Ingenieurfakultät Bau Geo Umwelt

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**- Biogenic Sulfuric Acid Corrosion in Sludge Digesters-**

**Characterization of the bacterial groups and the corrosion potential**

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„Gäbe es die letzte Minute nicht, so würde niemals etwas fertig.“

**Mark Twain**

# ABSTRACT

Biogenic sulfuric acid (BSA) corrosion, a serious and costly problem, usually affects sewerage infrastructure, but typical BSA damage patterns were also observed in the headspace of different full-scale digesters. Thus, this study aimed to verify BSA corrosion in digesters by (i) identifying the relevant bacteria participating in the concrete corrosion process, i.e. sulfate reducing and sulfur oxidizing bacteria (SRB and SOB), and (ii) analyzing the BSA corrosion potential. Additionally, chemical and microbiological sulfuric acid ($H_2SO_4$) experiments with both hardened cement paste representing a concrete binder and concrete specimens reflecting a digester wall were performed to better understand BSA corrosion.

First, digester sludge and biofilm samples from the concrete surface in the headspace of six corroded digesters were collected. SRB diversity within digester sludge was investigated by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) using the *dsrB* (dissimilatory sulfite reductase beta subunit) -gene. SOB analysis in biofilm samples was carried out by specific enrichment cultivation. PCR-DGGE was applied for in depth community composition analyses with the enriched cultures. BSA production of mixed and pure SOB cultures was tested under laboratory conditions and *in situ* by measuring the concrete sulfate content from the digester headspace and sludge zone. $H_2SO_4$ experiments with hardened cement paste and concrete at pH values 1.0 and 2.0 were performed over 28 days to analyze and compare concrete degradation to microbially generated $H_2SO_4$ (pH 1.3-2.4). Biogenic $H_2SO_4$ experiments were performed with *A. thiooxidans*, the key BSA producer originally isolated from digester headspace samples, for periods of 28 days, two, three and six months. To evaluate cement stone/concrete degradation, visual, physical (e.g. weight loss), and chemical parameters (e.g. neutralization depth) were applied using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDX).

Diversity studies revealed the presence of different uncultured SRB and a potential hydrogen sulfide ($H_2S$) production. Similar DGGE-profiles from the different sludge samples demonstrated that similar SRB species were present in the digesters. Cultivation yielded three pure SOB species originating from the digester headspace: *Acidithiobacillus thiooxidans*, *Thiomonas intermedia,* and *Thiomonas* perometabolis. The pure SOB species were also detected with PCR-DGGE in enriched mixed SOB cultures which showed a higher SOB diversity with additional acidophilic and neutrophilic SOB. Mixed SOB cultures achieved sulfate concentrations of 10-87 mmol/L after 6-21 days of

incubation (final pH 1.0-2.0), compared to 433 mmol/L after 42 days with pure *A. thiooxidans* cultures (final pH < 1.0), indicating a high BSA production potential. A significantly higher sulfate content (e.g. ten-times higher) in concrete specimens from the digester headspace compared to the sludge zone further indicated occurrence of *in situ* sulfur/sulfide oxidation.

In controlled biogenic corrosion experiments, *A. thiooxidans* produced high amounts of $H_2SO_4$ resulting in severe damage patterns on cement stone and concrete. Gypsum was identified as main corrosion product. Chemically and microbially produced $H_2SO_4$ resulted in similar corrosion patterns. The biogenic long-term experiments exhibited an increased deterioration over the first three months, but in the three- and six-month set-ups comparable damage patterns were observed. Concrete degradation primarily depended on $H_2SO_4$ production by *A. thiooxidans*, but no correlation with incubation periods was revealed. LA-ICP-MS and SEM/EDX proofed to be very suitable techniques to assess cement stone/concrete degradation. These techniques allowed elucidating the elemental distributions (e.g., C, Ca, Si, P, and S) in the corrosion layers and the corrosion products (e.g. gypsum). In particular, LA-ICP-MS analysis enabled a clear differentiation between corroded and non-corroded layers.

To conclude, the relevant SRB and SOB, responsible for the BSA process, were identified to be present in six different full-scale digesters. Their ability to produce $H_2SO_4$ and the significant corrosion potential of *A. thiooxidans* on both hardened cement paste and concrete samples revealed a high biogenic sulfuric acid corrosion potential in sludge digesters.

# ZUSAMMENFASSUNG

Biogene Schwefelsäure Korrosion (BSK) ist ein schwerwiegendes und kostspieliges Problem, das vor allem in der Abwasserkanalisation vorkommt. Das Auftreten von typischen BSK Schadensphänomen im Gasraum verschiedener Faulbehälter weist darauf hin, dass BSK auch eine Rolle in diesen Bauwerken spielen kann. Das Ziel dieser Arbeit war es, den BSK Prozess in Faulbehältern zu untersuchen. Diese Untersuchungen beinhalteten die Identifizierung der am Korrosionsprozess beteiligten Sulfat-reduzierenden und Schwefel-oxidierenden Bakterien (SRB und SOB) und die Analyse des Korrosionspotentials. Für ein besseres Verständnis des BSK Potentials im Faulbehälter wurden chemische und biogene Schwefelsäure ($H_2SO_4$) Versuche mit Zementstein, welcher als Bindemittel im Beton enthalten ist und Beton, das dominante Baumaterial von Faulbehältern, durchgeführt.

Zunächst wurden Faulschlamm- und Biofilmproben von der Betonoberfläche im Gasraum von sechs korrodierten Faulbehältern entnommen. Die SRB Diversität in den Faulschlammproben wurde mithilfe der Polymerase-Kettenreaktion und denaturierenden Gradienten-Gelelektrophorese (PCR-DGGE) unter Verwendung des *dsrB* (dissimilatorische Sulfit-Reduktase-beta-Untereinheit) -Gens untersucht. Die SOB wurden in spezifischen Flüssignährmedien angereichert und eine taxonomische Charakterisierung dieser Anreicherungskulturen erfolgte mithilfe der 16S rRNA PCR-DGGE und Sequenzanalyse. Unter Laborbedingungen wurde die Fähigkeit der SOB Misch- und Reinkulturen zur biogenen Schwefelsäureproduktion getestet. Des Weiteren wurde *in situ* der Sulfatgehalt von Betonproben aus dem Gasraum und der Schlammzone gemessen. Die chemischen $H_2SO_4$-Experimente mit Zementstein- und Beton wurden bei pH-Werten von 1,0 und 2,0 über einen Zeitraum von 28 Tagen durchgeführt, um den Korrosionsprozess zu analysieren und einen Vergleich zur biogen produzierten $H_2SO_4$ (pH 1,3 bis 2,4) herzustellen. Die biogenen $H_2SO_4$-Versuche wurden mit *A. thiooxidans* (isolierte Reinkultur aus dem Faulbehälter), dem Schlüsselorganismus der BSK, über einen Zeitraum von 28 Tagen, zwei, drei und sechs Monaten durchgeführt. Für die Charakterisierung der Zementstein- bzw. Betonkorrosion wurden die Proben optisch begutachtet und physikalische (z.B. Gewichtsverlust) und chemische Parameter (z.B. Neutralisationstiefe) bestimmt. Darüber hinaus wurden die Proben mithilfe der Laserablation induktiv gekoppelten Plasma-Massenspektrometrie (LA-ICP-MS) und Rasterelektronenmikroskopie (REM) mit energiedispersiver Röntgenspektroskopie (EDX) untersucht.

Die Diversitätsuntersuchungen identifizierten verschiedene nicht kultivierbare SRB in den Faulschlammproben, was auf ein Potential zur Schwefelwasserstoffproduktion ($H_2S$) hinweist. Vergleichbare DGGE-Profile in den verschiedenen Schlämmen zeigten, dass ähnliche SRB Spezies in den untersuchten Faulbehältern vorhanden waren. Mithilfe von kultivierungsbasierten Methoden wurden drei SOB Reinkulturen aus dem Faulbehälter Gasraum gewonnen: *Acidithiobacillus thiooxidans*, *Thiomonas intermedia* und *Thiomonas perometabolis*. Diese drei Bakterienarten wurden auch mit PCR-DGGE in den Mischkulturen nachgewiesen. Zusätzlich konnten noch weitere acidophile und neutrophile SOB identifiziert werden. Diese SOB Mischkulturen produzierten Sulfatkonzentrationen von 10-87 mmol/l nach 6-21 Inkubationstagen (finaler pH Wert: 1,0-2,0). In der *A. thiooxidans* Reinkultur wurden noch höhere Konzentrationen von bis zu 433 mmol/l nach 42 Tagen gemessen. Die Sulfatmessungen in den Betonproben aus dem Faulbehälter Gasraum zeigten höhere Werte (bis zu zehnmal so hoch) als die Betonproben der Schlammzone, was auf eine *in situ* Schwefeloxidation hinweist.

In den biogenen Korrosionsversuchen produzierte *A. thiooxidans* große Mengen an Schwefelsäure, welche zu starken Korrosionsschäden an Zementstein und Beton führte. Gips wurde als Hauptkorrosionsprodukt identifiziert. Die Korrosionsexperimente zeigten, dass sich vergleichbare Korrosionsschäden in Anwesenheit von chemisch und biogen erzeugter $H_2SO_4$ entwickeln. Bei den biogenen Langzeitversuchen wurde in den ersten drei Monaten ein Anstieg der Korrosion beobachtet, doch bei den drei- und sechs-Monate Versuchen wurden vergleichbare Schadensmuster beobachtet. Das Ausmaß der Korrosion war somit primär von der Schwefelsäureproduktion durch *A. thiooxidans* abhängig und korrelierte nicht mit den Inkubationszeiten. Die Korrosionsschäden konnten sehr zielgerichtet mithilfe von LA-ICP-MS und REM/EDX charakterisiert werden, da beide Methoden eine Analyse der Elementverteilung (z.B. C, Ca, Si, P und S) in den Korrosionsschichten sowie eine Identifizierung von Korrosionsprodukten ermöglichten. Darüber hinaus konnte mittels LA-ICP-MS zwischen korrodierten und nicht korrodierten Schichten unterschieden werden.

Schließlich konnten die am Korrosionsprozess beteiligten SRB und SOB in sechs verschiedenen Faulbehältern nachgewiesen werden. Ihre Fähigkeit zur $H_2SO_4$-Produktion und die großen Korrosionsschäden von *A. thiooxidans* auf Zementstein- und Betonproben deuten auf ein biogenes Schwefelsäure-Korrosionspotential in Faulbehältern hin.

# AFFIDAVIT

I hereby affirm that I wrote this PhD thesis independently and on my own without illegal assistance of third parties. To the best of my knowledge, all sources that I used to prepare that thesis are labeled as such. This thesis has not been received by any examination board, neither in this nor in a similar form.

Au i.d. Hallertau, June 20, 2016

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Bettina Huber

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# LIST OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| µ | micro |
| µg | microgram |
| AC | abiotic control |
| AD | anaerobic digestion |
| ASOB | acidophilic sulfur oxidizing bacteria |
| ATCC | American Type Culture Collection |
| BLAST | Basic Local Alignment Search Tool |
| BOD | biological oxygen demand |
| Bp | base pairs |
| BSA | biogenic sulfuric acid corrosion |
| $C_3A$ | calcium aluminate hydrate |
| CTAB | cetyltrimethylammonium bromide |
| d | days |
| DEV | Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung |
| Dg | digester |
| DGGE | denaturing gradient gel electrophoresis |
| DNA | deoxyribonucleic acid |
| DO | dissolved oxygen |
| DSMZ | Deutsche Sammlung von Mikroorganismen und Zellkulturen |
| *dsrB* | dissimilatory sulfite reductase beta-subunit |
| EDX | Energy dispersive X-ray spectroscopy |
| ENA | European Nucleotide Archive |
| g | centrifugal force |
| GGBS | ground granulated blast furnace slag |
| IC | ion exchange chromatography |
| ICP | inductively coupled plasma |
| L | liter |
| LA | laser ablation |
| m | milli |
| mg | milligram |
| MICC | microbial induced concrete corrosion |
| min | minute |
| mL | milliliter |
| mm | millimeter |

| | |
|---|---|
| MS | mass spectrometry |
| NSOB | neutrophilic sulfur oxidizing bacteria |
| OES | optical emission spectrometry |
| ORP | oxidation reduction potential |
| NC | negative control |
| PCR | polymerase chain reaction |
| PE | population equivalent |
| R | reactor |
| RNA | ribonucleic acid |
| rpm | revolutions per minute |
| RT | retention time |
| SDS | sodium dodecyl sulfate |
| SEM | scanning electron microscopy |
| SOB | sulfur/sulfide oxidizing bacteria |
| sp. | species |
| s | second |
| spp. | species pluralis |
| SRB | sulfur/sulfate reducing bacteria |
| SRT | sludge retention time |
| Tris | tris(hydroxymethyl)aminomethane |
| TUM | Technical University of Munich |
| UV | ultraviolet |
| vol% | volume percent |
| v/v | volume per volume |
| w/c | water to cement ratio |
| w/s | water to solid ratio |
| w/v | weight per volume |
| w/w | weight per weight |
| wt% | weight percent |
| WWTP | wastewater treatment plant |

# AUTHOR CONTRIBUTIONS

Chapters 4 to 7 in this work have been submitted or published in a similar form in the following peer-reviewed journals.

## Chapter 4

Huber, B., Drewes, J.E., Lin, K.C., König, R., Müller, E. 2014. Revealing biogenic sulfuric acid corrosion in sludge digesters: detection of sulfur-oxidizing bacteria within full-scale digesters. *Water Science & Technology*, **70**(8), 1405-1414.

Bettina Huber drafted the manuscript, designed, and performed the experiments. Kuan Chuan Lin helped in maintaining the laboratory experiments. Rolf König provided the digester samples, took the picture and provided information on the different sludge digesters. Jörg E. Drewes reviewed the manuscript. Elisabeth Müller conceived of the study and helped to review the manuscript. All authors read and approved the final manuscript.

## Chapter 5

Huber, B., Herzog, B., Drewes, J.E., Koch, K., Müller, E. 2016. Characterization of sulfur oxidizing bacteria related to biogenic sulfuric acid corrosion in sludge digesters. Revisions submitted to *BMC Microbiology*.

Bettina Huber drafted the manuscript, designed and carried out the laboratory experiments. Bastian Herzog, Jörg E. Drewes, and Konrad Koch reviewed and edited the manuscript. Elisabeth Müller conceived of the study and helped to review the manuscript. All authors read and approved the final manuscript.

## Chapter 6

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Bettina Huber drafted the manuscript, designed, and performed the biogenic sulfuric acid experiments. Harald Hilbig and Mariana M. Mago carried out the chemical sulfuric acid experiments and LA-ICP-MS analysis. Jörg E. Drewes reviewed the manuscript. Elisabeth Müller conceived of the study and helped to review the manuscript. All authors read and approved the final manuscript.

Chapter 7

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Bettina Huber drafted the manuscript, designed, and performed the biogenic sulfuric acid experiments. Harald Hilbig carried out the chemical experiments and LA-ICP-MS analysis. Jörg E. Drewes reviewed the manuscript. Elisabeth Müller conceived of the study and helped to review the manuscript. All authors read and approved the final manuscript.

# Chapter 1

Introduction

## 1.1 BRIEF HISTORY

Corrosion of concrete was first described in a sewer pipe in the late 19$^{th}$ century by Olmstead and Hamlin (Olmstead & Hamlin, 1900). In this study, the formation of a soft and chalky mass was observed on the corroded concrete surface of a sewer pipe. It was assumed that the corrosion resulted from chemical attack through sulfuric acid ($H_2SO_4$). The correlation between the presence of hydrogen sulfide ($H_2S$) in the sewer atmosphere and the production of sulfuric acid was noticed a few decades later (Wells et al., 2009). However, the formation of sulfuric acid was long considered as a purely chemical process, in which hydrogen sulfide produced under anaerobic conditions in wastewater is oxidized to sulfuric acid in the presence of oxygen (Revie & Uhlig, 2011; Wells et al., 2009). Finally, in 1945, microbiological influences became associated with concrete corrosion, when Parker isolated the highly acidophilic *Acidithiobacillus thiooxidans* (formerly known as *Thiobacillus concretivorans)* from corroded concrete sewer pipe material (Parker, 1945b; Parker, 1945c; Parker, 1951; Wells & Melchers, 2014). Seemingly, concrete deterioration included chemical and microbiological processes (Milde et al., 1983).

Microbial induced concrete corrosion (MICC) was not considered as major problem until a rapid deterioration of concrete was observed in the Hamburg sewer system in the early 1980s (Gu et al., 1998; Milde et al., 1983; Sand & Bock, 1984). Although newly installed concrete sewer pipes were coated with a thin layer of epoxy resin inhibiting microbial growth, severe corrosion occurred within a few years (Milde et al., 1983). Similar observations were made at this time in the United States (Gu et al., 1998; Kulpa & Baker, 1990; Mansfeld et al., 1991). The higher incidence of MICC was correlated with elevated wastewater temperatures resulting in enhanced bacterial growth, increased sulfur content in the sewage due to the use of sulfur containing detergents (Milde et al., 1983), and longer detention periods due to the increased length of sewer pipes which led to the formation of anaerobic zones in the wastewater and consequently to higher $H_2S$ production by sulfate reducing bacteria (SRB) (Sand et al., 1992; Wells & Melchers, 2015). In addition, governmental authorities reduced the discharge of chemicals into the sewage leading to significantly lower levels of biologically toxic metals (e.g. lead, cadmium, arsenic) (Gu et al., 1998; Mansfeld et al., 1991; Wells et al., 2009). As a consequence, microbial activity in the sewer systems as well as MICC significantly increased (Wells et al., 2009).

The higher incidence of MICC in sewer pipes led to an intensified research effort, especially in Germany (Diercks et al., 1991; Milde et al., 1983; Sand, 1987; Sand & Bock, 1984). Until now, a significant amount of research has been carried out to study biogenic sulfuric acid (BSA) corrosion in sewer systems and a variety of bacteria (Nica et al., 2000;

Okabe et al., 2007) and fungi (Cho & Mori, 1995; Gu et al., 1998; Nica et al., 2000) have since been linked to concrete corrosion. Table 1.1 shows some of the most commonly detected bacteria in samples from corroded concrete sewer pipe material. In most studies, *A. thiooxidans* was the dominant organism within heavily corroded concrete samples (Islander et al., 1991; Milde et al., 1983; Okabe et al., 2007; Sand & Bock, 1991).

Table 1.1 Microorganisms identified in corroded concrete sewer pipe material.

| **Organisms** | **References** |
|---|---|
| *Achromobacter xylosoxidans* | Okabe et al., 2007 |
| *Acidiphilium acidophilum* | Cayford et al., 2012; Okabe et al., 2007 |
| *Acidithiobacillus ferrooxidans (formerly Thiobacillus ferrooxidans)* | Maeda et al., 1999, Hernandez et al., 2002 |
| *Acidithiobacillus thiooxidans (formerly Thiobacillus thiooxidans, Thiobacillus concretivorans)* | Hernandez et al., 2002; Milde et al.,1983; Okabe et al., 2007; Sand and Bock, 1984; Vincke et al., 2001; Parker, 1945b |
| *Acinetobacter junii* | Okabe et al., 2007 |
| *Aeromicrobium erythreum* | Vincke et al., 2001 |
| *Brevundimonas subvibrioides* | Okabe et al., 2007 |
| *Frateuria sp.* | Okabe et al., 2007 |
| *Halothiobacillus neapolitanus (formerly Thiobacillus neapolitanus)* | Milde et al., 1983; Okabe et al., 2007; Sand and Bock, 1984 |
| *Ochrobactrum antropi* | Nica et al., 2000 |
| *Ochrobactrum tritici* | Okabe et al., 2007 |
| *Paracoccus alcaliphilus* | Okabe et al., 2007 |
| *Paracoccus aminophilus* | Okabe et al., 2007 |
| *Planococcus antarcticus* | Okabe et al., 2007 |
| *Pseudomonas pseudoalcaligenes* | Vincke et al., 2001 |
| *Sphingomonas sp.* | Vincke et al., 2001 |
| *Starkeya novella (formerly Thiobacillus novellus)* | Milde et al., 1983; Sand and Bock, 1984 |
| *Stenotrophomonas maltophilia* | Okabe et al., 2007; Vincke et al., 2001 |
| *Thiobacillus plumbophilus* | Okabe et al., 2007 |
| *Thiobacillus thioparus* | Parker, 1947 |
| *Thiomonas intermedia (formerly Thiobacillus intermedius)* | Milde et al., 1983; Okabe et al., 2007; Sand and Bock, 1984 |
| *Thiothrix sp.* | Okabe et al., 2007 |

## 1.2 CORROSION PROCESSES IN SEWER SYSTEMS

BSA corrosion is a complex and multi stage process involving chemical and biological aspects (Milde et al., 1983). Figures 1.1 and 1.2 give an overview of the abiotic and biotic processes that take place in a sewer pipe and show the progression of microbial corrosion over time (Wells et al., 2009). According to Islander et al. (1991) BSA corrosion in sewer systems proceeds in three stages which are described in the following:

1) Abiotic neutralization of the concrete surface

2) Colonization of the concrete surface by neutrophilic sulfur oxidizing bacteria (SOB)

3) Colonization of the concrete surface by acidophilic SOB

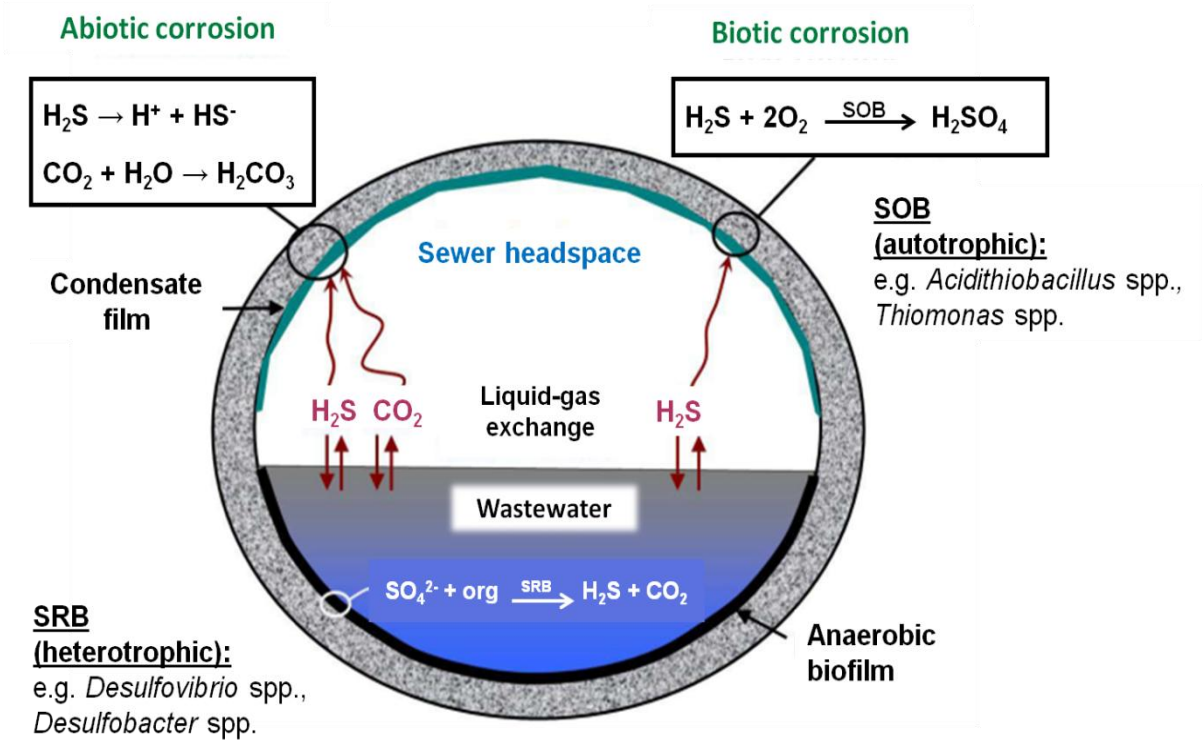



Figure 1.1 Schematic representation of the biochemical processes that contribute to the general corrosion that occurs within a concrete sewer pipe (adapted from Wells et al., 2009).

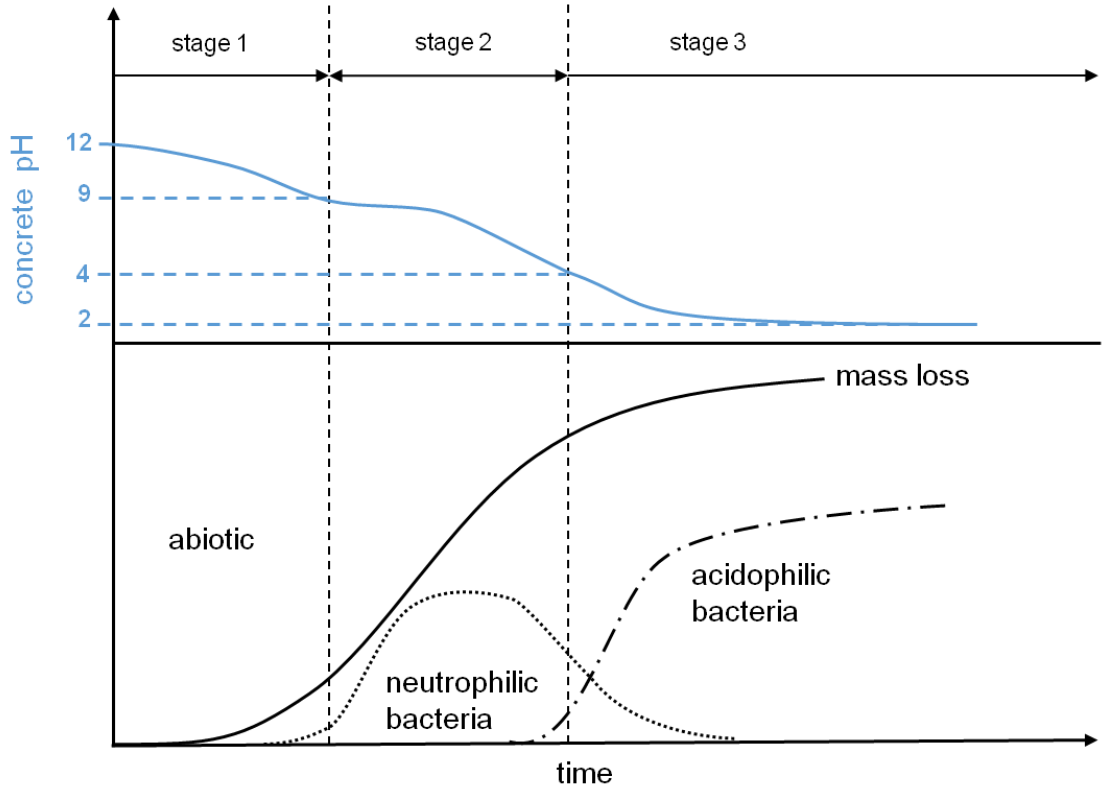



Figure 1.2 The three stages of BSA corrosion (adapted from Wells et al., 2009; Wells & Melchers, 2014).

Stage 1: Abiotic neutralization of the concrete surface

In stage 1, the calcium hydroxide ($Ca(OH)_2$) which accounts for up to 25% of new concrete structures dissolves in the concrete pore water resulting in a surface condensate film with highly alkaline pH values between 12 and 13 (Islander et al., 1991; Wells & Melchers, 2014). These high pH values hardly allow microbial growth on the concrete surface and therefore biological induced corrosion during this time is negligible (Wells & Melchers, 2014). However, sulfates and biodegradable organic carbon in the wastewater stream can be converted to hydrogen sulfide ($H_2S$) and carbon dioxide ($CO_2$) by sulfate reducing bacteria (SRB; e.g *Desulfovibrio* spp. and *Desulfobacter* spp.) growing in anaerobic biofilms below the waterline on the sewer walls (House & Weiss, 2014). These anaerobic biofilms mainly occur due to the slow flow of sewage (De Belie et al., 2004) and are typically 0.3-1.0 mm thick (House & Weiss, 2014). $CO_2$ and $H_2S$ are transferred into the sewer headspace due to turbulences or pH decrease and dissolve in the condensate layer on the sewer crown (De Belie et al., 2004). $CO_2$ can react with water to carbonic acid ($H_2CO_3$) and $H_2S$ re-dissociates to $HS^-$ and $H^+$ due to the high pH value of the concrete

surface (Roberts et al., 2002; Wells et al., 2009). These weak acids react with the alkaline components of concrete leading to an abiotic neutralization of the concrete surface to pH values around 9 (Wells et al., 2009; Wells & Melchers, 2014). However, the concrete deterioration during stage 1 is rather limited (see Figure 1.2) (Wells & Melchers, 2014). The abiotic oxidation of $H_2S$ may lead to the production of various sulfur compounds including thiosulfates, polythionic acids, sulfite, and elemental sulfur which can subsequently be metabolized in the following stages by the majority of sulfur oxidizers (Islander et al., 1991; Nica et al., 2000). The abiotic neutralization of concrete is a rather quick process and usually occurs within 56-90 days (Gutiérrez-Padilla et al., 2010; Okabe et al., 2007; Roberts et al., 2002; Wells & Melchers, 2014).

Stage 2: Colonization of the concrete surface by neutrophilic SOB

Once the pH value reaches values of around 9, microbial growth is not hindered any longer and due to nutrient, moisture and oxygen availability, neutrophilic sulfur oxidizing bacteria such as *Thiobacillus* spp. and *Thiomonas* spp. start colonizing the concrete surface (Roberts et al., 2002). These SOB grow in aerobic biofilms on the moist concrete surface and oxidize $H_2S$ and other reduced sulfur compounds (e.g. $S^0$, $S_2O_3^-$) to sulfuric acid, and thus, further reduce the pH value from ~9 down to 3.5-5.0 (Bielefeldt et al., 2009; Wells & Melchers, 2014). In an aggressive sewer environment (26°C, 98% relative humidity and 79 ppm $H_2S$ concentration), this pH reduction can be achieved within 18 months (Wells & Melchers, 2014). During stage 2, the concrete deterioration significantly increases (Figure 1.2).

Islander et al. (1991) described the succession of different SOB species. At pH values around 9 *Thiobacillus thioparus* begins with the oxidation of sulfides, elemental sulfur, and thiosulfate in order to produce sulfuric acid and polythionic acids (House & Weiss, 2014; Islander et al., 1991). The next species that colonize the concrete surface are *Thiobacillus novellus* (recently named *Starkeya novella*) and *Thiobacillus intermedius* (recently named *Thiomonas intermedia*) mainly using thiosulfate as substrate (House & Weiss, 2014). When the pH decreases to values of around 6, *Thiobacillus neapolitanus* (recently named *Halothiobacillus neapolitanus*) can develop in the aerobic biofilm oxidizing $H_2S$, sulfur and thiosulfate (Roberts et al., 2002). During stage 2, the thiosulfate pathway is dominant (Islander et al., 1991). When the pH decreases further, an abiotic conversion of thiosulfate to elemental sulfur takes place and the sulfur oxidation pathway becomes more and more important (Islander et al., 1991).

Stage 3: Colonization of the concrete surface by acidophilic SOB

When the pH value of concrete has dropped to levels of around 4, acidophilic SOB begin with the colonization of the concrete surface and the bacteria continue with the production of sulfuric acid (Roberts et al., 2002). *A. thiooxidans*, which uses elemental sulfur as preferential energy source (Robertson & Kuenen, 2006), was reported to be the key organism within this last step of corrosion (Islander et al., 1991). This acidophilic bacterium produces large amounts of sulfuric acid and was mostly found in heavily corroded concrete material (Diercks et al., 1991; Grengg et al., 2015; Milde et al., 1983; Okabe et al., 2007; Sand & Bock, 1984). Through the activity of acidophilic SOB, the pH of the concrete surface gradually decreases to values of 1.0-2.0 with such values being highly aggressive for all cement based materials (Scrivener & De Belie, 2013).

## 1.3 CONCRETE DETERIORATION

In all instances concrete consists of i) cement, ii) aggregates, and iii) water. Sometimes additional ingredients are included to further adjust the concrete characteristics. Aggregates which usually account for more than 60% of concrete (Scrivener & De Belie, 2013) can be involved in the concrete corrosion process. While acid inert siliceous aggregates ($SiO_2$) are usually not involved in the degradation process due to their insolubility in acids, reactive limestone aggregates ($CaCO_3$) may contribute to the corrosion (Zivica & Bajza, 2002). When sulfuric acid reacts with the concrete, two main reactions have to be considered (Van Tittelboom et al., 2013). On the one hand there is the volume expansion effect due to the reaction of sulfate ions with concrete constituents and on the other hand there is the dissolution effect by the hydrogen ions (Hewayde et al., 2007; Van Tittelboom et al., 2013). The sulfuric acid reacts with the main hydrated phases of Portland cement, calcium hydroxide $Ca(OH_2)$, calcium silicate hydrates (C-S-H) and with the soluble aggregates of concrete resulting in the formation of gypsum ($CaSO_4 \cdot 2H_2O$) and amorphous silicic acid ($Si(OH)_4$)) (Hewayde et al., 2007; Lavigne et al., 2016; Van Tittelboom et al., 2013). The reactions can be described as follows (Monteny et al., 2000):

$$Ca\,(OH)_2 + H_2SO_4 \rightarrow CaSO_4 \cdot 2\,H_2O$$

$$CaO \cdot SiO_2 \cdot 2H_2O + H_2SO_4 \rightarrow CaSO_4 + Si(OH)_4 + H_2O$$

The formation of gypsum is associated with a volume increase by a factor of 1.2-2.2 leading to an internal pressure increase and, as a consequence, to the weakening of the concrete structure (Attiogbe & Rizkalla, 1988; Monteny et al., 2001; Monteny et al., 2000; Wells et al., 2009).

However, it is assumed that the second reaction, in which gypsum crystals react with calcium aluminate hydrate ($C_3A$) of the cement binder to form ettringite ($3CaO{\cdot}Al_2O_3{\cdot}3CaSO_4{\cdot}32H_2O$), is much more detrimental (Monteny et al., 2000). Volume expansions by a factor ranging from 2-7 were reported in the literature (Attiogbe & Rizkalla, 1988; Monteny et al., 2000). The formation of ettringite causes internal cracks and pitting of the concrete finally resulting in disintegration of the concrete structure (Wells et al., 2009). The formation of ettringite can be described via the following reaction (Monteny et al., 2000):

$$3(CaSO_4 \cdot 2\,H_2O) + 3CaO \cdot Al_2O_3 \cdot 12\,H_2O + 14\,H_2O \rightarrow 3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O$$

Consequently, gypsum and ettringite are reported to be the main corrosion products (Scrivener & De Belie, 2013). However, the formation of gypsum is promoted at lower pH values (Gutberlet et al., 2015). During the corrosion process, a white and pulpy corrosion layer develops on the concrete surface (Scrivener & De Belie, 2013). The thickness of the corrosion layer increases with time, when more acid is formed by the SOB capable to react with the sound concrete (Zhang et al., 2008). Some researchers claim that the soft corrosion layer with its increased humidity and high porosity provides excellent growth conditions for microorganisms (Monteny et al., 2000; Scrivener & De Belie, 2013). It is assumed that the bacteria can penetrate the corrosion layer until they reach the sound concrete in order to produce more sulfuric acid close to the not yet corroded concrete (Monteny et al., 2000). This would lead to a microbial attack on concrete from the inside. A more detailed study focusing on microbial growth within the corrosion layer (Okabe et al., 2007) analyzed a 10 mm thick gypsum layer regarding the oxygen concentrations, pH value and distribution of the sulfur oxidizer *A. thiooxidans*. The highest activity of *A. thiooxidans* was detected on the outer surface layer only where oxygen and $H_2S$ concentrations were high. It was shown, that the diffusion of $H_2S$ and oxygen into the thick corrosion layer (only a few hundred micrometers) is quite limited. Therefore, the acid production seems to take place on the outer layer of the corroded concrete surface and no internal cracking of the concrete by microorganisms occurs (Okabe et al., 2007). If the

gypsum layer can serve as a growth matrix and moisture and nutrient supplier for the bacteria is still unclear and requires further investigations (Scrivener & De Belie, 2013).

## 1.4 FACTORS INFLUENCING CONCRETE CORROSION

The BSA corrosion process is extremely complex and strongly depends on the interactions between (sewer) environment (liquid and gas phase), microbial activity and building material (Alexander et al., 2013). One crucial factor for the development of BSA corrosion is the generation of $H_2S$ (Roberts et al., 2002). The formation of $H_2S$ is influenced by a variety of parameters, such as sulfate concentration in the wastewater, dissolved oxygen (DO) content, biochemical oxygen demand (BOD), temperature, pH value, or turbulences due to inadequate design of the sewer pipes (e.g. junction structures with colliding flows) (House & Weiss, 2014; Parande et al., 2006). The most important factor for the formation of $H_2S$ is the presence of sulfates and organic matter in the sewage which can be metabolized by SRB under anaerobic conditions (House & Weiss, 2014). For domestic wastewater, sulfate concentrations usually range between 0.4 and 2.1 mmol/L (Zhang et al., 2008). Low DO values (≤0.1 mg/L), high BOD values and high temperatures in the wastewater support the growth of SRB communities in anaerobic biofilms and consequently promote the reduction of sulfates to sulfides (House & Weiss, 2014; Parande et al., 2006). Furthermore, the pH value of wastewater has a major impact on the $H_2S$ concentration in the sewer atmosphere (House & Weiss, 2014). Since the pH value of municipal wastewater is around 7, $H_2S$ is mainly present at two sulfide species, $H_2S$ and $HS^-$ (Zhang et al., 2008). Around 25-35% of the dissolved sulfide exists as molecular $H_2S$ (Wells et al., 2009). Only $H_2S$ is transferred to the gas phase which depends on the pH value, temperature and hydraulic conditions in the wastewater (Yongsiri et al., 2004; Zhang et al., 2014). The liquid gas exchange of $H_2S$ increases with decreasing pH values, increased temperatures and higher turbulences (Vincke et al., 2001; Wells et al., 2009).

When sufficient $H_2S$ escaped into the sewer headspace, abiotic factors, such as temperature, and moisture influence SOB growth in the aerobic biofilm on the concrete surface (Wells & Melchers, 2015). Elevated temperatures and high moisture were reported to be beneficial for SOB growth leading to accelerating corrosion rates (Islander et al., 1991; Wells et al., 2009).

Additionally, the composition of the building material has an impact on the corrosion rate (Wells et al., 2009). The type of cement that is used for concrete production plays an

important role in acid resistance (Zivica & Bajza, 2002). For instance, previous studies have shown that high alumina cement is more resistant against biogenic sulfuric acid than ordinary Portland cement probably due to antibacterial effects of the aluminum (Scrivener & De Belie, 2013). Not only the cement type, but also the cement content and water-cement (w/c) ratio influence the corrosion rates (Hewayde et al., 2007; Monteny et al., 2000). Hewayde et al. (2007) reported higher corrosion rates when concrete with high cement content and low water to cement ratios were used. Usually, cement contents between 300 and 400 $kg/m^3$ are sufficient to achieve a low permeability and high acid resistance of concrete if the w/c value is below 0.5, and compaction and curing is appropriate (Zivica & Bajza, 2002). Furthermore, the neutralization capacity of concrete can be increased by the use of “sacrificial” limestone aggregates instead of acid resistant aggregates such as gravel (Scrivener & De Belie, 2013). These kinds of aggregates are “sacrificial”, because they are dissolved during acid attack which results in a local buffering environment protecting the cementitious binder (De Belie et al., 2004). When conducting laboratory conditions with chemical and biogenic sulfuric acid, the sacrificial aggregates showed a lower degradation depth compared to inert aggregates (De Belie et al., 2004).

## 1.5 ECONOMIC IMPACTS

BSA corrosion, especially in sewer systems, is a substantial challenge worldwide leading to economic losses of multibillions of dollars every year (Hewayde et al., 2007). In Germany, for instance, around 40% of the damage in concrete pipes and brick work sewers is caused by microbially produced sulfuric acid (Kaempfer & Berndt, 1999). It has been estimated that maintenance and repair of these damaged concrete structures costs around 100 billion dollars every year (Kaempfer & Berndt, 1999). In Flanders (Belgium), BSA corrosion breakdown of sewer pipes amount for 10% of the overall sewage treatment costs accounting for 5 million euro every year (Zhang et al., 2008). In Los Angeles County, about 10% of the corrosion in sewer pipelines can be attributed to BSA attack and the associated restoration costs run to about 400 million euro (Zhang et al., 2008). In Australia, losses due to MICC are in the range of 10-100 millions of dollars per year (Wells et al., 2009). The deterioration of concrete, especially in sewer pipes (see Figure 1.3), can be very severe and strongly depends on the availability of $H_2S$, moisture, and oxygen in the sewer atmosphere (Roberts et al., 2002). Corrosion rates of several millimeters per year have been reported for sewer pipes (De Belie et al., 2004; Mori et al., 1991; Vincke et al., 2002; Vollertsen et al., 2008).

