

Report.

Determination of the effectiveness of the product CoolPuck[®] in the mitigation of biofilms in HVAC-utilities

Arnhem, 17 October 2007



50762957-TOS/MEC 07-9125

**Determination of the effectiveness of the
product CoolPuck® in the mitigation of
biofilms in HVAC-utilities**

Arnhem, 17 October 2007

Author L.P. Venhuis
KEMA Technical & Operational Service

By order of Special Water Europe BV

author : L.P. Venhuis	07-10-17	reviewed : M.C.M. Bruijs	07-10-
B 25 pages 1 annexes	WSc	approved : A.G.L. Zeijesink	07-10-



© KEMA Nederland B.V., Arnhem, the Netherlands. All rights reserved.

It is prohibited to change any and all versions of this document in any manner whatsoever, including but not limited to dividing it into parts. In case of a conflict between the electronic version (e.g. PDF file) and the original paper version provided by KEMA, the latter will prevail.

KEMA Nederland B.V. and/or its associated companies disclaim liability for any direct, indirect, consequential or incidental damages that may result from the use of the information or data, or from the inability to use the information or data contained in this document.

The contents of this report may only be transmitted to third parties in its entirety and provided with the copyright notice, prohibition to change, electronic versions' validity notice and disclaimer.

CONTENTS

	page
SUMMARY	4
1 Introduction	5
1.1 Effective mitigation of microbial activity and biofilms	7
2 Effectiveness of the product coolpuck in optimazing microbial mitigation	9
2.1 Effectiveness of the CoolPuck® product itself	9
2.2 Effectiveness of the CoolPuck® product in combination with Na-hypochlorite.....	12
3 Discussion, Conclusions and Recommendations	16
REFERENCES	18
Appendix I Applied detection methods for effectiveness determination	21

SUMMARY

By order of the company Special Water Europe BV, KEMA tested the effectiveness of the additive CoolPuck® in its laboratory using a small scale test assembly based on the Dutch Standard for assessing the performance of treatment programmes for open recirculating cooling water systems (NEN 7420).

The product CoolPuck® is a man and environmental friendly dispersant, which implies that the product itself has no microbial biocidal effect. The mitigation effect should be introduced by an oxidative biocide, which in industrial application should be dosed in combination with the CoolPuck® product to become much more effective in mitigating microbial settlement, so called biofilms. Effective biofilm mitigation in recirculation cooling water systems and HVAC-utilities is vital for operational management without economic losses, jeopardizing material structural integrity by corrosion or human health risks due to pathogenic microbes.

KEMA tested the effectiveness of the product CoolPuck® for application in industrial cooling water systems and HVAC-utilities by stimulating biofilm formation in a small scale cooling water system equipped with heat exchanger and cooling tower, using River Rhine surface water. For accurate mitigation determination a biofilm activity monitoring system called BioGeorge and a deposit fouling monitor called DATS were applied, extended with a metabolic enzymatic method based on Adenosine-5'-triphosphate (ATP). The effectiveness of the CoolPuck® product was tested with and without Na-hypochlorite addition.

From the tests it is obvious that the CoolPuck® product, as additive to Na-hypochlorite, increases the mitigation activity of the Na-hypochlorite. A batch Na-hypochlorite dosing of 0.6 mg CL₂/L which was not effective in biofilm mitigation, became significantly effective after 6.0 mg/L CoolPuck® was added approximately 24 hours before Na-hypochlorite dosing. Dosing of the CoolPuck® product showed no effect on the initial pH and conductivity of the recirculation water and applying the CoolPuck® product itself resulted in precipitation of organic compounds and microbes from the water phase.

For the application of the CoolPuck® product as additive to Na-hypochlorite in industrial cooling water systems and HVAC-utilities, it is recommended to protect the cooling tower from direct gale exposure, so no foam will blow away. This can cause great concern to the neighbouring public. It is also recommended to mitigate the formation of biofilms preventative, this to minimize the chance for health risks for employees and neighbours related to pathogens like *Neagleria spp.*, *Acanthamoeba spp.*, and *Legionella pneumophila*.

1 INTRODUCTION

Surface water used for cooling industrial processes contains a wide scale of organic and inorganic substances as well as microbes. Surfaces exposed to surface water will directly be conditioned by adsorption of the organic materials and bacteria. Planktonic waterborne bacteria migrate to surfaces and attach by excretion of exopolymeric substances (xPS), forming gel like matrices in which the bacteria are enclosed. Eventually, areas of these matrices will join and form a contiguous biofilm. Microbial multiplication in the biofilm, incorporation of organic material and microbes from the water phase lead to an increase in the size and stability of the biofilm (see figure 1).

