Netherlands Enterprise Agency

Microbial Influenced Corrosion

Hollandse Kust (zuid) Wind Farm Zone

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Author: Fugro Engineers B.V.

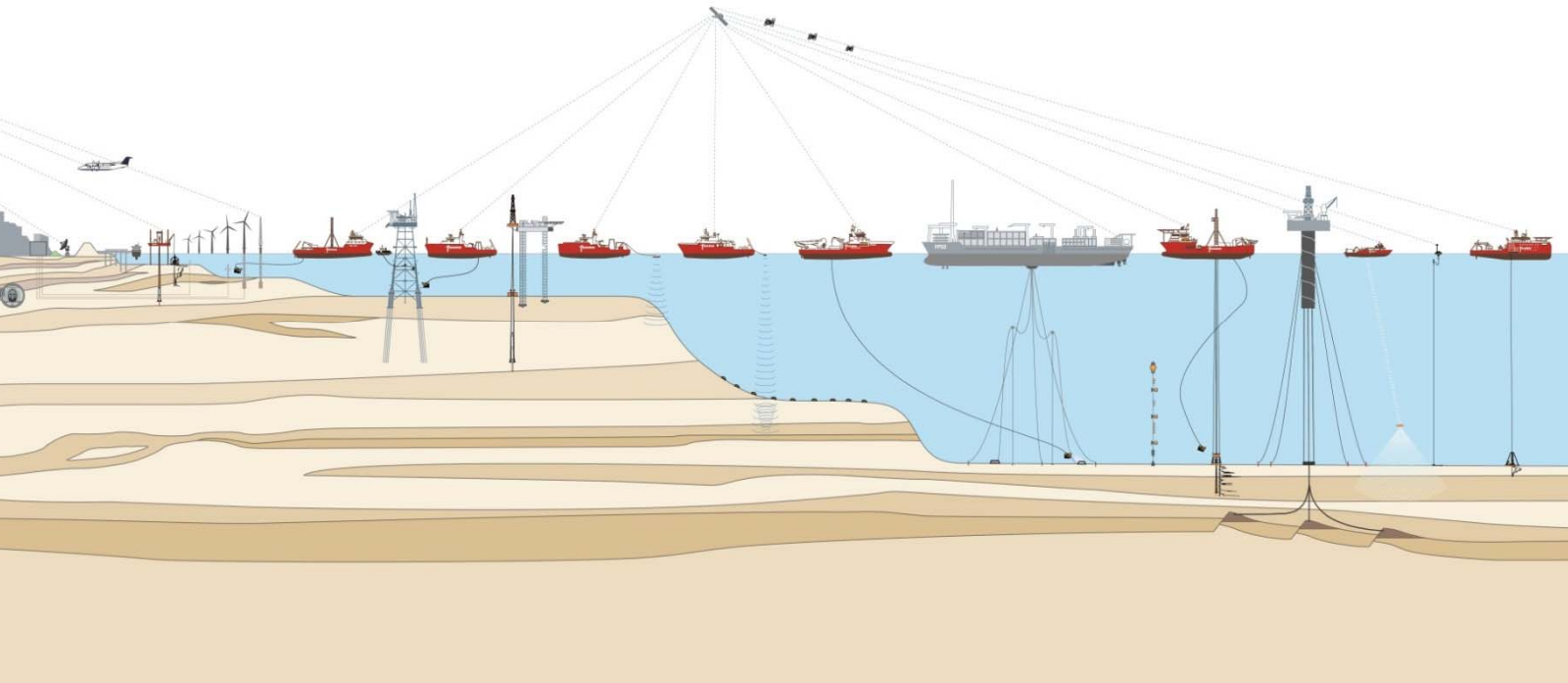
Technical Note

Microbial Influenced Corrosion
Hollandse Kust (zuid) Wind Farm Zone
Dutch Sector, North Sea

Client Reference WOZ1600020
Fugro Reference N6196/TN-MIC-2 (2)

Rijksdienst voor Ondernemend Nederland

Rijksdienst voor Ondernemend Nederland (RVO)



Technical Note Microbial Influenced Corrosion Hollandse Kust (zuid) Wind Farm Zone Dutch Sector, North Sea	
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1. PURPOSE AND SCOPE

This technical note considers marine soil conditions at the Hollandse Kust (zuid) Wind Farm Zone (WFZ) as environmental setting for microbial influenced corrosion (MIC) of steel foundations embedded in marine soil. The presented information is intended as input for assessment of environmental action(s) for durability limit states (ISO, 2008; ISO, 2015; DNV GL, 2016). It includes an assessment of possible MIC risk within the wider context of the Southern North Sea.