Figure 1.3 A heavily corroded piece of concrete originating from a sewer pipe in Germany. © Bettina Huber

## 1.6 OCCURRENCE OF BSA CORROSION IN OTHER CONCRETE FACILITIES

Although BSA corrosion is mostly found in sewage collection systems, the corrosion was reported to affect other types of concrete buildings (Roberts et al., 2002) such as wastewater treatment facilities (Redner et al., 1992), agricultural biogas plants (Koenig & Dehn, 2016), bridge structures (Vupputuri et al., 2015), and hydraulic facilities (Zherebyateva et al., 1991). No literature was found that describes BSA corrosion in sludge digesters. Due to the anaerobic conditions in a digester, one would expect the presence of SRB and $H_2S$ production, but the growth of aerobic SOB would come as a surprise. However, the occurrence of typical BSA corrosion damage patterns in the headspace of several digesters in Germany, characterized by washed out concrete surfaces and yellow deposits, were observed (Figure 1.4). These findings suggest that SOB might be present in the digester headspace inducing BSA production and concrete corrosion. To shed light on this lack of knowledge, the following section focuses on the fundamentals of anaerobic digestion.

Figure 1.4 Severe concrete corrosion observed within the headspace of two different digesters in Germany. © Weber-Ingenieure GmbH.

## 1.7 FUNDAMENTALS OF ANAEROBIC DIGESTION

Anaerobic digestion (AD) is a commonly used technique for the stabilization of wastewater sludge (Ramos et al., 2014). In Germany, approximately 1,450 wastewater treatment plants are equipped with an anaerobic sludge digester (Wolz et al., 2010). During AD, organic matter in the sludge is converted to biogas consisting mainly of methane ($CH_4$) and carbon dioxide ($CO_2$) (Nghiem et al., 2014). For the AD process, strictly anaerobic conditions (oxidation reduction potential (ORP) <-200 mV) are required (Appels et al., 2008). The main end product, $CH_4$, can be used as renewable energy source for the generation of heat and electricity (Rasi et al., 2007). Biogas produced from digester sludge typically contains 55-65% $CH_4$, 35-45% $CO_2$ and <1% nitrogen (Rasi et al., 2007). Furthermore, typical $H_2S$ concentrations generated during anaerobic digestion of activated sludge are in the range of 1,000 – 2,400 ppm (Nghiem et al., 2014; Ramos et al., 2014) which represents sufficient concentration for BSA corrosion. In sewer pipes, concrete corrosion was already observed at a concentration of only 0.5 ppm $H_2S$ (Weissenberger, 2002).

Generally, AD of organic matter consists of the following steps (Appels et al., 2008): hydrolysis, acidogenesis, acetogenesis and methonogenesis (see Figure 1.5).

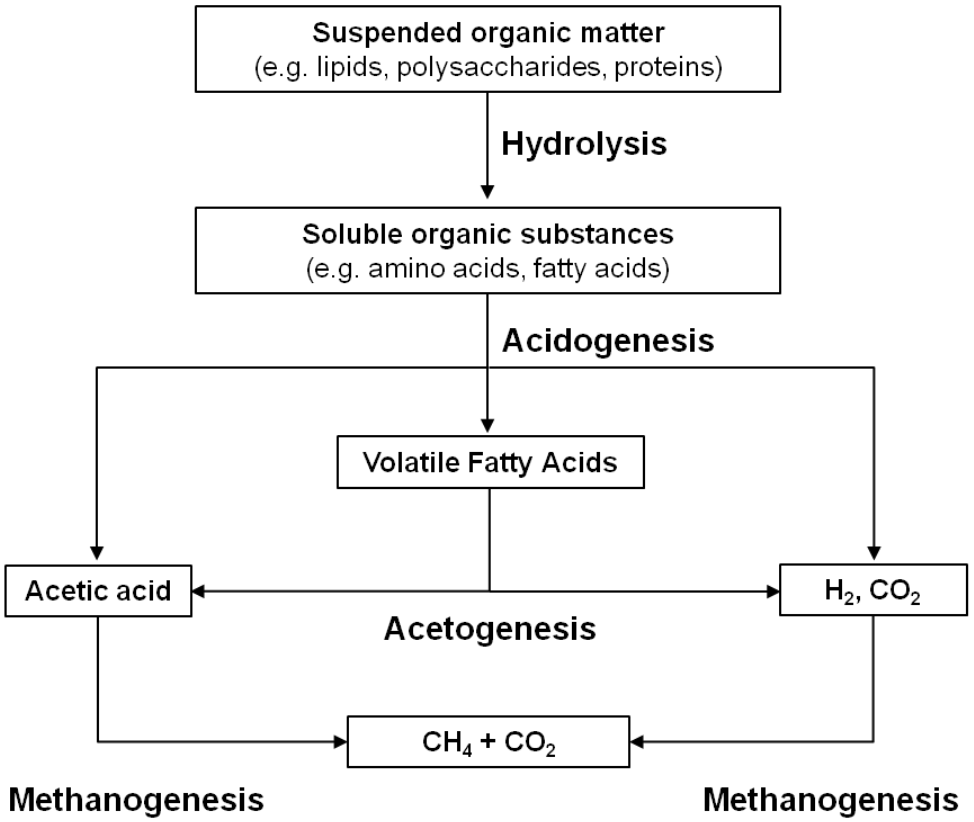



Figure 1.5 Steps in anaerobic sludge digestion (adapted from Appels et al., 2008).

During hydrolysis, insoluble organic matter as well as high molecular weight compounds (e.g., lipids, polysaccharides, proteins and nucleic acids) are transformed into soluble organic compounds such as amino acids and fatty acids (Appels et al., 2008). In the acidogenesis step, the compounds generated during the hydrolysis step are further transformed by the activity of acidogenic or fermentative bacteria in order to produce volatile fatty acids (VFA) and other by-products (e.g., ammonia, $CO_2$, and $H_2S$) (Appels et al., 2008). In the following step, acetogenesis, the higher organic acids and alcohols formed during acidogenesis can be used by acetogens (Appels et al., 2008). Acetic acid, $CO_2$ and $H_2$ are the main end products of this digestion process (Appels et al., 2008). The last step of AD is the methanogenesis, where $CH_4$ is generated by two different kinds of methanogens: the acetoclastic methanogens use acetate in order to produce $CH_4$ and $CO_2$, while the hydrogenotrophic methanogens convert $H_2$ and $CO_2$ to $CH_4$ (Demirel & Scherer, 2008).

# Chapter 2

## Research significance, hypotheses and organization of PhD thesis

## 2.1 RESEARCH SIGNIFICANCE AND HYPOTHESES

Several billions of dollars have to be spent for the repair and maintenance of sewage systems every year, whereupon the deterioration of concrete due to BSA production is one of the most serious and costly problems. Microbial induced corrosion is well described in sewer pipes, but little is known about this process within sludge digesters. Although the predominant anaerobic conditions within a digester should not support the activity of aerobic SOB, characteristic BSA corrosion damage patterns were observed in the headspace of several full-scale digesters. Therefore, the main aim of this doctoral dissertation was to verify BSA corrosion in six selected digesters under controlled laboratory conditions by i) identifying and characterizing the relevant bacterial groups (SRB and SOB) and ii) analyzing the BSA corrosion potential on hardened cement paste and concrete samples usually applied in the construction of full-scale digesters. Thereby, comparative studies with chemically and microbially produced sulfuric acid were carried out using laser ablation inductively coupled plasma mass spectrometry as novel assessment tool.

In the following, the two main objectives of the thesis with the corresponding research questions are described:

**Identification and characterization of BSA related microbial communities (SRB and SOB)**

*Research Question #1:* Can both SRB and SOB communities relevant for the BSA process also be identified in samples from sludge digesters?

*Research Question #2:* Are the identified SOB species able to produce sulfuric acid under controlled laboratory conditions and thus possess the potential to contribute to BSA corrosion in sludge digesters?

**Analysis of the BSA corrosion potential in laboratory experiments**

*Research Question #3:* Are there different corrosion degradation patterns on hardened cement paste samples reflecting a concrete binder and concrete samples representing a digester wall after purely chemically and biogenically produced sulfuric acid attack?

*Research Question #4:* Is there a linear relationship between concrete degradation and incubation time in microbially produced sulfuric acid?

These four research questions will be addressed by testing the following four hypotheses in a series of experiments (see Chapters 4-7 and Figure 2.1).

*Hypothesis #1:* The relevant bacteria, i.e. SRB and SOB, responsible for the BSA process can also be found in sludge digesters (see Chapter 4).

*Hypothesis #2:* The SOB species within the enriched cultures are able to produce sulfuric acid and might therefore contribute to BSA corrosion in sludge digesters (see Chapter 5).

*Hypothesis #3:* Chemically and microbially generated sulfuric acid can lead to comparable damage patterns on pure hardened cement paste and concrete (see Chapters 6 and 7).

*Hypothesis #4:* There is no linear increase in concrete deterioration with increasing incubation periods in microbially produced sulfuric acid (see Chapter 7).

| Research Topic | Hypothesis | Chapter | Journal Article |
|---|---|---|---|
| **Identification and characterization of BSA related microbial communities (SRB and SOB)** | **Hypothesis # 1:** The relevant bacteria, i.e. SRB and SOB, responsible for the BSA process can also be found in sludge digesters. | 4 | **Huber et al., 2014:** *Water Science & Technology*, 70(8), 1405-1414. |
| | **Hypothesis # 2:** The SOB species within the enriched cultures are able to produce sulfuric acid and might therefore contribute to BSA corrosion in sludge digesters. | 5 | **Huber et al., 2016:** Revisions submitted to *BMC Microbiology*. |
| **Analysis of the BSA corrosion potential in laboratory experiments** | **Hypothesis # 3:** Chemically and microbially generated sulfuric acid can lead to comparable damage patterns on pure hardened cement paste. | 6 | **Huber et al., 2016:** *Cement and Concrete Research* 87, 14-21. |
| | **Hypothesis # 3 :** Chemically and microbially generated sulfuric acid can lead to comparable damage patterns on concrete. **Hypothesis # 4:** There is no linear increase in concrete deterioration with increasing incubation periods in microbially produced sulfuric acid. | 7 | **Huber et al., 2016:** Submitted to *Cement and Concrete Research*. |

Figure 2.1. Overview of the two main objectives of the thesis and the associated hypotheses.

## 2.2 ORGANIZATION OF PHD THESIS

This PhD thesis is structured according to the two main research topics and four guiding hypotheses (Chapters 4-7; see Figure 2.1), respectively. First of all, a short overview of the material and methods is given in Chapter 3. The four hypotheses are tested in Chapters 4-7. For each chapter, the background, experimental approach, results and discussion and conclusions are given.

Chapters 4 and 5 describe the bacterial communities involved in the BSA corrosion process. While the focus of Chapter 4 is on the identification of both bacterial groups (SRB and SOB), the aim of Chapter 5 is on the characterization of SOB communities and on the analysis of their sulfuric acid production potential under laboratory conditions and *in situ* in sludge digesters.

In Chapters 6 and 7 the BSA corrosion potential is investigated under different laboratory conditions. Chapter 6 describes the influence of chemically and microbially produced sulfuric acid on hardened cement paste samples representing a concrete binder. Chapter 7 investigates the chemical and microbial sulfuric acid attack on concrete and analyzes the long-term effects of biologically produced sulfuric acid on concrete samples.

In Chapter 8, the major outcomes of the thesis are presented and an appropriate assessment of the results with regard to current literature is provided.

# Chapter 3

Material and methods

This chapter provides a short outline of the Materials and Methods used for addressing the two main research topics, namely: i) characterization of BSA related microbial communities (SRB and SOB), and ii) analysis of the BSA corrosion potential. Detailed information of each experimental approach is given in Chapters 4-7.

## 3.1 CHARACTERIZATION OF BSA RELATED MICROBIAL COMMUNITIES (SRB AND SOB)

For the detection and characterization of BSA-related bacterial groups, six different full-scale digesters in Germany showing indications of BSA corrosion in the digester headspace were investigated. The sludge digesters had an operation time of 23-52 years. Table 3.1 summarizes the design specifics of the six analyzed digesters (A-F). For the identification of SRB and SOB communities, digester sludge and biofilm samples from the corroded concrete surface in the digester headspace were collected. The different steps of digester sludge and biofilm processing are summarized in Figure 3.1. Furthermore, drilling dust samples of digesters A-C and E-F were taken from the concrete wall of the digester sludge zone and headspace to analyze the sulfate content in concrete and obtain information on the potential microbial sulfur oxidizing activity *in situ*.

Table 3.1 Overview of the six analyzed digesters (A-F).

| **Dg** | **Year of construction** | **WWTP PE** | **Digester volume [$m^3$]** | **RT [d]** | **Operating temperature [°C]** | **pH of digester sludge** | **Sampling dates (digester sludge/ biofilm)** |
|---|---|---|---|---|---|---|---|
| A | 1969 | 110,000 | 1,100 | 18 | 38 | 7.3 | April 2013<br>Feb. 2014 |
| B | 1974 | 83,000 | 1,150 | 25 | 37 | 6.9-7.1 | Sept. 2013 |
| C | 1980 | 83,000 | 1,000 | 27 | 38 | 7.7 | Sept. 2013 |
| D | 1990 | 30,000 | 2,000 | 35 | 40 | 7.5-7.6 | Sept. 2013<br>Feb. 2014 |
| E | 1963 | 10,000 | 320 | 60 | 30–33 | 7.0 | Nov. 2013<br>Feb. 2014<br>May 2015<br>Oct. 2015 |
| F | 1982 | 94,500 | 2,100 | 35 | 39 | 7.2 | Feb. 2014 |

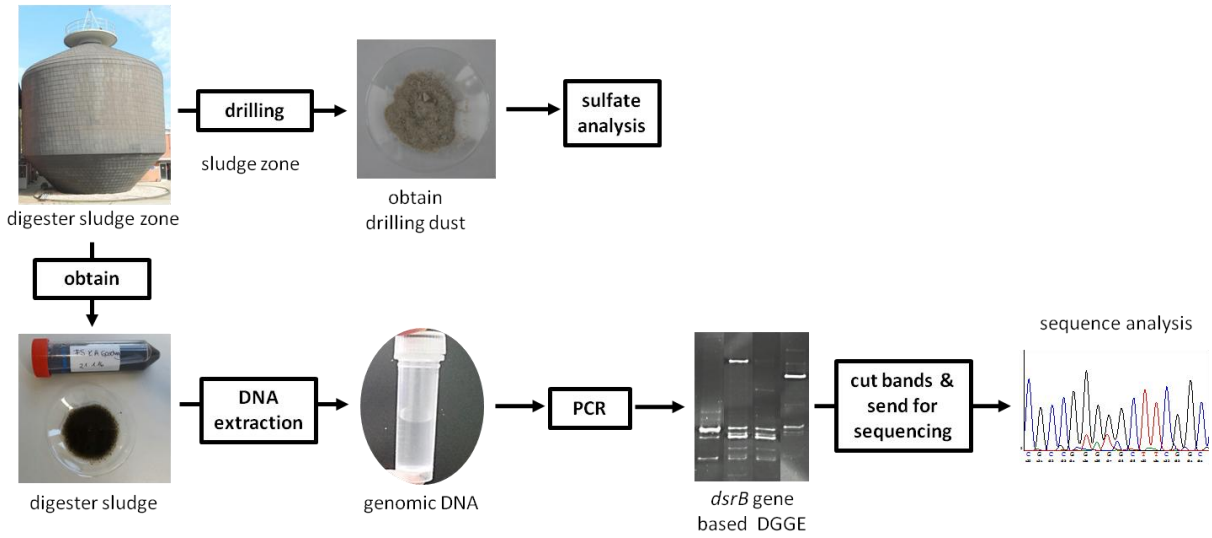
Dg = Digester, WWTP PE = Wastewater Treatment Plant Population Equivalent, RT = Retention Time

From each digester sludge sample, genomic DNA was isolated (Figure 3.1A). Subsequently, the SRB diversity was analyzed with polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) using the *dsrB*-gene (dissimilatory sulfite reductase beta subunit). This gene can be used as phylogenetic marker, since it encodes the dissimilatory sulfite reductase, a key enzyme in sulfate reduction, which is present in all known sulfate-reducing prokaryotes (Barton & Fauque, 2009; Grein et al., 2013). The ~350 bp gene fragment was separated on a denaturing polyacrylamide gel. The different bands obtained on the DGGE gel representing different SRB species were cut out, send for sequencing and phylogenetically analyzed.

For the investigation of the SOB diversity, the biofilm samples which were scratched off from the concrete wall of the digester headspace were enriched in specific liquid media containing elemental sulfur or sodium thiosulfate as only energy sources (Figure 3.1B). Autotrophic media were used, since the most SOB are obligate or facultative autotrophs (Robertson & Kuenen, 2006). Furthermore, the aim was to reduce unwanted growth of heterotrophic bacteria. SOB activity within the enrichment cultures was monitored by pH and sulfate measurements. A decreasing pH value in combination with an increasing sulfate concentration was an indicator for SOB growth. All enrichments that showed SOB activity were streaked onto the corresponding agar media to finally obtain pure cultures. SOB pure cultures were isolated and identified by 16S sequencing. Additionally, the microbial community structure within the mixed enriched cultures was analyzed with PCR-DGGE using universal bacterial primers (~550 bp fragment) and subsequent 16S sequence analysis.

From both zones (sludge zone and headspace), drilling dust samples were collected on the concrete walls in a depth of 0-40 mm in order to determine the sulfate content in the concrete specimens. A higher sulfate content in the digester headspace compared to the sludge zone was an indicator for sulfur/sulfide oxidation *in situ*.

A) Digester sludge zone




B) Digester headspace

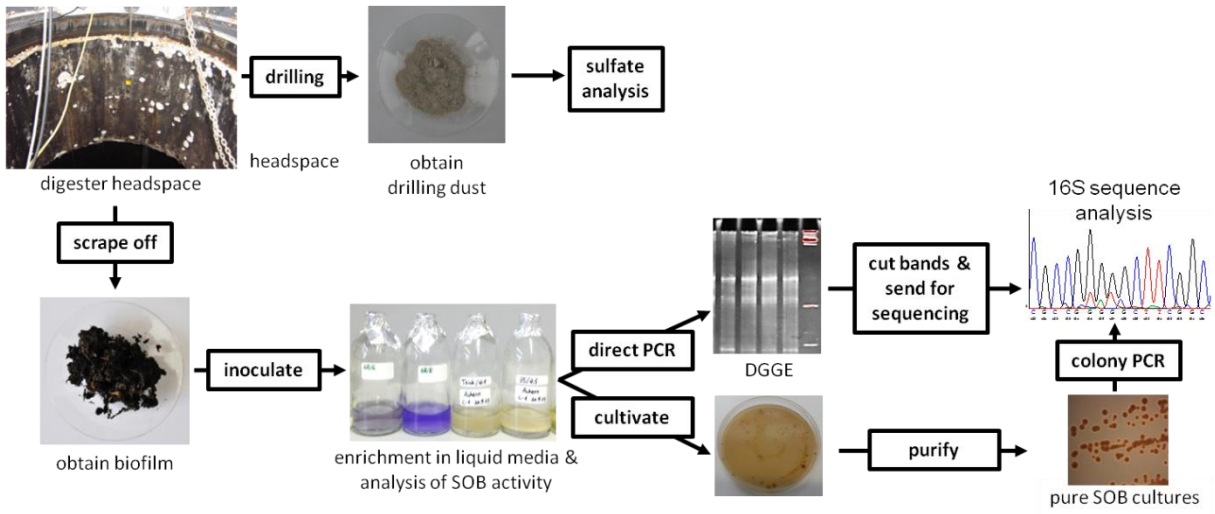



Figure 3.1 Different steps of sample processing for i) the identification and characterization of BSA related bacterial groups and ii) sulfate analysis of drilling dust originated from the concrete surface. A) SRB in the digester sludge samples and *in situ* sulfate measurement of the sludge zone. B) SOB communities in biofilm samples from the digester headspace and *in situ* sulfate measurement of the headspace.

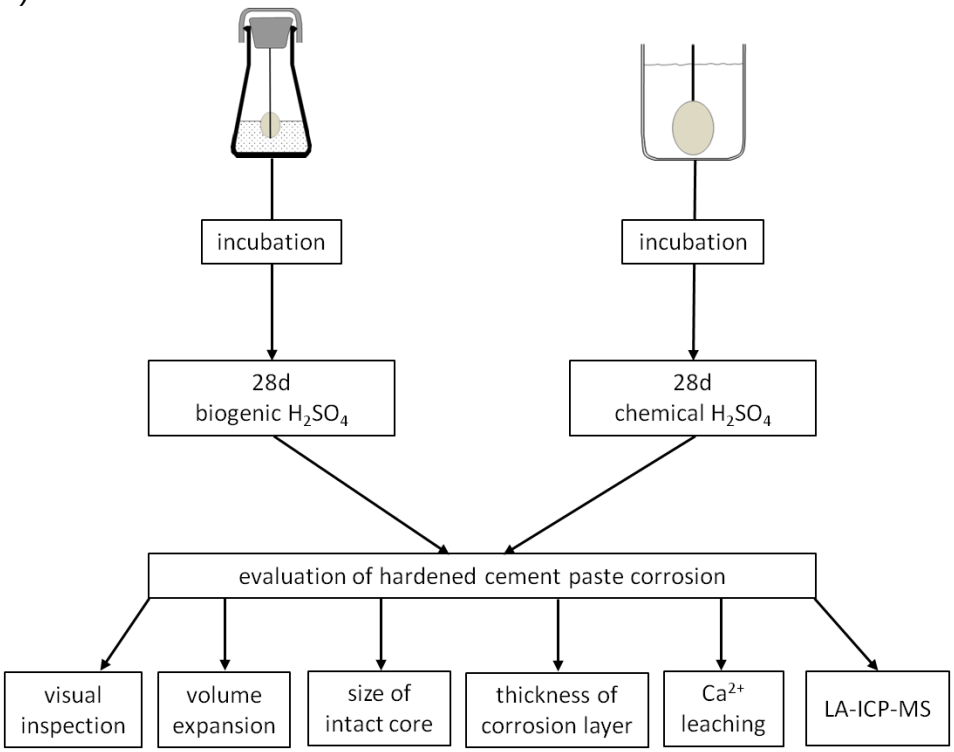
## 3.2 ANALYSIS OF THE BSA CORROSION POTENTIAL

For the analysis of the corrosion potential, the last stage of BSA corrosion, in which sulfuric acid is produced by SOB, was simulated. Biogenic and chemical sulfuric acid experiments were performed using hardened cement paste and concrete. The aim was to mimic a worst-case scenario promoting corrosion. Therefore, microbiological sulfuric acid experiments were set-up with the acidophilic *A. thiooxidans*, originally isolated from digester E, since this bacterium is able to produce high amounts of sulfuric acid lowering the pH to values of approximately 1.0. In addition, chemical autotitrator experiments with pure sulfuric acid were carried out at pH 1.0 and 2.0. The main focus was to study the impact of chemically and microbially produced sulfuric acid on hardened cement paste and concrete after 28 days of incubation in the corresponding sulfuric acid solutions. In addition, long-term biogenic sulfuric acid tests were performed with concrete samples for two, three and six months, to evaluate the corrosion behavior over time.

The hardened cement paste disks contained ground granulated blast furnace slag (80 wt.%; GGBS), a binder often used for structures in wastewater facilities. The concrete specimens had a water to cement ratio (w/c) of 0.5 and GGBS as cement type with low hydration heat and high sulfate resistance (CEM III/B 32.5 N-LH/SR). The cement paste used for the concrete samples was comparable to the pure cement paste. For the concrete samples, reactive limestone and inert quartz were used as aggregates. Finally, the concrete composition had a strength class of C30/37, which is widely used for the construction of sludge digesters.

For the experiments with hardened cement paste, chemical sulfuric acid experiments at two adjusted pH values (pH 1.0 and 2.0) and microbiological experiments with *A. thiooxidans* (pH 1.5-2.1) were performed over a period of 28 days (Figure 3.2A). Within the biotic set-ups, the sulfuric acid production by *A. thiooxidans* was controlled by pH and sulfate measurements. After 28 days of incubation, the cement stone degradation was evaluated using the following parameters: i) visual inspections of the specimens, ii) volume expansion, iii) size of intact cement stone core, iv) thickness of the corrosion layer, v) calcium leaching, and vi) laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

A)




B)

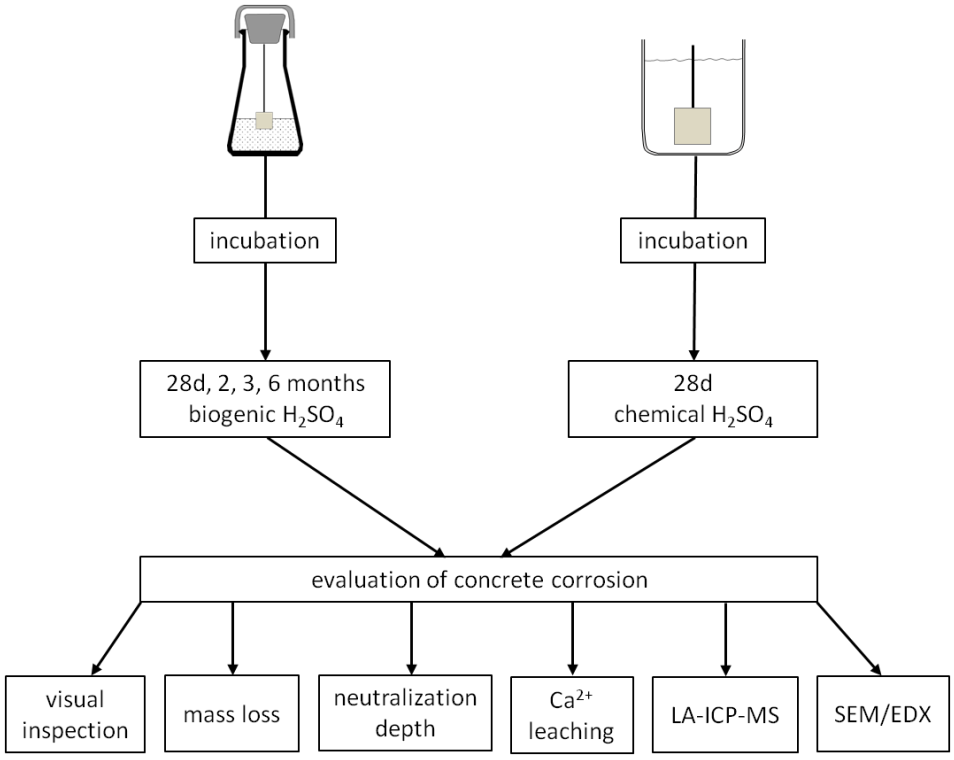



Figure 3.2 Experimental set-ups for the biogenic and chemical corrosion experiments with A) hardened cement paste samples and B) concrete specimens.

Chemical and biogenic experiments with concrete samples (Figure 3.2B) were performed under the same experimental conditions over a period of 28 days. Additionally, biogenic sulfuric acid experiments were carried for two, three and six months to study the long-term effect of microbially produced sulfuric acid on concrete. For the assessment of concrete deterioration a variety of parameters were used including i) visual inspections of the specimens, ii) weight loss, iii) neutralization depth, iv) calcium leaching, v) LA-ICP-MS, and vi) scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectroscopy.

# Chapter 4

## Revealing biogenic sulfuric acid corrosion in sludge digesters: Detection of sulfur oxidizing bacteria within full-scale digesters[1]

*Biogenic sulfuric acid (BSA) corrosion is a costly problem affecting both sewerage infrastructure and sludge handling facilities such as digesters. The aim of this study was to verify BSA corrosion in full-scale digesters by identifying the microorganisms involved in the concrete corrosion process, that is, sulfate reducing (SRB) and sulfur oxidizing bacteria (SOB). To investigate the SRB and SOB communities, digester sludge and biofilm samples were collected. SRB diversity within digester sludge was studied by applying polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) targeting the dsrB-gene (dissimilatory sulfite reductase beta subunit). To reveal SOB diversity, cultivation dependent and independent techniques were applied. The SRB diversity studies revealed different uncultured SRB, confirming SRB activity and $H_2S$ production. Comparable DGGE profiles were obtained from the different sludges, demonstrating the presence of similar SRB species. By cultivation, three pure SOB strains from the digester headspace were obtained including Acidithiobacillus thiooxidans, Thiomonas intermedia and Thiomonas perometabolis. These organisms were also detected with PCR-DGGE in addition to two new SOB: Thiobacillus thioparus and Paracoccus solventivorans. The SRB and SOB responsible for BSA corrosion were identified within five different digesters demonstrating that BSA corrosion is a problem occurring not only in sewer systems but also in sludge digesters. In addition, the presence of different SOB species was successfully associated with the progression of microbial corrosion.*

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[1] Huber, B., Drewes, J.E., Lin, K.C., König, R., Müller, E. 2014. Revealing biogenic sulfuric acid corrosion in sludge digesters: detection of sulfur-oxidizing bacteria within full-scale digesters. *Water Science & Technology*, **70**(8), 1405-1414.

## 4.1 INTRODUCTION

Corrosion of concrete due to biogenic sulfuric acid production is a well-known problem affecting the world's sewerage infrastructure and wastewater treatment with repair costs of several billions of dollars every year (Hewayde et al., 2007). In Germany alone, the estimated costs for the restoration of damaged sewer systems amount to 100 billion euro, of which 40% of the damage can be attributed to biogenic sulfuric acid corrosion (BSA) (Kaempfer & Berndt, 1998). For the BSA corrosion process, a complex microbial ecosystem involving anaerobic sulfate reducing as well as aerobic sulfur oxidizing bacteria (SRB and SOB, respectively) is required. In a sewer pipe, where anaerobic conditions can occur due to long detention periods or the slow flow of sewage, heterotrophic SRB (e.g., *Desulfovibrio* sp.) reduce sulfur compounds with organic substances as electron donors to hydrogen sulfide ($H_2S$). The $H_2S$ gas escapes into the sewer headspace and is subsequently converted abiotically to various partially reduced sulfur compounds in the condensate on the sewer crown (Roberts et al., 2002). In the presence of oxygen, autotrophic/mixotrophic SOB growing on the moist concrete surface (e.g., *Thiobacillus* sp.) finally oxidize these reduced sulfur compounds (e.g. $S_2O_3^-$, $S^0$) to sulfuric acid (Okabe et al. 2007). The sulfuric acid reacts with the cementitious material of concrete and corrosion products like gypsum ($CaSO_4 \cdot 2H_2O$) and ettringite ($3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O$) are formed (Bock & Sand, 1986; Wiener et al., 2006). The formation of these expansive compounds increases the internal pressure leading to cracks and pitting of the concrete (Aviam et al., 2004; Kaempfer & Berndt, 1998). In sewer systems corrosion rates of up to several millimeters per year are reported (De Belie et al., 2004; Mori et al., 1991; Vincke et al., 2002; Vollertsen et al., 2008).

While BSA corrosion is well studied in sewer pipes, a lack of understanding exists regarding corrosion processes in sludge digesters (for detailed information on anaerobic sludge digestion see Appels et al. (2008)) and in particular the role of BSA production. The predominant anaerobic conditions in a digester would suggest SRB activity in the presence of sulfur compounds, but no sulfuric acid production by SOB which require oxygen for growth. However, characteristic BSA corrosion damage patterns were also observed in different full-scale digesters in Germany (Figure 4.1). This might suggest that oxygen is locally available in the headspace ("hot spots") enabling the development of active SOB communities on the concrete wall. Due to the old age of many digesters imperfections may occur over time and oxygen might diffuse through existing cracks. Another possibility for the oxygen entry may be the desulfurization, where oxygen/air, usually in the range of 2–6 vol%, is added to the biogas (Appels et al., 2008). During this process *thiobacilli*, commonly present within digester sludge, convert the $H_2S$ to elemental

sulfur and sulfates reducing the $H_2S$ level from 3,000–50,000 ppm to 50–100 ppm (Appels et al., 2008).

The aim of this study was to analyze the hypothesis if the relevant bacteria, i.e. SRB and SOB, responsible for the BSA process can also be found in sludge digesters (see hypothesis # 1; Chapter 2), in order to verify concrete corrosion by biogenic sulfuric acid production in digester systems. For this purpose, digester sludge (SRB) and biofilms growing on the concrete surface (SOB) of five full-scale digesters in Germany with different degrees of corrosion damage were investigated regarding the occurrence of BSA-related microorganisms. Furthermore, a correlation between the presence of different SOB species and the progression of microbial concrete corrosion was revealed.

Figure 4.1 Concrete corrosion pattern observed in the headspace of digester E.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Environmental samples

Environmental samples, including digester sludge and biofilms removed from the concrete surface, were collected from five different digesters in Germany (A–E) exhibiting characteristic BSA damage patterns (see Figure 4.1). Further information on the investigated sludge digesters is given in Table 4.1.

From each digester outflow, fresh digester sludge samples comprising SRB were taken. Within the digester headspace, biofilm samples containing potential SOB were scratched off from the corroded concrete surface using a sterile spatula. Field sampling of the environmental samples was performed by Weber-Ingenieure GmbH. The obtained samples were directly transferred into sterile 50 mL tubes and stored at 4°C. The biofilm samples were immediately inoculated in specific liquid media as described below. For subsequent molecular biological analyses, both samples were stored at −20°C.

Table 4.1 Detailed information on the five sludge digesters A-E.

| Digester | Year of construction | PE[1] | Digester volume [$m^3$] | SRT[2] [d] | Temperature of digester sludge [°C] | pH of digester sludge | Sampling date |
|---|---|---|---|---|---|---|---|
| A | 1969 | 110,000 | 1,100 | 18 | 37.5-39.0 | 7.3 | April 2013<br>February 2014 |
| B | 1974 | 83,000 | 1,150 | 26-30 | 37.5-38.0 | 6.9-7.1 | September 2013 |
| C | 1980 | 83,000 | 1,000 | 26-27 | 38.1 | 7.7 | September 2013 |
| D | 1990 | 30,000 | 2,000 | 30-40 | 39.0-41.0 | 7.5-7.6 | September 2013<br>February 2014 |
| E | 1963 | 10,000 | 320 | 60 | 30.0-33.0 | 7.0 | November 2013<br>February 2014 |

[1] population equivalent
[2] sludge retention time

### 4.2.2 Enrichment and cultivation of SOB

The enrichment and cultivation was carried out using a variety of media, differing in pH and energy source. DSMZ medium 35 (pH 4.5) with elemental sulfur and DSMZ medium 68 (pH 6 and 8) with sodium thiosulfate ($Na_2S_2O_3$) as sole energy source were prepared according to Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (http://www.dsmz.de/home.html). ATCC medium #125 (pH 4.1) containing $(NH_4)_2SO_4$, $MgSO_4$, $FeSO_4$ and elemental sulfur as well as *Thiobacillus* medium (pH 4.1) with $(NH_4)_2SO_4$, $MgSO_4$, $FeSO_4$, and $Na_2S_2O_3$ as energy source were performed as described by Starosvetsky et al. (2013). Inoculation of liquid media was done under sterile conditions from 100 to 500 mg of freshly collected biofilm material that had been stored at 4°C for no longer than two days. Enriched SOB cultures were incubated at 30°C and shaken at 125 rpm. Mixed enriched SOB cultures were analyzed by polymerase chain reaction combined with denaturing gradient gel electrophoresis (PCR-DGGE) and phylogenetic analysis. Pure cultures were finally obtained by cultivation of SOB on the corresponding agar media and identified by colony PCR amplifying the nearly full-length 16S rRNA gene and phylogenetic sequence analysis.

### 4.2.3 Analysis of SRB and SOB diversity

#### 4.2.3.1 DNA extraction and PCR amplification of 16S rRNA and *dsrB* genes

Genomic DNA was isolated from 2 mL of digester sludge using a standard phenol-chloroform-isopropyl alcohol (25:24:1) and CTAB-buffer extraction method. The composition of the CTAB lysis buffer was as follows: 0.25 M Tris/HCl (pH 8.0), 1.60 M NaCl, 0.03 M EDTA, 1.00% (w/v) SDS, 0.05 M ammonium acetate, and 1.60% CTAB (w/v). For the amplification of the nearly full-length 16S rRNA gene (colony PCR) the universal bacterial primers 27f (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492r (5′TAC GGY TAC CTT GTT ACG ACT T-3′) were applied (Lane, 1991). For DGGE of mixed SOB cultures (direct PCR), the partial 16S rRNA gene fragment (~550 bp) was amplified using bacterial primers 27f and 517r (5′-GTA TTA CCG CGG CTG CTG GC-3′; (Furushita et al., 2003)). The temperature program for the primer sets 27f/1492r and 27f/517r was as follows: initial denaturation step of 95°C for 2 min, 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, elongation at 72°C for 40 sec, and final elongation at 72°C for 5 min. For SRB diversity analysis (PCR of extracted genomic DNA), the *dsrB* (dissimilatory sulfite reductase beta-subunit) gene fragment (~350 bp) was amplified with primers DSRp2060F (5′-CAA CAT CGT YCA YAC CCA GGG-3′ (Geets et al., 2006)) and DSR4R (5′-GTG TAG CAG TTA CCG CA-3′; (Wagner et al., 1998)). Thermal cycling was performed as described by Geets et al. (2006) with an initial denaturation step of 95°C for 2 min. In order to obtain stable melting behavior of the DNA fragments during DGGE, each forward primer contained a 40 bp GC-clamp at the 5′end (5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCG CCC CCG CCC CGG-3′; (Muyzer et al., 1993)). All primers were purchased from MWG Operon (Ebersberg, Germany). PCR was performed according to the GoTaq(R) G2 Hot Start Colorless Master Mix (Promega GmbH, Mannheim, Germany) in a total reaction volume of 25 µL.

#### 4.2.3.2 DGGE of 16S rRNA and *dsrB* gene fragments

DGGE was performed with the DCode$^{TM}$ Universal Mutation Detection System from Bio-Rad (München, Germany). Ten microliters of the PCR product which was obtained either from amplification of 16S rRNA or *dsrB* genes was loaded onto a 6% (w/v) polyacrylamide gel. Denaturing gradients ranged from 20 to 70%, where 100% denaturant is defined as 7 M urea and 40% (v/v) formamide. Electrophoresis was carried out at a constant voltage of 60 V for 16.5 h at 55°C. Interesting DGGE bands were excised and re-amplified using the corresponding primer sets (without GC-clamp).

#### 4.2.3.3 Sequencing and phylogenetic analysis

Sequences of 16S rRNA and *dsrB* genes were obtained either from colony PCR or re-amplification of excised DGGE bands. The obtained PCR products were purified with the innuPREP DOUBLEpure Kit from Analytik Jena (Jena, Germany) and sent to sequencing (MWG Operon, Ebersberg, Germany). Nucleotide sequences were analyzed with public databases using BLAST (Basic Local Alignment Search Tool; http://blast.ncbi.nlm.nih.gov /Blast.cgi) to identify the nearest phylogenetic neighbors.

## 4.3 RESULTS AND DISCUSSION

### 4.3.1 Characterization of SRB communities in digester sludge

The examination of sulfate reducing microbial communities within digester sludge was performed by *dsrB* gene-based PCR-DGGE. The *dsrB* gene can be used as functional marker for SRB, since it encodes the dissimilatory sulfite reductase, a key enzyme in sulfate reduction catalyzing the reduction of sulfite to sulfide (Klein et al., 2001). The SRB diversity studies within anaerobic digester sludge from digesters A–D revealed that quite similar DGGE fingerprinting profiles were produced from the different sludges (Figure 4.2(I)) demonstrating that comparable SRB communities were present in these digesters examined.

