



# CHALMERS

**Final report of the preliminary study of the project**  
**”The role of microbiological biofilm communities for degradation of  
sprayed concrete in subsea tunnels”**

2014

Britt-Marie Wilén & Frank Persson, Department of Civil and Environmental Engineering, Division of Water Environment Technology (WET), Per Hagelia, Norwegian Public Road Administration (NPRA)

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### **Background**

There are today over 30 subsea tunnels in Norway. These tunnels, built since the early 1980's, connect islands and peninsulas in the fjord dominated landscape. Several new subsea tunnels are also planned in the near future, within the ferry-free coastal route E39 project.

Sprayed concrete is used for rock support in road tunnels. To increase the elastic properties, the sprayed concrete is reinforced with fibers, commonly made of steel. Degradation of the sprayed concrete and its fibers due to chemical and microbial reactions can result in destabilization of the rock mass, leading to potential safety risks, increased costs for maintenance and reduced overall lifetime of the tunnel. In general the sprayed concrete is designed to last for more than 50 years but in many cases deterioration can be observed after less than 5 years.

A novel deterioration process has recently been discovered in several Norwegian subsea tunnels resulting in rapid degradation of the concrete matrix and destructive corrosion of the steel fibers in areas with leakages of saline groundwater. Thick microbial biofilms, with (putative) iron- and manganese oxidizing bacteria are involved in the degradation process. Investigation regarding effects on concrete in the Oslofjord tunnel and three other Norwegian subsea tunnels suggests the structural impact of biofilms on sprayed concrete varies a lot. As yet knowledge about the microbial communities involved is extremely poor. In order to understand and ultimately prevent such degradation processes, thorough biofilm microbiological and chemical studies are necessary. Microbial biofilm communities and their activities involved in degradation (corrosion) of manmade materials are however complex and require detailed investigations using molecular microbial methods in conjunction with thorough chemical measurements in order to avoid oversimplifications

The significance of sprayed concrete degradation caused by Mn and Fe bacteria in subsea road tunnels in Norway has been reported previously in a PhD thesis by Hagelia in 2011 and papers by Per Hagelia written during the period 2007-2013. Although the phenomena, typical deterioration reactions and characteristic environmental loads are now quite well established, the problem cannot be solved without a much closer look at the microbial communities involved. There is, hence, a need for involving microbiological expertise. This report summarizes the activities of the pre-study for the project 10 “The role of microbiological biofilm communities for degradation of sprayed concrete in subsea tunnels”.

## **Activities**

When the Norwegian Public Road Administration (NPRA) first initiated contacts with Chalmers for collaboration within the E39 project, the problem of degradation of reinforced sprayed concrete was mentioned as one critical problem that should be studied within the frame of a PhD project. To collect information and establish contacts between Chalmers and NPRA a series of activities were initiated.

### **Subsea Tunnel workshop at Chalmers 2014 02 24**

Frank Persson took part in the workshop, presented the research problem and ideas for a research proposal and got feedback on the proposed approaches for the planned PhD project.

### **Meeting in Oslo 2014 03 04**

#### Visit to NPRA head office in Oslo

Per Hagelia, Thomas Haverkamp, Mohammed Hoseini, Alf T. Kveen, Britt-Marie Wilén and Frank Persson met as NPRA for a full day of discussions of the problem with degradation of reinforced sprayed concrete. The meeting was intended as a start-up of the process of planning a PhD project.

The research and development projects at NPRA were briefly summarized by Per Hagelia and Alf T. Kveen to give everyone an overview of their activities. Per Hagelia gave in-depth background of the problem of microbially induced corrosion (MIC) and degradation of sprayed concrete, based on his research.

Thomas Haverkamp, dept. of Biosciences at the University of Oslo, summarized a preliminary study of the microbial communities in the sprayed concrete biofilms, with emphasis on details about the methods for analysis such as primer choice, DNA extraction methodology, metagenomics experiences etc. We have now access to the results of this preliminary study as background for setting up the experiments for the proposed PhD project.

Frank Persson and Britt-Marie Wilén presented their ideas so far about approaches for investigations, as well as introduced the competence and infrastructure within the group at Chalmers, including the collaborations with GU. A discussion followed about approaches for investigating MIC in sprayed concrete. Mohammed Hoseini also advised on how to formulate a PhD project within the Chalmers-NPRA collaboration.

#### Discussion of content of the project

After the meeting, Per H, Britt-Marie and Frank started to work on the PhD plan and set out a framework for the project including; criteria for choice of study sites, experimental set-ups, time plans, organization and supervision (Per H, FP and BMW will all be supervisors), external collaborations and the need for additional infrastructure (access to lab facilities in Oslo during sampling campaigns).

#### On-site visit in the Oslofjord test site

The Oslo meeting was planned to match the regular maintenance work in the Oslofjord tunnel, to get access to the test site (only accessible during tunnel maintenance). Per, Britt-Marie and Frank visited the test site between 10 pm and 2 am for sampling within the ongoing program at NPRA and for testing sampling methods for microbial community analysis, with regard to the different types of microbial biofilms present at different corrosion spots in the tunnel. Samples were also brought to Chalmers, where sample preservation methods were tested.



**Figure 1. Photos from left to right: the tunnel at the test site, one of the tunnel locations with extensive biofilm formation and close up of the biofilm on the sprayed concrete.**

### **Participation in Teknologidagene-Program for E39**

Britt-Marie and Per H participated in the conference “Teknologidagene” arranged by NPRA in the programme specially devoted to the E39 project (8-9 October 2014). The research proposal was presented by Britt-Marie.