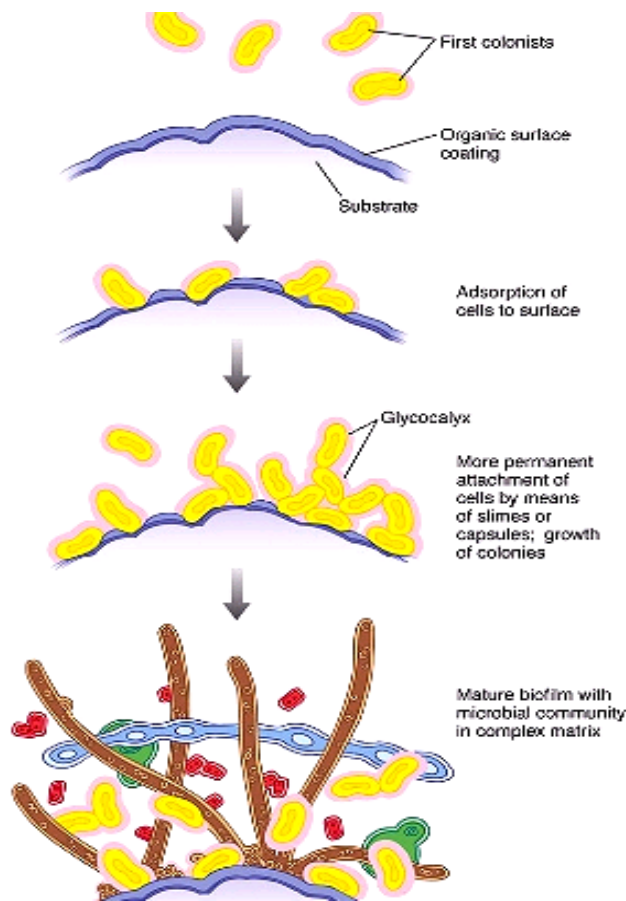


Figure 1 Overview of biofilm formation

By buffering their bacterial occupants from the bulk water, biofilms provide shelter and protection for microbes against varying physical environmental circumstances and biocides (Carpentier & Cerf, 1993). The biofilm also acts as a diffusive barrier, by which an oxygen gradient is developed. The oxygen is used in the surface layer of the biofilm, leading to an anaerobic environment on the conditioned surface underneath. As these various micro environments become established, there is an increased development of specific microbes that initiate and promote corrosion of metallic surfaces. Flemming (1994) showed that rather 'clean' cooling water utilities contain biofilms in which microbes concentrations are 5 till 6 times larger compared to microbes present in the planktonic water phase.

The following operational problems in cooling water utilities occur due to increased biofilm development.

– ***Decreased efficiency of heat transfer***

Deposition of biofilms on the walls of a heat exchanger impairs the heat transfer and stimulates the deposition of calcium salts. The thermal conductivity of a biofilm ($0.6 \text{ W m}^{-1} \text{ K}^{-1}$) is comparable with that of water (Flemming, 1994), thereby leading to a much higher heat transfer resistance.

– ***Increase of cooling water pumping costs***

Biofilms reduces the tube inside diameter and, hence, the effective cross-sectional area for flow. To achieve the same flow, the tube-side velocity must increase. This results in a pressure drop increase (Costerton et al., 1987), requiring greater pumping power to overcome the additional system head.

– ***Increased risk of macro-fouling***

Biofilms stimulate the settlement of macro-organisms like mussels, oysters and barnacles (Flemming & Schaule, 1994). The settlement of these macro-organisms leads to a substantial decrease in diameter of cooling water piping, thereby leading to even higher pumping costs and eventually leading to a forced outage for removal of macro-fouling debris.

– ***Increased corrosion***

Deposition of microbes on metallic surfaces can lead to an increase in the speed of corrosion of these metals. This type of corrosion is called microbial influenced corrosion (MIC). Through-wall penetration of piping and heat exchanger tubing as localised corrosion, at rates 10 to 1000 times more rapid than those normally encounters, can result. Accelerated corrosion may occur as the result of corrosive metabolic products such as sulphides, ammonia, organic acids, or mineral acids. Application of noble materials (such as Monel) and biotoxic materials (such as copper) in practice does not give any guarantee of MIC resistance, with the exception of titanium.

– **Increase of health risks**

Biofilms can also serve as hosts for pathogenic microbes. Mainly in open recirculation utilities with cooling tower, the activity and growth (increase in numbers) of microbes (by an increased water temperature and sufficient oxygen and nutritional supply) can increase to such an extent that it can cause increased health risks for employees and neighbours. The threat is particularly acute as biofilms can suddenly slough off the substrate and be released in massive amounts into an area such as a cooling tower, where the pathogens can become airborne through aerosols and come in contact with people. Human pathogens often connected with cooling towers are *Neagleria spp.*, *Acanthamoeba spp.*, and *Legionella pneumophila* (Tyndall, 1993). Numerous outbreaks of Legionnaires' disease have been tied to outbreaks from cooling towers and HVAC (air conditioning) units.

1.1 **Effective mitigation of microbial activity and biofilms**

Microbial settlement, so called biofilms, in cooling water utilities is often mitigated by chemical water treatment. Biocide dosing is mostly carried out by general dosing regimes, which however in most cases are not attuned to the mitigation of biofilms for the specific conditions of a specific cooling water system. However, it is the sessile organism, those attached to metallic surfaces, that influence corrosion and which are far less sensitive to biocides than planktonic microbes in the water phase. Concentrations of some biocides must be elevated 10 to 100 times in order to reach the same effectiveness (Holah, 1992). Little *et al.* (1994) showed that there is no relationship between planktonic counts and sessile counts. By controlling biofilm formation corrosion can be prevented and thick biofilms on piping or heat exchanger tube surfaces can be eliminated.

For effective mitigation, present general dosing regimes must be many times higher than what is necessary to control biofilms. This results in extra costs for biocides, unnecessary environmental impact, and increased corrosion by the excess of oxidising biocides. Special Water Europe B.V. developed an innovative product called CoolPuck[®]. This product is man and environmental friendly. It is a dispersant which implies that the product itself has no microbial biocidal effect. This mitigation effect should be introduced by an oxidative biocide, which in industrial application should be dosed in combination with the CoolPuck[®] product to become much more effective.

By order of the company Special Water Europe BV, KEMA tested the effectiveness of the additive CoolPuck[®] in its laboratory using a small scale test facility based on a Dutch

Standard for assessing the performance of treatment programmes for open recirculating cooling water systems (NEN 7420). This test assembly includes a recirculation cooling water system equipped with heat exchanger and cooling tower (figure 2). To monitor the effectiveness of mitigation activity, KEMA applied a specific biofilm activity monitoring system called BioGeorge and a less specific deposit fouling monitor called DATS. Both systems have shown to be effective for predicting biofilm formation, and are applied as continuous measuring devices for monitoring biocide effectiveness in industrial cooling water systems and HVAC-systems (Licina *et al.*, 1995; KEMA, 2001). For microbial detection of the water phase an enzymatic method based on Adenosine-5'-triphosphate (ATP) was applied. This report describes the results of the effectiveness of the product CoolPuck[®] as additive for the mitigation of biofilms in HVAC-utilities. The effectiveness of the product was tested with and without Na-hypochlorite addition (chapter 2) and conclusions are presented in chapter 3.