2. RESULTS

2.1 Key Results

The marine soil conditions at the Hollandse Kust (zuid) WFZ are assessed as benign (mild) for possible occurrence of MIC of steel foundations. The classification “benign (mild)” is based on

- The interpretation of results of a laboratory test programme performed on samples from the Hollandse Kust (zuid) WFZ;
- The soil profile in this WFZ, which is assessed as typical for the Southern North Sea.

It is recommended that service life prediction for MIC should be based on experience with existing steel structures in the Southern North Sea. ISO (2008) and DNV GL (2016) present general guidance. An alternative recommendation would be to perform site-specific in situ tests for qualitative and quantitative assessment of MIC, i.e. installation, keeping in-place and extraction of embedded steel coupons for monitoring of MIC. The in-place period should be one year or more.

The following sub-section provides important information for understanding and use of the key results. Section 3 provides supporting information.

2.2 Comments on Key Results

Comments on the key results are as follows:

- The future foundation structures considered for this technical note are driven piles, monopiles, and suction-installed caissons designed according to ISO (2015) or equivalent. Scour protection measures at and around a structure will be implemented. Each structure will be connected with one or more power cables embedded in the seabed.
- Current site conditions will change because of placement of scour protection measures at and around a structure. This technical note considers that any scour protection is such that the

environmental setting will remain largely unchanged from current free-field conditions. For example, the measures will not promote marine growth.

- Marine growth can lead to accumulation of organic material accelerating microbial growth, enhancing risk of MIC. However, in some cases, macro-fouling can delay corrosion and/or change a corrosion mechanism. Any protection effects will depend on the type of macro-fouling, chemistry, temperature setting and type of microorganisms.
- Any change of site conditions should be verified.

- This technical note subdivides the general environmental setting for MIC of a foundation structure as (1) active, (2) semi-active, and (3) static, broadly as function of depth below seafloor.
  - The active zone of the seabed is where a foundation is periodically exposed to seawater and subject to soil particle movement. Examples are any soil deposited and, possibly, subsequently eroded above the level of scour protection and any soil exposed by foundation-soil gapping below the level of scour protection. The gapping zone is probably extending to less than 2 m below scour protection (BSP). Generally, this zone shows highest microbial activity, both aerobic heterotrophic activity and sulphate reduction.
  - The semi-active zone shows significant groundwater movement around a foundation. For example, groundwater flow can be induced by temporary structure displacements that typically diminish with depth below seafloor, can be generated by wave pressure variations, and can be caused by pressure (currents) fields generated by large vessels. The semi-active zone is probably extending to less than about 10 m BSP.
  - No significant soil displacement and groundwater flow apply to the static zone.
  - The zone depths are indicative. They should be verified, if critical.
- The Hollandse Kust (zuid) WFZ shows sands up to depths varying between about 9 m below seafloor (BSF) and more than 50 m BSF. Organic soils and peaty zones are locally present below 10 m BSF (Fugro, 2016a to 2016f). This level (>10 m BSF) typically places these soil types in the static environmental zone. In this regard, it is noted that several areas of the Southern North Sea show organic soils and peaty zones within 5 m BSF.
- Results of a limited laboratory test programme (Appendix 1 to this technical note) provide some indication for potential of microbial communities within the active and semi-active zones.
  - The selected test methods (Total Bacteria, IRB, SRB, APB, SOB, IOB, and SFB) were for soil only, i.e. excluded a reference metallic object. Furthermore, measured microbial numbers represent functional groups of bacteria possibly involved in MIC. The results provide an indication of a microbial consortium for a sampling point at a specific time. Microbial numbers will change with time. Microbial numbers do not equal microbial rates and/or microbial activity. To the knowledge of the authors of this technical note, the relationship between microbial numbers and MIC has not been established in published scientific studies.
  - Detection limits are defined as 10 cells/g (MPN; all analyses), 90 cells/g (qPCR; IRB), 100 cells/g (qPCR; SRB and total bacteria). Refer to Appendix 1 for more details on methods of analysis.
  - Results of 13 of 17 tests on sand samples showed microbial numbers above detection limits. These 13 samples were taken between 2.5 m and 7.0 m BSF.
  - Results of two tests on (very) clayey sand samples showed all analysed microbial numbers as “not detectable”. These two samples were taken at 9.0 m and 9.2 m BSF.
  - Measured organic contents for the sands were below the laboratory detection limit. This limit is about 0.1 % by mass of dry soil.

