Microbiology in geothermal operations

Sabine Doddema[1], Floris Veeger[2], Elsemiek Croese[1]

[1] Microbial Analysis, Rozenburglaan 13, 9727 DL, Groningen, The Netherlands

[2] Veegeo bv Noordsingel 117, 3035 EM, Rotterdam, The Netherlands

doddema@bioclear.nl

Keywords: Low Enthalpy Geothermal Operations, Obstruction; MIC; QPCR; Next Generation Sequencing (NGS); MMM; Microorganisms; biocide.

ABSTRACT

A geothermal system faced serious injection problems due to obstruction two-and-a-half months after start-up. The obstruction was so severe that the operation was suspended and research was done in order to determine the cause. To solve the obstruction problem the system was treated downhole using acid and biocide. During this treatment dangerous amounts of H_2S were released. This study was initiated to understand the reason behind the obstruction and its treatment observations and to give options for treatment and prevention. The results show high amounts of bacteria in the obstructing material indicating that microorganisms caused the obstruction. The detected species were typical thermophilic species with optimal growth temperature at 40-60 ºC. The change in temperature, in combination with the used oxygen scavenger containing nutrients ideal for microbial growth and activity, is suspected to have caused microbial growth and thus obstruction. The H_2S formation was likely caused by a combination of biological FeS formation and the addition of acid during the cleaning operation. To prevent similar problems, treatments options were investigated. In conclusion biocide treatment is recommended, not only during but also before production is started. This strategy was applied to the injection pipe, the long term effect of this strategy was evaluated after two years.

A follow up study in similar systems in the Netherlands showed that various types of bacteria are present in the production water. The detected species could cause a variety of microbiology related problems including Microbiologically Influenced Corrosion (MIC) and degradation of corrosion inhibitor. The gathered information was used to assess the risks related with microbiology in geothermal operations.

1. INTRODUCTION

A geothermic system in the Netherlands faced serious injection obstruction problems two-and-a-half months after start-up. The obstruction was so severe that the operation was suspended and research was done in order to determine the cause. The conclusion was that the obstruction was caused by a combination of slime forming bacteria and iron and copper oxides/sulphides (possibly formed by bacteria). To solve the obstruction problem the system was treated downhole using acid and biocide. During this treatment dangerous amounts of H_2S were released. The hypothesis is that H_2S was formed by the activity of (sulphate reducing) bacteria and/or by the chemical reaction during the acid treatment. Due to QHSE (Quality, Health, Safety and Environment) regulations and lack of knowledge about what has happened in this system, there is the need to understand which processes have taken place and what can be expected in the future with regard to obstruction issues, H_2S formation and Microbiologically Influenced Corrosion (MIC). To understand the issues better, the microbiology in the injection well was identified and interesting bacterial targets were further used to quantify its activity. To prevent microbiology related issue in the future, biocide was dosed once monthly in the injection well with the aim to prevent microbiological growth. However, after several months the injection pressure started increasing again slowly. Although the injection pressure was well within the production requirements, after more than two years of operation, additional tests in the same but also other geothermal systems were done and further research was performed to understand the reason for this increase in pressure and to get a better understanding and control of risks related to microbiology and MIC threat based on the detected microbiology. This article described the results of this study.

2. METHODS

2.1 Geothermal System

The main geothermal system that was investigated for this study was a system addressing an aquifer in the Triassic sediments [Hardegsen, Detfurth, Volpriehausen sandstones] with a temperature of approximately 80-84 ºC. At injection the temperature can be as low as 30 ºC. The wells were approximately 2.5 km deep. The reservoir fluid is a highly saline brine with a dissolved gas ratio of 1 Sm3/m3 at surface consisting of >90%mol methane and ~4%mol of CO_2.

The wells for this geothermal system were constructed in the course of 2015-2016. The relevant steps of installation until obstruction and H_2S detection were as follows:

1) Water is pumped to the surface and stored in open basins.
2) Oxygen scavenger (sodium bisulphite) is added to the water before reinjection.
3) Water is pumped back into the pipes after finishing their installation.
4) The construction of the above ground installation is finished.
5) Approximately one month after reinjection of the water, the operation is started.
6) Obstruction increased gradually over the first 8 weeks of operation.
7) Samples and analysis of obstruction material show very high solubility in HCl.
8) HCl is injected and the flowback contains more than 150 ppm H_2S.