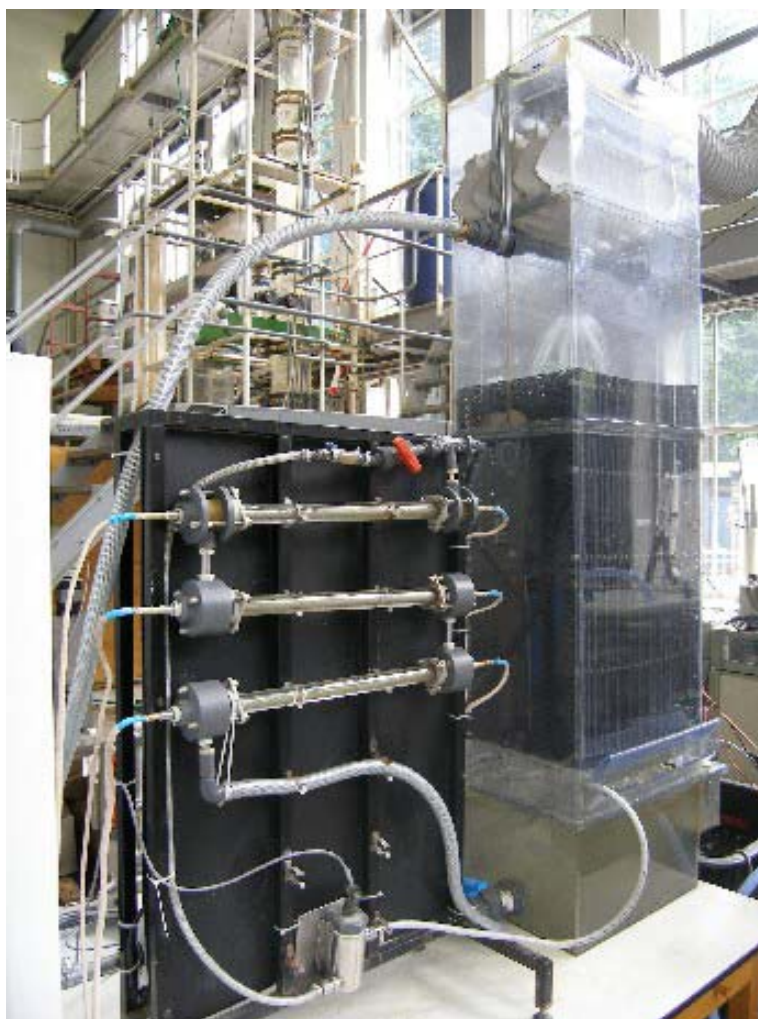


Figure 2 KEMA test facility

2 **EFFECTIVENESS OF THE PRODUCT COOLPUCK IN OPTIMIZING MICROBIAL MITIGATION**

For the determination of the effectiveness of the CoolPuck[®] product two tests were carried out in the KEMA test facility. In the first test the effect of the CoolPuck[®] product itself was determined. In the second test, the effectiveness of the CoolPuck[®] product in combination with Na-hypochlorite was tested.

The KEMA test facility used is designed for standard NEN 7420 - a test method for assessing the performance of treatment programmes for open recirculation cooling water systems. This test facility comprises a heat exchanger section and a cooling tower section in which surface water is recirculated. The core of the test assembly is a heat exchanger section comprising six test tubes. In these test tubes, heat is generated electrically by electrical heating elements which, via the tube wall, is dissipated to the surrounding water. This heated water is pumped from the heat exchanger to the cooling tower section, in which through partial evaporation of water, the heat absorbed is subsequently released to the environment

The KEMA test facilities volume was expanded by an 850 Liter vessel to provide more buffering capacity. The total water volume of the test facility during the tests is approximately 1 m³ with a recirculation velocity of 1000 – 1700 Liter per hour. For the accurate monitoring of biofilm formation and effectiveness of the product CoolPuck[®] a BioGeorge Activity Monitoring System and a DATS Deposit Fouling Monitor are installed within the test facility. Detailed information about these monitoring systems is presented in appendix A. Before starting the different tests, the overall test assembly will be cleaned in order to prevent contamination with products from previous tests or undesirable microbiological fouling.

2.1 **Effectiveness of the CoolPuck[®] product itself**

For the determination of the mitigation effectiveness of the CoolPuck[®] product itself, the product CoolPuck[®] was dosed in this experiment without Na-hypochlorite dosing. The KEMA test facility was operated from 01/08/07 till 29/08/07 for biofilm development, using surface water from the Rhine River. The test facility was operated with a constant flow velocity of 0.6 – 0.8 m/sec and a water temperature of 32°C. During the experiment water parameters as conductivity and pH were determined daily. At 29/08 significant biofilm settlement was available in the KEMA test facility detected by the DATS monitor (increase in HTR, figure 3) and BioGeorge monitor (increase in biofilm activity, figure 4).

At 29/08 (9:20 hours), a representative amount of CoolPuck[®] product (6.0 gram per 1000 Liter) was added to the cooling tower basin of the test facility. During the next days the microbial content of the water and the amount of biofilm was also monitored by ATP-measurement. Results show a significant decrease in planktonic microbial activity approximately 24 hours after CoolPuck[®] dosing (table 1). This decrease of planktonic microbial activity in the recirculation water was attended by sedimentation building up in the cooling tower basin. After CoolPuck[®] dosing no increase of free ATP was detected; the decrease of planktonic microbial activity was the result of both decreases in free and total ATP-measurements. CoolPuck[®] dosing results showed no mitigation effect on the biofilm, no significant decrease of heat transfer resistant by the DATS monitor (figure 3) and no significant decrease in biofilm microbial activity by ATP measurement was detected (table 1). After some period after the CoolPuck[®] dosing the HTR increase continues however after three days the HTR stabilised. During this HTR stabilisation period the BioGeorge system was detecting a decrease in biofilm activity (figure 4).