- Results of one test on a clay sample showed all analysed microbial numbers as “not detectable”. This sample was taken at 9.0 m BSF. In this regard it is noted that clay and clay-like soils can be expected to show less microbial activity compared to sands, for otherwise equal organic content. This is because of reduced diffusion capability for water and nutrients (e.g. Wong et al., 2004).
- Detectable microbial numbers were found for one sample classified as peat, sampled at 14.4 m BSF. This is as expected for the type of soil. If organic content is high, also sulphate reduction rates and associated concentration of sulphide can be high, increasing MIC risk.

- The Hollandse Kust (zuid) WFZ is 18 km to 36 km from the Dutch coastline, in the Southern North Sea. This distance implies that discharge of major river systems will have limited influence on variation in seawater salinity, temperature, nutrients and pollutants, compared to sites closer to river mouths and coast.

3. SUPPORTING INFORMATION

3.1 Scope of Study Programme

The main stages of the study programme included:

- Laboratory test programme (including sample selection, sampling, sample handling and sample transport); this stage formed initially the only part of the study programme;
- Interpretation of laboratory test results;
- Assessment of Hollandse Kust (zuid) WFZ site setting, including environmental setting;
- Recommendations for service life prediction for MIC.

3.2 Laboratory Test Programme and Sample Selection

Appendix 1 includes an overview of samples used in the testing programme. Refer to Plate 1 for a spatial overview of borehole locations from which samples were taken.

Comments are as follows:

- RVO (Client) defined the test programme, including test types, offshore borehole locations and depths for sampling.
- Samples were taken during a geotechnical site investigation at Wind Farm Sites (WFS) III&IV of the Hollandse Kust (zuid) WFZ (Fugro, 2016c and 2016d).
- Testing took place in an office-based laboratory specialized in corrosion analysis and -prevention (Endures, the Netherlands).

3.3 Sampling and Sample Handling

Samples were collected using a push sampler equipped with an internal PVC liner. Refer to Fugro (2016c and 2016d) for more details about sampling methods used.

Important stages in sample handling included:

- Removing cutting shoe from sampling tube
- Removing PVC liner from sampling tube

- Removing excess liner using a saw
- Geotechnical description and classification of top and bottom of sample
- Sealing top and bottom of liner using wax
- Closing top and bottom of liner using caps and tape
- Sample packaging, labelling and vertical storage in a temperature-controlled container on-site (temperature regulated between 1 °C and 4 °C; protection from direct sunlight)
- Off-loading of sample container from drilling vessel at port of demobilisation (IJmuiden, the Netherlands)
- Temperature-controlled road freight to specialized laboratory of Endures (Den Helder, the Netherlands)
- Removing tape, caps and wax from liner samples
- Sub-sampling from the centre part of a liner as soon as possible after arrival of liner samples in the laboratory
- Preparing sub-samples for the various MIC-related analyses
- Packaging and labelling of left-over sample material for disturbed preservation
- Transport of left-over material in labelled shipping containers to the Fugro geotechnical laboratory in Wallingford (UK)

The time between sampling and testing was less than 14 days.

3.4 Microorganisms

MIC is caused by the presence and activities of microorganisms, including bacteria and fungi. The extent of MIC depends on environmental setting, soil type(s), microorganisms and the material exposed to corrosion (Peabody, 2001; Lewandowski and Beyenal, 2009).

The presence of a higher content of organic material can favour the activity of APB and SRB. Measurements of organic content and sulphate-reduction rate of soil are important.

Microorganisms are present almost everywhere in soil, freshwater, seawater and air. This means that defining quantities of microorganisms in water or soil is not enough for prediction of MIC risk. It is important to (1) assess the interaction between microorganisms and metallic structures, (2) demonstrate that the presence of microorganisms accelerates the corrosion process, and (3) exclude other corrosion mechanisms from the analyses. Measurement of pH and sulphide concentration can be helpful: high pH generally means protective (unless concomitantly with high sulphide concentration) and high sulphide concentration generally means higher MIC risk.

