P-06-200

Oskarshamn site investigation

Triple control of microorganism content in flushing water used for drilling of KLX13A

Karsten Pedersen, Microbial Analytics Sweden AB

December 2006

Svensk Kärnbränslehantering AB
Swedish Nuclear Fuel
and Waste Management Co
Box 5864
SE-102 40 Stockholm Sweden
Tel 08-459 84 00
+46 8 459 84 00
Fax 08-661 57 19
+46 8 661 57 19

ISSN 1651-4416
SKB P-06-200

Oskarshamn site investigation

Triple control of microorganism content in flushing water used for drilling of KLX13A

Karsten Pedersen, Microbial Analytics Sweden AB

December 2006

Keywords: ATP, Bacteria, Disinfection, Laxemar, Simpevarp, Ultra violet light.

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 for a flushing water tracer attached on line in the flushing water system.

The numbers of cultivable bacteria in the flushing water were much too high in the first samples from 2006-05-24. A limit of 1,000 cultivable bacteria ml^{-1} has been set and all samples were above this limit. The data indicate that there was particulate matter in the sampled water from this date. Particles may give much higher bacteria counts than if water only is sampled. In addition, particles may shade bacteria from the UV light which will contribute to a high number. It is obvious that the flushing water system was dirty and needed to be cleaned, alternatively, the preceding cleaning (2006-05-17) was not well done. In contrary, the numbers of cultivable bacteria in the flushing water was at or below the limit of 1,000 bacteria ml^{-1} in the 2006-06-15 samples. This suggests that the flushing water system was clean this date. Numbers were above the limit in the HLX14 well but after the UV-treatment, lower numbers were obtained indicating that the UV unit was operational. With exception for the sampling point P4 next to the drilling machine, the last sampling occasion at 2006-08-17 showed values well below the limit of 1,000 bacteria ml^{-1}. Elevated numbers of cultivable bacteria were observed on this point at all three sampling occasions. It seems obvious that there was a source of contamination between point P3 and P4 which was not successfully cleaned by the cleaning procedure. Emphasis should be put on a more efficient cleaning of this part of the flushing water system in the future.

The adenosintrifosfat (ATP) concentrations were about twice as high at 2006-05-24 compared to 2006-06-15. ATP is a measure of both contaminating bacteria in the flushing water system and naturally occurring microorganisms in the flushing water well. The high concentration of ATP in the first analysis occasion correlates with high numbers of cultivable bacteria. The last sampling (2006-08-17) had the lowest concentrations of ATP, which correlates well with the lowest observed cultivable bacteria at this sampling date. There was no difference in ATP between P3 and P4, further suggesting that the repeatedly observed increase in cultivable bacteria was from an externally introduced source, or from a dirty spot in the system between P3 and P4.

Earlier controls of the flushing water system in Oskarshamn have revealed numbers of cultivable bacteria far above the limit for an acceptable system in some cases. This investigation demonstrated values both above and below the limit of acceptable numbers in the second analysis (2006-06-15), but much above acceptable numbers in the first (2006-05-24) analysis. It appears that cleaning efforts (2006-05-17) was not effective in the first part of the KLX13A drill campaign. However, the cleaning performed 2006-08-03 seems to have been effective as the values was well below the limit, except for P4.

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 till att kontrollera effektiviteten i rengöringsprocedurerna under borrning av KLX13A 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 från HLX14 låg högt över nivån för acceptabelt dvs över 1 000 bakterier ml^{-1} i prover från 2006-05-24. Erhållna data från detta analystillfälle tyder på att det fanns mycket partikulärt material i spolvattnet. Partiklar kan ge mycket högre bakterievärden jämfört med bara vatten utan patiklar. Dessutom innebär partiklar i vattnet att bakterierna delvis skuggas från UV-ljuset och överlever, vilket kan bidra till höga antal efter UV-enheten. Det är således uppenbart att spolvattensystemet var smutsigt 2006-05-24 och att rengöringen 2006-05-17 inte var tillräckligt väl genomförd. Antalet odlingsbara bakterier låg på, eller strax under under gränsen för acceptabelt vid provtagningen 2006-06-15. Antalet bakterier låg över gränsen på 1 000 bakterier ml^{-1} i HLX14 men föll till värden under gränsen efter UV-behandlingen. Det indikerar att UV-enheten fungerade som planerat. Den sista provtagningen utfördes 2006-08-17 och visade på de lägsta halterna bakterier av de tre undersökta tidpunkterna. En förhöjd halt bakterier observerades i provpunkt 4, före borrmaskinen i alla tre mätningarna. Det tyder på tillförsel av bakterier utifrån mellan punkt P3 och P4, eller på att någon del mellan P3 och P4 var smutsig.