Table 1 Overview of pH, conductivity and ATP results

Date	Time	Action	pH	Conductivity (µS/cm)	ATP (RLU/mL) - water phase -	ATP (RLU/cm ²) - biofilm -
17/08/2007	14:00		8,54	564	12217	-
24/08/2007	13:30		8,32	777	328755	-
27/08/2007	14:15		8,28	836	163803	22245
29/08/2007	8:45		8,45	919	178637	21543
	9:20	dosing product Coolpuck[®]				
	9:30		8,51	929	167770	-
	10:00		8,62	932	152975	-
	10:45		8,58	936	121085	-
	13:15		8,53	939	71285	-
	14:15		8,54	943	55650	-
	15:30		8,56	946	40756	-
30/08/2007	9:00		8,58	990	31260	21838
31/08/2007	8:50		8,65	874	56273	20472
02/09/2007	17:00		8,68	1067	60410	22169

- = no measurement performed

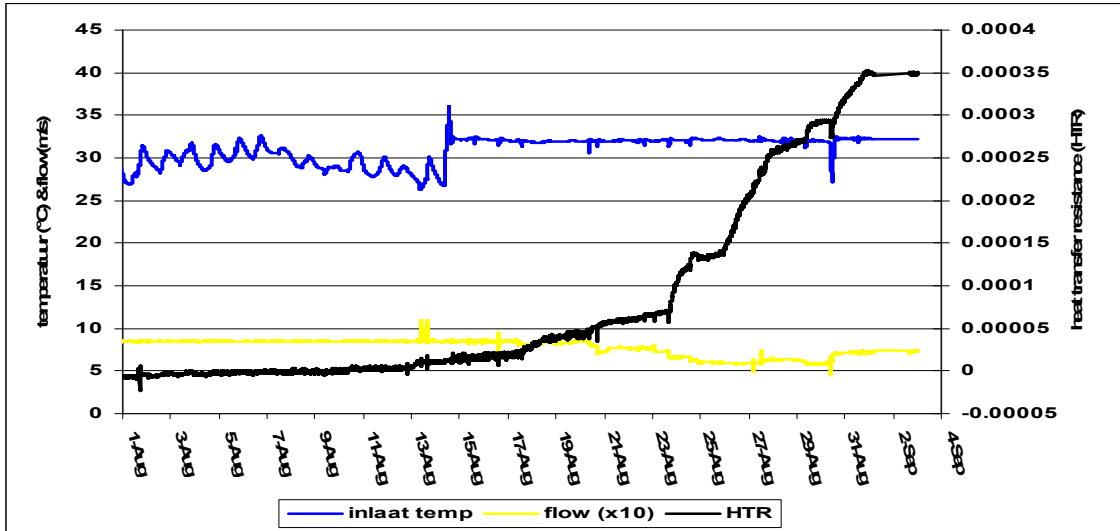


Figure 3 DATS monitors' flow (multiplied by 10); inlet temperature; and calculated heat transfer resistance (HTR). At August 15 the cooling of the cooling tower was switched from 'forced air' to 'natural draft' which resulted in a stable water temperature without day and night differences

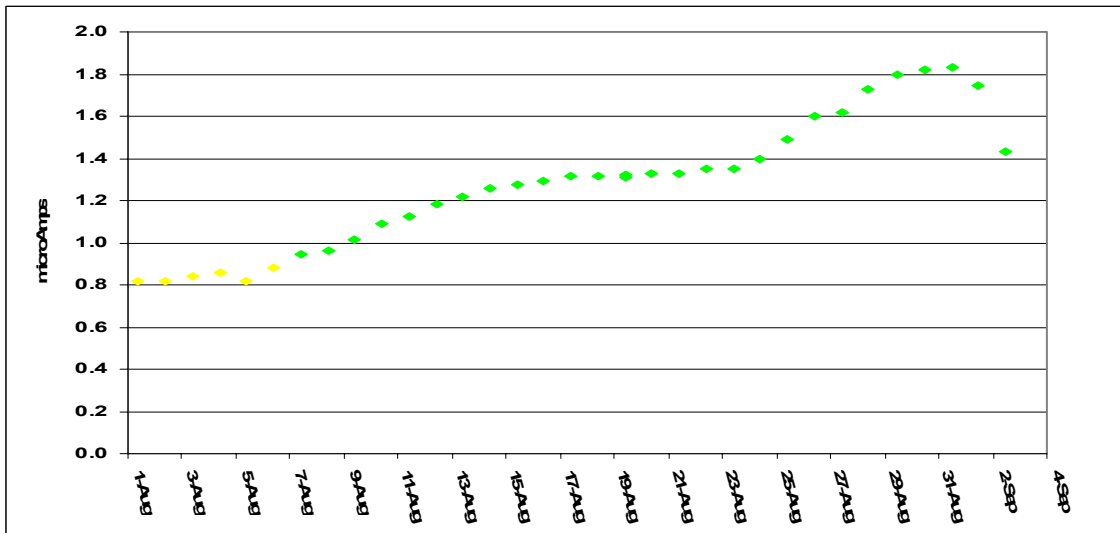


Figure 4: BioGeorge monitoring system 'Applied current' results. (system alarm indication was not set)

2.2 Effectiveness of the CoolPuck[®] product in combination with Na-hypochlorite

Determination of the effect of the product CoolPuck[®] with Na-hypochlorite dosing was tested in a second experiment. The test assembly was cleaned in order to prevent contamination with product or undesirable microbiological fouling. Development of biofilm was stimulated during the period 14/09 – 03/10, using surface water from the Rhine River. The test facility was operated with a constant flow velocity of 1.2 – 1.3 m/sec and a water temperature of 32°C. During the experiment water parameters as conductivity and pH were determined daily. At 3/10 significant biofilm settlement was available in the test facility detected by the DATS monitor (increase in HTR, figure 5) and BioGeorge monitor (increase in biofilm activity, figure 6)

At 3/10 (9:30 hours) a batch amount of Na-hypochlorite was dosed within the cooling tower basin to provide a Na-hypochlorite concentration within the system of 0.6 mg/L free oxidant (FO). This concentration lasted for three minutes because of the demand of the cooling water. The Na-hypochlorite total reduced oxidant (TRO) however lasted for one hour. TRO-concentration at the start was approximately 0.85 mg/L. During this period an increase in of free ATP was detected. Results show a decrease in planktonic microbial activity but no mitigation effect in biofilm activity (table 2). Na-hypochlorite dosing results showed no mitigation effect on the biofilm, no significant decrease of HTR by the DATS monitor (figure 5) and no significant decrease in biofilm microbial activity by ATP measurement was detected (table 2). However, the BioGeorge system was detecting a minor decrease in biofilm activity (figure 6).

