Report: case study – evaluation of peroxide treatment, UV and the combination of peroxide/UV for the controlling of bacteria and biofilms in cooling towers.

Laboratory for process microbial ecology and bio-inspirational management

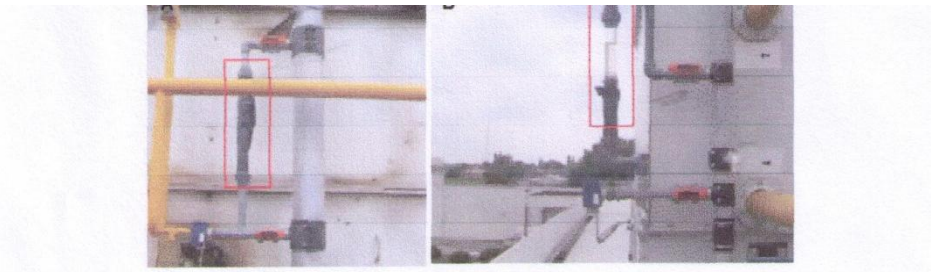
Situating

The treatment of process water in three separate cooling towers was followed in period of approximately one month in a meat producing company. Treatment (i.e. disinfection) of the cooling water should enable the company to drain water less frequently and therefore reduce water consumption. More specifically the study focused on cooling water used for cooling down certain processes in the company. This cooling water was further on cooled in three separate cooling towers and reused. The cooling water was treated with hydrogen peroxide, ultrasonic sound (US) or ultraviolet light (UV) combined with hydrogen peroxide. For each treatment the number of planktonic and biofilm associated bacteria was followed up.

Material and method

Two of the examined cool towers had a circulation volume of $1m^3$ (CT1 and CT2). The third cooling tower (CT3) had a lower recirculation volume ($0.8m^3$).

In the cooling water circuit a disinfection module and a biofilm monitor was installed. The biofilm monitor was introduced in the system by means of a bypass (figure 1). The biofilm monitors consists of a transparent tube with 30 polycarbonate rings. The biofilms developing on the inside of these rings were examined at fixed moments in time. The number of bacteria present in the biofilms was expressed in number of colony forming units (CFU) per cm^2. Beside the analysis of bacteria in the biofilm, also the number of bacteria remaining in the water (planktonic bacteria) was followed up (expressed in CFU/ml). From the several samples taken, the CFU was determined six times. For detailed description of the method reference is made to Vankerckhoven et al. (2009 and 2010), Hulsmans et al. (2010) and Verbessem et al. (2010).



Figuur 1: Plaatsing biofilmmonitoren (rood omkaderd) op de koeltoren KT1 (A) en koeltoren KT3 (B). Hierbij werd de biofilmmonitor telkens in bypass aangebracht, net voor de ingang in de condensor van de koeltoren. Deze monitor bestond uit een doorzichtige buis waarin 30 polycarbonaat ringen geplaatst werden.

Following water treatment methods were examined: hydrogen peroxide and hydrogen peroxide combined with UV and US. The hydrogen peroxide (EcoClearProx®, 42% H2O2) was stabilized with Sorbitol. In the first case an effective dose of hydrogen peroxide was applied and the reduction of the number planktonic and biofilm associated bacteria was determined. On day 15 the dose of peroxide was reduced to examine whether a lower dose is sufficient to control the number of bacteria, i.e. to avoid regrowth. Furthermore a low pressure UV lamp was used with an output of 47W. As US-reactor a low power US reactor (11W) was acquired and directly placed in the condenser. During the experiments it was chosen to first test a sufficiently high concentration of hydrogen peroxide. The cooling tower treated with hydrogen peroxide was injected with a dose of 200 ml per two hours. The cooling tower treated with combination of hydrogen peroxide/UV was dosed on flow basis, but both doses were comparable. After several weeks these doses were reduced tenfold, to examine eventual regrowth or control of bacteria. The absolute consumption of hydrogen peroxide was follow up during the experiment. Furthermore the effective concentration of hydrogen peroxide was determined using a spectroscopic method.

