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Oskarshamn site investigation

Control of microorganism content in flushing water used for drilling of KLX08

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Abstract

A system for disinfection of flushing water and continuous dosage of tracer for drilling fluids has been developed. It comprises an ultra violet (UV) radiation unit and a flow controlled dosing pump attached on line in the flushing water system.

This activity aimed to control the effect from cleaning on the drill water system during drilling of KLX08 and to determine the amount of microorganisms being introduced in the borehole during drilling. The second aim was to control the disinfection efficiency of the UV-unit during drilling.

The flushing water was above the acceptable limit, i.e. 1,000 cells ml^{-1}, during drilling of KLX08, at least at the day of sampling. Both the number of cultivable bacteria and the ATP concentrations were higher than during drilling of KAV04 but much lower than during drilling of KLX03.

Although there was no effect from the UV, it is still possible that the UV-unit was operative, if the source of contamination was located between the UV-unit and the sampling point after the UV-unit. It is plausible that the uranin solution may have had bacteria which were continuously dosed. It is recommended that the tracer solution is kept free from microbes.

In conclusion, it is possible to keep flushing water systems clean. However, doing so is a continuous process that requires repeated cleaning and control.

Sammanfattning

Ett system för anti-mikrobiell behandling av spolvatten för borrning har utvecklats. Systemet omfattar en UV-enhet samt en flödeskontrollerad dosering av spårämne på spolvattensystemet ”on line”.

Denna aktivitet syftade på att kontrollera effektiviteten i rengöringsprocedurerna under borrning av KLX08 samt att fastställa mängden mikroorganismer som introducerades till borrhålet i samband med borrningen. Ett ytterligare syfte var att kontrollera desinfektionsförmågan hos UV-enheten.

Bakteriehalten i spolvattnet låg över acceptabel nivå dvs 1 000 cells ml^{-1}, under borning av KLX08, åtminstone på dagen för provtagning. Antal bakterier och mängd ATP var båda högre än under borrning av KAV04 men lägre än under borrning av KLX03.

Det är fullt möjligt att UV-enheten fungerade som den skulle trots att resultaten indikerar motsatsen, om källan till kontamination ligger mellan UV-enheten och provpunkten efter enheten. En möjlig källa kan vara spårlösningen med uranin. Om den innehåller bakterier doseras ju bakterier kontinuerligt. Det rekommenderas att spårlösningen hålls fri från bakterier.

Sammanfattningsvis kan det konstateras att det är möjligt att hålla spolvattnet på en acceptabel nivå vad gäller mikrobinnehåll. Men för att göra det krävs kontinuerlig rengöring och kontroll.

Contents

1 Introduction

A system for disinfection of flushing water and continuous dosage of tracer for drilling fluids has been developed (Figure 1-1). It comprises an ultra violet (UV) radiation unit and a flow controlled uranin dosing pump attached on line in the flushing water system. It is known since earlier investigations that flushing water may introduce large number of contaminating microbes into the aquifers /Pedersen et al. 1997/. This should be avoided because it may cause errors in the suceeding investigations of geochemistry and mirobiology. The basic procedure to achieve a microbiologically approved flushing water system is to clean the drillwater system frequently. The UV-lamp should be kept clean and its proper efficiency should be continuously controlled. The uranin tank and mixture must be kept free from microbes. This is because some bacteria can grow on and degrade this tracer.

The drill water system was sampled at three points (Figure 1-1). The first sample (1) was taken directly after the drill water was pumped up from the borehole. This point gives the microbial content in the borehole and the hygienic status of the borehole pump. The second sample point was located after the UV-unit (2). This point should demonstrate the efficiency of the UV-unit and hygiene of the uranin dosage system. The numbers should here be significantly lower compared to the first sampling point. The last point (3) was located in the drilling machine. This point shows the hygienic status of the drill rig and the storage tank.

The results from a previous drill water investigation in Oskarshamn during drilling of KSH03 have been reported /Kalmus, 2004/ and KAV04 and KLX03 /Pedersen, 2005 in press/. This document reports the results gained during drilling of KLX08 (AP PS400-04-115). The system was sampled 2005-04-21. The drill water source was HLX10. The results have been reported to the SICADA database.