Corrosion is an electrochemical process, whereby both electron donor and electron acceptor mechanisms influence the corrosion rate. Strong correlation applies between microbial activity and availability of electron donors and electron acceptors. Metabolic activity of microorganisms is based on oxidation and reduction reactions. Aerobic organisms are using oxygen as terminal electron acceptor, whereas anaerobic organisms use several other compounds such as nitrate, manganese, iron or sulphate as electron acceptors for microbial respiration. Organic and inorganic material (e.g. hydrogen, hydrogen sulphide) can be utilised as energy source. Electron transport in microorganisms is a complex process, especially because different groups can vary in their use of electron acceptors in absence of oxygen or at different oxygen levels such as SRB (Borenstein, 1994).

Microorganisms have different metabolisms. They can convert different substrates and subsequently supply other groups of bacteria with the substrate they need in order to survive. An example for this is the sulphur cycle where SRB and SOB/TOB are active. Under anaerobic conditions, SRB reduce sulphate to hydrogen sulphide. Through chemical oxidation of H_2S, sulphur compounds are released which then become available for SOB/TOB. These groups of microorganisms are able to oxidize thiosulfate and elemental sulphur to sulphate which can result in sulfuric acid and, subsequent, metallic corrosion. Sulphate serves also as a substrate for SRB, which convert sulphate to hydrogen sulphide. A closed sulphur cycle is present if both groups of organisms are active. This setting can accelerate corrosion processes in the active zone where significant oxygenation can be expected.

Fermenting bacteria (acid producing bacteria) convert larger organic compounds to smaller compounds, such as organic acids, which can be further converted to e.g. SRB. If, due to the interaction of water and metal, molecular hydrogen is accumulating on an unprotected metal surface, SRB can further oxidize the hydrogen and produce the corrosive agent hydrogen sulphide for soils having significant organic content.

It is reported that some SRB use only iron, lactate or pyruvate for the reduction of sulphate. Reduction becomes slower in the presence of hydrogen sulphate. There are also SRB that use electrons directly from the metal surface without involvement of hydrogen to feed their sulphate- reducing system (Hang, 2003; Enning et al., 2012). This can accelerate corrosion processes, as de-polarisers in the medium are readily available, which in turn will enhance oxidation reactions (Ox of Fe). It is potentially interesting to try to detect the presence of these specific sulphate-reducing bacteria.

3.5 Environmental Setting Influencing MIC

Fugro (2016a to 2016f) present a general environmental setting for the Hollandse Kust (zuid) WFZ. This setting is expected to show some change with time due to external factors, including continuing expansion of regional infrastructure (including multiple wind farm developments) and climate change.

Water depths are between approximately 16 m and 28 m reduced to Lowest Astronomical Tide (LAT). This implies significant influence of (storm) wave action on the seabed.

Seawater is influenced by currents through the English Channel, with an average salinity >35 g/l and a temperature range of about 6 °C to 18 °C. Seawater salinity, temperature, nutrients and pollutants are also influenced by discharge of major river systems, such as the Rhine and Meuse. This implies potential for a wide variety of microbial activity.

The seabed at the Hollandse Kust (zuid) WFZ shows predominantly sand to at least 9 m BSF. The upper part of these sand strata is currently mobile, i.e. subject to reworking and displacement as a result of metocean conditions. This setting can enhance microbial activity.

The active zone is expected to show aerobic as well as anaerobic microbial activity. Sulphate reduction is expected to occur. No laboratory test results are available for this zone.

Analyses for microbial numbers on samples from deeper layers (approximately 3 m to 7 m BSF) only showed activity by anaerobic microorganisms. This depth range probably corresponds with the semi-

active zone in which sulphate-reducing and acid-producing processes can take place, whereas analysed samples from about 9 m BSF showed no detectable microbial activity.

An “indirect” indication for the presence of oxygen can be derived from the presence of identified species which need oxygen for their metabolism. Nevertheless, some microorganisms, which are categorized as aerobic microorganisms, have the capability of using other electron acceptors when oxygen is not present and can then survive. This means that detection of aerobic microorganisms in specific layers can only be used as an indirect indicator for the presence of oxygen and not as hard evidence that aerobic MIC or corrosion will take place.

