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# Forsmark site investigation

# Control of microorganism content in flushing water used for drilling of KFM06A

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# Control of microorganism content in flushing water used for drilling of KFM06A

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This report concerns a study which was conducted for SKB. The conclusions and viewpoints presented in the report are those of the author and do not necessarily coincide with those of the client.

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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 disinfection efficiency of the UV-unit during drilling of KFM06A.

The number of cultivable bacteria, sampled before the UV-unit, was low and at a good level. Although they were higher after the UV-unit, they still were below the level of approval, i.e. 1,000 cells ml<sup>-1</sup>.

It is 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 Uranine solution may have contained bacteria which were continuously dosed. It is recommended that the tracer solution is kept free from microbes.

## 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 "in line".

Denna aktivitet syftade till att kontrollera effektiviteten hos UV-enheten under borrning av KFM06A.

Antalet odlingsbara bakterier före UV-enheten var lågt och på en mycket acceptabel nivå. Även om det var högre efter enheten låg värdet fortfarande betryggande under högsta acceptabla nivå, som är 1 000 celler ml<sup>-1</sup>.

Det är fullt möjligt att UV-enheten fugerade som den skulle, 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.

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### 1 Introduction

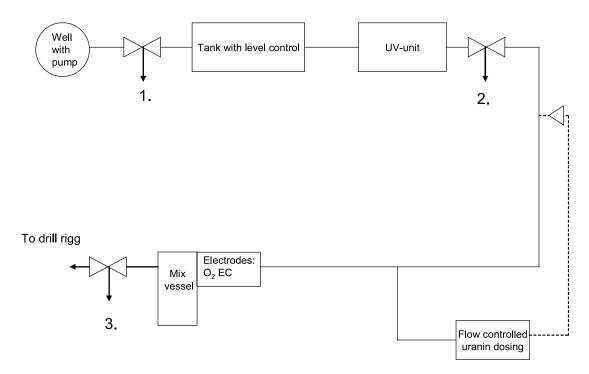
A system for disinfection of flushing water and continuous dosage of tracer for drilling fluids has been developed (Figure 1-1). It comprises an ultra violet (UV) radiation unit and a flow controlled Uranine dosing pump attached on line in the flushing water system. It is known since earlier investigations that flushing water may introduce a large number of contaminating microbes into the aquifers /Pedersen et al. 1997/. This should be avoided because it may cause errors in the succeding 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 Uranine tank and mixture must be kept free from microbes. This is because some bacteria can grow on and degrade this tracer.

The results from previous flushing water investigations in Forsmark during drilling of KFM01, 02, 04 and 05 have been reported /Pedersen, 2003; Pedersen and Kalmus, 2003; Hallbeck et.al. 2004/. This document reports the results gained during drilling of KFM06A. KFM06A was sampled 2004-09-21. A controlling document for performing the activity is presented in Table 1-1.

The flushing water system was sampled at two points (Figure 1-1). The first sample was collected before the UV-unit (point 1 in Figure 1-1). This point gives the microbial content in the borehole and the hygienic status of the borehole pump. The second point was located after the UV-unit and the dosing system (point 3 in Figure 1-1). This point should demonstrate the efficiency of the UV-unit and the numbers should be significantly lower compared to the first sampling point.

Table 1-1. Controlling document for performance of the activity.

Activity plan	Number	Version
Kontroll av bakterieförekomst i spolvatten vid borrning av KFM06A	AP PF 400-05-052	1.0



**Figure 1-1.** Schematic drawing of the flushing water system. Confer appendix in /Pedersen and Kalmus, 2003/ for a photographic image. Valves numbered 1) and 3) were used for sampling.

# 2 Objective and scope

A disinfection UV-unit have been introduced to minimize the amount of microbes in the flushing water system during drilling. This activity aimed to check the microbe content in the flushing water used for drilling of KFM06A and to control 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 2 positions in the flushing water line. Sampling was repeated once. This was done to understand the total variability over time included in the sampled water (short term fluctuations in water quality), and the sampling procedure.

#### 4 Execution

#### 4.1 General

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 air to the laboratory in Göteborg. The laboratory was prepared in the afternoon to analyse the samples as soon as they arrived. The inoculations and ATP measurements were finalized before evening the same day of arrival.

#### 4.3 Execution of field work

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

Table 4-1. Sampling times.

		Sampling point				
Sampling	Date	B1	B2	A1	A2	
KFM06A	040921	07:20	07:50	07:10	07:40	

## 4.4 Data handling/post processing

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

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

#### 4.6 Nonconformities

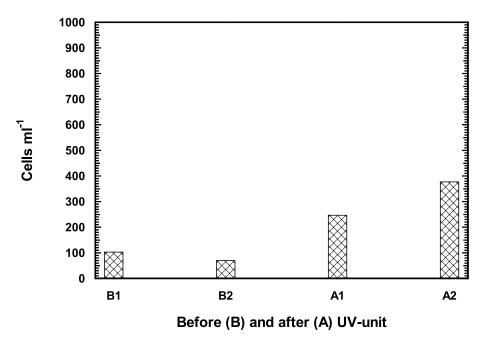
Nonconformities did not appear in this activity.

## 5 Results

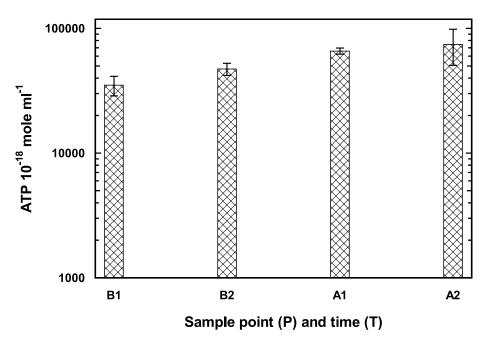
### 5.1 KFM06A drilling

There is no general upper limit that cannot 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 1,000 cultivable bacteria can easily be achieved if the system is kept clean. This number is, therefore, taken as the limit for approval of a clean flushing water system.

The cleaning routines of the flushing system during drilling of KFM06A were efficient. The numbers of cultivable bacteria was far below the limit of approval (Figure 5-1). The UV-unit seemed at a first glance not to work properly as the numbers after UV were significantly higher compared to before the unit. A source contamination was present between the two sampling points. However, it is possible that this point lies after the UV-unit. The general pattern obtained for cultivable bacteria was repeated in the ATP measurements (Figure 5-2), which supports the cultivation results.



**Figure 5-1.** Cultivable bacteria in the flushing water system during drilling of KFM06A. Sample points refer to the points 1 (B) and 3 (A) in 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 KFM06A Sample points refer to the points 1 (B) and 3(A) in Figure 1-1. Sampling times are given in Table 4-1. Bars give standard deviation for three ATP determinations.

## 6 Summary and discussions

The possible sources of microbial contaminations to a flushing water system are several. The flushing water well may have had dirty water. Fast pumping may stir up debris that can carry high numbers of bacteria. Finally, the trace solution can become contaminated.

The numbers of cultivable bacteria before the UV-unit was low and at a good level (Figure 5-1). Although they had increased after the UV-unit, they still were below the level of approval, i.e. 1,000 cells ml<sup>-1</sup>.

It is 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 (Figure 1-1). It is plausible that the Uranine solution may have had bacteria which were dosed. It is recommended that the tracer solution is kept free from microbes.

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