Halten ATP var ungefär dubbelt så hög 2006-05-24 som vid provtillfället 2006-06-15. ATP är ett mått på summan av kontaminerande bakterier i spolvattensystemet och på naturligt förekommande mikroorganismer i borrhålet varifrån spolvattnet tas. Den höga koncentrationen ATP 2006-05-24 korrelerar med uppmätta höga antal av odlingsbara bakterier vid samma tillfälle. Den sista provtagningen (2006-08-17) hade de lägsta halterna av ATP vilket stämmer bra med de låga halterna av odlingsbara bakterier som observerades samtidigt. Det förelåg ingen skillnad i ATP mellan P3 och P4 2006-08-17, vilket stärker antagandet att kontamination av odlingsbara bakterier sker mellan P3 och P4.

Tidigare analyser av spolvatten i Oskarshamn har visat på antal odlingsbara bakterier långt över gränsen för acceptabelt (1 000 bakterier ml^{-1}) i vissa prover. I denna P-rapport redovisas antal under gränsen för acceptabelt i den andra analysomgången (2006-06-15), men mycket över gränsen i den första omgången (2006-05-24). Det är uppenbart att rengöring inte varit effektiv i början av borrkampanjen. Däremot var rengöringen som gjordes 2006-08-03 effektiv eftersom antalet bakterier låg under gränsen, utom för P4. Denna renhetsnivå måste eftersträvas redan från första dagens borrning.

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 microorganisms 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 flushing water system frequently. The UV-lamp should be kept clean and its proper efficiency should be continuously controlled. The uranin tank must be kept free from microorganisms. This is because some bacteria can grow on and degrade this tracer.

The flushing water system was sampled at four points (Figure 1-1). The first sample (1) was taken directly after the flushing 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. The numbers of cultivable bacteria should here be significantly lower compared to the first sampling point. The third sample was taken directly after the uranin dosage system (3). The last point (4) was located in the drilling machine. This point shows the hygienic status of the drilling machine, mixing vessels, electrodes and the storage tank.

Results have been reported from previous flushing water investigations in Oskarshamn during drilling of KSH03 /Kalmus 2004/, KAV04 and KLX03 /Pedersen 2005a/, KLX08 /Pedersen 2005b/ and KLX11 /Pedersen 2006/. This document reports the results gained during drilling of KLX13A. The system was sampled 2006-05-24, 2006-06-15 and 2006-08-17. The flushing water source was HLX14. The results have been reported to the SICADA database.