During the experiments, apart from the CFU, several other relevant parameters were followed up. Conductivity and temperature were measured for each sample. ATP measurements are often used as fast and alternative method for determination of microbiological contamination. Adenosinetriphosphate (ATP) is a chemical compound rich in energy present in living cells and is therefore a measure for active biomass present in the sample. The ATP determination is based on the reaction of luciferine with the enzyme luciferase that occurs in the presence of free ATP. As consequence of this reaction light is emitted at a wavelength of 562 nm. This signal was measured by means of a luminosity meter and expressed in relative light units (RLU).

Finally the water consumption was measured by means of the water meter available at the site.

Results

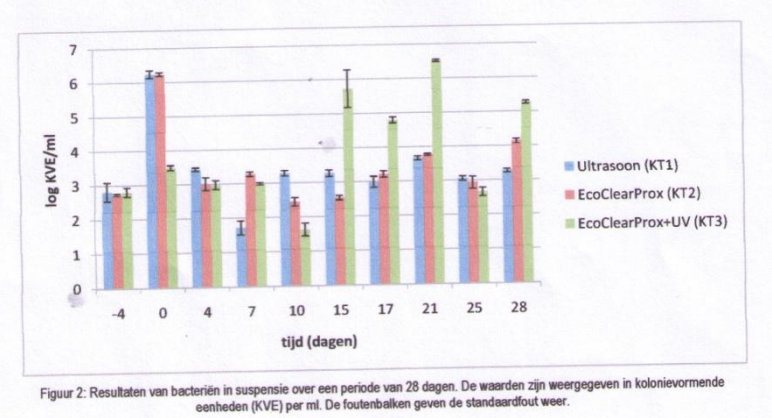
One cooling tower (CT1) was treated with ultrasound. The remaining cooling towers were treated with peroxide (CT2) or a combination of peroxide and UV (CT3). During the experiments problems have been observed with the water drain control of the cool towers. This electronic drain control was programmed by an external company and occasionally caused unexpected and undesired flows of water drainage, which affected the results. The influencing disturbances are mentioned within the discussion of the results. In the following the results of the most important parameters are discussed. Raw data of all followed parameters is included in the attachment.

3.1 Effectiveness for the treatment of planktonic bacteria.

The experiment was conducted over a period of 28 days. Before start of the experiments a water sample was taken in the cooling towers and the number of planktonic bacteria was determined to evaluate the effect of current disinfection treatments (day = -4). After samples were taken, the biofilm monitors in the cooling water circuit were taken into service and the disinfection treatment was stopped. This way the biofilm and in the biofilm monitor could grow during a period of 4 days. On day 0 a sample was taken and the number of planktonic and biofilm associated bacteria was determined. Afterwards the disinfection was restarted and on regular moments in time a water sample in the condenser was analyzed for the number of planktonic bacteria.

Treatment was first conducted during a period of 15 days. For CT1 this was a treatment with ultrasound. CT2 was injected with 200 ml of hydrogen peroxide after each period of 2 hours. CT3 was treated with UV in combination with hydrogen peroxide. Subsequently the US treatment was stopped on day 15; and the dose of hydrogen peroxide for CT2 and 3 were reduced with a factor 10.

From figure 2 it seems the number of planktonic bacteria after shutting down of the disinfection on day -4 significantly increases. Afterwards the disinfection in the 3 cooling towers was restarted as described above.



Figuur 2: Resultaten van bacteriën in suspensie over een periode van 28 dagen. De waarden zijn weergegeven in kolonievormende eenheden (KVE) per ml. De foutenbalken geven de standaardfout weer.

US seems to be an effective disinfection method for the removal of planktonic bacteria. A reduction of 3 log colony forming units (CFU) per ml was observed after 4 days. Subsequently the values remained relatively constant. This was caused by cleaning works within the condenser. Afterwards the number of planktonic bacteria increased again to about 3 log CFU/ml. On day 15 the US treatment was stopped. Only a limited or no growth of

planktonic bacteria was observed. This was explained because the water drain frequency of the condenser after day 15 was adjusted causing large amount of water drain, flushing away the bacteria.