4. ABBREVIATIONS

APB	Acid-producing bacteria
BSF	Below seafloor
BSP	Below scour protection
IOB	Iron-oxidizing bacteria
IRB	Iron-reducing bacteria
LAT	Lowest Astronomical Tide
MIC	Microbial influenced corrosion
MPN	Most probable number
qPCR	Quantitative polymerase chain reaction
SFB	Slime-forming bacteria
SOB	Sulfur-oxidizing bacteria
SRB	Sulfate-reducing bacteria
TOB	Thiosulfate-oxidizing bacteria
WFS	Wind farm site
WFZ	Wind farm zone

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6. USE OF THIS TECHNICAL NOTE

Fugro Engineers B.V. prepared this Technical Note according to a project specification determined by the Client. The content of this document is endorsed and reviewed by experts from Delft University of Technology (Faculty of Civil Engineering and Geosciences, Section of Materials and Environment).

Fugro understands that the presented information will be used for the purpose described above. That purpose was a significant factor in determining the scope and level of the services. If the purpose for which the presented information is used or the Client's proposed development or activity changes, this Technical Note may no longer be valid.

Document distribution is restricted to project participants approved by the Client.

This document supersedes Fugro (2016g). Furthermore, this document is supplementary to and must be read in conjunction with Fugro (2016a to 2016f) listed in the section titled 'References' of this Technical Note. The terms and conditions applicable to the referenced documents also apply to this Technical Note.

This document contains 21 pages and plates (including Appendix 1), the definitive version of which is held in Fugro's information system.

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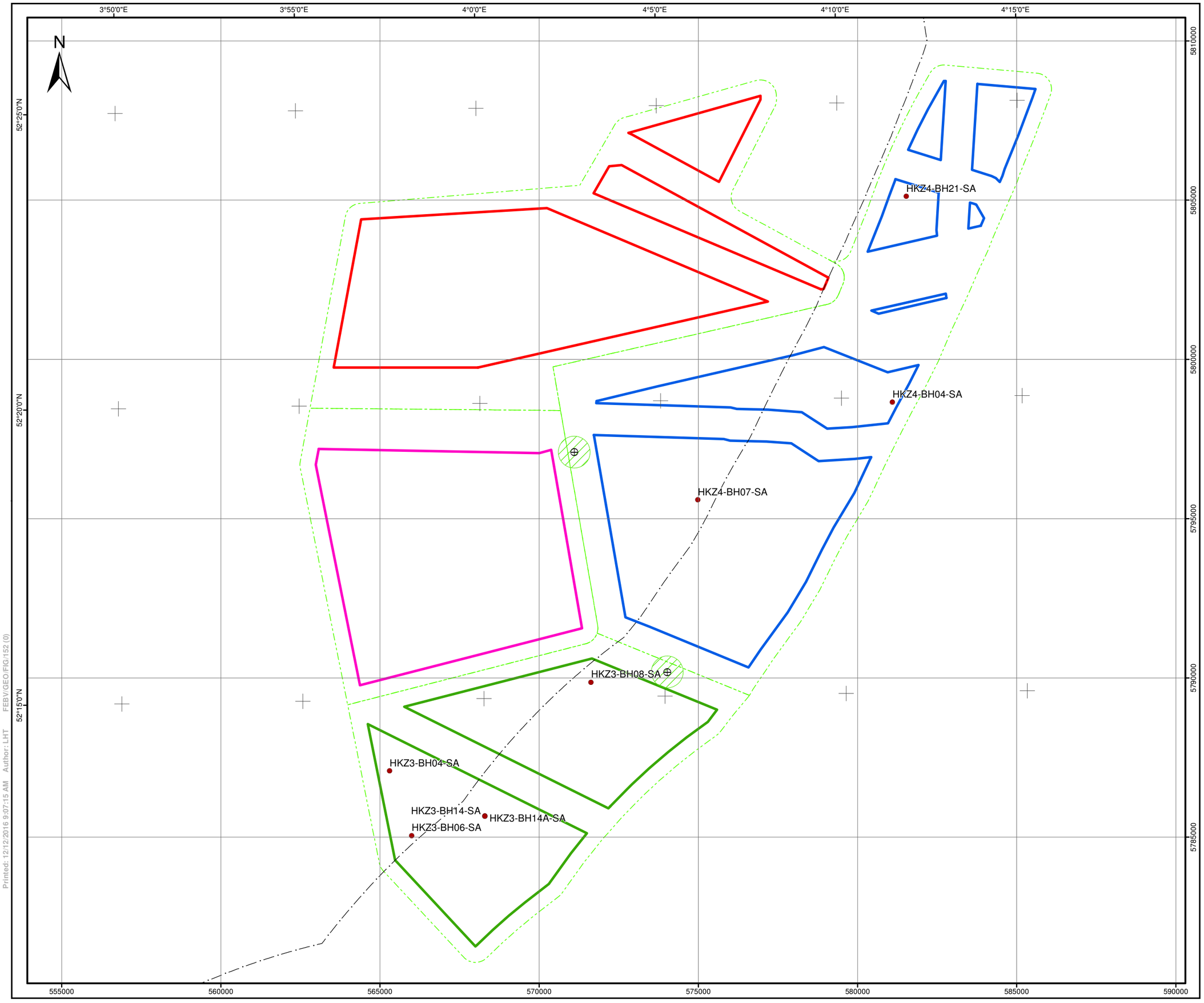