### **Establishment of collaborators**

Since this project involves different disciplines such as geochemistry, geology, ground water chemistry, microbiology, molecular biology and concrete, some contacts were taken to discuss the project and to initiate collaboration. The following contacts have been taken during 2013-2014:

Adj. Prof. Karsten Pedersen, CEE, GeoEngineering, Chalmers. Karsten has vast experience of geomicrobiology including methodology such as microsensors for chemical gradient measurements, which will be used within the project.

Prof. Karin Lundgren, CEE, Structural Engineering, Chalmers. Karin is leading the research group Concrete Structures at Chalmers, with projects about e.g. corrosion of reinforcement, with relevance for the proposed PhD project.

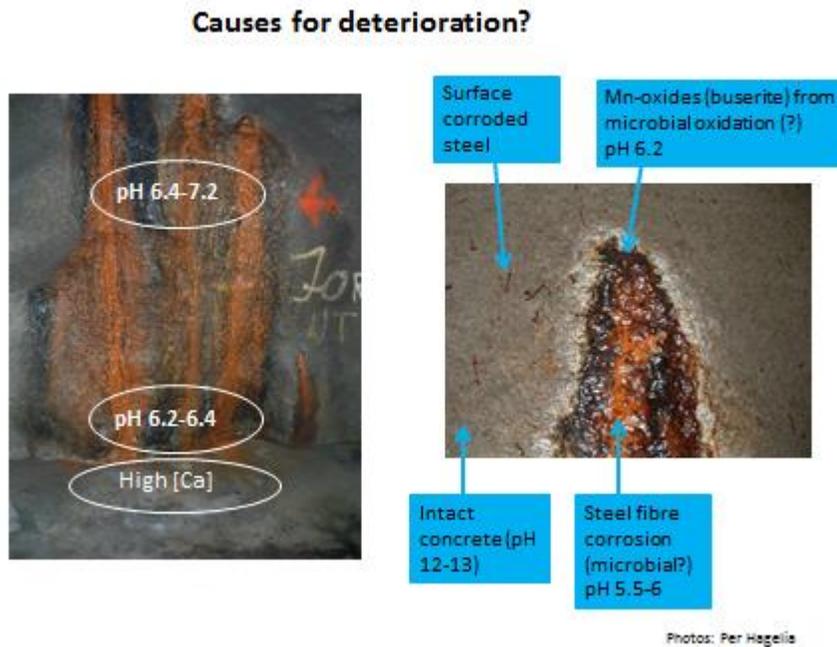
Prof. Malte Hermansson, CMB, Microbiology, University of Gothenburg is an expert in biofilm microbial ecology, including MIC. We also have access to the laboratory facilities at CMB, Microbiology for the proposed PhD project.

Also international collaboration, within the Mn and Fe microbiological /biomineral system is being established.

### **Research approach**

There are already some hypotheses for the causes of the degradation of the concrete based on literature and previous research by Per Hagelia (Figure 2) as well as in the available literature (Appendix 1). In this research plan, we propose studies of spatial and temporal patterns, using a suite of molecular microbial

methods and chemical measurements, for assessment of the composition, diversity, stratification and activity of the biofilm communities in relation to the associated degradation of concrete and fibers. Studies will be performed on site in the subsea tunnels as well as at defined conditions at laboratories (Figure 3). To be able to understand how the biofilm works it is important to measure the gradients of important parameters such as pH, H<sub>2</sub>S and O<sub>2</sub> concentrations, and oxidation-reduction potentials with microelectrodes (Figure 4). These measurements will mainly take place on site but also in small controlled systems in the lab where pieces of biofilm from the tunnel(s) are exposed to different environmental conditions, such as ground water chemistry, O<sub>2</sub> concentration etc, mimicking the conditions in tunnel.