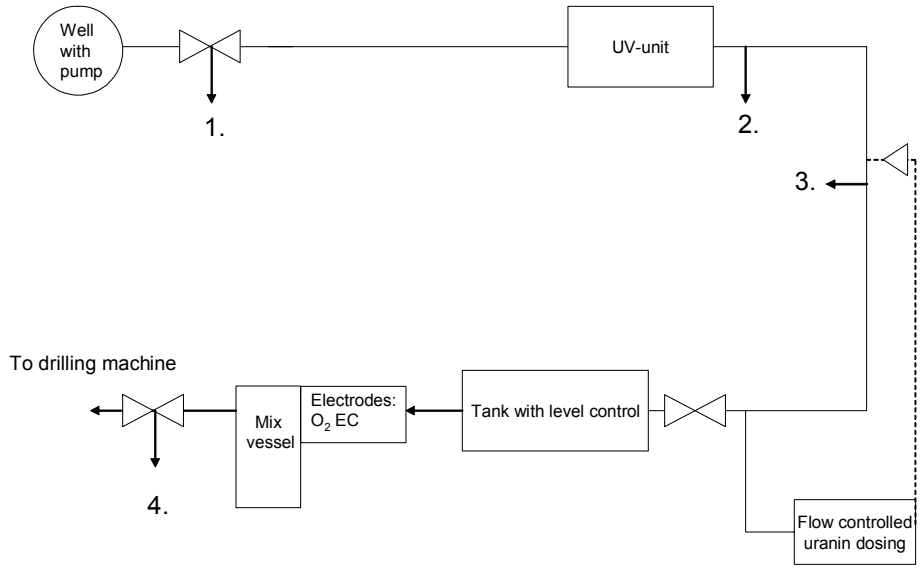

Figure 1-1. *Schematic drawing of the flushing water system.*

2 Objective and scope

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

1. Control of the effect from cleaning of the flushing water system during drilling of KLX13A. The performance of the cleaning procedure with reference to its ability to reduce potentially occurring microorganisms in the flushing water was analyzed.
2. Control of the disinfection efficiency of the UV-unit.
3. Control of microorganism content directly after the uranin dosage point.
4. Determination of the amount of microorganisms being introduced in the borehole during drilling.

The date of start of drilling was 2006-05-17. Cleaning and disinfection was undertaken the same day, following the document named "Rengöring av spolvattensystemet för ingående vatten på PO's borrplatser" by Nils Håkansson, dated 2004-03-10. The flushing water system was not in use between 5th to 16th of May and between 7th July and 3rd August 2006. It was cleaned again at start of the last drilling, 3rd August 2006.

3 Equipment

3.1 Description of equipment/interpretation tools

3.1.1 Sampling

Sampling was performed in 1 L sterile glass bottles on 4 positions in the flushing water line according to Figure 1-1. Sampling was repeated once to understand the total variability over time included in the sampled water (short term fluctuations in water quality), and the sampling procedure.

3.1.2 Number of cultivable bacteria

A plate count medium was constructed based on earlier work /Pedersen and Ekendahl 1990, Pedersen et al. 1997/. It was prepared as follows: Per litre of medium: peptone, 0.5 g; yeast extract, 0.5 g; starch (soluble), 0.25 g; sodium acetate, 0.25 g; $CaCl_2 \times 2H_2O$, 0.2 g; K_2HPO_4, 0.1 g; NaCl, 10 g, Non-chelated trace element solution according to Table 3-1, 1 ml; agar,15 g. pH was adjusted to 7.0. The medium was autoclaved at 121°C for 20 minutes, cooled to 50°C and poured in 15 ml portions in sterile Petri dishes. The produced agar dishes were allowed to dry for about 12 hours and then put in plastic bags until use.

Dilution tubes were prepared as follows: 18 ml Hungate tubes were supplied with 10 ml the following solution and autoclaved: NaCl, 10 g; K_2HPO_4, 0.1 g.

The samples in Table 4-1 were diluted in 10 times increments. From each dilution tube, 0.1 ml was spread with sterile glass loops on an agar dishes produced as described above.

3.1.3 Analysis of ATP

Groundwater for adenosintrifostat (ATP) extraction analysis was collected in triplicate from each sample point and transferred into sterile 50 mL Falcon centrifuge tubes. Extraction and analysis of ATP concentrations were based on the methods by /Lundin et al. 1986/ and /Lundin 2000/. ATP was extracted from 100 µL aliquots of groundwater within an hour of collection using 100 µL BS-buffer (BioThema ATP Biomass Kit HS, Sweden). After extraction, 100 µL of the ATP extract was mixed with 400 µL of HS-buffer (BioThema ATP Biomass Kit HS, Sweden) in a FB12/Sirius luminometer (Berthold, Germany). The total volume of groundwater in the sample then became 50 µl. Prior to each sample measurement, light emission of the

Table 3-1. Non-chelated trace element solution.