At 3/10 (13:10 hours) a representative amount of CoolPuck[®] product (6.0 gram per 1000 Liter) was added to the cooling tower basin of the test facility. Results show again a decrease in planktonic microbial activity approximately 19 hours after CoolPuck[®] dosing (see table 2). This decrease of planktonic microbial activity in the recirculation water was again attended by small sedimentation building up in the cooling tower basin. After CoolPuck[®] dosing no increase of free ATP was detected; the decrease of planktonic microbial activity was the result of both decreases in free and total ATP-measurements. CoolPuck[®] dosing results showed no mitigation effect on the biofilm, no significant decrease of heat transfer resistant by the DATS monitor (figure 5) and no significant decrease in biofilm microbial activity by ATP measurement was detected (table 2).

Table 2 Overview of pH, conductivity and ATP-results

Date	Time	Action	pH	Conductivity ($\mu\text{S/cm}$)	ATP (RLU/mL) - water phase -	ATP (RLU/cm ²) - biofilm -
14/09/2007	16:00		8,36	523	-	-
20/09/2007	8:30		8.58	649		
28/09/2007	8:00		8,69	854		
01/10/2007	15:30		8.75	957		
02/10/2007	13:00		8.68	1178	7710	
	15:00		8.54	1136	7225	
03/10/2007	9:20		8.54	1032	289285	10998
	9:30	dosing 0,6 mg/L Na-hypochlorite (FO)				
	9:40		-	-	207290	
	9:50		8.54	974	142840	
	10:00		-	-	100200	
	10:15		8.55	973	77010	
	10:25				67580	
	10:40		8.55	976	59470	10767
	13:00		8.58	977	65183	
	13:10	dosing product CoolPuck®				
	13:20		8.65	987	64080	
	14:00		8.65	990	64897	
	14:30		8.64	991	64535	
	15:00		8.63	993	62115	
	16:00		8.63	994	61805	
	17:00		8.62	996	62385	
04/10/2007	8:15		8.61	1009	39753	9128
	8:50	dosing 0,6 mg/L Na-hypochlorite (FO)				
	9:30		8.58	1015	0	
	11:30		8.53	1012	0	
	12:45		8.55	1004	0	25
	13:10	dosing product CoolPuck®				
	14:00		8.59	1013	0	
	16:15		8.59	1015	4015	
05/10/2007	10:00		8.62	1167	69470	3207
	10:30	dosing 0,6 mg/L Na-hypochlorite (FO)				
	10:40		8.64	1173		
	11:15		8.66	1170		
	12:00		8.65	1175	0	9

- = no measurement performed

At 4/10 (8:50 hours) a batch amount of Na-hypochlorite was dosed again within the cooling tower basin to provide a Na-hypochlorite concentration within the system of 0.6 mg/L free oxidant (FO). This FO-concentration lasted for seven minutes because of the demand of the cooling water. The Na-hypochlorite total reduced oxidant (TRO) however lasted for two hours. TRO-concentration at the start was approximately 0.85 mg/L. After Na-hypochlorite dosing a significant foam development occurred within the cooling tower basin and the buffer vessel (figure 7). The dosing resulted in a total mitigation of planktonic microbial activity in the recirculation water and a significant mitigation of biofilm microbial activity (see table 2). Also a significant HTR reduction was detected by the DATS monitor (figure 5). After this dosing the BioGeorge monitoring system detected a total mitigation of biofilm activity (figure 6).

Repeated procedure of CoolPuck® product dosing at 4/10 (13:10 hours) and batch amount of Na-hypochlorite dosing at 5/10 (10:30 hours) resulted in a total biofilm mitigation, detected by ATP-measurements and DATS monitoring system (figure 5).

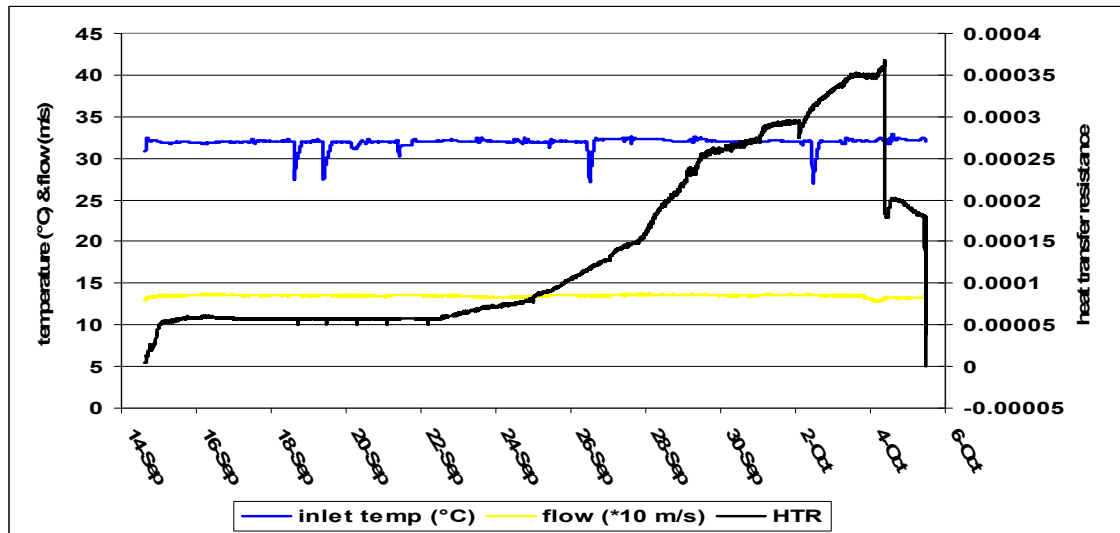


Figure 5 DATS monitors' flow (multiplied by 10); inlet temperature; and calculated heat transfer resistance (HTR)