**Figure 2. Possible causes for the deterioration of reinforced sprayed concrete.**

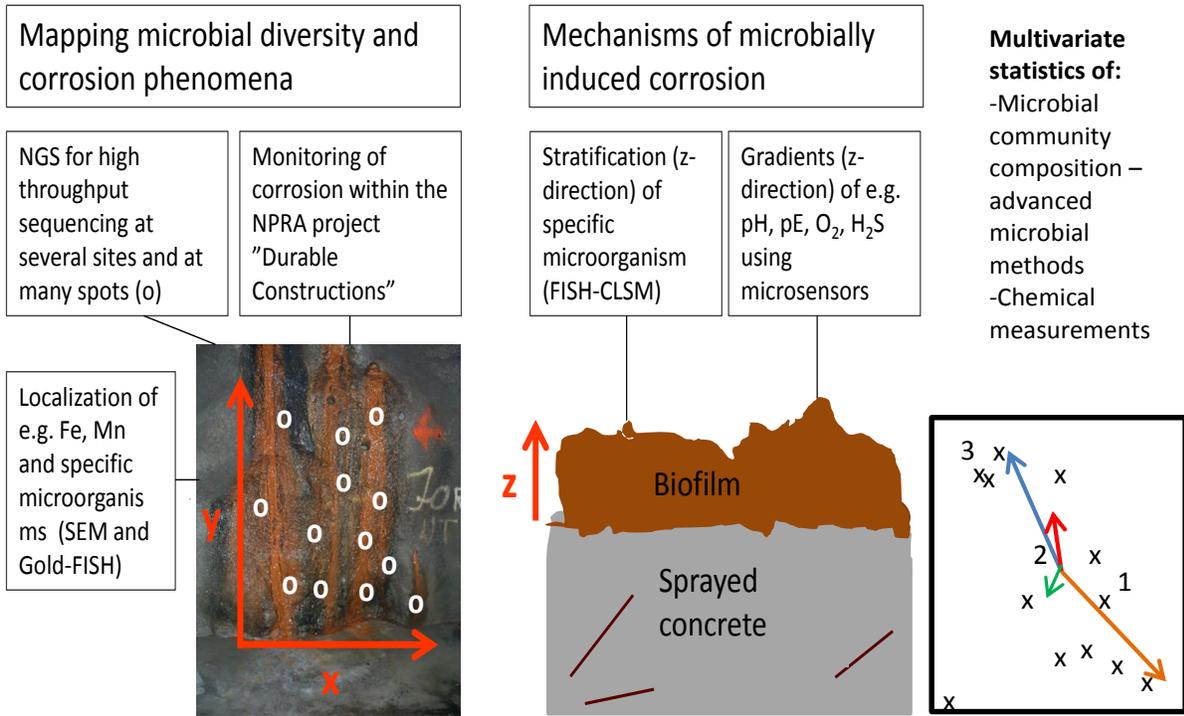


Figure 3. Experimental approach

### What role does the biofilm have?

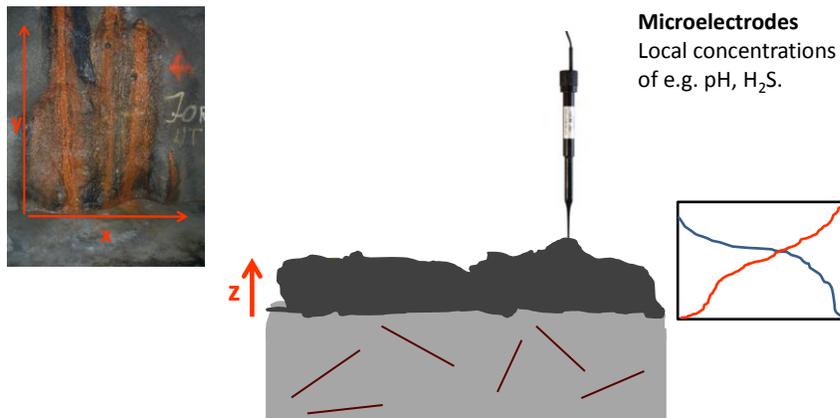


Figure 4. Measurement of gradients of environmental conditions inside the biofilm by microelectrodes.

## **Writing a PhD plan**

Based on the meetings, feedback and on-site visit we started to write a PhD project plan which was finished in time for the steering group meeting within the NPRA-Chalmers collaboration, August 2014. The PhD proposal is attached in Appendix 1.

We have already made a study plan with some suggested PhD courses included (Appendix 3).

## **Advertisement for a PhD position**

The advertisement for a PhD student was done mid December 2014 with deadline the 11<sup>th</sup> of January 2015 (Appendix 2). In total 24 applicants were registered and we are now in the process of selecting candidates for an interview. By the 20<sup>th</sup> of January all applications had been read and Frank and Britt-Marie had a selection meeting the 22<sup>nd</sup> of January and after that we have discussed with Per Hagelia who will be eventually called for an interview. This process is ongoing and we will start invite candidates for interviews at the end of January. The plan is to start the project in the beginning of April. The person we look for should have some knowledge in microbiology, building materials and/or some other insight into infrastructure. It would also be desirable with some knowledge in concrete chemistry. However, we realize that it is very hard to find such person so the most important is the interest in the topic and he or she has to study the areas where lack of knowledge exists.

## **Planned Purchase of equipment**

Since it is important to be able to measure on site own equipment for this must be purchased. We plan to buy equipment for measurement of profiles within the biofilm with microelectrodes from Unisense ([www.unisense.com](http://www.unisense.com)) in Denmark. We have experience in using this type of equipment and Unisense are leading on the market. We need to buy a control panel (measurement instrument) with computer, a micromanipulator that enables fine tuning of the movement of the electrode ( $\mu\text{m}$  scale) and a stereomicroscope which enables exact position of the electrodes at the upper part of the biofilm as well as electrodes. Altogether this equipment cost approximately 250 000 – 300 000 Nkr. Since we have not used all money available for the pre-study (ca 200 000 Nkr) we would like to use the remaining part for the purchase of this equipment.

## **Concluding remarks**

The project will start spring 2015 after a substantial literature review, site visit and discussions. As a start a PhD student will be selected and the microsensor equipment will be purchased. During 2015 sampling of the test sites will start and a methodology for sampling and preservation of the biofilm samples will be developed. The molecular techniques have been developed at WET where the same methods are applied for studies of mainly wastewater biofilm, flocs and granular sludge. The PhD candidate will start relatively soon to learn these techniques.

## References

Hagelia, P., *Deterioration mechanisms and durability of sprayed concrete for rock support in tunnels*. 2011, TU-Delft, The Netherlands. 205 pp. + appendices. <http://repository.tudelft.nl/view/ir/uuid%3Ad64b8ff8-8d68-4eea-a320-73148e9f1b15/>

## **APPENDIX 1.**

# **THE ROLE OF MICROBIOLOGICAL BIOFILM COMMUNITIES FOR DEGRADATION OF SPRAYED CONCRETE IN SUBSEA TUNNELS**

## **SUMMARY**

There are today over 30 subsea tunnels in Norway. These tunnels, built since the early 1980's, connect islands and peninsulas in the fjord dominated landscape. Several new subsea tunnels are also planned in the near future, including within the ferry-free coastal route E39 project.