Treatment with hydrogen peroxide in CT2 appeared to be sufficient for obtaining a reduction of the number of planktonic bacteria. Before the restart of the peroxide dosing a clear regrowth of the planktonic bacteria occurred up till a level of 6.2 log CFU/ml. Peroxide treatment during 4 days was sufficient to reduce the amount of planktonic bacteria to an acceptable level of ca. 3 log CFU/ml. After day 15 the peroxide dose was reduced by a factor 10 and a slight increase of planktonic bacteria was observed to a level of 4.1 log CFU/ml on day 28.

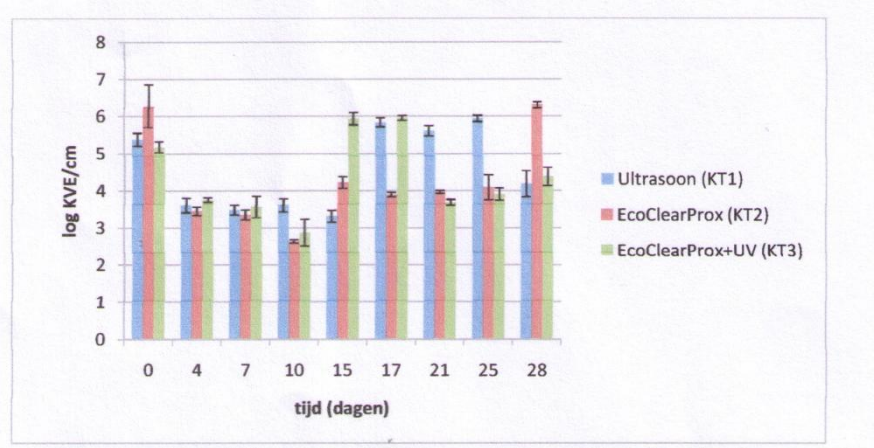
Regarding the treatment in CT3 compared to CT1 and CT2 it was clearly noticeable only a small increase of planktonic bacteria was observed. This is possible due to residual concentrations of peroxide of the treatment until day -4 that somewhat controlled the regrowth of planktonic bacteria. Furthermore this cooling tower was thoroughly cleaned just before the start of the experiments, which possibly affected the initial growth of planktonic bacteria.

On day 4 it was observed the number of planktonic bacteria slightly decreased below a level of 3 log CFU/ml. Due to a defect at the dosing pump the hydrogen peroxide was only dosed from day 7. This means the reduction on day 4 is only due to the UV treatment. After dosing from day 7 there was an additional reduction to a level of 1.6 log CFU/ml observed from day 10. On day 15 there was again a disturbance with the dosing pump, which explains the significant regrowth of planktonic bacteria. Also in the circuit of CT3 the peroxide dose was reduced by a factor 10. This resulted again in a slight increase.

3.2 Effectiveness for the treatment of biofilm.

Simultaneous to the sampling for planktonic bacteria, the biofilm associated bacteria were followed by means of biofilm monitors. After the installation of the biofilm monitors (day -4) the biofilm was able to grow during 4 days in the biofilm device. After sampling on day 0 it appeared in all three cooling water systems the biofilm had grown to about 5 – 6 log CFU/cm2. After sampling on day 0, disinfection in the 3 circuits was started again and the effect of US, hydrogen peroxide and the combination of hydrogen peroxide/UV on the biofilm was examined.

US resulted after 4 days of treatment in a reduction of 2 log CFU/cm2 to a level of 3.5 log CFU/cm2 (figure 3). Afterwards the number of biofilm associated bacteria remained relatively constant. This illustrates US is capable of partially removing biofilm and controlling it to an acceptable level.



Figuur 3: Resultaten van biofilm-geassocieerde bacteriën over een periode van 28 dagen. De waarden zijn weergegeven in kolonievormende eenheden (KVE) per cm². De foutenbalken geven de standaardfout weer.