After cleanout, glutaraldehyde based biocide was injected once a month for 24h, at 100 ppm. In addition to the use of a biocide the system was also treated using antiscalent – polycarboxylic acid based (pH 1.2-2.5), CO_2 - to compensate the CO_2 that is released when water gets above ground - and a corrosion inhibitor containing Bezyl C12 C16Alkyl-Dimethyl Ammonium Chloride; Talloil; DETA and Imidazoline Acetates. All was injected in the injection well except the corrosion inhibitor which was dosed into the production well and has been proved to enter the injection well too. With this treatment the system operated stable with a minor increase of injection pressure over 2 years. In 2018 additional research was performed and samples were collected.

2.2 Sample Collection

Samples for microbiological analysis were collected after the HCl cleanout from sludge from a bailer run in the injection well and from the injection and production water from the geothermal system in 2016. And additional samples were collected from scraper and bailer sludge from a bailer run performed in 2018 (see table 1). Furthermore were samples collected from production water of five additional geothermal systems in the Netherlands, addressing diverse geothermal earth layers (see table 2). All samples were conserved for microbial analysis directly when collected.

Table 1 Samples for QPCR on DNA and RNA.

Sample code	Sample type	Sampling date	Target group	Sample type
001A	Sludge from Pump Bailer run	May 2016	DNA and RNA	Solid
002A	Production water	May 2016	DNA and RNA	Liquid
003A	Injection water	May 2016	DNA and RNA	Liquid
001B	Bailer	August 2018	DNA	Solid
002B	Scraper	August 2018	DNA	Solid

Table 2: Samples used for identification analyses

Sample code	Sample type	Sampling month	Earth layer
001 (=002A)	Production water	June 2017	Triassic sandstone [bunter]
002	Production water	January 2018	Westlands Delft sandstone
003	Production water	Januari 2018	Rotliegend sandstone Heemskerk
004	Production water	Januari 2018	Westlands Delft sandstone
005	Production water	February 2018	Rotliegend sandstone
006	Production water	February 2018	Combination of Dinantien limestone and Condroz sandstone

From all samples DNA was extracted for QPCR. From the initial obstruction samples (001A) and from the water samples (002A and 003A) also RNA was extracted. QPCR was performed on DNA and RNA for those samples. The extracted DNA from sample 001A and 002A and samples 001-006 were also used for identification of the bacteria using Next Generation Sequencing (NGS).

2.3 Next Generation Sequencing (NGS)

To be able to get a better understanding of the origin of the obstruction material, the bacterial species that were present in the obstruction material and in the production water were identified using 16S bacterial Next Generation Sequencing (NGS) and in-house annotation software. To get a better understanding of the diversity of species present in production waters of different geothermal systems, additional samples from production water from other geothermal systems were analysed as well using this identification technique. The used approach consists of a series of steps that are specifically designed to access as much biological information as possible.

2.4 Quantitative PCR (QPCR)

Quantitative PCR (QPCR) was used to be able to quantitatively compare the different samples. QPCR can target specific predefined targets. QPCR was performed according to Heid at al. (1996) targeting total bacteria, archaea, *Halanaerobium*, *Desulfotomaculum* and sulphate reducing bacteria. The species specific analysis were designed based on the sequencing results from the obstruction material (sample 001A) and the most detected species in this sample. Analysis were performed on DNA (measuring living and dead cells) and RNA (measuring only active cells) which was extracted using a commercially available method.

2.5 Mineral Analysis

To understand the composition of the obstruction material better, X-ray diffraction (XRD) analysis was performed on the sample from the initial obstruction material (001A).

3. RESULTS

3.1 Identification of Microorganisms in Obstruction Material (NGS)

Of all identified species, a large portion matched with unclassified or uncultured bacteria. This is a common image of natural samples, however makes it difficult to deduce any functional information. To overcome this, the most related species is mentioned as results for those uncultured and unclassified species. This makes it possible to interpret the results since it provides information about the probable function of microbial species.

Figure 1 shows a circular diagram of the identified species in the initial obstruction scraper material from the geothermal system. The most prominent bacterial species that were detected are *Desulfotomaculum* species and *Halanaerobium* species. *Desulfotomaculum* is a sulphate reducing species which can reduce sulphate to hydrogen sulphide (Goorissen et al., 2003). These species are presumed to be the species responsible for hydrogen suphide formation in the injection well. They can form spores and are therefore able to survive harsh conditions such as high temperatures. Furthermore *Desulfotomaculum* species are able to utilize several carbon sources as carbon and energy source but are also able to grow autotrophically using carbon dioxide, which is present in the system. *Halanaerobium* species are anaerobic species which can grow under high salt concentrations (Booker et al., 2017). Some of this species are also able to reduce sulphate to hydrogen sulphide.