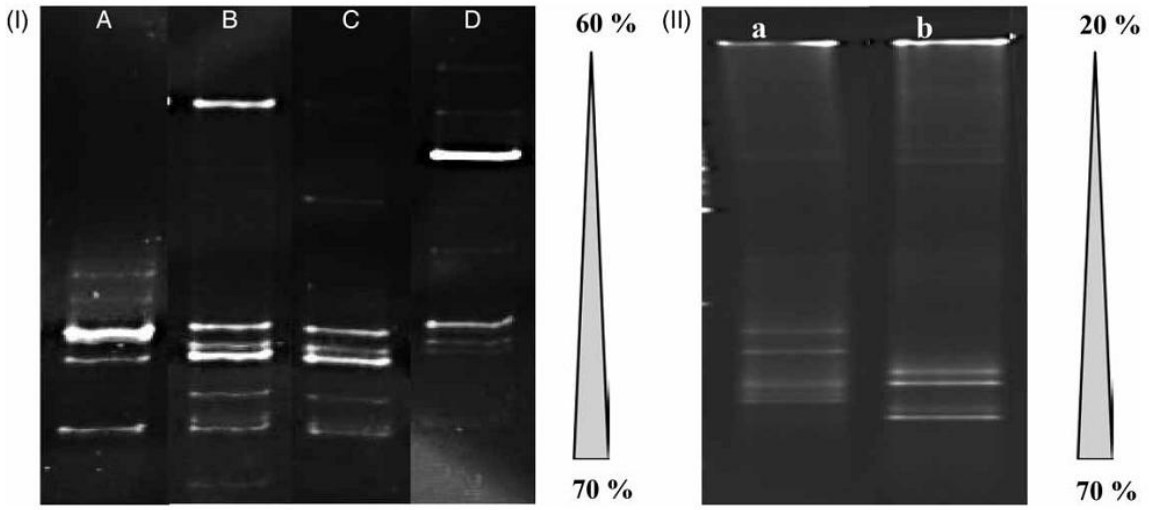



Figure 4.2 DGGE fingerprinting profiles. (I) *DsrB* gene-based DGGE of SRB detected in digester sludge from digesters A–D. (II) 16S rRNA gene-based DGGE profile of the biofilm sample (digester A) inoculated in DSMZ medium 35 containing $S^0$ (a) and DSMZ medium 68 containing $Na_2S_2O_3$ (b) as sole energy source. 6% polyacrylamide gel with denaturing gradients ranging from 60–70% (I) and 20–70% (II).

Sequence analysis of these DGGE bands revealed the presence of uncultured SRB (*Deltaproteobacteria*) in the digester sludges indicating SRB activity and $H_2S$ production. The conversion of sulfate to $H_2S$ by SRB is an initial step within the concrete corrosion process. Since the pH of digester sludge is normally slightly acidic to neutral (pH 6–7; (Derbal et al., 2009)), $H_2S$ is the dominant sulfide species. Due to its poor water solubility, the $H_2S$ will partition into the headspace (based on Henry's law) and re-partition into the condensate on the concrete surface because of the alkaline pH of the condensate layer (Roberts et al., 2002). At basic pH values, $H_2S$ is converted to $HS^-$ (pH 7–9.5) or $S^{2-}$ (pH > 9.5). When oxygen is available in the digester headspace, which might enter the system through cracks or desulfurization (see 4.1), the sulfide species chemically react to partially oxidized sulfur species (elemental sulfur, thiosulfate, and polysulfate species), which can be subsequently used as an energy source by SOB.

### 4.3.2 Analysis of enriched SOB cultures

Since the DNA extraction from the biofilm samples scratched off from the concrete surface resulted in insufficient mass, conventional cultivation techniques were applied. Previous cultivation studies have shown that sulfuric acid producing bacteria originating from concrete samples collected from a sewer pipe (Nica et al., 2000) and bridge support (Wei et al., 2010) could be successfully enriched using culture media. For the enrichment of a broad range of SOB a variety of culture media differing in pH and energy source were applied. As active SOB produce acid, pH decline was used as a positive indicator for growth. Within the mixed SOB cultures the acid producing activity was very high, since the pH within some liquid media decreased from 4.5 to 0.5 within 7 days (e.g., digester E). Similar observations were made with concrete samples collected from a bridge support, where microbes within the culture media were able to lower the pH from 6.5 to 2.5 within seven days (Wei et al., 2010). All enriched SOB liquid cultures exhibiting a pronounced pH decrease were used to cultivate and isolate pure SOB cultures on the corresponding agar media. Finally, three pure cultures originated from the headspace of digesters A, B, D, and E were gained and identified by colony PCR and sequence analysis (Table 4.2): *Acidithiobacillus thiooxidans, Thiomonas intermedia,* and *Thiomonas perometabolis.* The same three organisms were confirmed by PCR-DGGE and sequence analysis, from enriched SOB mixed cultures. The diversity study by DGGE identified even two additional SOB species for digesters A, B, and C which could not be cultivated on agar media: *Thiobacillus thiooparus* and *Paracoccus solventivorans* (Table 4.2). Finally, enrichment methods proved to be a good approach for cultivating and isolating different SOB species

although no information on the number of SOB cells actually present in the environmental samples is provided. Nevertheless, Wei et al. (2010) for instance, have demonstrated that *T. perometabolis* being the dominant acid producing bacterium within the enrichment medium was also the most frequently occurring within the original corroded concrete samples. Thus, dominant SOB species identified within the enriched cultures in the present study might also be the most abundant ones growing on the concrete surface of the digester headspace.

Table 4.2 Identification of various SOB species relevant for the BSA corrosion process in five different digesters in Germany (A-E).

| **Digester** | **Species** | **Detection method** | **Maximum identity [%]** | **pH optimum** | **Energy source** |
|---|---|---|---|---|---|
| A | *A. thiooxidans* | Pure culture: colony PCR | 99 | 2–4 | $Na_2S_2O_3$ |
| A | *P. solventivorans* | PCR-DGGE | 99 | 7–8 | $Na_2S_2O_3$ |
| B | *T. thioparus* | PCR-DGGE | 99 | 6–8 | $Na_2S_2O_3$ |
| B | *T. intermedia* | PCR-DGGE | 99 | 5.5–6 | $Na_2S_2O_3$ |
| B | *T. intermedia* | Pure culture: colony PCR | 100 | 5.5–6 | $Na_2S_2O_3$ |
| C | *P. solventivorans* | PCR-DGGE | 99 | 7–8 | $Na_2S_2O_3$ |
| D | *A. thiooxidans* | PCR-DGGE | 99 | 2–4 | $Na_2S_2O_3$ |
| D | *T. intermedia* | PCR-DGGE | 99 | 5.5–6 | $S^0$ |
| D | *T. intermedia* | Pure culture: colony PCR | 99 | 5.5–6 | $Na_2S_2O_3$ |
| D | *T. perometabolis* | Pure culture: colony PCR | 99 | 5.5–6 | $Na_2S_2O_3$ |
| E | *A. thiooxidans* | Pure culture: colony PCR | 99 | 2–4 | $Na_2S_2O_3$ |
| E | *T. intermedia* | PCR-DGGE | 99 | 5.5–6 | $Na_2S_2O_3$; $S^0$ |
| E | *T. perometabolis* | PCR-DGGE | 99 | 5.5–6 | $Na_2S_2O_3$; $S^0$ |

Furthermore, DGGE analysis revealed that the supply of different energy sources ($S^0$ or $Na_2S_2O_3$) caused a population shift within the enriched SOB cultures, although the SOB diversity remained constant (Figure 4.2(II), shown for digester A). *P. solventivorans* and *T. thioparus* could be only enriched in media containing $Na_2S_2O_3$ as the only source of energy (Table 4.2). *T. intermedia* and *T. perometabolis* were able to use either $Na_2S_2O_3$ or $S^0$. However, most identified SOB were detected in liquid media containing $Na_2S_2O_3$ as sole energy source. When additional sulfates such as $(NH_4)_2SO_4$, $MgSO_4$, and $FeSO_4$ were provided, no other SOB species could be cultivated. These results indicate that by applying different sulfate-based compounds no growth advantages can be achieved.

Generally, the process of microbial induced concrete corrosion (MICC) in a sewer pipe proceeds in a series of stages and a succession of microbial communities takes place (Bielefeldt et al., 2009; Roberts et al., 2002). The BSA process within sludge digesters is likely to occur in the same way. In a first step, bacteria in the digester sludge produce carbon dioxide, $H_2S$, and other gases with acidic properties leading to a gradual pH decrease of the concrete surface from an initial value of approximately 12 to 9. Once these abiotic processes have lowered the pH to around 9, microbial activity can take place and neutrophilic sulfur oxidizing bacteria (NSOB) start colonizing the concrete surface. *T. thioparus, T. neapolitanus, T. intermedia,* and *T. perometabolis* are typically considered as NSOB in sewer systems (Vollertsen et al., 2008; Wei et al., 2014). *T. thioparus* was reported to be the first to colonize new concrete surfaces, but disappears with ongoing corrosion (Wei et al., 2010). The NSOB oxidize sulfide, sulfur and thiosulfate and produce polythionic acids as well as sulfuric acid leading to a further pH decrease of the concrete matrix from approximately 9 to 3.5–5.0 (Bielefeldt et al., 2009). At pH values below 5, acidophilic sulfur oxidizing bacteria (ASOB), typically *A. thiooxidans*, continue sulfide oxidation by producing high amounts of $H_2SO_4$. The pH drops further to values of 0–1.5 (Diercks et al., 1991; Wei et al., 2014). *A. thiooxidans* is found to be the most dominant SOB in heavily corroded concrete (Diercks et al., 1991; Okabe et al., 2007). The different SOB species found in this study and their corresponding pH optima provide information on the progression of microbial corrosion. Especially in digesters A, D, and E, the detection of *A. thiooxidans* (pH optimum 2–4), the key organism in the BSA process (Diercks et al., 1991), provides evidence of an advanced BSA attack. In these digesters also the high acid producing activity within the enriched SOB cultures could be positively correlated to the degree of damage (Figure 4.1; shown for digester E). In digesters B and C, *T. thioparus, T. intermedia*, and *P. solventivorans* with pH optima of 5.5–8 were identified. The occurrence of these NSOB indicates a lower extent of corrosion. Although *P. solventivorans* was not mentioned in the context of BSA before, it seems to play a role

within the corrosion process as well, since it was detected in two digesters (A and C) and some *Paracoccus* species are capable of oxidizing sulfur compounds (Kelly et al., 2006). Finally, within this study, different active SOB were isolated from the biofilm that was taken from the headspace of different digesters. Since the SOB species found are strictly aerobic, oxygen which might come through imperfections or desulfurization must be available within the system initiating the BSA corrosion process.

However, the application of enrichment cultures and PCR-DGGE technique give no or only little information on the number of SOB cells originally present on the concrete surface of the digester headspace. To avoid the enrichment step, genomic DNA needs to be directly isolated from the biofilm samples. Since the isolation of genomic DNA has not been successful so far, probably due to low biomass concentration, the optimization of extraction techniques on low DNA content is required. In a further step, quantitative real-time PCR technique (qPCR) can be used for quantifying the different SOB species within the biofilm samples to better correlate the dominant SOB with the degree of concrete corrosion in sludge digesters.

## 4.4 CONCLUSIONS

The detection of the relevant bacteria involved in the BSA corrosion process (SRB and SOB) in five different digesters in Germany verified the proposed hypothesis (see hypothesis # 1; Chapter 2). The SRB diversity studies by *dsrB*-gene based DGGE revealed that different uncultured SRB (*Deltaproteobacteria*) were present in digester sludge indicating $H_2S$-producing potential. For the first time, SOB (*A. thiooxidans, T. intermedia, T. perometabolis, T. thiooparus,* and *P. solventivorans*) were identified in the digesters demonstrating that BSA corrosion is not only a problem prevalent for sewer pipes, but can also occur in sludge digesters. Conventional cultivation techniques for the enrichment and isolation of the SOB species *A. thiooxidans*, *T. intermedia*, and *T. perometabolis* proved to be very effective. With PCR-DGGE and sequence analysis the SOB communities in the mixed enriched SOB cultures could be described more accurately. In addition, the presence of SOB species characterized by an optimum growth at different pH could be correlated to the progression of microbial corrosion.

# Chapter 5

## Characterization of Sulfur Oxidizing Bacteria related to Biogenic Sulfuric Acid Corrosion in Sludge Digesters[2]

Biogenic sulfuric acid (BSA) corrosion damages sewerage and wastewater treatment facilities but is not well investigated in sludge digesters. Sulfur/sulfide oxidizing bacteria (SOB) oxidize sulfur compounds to sulfuric acid, inducing BSA corrosion. To obtain more information on BSA corrosion in sludge digesters, microbial communities from six different, BSA-damaged, digesters were analyzed using culture dependent methods and subsequent polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE). BSA production was determined in laboratory scale systems with mixed and pure cultures, and *in situ* with concrete specimens from the digester headspace and sludge zones. The SOB *Acidithiobacillus thiooxidans*, *Thiomonas intermedia*, and *Thiomonas perometabolis* were cultivated and compared to PCR-DGGE results, revealing the presence of additional acidophilic and neutrophilic SOB. Sulfate concentrations of 10-87 mmol/L after 6-21 days of incubation (final pH 1.0-2.0) in mixed cultures, and up to 433 mmol/L after 42 days (final pH <1.0) in pure *A. thiooxidans* cultures indicated huge sulfuric acid production potentials. Additionally, elevated sulfate concentrations in the corroded concrete of the digester headspace in contrast to the concrete of the sludge zone indicated biological sulfur/sulfide oxidation. The presence of SOB and confirmation of their sulfuric acid production under laboratory conditions reveal that these organisms might contribute to BSA corrosion within sludge digesters. Elevated sulfate concentrations on the corroded concrete wall in the digester headspace (compared to the sludge zone) further indicate biological sulfur/sulfide oxidation *in situ*. For the first time, SOB presence and activity is directly relatable to BSA corrosion in sludge digesters.

[2] Huber, B., Herzog, B., Drewes, J.E., Koch, K, Müller, E. 2016. Characterization of sulfur oxidizing bacteria related to biogenic sulfuric acid corrosion in sludge digesters. Revisions submitted to *BMC Microbiology*.

## 5.1 INTRODUCTION

Microbial deterioration of concrete by biogenic sulfuric acid (BSA) is a serious and common problem in wastewater treatment facilities. Worldwide, maintenance and retrofitting of degraded concrete structures costs several billions of dollars every year (Hewayde et al., 2007). BSA corrosion is a multistage process of sulfur/sulfate reducing (SRB) and sulfur/sulfide oxidizing bacteria (SOB). The first, anaerobic, step occurs when SRB reduce sulfate and other oxidized sulfur compounds to hydrogen sulfide ($H_2S$) (Cayford et al., 2012). $H_2S$ volatilizes and dissolves in the moist concrete surface (Okabe et al., 2007). The initial pH of concrete is approximately 12.0, a value hardly allowing microbial growth (Okabe et al., 2007). $H_2S$, $CO_2$ and other gases with acidic properties abiotically decrease the pH to values around 9.0 enabling the colonization of neutrophilic sulfur oxidizing bacteria (NSOB) such as *Thiobacillus* spp. and *Thiomonas* spp. (Roberts et al., 2002). These NSOB, oxidize $H_2S$ and other reduced sulfur compounds to sulfuric acid ($H_2SO_4$) and polythionic acids thus reducing the pH to around 3.5-5.0 (Bielefeldt et al., 2009). At pH 5.0 and below, acidophilic sulfur oxidizing bacteria (ASOB) such as *Acidithiobacillus thiooxidans*, continue sulfur oxidation by producing high amounts of sulfuric acid that decreases the pH to 1.0-2.0 (Diercks et al., 1991; Wei et al., 2014). $H_2SO_4$ reacts with the cement matrix leading to the formation of gypsum ($CaSO_4 \cdot 2H_2O$) and ettringite ($3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O$) (O'Connell et al., 2010). These expansive sulfate salts lead to internal cracks in the concrete and finally to structural failure (Wells & Melchers, 2015). Corrosion rates of several millimeters per year are reported for sewer pipes (Vollertsen et al., 2008). BSA corrosion, although well described in sewer pipes, is hardly investigated in sludge digesters where anaerobic conditions enable the growth of SRB and $H_2S$ production (Appels et al., 2008), but the occurrence of aerobic SOB, comes unexpected.

The hypothesis, that SOB species within the enriched cultures are able to produce sulfuric acid and might thus contribute to BSA corrosion in sludge digesters (see hypothesis # 2; Chapter 2), was evaluated in this study by identifying BSA-related bacteria and evaluating their corrosion potential. Biofilm from concrete surfaces, potentially containing SOB, was collected in the headspace of six different full-scale digesters at wastewater treatment plants in Germany. The digesters operated for 23 to 52 years and showed characteristic corrosion damage patterns (Figure 5.1). Conventional cultivation techniques showed the ability of the microbial biofilm community to produce sulfuric acid under controlled laboratory conditions. SOB, isolated and identified from enriched biofilm cultures, achieved a pH drop and sulfuric acid production thus indicating BSA corrosion potential. To characterize the community composition in-depth, polymerase chain reaction

denaturing gradient gel electrophoresis (PCR-DGGE) was applied while *in situ* sulfate measurements in concrete samples taken from the digester headspace and sludge zone provided further information on SOB activity.

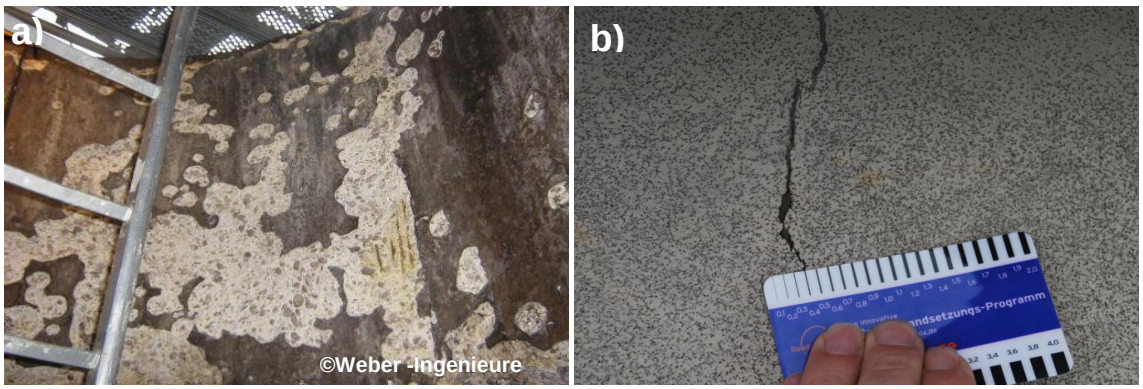



Figure 5.1 a) Digester headspace with severe concrete corrosion. b) Cracks (up to 0.4 mm) on the outside of a digester manhole-pit.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Sample Collection

Biofilm samples were collected from corroded headspace concrete surfaces of six different full-scale digesters at wastewater treatment plants in Germany (A-F). Detailed information about the digesters is provided in Table 5.1. Biofilms were sampled by Weber-Ingenieure GmbH (Pforzheim, Germany) from the concrete surfaces with a sterile spatula, transferred to a sterile 50 mL tube, and stored at 4°C for not longer than 48 h before inoculation in liquid media.

Table 5.1 Design characteristics of the six digesters A-F.

| Dg | Year of construction | WWTP PE | Digester volume [$m^3$] | RT [d] | Operating temperature [°C] | Sulfate in drilling dust headspace [% w/w] | Sulfate in drilling dust sludge zone [% w/w] |
|---|---|---|---|---|---|---|---|
| A | 1969 | 110,000 | 1,100 | 18 | 38 | 1.2 | 0.1 |
| B | 1974 | 83,000 | 1,150 | 25 | 37 | 0.5 | 0.4 |
| C | 1980 | 83,000 | 1,000 | 27 | 38 | 0.4 | 0.3 |
| D | 1990 | 30,000 | 2,000 | 35 | 40 | n.d.* | n.d.* |
| E | 1963 | 10,000 | 320 | 60 | 30–33 | 0.7 | 0.5 |
| F | 1982 | 94,500 | 2,100 | 35 | 39 | 0.6 | 0.2 |

Dg = Digester, WWTP PE = Wastewater Treatment Plant Population Equivalent, RT = Retention Time, *not determined

### 5.2.2 SOB enrichment and cultivation

Four culture media, differing in energy source and pH, were applied to cultivate a variety of SOB communities. DSMZ medium 35 (*Acidithiobacillus thiooxidans* medium) and DSMZ medium 68 (*Thiobacillus neapolitanus* medium) were prepared according to DSMZ instructions (http://www.dsmz.de/home.html). DSMZ medium 35 (pH 4.5) contained elemental sulfur and DSMZ medium 68 (pH 6.0 and 8.0) $Na_2S_2O_3$ as only energy sources. The other two media were prepared as described by Starosvetsky et al. (2013): ATCC medium #125 (pH 4.1) with elemental sulfur and *Thiobacillus* medium (pH 4.1) with $Na_2S_2O_3$ as sulfur sources. The enrichment of SOB in specific liquid media was performed as described in Chapter 4. All enrichment cultures that exhibited a significant pH decrease were transferred to corresponding solid media (agar concentration 1.5%). The colonies were separated according to their different morphologies and streaked onto fresh solid media until pure cultures were obtained after three repetitions. Pure cultures were sequenced while the microbial diversity of the enriched cultures was additionally analyzed by PCR-DGGE. Figure 5.2 graphically summarizes the applied steps for sample processing.

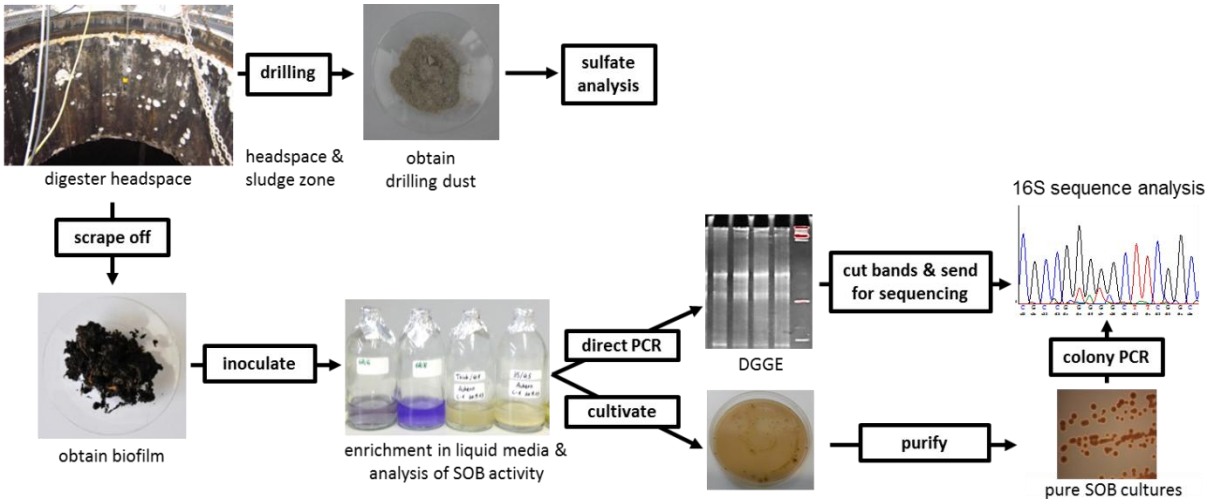



Figure 5.2 Different steps of sample processing.

### 5.2.3 PCR amplification of the 16S rRNA gene

For the identification of SOB pure cultures, colony PCRs were carried out using universal bacterial primers 27f (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492r (5′-TAC GGY TAC CTT GTT ACG ACT T-3′, (Lane, 1991)) amplifying the nearly full-length 16S rRNA gene. For DGGE analysis of the mixed SOB cultures, a 16S rRNA gene fragment (~550 bp) was amplified using bacterial primers 27f and 517r (5′-GTA TTA CCG CGG

CTG CTG GC-3′ (Furushita et al., 2003)), with the forward primer containing a GC-clamp (40 bp) at the 5´end (5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCG CCC CCG CCC CGG-3′ (Muyzer et al., 1993)). PCR conditions for the primers 27f/1492r and 27f/517r included an initial denaturation at 95°C for 2 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, elongation at 95°C for 30 s and final elongation at 72°C for 5 min. PCR primers were obtained from Eurofins MWG Operon (Ebersberg, Germany). PCR was carried out with a primus 96 cycler (PeqLab Biotechnologie GmbH, Erlangen, Germany) using GoTaq(R) G2 Hot Start Colorless Master Mix (Promega GmbH, Mannheim, Germany) according to manufacturer's instructions.

### 5.2.4 DGGE analysis

SOB-diversity studies in mixed cultures were performed with DGGE using 15 µl of the 550 bp PCR product. Separation was carried out with a 6% (w/v) polyacrylamide gel using the DCode™ Universal Mutation Detection System (Bio-Rad Laboratories, Munich, Germany). A denaturing gradient from 20% to 80% was used (100% denaturing solution defined as 7 M urea and 40% (v/v) formamide). Electrophoresis was performed at 55°C for 16.5 h at a constant voltage of 60 V. The polyacrylamide gels were stained with ethidium bromide (0.5 µg/mL) for 20 min, rinsed with Milli-Q-water (Millipore, Bedford, USA), documented under UV-light (312 nm), and the dominant bands cut out with a sterile scalpel. The DNA was eluted in sterile Milli-Q water (24 h, 37°C) and re-amplified using the primers 27f and 517r without GC-clamp.

### 5.2.5 16S rRNA sequencing

Purified PCR products (innuPREP DOUBLEpure Kit, Analytik Jena, Jena, Germany) were sequenced by Eurofins MWG Operon (Ebersberg, Germany). Sequences were assembled with Geneious 7.1.7 (http://www.geneious.com), analyzed with ENA (European Nucleotide Archive) sequence search (http://www.ebi.ac.uk/ena/search), and aligned with SINA 1.2.11 (Pruesse et al., 2012). Phylogenetic analyses were performed with MEGA6 (Tamura et al., 2013). Phylogenetic trees of nearly full length and 16S rRNA gene fragments were calculated based on the maximum composite likelihood method with 2,000 bootstrap replications (n= 2,000). Sequences obtained from pure cultures and DGGE bands were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) to get accession numbers.

### 5.2.6 SOB activity of mixed and pure cultures

Sulfuric acid production by SOB was measured under laboratory conditions via pH and sulfate monitoring. The pH was consistently tracked in all liquid cultures under sterile conditions using pH indicator strips (MColorpHast™, Merck Millipore, Billerica, USA), as its decline served as an indicator for SOB activity and growth. The sulfate concentration was measured in selected mixed cultures (batch cultures) after incubation of six to 22 days (Table 5.2). Sulfate measurements were carried out in the inoculated DSMZ medium 35 with elemental sulfur as the only energy source following German Standard Methods for the examination of water, wastewater and sludge (DEV, 2016).

A long-term sulfate measurement for 42 days in batch-configuration (DSMZ medium 35), monitored the BSA production of isolate DgE-1 (99% similarity with *A. thiooxidans,* LN864656).

### 5.2.7 *In situ* SOB activity monitoring

To gain information about the SOB activity *in situ*, the sulfate content on the digester concrete wall was determined (Table 5.1). One concrete composite sample consisting of three bores was taken from the headspace and the sludge zone from every digester showing characteristic corrosion damage patterns (washed out concrete surface). The concrete was sampled in form of drilling dust in a depth of 0-40 mm using a hollow drill (25 mm diameter) by Weber-Ingenieure GmbH (Pforzheim, Germany). For sulfate measurements, the drilling dust samples were thermally disintegrated at 80°C and 15% (v/v) hydrochloric acid followed by a photometrical analysis at 436 nm (Nanocholor 500 D, Macherey und Nagel, Germany). The analysis was performed by the laboratory “Dr. Michael Figgemeier-Baustoffanalyse & Bauphysik” (Ludwigsburg, Germany).

## 5.3 RESULTS

### 5.3.1 Enriched SOB cultures

The highest SOB diversities were obtained in *Thiobacillus* medium (pH 4.1) and DSMZ medium 68 (pH 6.0 and 8.0). The diversity assessment by PCR-DGGE with the mixed liquid cultures revealed different phylogenetic diversities in the six analyzed sludge digesters (Figure 5.3). 12 taxonomically distinct genera were found with PCR-DGGE. Highest diversities were observed in digesters D and E, with five and eight different genera, respectively. A lower microbial diversity was observed in the remaining four

digesters. Eight of the 12 genera are affiliated with sulfur-oxidizing bacteria and are marked in bold in the phylogenetic tree (Figure 5.3). Within the liquid cultures of all six digesters (Dg A-F), eight sulfur oxidizers were closely related to *Thiomonas* sp. (Dg A, B, D, E, F), *Delftia* sp. (Dg D and E), *Hyphomicrobium* sp. (Dg E), *Ancylobacter* sp. (Dg D), *Paracoccus* sp. (Dg A and C), *Mesorhizobium* sp. (Dg E), *Acidithiobacillus* sp. (Dg D and E), and *Alicyclobacillus* sp. (Dg B and E). The other four genera *Sphingomonas* sp. (Dg E), *Stenotrophomonas* sp. (Dg D), *Sphingobacterium* sp. (Dg C), and *Moraxella* sp. (Dg E) are non-SOB and might not be related to the sulfur cycle.

Table 5.2 pH and sulfate concentration measurements.

| Digester | Sample No. | Incubation time [d] | pH value | Final sulfate concentration [mmol/L] |
|---|---|---|---|---|
| A | 1 | 13 | 2.0 | 14 |
| A | 2 | 13 | 2.0 | 16 |
| B | 3 | 14 | 2.0 | 14 |
| B | 4 | 14 | 2.0 | 21 |
| D | 5 | 14 | 2.0 | 19 |
| D | 6 | 22 | 1.5 | 30 |
| D | 7 | 14 | 1.5 | 52 |
| E | 8 | 21 | 2.0 | 12 |
| E | 9 | 14 | 2.0 | 17 |
| E | 10 | 6 | 1.5 | 33 |
| E | 11 | 6 | 1.5 | 50 |
| E | 12 | 8 | 1.0 | 87 |
| F | 13 | 14 | 2.0 | 10 |
| F | 14 | 14 | 2.0 | 11 |
| F | 15 | 14 | 2.0 | 13 |

Analyses were performed in selected mixed enriched batch cultures after 6-22 days of incubation. DSMZ medium 35 (*A. thiooxidans* medium) with an initial pH value of 4.5 and elemental sulfur as sole energy source was used as culture medium.

Sulfate concentration measurements of specific mixed liquid cultures were used to analyze BSA production capacity (Table 5.2). The presence and laboratory-confirmed activity of different SOB was considered as BSA corrosion potential. For sulfate measurements, DSMZ medium 35 was used, because elemental sulfur was the only provided sulfur compound leading to an initial sulfate concentration lower than 0.5 µmol/L. The microbial community in this specific liquid medium, inoculated with biofilm from Dg E, reduced the pH from 4.5 to 1.0-1.5 (Table 5.2, No. 10-12) after six (No. 10 and 11) and eight days (No. 12) of incubation, respectively, while the sulfate concentration increased to 33-87 mmol/L indicating high sulfuric acid production. The main acid producers were

identified as *Acidithiobacillus* spp. and *Thiomonas* spp. (Figures 5.3 and 5.4). Dg D showed a high sulfuric acid production, too, and liquid cultures No. 6 and 7 reached sulfate concentrations of 30 mmol/L and 52 mmol/L after 22 days and 14 days of incubation, respectively. The sulfate concentrations in the other analyzed cultures fluctuated between 10 mmol/L and 21 mmol/L showing a lower BSA production, with the lowest (10-13 mmol/L) found in digester F, where only the NSOB *Thiomonas* sp. was detected (Figures 5.3 and 5.4).

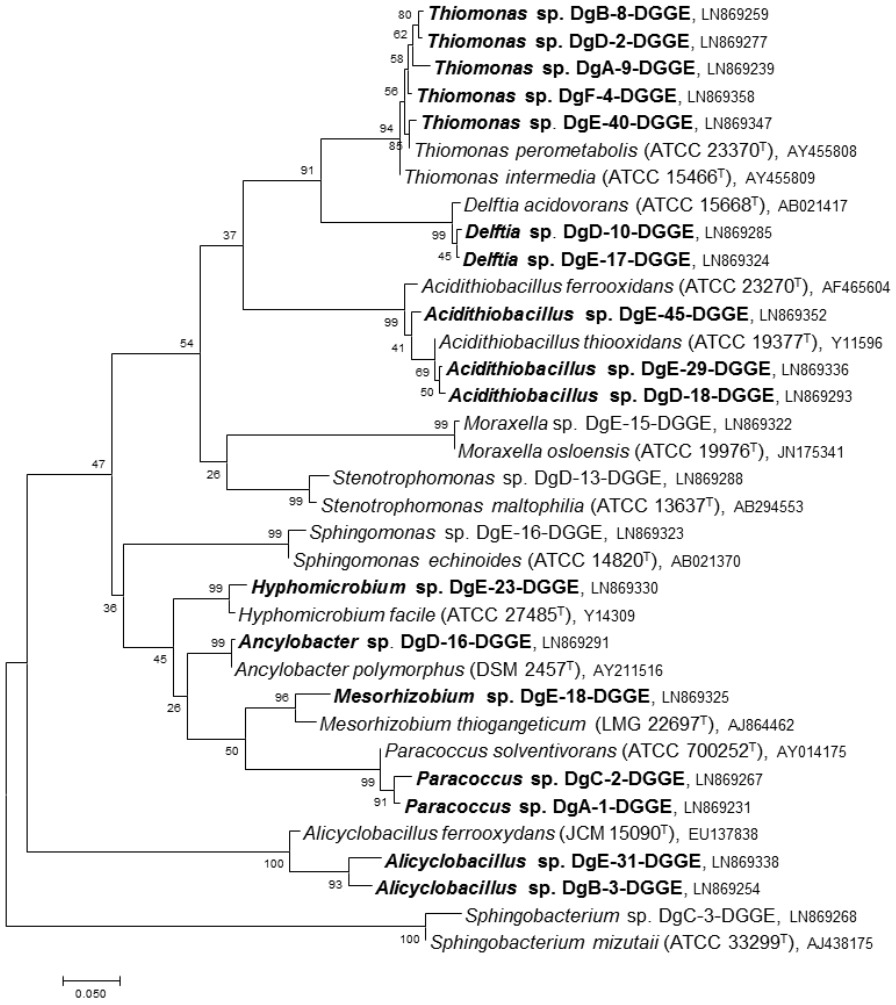



Figure 5.3 Maximum likelihood based phylogenetic tree showing the most dominant species in enrichment cultures from digesters (Dg) A-F regarding DGGE analyses (partially 16S rRNA gene sequences, 550 bp) and their respective type strains. Sulfur oxidizing genera are marked in bold. The evolutionary history was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-4546.7510) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5303)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted with MEGA6.

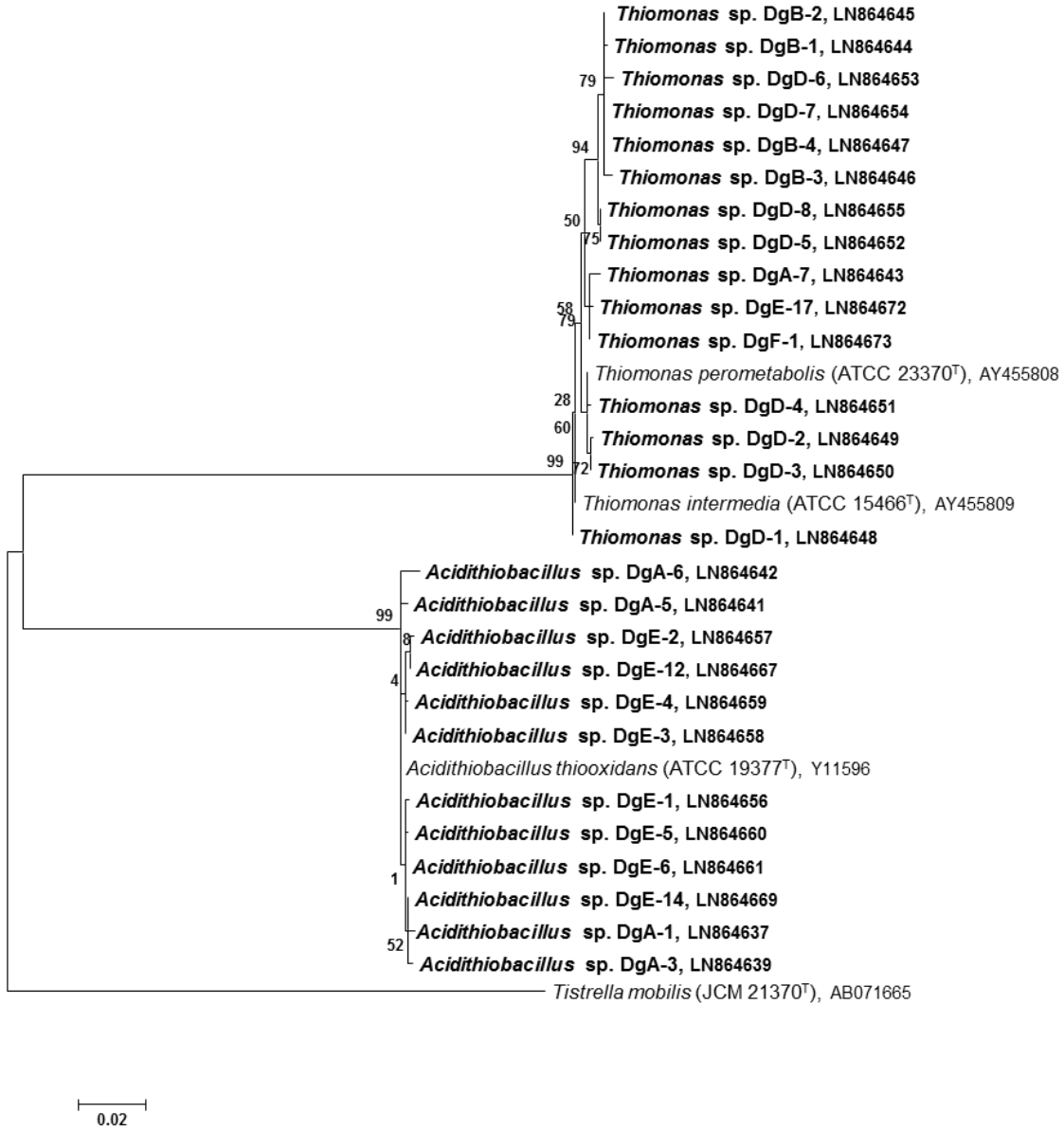



Figure 5.4 Maximum likelihood based phylogenetic tree of almost complete 16S rRNA gene sequences from pure SOB cultures obtained from digesters (Dg) A-F. Their respective type strains are indicated with a superscripted T. *Tistrella mobilis* served as out-group. Maximum likelihood calculations were based on the Tamura-Nei model (Tamura & Nei, 1993) and the tree with the highest log likelihood (-4678.6398) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3865)).

### 5.3.2 Pure SOB cultures

Enrichments that exhibited a significant pH decrease were streaked on their corresponding solid medium to isolate pure SOB. For isolation, DSMZ medium 68 (pH 6.0) and *Thiobacillus* medium (pH 4.1) showed the best results. Approximately 40 isolates that showed a pH decline on the agar medium (visible due to pH indicators) were identified by 16S rRNA gene sequence analysis. Three sulfur oxidizing species, associated with concrete corrosion, were closely related to *Acidithiobacillus thiooxidans*, *Thiomonas intermedia* and *Thiomonas perometabolis*. Their phylogenetic relation is displayed in Figure 5.4 In Dg A and E, the acidophilic *Acidithiobacillus* spp. and neutrophilic *Thiomonas* spp. were detected, whereas in Dg B, D and F only neutrophilic *Thiomonas* spp. were identified.

By applying different media, varying in initial pH and sulfur components, a variety of SOB species detected by DGGE in mixed culture (Figure 5.3) was obtained. Enrichment and cultivation of mixed cultures worked best in DSMZ medium 68 and *Thiobacillus* medium. For cultivation of pure *A. thiooxidans*, best growth occurred in DSMZ medium 35 (pH 4.5) with elemental sulfur as sole energy source. After an incubation period of two weeks, *A. thiooxidans* reduced the pH in DSMZ medium 35 from 4.5 to 0.5 indicating a high sulfuric acid production and BSA corrosion potential. For cultivation of pure *Thiomonas* spp., media with $Na_2S_2O_3$ and initial pH values of 4.0-6.0 showed best results (DSMZ 68 and *Thiobacillus* medium). *Thiomonas* spp. reduced the pH within the used DSMZ medium 68 from 6.0 to 2.5 after two weeks of incubation, also revealing a high acid production potential.