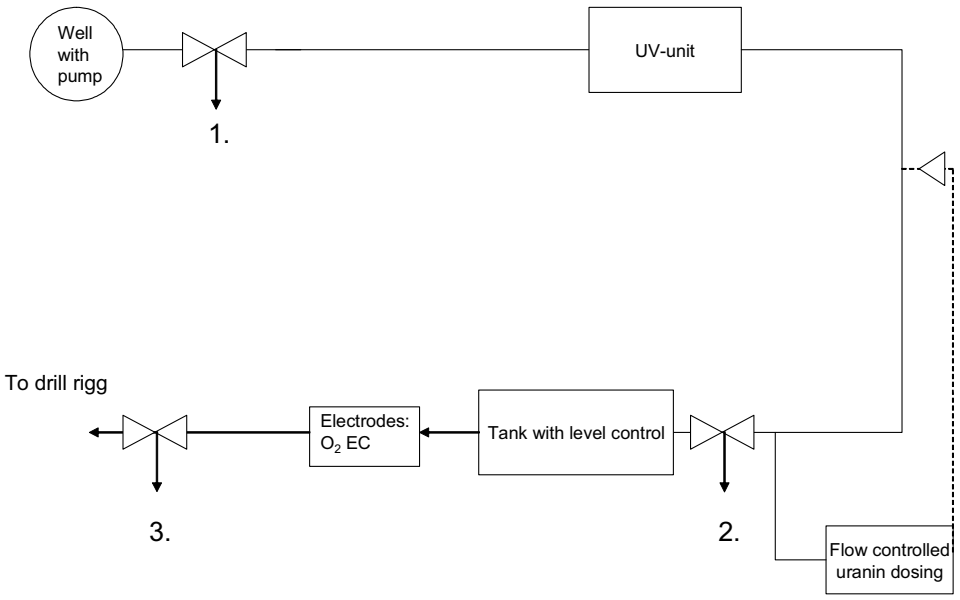

Figure 1-1. *Schematic drawing of the drill water system.*

2 Objective and scope

A washing/cleaning procedure and a disinfection UV-unit have been introduced to minimize the amount of microbes in the flushing water system during drilling. This activity aimed at:

1. Control of the effect from cleaning of the drill water system during drilling of KLX08. The performance of the flushing water treatment with reference to its ability to reduce potentially occurring microbes in the flushing water was analyzed.
2. Determination of the amount of microorganisms possibly being introduced in the borehole during drilling.
3. Control of the disinfection efficiency of the UV-unit.

3 Equipment

3.1 Description of equipment/interpretation tools

Standard cultivation equipment and procedures were employed as follows:

- Viable counts of microorganisms were analyzed in triplicates according to /Pedersen et al. 1997/, with R2A medium.
- ATP measurements were made in triplicates according to the method described in /Lundin, 2000/ using firefly luciferase enzyme.

Sampling was performed in 1 L sterile glass bottles on 3 positions in the drill water line. Usually sampling is repeated to understand the total variability over time included in the sampled water (short term fluctuations in water quality), and the sampling procedure. However, this time, misunderstandings at the site resulted in only on set of samples.

4 Execution

4.1 General

Six sterile 1 L bottles were sent in advance to the drill site.

4.2 Preparations

The samples were collected by personnel working at the drill site in the morning and sent by mail to the laboratory in Göteborg. The laboratory was prepared to analyse the samples as soon as they arrived. The inoculations and ATP measurements were finalized at noon the same day of arrival.

Sampling was executed at different times as listed in Table 4-1.

Table 4-1. Sampling times.

Sampling	Date	Sampling point					
		P1T1	P1T2	P2T1	P2T2	P3T1	P3T2
KLX08	050421	08.45	–	08.50	–	08.55	–

4.3 Data handling/post processing

The numbers obtained are directly transferred to the results section, without post processing.

4.4 Analyses and interpretations

Samples for cultivation are diluted and distributed on R2A medium agar dishes in triplets. The number of colonies is counted on all dilutions and parallels. The average of the triplet that lies between 30 and 300 colonies is taken as the value. This value should correspond well with the other triplets when the dilutions are taken into account.

The average ATP content of one living microbe in groundwater is about 10^{-18} mole. The ATP content can, therefore, be transferred to total amount of living microbes in the sample.

There is a general upper limit that should not be exceeded for the number of cultivable bacteria and ATP. Obviously, zero values would be best, but that is far from achievable under full scale field conditions. The simple recommendation is “the lower numbers the better”. Based on earlier experiences, a level of 1000 cultivable bacteria per litre can easily be achieved if the system is kept clean. At some sites levels of 100 cultivable bacteria per litre or less have been achieved. The number 1000 is, therefore, taken as the limit for approval of a clean flushing water system. A red line denotes this limit in the result Figures 5-1 and 5-2.

4.5 Nonconformities

Personnel at the site did only take one set of samples. The instructions indicated that two samples should be taken at different times.

5 Results

5.1 KLX08 drilling

The number of microorganisms in the flushing water was above the acceptable limits, i.e. 1000 cells ml^{-1}, during drilling of KLX08, at least at the day of sampling. Both the number of cultivable bacteria and the ATP concentrations were higher than during drilling of KAV04 (after cleaning) but lower that during drilling of KLX03.

Although there was no detectable effect from the disinfection with UV, it is still possible that the UV-unit was operative, if the source of contamination was located between the UV-unit and the sampling point after the UV-unit. It is plausible that the uranin solution may have had bacteria which were continuously dosed.