Component	Amount
Double distilled H_2O	987 mL
HCl (25%=7,7M)	12.5 mL
$FeSO_4$ x $7H_2O$	2.1 g
H_3BO_3	30 mg
$MnCl_2 \times 4H_2O$	100 mg
$CoCl_2 \times 6H_2O$	190 mg
$NiCl_2 \times 6H_2O$	24 mg
$CuCl_2 \times 2H_2O$	2 mg
$ZnSO_4 \times 7H_2O$	144 mg
$Na_2MoO_4 \times 2H_2O$	36 mg

400 µL HS-buffer without sample addition was allowed to diminish to less than 50 relative light units per second (rlu/s) in the luminometer. After addition of the ATP extracted sample, light emission was immediately determined using FB/Sirius PC Software quick measurement (Berthold, Germany). To account for attenuating effects from the sample on the enzymatic reactions (e.g. salinity, dissolved metals, sulphide), and to calibrate the luminometer readings, 10 µL of internal ATP-standard (0.1 µmole/L) was added to the extracted sample in the luminometer and light emission was measured again. All light emission measurements were performed three times. The equation used to calculate ATP-concentration in the sample was based on the average of three measurements as follows:

$$\text{pmol ATP / sample} = \frac{I_{smp} - I_{bkg}}{I_{(std + smp - bkg)} - I_{(smp - bkg)}} \qquad \text{(Equation 1)}$$

Where:

I = light intensity measured as relative light units

smp = sample

bkg = background with the HS buffer

std = standard

The amount of ATP ml^{-1} groundwater was calculated as:

amol/ml groundwater = pmol ATP/sample (from Equation 1) × SF × DF (Equation 2)

Where:

SF = shift factor from pmol to amol = 10^6

DF = dilution factor to obtain amount of ATP ml^{-} *=1,000/50*

4 Execution

4.1 General

Eight sterile 1 L bottles were sent in advance to the drill site per sampling occasion.

4.2 Preparations

The samples were collected by personnel working at the drill site in the morning and immediately sent by car 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 before 15.00 the day of sampling.

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

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 were diluted and distributed on nutrient medium agar dishes in triplets. The numbers of colonies were counted on all dilutions and parallels. The average of the triplet that lies between 30 and 300 colonies was taken as the value. This value should correspond well with the other triplets when all the dilutions were taken into account.

The average ATP content of one living microorganism in groundwater is about 10^{-18} mole. The ATP content can, therefore, be interpreted as the total amount of living microorganisms 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 1,000 cultivable bacteria ml^{-1} can easily be achieved if the system is kept clean. At some sites levels of 100 cultivable bacteria ml^{-1} or less have been achieved. The number 1,000 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, 5-2 and 5-3.

4.5 Nonconformities

None.

Table 4-1. Sampling times. P1 to P4 refer to sampling positions according to Figure 1-1. T1 and T2 represent sampling time one and two.

		Sampling point							
Sampling	Date	P1T1	P1T2	P2T1	P2T2	P3T1	P3T2	P4T1	P4T2
KLX13A	2006-05-24	06:18	06:43	06:25	06:45	06:28	06:48	06:31	06:55
KLX13A	2006-06-15	06:39	07:02	06:44	07:05	06:47	07:08	06:53	07:12
KLX13A	2006-08-17	06.20	06.45	06.34	06.47	06.37	06.49	06.40	06.52

5 Results

5.1 KLX13A drilling

The numbers of cultivable bacteria in the flushing water was too high in the 2006-05-24 samples (Figure 5-1). A limit of 1000 cultivable bacteria ml^{-1} has been set and all samples were above this limit. The flushing water from HLX 14 (P1) had the lowest number of cultivable bacteria, followed by the intermediate sample points P2 and P3. At the last sample point, P4, the numbers were very high. The numbers varied between time T1 and T2 at P2 and P3 (Figure 5-1). This is indicative of particulate matter in the sampled water. Particles may give much higher bacteria counts than if water only is sampled. In addition, particles may shade bacteria from the UV light which will contribute to a high number. As the trend in numbers of cultivable bacteria increase from P1 to P4, it is obvious that the flushing water system was dirty and needed to be cleaned, alternatively, the previous cleaning (2006-05-17) was not well done.

The numbers of cultivable bacteria in the flushing water were at or below the limit of 1,000 bacteria ml^{-1} in the 2006-06-15 samples (Figure 5-2). There was no strong difference in the numbers from P1 to P4. This suggests that the flushing water system was clean. The numbers were above the limit in P1 but after the UV-treatment (P2), the lowest numbers were obtained indicating that the UV unit was operational.