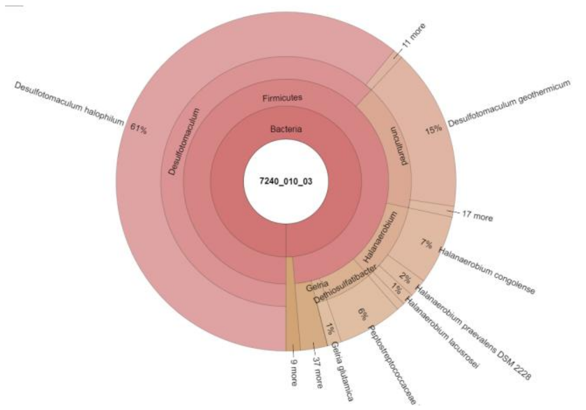

Figure 1: identified species in initial obstruction material

3.2 Identification of microorganisms in the production water (NGS)

To get a better understanding of the threat for microbiological issues and the origin of the detected microorganisms also samples were collected from the production water. Figure 2 shows the results of this identification.

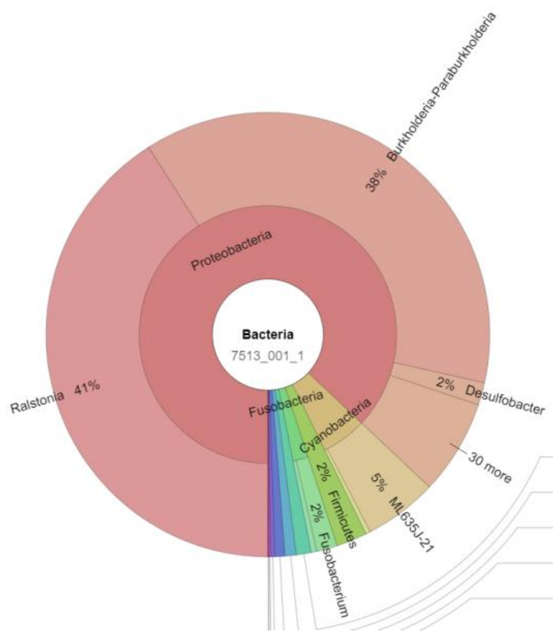

Figure 2: identified species from production water

The main identified species was *Ralstonia* which have been described to survive in environments with high concentration of metals and low concentration of nutrients. *Ralstonia* is a common species found in groundwater samples. This species is also identified in biofilms. It has been suggested that this species might be protective against corrosion fatigue, however solid prove of this has not been reported. Another well represented species in the production water smaple was *Burkholderia*. *Burkholderia* have been described as exopolymer-producing bacteria. Exopolymers have a slimy structure and are produced by bacteria that grow in a biofilm or aggregates. One of the functions of these exopolymers is to maintain nutrients in local conditions (prevent flushing away of the nutrients) which are therefore favourable for the microbe. This can include metabolic products such as acids which are influencing corrosion. Furthermore it has been reported that some *Burkholderia* could influence corrosion of metals (Simon and Kroneck, 2013). In addition to those species, the production water also contained several species (4% of total) which use sulphur related compounds (such as sulphate, sulphide, sulphite etc) for their energy gain. *Desulfotomaculum* and *Halanaerobium* species were also detected in the production water but in minimal relative amounts compared to the obstruction material.

Since a significant fraction of the identified species were sulphur metabolizing bacteria, there is a threat for growth and development of those species in the geothermal systems analysed here. Changes in conditions like addition of components or changes in temperature (which is inevitable for geothermal operations), can trigger growth of specific species and shifts in relative percentages of species.

3.2 Quantification of microorganisms in obstruction material and water (QPCR)

Based on the identification results QPCR analysis were developed for the most prominent bacterial species: *Desulfotomaculum* species and *Halanaerobium* species because those species can be directly related to H_2S formation and MIC. These analysis were performed on DNA and RNA. The unit for detection limits and analysis results is number of cells per millilitre (N/ml) cells or per gram (N/g), based on the assumption that 1 DNA- or RNA-copy is equal to 1 cell.

