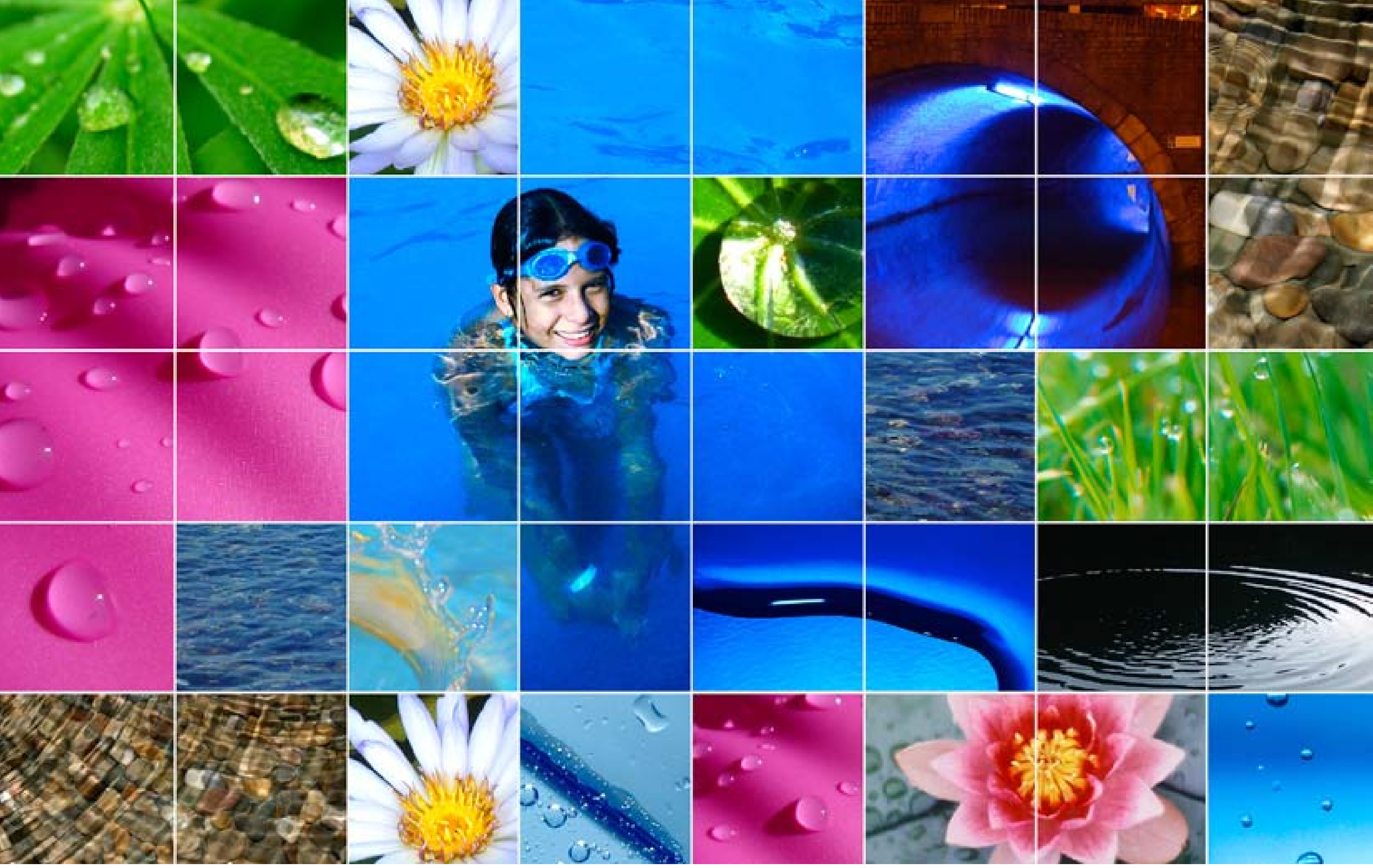

ATP-metry technology for Autocontrol of cooling circuits

→ APPLICATION NOTE #°2 - JANUARY 2008

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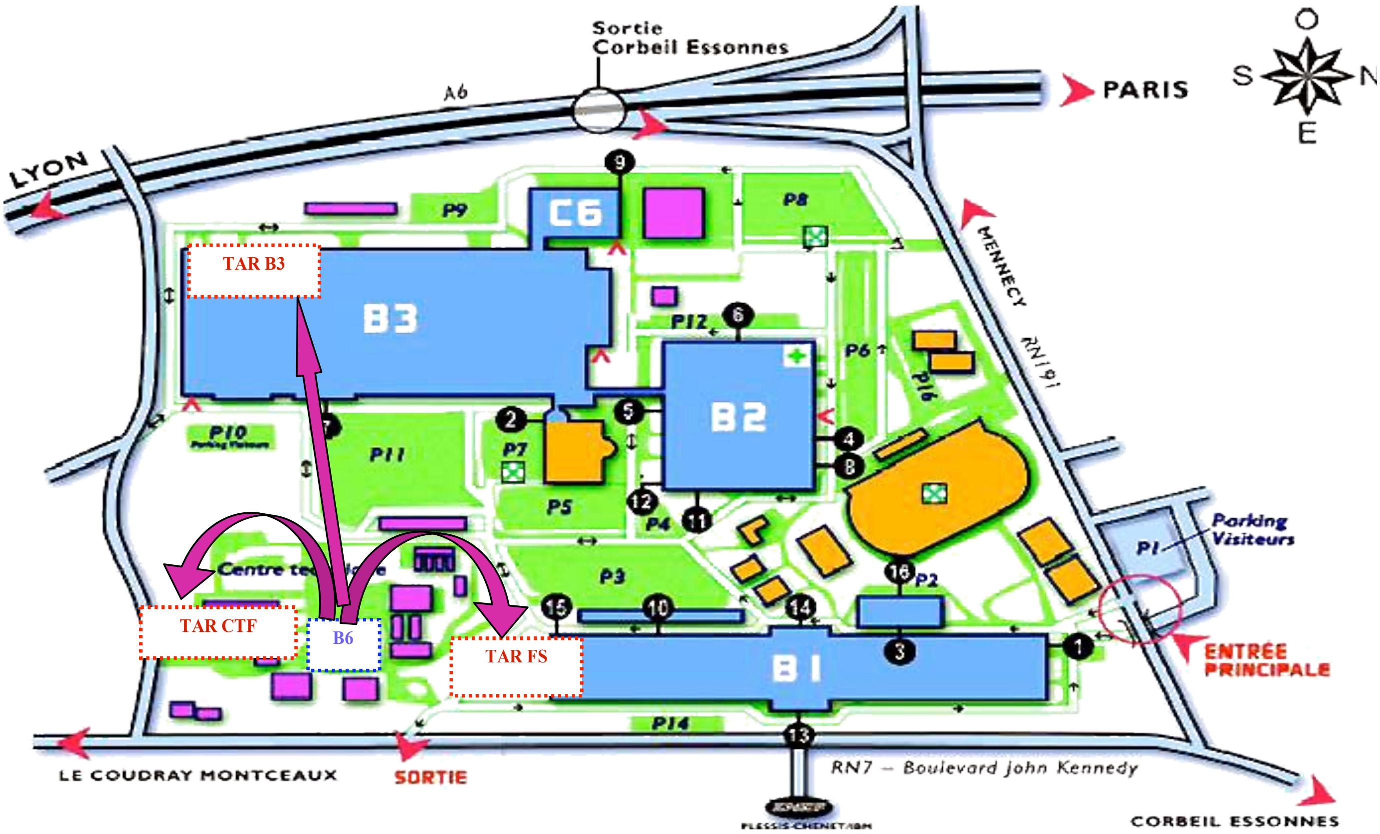
INTRODUCTION

Manufacturing semiconductors requires a production environment that is extremely stable in means of temperature and hygrometry. For production of chilled water needed to maintain the air conditioning systems of the clean rooms and of the general facilities, the Altis Semiconductor factory runs 19 cooling towers (CT) with a capacity of 115 cold MW, which are all subjected to authorization and spread over 14 networks situated throughout 3 geographical zones of the 135 acres wide site. Following to the decree of December 13th 2004, relative to the cooling installations using water dispersion in air flow and submitted to authorization under heading N° 2921, a great number of restrictions lead to a reinforced surveillance of installations. A switch off of these cooling towers, at Altis, would not only cause a problem of public health, but also lead to dramatical consequences for production.