The last sampling occasion at 2006-08-17 showed values well below the limit of 1,000 bacteria ml^{-1} with exception for the sampling point P4 next to the drilling machine (Figure 5-3). Elevated numbers of cultivable bacteria were observed on this point at all three sampling occasions. It seems obvious that there was a source of contamination between point P3 and P4 which was not successfully cleaned by the cleaning procedure. Emphasis should be put on a more efficient cleaning of this part of the flushing water system in the future.

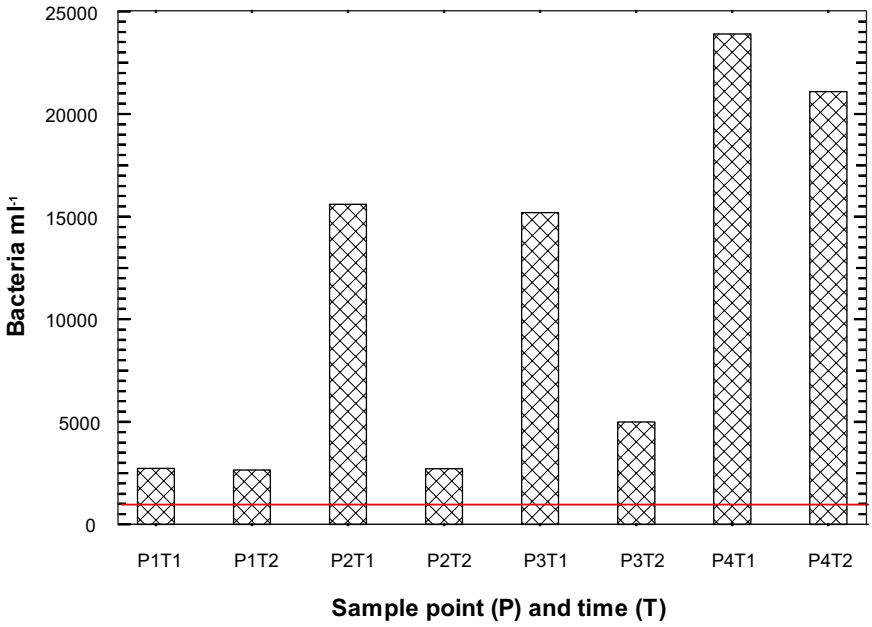

Figure 5-1. *The number of cultivable bacteria in the flushing water system during drilling of KLX13A in May 2006 (2006-05-24). Sample points refer to Figure 1-1. Sampling times are given in Table 4-1.*

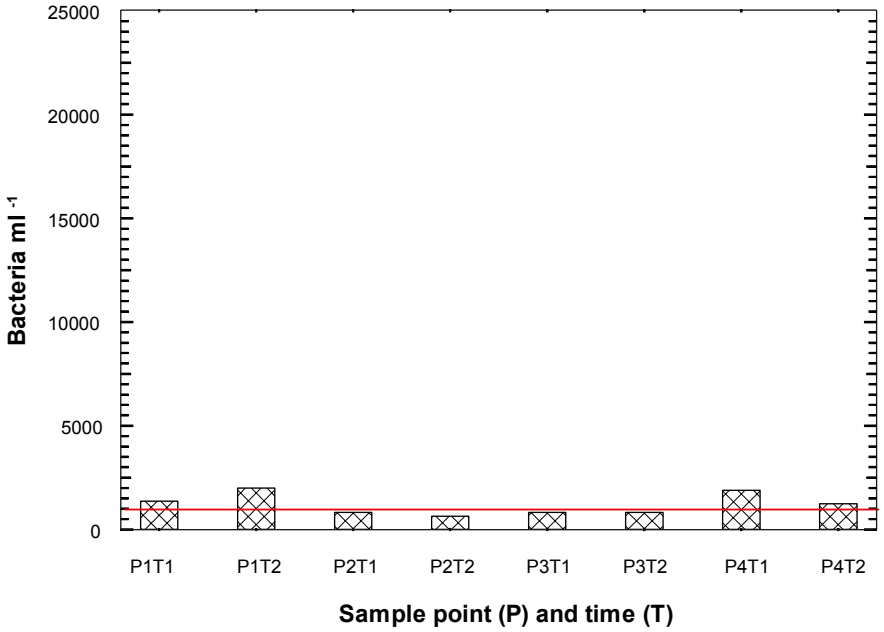

Figure 5-2. *The number of cultivable bacteria in the flushing water system during drilling of KLX13A in June 2006 (2006-06-15). Sample points refer to Figure 1-1. Sampling times are given in Table 4-1.*