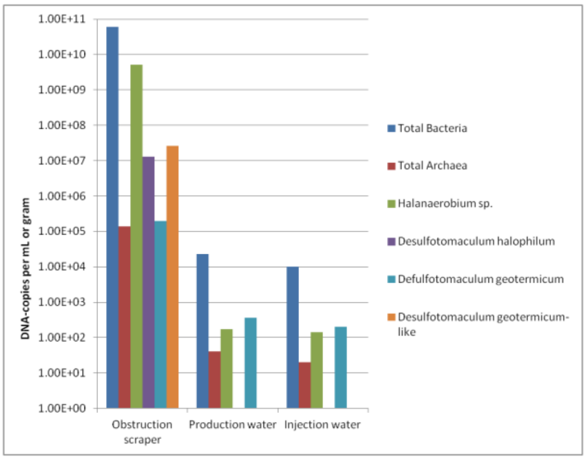

Figure 3 DNA QPCR results (presence)

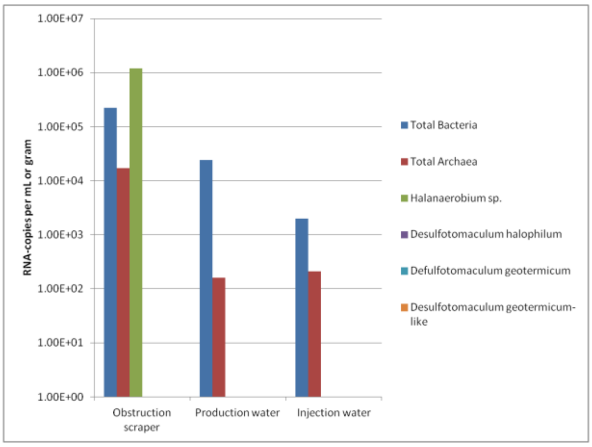

Figure 4 RNA QPCR results (activity)

The results of the QPCR analysis showed that much more (about 1000000 times more) bacteria were present in the obstruction material than in the water samples. Also some of the *Desulfotomaculum* species were only detected in the obstruction material and not in the water samples. The RNA analysis showed that also more bacteria were active in the obstruction material than in the water samples and that specifically *Halanaerobium* sp. were active in the obstruction material. The reason that no activity of *Desulfotomaculum* species was detected in the obstruction material could be due to the high detection limit for this sample.

3.3 Identification of microorganisms in different geothermal systems

The results of the identification of the bacteria in different geothermal productionwater samples (Figure 5) shows a diversity in the main detected species from the different geothermal operations. Remarkable is that there seems to be some similarity in sample 001 and 002, sample 003 and 004 and sample 005 and 006 (see figure 5), however, no clear correlation has been found between those sample couples. They originated from varying earth layers and also on the operational side no clear correlation was found. The main detected species in sample 001 and 002 was *Burkholderia* and *Ralstonia*; the main detected species in sample 003 and 004 was *Delftia*; and the main detected species in sample 005 and 006 was *Stenotrophomonas*.

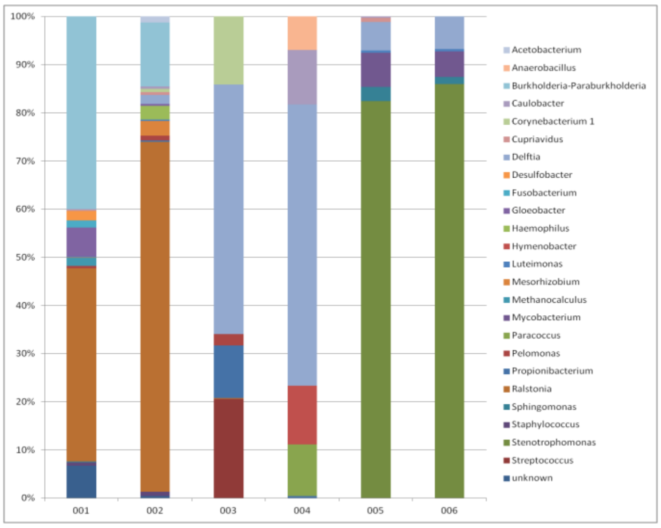

Figure 5 Identified species in production water of six different geothermal systems.

As described above *Burkholderia* have been described as exopolymer-producing bacteria and it has been reported that some *Burkholderia* could influence corrosion of metals. *Ralstonia* have been described to survive in environments with high concentration of metals and low concentration of nutrients. This species is also identified in biofilms. *Delftia* has been described to be involved in MIC before (Duncan *et al*., 2013) and can negatively affect the corrosion process of steel pipelines, leading to higher corrosion rates. It has been suggested that *Delftia* species can potentially degrade corrosion inhibitors (San et al. 2012). *Stenotrophomonas* has been described as aerobic bacterium (growing on oxygen). Oxygen is not expected to be present in geothermal systems, however, related species have also been isolated before from geothermal waters, which indicate that not all *Stenotrophomonas* species are aerobic. Furthermore are *Stenotrophomonas* species also able to form biofilms. Interestingly both *Burkholderia*, *Delftia* and *Paracoccus* (also found in sample 004), have been described to have sulfonate degrading capacity which is part of sulphite detoxification capacity, hence, they can withstand and survive high concentrations of sulphite, sulphate and sulphide (Simon and Nazir, 2013). In geothermal waters, sulphur related compounds are expected to be present which could be toxic to some microorganisms, but based on the above toxicity of sulphur components is not expected for those species. Those species have therefore higher chance of surviving and flourishing in high sulphur systems than other species.