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A novel deterioration process has recently been discovered in several Norwegian subsea tunnels resulting in rapid degradation of the concrete matrix and destructive corrosion of the steel fibers in areas with leakages of saline groundwater. Thick microbial biofilms, with (putative) iron- and manganese oxidizing bacteria are involved in the degradation process. Investigation regarding effects on concrete in the Oslofjord tunnel and three other Norwegian subsea tunnels suggests the structural impact of biofilms on sprayed concrete varies a lot. As yet knowledge about the microbial communities involved is extremely poor. In order to understand and ultimately prevent such degradation processes, thorough biofilm microbiological and chemical studies are necessary. Microbial biofilm communities and their activities involved in degradation (corrosion) of manmade materials are however complex and require detailed investigations using molecular microbial methods in conjunction with thorough chemical measurements in order to avoid oversimplifications. In this research plan, we propose studies of spatial and temporal patterns, using a suite of molecular microbial methods and chemical measurements, for assessment of the composition, diversity, stratification and activity of the biofilm communities in relation to the associated degradation of concrete and fibers. Studies will be performed on site in the subsea tunnels as well as at defined conditions at laboratories.

## **SPECIFIC AIM AND OBJECTIVES**

The overall aim of this project is to increase the understanding of the mechanisms involved in the biologically mediated corrosion of sprayed reinforced concrete in subsea tunnels. More specifically the objectives are to (1) investigate the biofilm composition in terms of microbial and chemical composition; (2) investigate the environmental conditions within the formed biofilms (e.g. pH, redox-potential, dissolved oxygen concentration and concentrations of sulfur species, nitrogen species etc.); (3) understand the biofilm evolution in terms of both time and space; (4) understand how different types of biofilms are formed depending on the prevailing environmental conditions at a particular site; (5) understand how the biofilm interacts with the under-laying sprayed concrete and eventually deteriorates it; (6) come up with possible solutions to prevent biofilm formation.

## **BACKGROUND (OVERVIEW OF THE RESEARCH AREA)**

Sprayed concrete reinforced with steel or synthetic fibre for rock support in subsea tunnels in Norway has been observed to deteriorate quickly with notable disintegration and corrosion of steel fibre within less than 5 years. Different mechanisms affect the cement paste matrix, which is mainly made up by calcium silicate hydrate (C-S-H). The degradation can be due to physical, chemical and biologically mediated mechanisms. This may destabilize the rock mass underneath and lead to increased maintenance costs but also risks. Sprayed concrete is exposed to different environmental loads such as water containing high  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{Mg}^{2+}$ , and  $\text{HCO}_3^-$  concentrations leading to both abiotic and biochemical reactions. The Mn-Fe biofilms have commonly caused acidification and weakening including material loss from sprayed concrete surfaces. This is due to extensive decalcification, with associated formation of thaumasite, calcite and brucite further inside. Formation of Mn-biominerals (buserite and todorokite) and Fe-compounds (ferrihydrite e.t.c.) play an important role in the chemical cycle. Calcium also leach from the concrete, forming calcite stalactites, as well as precipitations in the form of brucite, aragonite and gypsum which leads to further deterioration of the surface [1, 2]. Since concrete has a very porous structure with high surface roughness, access to water containing organic material together with an appropriate chemical composition can make microorganisms grow and colonise its surface, forming layers of biofilm.

Microbiologically induced concrete corrosion (MICC) is a well-known phenomenon that has been observed for a long time in for instance sewer systems [3]. Bacterial activities produce acidic compounds such as carbon dioxide and hydrogen sulphide which will reduce pH locally at the concrete surface. These conditions are favourable for sulphate oxidising bacteria (SOB) which oxidize hydrogen sulphide ( $\text{H}_2\text{S}$ ) to sulphuric acid ( $\text{H}_2\text{SO}_4$ ). These SOB are typically *Thiobacillus*. However, as pH drops there is a succession in SOB to species that can withstand low pH such as *Acidothiobacillus* [3]. Eventually the concrete loosens up, a process that can take 2-15 years. At this stage gypsum is formed (expansive gel) when  $\text{H}_2\text{SO}_4$  reacts with  $\text{Ca}(\text{OH})_2$  which leads to cracks in the concrete structure [4].

The steel fibres in concrete start to corrode very fast, especially when exposed to saline environments [5]. Chloride increases the solubility of  $\text{Fe}(\text{OH})_2$  which protects the steel surface from corrosion [6]. This leads locally to fast decrease in concrete strength. Abiotic corrosion of mild steel will occur when exposed to oxygen forming iron oxides [7]. Biofilm formation can lead to the formation of an anode underneath with little oxygen and a cathode in the aerated surfaces which will accelerate the corrosion even further. If the conditions are anaerobic, protons or hydrogen sulphide is reduced forming molecular hydrogen that can be used by sulphur reducing bacteria (SRB) as electron donor. Iron sulphide can be formed by SRB which will lead to further acidification. Reduced sulphide and ferrous iron are further oxidized with oxygen as terminal electron acceptor forming ferric oxide/hydroxides (rust) and elemental sulphur [7]. Due to concentration gradients of oxygen and pH in biofilms found in sewer pipes, SOB and SRB co-exist [8]. Stainless steel can also corrode due to alteration of the protective passive oxide layer made up of manganese oxides. It has been demonstrated that the manganese oxidizing bacteria (MOB) *Leptothrix discophora* can cause corrosion of stainless steel surfaces [9].  $\text{Mn}^{2+}$  is oxidised to  $\text{MnO}_2$  via  $\text{MnOOH}$ . It is hypothesised that  $\text{MnO}_2$  act as a cathode and is reduced to  $\text{Mn}^{2+}$  via  $\text{MnOOH}$ , which is recycled by MOB to  $\text{MnO}_2$ . In the Norwegian study a similar reaction was proposed to be responsible for the manganese oxidation. Bacteria with morphologies similar to *Leptothrix discophora* were observed. The  $\text{Fe}^{2+}$  was believed to be produced by *Gallionella ferruginea* (*betaproteobacteria*) and/or *Mariprofundus ferrooxidans* (*Zetaproteobacteria*)