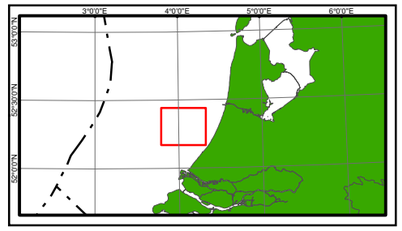
LEGEND:

- Borehole Location - Downhole Geotechnical
- 12 Nautical Mile Boundary
- Proposed Platform Location
- Outline Investigation Area
- Proposed Platform Exclusion Zone
- Wind Farm Site I
- Wind Farm Site II
- Wind Farm Site III
- Wind Farm Site IV

NOTES:

GEODETIC PARAMETERS:

DATUM	ETRS89
Ellipsoid	GRS80
Semi major axis	a = 6 378 137.000
Inverse flattening	1/f = 298.257222101
PROJECTION	UTM, Zone 31 North
Central Meridian (CM)	3° 00' 00" E
Latitude of Origin	0° 00' 00" N
False Easting	500 000 m
False Northing	000 000 m
Scale factor	0.9996
Units	metres / degrees



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DETAILED LOCATION PLAN

HOLLANDSE KUST (ZUID) WFZ
DUTCH SECTOR, NORTH SEA

Scale 1:110,000
at original A3 page size

0 1,000 2,000 3,000 4,000 metres

Fugro Report No. N6196/TN-MIC-2	Issue 2	Plate 1

APPENDIX 1: LABORATORY TEST REPORT

CONTENTS Reference

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Microbial influenced corrosion related sediment analysis - Hollandse Kust (zuid) Wind Farm Zone

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Sponsor	Fugro Engineers B.V. Prismastraat 4 2631 RT Nootdorp Netherlands
Project name	MIC sediment analysis
Project number	PROJ16066

Contents

1 Abbreviations

APB	Acid-producing bacteria
IOB	Iron-oxidizing bacteria
IRB	Iron-reducing bacteria
SFB	Slime-forming bacteria
SOB	Sulfur-oxidizing bacteria
SRB	Sulfate-reducing bacteria
MIC	Microbial influenced corrosion
MPN	Most probable number
qPCR	Quantitative polymerase chain reaction

2 Introduction

This report presents factual results of laboratory microbiological analyses of sediments collected from Wind Farm Sites III and IV of the Hollandse Kust (zuid) Wind Farm Zone.

The purpose of the laboratory test data is to provide input for microbial influenced corrosion (MIC) of steel foundations embedded in marine soil.

3 Materials and methods

3.1 Sample collection

Seventeen samples for MIC-related analyses were collected by Fugro using liner samples during a geotechnical drilling campaign. Liners were transported to the ENDURES laboratory under refrigerated conditions and pre-sampled. Sampling and sample handling procedures, including sample transport, have been performed in accordance with protocols designed jointly by Fugro and ENDURES.

An overview of all samples provided for analyses is presented in Table 1.