After sampling on day 15 the US reactor was switched off, after which the biofilm could redevelop. On day 17 the number of biofilm associated bacteria had increased to 6 log CFU/cm2. On day 28 again a decrease in biofilm associated bacteria was observed.

Also the treatment with hydrogen peroxide (CT2) resulted on day 4 in a significant decrease of biofilm associated bacteria. A maximum reduction of 3.6 log CFU/cm2 was observed on day 10. On day 15 again an increase was noticed. This is probably caused by a fault in the dosing pump which was identified on day 15. Furthermore the water in the condenser was not in circulation before the sampling, which could also have had an influence on the biofilm. The dosing pump was repaired and on day 15 the peroxide dose was cut by a factor of 10. Afterwards first the number of biofilm associated bacteria remained relatively constant. After day 25 a significant increase was observed.

In CT3 a combination of hydrogen peroxide with UV was applied. During the experiment frequently problems were noticed with the dosing pump and the water draining control, which complicates interpretation of the results. The combination of hydrogen peroxide and UV caused a significant decrease of 1.5 log CFU/cm2 in the number of biofilm associated bacteria on day 4, to a level of about 3.6 log CFU/cm2. This level is comparable to the level of the other cooling towers. On day 7 this amount remained relatively constant. However, on day 7 it was noticed that the dosing pump did not work properly and therefore until then no hydrogen peroxide had been injected. The effect of UV treatment alone resulted in a comparable effect with US or hydrogen peroxide. On day 7 the dosing pump was repaired, which could explain an additional reduction of 0.7 log CFU/cm2 in the number of biofilm associated bacteria on day 10. On day 15 a very large increase in the biofilm was observed. This increase was associated with a very large increase in conductivity, which possible influenced the results. From day 21 the number of biofilm associated bacteria decreased again

to a level of approximately 3.7 log CFU/cm2. On day 28 the level of biofilm in the cooling tower with hydrogen peroxide alone was significantly higher than in the tower with combined treatment of peroxide and UV. This could indicate the combination of peroxide and UV is capable of controlling biofilm growth at 10x lower dose, contradictory to the peroxide treatment alone. The hydrogen peroxide consumption on day 28 was in CT3 significantly lower than in CT2, while the number of biofilm associated bacteria remained under control, contradictory to CT2.

3.3 Follow up of the other parameters

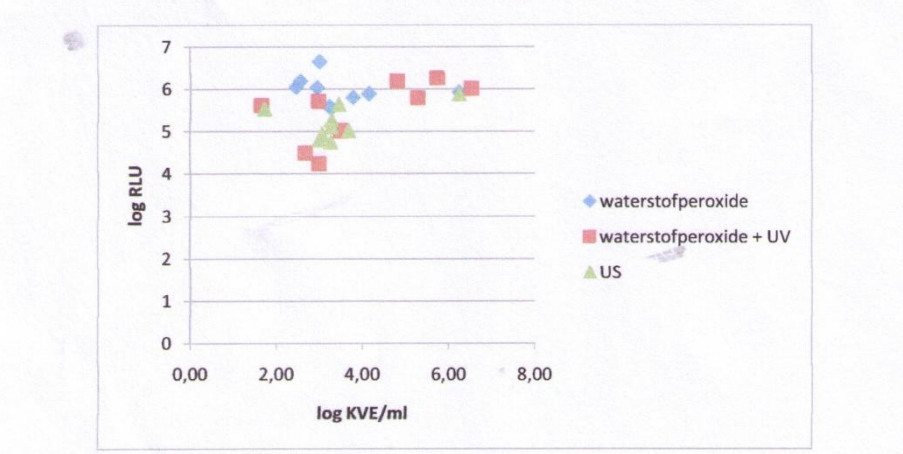
3.3.1 ATP measurements

ATP-measurements are often used as fast and simple to use alternative for classic microbiological units. Adenosinetriphosphate (ATP) is a chemical compound rich in energy which is present in living cells and is therefore a good measure for the amount of active biomass in a sample. The ATP determination is based on the reaction of luciferine with the enzyme luciferase, a reactor which occurs in the presence of free ATP. As consequence of this reaction, light is emitted at a wavelength of 562 nm. This signal is measured by means of a luminosity meter and expressed in relative light units (RLU). Based on this, the amount of ATP (expressed in pictogram pg) in the samples is calculated using an ATP calibration curve.