[1, 2]. The identification was, however, made on morphology only. It was also observed that iron and manganese accumulated on tunnel surfaces soon after concrete spraying. Iron oxidising bacteria (FeOB) are regulated to narrow redox boundary areas where there is Fe<sup>2+</sup> rich water at moderate levels of oxygen [10]. Chemical iron oxidation outcompetes the biological process at higher oxygen concentrations [11]. The understanding of the role played by FeOB in steel corrosion is incomplete [12]. FeOB within the *zeta-proteobacteria* have been found on carbon steel coupons in coastal seawater where the microbial communities were examined by clone libraries [12, 13].

The interactions between abiotic and microbial processes associated with concrete deterioration are complex and needs to be studied in situ conditions and modern molecular techniques have to be applied since most bacteria are not cultivable.

The Norwegian Public Road Administration (NPRA) established in 2010 a test site in the Oslofjord subsea tunnel at a location where severe deteriorations caused by microbial biofilm formation was observed on a 10-year old steel fibre reinforced sprayed concrete. At the location three sprayed concrete mixtures, including steel fibres and synthetic fibres, were sprayed manually on the tunnel wall. Additionally cast concrete samples of the same three concrete mixtures were made and exposed permanently in local ditch waters (ditch A and B) and air. The test site will be in operation at least beyond 2020. Ditch A receives tunnel drain water from a larger part of the tunnel. The ditch is fed by a steady source of representative water. Ditch B is provided with stagnant water from the period when spraying took place [5].

An additional test site will be investigated in parallel as the conditions are slightly different: the Frøya tunnel in Trondheim. Two locations will be investigated where intrusion of saline groundwater has occurred. At these sites biofilm formation has been observed locally with significant deterioration of the sprayed concrete. This tunnel was opened in 2000. Compared to the Oslofjord test site, the effect of the biofilm on cement paste degradation appear to be less and therefore it is of interest to elucidate the reasons behind this.

## **PROPOSED RESEARCH (RESEARCH DESCRIPTION)**

Degradation (corrosion) of manmade materials is often a combination of intricate biotic and abiotic reactions. Biodegrading and -corroding microbial communities are usually complex consortia with intricate interactions with their abiotic environment [14]. Most of the past investigations of microbiologically induced corrosions (MIC) has relied on cultivation to get bacterial isolates and has resulted in simplified models to reduce the complexity inherent in multi-species biocorroding biofilms [13]. Only a small fraction of environmental microorganisms are cultivable [15]. Hence, extrapolation of cultivation-based results to the real world has often failed to produce effective strategies for corrosion control [13]. In this research proposal, identification, diversity, localization and successional patterns will consequently be investigated with an array of molecular methods on-site in the subsea tunnel to prevent such oversimplifications. In parallel, controlled experiments will be performed in the laboratory where biofilm harvested from the test sites will be exposed to water with chemical compositions similar to the ones on-site to assess detailed degradation processes as well as biofilm development.



**Figure 1.** Photos from left to right: the tunnel at the test site, one of the tunnel locations with extensive biofilm formation and close up of the biofilm on the sprayed concrete.

### **PHASE I. COMPOSITION AND DIVERSITY OF BIOFILM CONSORTIA ON REINFORCED SPRAYED CONCRETE**

*The composition of the biofilm microorganisms will be investigated using next generation sequencing (NGS) at different areas on the sprayed concrete surfaces from the Oslofjord tunnel and the Frøya tunnel sites. Resulting compositional patterns will be compared with data on local environmental (corrosion related) factors, such as pH, redox potential and accumulation of corrosion products using obtained within the NPRAs sampling program in the project Durable Constructions. The potential outcome of this survey is (I) determination of the microorganisms present (II) understanding of the compositional changes at different locations (III) a map relating compositional changes with corrosion related environmental factors and (IV) it will provide basis for how to select parameters for the successional study at lab conditions (phase III).*

Complex microbiological communities have been revealed by molecular methods in biofilms related to microbiologically induced concrete corrosion (MICC) in sewers [3, 16] and on corroding metal surfaces [13, 17]. With the recent advents of NGS and metagenome analyses, complexity has further increased and potentially new players have been identified [18, 19].

Investigations of the composition of microorganisms on the sprayed concrete will be performed by NGS using the MiSeq Illumina platform. This enables in-depth amplicon sequencing of specific regions of the 16S rRNA gene of many (>96) parallel samples for rigid multivariate statistical analysis of composition and diversity [20]. An initial study of the biofilm community at the Oslofjord tunnel test site has recently been performed and the results of this study will be used for choice of methods and approaches for DNA extraction and –amplification [21]. For thorough phylogenetic characterization of major taxa present, clone libraries will be constructed for near full-length sequencing (Sanger) of the 16S rRNA gene, as well as of functional genes. This approach also provides us with the genomic information necessary for probe- and primer selection and construction used in phase II and III below.