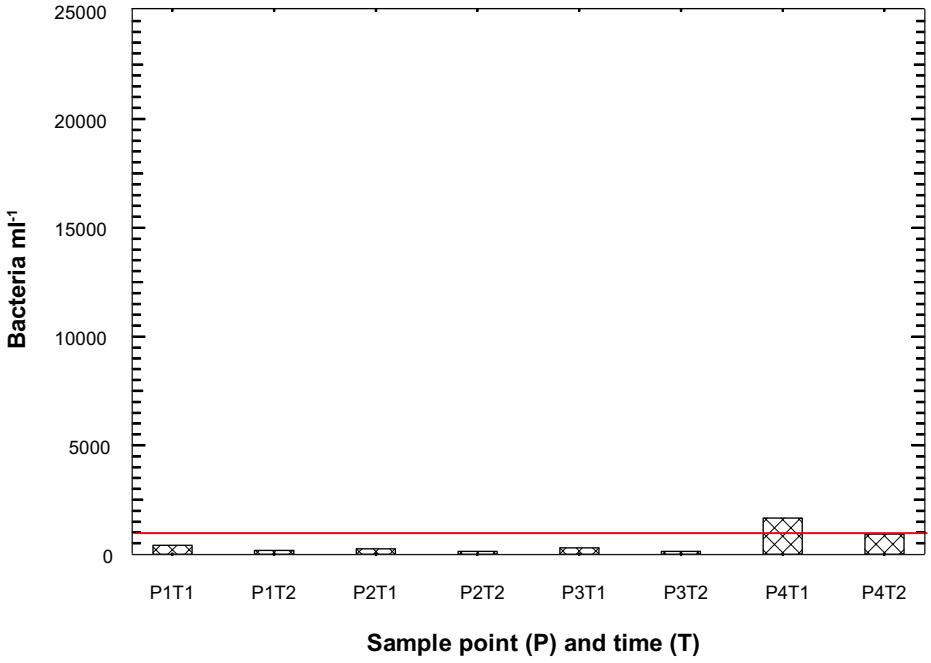

Figure 5-3. *The number of cultivable bacteria in the flushing water system during drilling of KLX13A in August 2006 (2006-08-17). Sample points refer to Figure 1-1. Sampling times are given in Table 4-1.*

The ATP microorganisms concentrations were about twice as high at 2006-05-24 (Figure 5-3) compared to 2006-06-15 (Figure 5-4). ATP correlates with the total number of bacteria. It is consequently a measurement of both contaminating microorganisms in the flushing water system and naturally occurring microorganisms in the flushing water well. The higher concentration of ATP in the first analysis occasion (2006-05-24) correlates with the high numbers of cultivable bacteria (Figure 5-1). The variation in ATP values between sampling points and time was low, and decreased little from P1 to P4. It is plausible that the UV-treatment kills bacteria, thereby lowering the ATP values.

The last sampling occasion (2006-08-17) had the lowest concentrations of ATP, which correlates well with the lowest observed cultivable bacteria a this sampling date. There was no difference in ATP between P3 and P4, further suggesting that the increase in cultivable bacteria is from an external introduced source, or a badly cleaned spot in the system between P3 and P4.