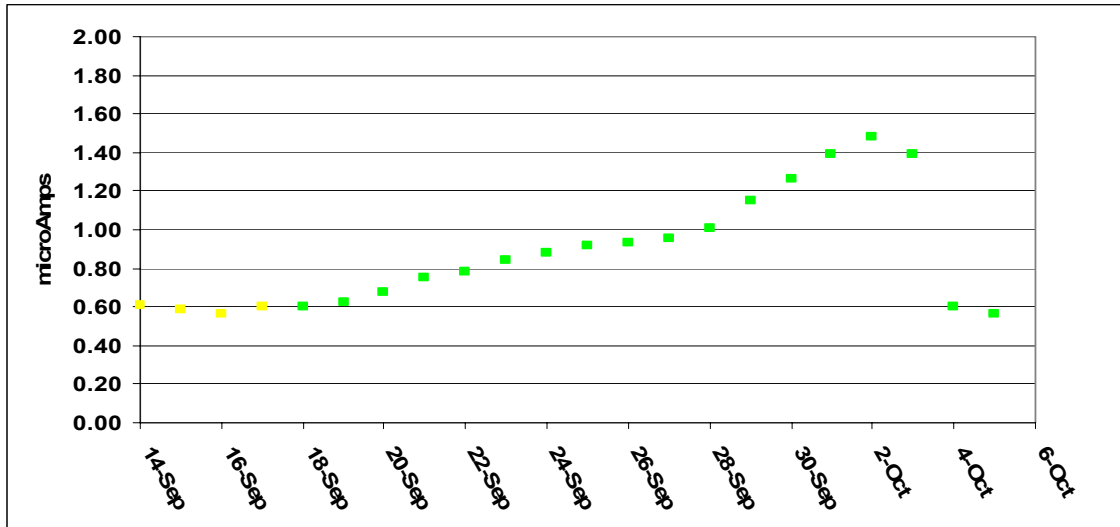


Figure 6 BioGeorge monitoring system 'Applied current' result (alarm indication was not set)

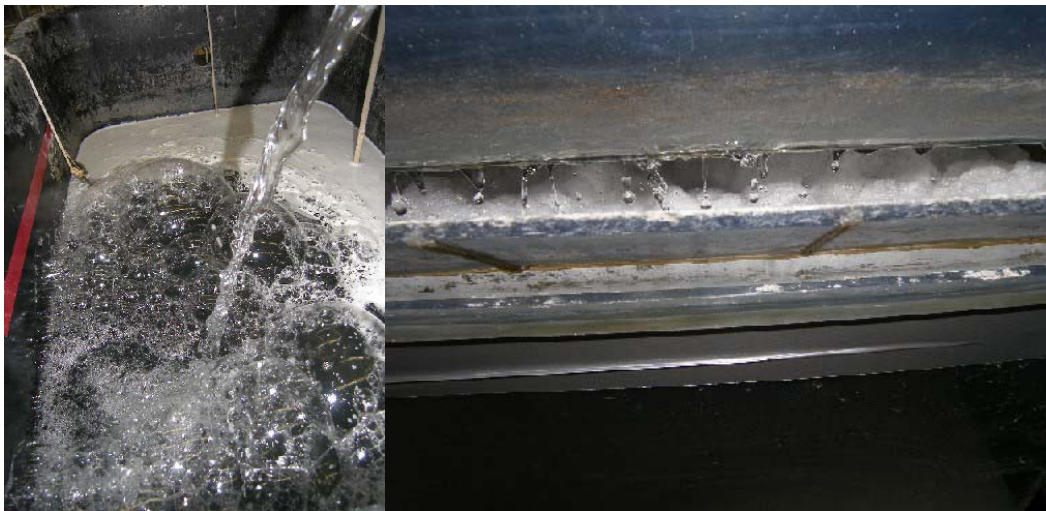


Figure 7 Pictures of foam development within the buffer vessel (left) and within the cooling tower basin (right)

3 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

From the effectiveness test results of the product CoolPuck® preformed by KEMA in a small scale open recirculation cooling water system, the following conclusions can be made:

The KEMA test facility designed for standard NEN 7420 - a test method for assessing the performance of treatment programmes for open recirculation cooling water systems – has been operated according test specifications during the test period. Significant biofilm formation was applied for accurate determine of the effectiveness of the product CoolPuck®.

Using the CoolPuck® product in the KEMA test facility showed no significant effect on the initial pH and conductivity of the recirculation water.

When applying the CoolPuck® as a single product in a cooling tower system, organic compounds and microbes from the water phase will precipitate in the parts of the recirculation system with low water velocity or stagnant zones. This precipitation results in a more clear water phase. According to the performed ATP-measurements, the CoolPuck® product itself seems to have no direct mitigation effect on attached biofilms. However, after three days the DATS monitor, which is detecting organics and biofilm, showed a stable HTR-value and the BioGeorge monitoring system, which is detecting the microbial activity at a metal surface, detected a decreasing signal. From the different monitoring values it can be concluded that although the CoolPuck® product itself seems to have no direct effect on the amount of attached microbes in the biofilm, the product however seems to destroy the 'structure' and 'viability' of the biofilm. This because the microbial growth and microbial processes which are responsible for causing Microbial Influenced Corrosion (MIC) seems to stagnate. From the performed tests it is not known if this result is a direct effect from the CoolPuck® product, or an indirect site-effect caused by the CoolPuck® product.

Effective biofilm mitigation in a cooling tower system can be applied by dosing the CoolPuck® product in addition to Na-hypochlorite. During the test a batch Na-hypochlorite dosing was unable to mitigate the biofilm significantly, however addition of 6.0 mg/L CoolPuck® approximately 24 hours before Na-hypochlorite dosing, increases the mitigation effectiveness of the batch Na-hypochlorite dosing to biofilm significantly. Two dosing procedures were necessary to clean the entire open recirculation cooling water system from microbial settlements.

Recommendations

It is recommended that in case of applying the CoolPuck® product as an additive to Na-hypochlorite in heavily fouled HVAC-utilities, the cooling towers should be protected from direct gale exposure. This is because major foam production will occur as rest product of microbial deterioration. Although the CoolPuck® product is man and environmental friendly, foam when blown away from a HVAC cooling tower system from a building roof can be of great concern to the neighbouring public.

By using the CoolPuck® product as an additive to Na-hypochlorite in HVAC-utilities and industrial cooling tower systems, it is still recommended to mitigate the formation of biofilms preventative, this to minimize the chance for health risks for employees and neighbours related to human pathogens like *Neagleria spp.*, *Acanthamoeba spp.*, and *Legionella pneumophila*.

REFERENCES

BORENSTEIN, S.W. & LICINA, G.L., 1998. Detecting microbiologically influenced corrosion using innovative monitoring and inspection techniques. Bron: Internet: <http://www.buglady.com/hotnews.html>.