Samples will be taken at different spots (in the x-y dimension) at the subsea tunnel sites. For identification of the relations between corrosion related (and other) environmental factors and the microbial community compositions, multivariate analysis will be performed using e.g. canonical correlation analysis and redundancy analysis [22], which has proven useful for this type of comparisons [23].

## PHASE II. LOCALIZATION AND STRATIFICATION OF MICROORGANISMS INVOLVED IN DETERIORATION OF SPRAYED CONCRETE

*In this part of the research, we will investigate the spatial distribution of the important corrosion related microorganisms (identified in phase I) in depth within the biofilms in relation to the micro-scale environmental conditions at biofilms from the different subsea tunnel sites. In biofilms, specific spatial localization and stratification of functional microbial groups is a common phenomenon. Often this is related to variations in pH, oxygen concentrations, availability of substrates etc., with depth in the biofilm, caused by the activities of the microorganisms and their surrounding environment. Furthermore, spatial separation of electron donors (anodes) and ultimate electron acceptors (cathodes) is pivotal for MIC and often occur with depth in the biofilms. Potential outcomes of the studies include (I) a linkage between the identified microorganisms and their activities at different depth in the biofilm (II) direct comparison of the microbial and chemical stratification in the biofilms from different sites with different corrosion characteristics.*

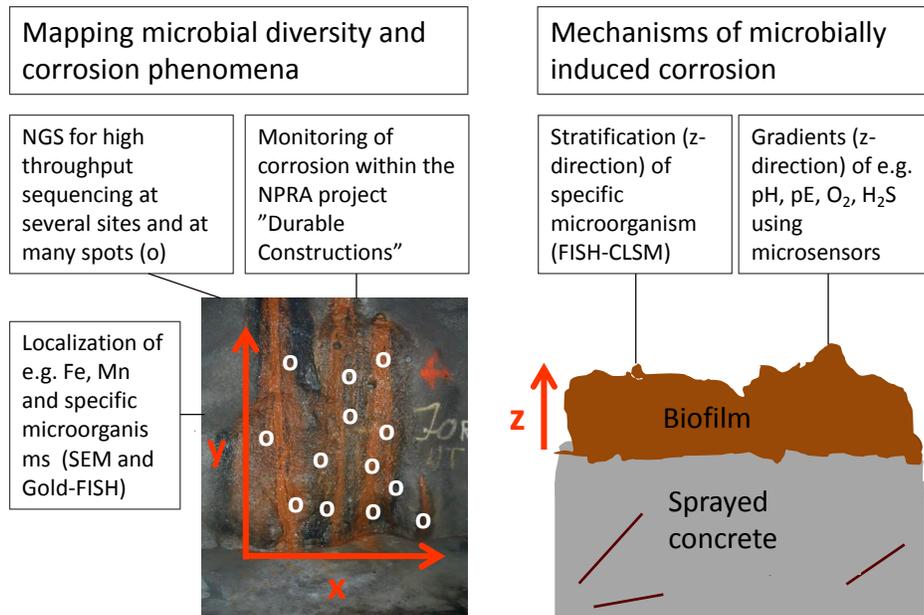
The localization of microorganisms is fundamental for corrosion phenomenon. Recent studies have e.g. shown distinct localization patterns of microbial organisms responsible for concrete sewer corrosion in depth (z-direction) within biofilms [8] as well as at different areas of the concrete surface (x-y direction) [19].

For quantification of specific microorganisms at different sites on the concrete surfaces (x-y direction) we will use quantitative PCR (qPCR) for 16S rRNA genes and for functional genes. qPCR allows high throughput quantification of target genes in environmental sample and has proven robust for quantification of changes in microbial consortia across temporal and spatial scales [24].

The spatial organization and stratification of microorganisms in biofilms (z-direction) will be investigated by fluorescence in situ hybridization (FISH) together with confocal laser scanning microscopy (CLSM) [25]. For retrieval of intact biofilms we will use cryoembedding and cryosectioning [26]. Acquired CLSM images will be analyzed by statistical image analysis methods to show distribution- and co-localization patterns of the biofilm microorganisms [27].

The gradients within the biofilm of concrete deterioration and corrosion relevant parameters such as oxygen, pH, H<sub>2</sub>S and redox will be investigated by microelectrodes. The use of microelectrodes in corrosion studies has e.g. shown the local sites of microbial activity in biofilms and corroded materials (in z-direction) and can also be used to calculate the flux of metabolic products, e.g. H<sub>2</sub>S, from the biofilm to the surrounding environment [8]. The combination of microelectrode measurements and FISH furthermore provide a link between the identity and localization of specific microorganisms and their function [3].

Further linkage between identity and activity of microbial community members can be provided by the novel method of combining FISH with scanning electron microscopy (SEM), called Gold-FISH [28]. By having the oligonucleotide probes conjugated to gold nanoparticles, simultaneous localization of FISH targeted populations and deposits of e.g. iron and manganese would be possible by SEM using EDS-mapping and would provide a novel way of linking identity and localization of target microorganisms with their surrounding abiotic environment, including potential electron donors and acceptors.