A long term sulfate measurement over 42 days in DSMZ medium 35, inoculated with *A. thiooxidans* isolate DgE-1 (LN864656; see Figure 5.4) from digester E, reached a sulfate concentration of more than 417 mmol/L (Figure 5.5). When comparing the sulfuric acid production of pure *A. thiooxidans* with the mixed enriched cultures, a similar trend was observed within the first days of incubation (Figure 5.5 and Table 5.2). After six days of incubation, the sulfate concentration in the mixed enrichments (33-50 mmol/L) was comparable to the sulfate concentration measured in the pure culture (~21 mmol/L). After an incubation period of 14 days, the sulfuric acid production in pure *A. thiooxidans* was significantly higher (130 mmol/L) than in the mixed cultures (10-52 mmol/L), and after 21 days, the difference between the mixed enrichments (12-30 mmol/L) and the pure *A. thiooxidans* (~208 mmol/L) was even greater.

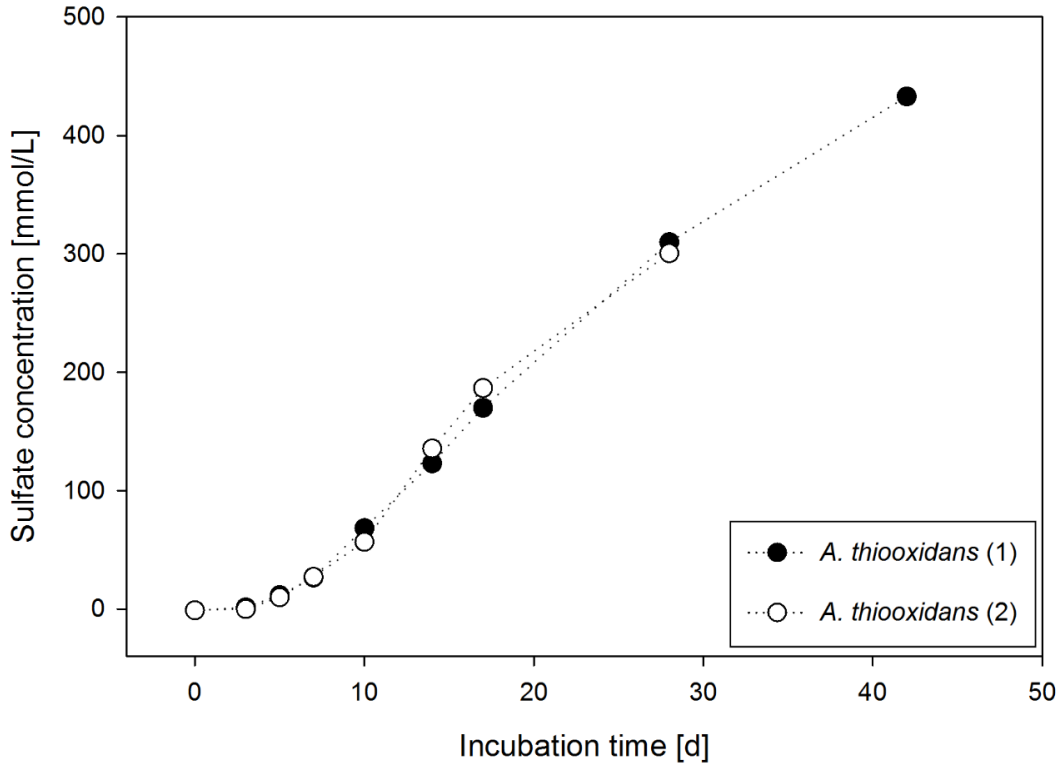



Figure 5.5 Final sulfate concentration in pure *A. thiooxidans* liquid batch culture (isolate Dg E-1) over 42 days. DSMZ medium 35 with an initial pH value of 4.5 and elemental sulfur as sole energy source was used. Error of the method was indicated by the manufacturer with 10%.

### 5.3.3 *In situ* BSA activity in sludge digester

All analyzed digesters (A-C and E-F) showed a higher sulfate content on the concrete surface of the digester headspace than in the sludge zone (Table 5.1). The highest difference was observed in Dg A, where the sulfate concentration in the headspace (1.2% w/w) was more than ten-times higher than in the sludge zone (0.1% w/w), potentially resulting from the activity of the ASOB *A. thiooxidans* found in this digester (Figure 5.4).

## 5.4 DISCUSSION

### 5.4.1 Cultivation and isolation of active SOB communities

SOB within the biofilm samples were specifically enriched to test their sulfuric acid production activity. Although cultivation dependent techniques may not be appropriate to draw a comprehensive picture of the microbial community, they are the only and still

powerful method to investigate the capability of sulfuric acid production and BSA corrosion potential under defined conditions. By enrichment in specific media, different SOB species were purified (Figure 5.4). A variety of BSA related bacteria were identified in the liquid cultures (Figure 5.3), and their sulfuric acid production capacity was demonstrated under laboratory conditions (10-433 mmol/L).

For SOB purification different media with elemental sulfur and sodium thiosulfate as only energy sources were applied to obtain a high SOB diversity. Thiosulfate, the most frequently used substrate for SOB cultivation, is, in contrast to elemental sulfur, highly water soluble and stable over a broad pH range (Robertson & Kuenen, 2006). In addition, autotrophic media were utilized as the main contributors to corrosion, e.g., *Acidithiobacillus* spp. and *Thiomonas* spp., are known obligate or facultative autotrophs. Furthermore, such media suppress the growth of unwanted heterotrophic bacteria, which are most likely dominant in the biofilm sample. One major problem in obtaining selective enrichment of chemolithoautotrophic organisms is the contamination through organic compounds (Robertson & Kuenen, 2006) resulting in the detection of non-SOB species. Sources for heterotrophic contaminants are i) contaminated water or chemicals used for media preparation, ii) trace amounts of soluble organic material in almost all agar brands, and iii) secretion of organic compounds by obligate chemolithotrophs (Robertson & Kuenen, 2006). Apart from the identification of a few heterotrophic non-SOB species, the application of selective culture media allowed to specifically enrich the organisms of interest (Figures 5.3 and 5.4). This shows the potential of highly selective media as the desired organisms grew best under these conditions. However, a more comprehensive picture of the SOB diversity within the liquid media was drawn by PCR-DGGE (Figure 5.3). It has to be mentioned, that only the combination of cultivation dependent and independent (PCR-DGGE) techniques revealed a variety of taxonomically different sulfur oxidizers that can be classified in acidophilic and neutrophilic sulfur oxidizing bacteria (ASOB and NSOB) as well as non-SOB.

### 5.4.2 Acidophilic sulfur oxidizing bacteria (ASOB)

*Acidithiobacillus* spp. and/or *Alicyclobacillus* sp. were identified in enriched cultures of Dg A, B, D and E (Figures 5.3 and 5.4) and are known to produce sulfuric acid from reduced sulfur compounds (Rohwerder & Sand, 2007). The identification of ASOB in the apparently neutral digester environment suggests that pH gradients and “acidic microniches” might be present, especially in the biofilm of the digester headspace (Robertson & Kuenen, 2006).

All members of the genus *Acidithiobacillus* are obligate acidophiles and characterized by chemolithoautotrophic growth (Robertson & Kuenen, 2006). Pure *A. thiooxidans* with a growth optimum at pH 2.0-4.0 can be cultivated in acidic media with elemental sulfur as the only nutrient (Robertson & Kuenen, 2006; Yin et al., 2014), as has been confirmed in this study as well. *A. thiooxidans,* found in Dg A, D and E, is a key organism for BSA corrosion, because it has been the most dominant species in heavily corroded concrete samples (Diercks et al., 1991; Okabe et al., 2007). *A. thiooxidans* can produce high amounts of sulfuric acid and grows at pH values as low as 0.5 (Diercks et al., 1991; Robertson & Kuenen, 2006). In this study, *A. thiooxidans* produced a sulfuric acid concentration of 4% (Figure 5.5). Cwalina (2008) stated that biogenic $H_2SO_4$ in concrete pores may even reach 10%.

*Alicyclobacillus* sp. was detected within the enrichment cultures of Dg D and E using PCR-DGGE. A few members of the genus *Alicyclobacillus* have been described as sulfur- and ferrous-oxidizing (Guo et al., 2009). A study by Vupputuri et. al. (2013), analyzing the microbial diversity on concrete surfaces from deteriorated bridge structures, revealed that *Alicyclobacillus* sp. was the most dominant sulfur oxidizing acid producer that reduced the pH value of the culture medium from 6.7 to 2.8.

### 5.4.3 Neutrophilic sulfur oxidizing bacteria (NSOB)

The presence of *Thiomonas intermedia* and *Thiomonas perometabolis,* obtained in pure (Figure 5.4), was already described in corroded concrete samples (Gomez-Alvarez et al., 2012; Okabe et al., 2007; Wei et al., 2010). A study by Wei et al. (2010) using liquid cultures inoculated with corroded material from a bridge support, found *T. perometabolis* as the dominant acid producer.

Another NSOB, *Paracoccus* sp., occurred in liquid cultures of Dg A and C and is known to oxidize reduced sulfur compounds (e.g. thiosulfate and elemental sulfur) to generate energy for autotrophic growth (Kelly et al., 2006).

The genera *Ancylobacter, Mesorhizobium*, *Hyphomicrobium* and *Delftia* comprise sulfur/sulfide oxidizing species, but are not typically mentioned in the context of BSA corrosion. Growth tests with *Ancylobacter aquaticus* showed its ability to grow chemolithoautrotrophically when thiosulfate was provided as only energy source (Stubner et al., 1998). For *Mesorhizobium thiogangeticum*, originally identified in rhizosphere soil, chemolithoautotrophic growth was observed with $Na_2S_2O_3$ and $S^0$ (Ghosh & Roy, 2006). *Hyphomicrobium* sp. is known for its oxidation of hydrogen sulfide to elemental sulfur

(Mohapatra et al., 2008). SOB, isolated from a rice field soil, were closely related to *Delftia* sp. (Graff & Stubner, 2003) indicating its ability for sulfur-oxidation.

### 5.4.4 Non-SOB species

Other heterotrophic microorganisms not commonly associated with sulfur oxidation and thus termed non-SOB, e.g. *Sphingobacterium* sp., *Sphingomonas* sp., and *Stenotrophomonas* sp., were detected in this study as well. The identification of heterotrophic non-SOB in the enrichment cultures was probably due to their presence in the original biofilm. A contamination with organic residues from the biofilm sample may have enabled the growth of heterotrophic non-SOB in the culture media. Furthermore, many obligate chemolithotrophic sulfur oxidizers produce organic substances that could be subsequently utilized by heterotrophs (Robertson & Kuenen, 2006) leading to the growth of non-SOB species. However, the non-SOB detected in this study might still play an important role because their presence was already reported in several samples of corroded concrete originating from different sewer pipes. SOB might interact with non-SOB in the biofilm matrix where excreted metabolites could serve as nutrients for non-SOB or vice versa. *Sphingobacteriales*, for instance, are dominant in microbial induced concrete corrosion layers (Cayford et al., 2012) and *Sphingomonas* sp. was detected in corroded sewer pipes above the water level (Vincke et al., 2001). *Stenotrophomonas maltophilia* was found in slightly corroded concrete material but was also observed in the surrounding of the steel bar (Okabe et al., 2007; Vincke et al., 2001).

### 5.4.5 Oxygen availability and BSA corrosion in sludge digesters

In contrast to sewer pipes, oxygen availability in sludge digester is rather limited (Appels et al., 2008; Rasi et al., 2007) and thus, crucial for BSA corrosion. However, in this study typical BSA corrosion damage patterns, characterized by washed out concrete surfaces, were observed in the headspace of several digesters (see Figure 5.1), indicating SOB activity resulting in BSA production and corrosion. Thus oxygen carriers must be at least available in small patches fostering the growth of sulfur-oxidizing communities. Many sulfur oxidizers can grow in niches, where sulfide and oxygen coexist (Robertson & Kuenen, 2006). When oxygen, even at low levels, is available, sulfur oxidizers can spontaneously oxidize sulfide. Very high turnover rates of sulfide were reported even at extremely low concentrations of sulfide and oxygen ($< 10^{-6}$ mM) (Robertson & Kuenen, 2006). It is supposed that oxygen availability in microniches might be sufficient for SOB

activity and enable the oxidation of sulfur compounds to sulfate or sulfuric acid. Thermodynamically, oxidation of sulfuric compounds is always favored compared to the oxidation of methane, as is applied in biological *in situ* desulfurization in digesters. The predominant anaerobic conditions in a sludge digester promote the growth of SRB communities and consequently sulfur/sulfate reduction (continuous lines, Figure 5.6). In case of local oxygen availability, especially in the digester headspace biofilm, it is assumed that sulfur/sulfide oxidation by SOB takes place (dashed lines, Figure 5.6).

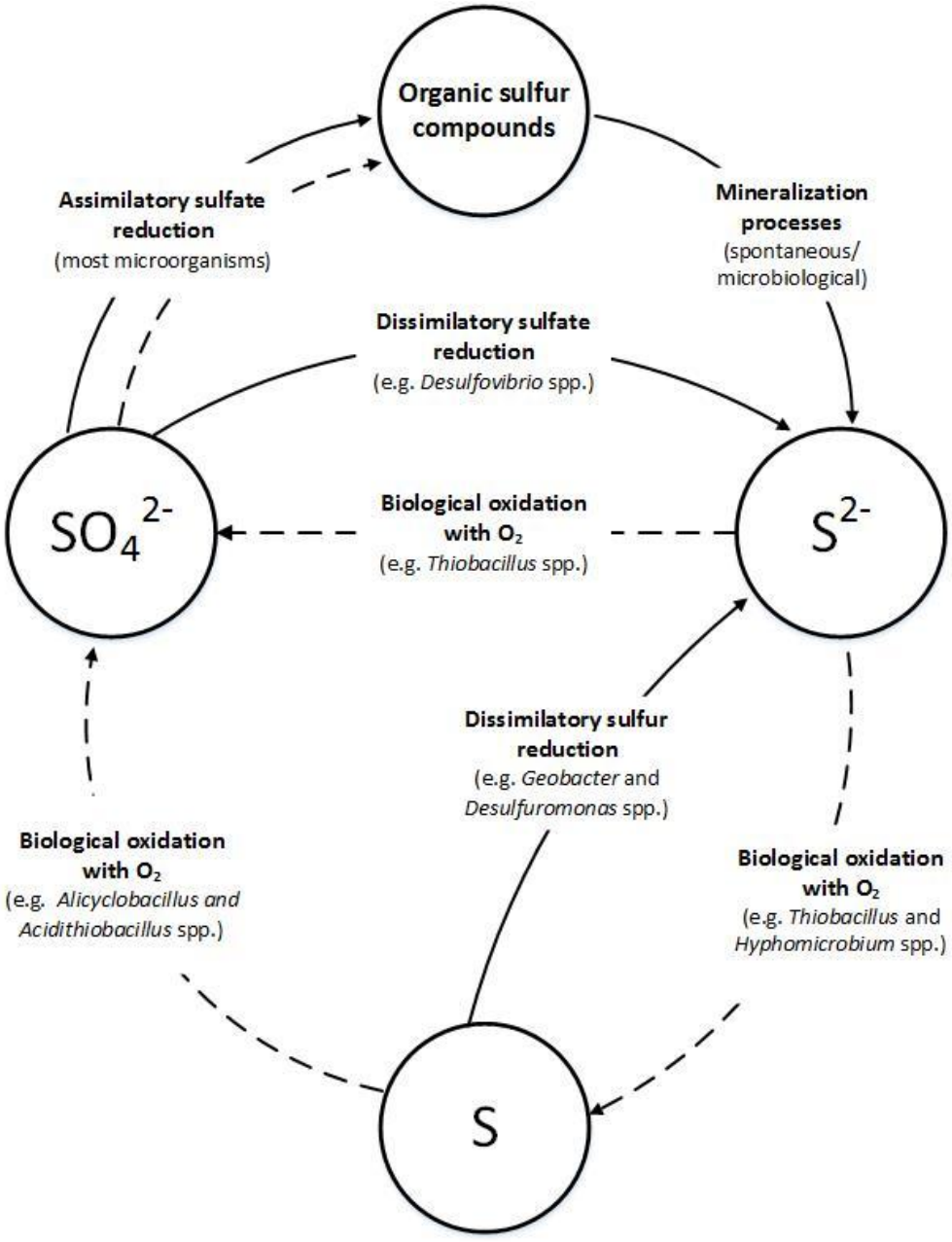



Figure 5.6 Potential microbial sulfur cycle (adapted from Bos and Kuenen, 1983) in a digester system. Predominant anaerobic conditions are marked with a continuous line; potential aerobic reactions are shown as dashed lines. A few organisms found in this study are provided.

Under anaerobic conditions, sulfate, which is commonly found in sewage sludge (Appels et al., 2008), can be reduced to sulfide ($S^{2-}$) by anaerobic SRB (e.g. *Desulfovibrio* spp.). $S^{2-}$ can be abiotically or biotically converted by, e.g. *Hyphomicrobium* sp., to elemental sulfur (Lee et al., 2000; Mohapatra et al., 2008). Elemental sulfur can either be further oxidized by other SOB species (e.g. *Alicyclobacillus*, and *Acidithiobacillus* spp.) in case of oxygen availability, or might be reconverted under anaerobic conditions to sulfide by SRB such as *Geobacter* spp.. The concurrent detection of SRB and SOB in biofilm samples from sludge digesters (see Chapter 4) indicates that these two groups might interact to oxidize and reduce sulfur compounds. Increased sulfate concentrations on the concrete wall of the digester headspace (compared to the sludge zone) provided further evidence for biological sulfur oxidation in the headspace. However, only a steady sulfur/sulfide oxidation over years to decades could result in the characteristic corrosion damage pattern as shown in Figure 5.1.

## 5.5 CONCLUSIONS

This study revealed the presence of different SOB species on the headspace concrete wall of six sludge digesters. The underlying hypothesis that SOB within the enriched cultures are capable to produce BSA under laboratory conditions and therefore potentially contribute to BSA corrosion in sludge digesters was confirmed (see hypothesis # 2; Chapter 2), especially as elevated sulfate concentrations on the concrete walls of the digester headspace were measured. However, further investigations on the availability of oxygen carriers, sulfide turnover rates and SOB activity in digester systems are vital to finally draw a conclusive picture about the BSA production *in situ*.

# Chapter 6

## Comparative analysis of biogenic and chemical sulfuric acid attack on hardened cement paste using Laserablation-ICP-MS[3]

*The aim of this study was to investigate the relative role of chemically and microbially derived sulfuric acid ($H_2SO_4$) corrosion on hardened cement paste representing a concrete binder. Cement stone disks were exposed to chemical $H_2SO_4$ (pH 1.0 and 2.0) and biological $H_2SO_4$ (pH 1.5-2.1). After 28 days, the degree of damage was evaluated by common visual-physical parameters and laser ablation ICP-MS as a novel evaluation tool to assess changes in elemental distributions. The results revealed a pH-dependent degree of damage. The 4.0 mm thick disk at pH 1.0 was completely corroded. For the disks exposed to biogenic and chemical $H_2SO_4$ at pH 2.0 an intact core remained with a similar thickness of the corrosion layer (1.8-2.0 mm) and sulfuric acid penetration depth (1.1-1.3 mm). Since the elemental distribution was similar in the corroded layer independent of applying biological or chemical $H_2SO_4$, no obvious differences between the two acid attacks were revealed.*

[3] Huber, B., Hilbig, H. Mago, M.M., Drewes, J.E., Müller, E. 2016. Comparative analysis of biogenic and chemical sulfuric acid attack on hardened cement paste using Laserablation-ICP-MS. *Cement and Concrete Research* **87**, 14-21.

## 6.1 INTRODUCTION

With an annual consumption rate of approximately 6 billion tons, concrete is one of the most frequently used construction materials in the world (Revie & Uhlig, 2011). Therefore, the deterioration or corrosion of concrete has enormous economic impacts. Worldwide, the costs for maintenance and retrofitting of damaged concrete structures amount to multibillion dollars every year. In the United States alone, 390 billion dollars have to be invested in the next 20 years to maintain existing wastewater facilities and construct new infrastructure (Gutiérrez-Padilla et al., 2010). Concrete is prone to material deterioration through physical and mechanical impacts, but chemical impacts such as acid induced corrosion is one of the major factors for reduced lifetime. The spectrum of acidic media which are able to react with concrete is broad and involves i) gaseous pollutants such as carbon dioxide, sulfur dioxide and nitrogen oxides, ii) organic acids such as lactic or acetic acid, and iii) mineral acids such as hydrochloric or sulfuric acid (Alexander et al., 2013; Beddoe & Hilbig, 2009; Zivica & Bajza, 2001). A significant and frequently occurring problem is the microbial induced concrete corrosion (MICC) through biogenic produced sulfuric acid. Sulfuric acid ($H_2SO_4$), generated by sulfur/sulfide-oxidizing bacteria (SOB; e.g. *Acidithiobacillus* spp. and *Thiomonas* spp.), has been identified as corrosive agent in sewage collection systems (Diercks et al., 1991; Parker, 1945a; Sand & Bock, 1984), wastewater treatment and sludge handling facilities (see Chapter 4), cooling towers and hydraulic facilities (Zherebyateva et al., 1991), as well as bridge structures (Vupputuri et al., 2015; Wei et al., 2010). In Germany, around 40% of the damage in concrete pipes and brick work sewers has been attributed to biogenic sulfuric acid corrosion (Kaempfer & Berndt, 1999).

The sulfuric acid attack on concrete is a combination of dissolution and expansion effects (mainly caused by sulfate) that result in instability of the cement stone matrix. During the degradation process, sulfuric acid reacts with the main constituents of Portland cement hydrates: portlandite ($Ca(OH)_2$) and calcium silicate hydrate phases (C-S-H) (Gutberlet et al., 2015). As a result, $Ca(OH)_2$ and C-S-H phases dissolve, whereas gypsum ($CaSO_4 \cdot 2H_2O$) and ettringite ($3CaO \cdot Al_2O_3 \cdot 3CaSO_4 \cdot 32H_2O$) precipitate. Under acidic conditions at pH value ≤ 2.0, the formation of gypsum is promoted (Gutberlet et al., 2015). If used, carbonatic aggregates will react in a similar manner. Thereby, a soft white corrosion layer develops on the concrete surface which thickens over time (Wells et al., 2009). The conversion of concrete to gypsum and ettringite is accompanied by a volume and pressure increase subsequently leading to internal cracking and structural failure of the concrete. In sewage systems corrosion rates of up to several millimeters per year have been reported (De Belie et al., 2004; Mori et al., 1991; Vollertsen et al., 2008).

The assessment of concrete durability and other cement-based building materials is of great importance for the structural integrity of the corresponding building structure. Thus, a lot of research has been carried out to study concrete corrosion processes in lab-scale experiments and *in situ*. Since biogenic sulfuric acid corrosion is a complex and long-term process, usually accelerated chemical tests on concrete or hardened cement paste are performed for the evaluation of different damage patterns (Gutberlet et al., 2015; Monteny et al., 2000; Mori et al., 1991). However, significant differences between a purely chemical sulfuric acid attack and biogenic sulfuric acid corrosion were reported in previous studies (Alexander & Fourie, 2011; Ehrich et al., 1999; Monteny et al., 2001; Monteny et al., 2000; Wells et al., 2009). Monteny et al. (2000) observed more detrimental effects on the concrete through biogenic sulfuric acid compared to a solely chemical $H_2SO_4$ attack. The authors suggested that the increased porosity and high humidity in the soft corrosion layer provides excellent microbial growth conditions accelerating the corrosion process. In contrast, a study by Okabe et al. (2007) suggests that the thick corrosion layer hinders bacterial growth. Their analysis of pH, oxygen concentration and microbial activity in corroded concrete revealed the highest abundance and activity of *A. thiooxidans* in the surface layer only. With increasing depth, the abundance decreased logarithmically due to oxygen and $H_2S$ transport limitations indicating that the sulfuric acid production occurs mainly on the outer concrete surface. Consequently, the sulfuric acid must penetrate through the gypsum layer in order to reach and attack the intact concrete.

Clearly a knowledge gap exists regarding potential differences of chemically and biogenically produced sulfuric acid leading to the hypothesis that chemically and microbially generated sulfuric acid can lead to comparable damage patterns on pure hardened cement paste (see hypothesis # 3; Chapter 2). Furthermore, it appears uncertain if experiments with pure chemical sulfuric acid can really represent microbial induced sulfuric acid attack on cement stone or concrete. Therefore, this paper highlights the influence of chemically and biologically derived sulfuric acid on hardened cement paste containing ground granulated blast furnace slag (GGBS), a binder mainly used for structures in wastewater facilities. To minimize artifacts of the aggregate itself, hardened cement paste was used instead of concrete. Cement stone disks were either exposed to a culture medium containing the sulfuric acid producer *Acidithiobacillus thiooxidans* generating a pH value of 1.5-2.1 or treated with pure sulfuric acid at two adjusted pH values of 1.0 and 2.0. During incubation, the sulfuric acid production by *A. thiooxidans* was measured and used as a parameter for microbial activity and –growth. After 28 days, the damage patterns were evaluated by visually inspecting the specimens and by determining a variety of physical parameters, such as thickness of the corrosion layer,

size of intact cement stone core, and volume expansion of the samples. In addition, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was applied as a novel evaluation tool to determine the distribution of carbon, calcium, silicon, sulfur and phosphorus in the cross section of the cement stone samples.

To our knowledge, this is the first comparative study analyzing biogenic and chemical sulfuric acid corrosion on hardened cement paste by means of LA-ICP-MS.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Hardened cement paste samples

A binder paste was prepared at a water to solid ratio (*w*/s) of 0.4 using 20 wt.% ordinary Portland cement CEM I 42.5 R, (20.6 wt.% $SiO_2$, 61.9 wt.% CaO, 5.2 wt.% $Al_2O_3$, 3.5 wt.% $Fe_2O_3$, LOI 1.5 wt.%), 80 wt.% ground granulated blast furnace slag (GGBS; 34.1 wt.% $SiO_2$, 38.8 wt.% CaO, 11.5 wt.% MgO, 11.2 wt.% $Al_2O_3$, 0.7 wt.% $Fe_2O_3$) and deionized water. Immediately after mixing, the paste was poured into cylindrical 30 mm diameter polyethylene vials that were vibrated and sealed with a cap. After 90 days at 20°C, the hardened cylinders were cut into 3.0 mm and 4.0 mm thick cement stone disks using a high-precision saw. While 3.0 mm thick disks were used in the standard acid experiments in the autotitrator (Gutberlet et al., 2015), the 4.0 mm thick disks were used for the expected more severe attacks in the pH 1.0 experiment in the autotitrator and in the biogenic experiments.

### 6.2.2 Biogenic sulfuric acid experiments

Biogenic sulfuric acid experiments with *A. thiooxidans* were carried out in 500 mL glass flasks (Schott Duran®, Germany) containing 200 mL liquid nutrient medium (Figure 6.1). Each flask (reactor) contained one 4.0 mm cement stone disk that was weighed and sterilized overnight under UV-light to prevent initial microbial contamination. The cement stone disk was fixed with a corrosion resistant wire to a rubber stopper and vertically submerged to three-quarters. A specific liquid medium (DSMZ medium 35) with an initial pH value of 4.5 and elemental sulfur as the only energy source was used as culture medium as it is recommended for the cultivation of *Acidithiobacillus* spp. by the German Collection of Microorganisms and Cell Cultures and promoted high growth of *A. thiooxidans* in pre-experiments (see Chapters 4 and 5). Furthermore, the low pH values within the biotic experiments promote the oxidation of elemental sulfur (Islander et al.,

1991) justifying the use of elemental sulfur as the reduced sulfur source instead of thiosulfate. Hydrogen sulfide was not an option due to its toxicity and high abiotic reactivity (Peyre Lavigne et al., 2015). DSMZ medium 35 was prepared according to DSMZ instructions (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium35.pdf). One liter contained 1.9 mmol $NH_4Cl$, 22.0 mmol $KH_2PO_4$, 0.5 mmol $MgCl_2 \cdot 6H_2O$, 1.0 mmol $CaCl_2 \cdot 2H_2O$, and 311.8 mmol sulfur (powder).

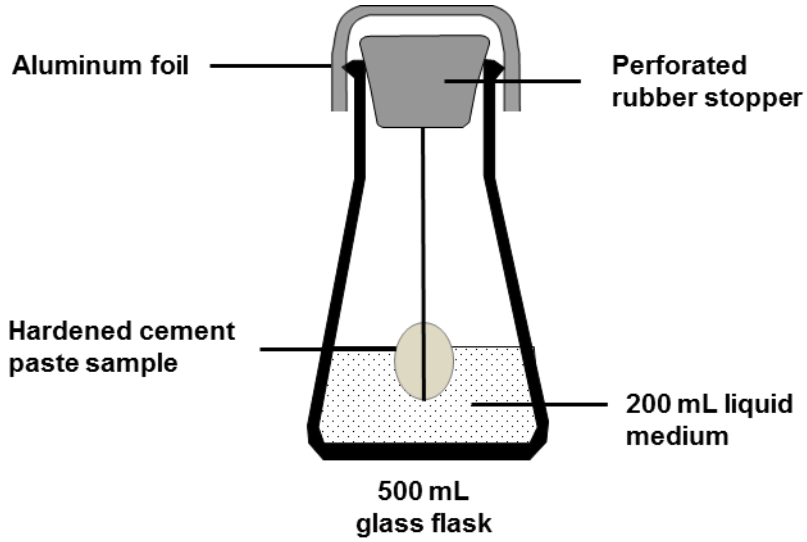



Figure 6.1 Setup of the biogenic sulfuric acid corrosion test.

The experiment was carried out in two parallel set-ups, reactor 1 and 2 (R1 and R2). The liquid medium of both reactors R1 and R2 were inoculated with pure *A. thiooxidans* (Accession No.: LN 8646660) originally isolated from a corroded concrete surface within the headspace of a sludge digester as described in Chapter 5. For inoculation of the reactors, approximately 10 µl of *A. thiooxidans* pure culture was taken from an agar plate with an inoculation loop. Then, the flasks were closed with a rubber stopper containing holes to allow oxygen transfer. The rubber stopper was covered with sterile aluminum foil to avoid contamination. The reactors were pre-incubated at 30°C and shaken at a constant speed of 125 revolutions per minute (rpm) to prevent nutrient and oxygen limitation until bacteria growth was visible by means of increasing turbidity and pH decrease. The acid production within the liquid medium was monitored via pH measurements using a pH electrode (WTW GmbH, Weilheim, Germany). After the pH of the medium decreased from pH 4.5 to 1.9, the cement disks were mounted as described above and incubated in the bacterial solution for 28 days (30°C, 125 rpm). The tests were carried out in semi-continuous draw and fill operated mode to supply nutrients for the

active growth of *A. thiooxidans*. For that 20 mL of the culture medium was replaced with fresh medium twice a week. The withdrawn liquid sample was frozen at -20°C for sulfate and calcium measurements (see 6.2.4.1). An abiotic control reactor (AC) containing 200 mL culture medium without *A. thiooxidans* was set up under the same conditions to analyze the influence of the medium. After 28 days, the hardened cement paste samples were removed from the liquid, dried at 40°C until weight constancy, documented photographically, and analyzed with LA-ICP-MS (see 6.2.4.2). Only the part that was submerged in the liquid medium was analyzed.

### 6.2.3 Chemical sulfuric acid experiments in the autotitrator

The sulfuric acid induced degradation process was studied at a constant pH of 1.0 and 2.0 using an automatic titrator with an automatic sample changer. It was therefore not necessary to renew the acid solution during storage in order to maintain acid strength with all diluted ions comparable to the biogenic experiments. The sample storage followed the procedure described by Gutberlet et al. (2015), where the structures of the corroded layers of cement stone samples after acid storage at pH 4.0, 3.0 and 2.0 were investigated: For each sample position, three 3.0 mm thick disks were weighed and placed in a special sample holder in a capped beaker with 150 mL acid that was stirred with a magnetic stir bar. The pH of the acid solution for each sample position was measured and re-adjusted at intervals of ten minutes by titrating with 0.5 M $H_2SO_4$. The added acid volume was recorded continuously. Because of the limited volume of the beaker, several times 30 mL of the solution was removed and collected. At the end of the experiment, the collected solution was analyzed to quantify the dissolved sulfate and calcium (see 6.2.4.1). Because of the expected stronger chemical reaction at pH 1.0 a 4.0 mm thick disk was inserted to avoid a complete destruction after 28 days of storage. To reduce the expected higher acid consumption at pH 1.0 only one disk was inserted. After 28 days, the three disks of the pH 2.0 experiment and the one disk of the pH 1.0 experiment were removed from the acid, dried at 40°C until weight constancy, documented photographically, and analyzed with LA-ICP-MS.

### 6.2.4 Chemical analyses

#### 6.2.4.1 Sulfate and calcium measurements and determination of acid penetration depth

The potential sulfuric acid production by *A. thiooxidans* within the culture media was analyzed via sulfate measurements by liquid ion exchange chromatography (ICS-1000 ion

chromatography system, Dionex Corporation, USA). The determination was carried out according to DIN EN ISO 10304-1 and followed German Standard Methods for the examination of water, wastewater and sludge (DEV, 2016). Since in the chemical sulfuric acid experiments sulfate was the only sulfur containing species, sulfate was determined in the corrosion solution via total sulfur measurements with ICP-OES (optical emission spectrometry, Jobin Yvon Ultima II).

Calcium was determined in the corrosion solutions with ICP-OES (Jobin Yvon Ultima II).

The sulfuric acid penetration depth was determined with phenolphthalein according to DIN EN 14630:2007-01.

#### 6.2.4.2 Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)

Hardened cement paste samples, which were exposed for 28 days to biogenically and chemically produced sulfuric acid solutions, were analyzed by LA-ICP-MS. To facilitate the measurement, the samples were embedded in low viscosity epoxy injection resin (Sika® Injection-451, Sika Ltd., New Zealand), cut with a high-precision saw (cross section) and dried for one day at 40°C before analysis.

The instrument consisted of two main components. In the laser ablation system (NWR 213, New Wave Research, Inc., USA), material from the sample surface was removed with a high-performance neodymium-doped yttrium aluminum garnet laser (213 nm). Ten lines across the cross section of the samples were ablated. 50 µm laser spots with a pulse rate of 20 Hz and a feed rate of 100 µm/s were applied. The ablated material was transported via helium as carrier gas into the inductively coupled plasma mass spectrometer (ICP-MS; quadrupole-ICP-MS NexION 300D, Perkin Elmer, USA). The material was ionized in the ICP-flame and subsequently analyzed in the MS system (O'Connor, 2007). The distributions of carbon, calcium, silicon, sulfur and phosphorus were determined. For LA-ICP-MS calibration a half of a cement stone disk in original state was embedded, cut and ablated in the same way as the investigated samples.

## 6.3 RESULTS AND DISCUSSION

The initial two sections (6.3.1 and 6.3.2) focus on the sulfuric acid production in the biotic set-up and sulfuric acid consumption in the chemical set-ups. The results are discussed separately in order to illustrate the comparability of the two test systems. In section 6.3.3, the extent of corrosion is evaluated in both chemical and biotic set-ups for a better

comparison of the damage patterns. First the assessment of the cement stone corrosion is evaluated using visual and physical parameters (6.3.1) followed by an analysis of LA-ICP-MS results of various cross sections of the hardened cement paste disks (6.3.2).

### 6.3.1 Sulfuric acid production within biotic set-up

Since microbial induced concrete corrosion (MICC) is a multi-stage and slow process, accelerated laboratory tests were performed by incubating hardened cement paste samples with a large surface/volume ratio in semi-continuous fed batch test with the pure culture *A. thiooxidans* isolated from biofilm originating from corroded concrete surface of digester headspace (see Chapter 5). The experimental setup mimics the last, but decisive step, within the MICC process where sulfuric acid produced by sulfur-oxidizing bacteria (SOB) reacts with the cement stone components of the concrete finally leading to its deterioration. The acidophilic sulfur oxidizing *A. thiooxidans* was used as it was found to be the dominant organism in heavily corroded concrete and its key role in the corrosion process (Diercks et al., 1991; Okabe et al., 2007). It is known that *A. thiooxidans* is able to produce high amounts of sulfuric acid reducing the pH to values of 0.5-2.0 (Aviam et al., 2004; Diercks et al., 1991; Okabe et al., 2007; Robertson & Kuenen, 2006). The aim of the present study was to achieve a high and continuous microbial sulfuric acid production in order to create a worst case scenario providing a comparable set-up to the solely chemical experiments. To ensure that the amount of elemental sulfur was not limiting, the test was operated in draw and fill mode feeding the bacteria culture regularly with fresh medium (addition of a total amount of 113 mmol sulfur). Untreated hardened cement paste effects a high pH of 11-13 that might inhibit bacterial growth (Aviam et al., 2004). Thus, a pre-incubation of *A. thiooxidans* at 30°C and 125 rpm without cement stone disks was carried out until the pH in the liquid medium decreased from an initial value of 4.5 to 1.9 indicating a stable and vital bacterial community. After two weeks pre-incubation the pH reduction was achieved and the cement stone disk was added to the bacteria culture.

After the addition of the cement stone disks, the sulfuric acid production by *A. thiooxidans* was monitored via pH and sulfate analyses in the liquid medium. A decreasing or consistent pH in combination with an increasing sulfate concentration indicated sulfuric acid production and microbial growth. During the experiment, the pH in the liquid cultures remained at values of 1.5-2.1 (see Figure 6.2). The pH of the abiotic control increased within the first five days of incubation from 4.5 to 6.5 due to the alkalinity of the hardened cement paste disk and remained quite stable (pH 6.3-6.6) for the duration of the experiment. Figure 6.2 displays the sulfate concentration over time for the biogenic

sulfuric acid reactors (R1 and R2) and the abiotic control (AC) after the addition of the cement stone disks. At the beginning of the cement corrosion experiment (day 0), a sulfate concentration of 43 mmol/L and 42 mmol/L was measured for R1 and R2, respectively. After 28 days of cultivation, the sulfate concentration reached values of 117 mmol/L (R1) and 104 mmol/L (R2) which corresponds to an absolute amount of sulfate of 23 mmol (R1) and 21 mmol (R2) in 200 mL culture medium. The increasing amount of sulfate in both reactors as well as the constantly low pH revealed a continuous microbial production of sulfuric acid confirming that the bacteria within the liquid medium were active over a period of 4 weeks. The hardened cement paste disks were continuously exposed to biogenic sulfuric acid and a fast degradation of the cement paste was visible (Figure 6.4). The sulfate concentration of the abiotic control was both lower than 1 µmol/L at the beginning and at the end of the experiment (Figure 6.2). This indicates that no, or only very little, sulfate emerged from the hardened cement disk. No contamination with bacteria occurred in the abiotic control set-up. Calcium values of the abiotic control remained below 1 mmol/L during the 28 days of incubation (Figure 6.2). Within the biotic reactors, the calcium concentration increased within the first five days to 13 mmol/L and remained at this low level (11-14 mmol/L) during the whole experiment. These steady calcium values indicate that calcium released from the hardened cement paste has been immediately precipitated as gypsum. During the 28 days of cultivation, 113 mmol sulfur was added via the medium to the experiment leading to an amount of 113 mmol sulfate providing the bacteria would have used it as energy source and oxidized it all. Usually, autotrophic sulfur oxidizers such as *A. thiooxidans* are able to completely oxidize reduced sulfur compounds to sulfate (Robertson & Kuenen, 2006). However, within this set-up it was determined that the elemental sulfur which was provided as sole energy source was only utilized by approximately 70% by the bacteria. Assuming a yield of 70%, the resulting sulfate (79 mmol) would be still much higher than actually determined in solution at the end of the experiment (23 mmol and 21 mmol) likely suggesting gypsum precipitation. Similar experiments with *A. thiooxidans* in DSMZ medium, but without cement stone samples, revealed sulfate concentrations of 301-311 mmol/L and pH values ≤0.5 after 28 days of incubation (see Chapter 5). These significant higher sulfate concentrations and lower pH values further indicate that huge amounts of gypsum may have been precipitated in the biotic corrosion experiments and/or microbial growth is enhanced without cement stone.