BUCHANAN, R.A. *et al.*, 1991. Fundamentals of coupled electrochemical reactions as related to microbially influenced corrosion, Microbially influenced corrosion and biodeterioration, ed. N.J. Dowling, University of Tennessee, 1991.

CARPENTIER, B. & Cerf, O., 1993. A review: biofilms and their consequences, with particular reference to hygiene in the food industry., J. Appl. Bacteriol. vol. 75, pp. 499-511.

COSTERTON, J.W. *et al.*, 1987. How bacteria Stick, Scientific American 238, pp. 86-95.

DEXTER, S.C. & Zhang, H.-J., 1991. "Effect of biofilms, sunlight, and salinity on corrosion potential and corrosion initiation of stainless alloys", EPRI NP-7275, EPRI, Palo Alto, CA, May, 1991.

DUQUETTE, D.J. & RICKER, E.R., 1986. Electrochemical Aspects of Microbiologically Induced Corrosion. Biologically Induced Corrosion, NACE-8, National Association of Corrosion Engineers, Houston, TX, 1986, pp. 121-130.

FLEMMING, HC., 1994. Biofouling, Microbial deterioration of materials, Werkstoffe und Korrosion Vol. 45, pp. 29-39.

FLEMMING & SCHAULE, 1994. Bekämpfung von biofouling in wässrigen Systemen.', Werkstoffe und Korrosion Vol. 45, pp.40-3.

GUEZENNEC. J., 1991. Microbially Influenced Corrosion and Biodeterioration (Eds. N.J. Dowling, M.W. Mittleman and J.C. Danko), 1991. University of Tennessee.

HERBERT, R.A., 1990. Methods for enumerating microorganisms and determining biomass in natural environments, Methods in Micobiology Vol. 22, pp. 1-39.

HOLAH, J.T., 1992. Industrial monitoring: hygiene in food processing. In: Melo, L.F., Bott, T.R., Fletcher, M., Capdeville, B., (eds.) Biofilms-Science and Technology. Kluwer Academic Publications, Dordrecht, The Netherlands, pp. 645-659.

KEMA, 2000a. (L.P. Venhuis) 'Optimization of Isothiazolinone in the mitigation of biofilm formation of a fresh water open cooling water system with cooling tower in the city of Gouda, the Netherlands', confidential KEMA report 50050839-KPS/MEC 00-5785 (Dutch language).

KEMA, 2000b. (L.P. Venhuis) 'Optimization of ozone in the mitigation of biofilm formation of a fresh water open cooling water system with cooling tower in the city of Rotterdam, the Netherlands', confidential KEMA report 99580060-KPS/MEC 00-6036 confidential Dutch language).

KEMA, 2001. (L.P. Venhuis) 'Mitigation of microbial activity and microbial influenced corrosion in the cooling water system of Hydro Agri Sluiskil BV the Netherlands', confidential KEMA report 50050936.000 01-6132 (Dutch language).

LICINA, G.J. & Nekoksa, G., Microbiologically Influenced Corrosion Testing (Eds. J.R. Kearns, B.J. Little), 1993. American Society for Testing and Materials, ASTM STP-1232, 118.

LICINA, G.J. & Nekoksa, G., 1995. "On-line monitoring of biofilm formation for the control and prevention of microbially influenced corrosion.", International Symposium on Microbially Influenced Corrosion, 8-10 May 1995, New Orleans, LA.

LICINA, G.J., Willertz, L.E., Swoyer, B.M., Tombaugh, R.S, The Ninth EPRI Service Water System Reliability Improvement Seminar, 1996. Electric Power Research Institute, Palo Alto, CA.

LICINA, G.J. & Venhuis, L.P., 2003. "Biocide optimization using an on-line biofilm detector", Journal of the international water conference at October 20 -24, 2002, Volume III, Issue 3, The conference Issue, pp.11 -16.

LITTLE, B.J. *et al.*, 1994. Tri-Service Conference on Corrosion.

NALEPA *et al.*, 1996. "Minimization of corrosion using activated sodium bromide in a medium-size cooling tower", Materials Performance, July, pp. 42-46, 1996.

NEKOKSA, G. & Gutherman, B., "Cathodic protection criteria for controlling microbially influenced corrosion in power plants", EPRI NP-7312, Electric Power Research Institute, Palo Alto, CA, 1991.

NEN 7420 'Industrial cooling water. Assessment of the performance of treatment programmes under standard conditions.', Netherlands Standard Institute, 1st edition, September 1996.

TYNDALL, R.L., 1993. Presence of pathogenic micro-organisms in power plant cooling waters. Final report for October 1, 1981, to June 30, 1983. ORNL Report Publication No. 2208.

APPENDIX I APPLIED DETECTION METHODS FOR EFFECTIVENESS DETERMINATION

For the determination of microbial mitigation effectiveness in industrial cooling water systems and HVAC-utilities, KEMA Nederland BV applies several detection methods. The methods used in this CoolPuck® effectiveness testing are the following and described in detail underneath.

- BioGeorge™ Biofilm Activity Monitoring System of the company Structural Integrity Associates, Inc., San Jose (CA), USA.
- DATS™ Deposit Fouling Monitoring System of the company Bridger Scientific, Inc., Sandwich (MA), USA.
- Adenosine-5'-triphosphate (ATP) testing kits of the company Celsis International BV, Landgraaf, The Netherlands.

The BioGeorge on-line monitoring system

The BioGeorge™ system is an electrochemical biofilm activity monitoring system (Licina *et al.*, 1993; Licina *et al.*, 1996) which has been developed, provides on-line and real-time indications of biofilm activity on typical metallic surfaces. It has been shown to be effective for indicating biofilm activity and, applied as a continuous device to measure biocide effectiveness (Nekoksa *et al.*, 1991). The BioGeorge™ system is operated in such way that biofilm formation is encouraged to form more rapidly on the probe's surface than on plant piping and heat exchanger tubes. As a result, maintaining the probe in a clean condition will assure clean heat exchangers are piping.