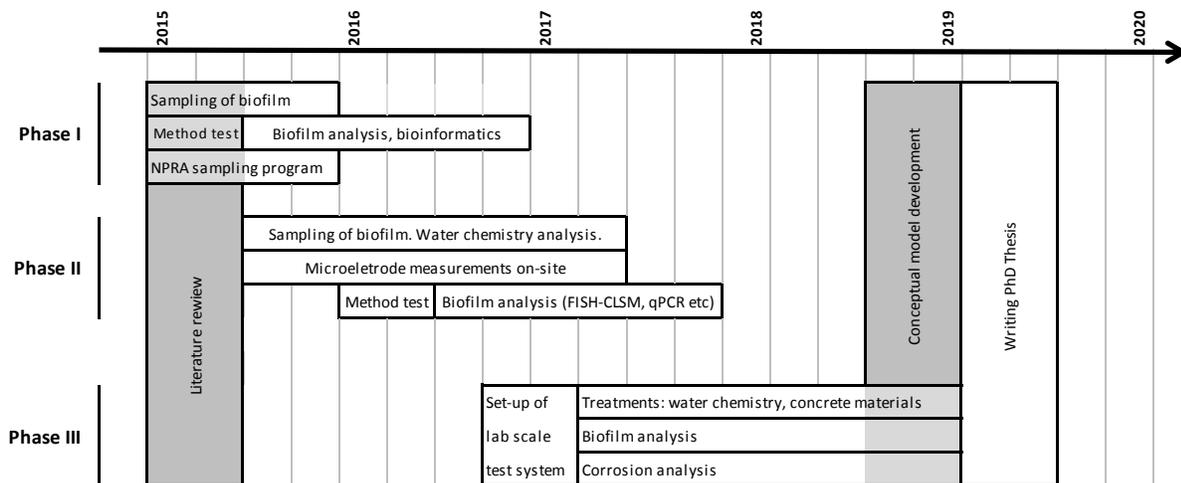
**Figure 2.** Example of approaches used in the proposed research. Photo to the left of biofilms at the Oslofjord tunnel test site (Per Hagelia). Schematic image to the right of biofilm on top of the reinforced sprayed concrete.

### PHASE III. SUCCESSIONAL PATTERNS OF CONCRETE DEGRADING MICROBIAL COMMUNITIES

*In order to understand the formation and mechanism of degradation of sprayed reinforced concrete, temporal studies will be performed on pristine surfaces at defined conditions at the lab at Chalmers. Different treatments include water chemistry data (salinity, redox etc.), obtained by the measurements at the different tunnel sites, as well as different concrete reinforcement materials (steel and polypropylene fibers). Here the composition, localization and stratification of the biofilm microorganisms in relation to the degrading activities will be followed in time-series. One important potential outcome is the establishment of critical factors (abiotic and/or microbial) for the development of degradation of reinforced sprayed concrete.*

The methods and approaches outlined in Phase I and II will be used in the study of temporal patterns of microbial communities and degradation of concrete and fibers.

Ultimately the body of information and evidence acquired in phase I-III will be used for development of a conceptual model, which takes the spatial and temporal patterns of biofilm compositions and corrosion on different materials into account.



**Figure 3.** Overview of the activities within the research project.

## COURSES

A PhD student should take a number of courses of 120 credits during their training. Courses include more general competence development to become a researcher such as information literacy, career planning, scientific writing and pedagogy as well as specific competence development. For this project, several in-depth courses in e.g. microbial methods, biotechnology, bioinformatics, and geochemistry will be part of the training program.

## ORGANIZATION

The research will be conducted by a PhD student at Water Environment Technology (WET), dept. of Civil and Environmental Engineering, Chalmers University of Technology, together with supervisors and other collaborators.

### Supervisors

Prof. Britt-Marie Wilén (WET) is leading the group within the field of wastewater treatment at WET. Britt-Marie has vast experience in environmental biotechnology with special expertise in wastewater processes.

Assistant Prof. Frank Persson (WET) is a specialist in microbial ecology of biofilm processes in drinking water, seawater and wastewater.

Senior Engineer Per Hagelia at NPRA, Norway, is an expert in concrete degradation mechanisms with a PhD on this topic. Per is also the initiator of the proposed PhD project.

### THE JOINT RESEARCH GROUP

The PhD student will be integrated in a joint biofilm research group at WET, Chalmers and CMB, University of Gothenburg (Bioresource Labs). The following group members have particular relevance for the project:

Prof. Malte Hermansson, dept. of Chemistry and Molecular Biology (CMB), University of Gothenburg is an expert in biofilm microbial ecology, including MIC.

Assistant Prof. Oskar Modin (WET) is an expert in bio-electrochemical systems.

The members of the Bioresources Labs have together a long experience in working with microbial aggregates and biofilms within wastewater treatment but also drinking water treatment and marine environments. This background is relevant since many of the mechanisms and processes involved are to a large extent similar to the biofilm formation to be studied in this project. Our expertise includes aggregation behavior of microbial cells, surface and colloidal chemistry, microbial conversion mechanisms of organic matter, nutrients and inorganic compounds such as iron and manganese. Besides the group has vast experience in molecular tools relevant to the proposed project. At the moment we run projects together within the field of aerobic granular sludge, anammox biofilms, nitrification biofilms, bioelectrochemical cells.

#### **EXTERNAL COLLABORATIONS**

Prof. Karin Lundgren, Civil and Environmental Engineering, Chalmers, leads a research group on concrete structures, with emphasis on e.g. deterioration processes. This group has an important competence for our project, which is available within the same department at Chalmers.