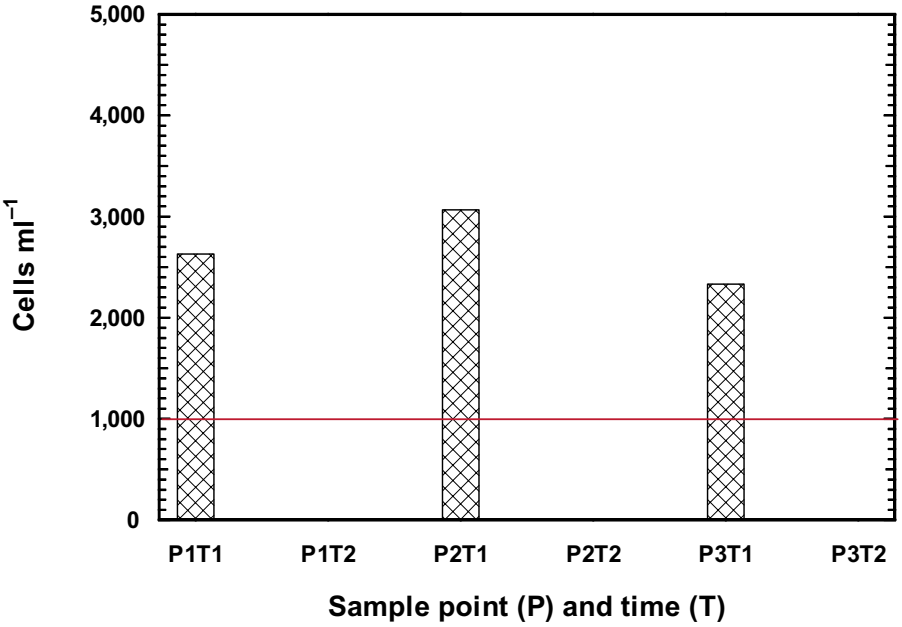

Figure 5-1. *The number of cultivable bacteria in the flushing water system during drilling of KLX08. Sample points refer to Figure 1-1. Sampling times are given in Table 4-1.*

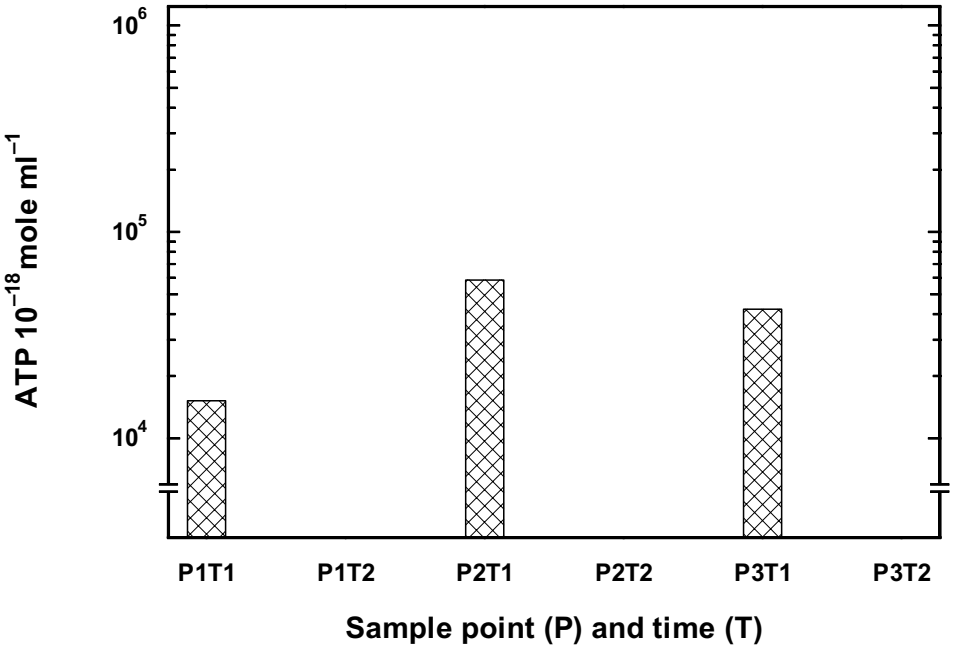

Figure 5-2. *The concentration of ATP in the flushing water system during drilling of KLX08 Sample points refer to Figure 1-1. Sampling times are given in Table 4-1.*

6 Conclusions

The high numbers of microorganisms in KLX08 flushing water are probably caused by high particle concentrations in the system. This can, however, not be concluded because of the lack of a second sampling time. The lack of repeated sampling limit the information value of this test significantly.

It is recommended that the sampling procedure with two sampling occasions separated by about 20 minutes is followed in the future.

It is also recommended that the tracer solution is kept free from microbes.

In conclusion, it is possible to keep flushing water systems clean. However, doing so is a continuous process that requires repeated cleaning and control.

References

Lundin A, 2000. Use of firefly luciferase in ATP-related assays if biomass, enzymes and, metabolites. Methods in ezymology 305, 346–370.

Kalmus A, 2004. Oskarshamn site investigation Control of flushing water used for drilling 2003-11-25, pp 1–20. SKB, P-04-32. Svensk Kärnbränslehantering AB.

Pedersen K, Hallbeck L, Arlinger J, Erlandson A-C, Jahromi N, 1997. Investigation of the potential for microbial contamination of deep granitic aquifers during drilling using 16S rRNA gene sequencing and culturing methods. *J Microbiol Methods* 30, 179–192.

Pedersen K, 2005. Oskarshamn site investigation. Control of microorganism content in flushing water used for drilling of KAV04 and KLX03. P-05-86 (in press).