3.3.1 Sulphur related processes

In the main studied geothermal system used for this study (from which all A and B samples and sample 001 originated), serious obstruction and hydrogen sulphide formation was observed. The main bacteria that were observed in this obstruction material (sample 001A) were sulphur metabolizing bacteria. As a comparison to this sample and to get an idea if similar problems could be expected in other geothermal systems, the sulphur metabolizing population in each sample was observed. Sulphur metabolizing species are, among others, sulphate reducing species which can produce hydrogen sulphide and with that iron sulphide if iron (Fe) is available. Hydrogen sulphide is a toxic gas. Iron sulphides can cause obstruction and can release hydrogen sulphide during cleaning with acids, which was the suspected problem in the main system under investigation here.

In all studied systems sulphur metabolizing species were detected. The relative abundance of sulphur metabolizing bacteria ranged from 0.02% to 4.1%. An overview of all percentages of sulphur metabolizing bacteria per sample is given in table 3.

Table 3: fraction of sulphur metabolizing species in each sample

Sample	Earth layer	% of sulphur metabolizing bacteria
001	Trias sandstone [bunter]	4.10%
002	Westlands Delft sandstone	0.70%
003	Rotliegend sandstone Heemskerk	0.10%
004	Westlands Delft sandstone	0.20%
005	Rotliegend sandstone	0.02%
006	Combination of Dinantien limestone and Condroz sandstone	0.03%

3.4 Mineral analysis

XRD analysis showed high relative amount of minerals which can be expected from the adrresed aquifer such as calcite and quartz. Interestingly also chalcopyrite ($FeCuS_2$), pyrite (FeS) and magnetite (Fe_3O_4) which are indications of corrosion of iron. In addition the sulphide containing elements are an indication that sulphide is formed, presumably by activity of microorganisms. Dissolved iron was also determined in the water. The iron concentrations in the water were somewhere between 76 and 19 mg/L.

3.5 Treatment strategy

As a treatment strategy to control microbiology it was chosen to use a glutaraldehyde based biocide. This biocide was injected once a month for 24h, 100 ppm. In addition to the use of a biocide the system was also treated using antiscalent, CO_2 (to compensate the CO_2 that is released when water gets above ground) and a corrosion inhibitor. All was injected in the injection well except the corrosion inhibitor which was dosed into the production well and has been proved to enter the injection well too. After several months of operation the injection pressure started to increase again. This time the increase was much smaller than in the initial startup. Samples were collected again almost 2 years later in the injection well. Black slurry came back from the scraper. This material was highly soluble in toluene, suggesting an organic origin.

Microbiological analysis were performed to understand the origin of the obstruction better.

3.6 Microbiology after biocide treatment

After more than 2 years of biocide treatment samples were collected again using a bailer and scraper. The bailer and scraper material was analysed using QPCR. The results are shown below (figure 6):

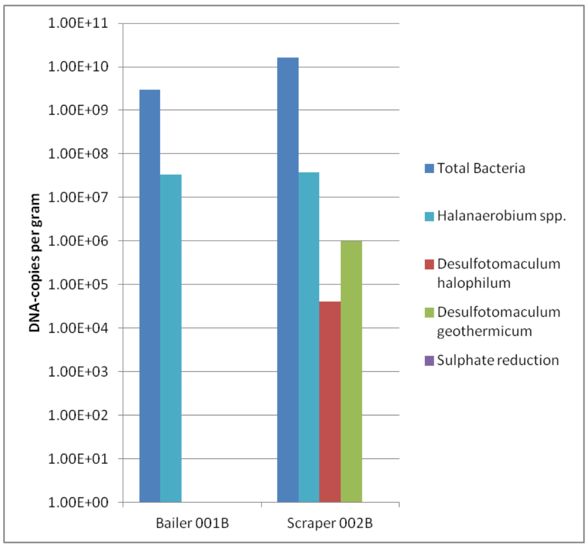

Figure 6 DNA QPCR results (presence) after two years of biocide treatment

The QPCR analysis show that high amount of bacteria are present in the bailer and scraper material after two years of operation and monthly dosage of biocide. The amount of bacteria is as high as can be expected from a bacterial culture and it is therefore expected that a significant biofilm developed on the internal surface of the injection pipe. Also high amounts of *Halanaerobium* species were detected in both the bailer and scraper sample and *Desulfotomaculum* also in the scraper sample but not in the bailer sample. *Halanaerobium* and *Desulfotomaculum* species are both species which can produce H_2S and with that cause corrosion of iron containing structures. The presence of high amount of bacteria after two years of biocide dosage is an indication that the currently used biocide treatment did not prevent microbiological growth in the system.