Prof. Karsten Pedersen, Civil and Environmental Engineering, Chalmers, is an authority within the field of geomicrobiology in deep underground aquifers.

Dr. Robert Almstrand, dept. of Civil and Environmental Engineering, Colorado School of Mines, is a specialist in advanced biofilm hybridization- and visualization methods.

Assoc. Prof. Holger Daims, dept. of Microbial Ecology, University of Vienna, is a leading authority in the field of CLSM and digital image analysis.

## **METHODOLOGY AND EQUIPMENT**

#### **QUANTITATIVE PCR (qPCR)**

A number of primers for taxonomical and functional genes are available for identification and quantification of corrosion related microorganisms, including iron oxidizing *zeta-proteobacteria* (FeOB), manganese oxidizing bacteria (MOB) such as *Leptothrix* sp., sulfate reducing bacteria (SRB) and sulfate oxidizing bacteria (SOB) [12, 13, 29]. At WET and CMB we have thorough experience of using qPCR for identification and quantification of gene targets in environmental samples [30, 31] [32].

#### **NEXT GENERATION SEQUENCING (NGS)**

Sequencing on the MiSeq Illumina platform will be performed at the Genomics core facility at the Sahlgrenska Academy. At WET we are presently using MiSeq for analyzing the V4 region of the 16S rRNA gene in multiple environmental samples (96 samples per run).

#### **FLUORESCENCE IN SITU HYBRIDIZATION AND CONFOCAL LASER SCANNING MICROSCOPY (FISH-CLSM)**

At the joint research group at WET and CMB, GU we have long tradition of using FISH-CLSM and digital image analysis, e.g. [26, 33]. Oligonucleotide FISH probes are available for a number of corrosion related microorganisms including SOB, SRB, FeOB and MOB, for details see ProbeBase [34]. For novel microorganisms, probes can be designed, as long as sequence information is available. CLSM will be performed at the Core center for Cellular Imaging at the Sahlgrenska Academy, where we have a long-standing collaboration. The method Gold-FISH will be set-up in association with Robert Almstrand,

Colorado School of Mines, where they recently have implemented this method for SEM of environmental samples.

#### **MICROELECTRODE MEASUREMENTS**

Measurements of gradients of pH, oxygen, H<sub>2</sub>S and redox will be carried out using Unisense microelectrodes (Unisense A/S, Denmark). At WET we have previous experience of microelectrode measurements, see e.g. [35], but have no access to equipment which can be operated in the field. Since microelectrode measurements need to be performed on the actual biofilm in the tunnels, we plan to purchase such equipment within this project.

#### **SCANNING ELECTRON MICROSCOPY (SEM)**

At WET we have used SEM for biofilm visualization in several projects, most recently on anodes and cathodes from microbial fuel cells, in association with the dept. of Chemical and Biological Engineering at Chalmers. SEM enables imaging of samples at their normal state, i.e. wet and uncoated and both organic and inorganic elements can be visualized at high resolution. To the extent necessary SEM data from Norwegian work may be included.

#### **THE NPRA DURABLE CONSTRUCTIONS SAMPLING PROGRAM**

Extensive sampling and analysis to evaluate the extent of corrosion at the Oslofjord tunnel test site will be performed during 2015, after 5 years of exposure. Besides analysis of water composition, pH, redox potential etc. analyses include sulfate, chloride, nitrogen species, TOC as well as a range of elements including heavy metals by ICP-MS. Moreover, exposed concrete test samples and concrete from sprayed concrete test fields will be tested for standard concrete properties. Samples of precipitates, concrete debris etc. will be analyzed by SEM and XRD as outlined in [5]. The PhD candidate at Chalmers will have full access to these results as well as other results from the R&D program “Durable constructions”.

#### **PLAN FOR SCIENTIFIC DELIVERABLES**

Results from the research project will be presented in international peer-reviewed journals and at international conferences.

#### **DESCRIPTION OF THE SOCIETAL VALUE OF THE RESEARCH**

In Norway there are more than 30 subsea tunnels and more are planned. In most of them, sprayed concrete is applied to increase safety. Also worldwide, many such tunnels exist. Building and maintenance of tunnels is very costly and deterioration of the sprayed concrete is therefore a major concern. The understanding of the degradation of steel reinforced concrete due to biofilm formation is very limited and increased knowledge in this area is therefore needed. Due to its complexity it is necessary to work multi-disciplinary, including studies of the microbial community structure by using advanced high throughput molecular techniques in combination with geochemical analysis and other tools such as microsensors to get a comprehensive picture of the prevailing conditions under different environmental loads. This will ultimately increase the understanding for how to prevent biofilm formation on the sprayed concrete surface and hence reduce the deterioration of the material.

The project will also contribute to the build-up of a strong research group within this area both in Norway and Sweden and it will also lead to the education of one PhD. Besides, master students will be recruited for the project as it requires extensive sampling.

## PLAN FOR COMMUNICATION WITH STAKEHOLDERS

To be able to increase knowledge among the stakeholders and end users, it is important to communicate the results from research projects performed at the university. The results will be continuously presented at the homepage for the Vegvesen-E39 project (under construction). Apart from this the results will be presented in different branch magazines both in Norway and Sweden. One example is the magazine *Byggeindustrien* where an article has been published this year about our planned project (Bakterier angriper sprøytebetong, nr 11, 2014). Seminars will also be held at Chalmers and at Statens Vegvesen (Norwegian Public Road Administration) in Norway.

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