Table 1 Overview of samples provided for MIC-related analyses

Endures Sample ID	Location	Fugro Sample ID	Depth [m BSF]*	Date of Sampling	Date of Delivery to Laboratory
1	HKZ3-BH04-SA	16	9.00	18-Aug-2016	22-Aug-2016
2	HKZ3-BH06-SA	19	9.00	16-Aug-2016	22-Aug-2016
3	HKZ3-BH06-SA	19	9.20	16-Aug-2016	22-Aug-2016
4	HKZ3-BH08-SA	04	2.50	01-Sep-2016	09-Sep-2016
5	HKZ3-BH08-SA	08	5.00	01-Sep-2016	09-Sep-2016
6	HKZ3-BH08-SA	11	7.00	01-Sep-2016	09-Sep-2016
7	HKZ3-BH14-SA	06	3.00	17-Aug-2016	22-Aug-2016
8	HKZ3-BH14-SA	09	5.00	17-Aug-2016	22-Aug-2016
9	HKZ3-BH14-SA	13	7.00	17-Aug-2016	22-Aug-2016
10	HKZ3-BH14A-SA	01	5.00	17-Aug-2016	22-Aug-2016
11	HKZ4-BH04-SA	07	3.00	05-Sep-2016	09-Sep-2016
12	HKZ4-BH04-SA	10	5.00	05-Sep-2016	09-Sep-2016
13	HKZ4-BH04-SA	14	7.00	05-Sep-2016	09-Sep-2016
14	HKZ4-BH07-SA	22	14.40	08-Sep-2016	09-Sep-2016
15	HKZ4-BH21-SA	04	3.00	07-Sep-2016	09-Sep-2016
16	HKZ4-BH21-SA	06	5.00	07-Sep-2016	09-Sep-2016
17	HKZ4-BH21-SA	09	7.00	07-Sep-2016	09-Sep-2016
*BSF: below seafloor					

3.2 MPNs

Presence and activity of MIC related microorganisms were determined using the Most Probable Number (MPN) method. Sediment samples taken from liners were inoculated and diluted in appropriate growth media, specific for the following types of microorganisms: Iron-oxidizing microorganisms (IOB), iron-reducing microorganisms (IRB), sulfur-oxidizing microorganisms (SOB) and sulfate-reducing bacteria (SRB), acid-producing bacteria (APB) and slime-forming bacteria (SFB).

3.3 Quantitative real-time polymerase chain reaction (qPCR)

These are genetic level-based techniques which can provide information on microbial populations without the need of cultivation. The method is based on the amplification of target DNA fragments which are unique of certain microbial

species or microbial processes (e.g. sulfate reduction). The main difference with classical PCR is that in this technique it is only possible to assess the end product of the reaction which does not distinguish well the quantity of the target sequence amplified. In contrast, in qPCR the amplification product can be quantified in every cycle of the amplification process. This provides quantitative information of the presence of the target organism or process.

The same samples as for MPNs were analysed by qPCR for three target organisms/activities: total bacteria, sulfate reducing activity and iron reducing bacteria.

4 Comments on Results

Results of culture-based methods (MPN) and molecular-based methods (qPCR) are summarized in Table 2. Colours used in the table indicate samples from one corresponding borehole.

IRB, SRB and APB are anaerobic microorganisms and highlighted in red in Table 2, to point out their possible relevance for MIC in sediment samples especially for deeper layer samples in which oxygen is limited or not present. SOB, IOB and SFB are aerobic microorganisms and to be expected in environments in which oxygen is present (first metres below seafloor).

SRB are traditionally implicated in accelerated corrosion by the production of H_2S whereas IRB destabilize iron oxides, altering in this way abiotic corrosion processes. APB create acidic microenvironments under anaerobic conditions due to acid production as consequence of the conversion of organic compounds.

SOB are participating (together with SRB) on the cycling of sulfur in natural environments. They can affect corrosion either by acid production or by their interaction with SRBs, especially in intermittently aerated environments. IOB are implicated in corrosion, especially by acidification processes and can interact with IRB on the iron cycle.

SFB are actively consuming oxygen and producing high amounts of slime. SFB are aerobic microorganisms which are widespread in nature. Although not directly involved in corrosion processes, SFB have an important role on the development of anaerobic conditions. Anaerobic conditions under biofilms of aerobic bacteria allow the survival and activity of APB, IRB and SRB. Therefore, SFB are important indicators.

qPCR results for SRB show higher numbers of microorganisms per gram sediment as compared to MPNs (growth culture-based methods). The reason for this could be that some microorganisms resist cultivation using standard methods.

IRB were positive via the MPN method but not detectable via qPCR (*Geobacter primer*) which can be explained by the use of the very specific DNA primer for this group of microorganisms.

Total bacteria numbers are in some cases lower as the positive result for SRB (q-PCR detection) which can be explained by the fact that SRB can be part of the species bacteria or archaea. In the present test we determined only bacteria and no archaea.