4. DISCUSSION AND CONCLUSIONS

the course of two years several analysis were performed on a geothermal system in the Netherlands to get a better understanding of microbiology in these systems and the possible treatment strategies to prevent microbiology related problems.

4.1 Microbiologically Influenced Corrosion (MIC)

Microorganisms can be involved in corrosion of piping systems. One of the best known groups of microorganisms which are involved in corrosion are the sulphur metabolizing bacteria. *Halanaerobium* and *Desulfotomaculum* are sulphur metabolizing species. These species were present in all samples analyzed here, before and after biocide dosing. These species produce hydrogen sulphide or sulphuric acid which can cause damage of the pipes or they can directly damage the pipe material. Not only sulphur metabolizing bacteria can influence corrosion, many other species are related to MIC. MIC occurs often on surfaced where biofilms are formed. Due to the formation of the biofilm local conditions are altered which can create favourable conditions for MIC to occur. In the samples analyzed for this project many MIC related species were detected which is an indication that MIC might be a significant issue in those systems.

Sulphur metabolizing bacteria are related to MIC but also to precipitation of iron (copper) sulphide which can cause sudden release of hydrogen sulphide if acid is used for cleaning. Sulphur metabolizing species have been detected to some extent in all geothermal production waters analyzed here. Those species can only grow if sulphur or sulphate is present. However, in geothermal water it is expected that a sulphur source is available.

To determine if MIC is indeed happening in geothermal pipes more studies, including coupon studies, are necessary to understand if the high potential of MIC also causes MIC to occur. Surface thickness measurements can help to get an overall view of corrosion.

4.2 Microbiological Growth

High numbers of bacteria have been detected in obstruction material and in scraper material from the injection well, also after several years of biocide treatment. The identified species have been related to H_2S formation and MIC. Therefore the threat for MIC in this system is expected to be high, not only in the injection well but also in other parts of the geothermal system such as the heat exchanger and pumps, since the bacteria originate from the production well.

The production water seems to have a different microbiological composition and lower amount of bacteria than the obstruction and scraper material, therefore growth is expected to have occurred in the system. Changes in water temperature or additives could trigger growth of bacteria.

The scraper material (sample 002B) is expected to contain corrosion inhibitor since this is applied and expected to form a film on the inside of the pipes. The corrosion inhibitor consist of a combination of chemicals among which several components which have been described to be biologically degradable (Tezel and Pavlostathis, 2015; Rajasekar *et al*., 2007; Dwyer and Tiedje, 1983). However, the exact mode of degradation and the related species is unclear. Presence of high amount of bacteria in the scraper from the pipe suggests that the microorganisms can survive and potentially also grow in this film formed by the corrosion inhibitor.

The main identified microorganisms in the obstruction material are able to produce H_2S and could therefore be responsible for H_2S formation from the injection well. The high amounts of H_2S that have been formed, raised the question if the H_2S production could have been caused by microbial activity only.

Evaluating the biological and chemical composition of the production and injection water, there seem to be three options for H_2S release. Purely biological by sulphate reducing micro-organisms. Purely chemical by precipitated pyrite (FeS) and chalcopyrite ($FeCuS_2$), or a combination of biological and chemical processes.

The first option of purely biological process would require sulphate reducing microorganisms and a sulphate source (or another form of partly oxidized sulphur, like thiosulphate or biosulphide). Both were present in the obstruction material. However, you would also expect a slow increase in H_2S release because the more biology grows, the more H_2S will be released. A sudden release of H_2S could be expected if the system is under pressure and the pressure is released, which happens when the acid flows back to the surface during the backwash after the treatment. This option would require sufficient carbon source (in amounts similar to the sulphate), which seems to be present in sufficient amount, either as methane, or heavier hydrocarbons, but possibly also as a result of the available CO_2. Along this route, the sulphate present explains the high amount of biomass formed.

A purely chemical process would require chemical FeS and $FeCuS_2$ formation in the injection well and thus FeS or $FeCuS_2$ formation trough changing conditions from production towards injection well. When production water is pumped to the surface CO_2 is released due to changes in pressure. This effects the pH and might cause precipitation of FeS or $FeCuS_2$. Further, sodium bisulphide was added to this system as oxygen scavenger. Sodium bisulphide should, however, have reacted with the oxygen in the water and not with other chemicals.