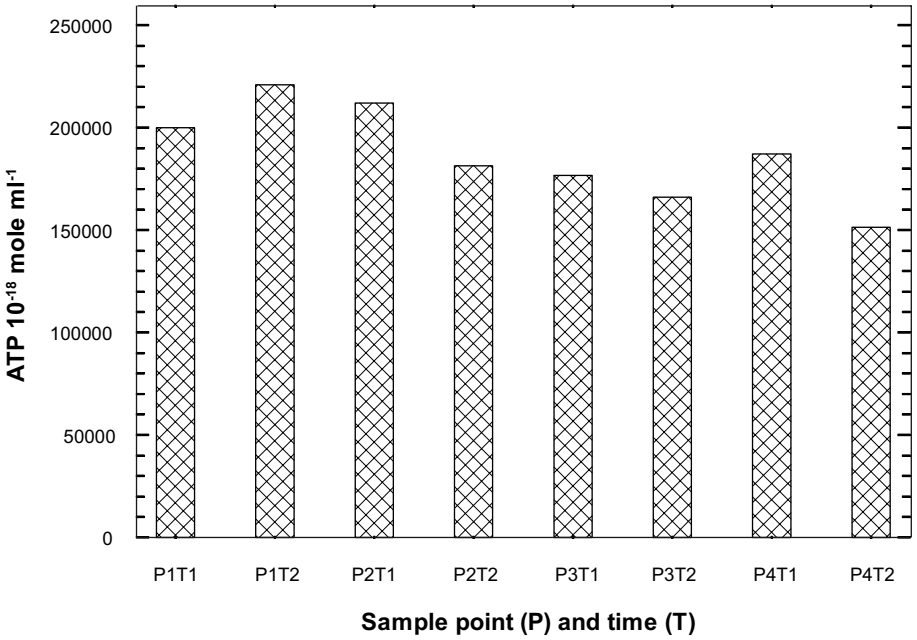

Figure 5-4. *The concentration of ATP in the flushing water system during drilling of KLX13A in May 2006 (2006-05-24). Sample points refer to Figure 1-1. Sampling times are given in Table 4-1.*

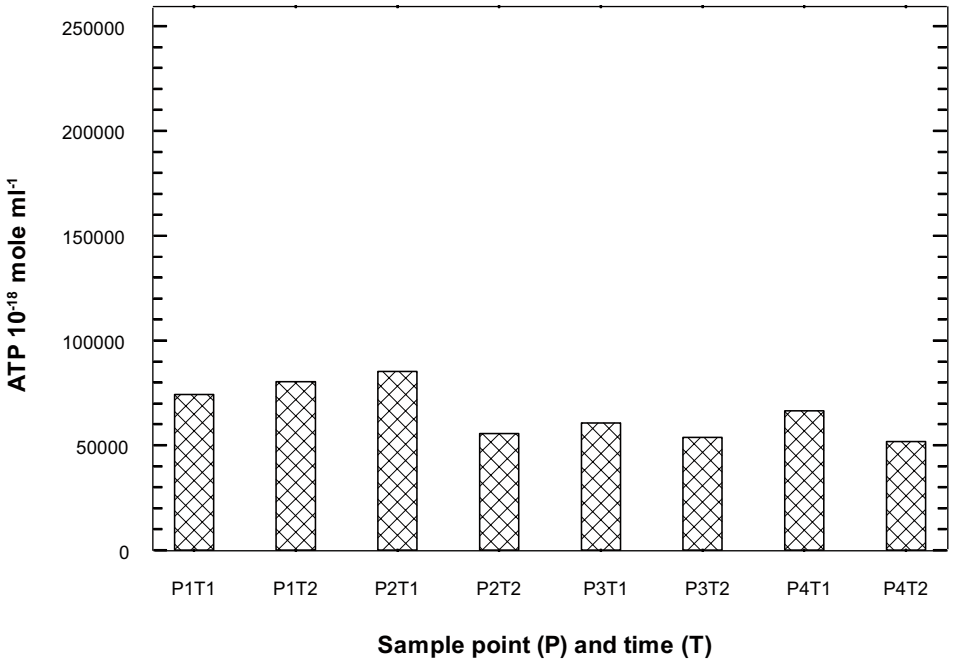

Figure 5-5. *The concentration of ATP in the flushing water system during drilling of KLX13A in June 2006, 20(06-06-15). Sample points refer to Figure 1-1. Sampling times are given in Table 4-1.*