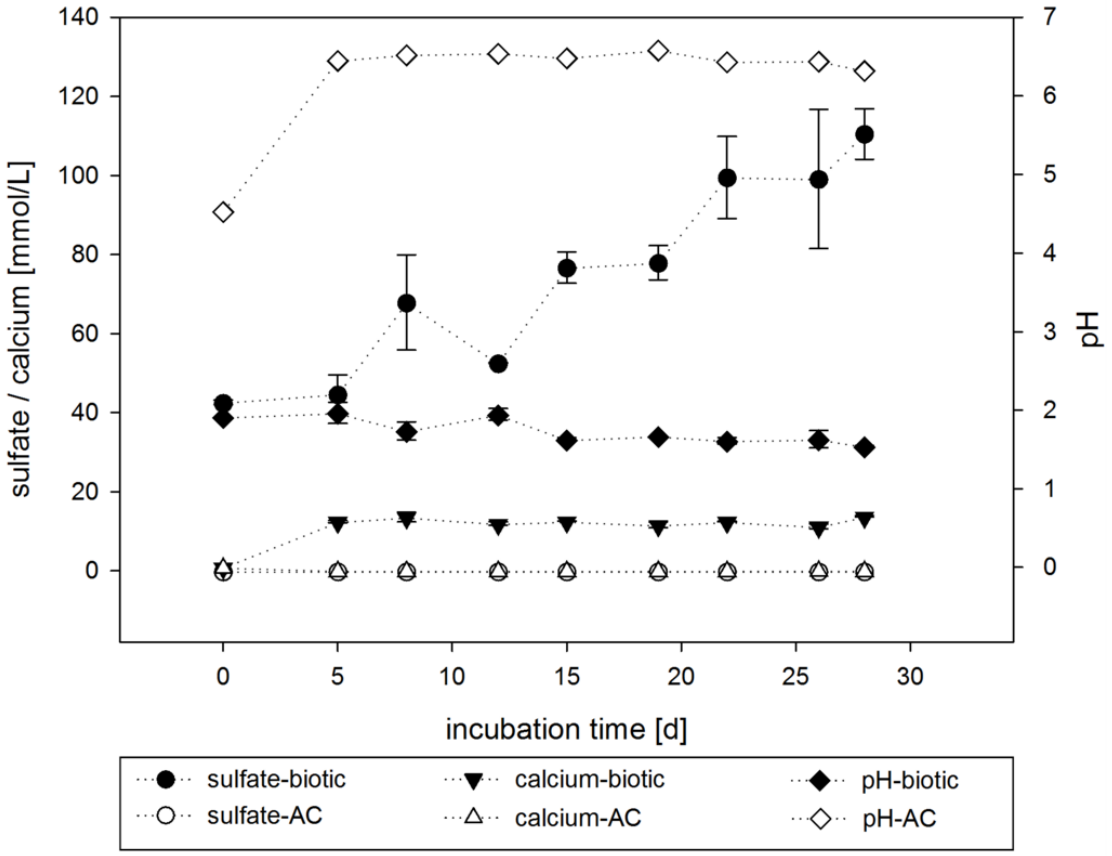



Figure 6.2 pH value, sulfate and calcium concentrations measured in the liquid medium of the biotic sulfuric acid reactors R1 and R2 (mean values with standard deviations) and the abiotic control (AC) after the addition of the cement stone disk. At each data point 10% of the culture solution was replaced with fresh medium.

Generally, the sulfate concentrations measured in this study (42-117 mmol/L) were significantly higher than those described in previous studies (De Belie et al., 2004; Monteny et al., 2001; Vincke et al., 2002). When conducting similar microbiological experiments, De Belie et al. (2004) reported maximum sulfate concentrations of 21-42 mmol/L while Monteny et al. (2001) as well as Vincke et al. (2002) measured values up to 26 mmol/L. Their microbiological test procedure was carried out in four cycles of 17 days using a mix of *Thiobacillus* cultures instead of pure *A. thiooxidans* culture. The high initial sulfate concentrations (42 mmol/L) in the present study resulted from the fact that *A. thiooxidans* was pre-cultivated before the cement stone disks were added. Nevertheless, the sulfuric acid production in the liquid medium was much higher in this study as compared to others. One reason might have been the continuous nutrient supply in form of fresh medium to avoid the energy source limitation promoting a high bacteria growth and activity. These conditions reflect a worst case scenario and allow a

comparison to the chemical set-up. In addition, the usage of an acidophilic bacterium that is capable of producing large quantities of sulfuric acid, instead of a mix of *Thiobacillus* cultures, might have been another factor explaining the elevated sulfate content.

### 6.3.2 Acid consumption within chemical set-up

For comparative studies of microbiological and chemical sulfuric acid attack patterns, equal or closely similar pH values and sulfate concentrations should be used. The biogenic sulfuric acid corrosion experiments already revealed that *A. thiooxidans* was able to reduce the pH in the medium to values of 1.5-2.1 and these low pH values remained stable over a period of 28 days (see 6.3.1). To compare biogenic with chemical corrosion, experiments were performed by incubating hardened cement paste disks in pure sulfuric acid solutions with constant pH values of 1.0 and 2.0. Over 28 days, 172 mL of 0.5 M acid was titrated to maintain pH 1.0 (one disk) whereas a total of 167 mL was necessary to keep a pH value of 2.0 (three disks). Normalizing the acid consumption to the surface of the stored samples, 9.6 mL/cm$^2$ and 3.3 mL/cm$^2$ of total acid was consumed (see Figure 6.3). Concerning the total acid input, 94 mmol and 84 mmol sulfate was added to the set-ups. While only 68 mmol and 44 mmol were recovered in the solution after the experiment, likely the difference must have been precipitated as gypsum. Due to this saturation conditions, it could be assumed that a sulfate concentration in the system similar to the range of the microbiological tests (42-117 mmol/L) was achieved. The calcium concentration within the pH 1.0 corrosion solution (one disk) was 20 mmol/L, while for the pH 2.0 solution a concentration of 32 mmol/L (three disks) was determined. These values are in a similar range as compared to the biotic set-ups.

Finally, the experimental conditions in the chemical and biological set-up are similar due to related pH values, sulfate and calcium concentrations making the two different test systems comparable.

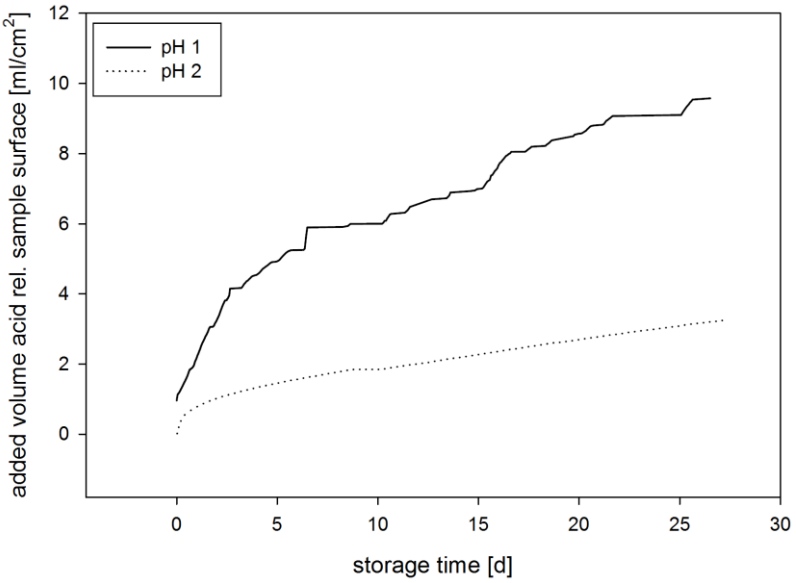



Figure 6.3 Consumption of 0.5 M sulfuric acid in relation to the sample surface during the chemical titrator experiments to keep the pH at 1.0 and 2.0, respectively.

### 6.3.3 Cement stone corrosion within both chemical and biotic set-ups

#### 6.3.3.1 Visual-physical parameters

After 28 days of exposure to different sulfuric acid solutions, the samples of both biotic and chemical tests showed clear signs of corrosion (Figure 6.4, B-D). The surface was covered in a thick white corrosion layer that exhibited low mechanical strength. No signs of degradation were observed for the abiotic control (Figure 6.4 A). However, a thin layer of salt crystals was visible on the surface resulting from calcium phosphate precipitation due to the high phosphate concentration in the medium (see also 6.3.3.2).

Besides visual inspections, the extent of corrosion was determined by a combination of physical parameters: i) size of the intact cement core, ii) thickness of the corrosion layer, and iii) volume expansion of the specimens (Table 6.1). For the determination of these parameters, a cross-sectioning of the sample disks was necessary (see Figure 6.4, bottom). Generally, a correlation between the pH and the degree of damage was observed. The specimen exposed to chemical sulfuric acid at pH 1.0 exhibited the highest degree of degradation, while the samples exposed to pH 2.0 and the ones within the microbially produced sulfuric acid (pH 1.5-2.1) revealed a slightly lesser damage pattern. The extent of corrosion was particularly reflected in the corresponding cross sections (Figure 6.4, bottom). The cross section of the pH 1.0 sample clearly showed that the 4.0 mm thick disk was completely corroded. The sample consisted of a continuous corrosion layer and no intact cement stone remained. Since already parts of this layer

broke off, the thickness of the corrosion layer could not be determined exactly, but was at least 1.8 mm thick (Table 6.1). For the samples exposed to pH 2.0 (3.0 mm disks) and microbially sulfuric acid (4.0 mm disks), an intact cement stone core of 0.5 mm and 1.6 mm on average remained, respectively (Figure 6.4 B and D, bottom, Table 6.1). Considering the different size of the sample disks, the thicknesses of the corroded layers were comparable. The average corrosion layer for the biogenic sulfuric acid and for the chemical sulfuric acid at pH 2.0 was determined to be 1.9 mm and 2.0 mm thick, respectively (Table 6.1).

Table 6.1 Thickness of hardened cement paste disks before and after sulfuric acid attack, intact cement core, size of the corrosion layer, and acid penetration depth (determined with phenolphthalein) of the cement disk samples after 28 days of incubation in the different solutions.

| | **abiotic control** | **biotic experiments** | | **chemical experiments** | |
|---|---|---|---|---|---|
| | **AC** | **R1** | **R2** | **pH 1** | **pH 2** |
| Initial thickness | 4.0 mm | 4.0 mm | 4.0 mm | 4.0 mm | 3.0 mm |
| Thickness after acid attack | 4.0 mm | 5.2 mm | 5.3 mm | 3.5 mm* | 4.4 mm |
| Intact cement stone | 4.0 mm | 1.4 mm | 1.8 mm | 0.0 mm | 0.5 mm |
| Corrosion layer | 0.0 mm | 1.9 mm | 1.8 mm | 1.8 mm | 2.0 mm |
| Acid penetration depth | 0.0 mm | 1.3 mm | 1.1 mm | ≥2.0 mm | 1.3 mm |

* part of the corrosion layer is completely destroyed

Furthermore, an increased disk volume was noticed during sulfuric acid exposure experiments (Figure 6.4, B-D and Table 6.1). Previous studies reported that the formation of gypsum was associated with a 1.2-2.2 times volume increase (Monteny et al., 2001; Monteny et al., 2000), while in this study an increase in volume by a factor of 1.3 and 1.5 was observed for the biogenic sulfuric acid test and for chemical experiments at pH 2.0 during an incubation of 28 days, respectively. The volume expansion of the sample disk stored at pH 1.0 could not be determined exactly, since part of the corrosion layer was mostly destroyed after a period of acid exposure of 28 days. The size of the abiotic control remained unchanged.

Besides the parameters already mentioned, weight loss is another physical parameter, to assess concrete degradation. A study by Vincke et al. (2001) analyzing the influence of

chemical and biological sulfuric acid on Portland cement samples revealed twice as high mass losses and sample thicknesses for the chemically treated samples compared to the microbiologically treated samples (Scrivener & De Belie, 2013; Vincke, 2001). Because of the high degree of corrosion in our experiments with undefined mass loss of particles and precipitations in the storage solutions no reliable values could be achieved. Mass-loss is often used as sole indicator for corrosion and various values ranging from 1.8% up to 31.0% are reported in literature (Aviam et al., 2004; Sand & Bock, 1987; Sand & Bock, 1984; Schmidt et al., 1997). However, the formation of gypsum can also increase the weight, particularly in the beginning when sulfuric acid penetrates the cement stone and the corrosion products compact the pores. Since mass loss occurs only after an extended time period of the experiment, this parameter can be a significant parameter at later stages of degradation, especially when the softened material on the surface is first removed by cleaning actions. For short-term experiments, however, weight loss may not suffice to describe concrete corrosion and further chemical investigations such as LA-ICP-MS are required in order to draw a more conclusive picture. With LA-ICP-MS the element distributions in the cross section of the sample disks can be determined. The analysis of certain elements playing a role within the corrosion process (e.g. calcium, silicon, sulfur) provides further information on the degree of damage.

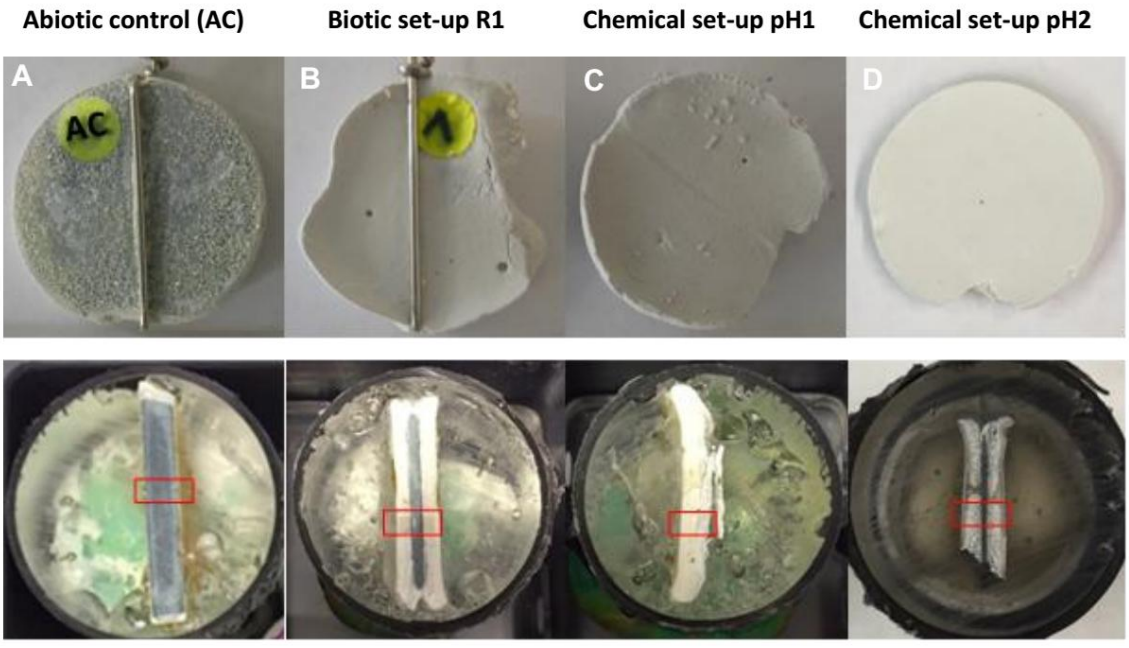



Figure 6.4 Hardened cement paste samples after storage in the different sulfuric acid solutions for 28 days (top) and their corresponding cross sections (bottom). Area where LA-ICP-MS was performed is marked in red. A: abiotic control (AC), B: biogenic sulfuric acid reactor R1 (representative for R2), C: chemical sulfuric acid at pH 1.0, and D: chemical sulfuric acid at pH 2.0.

#### 6.3.3.2 LA-ICP-MS analysis

Besides the visual-physical parameters already described, laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) was applied as an evaluation tool for assessing cement stone degradation. Usually, LA-ICP-MS allows to analyze qualitatively and quantitatively almost all elements of the periodic system. However, within this study, only semi-quantitative statements can be made due to large differences in the surface properties of the corroded and non-corroded area of the cement stone samples and the resultant dissimilar ablation behavior. Different surface properties are leading to different ablation depths and accordingly to uncertain sample material weight for analysis. For the evaluation of cement stone corrosion, five elements were selected: carbon, calcium, silicon, sulfur, and phosphorus. Carbon was selected to monitor the influence of the epoxy injection resin used for embedding the cement stone disks. Calcium and silicon, the main constituents of hardened cement paste, served as indicator for the corrosion stage of the cement stone because a loss of these elements demonstrates the destruction of the cement stone matrix (Aviam et al., 2004). There are two effects of the sulfuric acid attack on the cement stone matrix, the leaching effect of the acid and the precipitation effect of the sulfate. While in principle acid solubility of calcium is high, it is low for silicon. Calcium is withdrawn from $Ca(OH)_2$ and C-S-H and silicon is accumulated in the corrosion layer due to the formation of amorphous silicic acid (silica gel). A sole acid attack would lead to a weak corroded layer consisting of silica gel low in calcium content (Gutberlet et al., 2015). On the other side the formation of gypsum ($CaSO_4 \cdot 2H_2O$) in the pore area of the silica gel, leads to a densification and expansion of the corroded layer. If the expansion is too high, cracks will develop and destabilize the corroded layer. Sulfur provided additional information on the penetration of sulfuric acid into the cement stone. Phosphorus was monitored to investigate a potential influence of high phosphate concentrations on cement stone degradation which was provided in the nutrient medium to optimize bacterial growth.

The results of the element distribution in the cross sections of the untreated sample/negative control (NC), abiotic control (AC), biogenic (R1) and chemical set-ups (pH 1.0 and 2.0) are illustrated in Figure 6.5 after 28 days of exposure to the corresponding solutions. Since the two disks of the biogenic reactors R1 and R2 and the three pH 2.0 disks revealed the same LA-ICP-MS profiles, respectively, only results of one sample disk is shown representative for the others. For all samples, signs for a penetration of the epoxy injection resin into the corroded cement paste were observed which is visible in the carbon measurements (Figure 6.5).

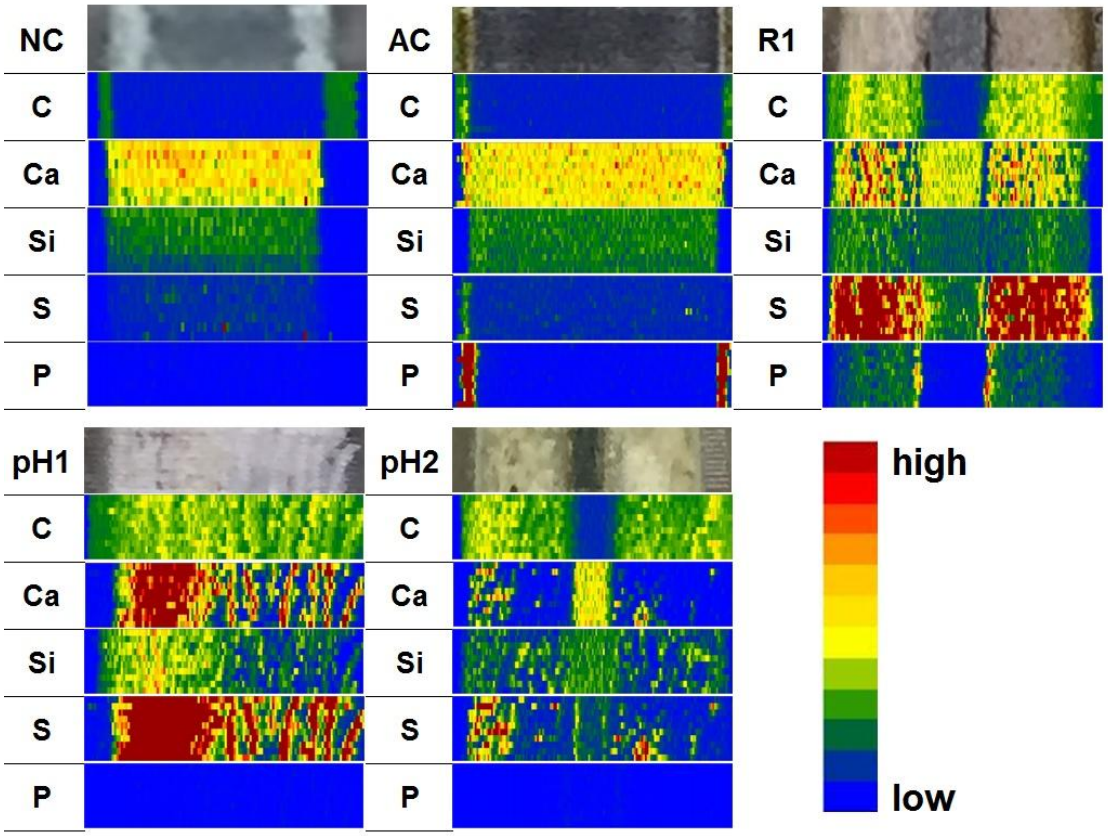



Figure 6.5 Magnification of the cross-sectional area from Figure 6.4 (red marked areas) used for LA-ICP-MS analysis and corresponding elemental distributions as a false color representation (C, Ca, Si, S and P) for the abiotic control (AC), biotic set-up R1 (representative for R2) and chemical set-ups at pH 1.0 and pH 2.0 after 28 days of incubation in the corresponding solutions. An untreated sample (negative control, NC) is shown as reference.

The untreated cement stone sample (NC), which was neither exposed to the culture medium nor to any sulfuric acid solution, showed a homogenous distribution of calcium, silicon and sulfur across the analyzed area. Phosphorus was not detected. Generally, the concentrations for calcium and silicon were significantly higher than that for sulfur. This is due to the fact that calcium and silicon (part of C-S-H phases) are the main components of original hardened cement paste, whereas sulfur constitutes only a minor part of cement stone (in ettringite and monosulfate).

The abiotic control (AC), exposed for 28 days to the nutrient medium without biomass, showed again a homogenous distribution of the three elements Ca, Si and S over the analyzed area while an accumulation of phosphorus was determined at the outer surface

indicating some degree of calcium phosphate precipitation (thickness about 100 µm) due to the high phosphate concentration of 22 mmol/L $KH_2PO_4$ in the medium. The poor water solubility of calcium phosphates (Hagmeyer, 2011) promoted the formation of calcium phosphate crystals on the surface of the abiotic control (pH ≥ 6.0). In contrast to the abiotic control, within the biotic experiments the produced sulfuric acid inhibited the formation of calcium phosphate crystals due to the high solubility of these crystals in diluted acid. Calcium phosphate precipitation was already observed by Aviam et al. (2004) when incubating hardened cement paste samples for 39 days in a medium containing 29 mmol/L $KH_2PO_4$. The authors (Aviam et al., 2004) stated also that the precipitation itself is minor and seems to have no influence on the structural stability of the cement stone, since no degradation was observed for the control samples without bacteria growth.

The sample disk (R1) exposed to biogenic sulfuric acid, exhibited significantly higher concentrations of sulfur and phosphorus in the corrosion layer than in the intact core zone. High sulfur and calcium concentrations in the corrosion layer indicated the formation of gypsum ($CaSO_4 \cdot 2H_2O$). The average sulfuric acid penetration depth for the biogenic sulfuric acid reactors was 1.2 mm (Table 6.1). Nevertheless, an almost intact core remained (1.6 mm on average), visible due to the occurrence of lower concentrations of sulfur and phosphorus. The high phosphorus concentrations in the corrosion layer originated from the phosphate concentration in the medium. An accumulation of phosphorus was observed at the reaction front (degraded layer/undamaged cement stone interfacial region) indicating calcium phosphate precipitation due to its lower solubility with rising pH value on the un-corroded cement stone surface. However, no penetration of external phosphorus was observed in the intact matrix zone.

The 4.0 mm-thick disk stored in chemical sulfuric acid at pH 1.0 was completely corroded (see Figure 6.4), confirmed with both visual-physical means and by LA-ICP-MS (Figure 6.5). The sample was characterized by a more or less inhomogeneous distribution of calcium, silicon and most important sulfur at high level across the analyzed area. These findings confirm the complete acid penetration (≥ 2.0 mm, Table 6.1) into the cement stone. In contrast to the biogenic sulfuric acid reactors with an average intact core size of 1.6 mm, no intact core was visible for the pH 1.0 disk. Since the sample at pH 1.0 (and pH 2.0) was not exposed to nutrient medium containing high phosphate concentration (as the biogenic sulfuric acid samples), no phosphorus accumulation was observed.

For the samples at pH 2.0 (3.0 mm disks), the elemental distribution was similar to the distribution found after the biogenic attack, but for calcium and sulfur at lower level. While the visual-physical observations revealed almost equal damage patterns for the biogenic

and sulfuric acid attack at pH 2.0, the LA-ICP-MS profiles indicated a slightly lower extent of corrosion for the pH 2.0 samples due to the lower concentrations of calcium and sulfur in the corrosion layer. However, the degree of corrosion was significantly less compared to the sample exposed to chemical sulfuric acid at pH 1.0. The acid penetration depth (Table 6.1) for the pH 2.0 samples (1.3 mm) was much lower compared to pH 1.0 (≥ 2.0 mm). Furthermore, an intact core part of 0.5 mm remained in the middle of the cement stone disk, further indicating a lower extent of corrosion at pH 2.0. The lower level of calcium and sulfur in the corroded layer of the pH 2.0 samples is in agreement with the lower sulfate input (total acid consumption of 9.6 mL/cm$^2$ and 3.3 mL/cm$^2$ for pH 1.0 and pH 2.0, respectively).

## 6.4 CONCLUSIONS

For the assessment of cement stone degradation, visual-physical parameters provided a first insight into the corrosion damage of the samples. However, within this study, the determination of one single parameter was not sufficient enough and thus a combination of different parameters was necessary. The results obtained with the novel evaluation tool LA-ICP-MS verified the findings using visual-physical means supporting a pH-dependent degree of damage. However, more in depth-results were gained with LA-ICP-MS due to semi-quantitative analyses of the elemental distribution in the corrosion layers of the hardened cement paste samples. The compositions of the corroded layers of the biogenic attack and the pH 1.0 sample were similar with the exception of the phosphorus precipitation. It is assumed, that the biogenic attack lies in between pH 1.0 and pH 2.0, which is in agreement with the measured averaged pH value of 1.7. The acid penetration depth of the biogenic samples is in the range of the pH 2.0 sample. That might be caused by the precipitation of calcium phosphate retarding the penetration of the sulfuric acid.

No obvious differences between biologically and purely chemically produced sulfuric acid were observed on the hardened cement paste samples within these test systems confirming the proposed hypothesis (see hypothesis # 3; Chapter 2). This suggests that the thick corrosion layers of 1.8-2.0 mm did not support microbial growth probably due to oxygen and nutrient transport limitations confirming the observations by Okabe et al. (2007). In our system, microbial growth appears to take place on the outer corroded surface only and in the surrounding culture medium, respectively. Since no internal attack through microbially produced sulfuric acid takes place, the availability of the sulfuric acid is comparable to the purely chemical attack. In both setups the sulfuric acid must diffuse through the thick corrosion layer to reach and attack the intact cement stone core. The

comparability of the two acid attacks is reflected in the development of similar corrosion damage patterns independent of using biologically or chemically produced sulfuric acid. However, this study focused only on the last step of MICC, where the sulfuric acid produced by the SOB reacts with the hardened cement paste. Other processes, such as the colonization or selection of the microbes on the cement stone, were not considered which also depends on the bioreceptivity of the used material (e.g. in presence of biocidal compounds).

Additional experiments will be performed with concrete samples and LA-ICP-MS as evaluation method to further prove the applicability of this technique and to better understand the biogenic sulfur attack on concrete structures.

# Chapter 7

## Evaluation of concrete corrosion after short- and long-term exposure to chemically and microbially generated sulfuric acid[4]

*Concrete samples were incubated for 28 days (short-term) in microbially derived $H_2SO_4$ (pH 1.3-2.4) and purely chemically generated $H_2SO_4$ (pH 1.0 and 2.0) to investigate the differences between the two acid attacks. Additionally, long-term biogenic experiments were performed over two, three and six months to evaluate the concrete corrosion behavior over time. Concrete corrosion was evaluated by visual, physical and chemical parameters, including laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The 28-day experiments revealed a pH-dependent degree of damage. No obvious differences between biologically and chemically generated $H_2SO_4$ were observed due to similar elemental distributions in the corrosion layers. For the long-term experiments, the extent of damage primarily depended on the amount of $H_2SO_4$ produced by A. thiooxidans, which varied within the different set-ups. No linear relation between the degradation and incubation periods was observed. In all set-ups, gypsum was the main corrosion product and no microbial growth was observed within the corrosion layer.*

[4] Huber, B., Hilbig, H., Drewes, J.E., Müller, E. 2016. Evaluation of concrete degradation after short- and long-term exposure to chemically and microbially generated sulfuric acid. Submitted to *Cement and Concrete Research*.

## 7.1 INTRODUCTION

Concrete is one of the most commonly used building materials in the world. It is applied for the construction of various infrastructures, such as highways, bridges, tunnels, wastewater and sludge handling facilities (House & Weiss, 2014; Revie & Uhlig, 2011). Approximately 6 billion tons of concrete are used every year (Revie & Uhlig, 2011). When concrete structures are exposed to acidic environments deterioration of concrete occurs (Miyamoto et al., 2014). Acid attack on concrete can be extremely diverse and ranges from gaseous pollutants (e.g. carbon dioxide, sulfur dioxide) over organic acids (e.g. lactic or acetic acid) to mineral acids (e.g. hydrochloric or sulfuric acid) (Alexander et al., 2013; Beddoe & Hilbig, 2009; Zivica & Bajza, 2001). Thereby, the corrosion of concrete through microbially produced sulfuric acid, particularly occurring in sewage and wastewater treatment systems, is a major problem (Peyre Lavigne et al., 2015) leading to financial losses of multibillions of dollars every year (Hewayde et al., 2007). Biogenic sulfuric acid (BSA) corrosion significantly reduces the lifetime of concrete structures, from estimated 100 down to 30-50 years (Grengg et al., 2015). In severe cases the lifespan can be even reduced to 10 years (Grengg et al., 2015; Jensen, 2009). BSA corrosion in a sewer pipe is a complex multistage process involving chemical and microbiological reactions (Monteny et al., 2001). In a first step, when anaerobic zones develop in the sewage due to the slow flow, sulfur/sulfate reducing bacteria reduce sulfate to hydrogen sulfide ($H_2S$) (Monteny et al., 2000; Okabe et al., 2007). The $H_2S$ gas escapes into the sewer headspace due to volatilization and dissolves in the moist concrete surface on the sewer crown (Okabe et al., 2007), where it can react with oxygen to elemental sulfur (De Belie et al., 2004; Monteny et al., 2001). Aerobic sulfur oxidizing bacteria (e.g. *Acidithiobacillus thiooxidans*) metabolize elemental sulfur and other reduced sulfur compounds (e.g. $S_2O_3^-$) to sulfuric acid ($H_2SO_4$) finally leading to the deterioration of concrete (De Belie et al., 2004; Okabe et al., 2007).

When sulfuric acid reacts with concrete, two main aspects have to be considered: i) the reaction with the sulfate ion and ii) the action of the hydrogen ion (Van Tittelboom et al., 2013). The first step involves a reaction between sulfuric acid and calcium hydroxide ($Ca(OH)_2$) in the concrete resulting in the formation of gypsum ($CaSO_4 \cdot 2H_2O$) (De Belie et al., 2004; O'Connell et al., 2010; Van Tittelboom et al., 2013). This expansive corrosion product leads to a volume increase of concrete by a factor of 1.2-2.2 (Monteny et al., 2001; Monteny et al., 2000). A soft and white corrosion layer develops on the concrete surface which increases over time (Wells et al., 2009). Subsequently, gypsum reacts with calcium aluminate hydrate ($C_3A$) to form ettringite (De Belie et al., 2004). The formation of ettringite is associated with a even greater volume expansion than the formation of

gypsum (Monteny et al., 2000) finally leading to increasing internal pressure, cracking and weakening of the concrete structure (Joseph et al., 2012; Sun et al., 2016; Vincke et al., 2002). Under acidic conditions (pH ≤ 2.0), the precipitation of gypsum is dominant (Gutberlet et al., 2015).

Previous studies revealed large differences on concrete corrosion between purely chemical sulfuric acid tests and experiments including microbially produced sulfuric acid (Alexander & Fourie, 2011; Ehrich et al., 1999; Monteny et al., 2001; Monteny et al., 2000; Wells et al., 2009). Monteny et al. (2000), for instance, found a higher extent of concrete corrosion through BSA production. It has been assumed that the soft corrosion layer characterized by an increased porosity and a high humidity provides excellent conditions for microbial growth (Monteny et al., 2000). The corrosion process is accelerated when bacteria penetrate into the corrosion layer and sulfuric acid production can take place near the undamaged concrete surface. On the other hand, Okabe et al. (2007) showed that oxygen and nutrient availability and thus microbial growth is limited in the thick corrosion layer.

Although a lot of research has been performed on sulfuric acid attack on cement stone and concrete previously, little is known about potential differences of chemically and microbially generated sulfuric acid. We hypothesize that chemical and biogenic sulfuric acid attack can lead to comparable damage patterns on concrete (see hypothesis # 3; Chapter 2). We proposed an additional hypothesis, namely that there is no linear increase in concrete deterioration with increasing incubation periods of microbially produced sulfuric acid (see hypothesis # 4; Chapter 2).

Biogenic experiments with *A. thiooxidans*, able to produce pH values around 1.0, were carried out over 28 days, two, three and six months to investigate the concrete behavior over time. The sulfuric acid production by *A. thiooxidans* was monitored via pH and sulfate measurements to ensure microbial activity. Chemical experiments were performed over 28 days at two constant pH values, 1.0 and 2.0, to study the differences between biogenic and chemical sulfuric acid attack on concrete. After the corresponding time periods, the extent of concrete corrosion was evaluated by a combination of different parameters i) visual inspections, ii) weight loss, iii) neutralization depth, iv) calcium leaching), v) laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), and vi) scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analysis. LA-ICP-MS was already used in a previous study analyzing chemical and biological $H_2SO_4$ attack on hardened cement paste samples (see Chapter 6). Within this study, the applicability of this method was tested for concrete which is more complex than hardened cement paste due to the presence of aggregates.

## 7.2 MATERIALS AND METHODS

### 7.2.1 Concrete specimens

Concrete specimens were prepared by BBQ Bautechnik Süd GmbH & Co. KG (Stuttgart, Germany) with a water to cement ratio (*w/c*) of 0.5 (cement content = 350 kg/m$^3$). A blast furnace slag cement CEM III/B 32.5 N-LH/SR with low hydration heat and high sulfate resistance according to DIN EN 197-1, NBN B12-108 and DIN 1164 was used. The aggregate was a mixture of reactive limestone ($CaCO_3$) and inert quartz ($SiO_2$) with a maximum grain size of 16 mm. The used concrete composition had a strength class of C30/37, which is frequently used for the construction of wastewater facilities such as sludge digesters. For the biological and chemical 28-day ("short-term") experiments the concrete was cut into 20x20x10 mm$^3$ specimens. For the long-term experiments cubes with 20 mm edge length were used. The narrower specimens for the short-term experiments were necessary due to the chemical experiment device. All concrete specimens were weighed prior to experiments.

### 7.2.2 Biogenic sulfuric acid experiments with *A. thiooxidans*

To analyze the impact of microbially produced sulfuric acid on concrete, batch experiments of different time scales with *A. thiooxidans* were carried out. Experiments were performed over periods of 28 days, two, three and six months to evaluate the concrete behavior over time. Additionally the focus of the 28-day experiments was to correlate them to purely chemical sulfuric acid experiments over the same time period of exposure. Table 7.1 provides an overview of the biogenic experiments, corresponding abiotic control set-ups, and analyzed parameters.

Table 7.1 Overview of the examination parameters for the short-term biogenic and chemical set-ups and long-term biogenic experiments and the corresponding abiotic control set-ups.

| | **short-term 28 d biogenic set-ups** | | **short-term 28 d chemical set-ups** | | **long-term biogenic set-ups** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **set-ups** | biogenic reactor<br>bio-R-28d | abiotic control<br>AC-28d | chemical reactor<br>ch-R-pH2 | chemical reactor<br>ch-R-pH1 | 2 months biogenic reactor<br>bio-R-2M | 2 months abiotic control<br>AC-2M | 3 months biogenic reactor<br>bio-R-3M | 3 months abiotic control<br>AC-3M | 6 months biogenic reactor<br>bio-R-6M | 6 months abiotic control<br>AC-6M |
| **replicates** | R1-R5 | AC1 | R1-R2 | R1-R2 | R1-R3 | AC1 | R1-R3 | AC1 | R1-R3 | AC1 |
| **inoculum** ***A. thiooxidans*** | + | - | - | - | + | - | + | - | + | - |
| **concrete specimen size** | 20x20x10 $mm^3$ | 20x20x10 $mm^3$ | 20x20x10 $mm^3$ | 20x20x10 $mm^3$ | 20x20x20 $mm^3$ | 20x20x20 $mm^3$ | 20x20x20 $mm^3$ | 20x20x20 $mm^3$ | 20x20x20 $mm^3$ | 20x20x20 $mm^3$ |
| **analyses bulk phase** | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium | pH<br>sulfate<br>calcium |
| **analyses cement specimen** | visual inspection<br>mass loss<br>neutralization depth<br>LA-ICP-MS | visual inspection<br>mass loss<br>neutralization depth<br>LA-ICP-MS | visual inspection<br>neutralization depth<br>LA-ICP-MS | visual inspection<br>neutralization depth<br>LA-ICP-MS | visual inspection<br>mass loss<br>neutralization depth<br>LA-ICP-MS<br>SEM/EDX | visual inspection<br>mass loss<br>neutralization depth<br>LA-ICP-MS<br>SEM/EDX | visual inspection<br>mass loss<br>neutralization depth | visual inspection<br>mass loss<br>neutralization depth | visual inspection<br>mass loss<br>neutralization depth<br>LA-ICP-MS<br>SEM/EDX | visual inspection<br>mass loss<br>neutralization depth<br>LA-ICP-MS<br>SEM/EDX |

All experiments were set up in 500 mL glass flasks (reactors) and performed as described in Chapter 6 (Figure 7.1A). Each reactor contained 200 mL medium and one concrete specimen. The sample was weighed and fixed with a corrosion resistant wire (see Figure 7.1B) to a rubber stopper. After sterilization of the concrete surface with UV light (overnight), the specimen was submerged to three-quarters into the liquid medium. DSMZ medium 35 was used as culture medium, as it showed good growth for *A. thiooxidans* in previous studies (see Chapters 4 and 5). Furthermore, the medium is recommended for the cultivation of *Acidithiobacillus* spp. by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen- German Collection of Microorganisms and Cell Cultures). The culture medium was prepared according to DSMZ instructions (https://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium35.pdf) with an initial pH value of 4.5 and elemental sulfur as the only energy source. One liter medium contained 1.9 mmol $NH_4Cl$, 22.0 mmol $KH_2PO_4$, 0.5 mmol $MgCl_2 \cdot 6\ H_2O$, 1.0 mmol $CaCl_2 \cdot 2\ H_2O$, and 311.8 mmol sulfur (powder).

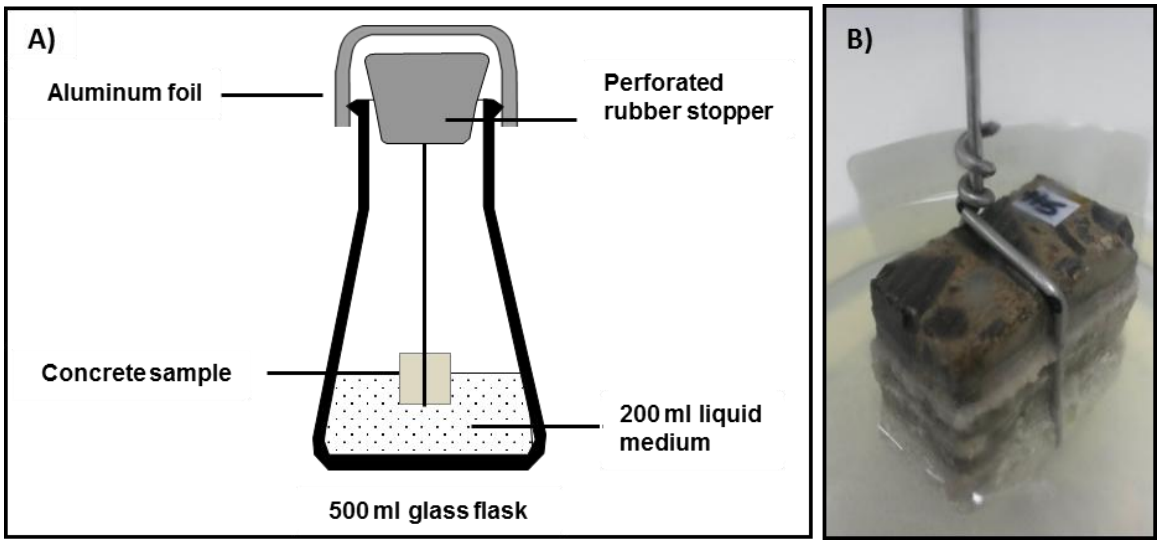



Figure 7.1 A) Setup of the biogenic sulfuric acid corrosion tests (adapted from Chapter 6). B) Concrete specimen of the six-month experiment after 38 days of incubation in the bacterial solution.