A combination of a biological and chemical process seems to be the most logical explanation. FeS/ $FeCuS_2$ could have been formed by biology. Iron concentrations in the water were somewhere between 76 and 19 mg/L. In the sample from the bailer not a lot of FeS and some $FeCuS_2$ has been found. In the pump FeS was found but only 1% of the total mineral composition of the bailer product. Iron could also have been dissolved from the stainless steel injection pipe by iron reducing bacteria. In addition to H_2S production, *Halanaerobium* (one of the major detected species in the sample) is also able to reduce iron (Paul et al., 2014). The combination of dissolved iron and sulphate/sulphide reduction can cause deposition of FeS and $FeCuS_2$.

In conclusion, the most likely scenario is that H_2S formation was caused by the following steps:

1. Decrease of temperature allowed enrichment of sulphate reducing species and microbial growth in general. The addition of sodium bisulphide as oxygen scrubber increased the growth potential of this group of species since sodium bisulphide can be used as nutrient source for sulphate reducing species.

2. In combination with iron reducing species and dissolved iron, FeS and $FeCuS_2$ was formed by the present biology. Two possible routes are available, for the next step, that may both have occurred. First, the sulphate reduction happened heterotrophically, producing H_2S, which reacted chemically with the iron of the well wall, forming FeS/ $FeCuS_2$ and H_2. This H2 may have served as a substrate for other bacteria. In the second scenario, the sulphate reduction was coupled to iron oxidation directly, making it a fully biological scenario. The resulting production of FeS is the same in both cases. $FeCuS_2$ could have been formed by similar reactions in the presence of copper. Copper is present in the production water itself and in minimal amounts also in the pipe material but in both cases iron is involved in the formation of the minerals. The amount of iron in the water seems to be too small to account for all the observations, implying the material of the injection well itself is a necessary component.

3. By addition of HCl, FeS and $FeCuS_2$ was dissolved and H_2S was released in high amounts at once. Biological H_2S formation and iron (copper) sulphide formation is a strong indication for MIC.

4.3 Control measures

To prevent issues like obstruction, H_2S formations and microbiologically induced corrosion (MIC) the microbiology needs to be controlled in geothermal systems. Control measures that can be taken are:

4.3.1 Corrosion inhibitor

Corrosion inhibitors are normally dedicated to inhibit chemical corrosion, however, they have been described to also suppress MIC development (Duncan et al., 2013). With the fact that corrosion inhibitors can reduce the MIC risk, it is possible that the used corrosion inhibitor can also reduce the MIC risk in the geothermal systems, however, corrosion inhibitors are not considered sufficient for microbial control in geothermal systems since they do not control microbial growth and with that do not prevent clogging or precipitation of iron sulphide. In addition, it has been described that corrosion inhibitors can be biologically degraded (Tezel and Pavlostathis, 2015; Rajasekar *et al*., 2007; Dwyer and Tiedje, 1983). In this study, scraper material, collected after applying corrosion inhibitor for more than two years, showed to consist of a corrosion inhibitor like material (most likely the inhibitor) and high amounts of bacteria.

Those microorganisms seem to be different than the ones identified in the production water and therefore have likely been growing somewhere in the system. If the corrosion inhibitor is microbiologically degraded is speculative, but it should be considered that the corrosion inhibitor can serve as nutrient source for microbial growth.

4.3.2 Prevention of Microbiological Growth

The most used microbial control measure in the Netherlands, at the moment consists of dosing the biocide glutaraldehyde into the injection well. This case showed that even when applying monthly shock treatment of glutaraldehyde, the microbiology can still develop in the injection well. Optimizing the biocide regime is necessary to control microbiological growth better.

Non-biocide options such as removing nutrients or changing growth conditions such as pH or oxygen or other disinfection methods that are used in other applications such as membrane filtration, UV treatment or ozone treatment seem not to be realistic and have not been applied for geothermal operation so far. If considering other options than biocides, this should be tested thoroughly before consideration in practice.

Besides preventing growth or activity of microorganisms that are already present in a geothermal system, it is important to consider and prevent contamination of microorganisms or favourable conditions for microorganisms by introducing microorganisms or nutrients during construction.

In the case that there is no steel surface where MIC can take place, on those surfaces corrosion and also MIC is eliminated. Although there are many other types of microbiological related processes this could help in controlling MIC in particular. Geothermal operators are looking into options to fully reline wells and facilities with Glass Reinforced Epoxy Pipes to avoid corrosion at all. This would only help for corrosion and MIC in the wells itself. Other metallic parts, such as heat exchanger, which are in contact with the geothermal water still face a high threat for MIC without any control measures.