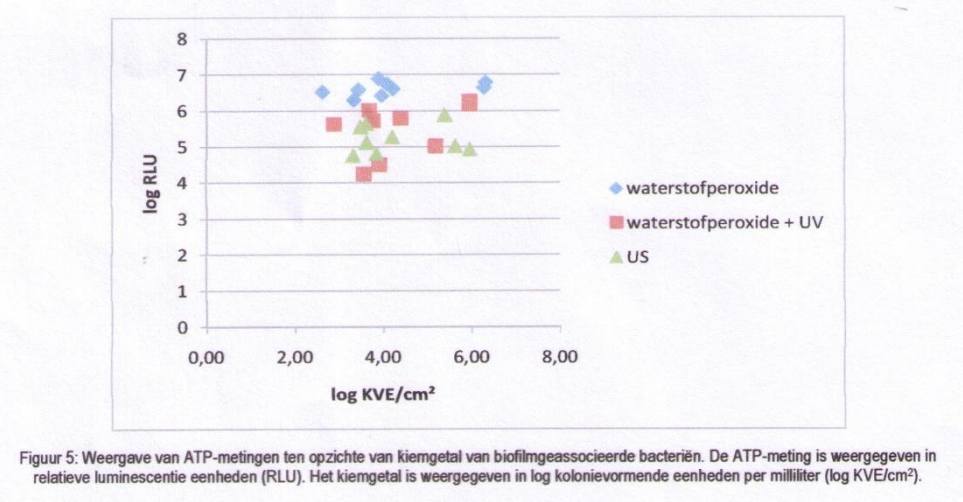
Within this case study it was also tried to correlate the ATP-measurements to a classic plate method (figure 4 and 5). For this the water samples were analyzed by means of the plate count as described above and compared to the ATP measurements.

For the planktonic nor for the biofilm associated bacteria no correlation was found between the ATP content and the observed CFU/ml or CFU/cm2. This indicates the ATP measurements as examined within this case study are not a scientifically correct method to follow the microbial population.

These results confirm the results obtained in a previous IWT project (TETRA/50073 – Treatment of water in closed circuits: ultrasonic versus traditional techniques).



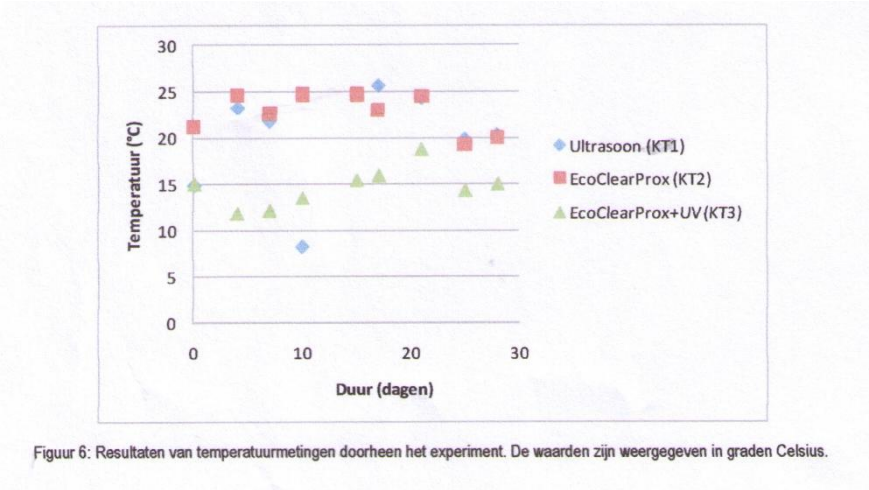
Figuur 4: Weergave van ATP-metingen ten opzichte van kiemgetal van planktonische bacteriën. De ATP-meting is weergegeven in relatieve luminescentie eenheden (RLU). Het kiemgetal is weergegeven in log kolonievormende eenheden per milliliter (log KVE/ml).