The BioGeorge™ utilises electrochemical methods for biofilm monitoring functions. Active biofilms have been shown to modify the rate of cathodic half-reactions on passive metals exposed to seawater, brackish waters or fresh waters (Duquette *et al.*, 1986; Buchanan *et al.*, 1991). Several different types of microbial interaction may produce the accelerated localised corrosion typical of MIC. Accelerated corrosion may occur as a result of corrosive metabolic products or as a result of the active participation in reactions that produce corrosion. In addition, Nekoksa (1991) and Guezennec (1991) have shown that while cathodic protection of structures minimised corrosion, the number of microbes was actually greater on the protected metals than on unpolarised structures. These two effects were utilised to produce the BioGeorge™ system.

The BioGeorge™ probe consists of two sets of identical metallic disks, separated from each other by an insulating epoxy. The standard cylindrical probe design uses a threaded pipe plug of nominal 2-in. (50mm) size. The probe is connected to an integrated electronic unit (controller), see Figure 1. The controller periodically cathodically polarises one set of disks (electrode) relative to the other for a short period each day. The electrodes are connected through a precision resistor during the remaining periods. By monitoring the current required to achieve the pre-set potential over a period of days or weeks, the influence of biofilms on operative half-reactions can be detected. The ‘gentle’ cathodic polarisation stimulates electrochemical conditions similar to those resulting from local anodic sites (e.g., inclusions or weldments) and produces local environments that result in differences in the types and numbers of microbes present on each electrode (Nekoksa *et al.*, 1991; Dexter *et al.*, 1991)



Figure 1 Overview of the BioGeorge on-line monitoring system

The applied current (I_a), the current which flows when the external power source is on, is tracked daily. When a biofilm becomes established on the probe surfaces, this applied current exhibits a distinct increase. Examinations of the trend of applied current thus provide a rapid, real time indication of biological activity on the probe surface.

The generated current (I_{gen}), the current that flows through the shunt resistor, which connects the electrodes when the power source is off, is monitored at all other times. Initially, the generated current is zero or very near zero as would be expected for two nominally identical electrodes exposed to the same environment. However, as a biofilm forms and electrochemical changes the local environments on the electrodes of different polarity, current will flow between the electrodes, even when no external potential is applied.

Deviations of either the applied current or generated current from their steady baseline values (i.e., when the probe is known to be clean) provide an indication of microbial fouling. The magnitude of the deviation of the applied current or generated current from the base line values provides an indication of the magnitude of the biofilm activity. In case of an inactivation of the biofilm by the application of an effective chemical, thermal or mechanical treatment, the applied current and the generated current will return to their initial values (Borenstein & Licina, 1998). For fresh water applications, stainless steel discs have been used successfully (Nalepa *et al.*, 1996). Titanium discs have been shown to provide excellent service in both fresh, brackish and saline waters (KEMA, 2000a; KEMA, 2000b; Licina & Venhuis, 2003). So, indications of biofilm activity are based upon trends, often over a long term, as opposed to instantaneous readings.

The Deposit Accumulation Testing System:

The DATS™ Fouling monitor is a microprocessor based, data acquisition system designed to control, monitor and report all parameters necessary to perform heat transfer analysis (Figure 2). As deposits, i.e. scaling, microbial slime, or sediments accumulate on the surface of heat exchangers; these systems become thermally insulated, leading to much higher Heat Transfer Resistance (HTR). The DATS™ Fouling Monitor is using this principle to allow the customer to analyse fouling for specific process conditions, necessary for efficient fouling management programs.

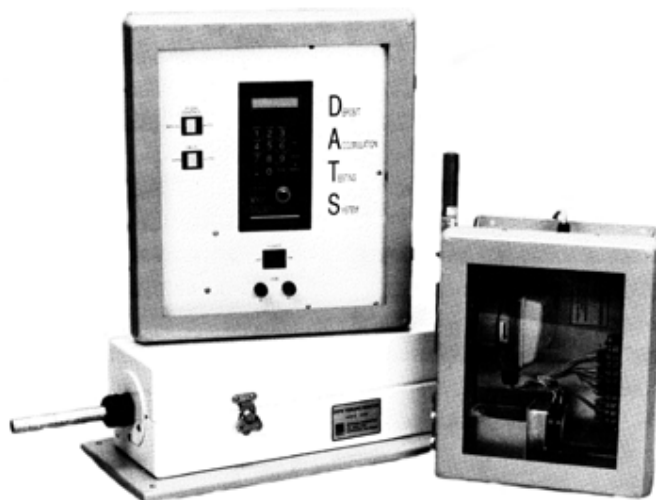


Figure 2 Overview of the DATS™ Fouling Monitor System

The DATS™ system is designed to simulate the geometry and heat flux of a shell and tube heat exchanger, where the cooling fluid circulates on the tube side. An electrical heating element is mechanically bounded to the exterior side of a customer-specified tube, and simulates heat application by the shell side fluid or gas. Precise measurements of the thermal gradient across the fluid-tube-heater system establish the heat transfer relationship. All heat transfer data is then provided as an analogue output signal to tie in with the customers' equipment. The HTR is set to zero during the DATS™ zero/HRT operation (clean tube surface). With time, the HTR increases. This increase of HTR value gives an indication of the reduced capacity or efficiency of a heat exchanger. Changes in HTR over time provide an accurate measure of chemical and biological fouling.

Adenosine-5'-triphosphate (ATP) testing:

The effectiveness of the Coolpuck product in the mitigation of sessile microbes (biofilm) and planktonic waterborne microbes was also determined by using an enzymatic detection method based on adenosine-5'-triphosphate (ATP). ATP-analyses have been carried out on river Rhine (intake) water, KBM effluent (outlet) water and on the sampling plates within the different KBM's. ATP is the general energy carrier in living organisms. Microbes require a continual supply of energy in order to function. However, before the energy can be used, it is first transformed into a form, which the organism can handle easily. This special carrier of energy is the molecule adenosine-5'-triphosphate, or ATP. The presence of ATP can be detected by bioluminescence detection method. The ATP is extracted by a total ATP extractant and then measured in a Lumino meter after addition of luciferin.

The amount of light produced correlates with the amount of ATP in the sample, expressed as Relative Light Units (RLU). This ATP-concentration correlates with the amount of microbial activity present in a sample (Herbert, 1990). ATP-concentrations are detected by using the 'Microbial Biomass Test Kit' and the 'Hygiene Monitoring Kit'.