For inoculation of the liquid medium, pure *A. thiooxidans,* originally isolated from the corroded concrete wall of a sludge digester (see Chapter 5) was used. The reactors were closed with a perforated rubber stopper to allow oxygen entry and additionally covered with aluminum foil to prevent contamination. Since the high pH value of the concrete samples inhibited initial growth of the acidophilic *A. thiooxidans*, all reactors were pre-

incubated at 30°C and 125 rpm until microbial growth was observable by turbidity and pH decrease. The pH of the medium was measured periodically with a pH electrode (WTW GmbH, Weilheim, Germany). The concrete samples were added to the solution after the pH decreased to values around 2 and incubated in the culture medium for 28 days (short-term experiments), two, three and six months (long-term experiments).

To provide optimal nutrient conditions for *A. thiooxidans* and prevent negative effects by the accumulation of metabolites in the solution, the experiments were performed in semi-continuous draw and fill operated mode. Therefore, ten percent of the liquid medium was replaced with fresh medium twice a week. The withdrawn solution was stored at -20°C for subsequent chemical analyses such as sulfate and calcium.

#### 7.2.2.1 Short-term experiments (28 days)

Short-term experiments were carried out with 20x20x10 $mm^3$ concrete specimens in five parallel set-ups, reactor 1 to 5 (R1-R5) over a period of 28 days. An abiotic control (AC) without *A. thiooxidans* was set-up under the same conditions to study the impact of the medium. After 28 days, the concrete samples were removed from the bacterial solution, washed with Milli-Q-water (Merck Millipore, Darmstadt, Germany) and dried at 40°C. Then, the weight and neutralization depth of the concrete specimen were determined. The samples were photographed and the element distribution was analyzed with LA-ICP-MS.

#### 7.2.2.2 Long-term experiments (two, three and six months)

Long-term experiments were carried out for 62 days (“2 months”), 93 days (“3 months”) and 187 days (“6 months”) with 20x20x20 $mm^3$ concrete specimens. Each approach was set up in triplicates (R1-3) including an abiotic control reactor (AC) as described in section 7.2.2.1. The two- and three-month experiments were started at the same time and therefore the same inocula were used. Since the six-month experiment was set-up later, other bacterial cultures were used for inoculation. To maintain an active bacterial community over the whole experimental phase, the culture medium was completely replaced at certain time intervals with *A. thiooxidans* pre-incubated medium (pH around 2). For the two- and three-month experiments, the solution was exchanged after 42 days. During the six months, the solution was renewed three times: on day 38, 83 and 125. After the corresponding time periods (two, three and six months), the concrete samples were removed from the bacterial solution, washed with Milli-Q-water (Merck Millipore, Darmstadt, Germany), and dried at 40°C until weight constancy. Afterwards, the samples

were photographed and the concrete corrosion was analyzed by mass loss determination, neutralization depth measurement and with LA-ICP-MS analysis. In addition, SEM and EDX analysis were performed for the concrete samples of the two- and six-month experiments.

### 7.2.3 Chemical short-term experiments (28 days)

Chemical short-term experiments were carried out over a period of 28 days in order to compare the degradation process by biogenically and chemically produced sulfuric acid. Therefore, concrete samples with a size of 20x20x10 $mm^3$, being the maximum possible size for this device, were exposed to sulfuric acid solutions with a constant pH value of 1.0 and 2.0 using an autotitrator. For each set-up (pH 1.0 and 2.0), two concrete samples were analyzed. The incubation of the samples in sulfuric acid followed the procedure described previously (Gutberlet et al., 2015). Each concrete specimen was placed in a special sample holder positioned in a capped beaker with 150 mL sulfuric acid. The solution was continuously stirred with a magnetic stir bar. The pH of the acid solution was determined for each sample position and readjusted every ten minutes by titrating 0.5 M $H_2SO_4$ and 0.1 M $H_2SO_4$ to maintain pH 1.0 and pH 2.0, respectively. The acid consumption was recorded continuously. Due to the limited capacity of the beaker, 30 mL of the corrosion solution were removed at certain intervals. The withdrawn solutions were collected and stored at 4°C until sulfate and calcium measurements. After 28 days of storage, the concrete specimens were removed from the sulfuric acid solution and dried at 40°C until weight constancy. Afterwards, the samples were photographed and the extent of damage was evaluated by LA-ICP-MS. Table 7.1 provides an overview of the chemical set-ups and analyzed parameters.

### 7.2.4 Chemical analyses

#### 7.2.4.1 Sulfate and calcium measurements

Sulfate measurements in the culture medium of the biogenic sulfuric acid experiments were carried out regularly to monitor the potential sulfuric acid production by *A. thiooxidans*. The dissolved sulfate was determined with liquid ion exchange chromatography (ICS—1000 ion chromatography system, Dionex Corporation, USA) according to German Standard Methods for the examination of water, wastewater and sludge (DEV, 2016).

In the chemical sulfuric acid experiments sulfate was the only sulfur species and was therefore measured in the corrosion solution via total sulfur measurements using inductively coupled plasma optical emission spectrometry (ICP-OES, Jobin Yvon Ultima II).

The dissolved calcium concentration was measured in the bulk phase of the chemical and biological set-ups using ICP-OES (Jobin Yvon Ultima II).

#### 7.2.4.2 Determination of neutralization depth

The neutralization depth of all concrete samples was determined on the cross sectional area with phenolphthalein according to DIN EN 14630:2007-01.

#### 7.2.4.3 Determination of mass loss

To determine the mass loss of the concrete samples, the weight of the untreated samples was documented at the beginning of the experiments using an analytical balance with an accuracy of 0.1 mg (Sartorius AG, Göttingen, Germany). After exposure to the different sulfuric acid solutions, the concrete samples were removed from the solutions and rinsed with Milli-Q-water (Merck Millipore, Darmstadt, Germany) in order to remove residual bacteria and sulfuric acid. Afterwards, the samples were dried at 40°C until weight constancy. The samples were weight out again and the difference in weight was documented.

#### 7.2.4.4 Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)

To evaluate the extent of concrete degradation, the corroded concrete samples of the biogenic and chemical short-term (28 days) and biogenic long-term experiments (two- and six-month set-ups) and the abiotic control of the biotic set-ups were analyzed with LA-ICP-MS as described in Chapter 6. Prior to analysis, the samples were cut transversally with a high-precision saw and dried at 40°C for one day. Ten lines across the cross section of the concrete specimens were ablated using the following settings in the laser ablation system (NWR 213, New Wave Research, Inc. USA): 50 µm laser spots with a pulse rate of 20 Hz and a feed rate of 100 µm/s. The ablated material was ionized in the inductively coupled plasma (ICP) and analyzed in the mass spectrometer (MS; quadrupole-ICP-MS NexION 300D, Perkin Elmer, USA). An untreated concrete sample was ablated in the same way and served as reference (negative control, NC). Due to the irregular surface of

the corroded and non-corroded samples and the resultant dissimilar ablation behavior only semi-quantitative statements could be made about the elemental distributions. Within this study the distribution of carbon, calcium, silicon, sulfur and phosphorus was analyzed.

### 7.2.5 Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX)

Concrete samples of the biogenic long-term experiments (two and six months) and the abiotic control set-ups were additionally analyzed with SEM and EDX. Therefore, the sample surfaces were coated with a thin layer of gold to avoid charging. The specimens were imaged with a scanning electron microscope (Jeol JSM -5900L V, Japan) at an accelerating voltage of 15kV. Elements were analyzed over the displayed area with an energy dispersive X-ray detector (Röntec, Germany).

## 7.3 RESULTS AND DISCUSSION

In natural environments, concrete corrosion due to microbially produced sulfuric acid is a multi-stage and slow process (Aviam et al., 2004). Since corrosion rates are in the range of 1 mm to a maximum of 5 mm per year it would require several years to study the durability of concrete *in situ* (Monteny et al., 2000). Thus, in most cases accelerated tests in the laboratory are performed. Within this study, 28-day biogenic and chemical experiments and long-term biogenic experiments (two, three and six months) were carried out to simulate the last, but decisive step of BSA corrosion, where sulfuric acid produced by SOB reacts with $Ca(OH)_2$ and C-S-H phases of concrete as well as with the carbonates of the aggregates finally resulting in instability of the concrete matrix. The main aim of the biological experiments was to simulate a worst-case scenario allowing a comparison to the purely chemical experiments. Therefore, the acidophilic *A. thiooxidans,* a bacterium that is able to generate high sulfuric acid concentrations and low pH values (0.5-2.0), was used (Robertson & Kuenen, 2006). *A. thiooxidans* is reported to be the key protagonist within the corrosion process and mostly found in heavily corroded concrete material (Islander et al., 1991; Sand & Bock, 1991; Vincke et al., 2001). *A. thiooxidans* used for inoculation of the biotic set-ups was an isolate from the corroded concrete surface of a sludge digester headspace (see Chapter 5). The pH optimum of *A. thiooxidan*s is between 2.0 and 3.5 (Pokorna & Zabranska, 2015). Since fresh concrete has an initial pH value of 12.0-13.0 (Jiang et al., 2014; Joseph et al., 2012), bacterial growth in the culture medium was inhibited by the addition of the concrete specimens. Thus, a pre-incubation with

*A. thiooxidans* was carried out until the pH of the culture medium decreased from 4.5 to around 2.0. This pH decrease in combination with an increasing sulfate concentration was an indicator for an active microbial community and the concrete samples were added to the bacterial solution. To provide optimal growth conditions for *A. thiooxidans* and ensure a continuous sulfuric acid production ten percent of the medium (20 mL) were replaced with fresh medium twice a week. For the long-term experiments, the medium was completely replaced at certain time intervals with *A. thiooxidans* pre-incubated medium to prevent negative effects by the accumulation of metabolites.

After exposure to the different sulfuric acid solutions, the extent of concrete degradation was evaluated by the following parameters (Table 7.1): i) visual inspection of the specimens, ii) determination of mass loss iii), determination of neutralization depth, iv) calcium measurements in the bulk phase, v) analysis of elemental distribution by LA-ICP-MS, and vi) SEM and EDX analysis (biotic two- and six-month experiments). A combination of visual, physical and chemical parameters was used, since one single parameter is often insufficient for the characterization of concrete degradation (Van Tittelboom et al., 2013). Visual inspections of the specimens provided insights into changes of the concrete surface (e.g., formation of a corrosion layer or salt crystals) and first qualitative data could be obtained. Weight loss was used to quantitatively describe the extent of damage. The neutralization depth was determined across the cross section of the concrete specimens with the pH-indicator phenolphthalein, which is colorless at pH < 8.2 and turns pink in basic environment such as fresh concrete (pH ~12.0). Furthermore, calcium was determined in the bulk phase, because the leaching of calcium from the concrete is an indicator for structural failure. LA-ICP-MS was used to i) characterize the concrete structure (aggregates and cement part) and ii) characterize changes in concrete composition (e.g. leaching of certain elements and identification of corrosion products) during sulfuric acid attack. Normally, with LA-ICP-MS qualitative and quantitative results about the elemental distribution can be gained. However, the different sample surface properties and the resultant dissimilar ablation behavior allowed only semi-quantitative results within this study. For the assessment of concrete corrosion, the distribution of carbon, calcium, silicon, phosphorus and sulfur was analyzed. Carbon and silicon displayed the presence of carbonatic and siliceous aggregates, respectively. Calcium provided additional information on the distribution of carbonates. Furthermore, calcium leaching and gypsum precipitation were indicators for the deterioration of concrete. Sulfur was analyzed to get information on the sulfuric acid penetration depth and detect gypsum precipitation in the corrosion layer. Phosphorus was studied due to the high phosphate concentration in the culture medium (22 mmol/L $KH_2PO_4$). The concrete

samples of the two- and six-month experiments were additionally analyzed with SEM and EDX to obtain further information on the microstructure and element distribution of the concrete samples.

### 7.3.1 Biological and chemical short-term experiments (28 days)

#### 7.3.1.1 Sulfuric acid production within biogenic reactors

After the addition of the concrete samples, the pH value and the dissolved sulfate concentration were determined twice a week to monitor the potential sulfuric acid production by *A. thiooxidans*. An unchanged or decreasing pH value in combination with an increasing sulfate concentration in the medium was an indicator for sulfuric acid production and bacterial activity. At the start of the experiment, the average pH value in the biogenic reactors R1-R5 was 2.4 (Figure 7.2). After adding the concrete specimens, the pH remained unchanged during the next two days and then decreased steadily. After 28 days of incubation, an average pH value of 1.3 was determined indicating acid production. Normally, the dissolution of concrete leads to a neutralization of the produced acid, even when *A. thiooxidans* communities are well established (Scrivener & De Belie, 2013). However, within this study, the pH decreased over time indicating that the sulfuric acid production by *A. thiooxidans* was higher than the neutralization capacity of the concrete. The pH of the abiotic control increased from an initial value of 4.5 to 6.3 after nine days of incubation due to the high pH value of the concrete and remained stable at this level until the end of the test (pH 6.1-6.3).

Besides the pH value, the dissolved sulfate concentration in the culture medium was determined over 28 days (Figure 7.2). Since the bacterial solution was pre-incubated with *A. thiooxidans*, the initial sulfate concentration was 20 mmol/L. Over the course of the experiment, the sulfate concentration increased continuously within the five biogenic sulfuric acid reactors (R1-R5). After 28 days, an average concentration of 102 mmol/L was determined. This corresponds to a final sulfate amount of 20 mmol in the applied 200 mL culture medium. During the 28 days, a total amount of 113 mmol sulfur was added to each set-up. If the bacteria would have oxidized all the provided sulfur, a concentration of 113 mmol sulfate within the 200 mL culture medium must have been detected at the end of the test. The lower detected sulfate amount of 20 mmol can be explained by the observations that i) the bacteria did not use all of the provided sulfur and ii) parts of the sulfate precipitated also as gypsum (see LA-ICP-MS, Figure 7.5). Generally, the increasing sulfate concentration and decreasing pH value indicated a continuous sulfuric acid production and microbial growth within the biogenic reactors over a period of 28

days. The sulfate concentration within the abiotic control was under the detection limit of 1.0 µmol/L. Consequently no sulfate leached out from the concrete.

For the simulation of biogenic sulfuric acid corrosion, it is important to achieve sulfate concentrations in the test systems that are comparable with the environment. In sewer pipes, for instance, sulfate concentrations of 94 mmol/L are quite common (Monteny et al., 2000). Therefore, the sulfate concentrations measured in the biotic set-ups (20-102 mmol/L) were in the range of those found in sewer systems and thus might also represent the amount of sulfuric acid produced *in situ*.

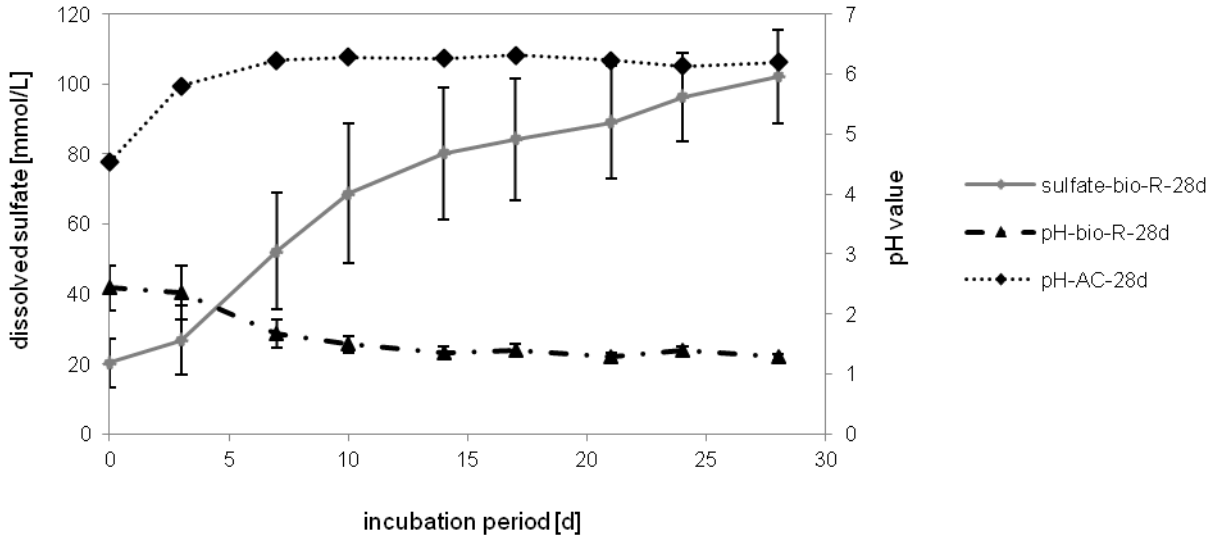



Figure 7.2 Average pH value and sulfate concentration with the corresponding standard deviation measured in the bulk phase of the biogenic reactors R1-R5 (sulfate/pH-bio-R-28d) and the abiotic control (pH-AC-28d) after the addition of the concrete samples. At each data point 10% of the culture solution was replaced with fresh medium.

#### 7.3.1.2 Sulfuric acid consumption within chemical set-ups

Autotitrator experiments with pure sulfuric acid at two constant pH values (pH 1.0 and pH 2.0) were carried out for 28 days to compare biological and chemical sulfuric acid corrosion on concrete. For the chemical experiments pH 1.0 and pH 2.0 were selected, because the average pH values generated by *A. thiooxidans* in the bacterial solution were between 1.3 and 2.4. The amount of sulfuric acid necessary to maintain a pH value of 1.0 and 2.0 was normalized to the sample surface (Figure 7.3). After 28 days, 4.7 mmol$H^+$/$cm^2$ (pH 1.0) and 1.0 mmol$H^+$/$cm^2$ (pH 2.0) of total acid were consumed by

the concrete. Considering the total acid input, 52 mmol (pH 1.0) and 16 mmol (pH 2.0) sulfate were added which is comparable to the total sulfate concentration of 20 mmol produced by *A. thiooxidans* in the biotic set-ups. In the corrosion solutions of the chemical set-ups 40 mmol (pH 1.0) and 10 mmol (pH 2.0) were recovered, the rest has likely been precipitated as gypsum (see LA-ICP-MS; Figure 7.5).

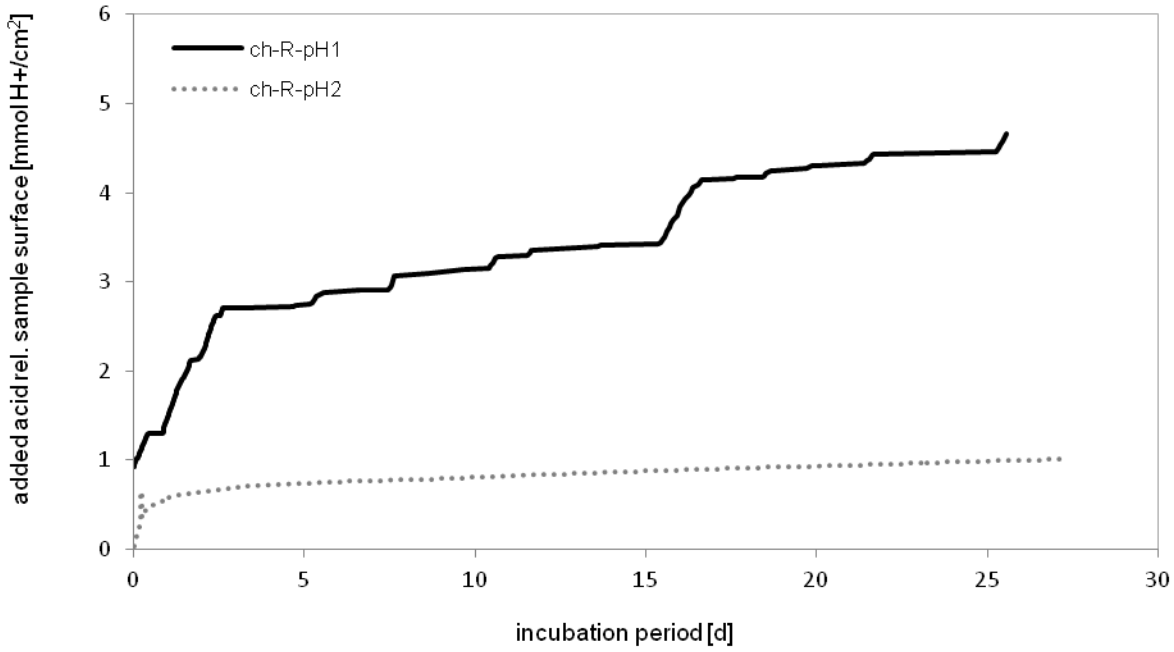



Figure 7.3 Amount of sulfuric acid, normalized to the sample surface, necessary to maintain the pH at 1.0 and 2.0 in the chemical set-ups.

### 7.3.1.3 Concrete corrosion within biogenic and chemical set-ups

#### 7.3.1.3.1 Visual inspections of the concrete specimens

After 28 days of incubation in biogenic (bio-R-28) and chemical sulfuric acid (ch-R-pH1 and ch-R-pH2), the concrete samples were optically corroded (Figure 7.4). To illustrate the extent of damage, an untreated sample (NC) is shown for comparison. All samples exposed to sulfuric acid were covered with a thin corrosion layer showing low mechanical strength. The concrete deterioration was highest in the areas of blast furnace slag cement stone. When comparing the chemical and biogenic set-up, the visual concrete degradation was highest for the chemical pH 1.0 samples, followed by the biogenic reactors (pH 1.3-2.4) and chemical pH 2.0 samples. Consequently, a correlation between the pH and the extent of damage was noticed. No degradation was observed for the abiotic control. Nevertheless, a thin layer of salt crystals was detected on the concrete surface (Figure 7.4; AC-28d). The formation of these salt crystals was identified as calcium phosphate

crystals by LA-ICP-MS analysis, and resulted from the high phosphate concentration in the medium.

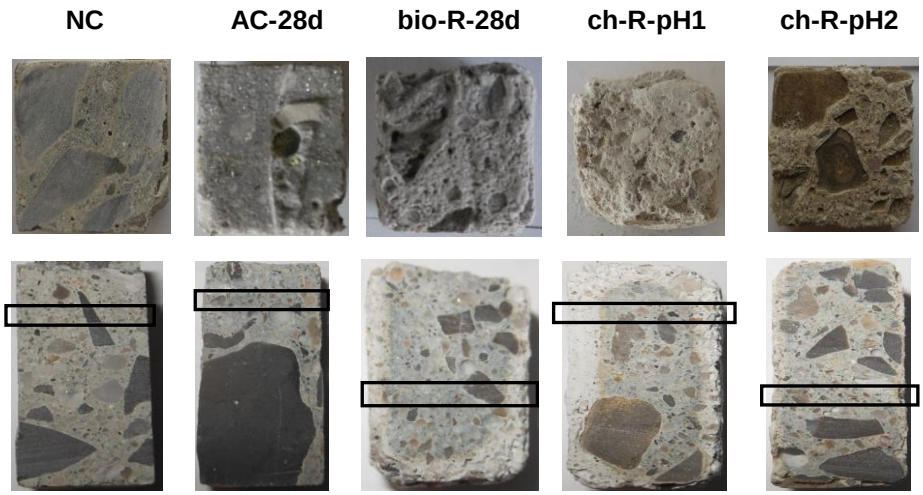



Figure 7.4 Top: Photos of the concrete samples after 28 days of incubation in the culture medium (AC-28d), bacterial solution (bio-R-28d), and purely chemical sulfuric acid at pH 1.0 (ch-R-pH1) and pH 2.0 (ch-R-pH2). Only one concrete specimen of each set-up is shown representative for the others. An untreated concrete sample (negative control-NC) is shown as reference. Bottom: Cross section of the corresponding concrete samples displayed above. Black marked regions show the area where LA-ICP-MS was carried out.

#### 7.3.1.3.2 Weight loss and neutralization depth

The mass loss and neutralization depth of the biogenic and chemical short-term experiments is given in Table 7.2. For the biogenic set-ups a weight loss of 2.1-4.8% was determined after 28 days of incubation in microbially produced sulfuric acid. The weight of the abiotic control was even slightly higher at the end of the test with a negative mass loss value of -0.7%. This may be due to densification of the concrete matrix through the formation of calcium phosphates. The neutralization depth of the abiotic control was 0.0 mm, which means that the leaching of the concrete with only the culture medium caused no decrease of the pH lower than 8.2 during 28 days. For the biogenic set-ups an average penetration depth of 1.2 mm was determined after 28 days of incubation, which is comparable to the chemical set-ups at pH 2.0 (1.3 mm). The highest neutralization depth with 2.0 mm was determined for the pH 1.0 samples (Table 7.2). The neutralization depth provided quantitative data about the extent of damage and confirmed the visually observed damage pattern.

Table 7.2 Mass loss and neutralization depth of the short-term biogenic and chemical experiments.

| | **Biogenic set-ups** | | | | | | **Chemical set-ups** | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | AC 28d | bio-R1-28d | bio-R2-28d | bio-R3-28d | bio-R4-28d | bio-R5-28d | ch-R1 pH 1 | ch-R2 pH 1 | ch-R1 pH 2 | ch-R2 pH 2 |
| **Mass loss [%]** | -0.7* | 4.8 | 4.8 | 2.1 | 3.4 | 4.7 | n.d. | n.d. | n.d. | n.d. |
| **Neutralization depth [mm]** | 0.0 | 1.2 | 0.6 | 1.6 | 1.6 | 1.2 | 2.0 | 2.0 | 1.0 | 1.5 |

*Negative values indicate an increase in weight; n.d.= not determined

#### 7.3.1.3.3 Calcium concentration in the bulk phase

Calcium measurements in the corrosion solutions were carried out to monitor calcium leaching from the concrete. For the abiotic control, calcium concentrations ≤1 mmol/L were determined. This means that no or only little calcium emerged from the concrete specimens during incubation in the culture solution without bacteria. After the addition of the concrete specimens into the biogenic sulfuric acid reactors, the calcium concentration in the bulk phase immediately increased from an initial value of 1 mmol/L to 9 mmol/L indicating calcium leaching from the concrete. Afterwards, the calcium concentration remained between 9 and 10 mmol/L. Compared to the abiotic control, the calcium concentration in the biotic reactors was at least ten times higher suggesting the dissolution of Ca-bearing phases of the cementitious matrix. Leaching of calcium was also observed within the chemical experiments. The calcium concentrations determined in the corrosion solutions after 28 days ranged from 19 mmol/L (pH 2.0) to 22 mmol/L (pH 1.0) and were therefore little higher than the calcium concentration of the biogenic experiments (9-10 mmol/L).

#### 7.3.1.3.4 LA-ICP-MS

Figure 7.5 shows the distribution of the five selected elements across the cross section of the negative control (NC), abiotic control (AC-28d), biogenic (bio-R1-28d) and chemical set-ups (ch-R1-pH1 and ch-R1-pH2) after 28 days of exposure to the corresponding solutions. The five replicates (R1-R5) of the biogenic experiments and the two replicates of the chemical set-ups showed similar LA-ICP-MS results, respectively. Therefore, only one LA-ICP-MS profile is shown for each set-up.

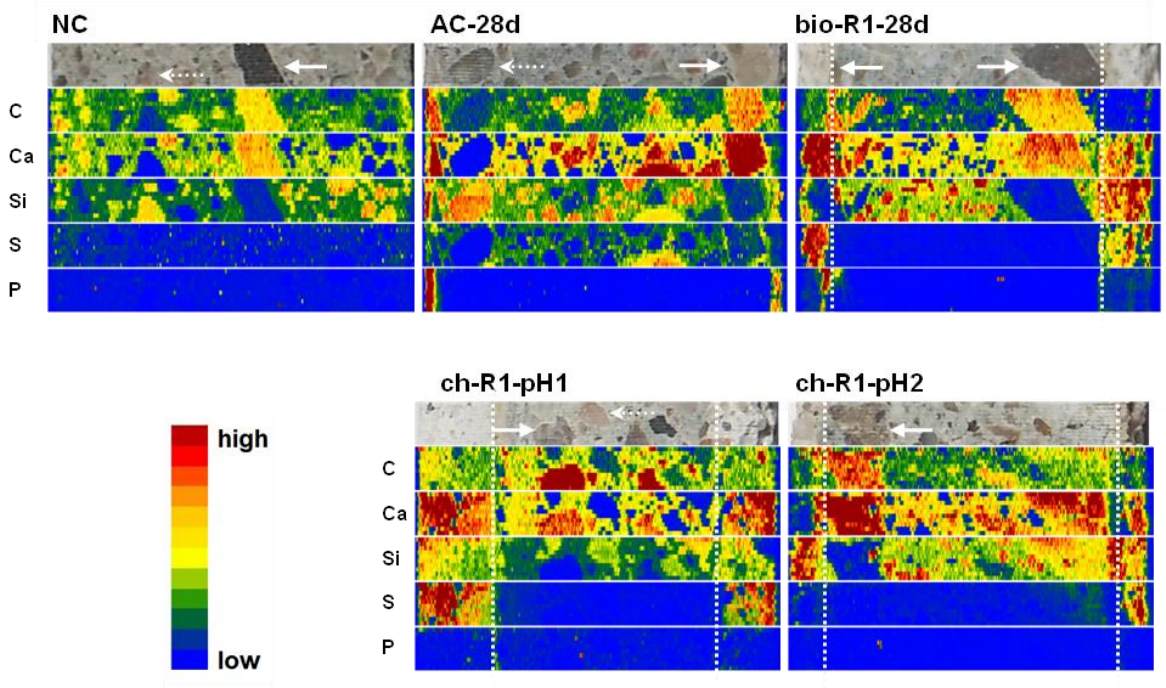



Figure 7.5 Top: Magnified view of the black marked areas (cross section) from Figure 7.4 analyzed by LA-ICP-MS. Bottom: LA-ICP-MS results showing the distribution of carbon (C), calcium (Ca), silicon (Si), phosphorus (P), and sulfur (S) across the cross section of the negative control (NC), abiotic control (AC-28d), biogenic (bio-R1-28d) and chemical set-ups (ch-R1-pH1 and ch-R1-pH2). Siliceous (dotted arrows) and carbonatic aggregates (continuous arrows) are exemplarily marked in the cross sectional area. The dotted lines show the transition zone of degraded and non-attacked concrete. The degraded part is defined as corrosion layer.

Concrete mainly consists of i) cement paste and ii) aggregates, which account for more than 60% (Scrivener & De Belie, 2013). Two different aggregates were used for concrete preparation: inert siliceous aggregate and reactive limestone.

Generally, high carbon and calcium concentrations indicate the presence of carbonates, whereas high silicon amounts give information about siliceous aggregates. For the untreated sample (NC), a homogenous elemental distribution was observed in the small areas with cement paste. Although a comparable distribution was found in untreated pure cement paste (see Chapter 6), care must be taken when comparing concrete with pure cement paste. Due to the presence of aggregates, an interfacial zone exists between cement paste and aggregates (Van Tittelboom et al., 2013) leading to different microstructures in pure cement paste and concrete. Besides the quite homogenous distribution in the areas with cement paste, a huge carbonatic aggregate could be

detected in the middle of the cross section (continuous arrow, Figure 7.5, NC) visible due to the high carbon and calcium concentrations in this part. Furthermore, a few smaller siliceous aggregates were found in between the concrete sample (dotted arrow, Figure 7.5, NC). The LA-ICP-MS profile of the abiotic control (AC), which was incubated for 28 days in the culture medium without *A. thiooxidans*, was similar to the profile of the negative control showing homogenous elemental distribution in the areas of cement paste and the presence of carbonatic (continuous arrow, Figure 7.5, AC) and siliceous aggregates (dotted arrow, Figure 7.5, AC). However, high calcium (on the left side) and phosphate concentrations (on both sides) were observed on the outer edges of the concrete specimen indicating calcium phosphate precipitation. The formation of these salt crystals was already observed visually and could be identified as calcium phosphate by LA-ICP-MS analysis. The precipitation is due to the high phosphate concentration in the culture medium. Since the formation of calcium phosphate is enhanced at higher pH values, the precipitation was primarily noticed for the abiotic control (pH >6.0) and on the reaction front (degraded concrete/undamaged concrete interfacial region) of the concrete samples incubated with *A. thiooxidans*. Calcium phosphate formation on surface of concrete and hardened cement paste specimens was already observed in experiments using culture media with $KH_2PO_4$ (22-29 mmol/L) (see Chapter 6 and Aviam et al., 2004). However, the formation of calcium phosphates seems to have no negative effects on concrete degradation. Aviam et al. (2004) suggested that the formation of calcium phosphates would have even increased the stability of the concrete matrix due to the densification of concrete pores. Furthermore, no accumulation of sulfur was detected with LA-ICP-MS on the outer concrete surface, which means that no sulfur from the medium penetrated into the sample. This is also in agreement with the detected neutralization depth of 0.0 mm.

LA-ICP-MS results of the concrete samples incubated in the biogenic reactors showed high concentrations of sulfur and calcium in the corrosion layer indicating the formation of the corrosion product gypsum ($CaSO_4 \cdot 2\ H_2O$). From both sides of the cross section, the sulfuric acid, visible on the higher sulfur concentration in the corrosion layer, permeated the concrete until carbonates appeared (continuous arrows, Figure 7.5, bio-R1-28d). An accumulation of silicon in the corrosion layer was observed which may be due to the formation of amorphous silicic acid (silica gel). Furthermore, phosphorus was detected at the reaction front resulting from the high phosphate concentration in the medium. The accumulation at the transition zone is due to the lower solubility of phosphates with increasing pH values on the non-attacked concrete surface. The concrete samples exposed to purely chemical sulfuric acid at pH 1.0 and 2.0 showed similar degradation

patterns with clear gypsum formation and silicon accumulation in the outer regions of the concrete and infiltration of sulfuric acid. However, for pH 1.0 a larger corrosion layer and thus a higher extent of corrosion were analyzed in the LA-ICP-MS profile. The sulfuric acid penetration depth was higher compared to the biotic and chemical pH 2.0 set-ups visible due to the higher calcium and sulfur concentrations in the corrosion layer. This is also in agreement with the higher neutralization depth of 2.0 mm detected for chemical set-ups at pH 1.0 (Table 7.2). Phosphorus was not detected on the concrete surface exposed to chemical sulfuric acid, because these concrete samples were not exposed to the culture medium containing high amounts of phosphorus.

Finally, chemical analyses of the concrete samples by LA-ICP-MS verified the findings obtained with visual and physical means supporting a pH-dependent degree of damage. Nevertheless, with LA-ICP-MS more in depth results were obtained due to semi-quantitative results concerning the elemental distributions of the concrete. LA-ICP-MS enabled the identification of the corrosion layer and clearly showed the transition zone of degraded and un-damaged concrete. Due to the analyses of the elemental distributions, gypsum could be identified as main corrosion product and also calcium phosphate precipitation was detected in the abiotic control reactor. The penetration depth of sulfuric acid determined by pH indicator phenolphthalein was confirmed by the identification of sulfur in the corrosion layer. Furthermore, siliceous and carbonatic aggregates could be identified and their distribution across the cross section of the sample was visible.

### 7.3.2 Biological long-term experiments (two, three and six months)

#### 7.3.2.1 Sulfuric acid production within biogenic reactors

The sulfuric acid production (sulfate concentration and pH value) of the long-term experiments is shown in Figure 7.6 (A and B). The same starting inocula were used for the two- and three-month experiments leading to similar sulfuric acid production and comparable pH trend for both set-ups. During cycle 1 (0-42 days), the sulfate concentration increased from an initial value of 23 mmol/L to 60 mmol/L. Within this time period, the pH decreased from 2.8 (day 0) to 1.4 (day 42). On day 42, the medium of both set-ups (two and three months) was exchanged with *A. thiooxidans* pre-incubated medium exhibiting an initial sulfate concentration of 20 mmol/L and a pH value of 1.7. After starting cycle 2, the sulfate concentration of both set-ups highly increased and reached values of 110 mmol/L after 62 days of incubation (end of two-month experiment). The corresponding pH values decreased and a pH value of 1.1 was measured for the two-month experiment after 62 days, while an average pH of 1.3 was determined for the three-

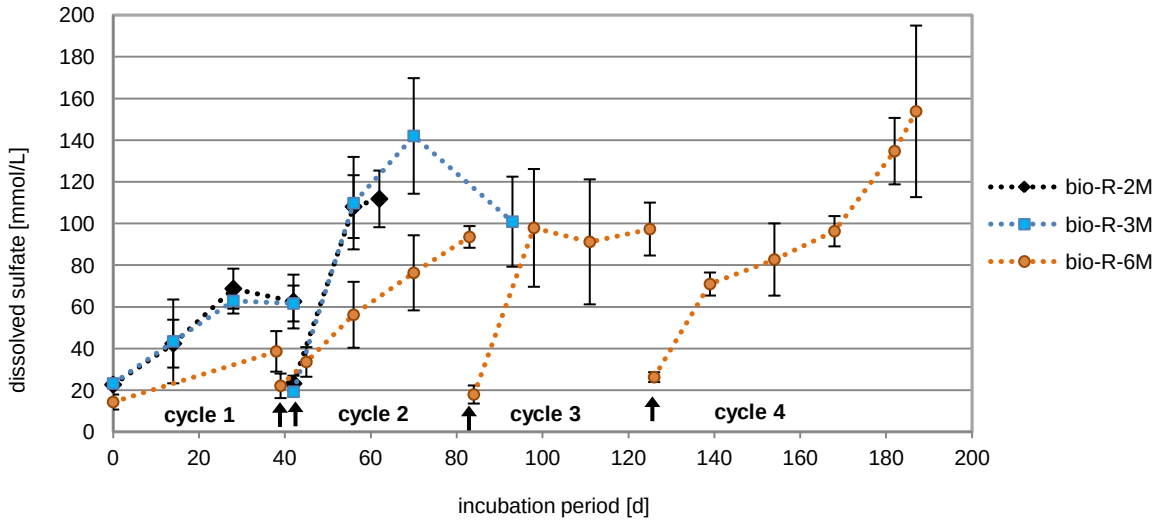
month experiment (day 63). In the further experimental course of the three-month set-ups, the sulfate concentration increased to 142 mmol/L and reached an average pH of 1.2 after 70 days. Subsequently, the concentration decreased to 110 mmol/L, which may be due an inactive/dying microbial community. Generally, the sulfate concentrations of cycle 2 (42-93 days) were significantly higher than in cycle 1 (0-42 days). This is likely due to the use of another (more active) starting inoculum.

During the six-month experiment, the medium was exchanged three times leading to four cycles: 0-38 days (cycle 1), 39-83 days (cycle 2), 84-125 days (cycle 3), and 126-187 days (cycle 4). Within the first cycle the sulfate concentration ranged between 14 and 39 mmol/L (pH 2.0). For cycle 2 and 3, 18-97 mmol sulfate per liter were determined in the culture medium. During this cycle the average pH value decreased from an initial value of 2.3 (cycle 2) and 1.8 (cycle 3) to 1.2. For the first three cycles lower sulfate concentrations were determined in comparison to the sulfate amount of the two- and three-month experiments. For cycle 4, which lasted 61 days, the highest production of *A. thiooxidans* was noticed with sulfate concentrations of 154 mmol/L and an average pH value of 1.1. In contrast to cycle 2 of the three-month experiment, where the bacteria already died after 30 days of incubation, a sharp increase of sulfate was noticed in cycle 4 of the six-month set-up after 40 days of incubation. This shows that the microbial community could be still very active and indicates that the sulfuric acid production was primarily dependent on the inoculum used.

The sulfate concentration within the abiotic control reactors was lower than 1.0 mmol/L. The pH within the abiotic control increased from an initial value of 4.5 to values between 6.3 and 6.7.

While the sulfate concentrations measured for the two- and three-month set-ups were quite similar, a different trend of sulfuric acid production was noticed for the six-month experiment. Even within the different cycles various trends were observed. The amount of sulfuric acid produced is directly dependent on the starting inoculum applied. When exactly the same bacterial culture was used for inoculation, e.g. for the two- and three-month experiments (start of cycle 1 and 2), the sulfuric acid production was comparable. Since the six-month experiment was carried out at a different time schedule a new bacterial culture had to be used leading to a different sulfate development. These results indicate that the biogenic sulfuric acid experiments were reproducible when equal activity of bacterial culture (e.g. use of same starting inoculum) prevailed.