Microbial activity was mainly detected in the sediment up to 7 m of the sampled locations. In these sediments, SRB and APB were regularly found in moderate numbers except of borehole HKZ4-BH21-SA where only APB were detected. IRB were only detected in borehole HKZ4-BH04-SA but in low numbers.

IOB were not present in the tested samples whereas SOB gave a positive signal in sample 7, 8, 10, 11 and 12 from borehole HKZ3-BH14(A)-SA and HKZ4-BH04-SA. Although, SOB gave a positive signal, they were not quantifiable.

Samples from deeper sediments below 9m were not microbiologically active. This was confirmed by qPCR. DNA was not extractable from deeper samples that mainly had a clay-like sediment structure.

The only exception for a positive signal in microbial activity for deeper layer samples is sample number 14 (HKZ4-BH07-SA). This sample originates from 14.4 m depth and showed activity for SRB, IRB and APB. This sample had a peat like structure and was different compared to all other sediment samples which had a sand- or clay-like structure.

Table 2. Determination of MIC-relevant microorganisms from the Wind Farm Sites III and IV of the Hollandse Kust (zuid) wind Farm Zone. Colours indicate samples from one corresponding borehole. IRB, APB and SRB are highlighted in red to point out their possible relevance for MIC.

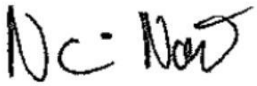
				Type of microorganism or metabolism					
No	**Borehole name**	**Depth [m]**	**Total bacteria [cells/g] (qPCR)**	**IRB [cells/g] (MPN/ qPCR)**	**SRB [cells/g] (MPN/ qPCR)**	**APB [cells/g] (MPN)**	**SOB [cells/g] (MPN)**	**IOB [cells/g] (MPN)**	**SFB [cells/g] (MPN)**
1	HKZ3-BH04-SA	9.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	HKZ3-BH06-SA	9.00	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	HKZ3-BH06-SA	9.20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	HKZ3-BH08-SA	2.50	n.d.	n.d.	2E+02/ 6E+02	1E+02	n.d.	n.d.	n.d.
5	HKZ3-BH08-SA	5.00	n.d.	n.d.	n.d./ 2E+02	1E+02	n.d.	n.d.	n.d.
6	HKZ3-BH08-SA	7.00	1E+04	n.d.	n.d./ 2E+02	n.d.	n.d.	n.d.	n.d.
7	HKZ3-BH14-SA	3.00	9E+04	n.d.	6E+02	1E+02	+	n.d.	2E+03
8	HKZ3-BH14-SA	5.00	8E+04	n.d.	1E+02/ 6E+03	1E+02	+	n.d.	n.d.
9	HKZ3-BH14-SA	7.00	5E+03	n.d.	2E+02/ 3E+04	n.d.	n.d.	n.d.	n.d.
10	HKZ3-BH14A-SA	5.00	3E+02	n.d.	1E+02/ 9E+03	1E+02	+	n.d.	n.d.
11	HKZ4-BH04-SA	3.00	2E+03	n.d.	n.d.	9E+01	+	n.d.	1E+02
12	HKZ4-BH04-SA	5.00	1E+04	9E+01 /n.d.	1E+02/ 1E+03	1E+02	+	n.d.	n.d.
13	HKZ4-BH04-SA	7.00	3E+05	9E+01 /n.d.	1E+02/ 4E+03	1E+02	n.d.	n.d.	n.d.
14	HKZ4-BH07-SA	14.40	3E+05	1E+02 /n.d.	2E+02/ 3E+05	1E+03	n.d.	n.d.	n.d.
15	HKZ4-BH21-SA	3.00	n.d.	n.d.	n.d.	9E+01	n.d.	n.d.	n.d.
16	HKZ4-BH21-SA	5.00	n.d.	n.d.	n.d.	9E+01	n.d.	n.d.	n.d.
17	HKZ4-BH21-SA	7.00	5E+03	n.d.	n.d.	1E+02	n.d.	n.d.	n.d.
		n.d. = not detectable, in the case for IRB and SRB if only one result is given it is applicable for both MPN and qPCR; + = positive but not quantifiable							

5 Signature

Den Helder, May 16th, 2017

Mr. S.J. Buter
Managing Director of ENDURES B.V.

ENDURES B.V.



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