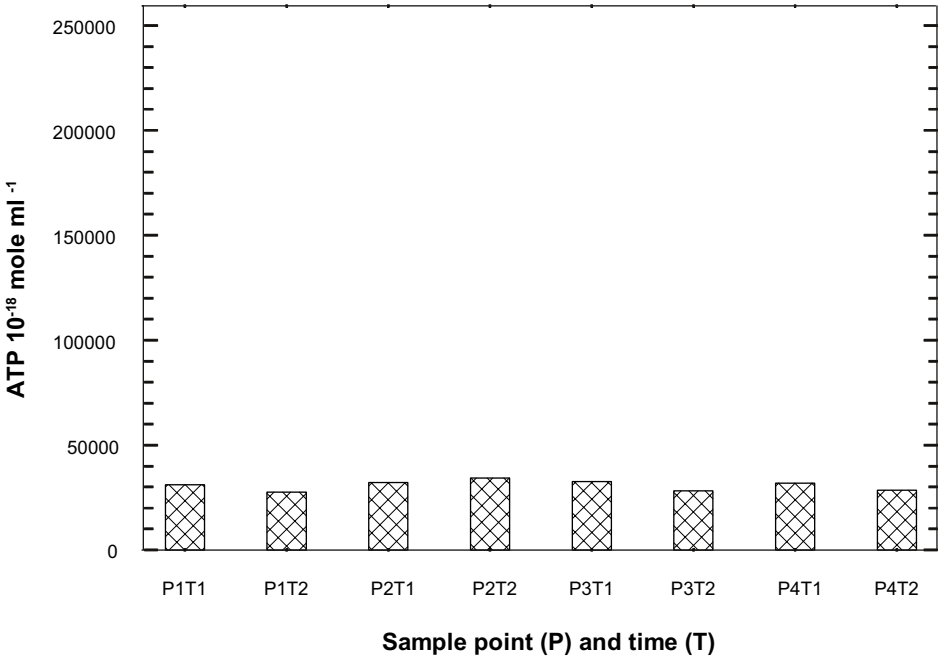

Figure 5-6. *The concentration of ATP in the flushing water system during drilling of KLX13A in August 2006 (2006-08-17). Sample points refer to Figure 1-1. Sampling times are given in Table 4-1.*

6 Conclusions

Earlier controls of the flushing water system in Oskarshamn have revealed numbers of cultivable bacteria far above the limit for an acceptable system (Pedersen 2005a and 2005b, 2006) in some samples. This investigation showed values at or below the limit for acceptable numbers in the second analysis (2006-06-15), but much above the limit for acceptable numbers in the first (2006-05-24) analysis. It appears that cleaning efforts (2006-05-17) was not effective in the beginning of the drill campaign. This is obvious from Figure 5-1 where low numbers of bacteria were obtained in the flushing water well and high numbers were found in the flushing water system. The only possible source for this contamination is consequently a dirty flushing water system. The repeated cleaning 2006-08-03 seemed to have been successful as judged by the sampling 2006-08-17, possibly with exception for the part between the uranin dosage point and the drilling machine. The levels of cultivable bacteria in 2006-06-15 and 2006-08-17 were good and should be aimed at from day one during drilling. The decrease in numbers of bacteria from the first (2006-05-24) to the second (2006-06-15) and finally to the lowest number in the last (2006-08-17) sampling occasion may be due to wash out of bacteria by flushing water from the well. If the numbers of bacteria were low in the flushing water well, as indicated in Figure 5-1, 5-2 and 5-3, this water may after some time replace the contaminated water in the flushing water system. Thereby will the numbers of bacteria in the flushing water decrease to levels close to the numbers in the flushing water well as shown in Figure 5-3 (with exception for possibly a dirty point close to the P4 sampling location).

References

Lundin A, Hasenson M, Persson J, Pousette Å, 1986. Estimation of biomass in growning cell lines by adenosine triphosphate assay. Methods in Enzymology 133, 27–42.

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-04-38. Svensk Kärnbränslehantering AB.

Pedersen K, Ekendahl S, 1990. Distribution and activity of bacteria in deep granitic groundwaters of southeastern Sweden. Microb. Ecol. 20, 37–52.

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, 2005a. Oskarshamn site investigation. Control of microorganism content in flushing water used for drilling of KAV04 and KLX03. pp 1–15 SKB P-05-86. Svensk Kärnbränslehantering AB.

Pedersen K, 2005b. Oskarshamn site investigation. Control of microorganism content in flushing water used for drilling of KLX08. pp 1–15 SKB P-05-147. Svensk Kärnbränslehantering AB.

Pedersen K, 2006. Oskarshamn site investigation. Control of microorganism content in flushing water used for drilling of KLX11A. pp 1–20 SKB P-06-18. Svensk Kärnbränslehantering AB.