A)




B)

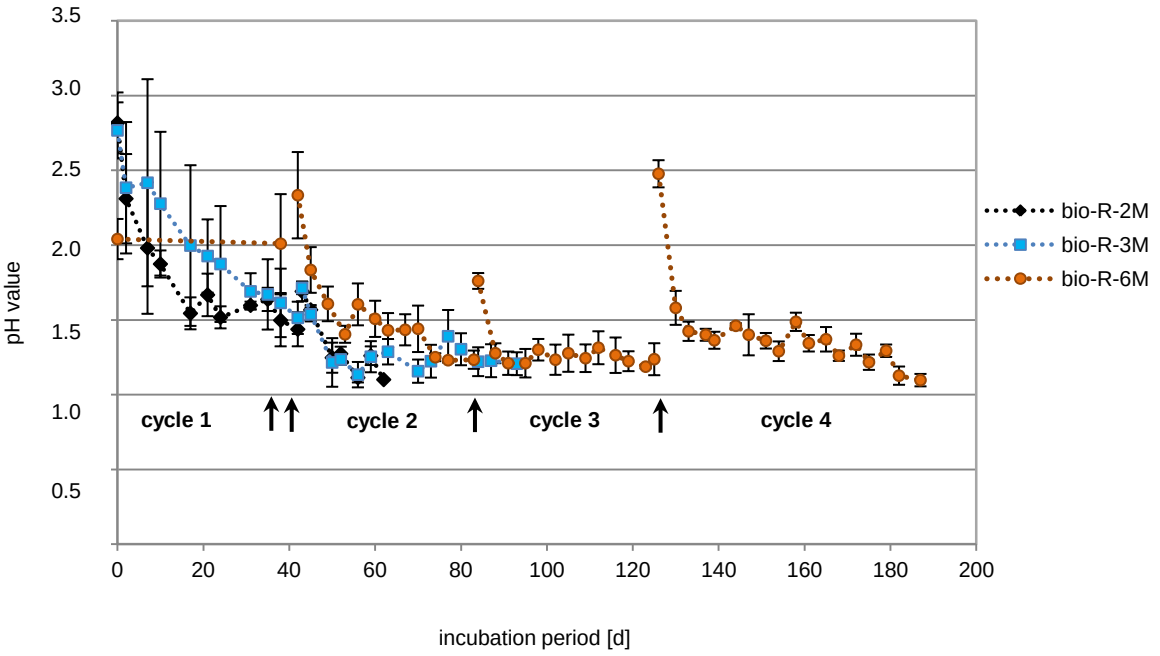



Figure 7.6 Average sulfate concentrations (A) and pH values (B) with corresponding standard deviations measured within the biogenic sulfuric acid reactors (R1-R3) of the two- (bio-R-2M), three- (bio-R-3M) and six-month (bio-R-6M) experiments. Arrows indicate medium replacement with *A. thiooxidans* pre-incubated medium after 39 days for the two- and three-month experiments (bio-R-2M and 3M) and after 42, 83 and 126 days for the six-month set-up (bio-R-6M). At all the other data points 10% of the culture solution was replaced with fresh medium.

#### 7.3.2.2 Concrete Corrosion

##### 7.3.2.2.1 Visual inspections of the concrete samples

Visual inspections of the concrete samples exposed to microbially produced sulfuric acid for two, three and six months showed a corroded surface with a washed out concrete look (Figure 7.7). The highest degradation was observed for the concrete samples of the three- and six-month experiments, because from these samples larger aggregates broke off. For all abiotic control reactors a thin layer of salt crystals (verified as calcium phosphate by LA-ICP-MS and EDX analysis) was observed on the concrete surface.

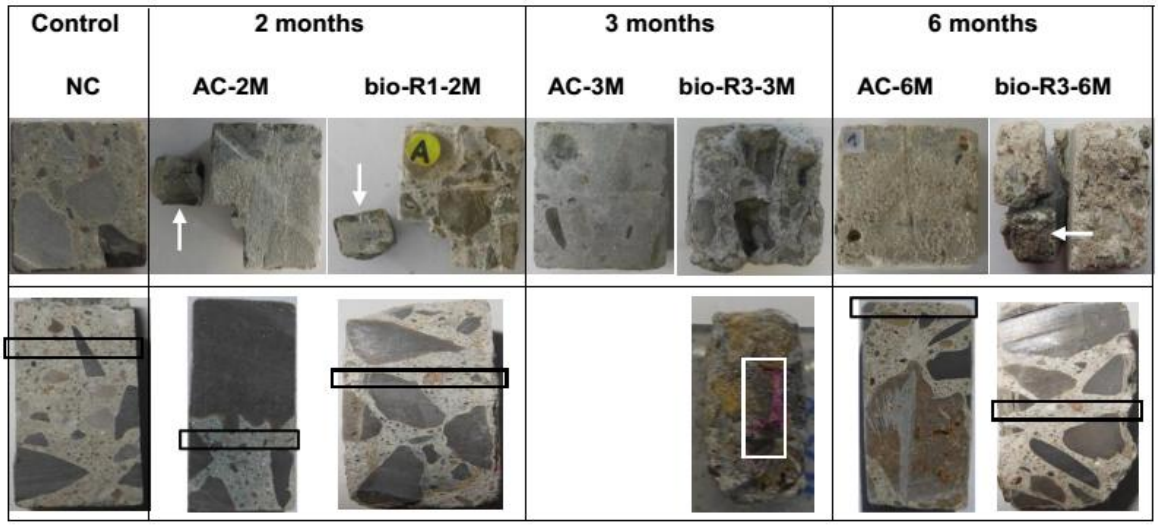



Figure 7.7 Top: Concrete samples after exposure in biogenic sulfuric acid solutions of the two- (bio-R1-2M), three- (bio-R3-3M), and six-month set-ups (bio-R3-6M) and corresponding abiotic control reactors (AC-2M/3M/6M). One corroded concrete sample of each set-up was chosen representative for the others. An untreated sample (NC) is shown as reference. From the part that was cut out (see arrows) from AC-2M, bio-R1-2M and bio-R3-6M SEM and EDX analyses were performed. Bottom: Cross section of the corresponding concrete samples shown above with black marked areas where LA-ICP-MS was carried out. For the three-month set-up no LA-ICP-MS was carried out. Instead, a fragment is shown with phenolphthalein results. The pink area (white box) shows the alkaline part of the concrete (pH> 8.2).

##### 7.3.2.2.2 Weight loss and neutralization depth

The weight loss and neutralization depth measured for all samples of the long-term experiments are given in Table 7.3. The two-month set-up showed a weight loss of 1.1-4.8% and an average neutralization depth of 2.3 mm. The weight loss and neutralization depth for the three- and six-month experiments were significantly higher than for the two-

month set-up. After three months, a loss in mass of 4.6-17% was determined. The neutralization depth was 3.1 mm on average (see Figure 7.7; bio-R3-3M). For the six-month set-up, the determined weight loss (6.3-18.2%) and neutralization depth (3.0 mm) were similar to the values measured after three months indicating a comparable extent of corrosion. The two corrosion parameters suggest the lowest extent of corrosion for the two-month experiments, followed by the three- and six-month set-ups. Although a higher extent of damage was expected after six months due to the longer exposure time to sulfuric acid, the degradation after three months showed comparable values. This may be due to the lower sulfate concentrations detected in the six-month set-ups during the first three cycles. Consequently, concrete degradation is primarily dependent on the amount of sulfuric acid produced by *A. thiooxidans* and does not necessarily depend on the incubation time.

Table 7.3 Mass loss and neutralization depth of the long-term biogenic experiments.

| | **Biogenic set-ups 2 months** | | | | **Biogenic set-ups 3 months** | | | | **Biogenic set-ups 6 months** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AC | R1 | R2 | R3 | AC | R1 | R2 | R3 | AC | R1 | R2 | R3 |
| **Mass loss [%]** | -0.6* | 2.0 | 1.1 | 4.8 | -0.1* | 4.6 | 10.7 | 17.0 | -1.2* | 7.1 | 6.3 | 18.2 |
| **Neutralization depth [mm]** | 1.0 | 2.5 | 2.5 | 2.0 | 2.0 | 3.4 | 3.0 | 3.0 | 2.0 | 3.0 | 3.0 | 3.0 |

* negative values indicate an increase in weight

Although the corrosion conditions (e.g., sulfuric acid production and neutralization depth) within the parallel set-ups were comparable, a high fluctuation in weight loss was noticed for the parallel approaches. In contrast to cement stone, concrete is very inhomogeneous due to the presence of aggregates. The distribution of aggregates varies resulting in different compositions of the analyzed concrete samples. When aggregates break off during sulfuric acid attack (“pop outs”), a higher weight loss occurs resulting in a higher extent of corrosion.

For all abiotic control reactors a slight increase in weight was observed. This may be due to the formation of calcium phosphates (see LA-ICP-MS) leading to a densification of the concrete matrix. The mass increase was highest for the six-month abiotic control which may be due to the fact that the concrete specimen had the highest exposure time in the culture medium. When the medium was replaced, also new potassium dihydrogen

phosphate (approx. 22 mmol/L) was provided and more phosphate was available for calcium phosphate precipitation. For the abiotic control set-ups, exposed for two, three and six months into the culture medium, a neutralization depth of 1.0-2.0 mm was determined. This indicates that the concrete pH within this determined depth is lower than 8.2. Since the culture medium had a pH value of 6.1-6.7 the long exposure probably reduced the surface concrete pH value. However, no sulfur penetrated into the concrete (see LA-ICP-MS) which means that no sulfuric acid attack occurred.

#### 7.3.2.2.3 Dissolved calcium concentration

The calcium concentration of the long-term experiments was measured at the end of each cycle. The calcium dissolved in the solution increased from an initial value of 1 mmol/L to 9-14 mmol/L during each cycle for all set-ups indicating calcium leaching and structural failure of the concrete matrix. For the two-month set-up calcium concentrations of 11 mmol/L (cycle 1) and 12 mmol/L (cycle 2) were determined. The concentration for the three-month experiment varied from 13 mmol/L for the first cycle to 9 mmol/L for the third cycle. The lower calcium concentration at the end of the three-month experiment is probably due to the inactive/dying microbial community. When no sulfuric acid is produced by *A. thiooxidans*, less calcium is leached out from the concrete. For the six-month experiment a comparable calcium amount (10 to 11 mmol/L) was detected in the bulk phase during the first three cycles and a slightly higher calcium (14 mmol/L) leaching was observed for cycle 4. This is in correlation with the higher sulfuric acid production during this cycle leading to higher calcium leaching from the concrete.

The calcium concentration of the abiotic control was lower than 1 mmol/L which shows that no or only very little calcium emerged from the concrete. The calcium concentrations of the long-term experiments during the different cycles were comparable to the short-term experiments.

#### 7.3.2.2.4 LA-ICP-MS analysis

Besides visual and physical means, LA-ICP-MS was used for the assessment of concrete degradation. Figure 7.8 shows the cross section of the negative control (NC), abiotic control (AC-2M) and biogenic reactor of the two-month experiment (bio-R-2M) as well as the abiotic control (AC-6M) and biogenic reactor of the six-month experiment (bio-R-6M). For each set-up only one corroded concrete sample is shown representative for the others. No LA-ICP-MS analysis was performed from the three-month experiments, since the extent of corrosion was comparable to the six-month set-up.

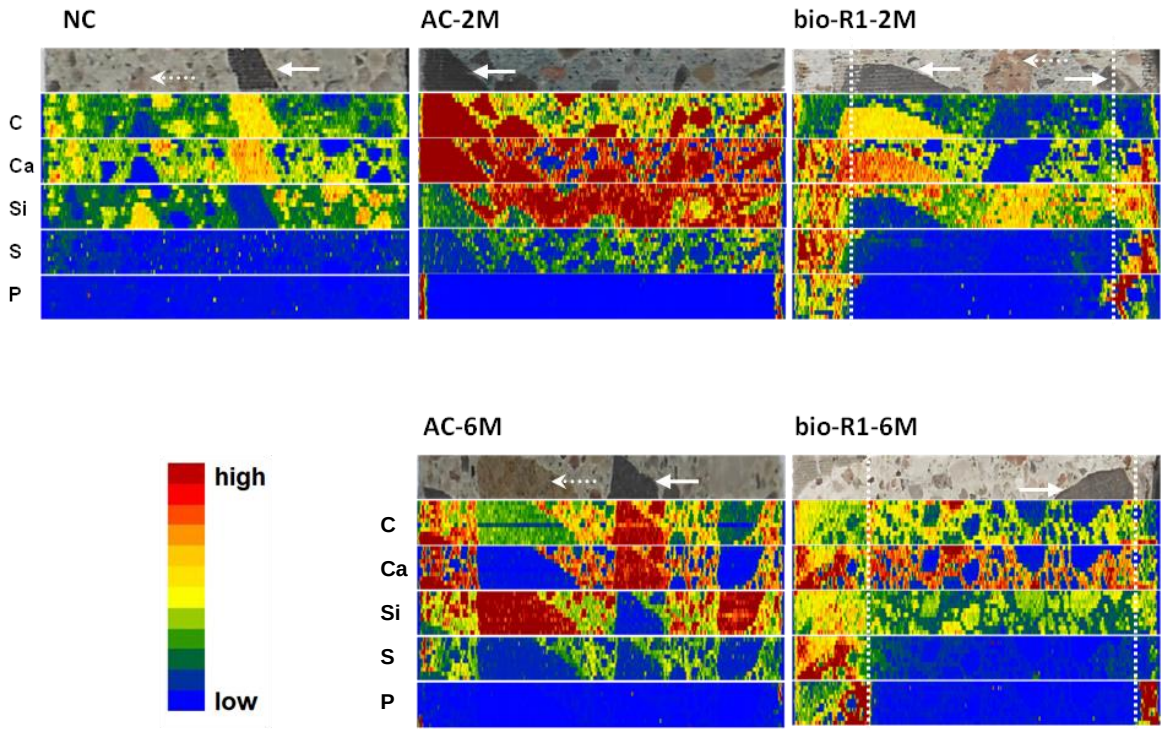



Figure 7.8 Cross section from Figure 7.7 with the corresponding elemental distribution across the displayed area. LA-ICP-MS profiles are shown for an untreated negative control (NC), one representative biotic (bio-R1-2M and bio-R1-6M) and abiotic reactor (AC-2M and AC-6M) of the two- (2M) and six-month (6M) set-up. Siliceous (dotted arrows) and carbonatic aggregates (continuous arrows) are exemplarily marked in the cross sectional area. The dotted lines show the transition zone of degraded and non-attacked concrete. The degraded part is defined as corrosion layer.

All LA-ICP-MS profiles clearly show the distribution of carbonatic (continuous arrows) and siliceous aggregates (dotted arrows) across the cross section of the samples. Due to the inhomogeneous distribution of aggregates each concrete sample looked differently. The abiotic control of the two-month experiment showed a huge carbonate on the left side of the sample (continuous arrow, Figure 7.8, AC-2M) visible due to the high carbon and calcium concentrations. Furthermore, the specimen comprised a few smaller carbonatic and siliceous aggregates. For the six-month abiotic reactor a huge siliceous aggregate (dotted arrow, Figure 7.8, AC-6M) was identified due to high silicon and low carbon and calcium concentrations in this part. On the right, next to the silicate, a carbonatic aggregate can be observed (continuous arrow, Figure 7.8, AC-6M). For both abiotic control reactors high phosphorus (especially for AC-2M) and calcium concentrations were observed on the outer concrete surface indicating calcium phosphate precipitation leading to a densification of the concrete samples which might be responsible for weight increase

(see Table 7.3). However, for all abiotic controls no penetration of sulfur was observed confirming that no sulfuric acid penetrated into the concrete.

The elemental distribution of the concrete sample exposed for two months in microbially produced sulfuric acid (bio-R-2M) analyzed by LA-ICP-MS were similar to the biogenic 28-day set-up, but showing a slightly thicker corrosion layer. High concentrations of calcium and sulfur in the corrosion layer were detected indicating gypsum precipitation. The sulfuric acid attack extends until carbonates appear on both sides of the concrete sample (continuous arrows, Figure 7.8, bio-R1-2M). In addition, high phosphorus concentrations were observed especially on the right side in the corroded part of the concrete due to calcium phosphate precipitation at the transition zone of damaged and non-attacked concrete. The detected higher silicon concentration in the corrosion layer indicates the formation of silicic acid (silica gel). For the six-month set-up, the elemental distribution of Ca, Si and P was similar to the distribution found after two months, but with higher penetration depths of sulfur on the left side of the cross section. On the right side, the presence of the carbonate aggregates (continuous arrow, Figure 7.8, bio-R1-6M) seemed to have reduced the acid penetration into the concrete. Finally, the detected damage pattern by LA-ICP-MS was highest for the six-month set-up, followed by the two-month and 28-day experiment.

#### 7.3.2.2.5 SEM and EDX analysis

Furthermore, SEM and EDX analyses were performed from concrete samples of the biotic two- and six-month experiments. For comparative analysis SEM and EDX were also performed from an untreated sample (NC) and the abiotic control of the two-month set-up (AC-2M). The SEM and EDX analyses of the untreated concrete showed the presence of silicon, calcium and aluminum (Figure 7.9A). This finding is not surprising, since the major oxides in cementitious material are $SiO_2$, CaO and $Al_2O_3$ (Scrivener & De Belie, 2013). No sulfur was detected. A dense layer of calcium phosphate crystals is visible on the surface of the abiotic control incubated for two months in the culture medium (SEM micrograph and EDX analysis, Figure 7.9B). This kind of precipitation was already observed visually for all abiotic control set-ups and was also identified as calcium phosphate by LA-ICP-MS. The surface of the concrete sample of the two-month experiment was obviously corroded (see Figure 7.10A and B) and EDX analysis (Figure 7.10B) revealed gypsum precipitation ($CaSO_4 \cdot 2H_2O$) and the detection of silicon and oxygen indicated the presence of siliceous aggregates ($SiO_2$). The surface of the concrete sample of the six-month experiment showed siliceous aggregates covered with a thin layer of gypsum (Figure 7.11A and B).

Compared to the two-month experiment, the gypsum layer seemed to be reduced. This can be an indicator for a higher degree of damage, because at early stages of degradation, the formation of gypsum leads to a densification and expansion of the corroded layer. If the acid attack continues, the expansion will be too high and cracks develop resulting in a destabilization of the corrosion layer and loss of concrete material.

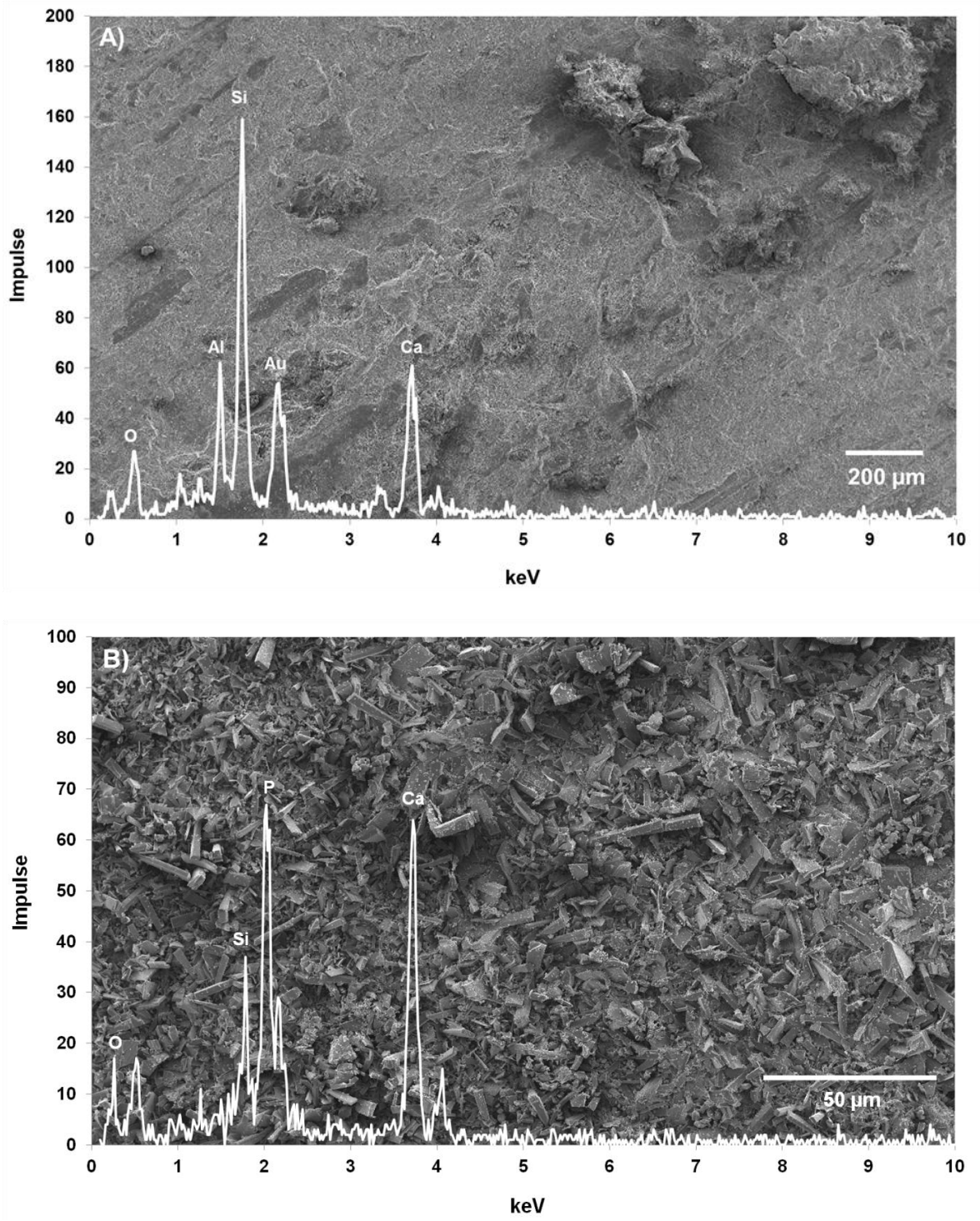



Figure 7.9 A) SEM and EDX analyses of an untreated sample and B) abiotic control exposed for two months in the culture medium. A dense layer of calcium phosphate crystals can be observed for the abiotic control.

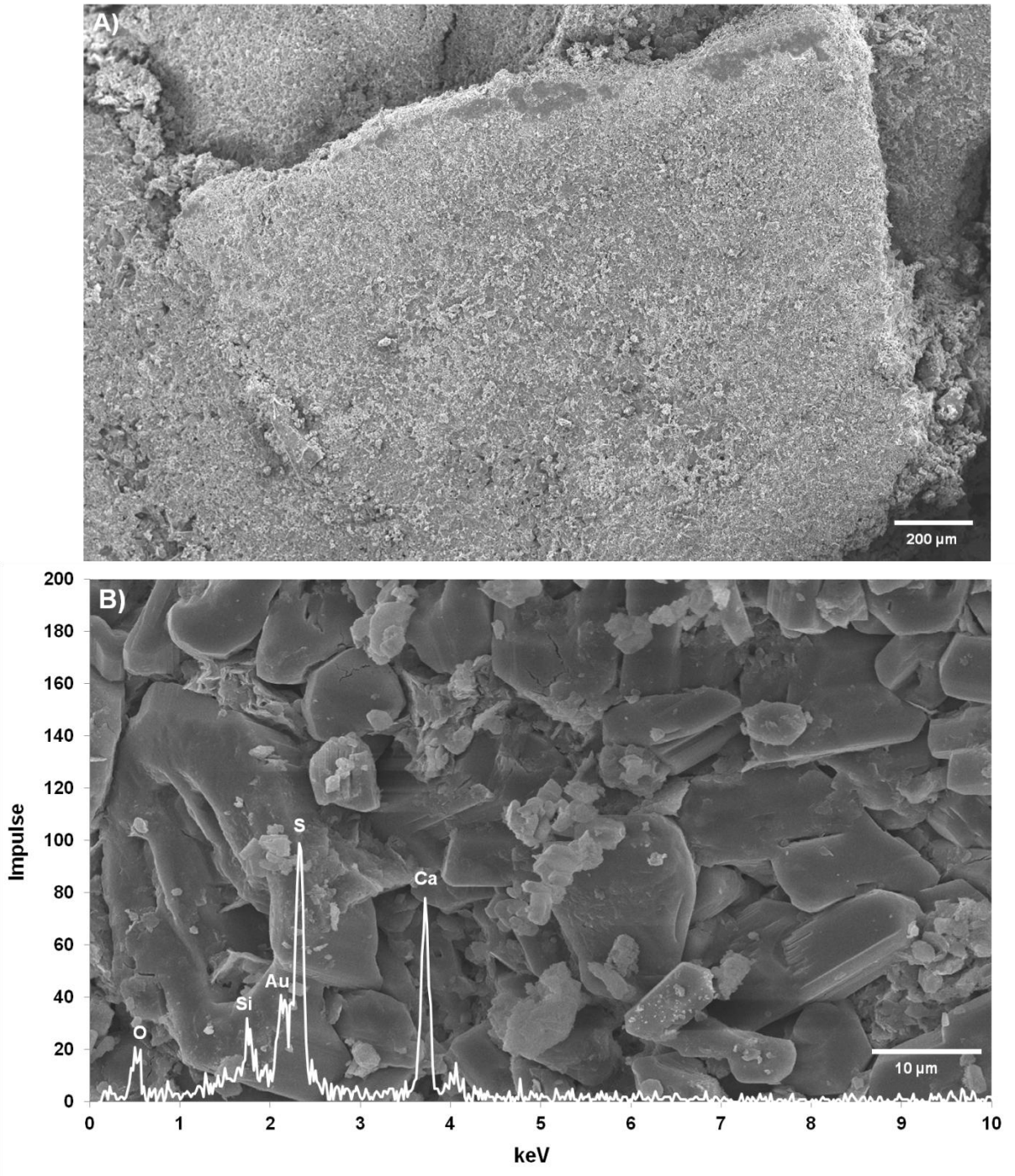



Figure 7.10 A) SEM micrograph of the concrete sample exposed for two months in biogenic sulfuric acid and B) detailed view of A. Element analysis suggests gypsum precipitation ($CaSO_4 \cdot 2H_2O$) and the presence of siliceous aggregates ($SiO_2$).

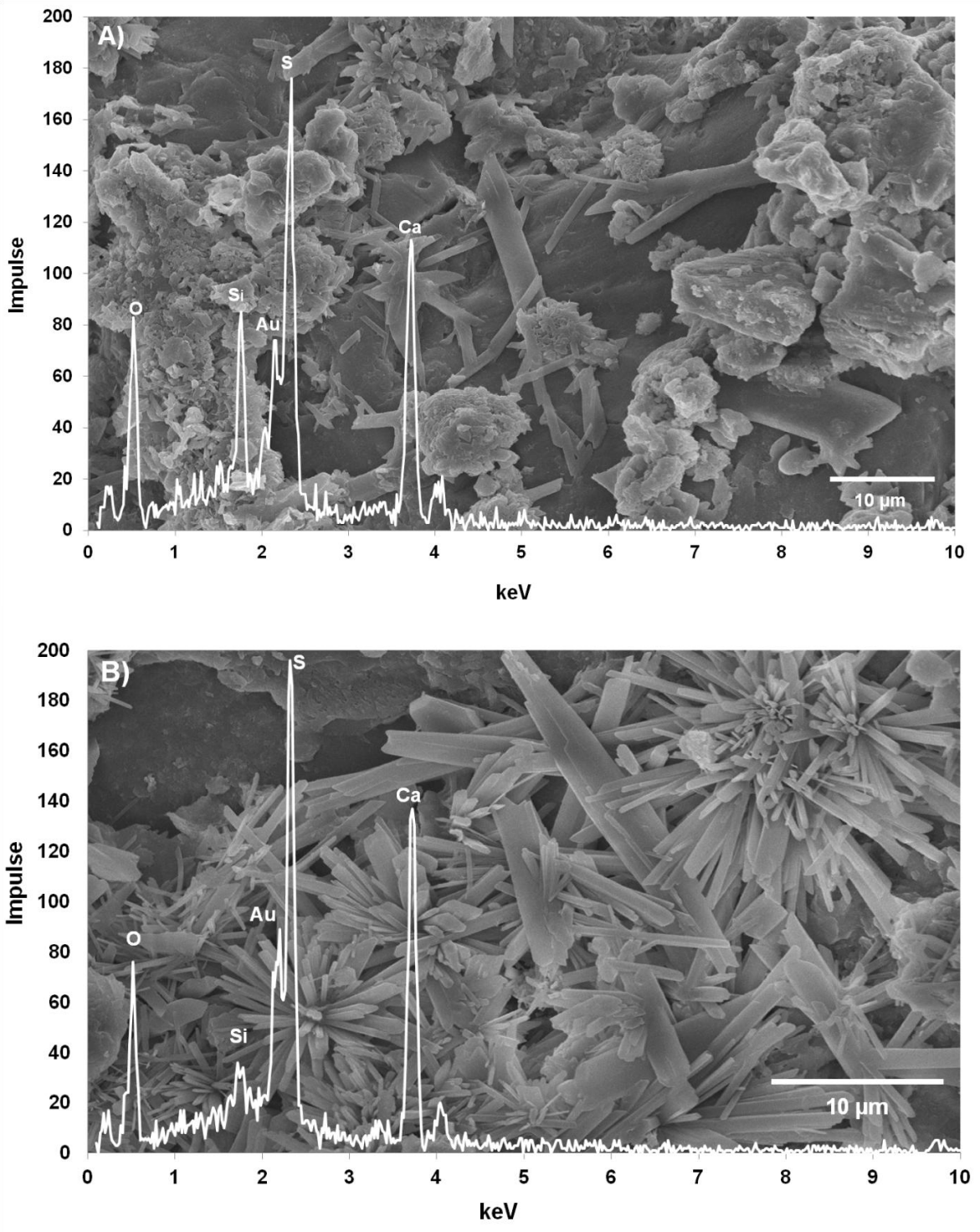



Figure 7.11 Concrete sample of the six-month experiment (detailed view) shows A) siliceous aggregates covered with a thin gypsum layer and B) dense layer of gypsum crystals over the entire area.

### 7.3.3 Concrete corrosion process

The present study mimics the last step of the multiplex BSA corrosion process, where sulfuric acid reacts with the main components of concrete. The used concrete mainly consists of i) calcium hydroxide ($Ca(OH)_2$) and calcium silicate hydrate (C-S-H) phases (blast furnace slag cement) and ii) inert siliceous ($SiO_2$) and reactive carbonatic ($CaCO_3$) aggregates. For both biotic and chemical test systems similar corrosion damage patterns were observed characterized by the formation of a thin white corrosion layer consisting of gypsum and amorphous silicic acid (silica gel). During the degradation process sulfuric acid dissolves calcium hydroxide and calcium silicate hydrates, the main constituents of the cement stone, resulting in gypsum precipitation and silica gel production. Additionally, the carbonates of the aggregates were dissolved precipitating gypsum and generating $CO_2$. The precipitation of ettringite, which is developed by the reaction of gypsum with calcium aluminate hydrate ($C_3A$), played a minor role. This may be due to the use of high sulfate resistant cement with limited $C_3A$ content (De Belie et al., 2004). Furthermore, the low pH values (pH ≤ 2.0) in the present study enhanced the precipitation of gypsum which was also observed by Gutberlet et al. (2015). In Chapter 6, short-term biotic and chemical autotitrator tests were performed under the same experimental conditions with pure hardened cement paste. The cement paste contained 80 wt% blast furnace slag cement and was comparable to the cement type used in this study. For both hardened cement paste and concrete exposed for 28 days to *A. thiooxidans* culture and chemical sulfuric acid at pH 1.0 and pH 2.0 a comparable penetration of sulfuric acid was determined. However, for the chemical test a twice (pH 1.0) and three times (pH 2.0) higher acid consumption was determined for the pure cement stone compared to the concrete samples. The lower acid consumption for the concrete samples is probably due to the presence of aggregates, which constitute more than 60% of the concrete (Scrivener & De Belie, 2013). Especially acid inert siliceous aggregates reduce the acid uptake of concrete, since they are not involved in the degradation process (Zivica & Bajza, 2002). In contrast to the concrete samples, a thicker corrosion layer consisting of gypsum and silica gel and a higher volume expansion was observed for the hardened cement paste specimens after 28 days of incubation in both biogenically produced $H_2SO_4$ and chemically generated $H_2SO_4$ at pH 2.0 (see Chapter 6). The lower volume expansion for the concrete samples is probably due to the presence of aggregates. A study by Bentur and Cohen (Bentur & Cohen, 1987) showed that the expansion in the cement paste-aggregate interfacial zone is limited. The reason of the lack of a thick layer of gypsum on the surface of the concrete samples may be the reaction of the acid with the carbonate

aggregates. During this reaction gypsum and $CO_2$ are developed whereas the gas $CO_2$ may prevent a gypsum precipitation on the surface.

The presence of inert siliceous and reactive carbonate aggregates in concrete samples reduce gypsum formation and volume expansion which cause a lower internal pressure leading to a retardation of concrete deterioration compared to pure cement stone. However, the dissolution of cement matrix ($Ca(OH)_2$) and calcium silicate hydrate (C-S-H) phases might break off the aggregates weakening the concrete structure.

## 7.4 CONCLUSIONS

Within this study, the last step of BSA corrosion, where sulfuric acid produced by SOB leads to the degradation of concrete, was simulated by microbiological tests using *A. thiooxidans* culture and chemical tests. In the corrosion solutions of the short-term biogenic and chemical experiments (28 days of incubation) similar pH values, sulfate and calcium concentrations were measured indicating comparable corrosion conditions. The comparability of the two test systems was also reflected in the development of similar damage, which verifies the hypothesis proposed in this study (see hypothesis # 3; Chapter 2). Semi-quantitative analysis of the elemental distribution by LA-ICP-MS revealed the same composition of the corrosion layers with the exception of calcium phosphate precipitation in the biogenic reactors. Concerning the deterioration of concrete, a pH dependent degree of damage was noticed. The severest concrete degradation was observed for the samples in chemical sulfuric acid at pH 1.0, followed by the biogenic set-ups (average pH 1.6), and chemical set-up at pH 2.0. These results indicate that, the availability of sulfuric acid is comparable to the purely chemical sulfuric acid attack. In both experimental set-ups the sulfuric acid reacts with the concrete surface. After the development of corrosion products sulfuric acid must penetrate through the gypsum layer to reach and attack the untreated concrete. In our experiments, the bacteria seem to have no growth advantages in the gypsum layers probably due to limited oxygen and nutrient supply. It´s assumed that bacterial growth takes place on the outer layer of the corroded concrete surface and in the culture medium, respectively. Therefore, no internal cracking of the concrete by the microorganisms seems to take place.

For the long-term biogenic experiments (two to six months), an increasing extent of concrete degradation could be observed within the first three months. However, the specimens exposed for three and six months in biogenic sulfuric acid revealed a comparable degree of damage. Since the corrosion strongly depends on the amount of sulfuric acid produced, the stagnant corrosion may be due to the lower activity of

*A. thiooxidans* within the six-month set-ups. This result confirms the hypothesis (hypothesis # 4; Chapter 2) that concrete corrosion is not a linear process over time and different factors e.g. microbial growth influence the degradation process.

The determination of visual and physical parameters (e.g. weight loss) provided first insights into the degradation of concrete. However, chemical analyses such as LA-ICP-MS and EDX showed more in depth-results concerning the composition of the corrosion layers. LA-ICP-MS and EDX analyses allowed the identification of gypsum as main corrosion product and showed that calcium phosphate precipitation occurred within the abiotic control reactors. Especially, LA-ICP-MS proved to be a very effective tool for the assessment of concrete degradation due to semi-quantitative analyses of the elemental distributions in the concrete. This method enables *in situ* detection of non-corroded siliceous and carbonatic aggregates and corrosion products such as gypsum. Consequently, for a detailed characterization of concrete degradation, chemical analyses of the concrete, such as LA-ICP-MS or EDX measurements, should be included.

When comparing the corrosion process of pure hardened cement paste and concrete samples two main differences were observed. On the one hand no significant volume expansion was observed for the concrete samples and on the other hand the corrosion layer of the concrete specimens was much thinner and more instable. The limited volume expansion may be due to the presence of aggregates. When the sulfuric acid reacts with the carbonatic aggregates no stabilizing silica gel but $CO_2$ is formed which might reduce gypsum formation on the concrete surface.

# Chapter 8

Discussion

This dissertation was divided into two key research objectives (see also Chapter 2): i) identification and characterization of BSA related microbial communities (SRB and SOB), and ii) analysis of the corrosion potential in laboratory experiments. For each research objective two hypotheses were proposed and tested in a series of experiments (see Chapters 4-7).

The first hypothesis of this dissertation was to examine if SRB and SOB communities can be also found in sludge digesters (see Chapters 2 and 4) in order to reveal their role in BSA corrosion potentials in full-scale digesters. Therefore, six different digesters showing signs of corrosion in the digester headspace were investigated regarding the presence of the SRB and SOB bacterial groups that are most likely involved in the corrosion process. The results obtained using targeted microbiological and biomolecular methods revealed, that SRB are present in the digester sludge while SOB were found in the biofilm samples taken from the digester headspace verifying the first hypothesis. With the second hypothesis the ability of pure and mixed SOB cultures to produce sulfuric acid was analyzed under controlled laboratory conditions (see Chapters 2 and 5). The SOB within the enriched cultures were capable of producing sulfuric acid revealing that the detected SOB possess a BSA production potential indicating a BSA corrosion potential in the examined sludge digesters (confirmation of hypothesis #2). The first research objective with the associated hypotheses #1 and #2 are discussed in Chapters 8.1 and 8.2.

The third hypothesis focused on the investigation of the corrosion potential of microbially and chemically derived sulfuric acid on both hardened cement paste and concrete (Chapters 2, 6 and 7). Results from well-defined corrosion experiments under controlled conditions revealed high corrosion potentials of *A. thiooxidans*, originally isolated from digester E on both sample types. The comparative studies of biogenic and chemical sulfuric acid revealed similar corrosion damage patterns and no obvious differences could be determined between these two acid attacks (verification of hypothesis #3). Additionally, long-term experiments over a period of two, three and six months with microbially generated sulfuric acid revealed that there is no linear increase in concrete deterioration with increasing incubation periods confirming hypothesis #4 (see Chapters 2 and 7). The second research objective with the corresponding hypotheses #3 and #4 are discussed in Chapter 8.3.

## 8.1 IDENTIFICATION OF SRB AND SOB WITHIN DIFFERENT SLUDGE DIGESTERS AND THEIR IMPACT ON BSA CORROSION

SRB are omnipresent in anoxic environments, where they can degrade organic matter by utilizing sulfate as terminal electron acceptor to generate sulfide (Muyzer & Stams, 2008). Sulfide can be incorporated into sulfur containing enzymes and amino acids (Muyzer & Stams, 2008). SRB are a phylogenetically and metabolically diverse group (Barton & Fauque, 2009) and inhabit natural and engineered ecosystems (Muyzer & Stams, 2008), such as marine (Ravenschlag et al., 2000) and freshwater sediments (Sass et al., 1998), sludge digesters of wastewater treatment plants (Ben-Dov et al., 2007; Dar et al., 2005; Dar et al., 2007), oil fields (Nilsen et al., 1996), and acid mine drainage (Sen & Johnson, 1999). Currently, over 220 SRB species, belonging to 60 genera have been identified (Barton & Fauque, 2009). By means of 16S rRNA sequence analyses (see Figure 8.1), the SRB can be divided into seven phylogenetic lineages consisting of five bacterial and two archaeal phyla (Muyzer & Stams, 2008). The following bacterial phyla are described in literature (Muyzer & Stams, 2008): *Proteobacteria* represented by the class *Deltaproteobacteria*, *Firmicutes* composes three endospore-forming SRB genera within the class *Clostridia*, and the three phyla lineages *Nitrospirae*, *Thermodesulfobacteria* and *Thermodesulfobacteriaceae* represent thermophilic sulfate reducers. In this work, the SRB diversity studies of the digester sludge samples revealed different uncultured SRB belonging to the *Deltaproteobacteria* (see Figure 8.1), the largest group within the seven lineages comprising approximately 23 genera (Muyzer & Stams, 2008).