To control microbiology in geothermal systems it is important to setup a case specific and well tested treatment regime and to regularly monitor if the control measures are still effective. Microorganisms can adjust and change over time, therefore, treatment regimes need to be adjusted accordingly. Results from this study has shown a diversity of different bacterial species in different geothermal systems. Therefore it can be concluded there is a risk for microbiology related issues in all these systems. Based on the diversity of detected species, the specific risks should be evaluated for each specific situation.

Changes in water temperature could trigger growth of bacteria, however treatment of only the injection well seems not to be sufficient since bacteria seem to originate from the production well and can also grow and adhere to for example the internal surface of the heat exchangers.

The present study was performed on a geothermal system in the Netherlands. In the Netherlands geothermal systems operate at relatively low temperature 75-85 ºC production temperature. It is not known if similar problems can occur when higher temperature production waters (e.g. 120-150 ºC) are used. Microorganisms might be less resistant to survive and grow in higher temperatures.

CONCLUSIONS

- During a 2,5 month period the casing was unprotected in high corrosive environment and bacteria and iron oxides, iron sulphides (FeS) and iron-copper sulphides ($FeCuS_2$) were identified suspected to have caused the obstruction.
- High amount of bacteria in the obstruction material indicating that microorganisms caused the obstruction of the geothermal system under investigation.
- Identification of the microorganisms in the production water showed that several MIC related species are already present in the production water.
- The detected species were typical thermophilic species with optimal growth temperature at 40-60 ºC.
- The H_2S formation was likely caused by a combination of biological FeS and $CuFeS_2$ formation (due to Microbiologically Influenced Corrosion; MIC) and the release of H_2S by the addition of acid during cleaning.
- There is a risk for microbiological growth and MIC in all analysed geothermal systems since microorganisms are present in significant amount.
- To prevent this type of issues biocide treatment was applied, however, further investigation showed that microorganisms were still able to grow in the system.
- Continuous monitoring and adjustment of kill strategies are essential for safe operation.

REFERENCES

Heid, C.A., J. Stevens, K.J. Livak and P.J. Wlliams. “Real time quantitative PCR.” Genome research 6 (1996) pp. 986 – 994.

Goorissen H.P., et al. “Isolation of thermophilic Desulfotomaculum strains with methanol and sulfite from solfataric mud pools, and characterization of Desulfotomaculum solfataricum sp. nov.” Int J Syst Evol Microbiol. 53 (2003) pp. 1223-9.

Booker A.E., et al. “Sulfide Generation by Dominant Halanaerobium Microorganisms in Hydraulically Fractured Shales”. mSphere 5 (2017) p. e00257.

Simon J. and P. M. Kroneck, "Microbial Sulfite Respiration," Advances in Microbial Physiology, 62 (2013) pp. 45-117.

Duncan K. E., B. M. Perez-Ibarra, G. Jenneman, J. B. Harris, R. Webb and K. Sublette, "The effect of corrosion inhibitors on microbial communities associated with corrosion in a model flow cell system," Appl Microbiol Biotechnol (2013) pp. 1-12.

San N.O., H. Nazır and G. Dönmez, "Microbiologically influenced corrosion of NiZn alloy coatings by Delftia acidovorans bacterium," Corrosion Science 64 (2012) pp. 198-203.

Tezel U. and S.G. Pavlostathis, "Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology" Current Opinion in Biotechnology 33 (2015) pp. 296–304.

Rajasekar A., S.Maruthamuthu, N.Palaniswamy and A.Rajendran, "Biodegradation of corrosion inhibitors and their influence on petroleum product pipeline" Microbiological Research 162/4 (2007) pp. 355-368.

Dwyer D.F. and J.M. Tiedje. “Degradation of Ethylene Glycol and Polyethylene Glycols by Methanogenic Consortia.” Applied an Environmental Microbiology, (1983) pp. 185-190.Author, A. and Author, B.: Example of a conference paper, *Proceedings of the European Geothermal Congress 2007,* Unterhaching, Germany, (2007), paper #001, 1-6.

Paul V. G., Minteer S. D., Treu B. L., Mormile M. R. “Ability of a haloalkaliphilic bacterium isolated from Soap Lake, Washington to generate electricity at pH 11.0 and 7% salinity.” Environ. Technol. 35 (2014) pp:1003–1011

Acknowledgements

The authors would like to thank the all operators that provided samples and input information. This project has been realized within the framework of Kennisagenda Aardwarmte from the Dutch ministry of LNV, “LTO Glaskracht Nederland” and the program “Kas als Energiebron”.