Figuur 5: Weergave van ATP-metingen ten opzichte van kiemgetal van biofilmgeassocieerde bacteriën. De ATP-meting is weergegeven in relatieve luminescentie eenheden (RLU). Het kiemgetal is weergegeven in log kolonievormende eenheden per milliliter (log KVE/cm²).

3.3.2 Temperature

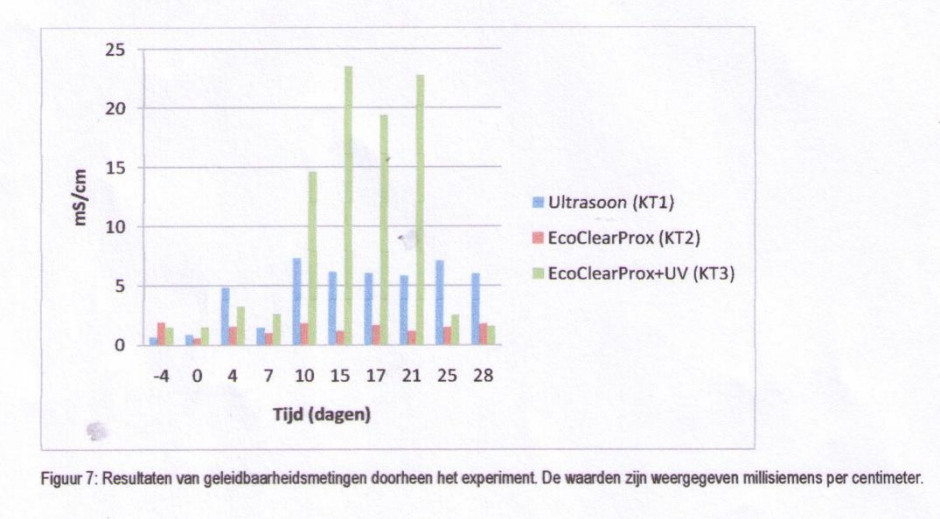
During the experiments, temperature in the cooling towers was monitored. This can have an influence on the microbial population present within the cooling towers. During the first 15 days, temperature within the cooling towers remained within an acceptable range. On day 7 a temperature decrease was observed in CT1. This is explained by works on the condenser, which exposed the temperature sensor to the air. During the second experiment the temperature decreased with about 5 degrees between day 0 and 4. This is caused by a lower ambient temperature. Later on temperature remained fairly constant.



Figuur 6: Resultaten van temperatuurmetingen doorheen het experiment. De waarden zijn weergegeven in graden Celsius.

3.3.3 Conductivity

Within the parameters that were monitored, the conductivity is an important factor for the water drain control of the cooling towers. For this reason this influencing factor was monitored throughout the experiment. The observed conductivity indicated from day 10 problems existed in CT3: suddenly the conductivity fluctuated around 20 mS/cm. Because of this increased conductivity no conclusions could be made for the second experiment for this cooling tower. For CT1 after the cleaning of the condenser also a slight increase of conductivity was observed. The conductivity of CT2 remained nearly constant throughout the whole experiment.



Figuur 7: Resultaten van geleidbaarheidsmetingen doorheen het experiment. De waarden zijn weergegeven millisiemens per centimeter.

4 Conclusions

Based on the results obtained in the case study following preliminary results could be made:

- The dose of 200ml of hydrogen peroxide per 2 hours is capable to annihilate and control both planktonic and biofilm associated bacteria.
- Reduction of this dose by a factor of 10 results in regrowth of the bacteria and the biofilm.
- Combination of UV with hydrogen peroxide results in an additional effect for both biofilm associated and planktonic bacteria
- US is capable of controlling both biofilm-associated and planktonic bacteria. With this method a stable level is reached which remains constant over time.

Due to repeated problems with the dosing pump and the water draining control, the results need to be interpreted carefully. To further support these conclusions, further research is required.