All known SRB possess the *dsrB* gene, which encodes the dissimilatory sulfite reductase (Wagner et al., 1998), the key enzyme in sulfate reduction catalyzing the reduction of sulfite to sulfide. Generally, the use of functional genes encoding important enzymes in the sulfate reduction pathway was reported to be a powerful tool as a phylogenetic marker for SRB (Muyzer & Stams, 2008). This was also proven to be effective in this study by identifying SRB communities in digester sludge samples applying PCR-DGGE and subsequent sequence analysis using the *dsrB*-gene. In contrast to the SOB where an enrichment cultivation step was necessary, the SRB could be directly identified in the digester sludge samples by genetic profiling. This suggests that the SRB are abundant within the digester sludge community. This finding is not surprising since organic carbon and sulfate are usually present in wastewater providing optimal growth conditions for SRB. Finally, the detection of different SRB in the digester sludge samples indicated SRB activity and $H_2S$ production, which can be used as energy source by chemolithotrophic SOB oxidizing sulfide to sulfate.

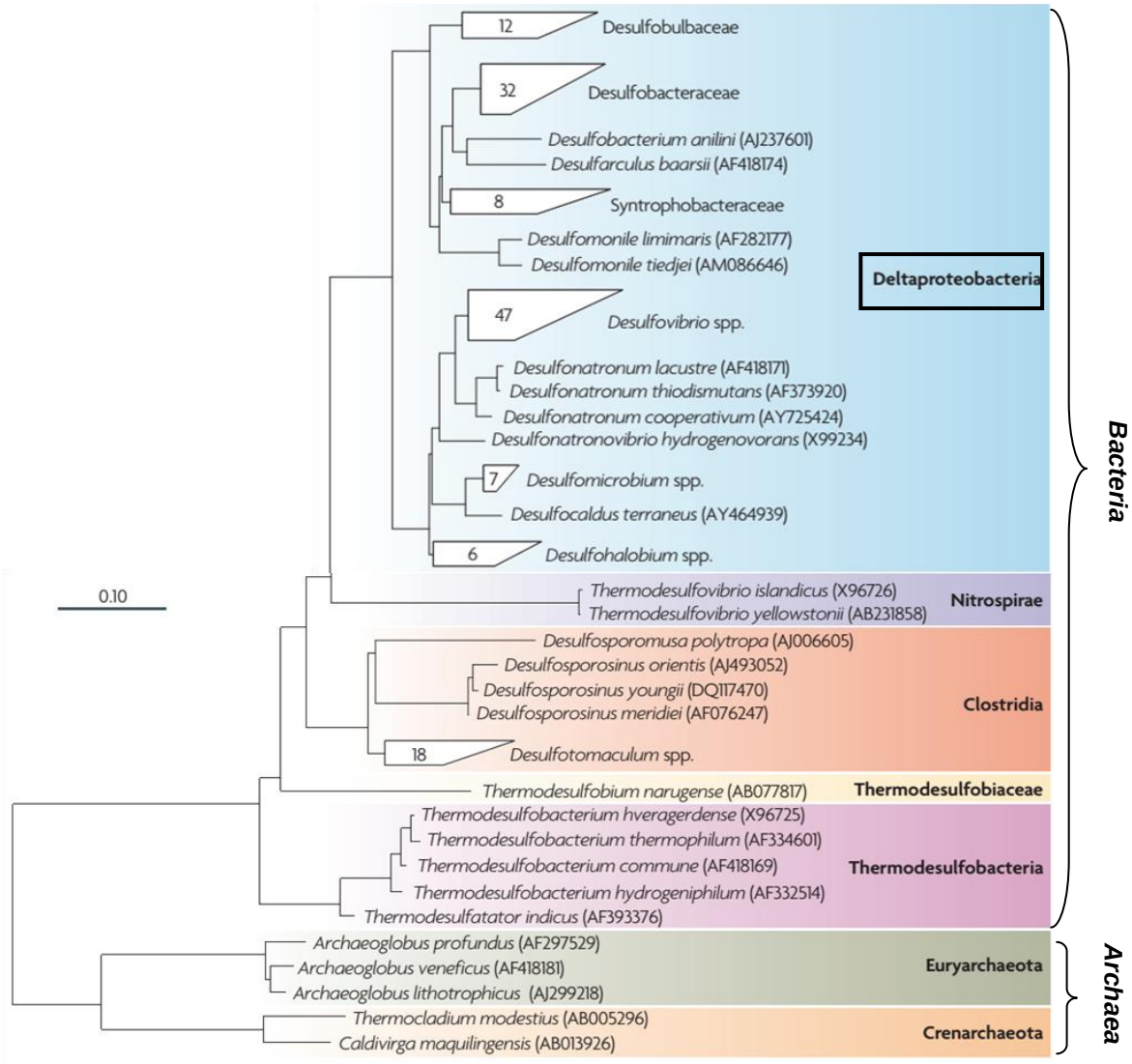



Figure 8.1. Phylogenetic tree based on nearly complete 16S rRNA sequences of described SRB species (Muyzer & Stams, 2008). The numbers in the collapsed clusters represent the number of different SRB species within the corresponding group. Scale bar= 10% sequence difference. The SRB identified within this work could be attributed to the *Deltaproteobacteria* (framed).

The oxidation of hydrogen sulfide to sulfate is an important reaction in the global sulfur cycle (Friedrich et al., 2001). Inorganic sulfur compounds are ubiquitous and mainly available as sulfides ($H_2S$ and metal sulfides), polysulfides, elemental sulfur, sulfite, thiosulfate, and polythionates (tri, tetra and penta) (Mohapatra et al., 2008). The oxidation of inorganic sulfur compounds can be carried out by a phylogenetically diverse group of sulfur oxidizing prokaryotes (Friedrich et al., 2001). Within the *Archaea*, the capability for

inorganic sulfur compound oxidation is restricted to the order *Sulfolobales* (Fuchs et al., 1996; Mohapatra et al., 2008). Within the *Bacteria*, sulfur oxidation can be performed by aerobic lithotrophs or by anaerobic phototrophs (Mohapatra et al., 2008; Peck & LeGall, 1994). The focus within this thesis was on the chemolithotrophic SOB, which are primarily associated with concrete corrosion.

For the identification of SOB communities in biofilm samples taken from the digester headspace, an enrichment cultivation was used, since diversity studies by PCR-DGGE within the original biofilm samples revealed no or only a few SOB species. This suggests that SOB abundance within the biofilm samples is rather low. Similar observations were reported by Okabe et al. (2007) when analyzing biofilm samples from a corroded concrete sewer pipe with fluorescence *in situ* hybridization techniques. They found that more than 95% of the microbial community was non-sulfur oxidizing. Even when concrete corrosion was already advanced and the concrete pH value was around 2, the non-SOB accounted for approximately 50% of the total community. These non-SOB species were mainly related to heterotrophic bacteria and the composition changed during the different corrosion stages. Within this work, also heterotrophic bacteria were found (see Chapter 5) leading to the assumption that an interaction between SOB and non-SOB species may take place. Metabolites produced by the SOB might be used by non-SOB or vice versa. *Acidithiobacillus*, for instance, is known to produce self-inhibitory organic compounds (Cho & Mori, 1995; Peccia et al., 2000; Vincke et al., 2001), which may be subsequently used by heterotrophs.

For SOB cultivation, specific autotrophic media containing $S^0$ or $Na_2S_2O_3$ as sole energy sources were used in order to prevent an overgrowth by heterotrophic bacteria. Finally, the application of enrichment cultivation in combination with PCR-DGGE allowed the identification and characterization of a variety of SOB species. This underscores the importance of choosing highly selective media as exactly the organisms of interest were cultivated. Although cultivation dependent techniques may fail to display a comprehensive picture of the bacterial community, they are still a very suitable way to study the capability of BSA production. However, care must be taken when drawing assumptions regarding the dominance structure in SOB communities. The dominant species identified in the enriched cultures are most likely not the dominant ones growing on the corroded concrete surface. Although the SOB might not be the most abundant organisms striving in the biofilm samples, they can be highly active under appropriate growth conditions (i.e., oxygen and nutrient availability; see also Chapter 8.3) and might induce severe corrosion damage patterns as was shown by Okabe et al. (2007).

According to Islander et al. (1991), five different “thiobacilli” play an important role within the BSA corrosion process in sewer pipes and a succession of these bacteria as a function of the pH value takes place (see Figure 8.2). Three of the five “thiobacilli” were also found within the biofilm samples of the digester headspace being *Thiobacillus thioparus*, *Thiomonas intermedia*, and *Acidithiobacillus thiooxidans*. This indicates that the BSA corrosion process within sludge digesters might proceed in a similar way as the one occurring in sewer pipes.

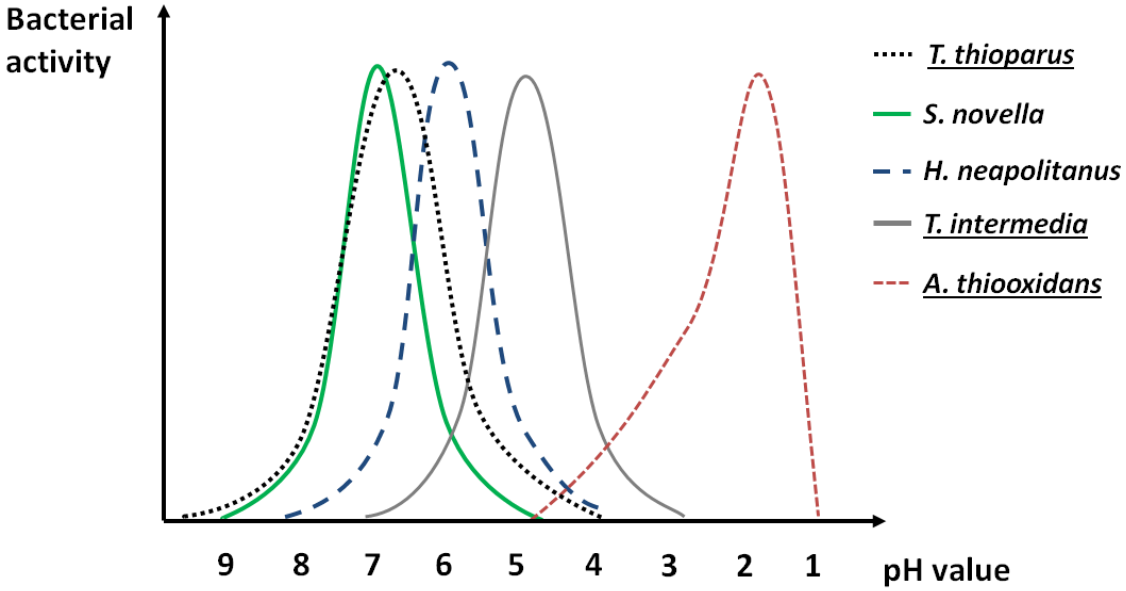



Figure 8.2. Activity of five different SOB species frequently found in corroded concrete sewer pipes as a function of pH (adapted from Scrivener & De Belie, 2013). The underlined species were found in the biofilm samples of the corroded concrete material originating from the digester headspace samples targeted in this study.

When the pH of the concrete surface is around 9 (stage 2 of BSA corrosion), *T. thioparus,* which was identified in digester B (Figure 8.3), begins to colonize the concrete surface (Islander et al., 1991), but disappears with ongoing corrosion (Wei et al., 2010). *T. thioparus* produces polythionic acids resulting in an additional pH reduction of the concrete surface (Islander et al., 1991). This enables the growth of other SOB, such as *Starkeya novella* (formerly *Thiobacillus novellus*) and *Halothiobacillus neapolitanus* (formerly *Thiobacillus neapolitanus*). Although *S. novella* and *H. neapolitanus* were found in corroded sewer pipe material (Milde et al., 1983), these two bacteria were not detected in this study. Subsequently, *Thiomonas intermedia* (formerly *Thiobacillus intermedius*), which has a pH optimum of 5.5-6.0 (Robertson & Kuenen, 2006) can establish on the

concrete surface. *T. intermedia* and *T. perometabolis*, which are closely related (Wei et al., 2010), were found in samples from five of the six digesters (Figure 8.3) and were the most dominant organisms in the enrichment cultures. Both SOB were able to reduce the pH value within the liquid medium containing $Na_2S_2O_3$ as sole energy source from 6.0 to 2.5 after two weeks of incubation indicating high sulfuric acid producing potential. While *T. intermedia* is long known for its role in the corrosion process (Islander et al., 1991; Milde et al., 1983), *T. perometabolis* was recently reported to be one of the dominant acid producers in corroded concrete samples originating from a bridge support (Wei et al., 2010). Some of the digester isolates were closely related to the *Thiomonas intermedia* K12 strain, that is able to use tetrathionate under aerobic and anaerobic conditions enabling the bacterium to survive phases of limited oxygen supply (Wentzien & Sand, 2004). Under oxic conditions, tetrathionate is oxidized, while a tetrathionate disproportionation takes place under anoxic conditions (Wentzien & Sand, 2004). Consequently, *Thiomonas intermedia* K12 is able to grow under aerobic and anaerobic conditions in both pathways generating trithionate and sulfate in varying amounts (Wentzien & Sand, 2004).

Once the pH is below 5, stage 3 of BSA corrosion begins, when acidophilic bacteria, especially *A. thiooxidans* establish on the concrete surface leading to a further pH drop (Milde et al., 1983). Since also acidophilic bacteria were found within the corroded concrete material of the digester headspace, it is assumed that the neutrophilic sulfur oxidizers already reduced the pH of the digester concrete surface to values around 4-5 or pH gradients with acidic microniches are present (Robertson & Kuenen, 2006). *A. thiooxidans*, the protagonist within the corrosion process, was identified in Dg A, D and E (Figure 8.3) and could also be obtained as pure culture. An isolate from Dg E was able to produce 433 mmol/L sulfate (final pH <1.0) after 42 days of incubation in a medium with elemental sulfur as sole energy source indicating a very high sulfuric acid production potential. Furthermore, *A. thiooxidans*, originally isolated from digester E was used for biotic corrosion experiments with both hardened cement paste and concrete samples (see Chapters 6 and 7). For both specimens severe corrosion damage patterns were already observed after 28 days of incubation in BSA indicating a very high corrosion potential that can be tracked back to the activity of this bacterium.

Furthermore, the presence of neutrophilic and acidophilic SOB species with certain growth and pH optima (see Figure 8.3) might provide information on the progression of BSA corrosion in the corresponding digester conditions. In digesters A, B, D and E, where neutrophilic and acidophilic SOB, such as *Acidithiobacillus* spp. and/or *Alicyclobacillus* spp. were detected, the corrosion process might be already in a more

progressed state compared to the digesters with only neutrophilic SOB, such as *Thiomonas* spp. and *Paracoccus* spp. (Dg C and F). Particularly in Dg A, D and E the identification of *A. thiooxidans*, which has been reported to be the dominant organism in heavily corroded concrete material (Milde et al., 1983; Okabe et al., 2007), provides evidence of an advanced attack. In contrast, on concrete structures showing a negligible extent of corrosion, no *A. thiooxidans* growth was detected (Sand & Bock, 1991). While the digesters characterized by neutrophilic SOB are likely in stage 2 of the BSA corrosion process, the digesters with both neutrophilic and acidophilic bacteria are in transition from stage 2 to stage 3 (Figure 8.3). Further information on the *in situ* SOB activity was obtained by sulfate measurements of concrete samples obtained from the digester sludge zone and headspace. In all digesters, higher sulfate concentrations were measured in the digester headspace samples compared to the sludge zone. In Dg A, for instance, the sulfate content in the digester headspace sample was more than ten times higher compared to the sludge zone indicating sulfate/sulfuric acid production in the headspace. These findings correlate with the detection of the high sulfuric acid producer *A. thiooxidans* in this digester.

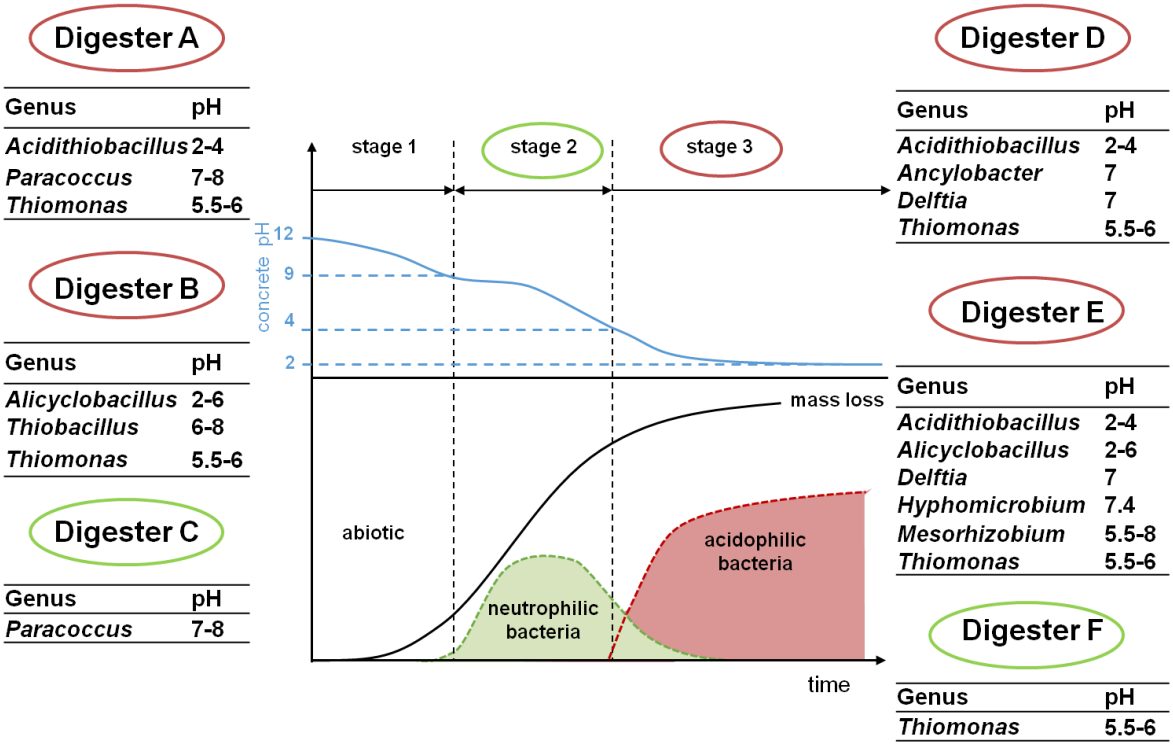



Figure 8.3. Correlation of sulfur oxidizing genera found in the present study and the potential progression of BSA corrosion in the six analyzed digesters.

## 8.2 POTENTIAL ROLE OF BSA CORROSION IN SLUDGE DIGESTER

Concrete corrosion starts when imperfections in the concrete coating occur due to long operation periods of the digesters. Subsequently, a reaction with the sound concrete becomes possible. This step might take decades to occur and is thus far slower than commonly observed in sewer pipes. In the following, the potential processes of BSA corrosion within a sludge digester as illustrated in Figure 8.4 will be discussed.

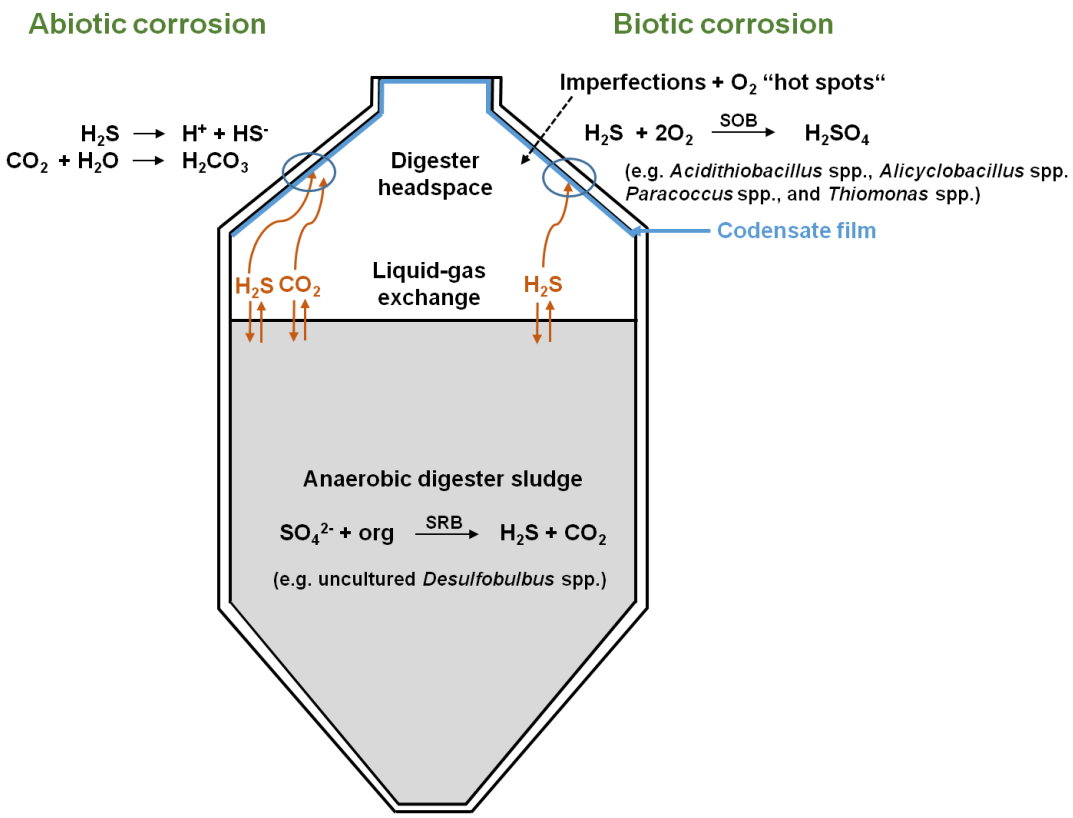



Figure 8.4. The potential steps of BSA corrosion and the relevant SRB and SOB found in the present study within a sludge digester.

BSA corrosion is a very complex process requiring both the activity of SRB and SOB communities. In a first step, sulfate that is commonly found in wastewater and consequently in sewage sludge (Appels et al., 2008), is reduced by the activity of SRB under anaerobic conditions to sulfide and $CO_2$ (Figure 8.4). As the pH value in digester sludge is usually between 6 and 7 (Derbal et al., 2009), sulfur is mainly present in the form of $H_2S$. Typical $H_2S$ concentrations in biogas generated during anaerobic digestion of sewage sludge are in the range of 1,000 – 2,400 ppm (Nghiem et al., 2014; Ramos et al.,

2014). These $H_2S$ concentrations are much higher compared to an aggressive sewer environment with $H_2S$ concentration of 79 to 84 ppm (Wells & Melchers, 2014; Grengg et al., 2015). However, due to the conditions in a digester, BSA occurs in a far more subtle way than in sewer systems, although high $H_2S$ levels might sustain a significantly higher corrosive action. Both, $H_2S$ and $CO_2$, are transferred from the liquid to the gas phase. The temperature in the digester headspace (30-55°C; (DWA, 2011)) is higher than the temperature on the concrete wall resulting in the formation of a condensate layer (Hanekrad et al., 2014), especially as the relative humidity of biogas is between 90 and 100% (DWA, 2011). The gases that escaped into the digester headspace may dissolve in the condensate film on the concrete wall and react with alkaline components (e.g. calcium hydroxide) of the concrete finally leading to an abiotic reduction of the highly alkaline concrete surface over time (Wells et al., 2009). This reaction on the concrete surface represents stage 1 of a potential BSA corrosion (illustrated in Figure 8.4).

Once the abiotic corrosion processes have lowered the pH value of the concrete surface to around 9, microbial growth can take place in the digester headspace. As described in Chapter 1.2 for sewer systems, the concrete surface is colonized by a succession of different neutrophilic and acidophilic SOB. The development of SOB communities depends on a variety of factors, such as humidity, temperature, nutrient ($CO_2$ and $H_2S$), and oxygen availability in the headspace (Wells & Melchers, 2015). In contrast to sewer pipes, the oxygen availability in the digester headspace is quite limited with values around 0-1 vol% (DWA, 2011). However, most SOB species found in this work (e.g., *T. thioparus*, *A. thiooxidans*) need oxygen for growth (Goto et al., 2003; Syed et al., 2006). This leads to the assumption that somehow, oxygen carries must, in one way or another, be present in the digester headspace (“hot spots”) where they foster the growth of sulfur oxidizing communities. However, almost nothing is known about oxygen penetration pathways into digester systems or oxygen concentrations *in situ* justifying the need for further investigations. If oxygen is available, it is probably present at low concentrations, highly localized and immediately consumed by the microbes, thus it might escape detection by common comprehensive biogas measurements. Nevertheless, many sulfur oxidizers can spontaneously start with the oxidation of sulfide even when sulfide and oxygen are present at only low concentrations ($10^{-6}$ mM) (Robertson & Kuenen, 2006). Therefore, it is assumed that the availability of oxygen in microniches (“hot spots”) is sufficient for the activity of SOB. In the following four hypotheses on oxygen availability in the digester are proposed and discussed:

**i)** Oxygen might diffuse into the system through cracks or imperfections that arose over time with the aging of most sludge digesters. The average age of the digesters

investigated within this study was 37 years and most digesters already showed cracks of up to 0.4 mm on the outside of the digester manhole-pit (see Figure 5.1).

**ii)** Oxygen might be provided by the concrete itself. Once the concrete coating is damaged through abiotic corrosion processes, the concrete might be an oxygen supplier for the bacteria. Even when concrete was optimally compacted, the air content in fresh state is between 1 and 2 vol-% (Dehn et al., 2003). With decreasing maximum grain size the amount of air voids in the fresh concrete even increases up to 6 vol-% (Dehn et al., 2003). Thus, little amounts of oxygen might become available during corrosion processes for the bacteria enabling sulfur/sulfide oxidation.

**iii)** Desulfurization, a process that adds air (2-6 vol-%) to the digester headspace in order to reduce the $H_2S$ level of the biogas (Appels et al., 2008). During this process the supplied oxygen is used by bacteria of the genus *Thiobacillus* for the oxidation of $H_2S$ to elemental sulfur and sulfates (Appels et al., 2008). Latter can potentially contribute to the BSA corrosion process within the digester headspace. *In situ* desulfurization was not carried out in the digesters investigated in this study, but might possess the potential to cause BSA corrosion damage patterns in other sludge digesters using this method.

**iv)** Oxygen may be generated by the reduction of $CO_2$ to $CH_4$. While the phenomena of increasing $CH_4$ productivity due to $CO_2$ enrichment are reported in several studies (Fernández et al., 2014; Koch et al., 2016), the underlying mechanisms are still far from being completely elucidated. One possibility might be the simultaneous reduction of $CO_2$ to $CH_4$ and oxidation of $H_2S$ to $SO_3^{2-}$ or $SO_4^{2-}$. Ochi and Sato (1992), for instance, have shown that the methane gas production increased by maintaining a high $CO_2$ concentration. At the same time it was observed that with increasing $CH_4$-concentrations the sulfides within the digester sludge decreased. It was assumed that SOB inhabit the digester sludge and a biological oxidation-reduction reaction of sulfides exists in the sewage sludge digestion. To prove whether the $H_2S$ oxidation is coupled to the $CO_2$ reduction, further experiments need to be carried out in this field.

Despite all these hypotheses, it remains unclear how oxygen can penetrate the concrete and get into the digester system or how much oxygen is really available for bacterial *in situ* activity. Since BSA corrosion is a very complex and long lasting process, damage patterns might first occur after decades of continuous operation. The digesters investigated within this study had an operation time of 23 to 52 years. In most digesters,

the concrete coating was already damaged and cracks on the outside of the digester manhole pits were observed. Since no *in situ* desulfurization was applied in any of the six digesters investigated, oxygen most likely penetrated the system through cracks or imperfections. At the same time, oxygen might have been available for the bacteria through uncovered concrete. It is assumed that a combination of the first two hypotheses took place in the six digesters.

In case of oxygen availability in microniches, sulfur/sulfide oxidation by SOB might take place in the digester headspace. SOB colonization and BSA formation might be restricted to local areas and strongly depend on the simultaneous presence of oxygen and $H_2S$. SOB groups are commonly present in wastewater and consequently in digester sludge (Appels et al., 2008). They might reach the digester wall through changing filling levels of the digester sludge. Furthermore, many SOB species, e.g. *Thiomonas* spp. and *Acidithiobacillus* spp. are also motile by means of one or more flagella and could consequently relocate themselves to the digester headspace (Kelly & Wood, 2000; Robertson & Kuenen, 2006). Under aerobic conditions, $H_2S$ and other reduced sulfur compounds can be used by neutrophilic *Thiomonas* spp. to produce sulfuric acid and polythionic acids leading to a gradual pH decrease from 9.0 to 3.5 on the concrete surface (Roberts et al., 2002) (stage 2 of the BSA corrosion, Figure 8.3). Depending on available oxygen concentrations, different end products are formed by the SOB (Díaz et al., 2010). Thereby, sulfur formation is enhanced when low levels of oxygen are available (Fortuny et al., 2008; Ramos et al., 2014). The oxidation reactions can be described as follows (Díaz et al., 2010; Madigan et al., 2009):

$H_2S + 0.5\ O_2 \rightarrow S^0 + H_2O$ ΔG° = -209.4 kJ/reaction

$S^0 + 2H_2O + 1.5\ O_2 \rightarrow SO_4^{2-} + 2H^+$ ΔG° = -587.1 kJ/reaction

$H_2S + 2\ O_2 \rightarrow SO_4^{2-} + 2H^+$ ΔG° = -798.2 kJ/reaction

After the pH value of the concrete surface decreased to ~4, stage 3 (Figure 8.3), the last step of BSA corrosion, begins (Wells & Melchers, 2014). Acidophilic SOB such as *A. thiooxidans* continue to produce high sulfuric acid concentrations and the damage of the concrete structure increases (Gutiérrez-Padilla et al., 2010). However, only a steady sulfur/sulfide oxidation and sulfuric acid production within the digester surface over years to decades could result in characteristic corrosion damage patterns as shown for digester E with an age of 52 years (Figure 4.1).

## 8.3 ANALYSIS OF THE BSA CORROSION POTENTIAL IN LABORATORY EXPERIMENTS

### 8.3.1 Experimental test systems

Since BSA corrosion is a slow process with annual corrosion rates of 1-5 mm for sewer pipes (Mori et al., 1991), it would require several years to study the durability of new concrete and other cement-based building materials *in situ* (Gutiérrez-Padilla et al., 2010). In a sludge digester, the corrosion process is even expected to be much slower due to restricted oxygen availability in the headspace and therefore limited BSA production. Furthermore, concrete corrosion in sludge digesters can first occur when the coating is damaged. Depending on the coating applied and the digester conditions (e.g. $H_2S$ and $O_2$ availability) this might last a few decades. Since sludge digesters are also closed systems, the accessibility to a sludge digester is much more difficult than accessing a sewer pipe. Therefore, accelerated laboratory experiments instead of *in situ* tests were carried out to analyze the durability of hardened cement paste and concrete against chemically and microbially derived sulfuric acid.

The applied short-term (28 days) and long-term (two, three and six months) test systems simulated the last step of the multiplex BSA corrosion process, where sulfuric acid reacts with the main components of concrete. For this, chemical and biological laboratory experiments were performed, since previous studies reported large differences between purely chemical sulfuric acid and microbially produced sulfuric acid damage patterns (Alexander & Fourie, 2011; Ehrich et al., 1999; Monteny et al., 2001; Monteny et al., 2000). Monteny et al. (2000) revealed a higher extent of corrosion on concrete in case of BSA production compared to purely chemical sulfuric acid attack. The authors claimed that the corrosion layer provides optimal growth conditions for the bacteria due to its porosity and high moisture content. Therefore, it was assumed that the bacteria penetrate into the corrosion layer and produce sulfuric acid right next to the sound concrete. This would lead to a microbial attack on concrete from the inside while the chemical sulfuric acid attack might be hindered by the thick gypsum layer that would function as an extra barrier to retard concrete corrosion. A different result was shown in the investigation by Okabe et al. (2007). They measured the pH value, oxygen levels, and the abundance of *A. thiooxidans* in the corrosion layer and showed that bacterial growth and activity takes place on the outer concrete surface only due to limited oxygen and nutrient availability in the corrosion layer itself. They concluded that sulfuric acid is only produced on the external concrete surface and no bacterial growth in the corrosion layer might occur. In this case, the BSA attack would be comparable to a purely chemical sulfuric acid attack, since in both cases, the sulfuric acid must penetrate through the entire corrosion layer to

reach and damage the intact concrete core. Due to such conflictive discussions in the literature, this work took a closer look at the potential differences of chemically and microbially produced sulfuric acid. For the biotic set-ups, *A. thiooxidans,* originally isolated from Dg E, was used, since this bacterium was able to produce huge amounts of sulfuric acid (up to 433 mmol/L; final pH < 1.0), and therefore showed a high corrosion potential. Moreover, *A. thiooxidans* is considered to be the key player within the concrete corrosion process (Milde et al., 1983; Sand & Bock, 1984). Optimal growth conditions were provided for *A. thiooxidans* to simulate a worst-case scenario and compare the BSA attack to experiments representing purely chemical conditions. For the chemical set-ups, the pH was kept at a constant value of 1.0 and 2.0 using automatic titration that was comparable to the pH values generated by *A. thiooxidans* (1.1-2.8).

The described chemical and biological sulfuric acid experiments were first performed with hardened cement paste, because during the corrosion process sulfuric acid mainly reacts with the cementitious phases of concrete such as calcium hydroxide ($Ca(OH)_2$) and calcium silicate hydrate phases (C-S-H) (Lavigne et al., 2016). Hardened cement paste samples with 20 wt% ordinary Portland cement and 80 wt% blast furnace slag cement were used, because this binder is mainly used for concrete structures in wastewater facilities such as sludge digesters. In a second approach, the impact of biogenic and chemical sulfuric acid experiments was studied with the more complex concrete that is used for constructing digester walls. The used concrete mainly consisted of i) calcium hydroxide ($Ca(OH)_2$) and calcium silicate hydrates (C-S-H) (blast furnace slag cement) and ii) inert siliceous and reactive carbonatic aggregates. Siliceous aggregates are insoluble and usually are not involved in the corrosion process, whereas soluble carbonates are dissolved by acid and hence contribute to concrete deterioration (Zivica & Bajza, 2002). The used concrete composition had a strength class of C30/37, which is nowadays used for the construction of a new sludge digester.

The evaluation and description of cement stone and concrete degradation was carried out by analyzing a variety of different parameters, since one parameter is often not sufficient for the characterization of concrete deterioration (Van Tittelboom et al., 2013). In addition to visual inspections of the specimens, the cement stone/concrete corrosion was evaluated by the following parameters: i) mass loss, ii) neutralization depth, iii) calcium leaching, iv) volume expansion, v) size of the intact core, vi) thickness of the corrosion layer, vii) LA-ICP-MS, and viii) SEM and EDX (for detailed information see Figure 3.2). For sulfuric acid corrosion tests, weight loss is commonly used as evaluation tool (Van Tittelboom et al., 2013) and values between 1.8-31.0% can be found in literature (Aviam et al., 2004; Sand & Bock, 1987; Sand & Bock, 1984). However, the weight can also

increase, especially in the beginning of the concrete degradation process when corrosion products compact the pores. Therefore, mass loss can be only used at later stages of degradation, when the gypsum layer disintegrates from the specimens and mass loss occurs (Van Tittelboom et al., 2013). Therefore, additional methods are required to characterize corrosion damage patterns and to provide more in-depth results. In the present study, LA-ICP-MS and EDX were applied to determine element distribution within the corrosion layer and the non-corroded area. LA-ICP-MS technique, which was used as novel tool for the assessment of cement stone/concrete degradation, is generally applied to obtain qualitative and quantitative data of almost all elements of the periodic system. However, within the present study, only semi-quantitative analyses were possible due to the irregular sample surfaces and the associated different ablation behavior. Both LA-ICP-MS and EDX allowed the identification of gypsum as main corrosion product. Additionally, LA-ICP-MS enabled the differentiation between corroded and non-corroded layers and clearly showed the distribution of siliceous and carbonatic aggregates. For a detailed characterization of cement stone and concrete degradation, chemical analyses such as LA-ICP-MS or EDX measurements are essential to draw a conclusive picture about the extent of corrosion.

### 8.3.2 Corrosion process

During sulfuric acid attack, calcium hydroxide and calcium silicate hydrate phases, the main constituents of cement paste, as well as the carbonatic aggregates in concrete were dissolved by acid resulting in gypsum precipitation and amorphous silica acid (silica gel) production. Monteny et al. (2000) claimed that during BSA corrosion both, ettringite and gypsum, are formed and that the precipitation of ettringite is even more destructive to concrete than the formation of gypsum. However, within this work, only gypsum and silica gel have been identified in the corrosion layers of both chemical and microbiological sulfuric acid experiments independent of applying hardened cement paste or concrete. No ettringite that is typically formed by the reaction of gypsum with calcium aluminate hydrate ($C_3A$) could be detected in the corrosion layers analyzed with LA-ICP-MS and SEM/EDX. This is in accordance with the findings of Davis et al. (1998) where no ettringite was found in the outer corrosion layers of a concrete sewer pipe. They identified only little amounts of ettringite in the deeper sections of concrete (Davis et al., 1998), where the pH value was probably alkaline enough to maintain the stability of ettringite (O'Connell et al., 2010). Since ettringite is instable at pH values < 10.6 (St John et al., 1998), the lack of ettringite in this study is probably due to the very low pH values in the experimental set-ups (pH 1.0-2.8). Consequently, the formation of gypsum is usually enhanced in acidic

environments, as was also noticed by Gutberlet et al. (2015). Moreover, within this work, concrete samples with high sulfate resistant cement, possessing low $C_3A$ contents (De Belie et al., 2004), were used. Due to the limited availability of $C_3A$, ettringite formation plays only a subordinate role in such types of concrete.

For both acid attacks (chemical and microbiological) and both sample types (hardened cement paste and concrete) gypsum has been identified as the main corrosion product while ettringite formation was negligible. However, two main differences could be observed between hardened cement paste and concrete samples: i) the volume expansion for the hardened cement paste samples was much higher compared to the concrete specimens, and ii) a much thicker gypsum layer precipitated on the surface of the hardened cement paste compared to the concrete. The volume expansion in concrete is limited in the cement paste aggregate interfacial especially in the presence of inert siliceous aggregates. The thinner gypsum layer on the concrete surface may be due to the formation of $CO_2$, which is produced by the reaction of sulfuric acid with the carbonatic aggregates. In contrast to the hardened cement paste samples, the $CO_2$ gas evolution within the concrete may reduce gypsum precipitation on the concrete surface and destabilize the corrosion layer. Further studies are needed to clarify that aspect by focusing on the reaction of the carbonates with the sulfuric acid and the effect of $CO_2$ formation on the corrosion layer. Finally, both hardened cement paste and concrete showed a different corrosion behavior regarding the volume expansion and the thickness of the gypsum layer. Both, inert siliceous and reactive carbonatic aggregates accounting more than 60% of the concrete, (Scrivener & De Belie, 2013) play a decisive role in the concrete corrosion process. The reduced gypsum formation and low volume expansion observed slow down the concrete deterioration. However, the dissolution of the cement binding matrix of the concrete might break off the aggregates weakening the concrete structure and increasing mass loss.

For the biogenic long-term experiments which were carried out to test the durability of concrete against microbially produced sulfuric acid over a period of two, three and six months, similar corrosion products as described above were determined. The extent of deterioration increased within the first three months, which was visible due to the increasing neutralization depths and weight losses. The higher weight loss of the three-month set-up was induced by the break off of aggregates. For the concrete samples of the six-month experiment similar damage patterns, mass losses, and neutralization depths were revealed as for the concrete exposed for three months to biogenic produced sulfuric acid. These results indicate that the concrete deterioration is not a linear process over

time. Instead, the corrosion primarily depends on the amount of sulfuric acid produced by *A. thiooxidans* at a certain timepoint.

Within this work, similar corrosion damage patterns were identified between chemical and biological sulfuric acid attack on both hardened cement paste or concrete. For the biotic and chemical set-ups the corrosion layer consists of gypsum and amorphous silicic acid (silica gel) and no internal cracking of the concrete by the bacteria was observed. This led to the assumption that bacterial growth occurred exclusively on the outer cement stone/concrete surface as well as in the surrounding culture medium. The availability of sulfuric acid would be thus comparable to the purely chemical sulfuric acid attack. In both set-ups, the acid first reacts with the hardened cement paste/concrete surface. After corrosion products are formed, sulfuric acid must penetrate through the gypsum layer to reach and corrode the untreated concrete. The bacteria seem to have no growth advantages in the gypsum layers probably due to limited oxygen and nutrient supply as was already observed by Okabe et al. (2007). Consequently, the experimental set-up applying chemical sulfuric acid served as a good model system to simulate the last step of a potential BSA corrosion (worst case scenario), but in a highly simplified laboratory experiment.

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