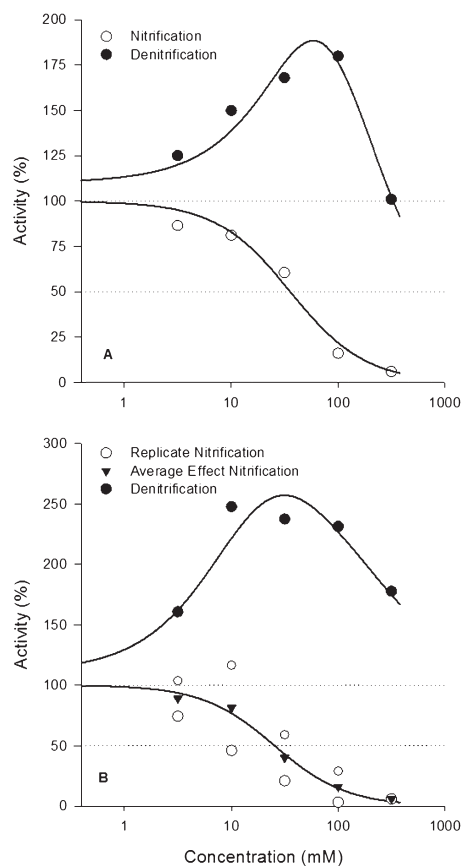


Fig. 8. Effect of MEA (A) and MEA based reclaimer waste (B) on nitrifying and denitrifying biofilm. Note differences in scaling of the activity.

nitrogen removal has not been reported yet. This knowledge gap needs to be closed for further evaluation. Furthermore, we have successfully shown how the composition of the biofilm may influence the sensitivity and stability of the nitrification process. These data are vital for applying the process of biological nitrogen removal on such waste streams.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijggc.2015.12.023.

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Contributions



STATEMENT FROM CO-AUTHOR

(cf. section 10.1 in the PhD regulations)

Ingrid Annemarie Henry applies to have the following thesis assessed:

Biological Nitrogen Removal of Effluents from Amine-based CO₂ Capture Plants – Screening, Feasibility and Limitations

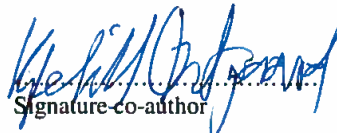
The statement is to describe the work process and the sharing of work and approve that the article may be used in the thesis.

Statement from co-author on article:

Hauser, I., Einbu, A., Svendsen, H. F. & Østgaard, K. 2014. **Biological treatment of effluents generated by amine based CO₂-capture plants**. Journal of Energy Challenges and Mechanics 1 (4), 1-5.

This paper was written by IH and edited by HS, AE and KØ. IH finalized the paper. This article may be used in the thesis.

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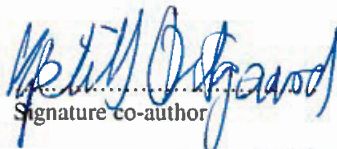

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Signature co-author

Statement from co-author on article:

Østgaard, K., Shuai, W., Henry, I. A., Sposob, M., Kowarz, V., Haugen, H. H. & Bakke, R. 2016. **Syringe test screening of microbial gas production activity: Cases denitrification and biogas formation**. Submitted to the Journal of Microbiological Methods.

The experimental work for the denitrification with different substrates and inocula in this paper was carried out by IH. IH was involved in the planning, in collecting various inocula, running the experiments, as well as analysing and interpreting the data. Experiments in the context of error sources were done by VK, while KØ, HS and AE contributed significantly to the planning of those experiments. All biogas experiments were planned and carried out by MS, WS, HHH and RB. This paper was written by KØ, edited by IH and all other co-authors. KØ finalized the paper. This article may be used in the thesis.

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Place, date


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Statement from co-author on article:

Henry, I. A., Kowarz, V. & Østgaard, K. 2016. **Aerobic and anoxic biodegradability of amines applied in CO₂-capture.** Submitted to the International Journal of Greenhouse Gas Control.

The experiments of this paper were planned by IH and KØ. IH was responsible for running the aerobic experiments with VK, while VK conducted the anoxic experiments during her Master Thesis under IHs guidance. The data was analysed by IH and interpreted with KØ with contributions from VK. The paper was written by IH, edited by KØ and finalized by IH. This article may be used in the thesis.

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Place, date

Kjetill Østgaard

Signature co-author

Statement from co-author on article:

Hauser, I., Colaço, A., Skjæran, J., Einbu, A., Østgaard, K., Svendsen, H. & Cervantes, F. 2013. **Biological N Removal from Wastes Generated from Amine-Based CO₂ Capture: Case Monoethanolamine.** Applied Biochemistry and Biotechnology 169, 1449-1458.

The experiments of this paper were planned by KØ, HS and AE. The experiments were run during the Master Thesis of AC and JAS, who conducted the experiments. IH was responsible for data analysis and data interpretation with FJC and KØ. IH was responsible for writing the manuscript, while FJC and KØ edited the paper. IH finalized the paper. This article may be used in the thesis.

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Place, date

Kjetill Østgaard

Signature co-author

Statement from co-author on article:

Hauser, I., Einbu, A., Østgaard, K., Svendsen, H. & Cervantes, F. 2013. **Biodegradation of amine waste generated from post-combustion CO₂ capture in a moving bed biofilm treatment system.** Biotechnology Letters 35, 219-224.

The experiments of this paper were planned by KØ, AE and HS. IH carried out the experimental work during her Master Thesis. IH was responsible for data analysis and data interpretation with FC and KØ. IH was responsible for writing the manuscript, while FC and KØ edited the paper. IH finalized the paper. This article may be used in the thesis.

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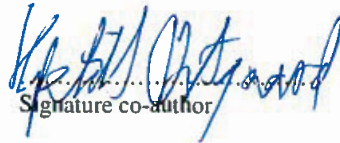
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Statement from co-author on article:

Henry, I. A., Einbu, A., Svendsen, H. F., Bakke, I. & Østgaard, K. 2016. **Inhibition factors in biofilm N removal systems treating wastes generated by amine based CO₂ capture.** International Journal of Greenhouse Gas Control 45, 200-206.

The experimental planning of this paper was done by IH, KØ and IB. Experiments were run by IH, whereof 2 toxicity tests of MEA were run by AC and one for AMP by JAS. IB was responsible for analysing the sequencing data. Other to that, IH was responsible for data analysis and interpretation with KØ and IB. IH wrote the manuscript, while HS, AE and KØ edited it. IH finalized the paper. This article may be used in the thesis.

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