19 Cooling towers, 14 circuits : 115 MW cold water
stop/restart according to climatical conditions



Altis facility, location of installations

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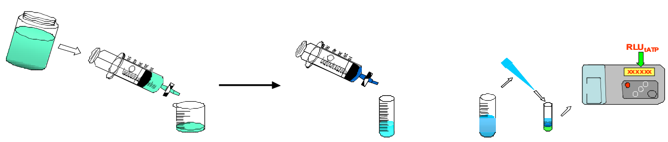
Many actions have been carried out to control Legionella risks on one side, but also to decrease in a significant way water consumption, chemical products and environmental impact of treatments applying to the ISO 14001 guidelines.
For these reasons, in 2005, a recycling program has been established for the ultrapure waters representing the major part of makeup water for the cooling towers, together with a centralized treatment and steady monitoring. Installations that are growing old can be followed up by treatment procedures adapted to each situation, in the same way as a good practical operation and maintenance.
The operations carried out lead to a saving of several tons of chemical products and of 190.000m^3 of water per year (10% of the annual consumption of the facility), ¼ of which is not pumped anymore from natural environment. The new monitoring tools set up (PCR, ATP) as autocontrol enabled the control of a deviating process. The Legionella culture excesses (between 10^3 et 10^4 UFC/l) decreased this way from 19.2% in 2005 to 2.8% in 2006 and to 1.3% up to now in 2007.
Among the used methods for microbiological follow up of cooling towers, a new technology recently appeared on the market : kits dedicated for microbiological control of any water network and based on a second generation of ATP- metry (ATP=Adenosine Triphosphate) assays as an indicating measure of active microorganisms. Concerned by optimization of monitoring and with regards to the permanent research for process control tools, this new ATP-metry technology has been tested at Altis premises during 1 month, as a new tool for microbiological follow up of the cooling tower circuits.

Use of Quench Gone Aqueous (QG™) & Total Control Microbiology (TCM™)

Adenosine Triphosphate is an essential molecule for cellular life because it is the energy form immediately used for the needs of the cell, the source of energy mostly being Glucose as the most easily transformed and the most available for autotrophic and heterotrophic organisms.
Therefore, ATP is a molecule found in all animal and vegetal substances.
Three types of ATP can be differentiated:
- Intracellular ATP (cATP) = living organisms
- Extracellular ATP (dATP) = dead organisms or bacteria in state of programmed death
- Total ATP (tATP) = cATP + dATP

This study was performed using a second generation of ATP measurements. The chemical reaction used in the method is the same as in nature, when Fireflies produce light. The principle of the measurement relies on the count of photons produced by the action of an enzyme, luciferase, emitting photons in the process of hydrolysis of an ATP molecule: this phenomenon is called bioluminescence. The intensity of emitted light is measured with a luminometer.

$$\text{ATP} + \text{luciferin} + O_2 \xrightarrow[\text{luciferase}]{Mg^{++}} \text{AMPP} + PP_i + \text{oxyluciferin} + \textbf{LIGHT}$$



1. Filtration   2. Extraction   3. Result

The water sample volume for the cooling towers analysis is 50ml.
Intracellular ATP is obtained after filtration of the sample (elimination of dATP) and lyses of bacteria retained on the filter for release of ATP.
The quantity of produced light is directly proportional to

active biomass: in fact, the main interest of this second generation of ATP kits is the possibility to convert results expressed in RLU (Relative Light Unit) in pg ATP/ml or Equivalent microbials/ml. There is a consensus that 1pg of ATP corresponds to 1.000 microorganisms. A calibrated added volume of ATP (Ultracheck™), stable over time, offers the possibility to compare historical results in ATP rather than in RLU as was the situation with the first generation of ATPmetry.

Two ATPmetry kits were tested during the study:

- Quench-Gone™ Aqueous (QGA™, Aqua-tools.com) kit: a tool for monitoring active biomass through intracellular ATP measurement
- Total Control Microbiology (TCM™, Aqua-tools.com): a tool for specific evaluation of biomass stress (BSI™) through the measurement of total ATP (tATP) and extracellular ATP (dATP), used during change of process (BSI = dATP/tATP)

The qualification of the measures on the cooling circuits was performed in the following way:

- Follow-up of intracellular ATP on 10 cooling circuits with 3 different continuous oxidizing biocide treatments;
- Evaluation of Biomass Stress Index during the change of the continuous biocide treatment;
- Follow-up of intracellular ATP on a cooling tower circuit treated by oxidizing biocide shocks;
- Evaluation of the efficiency of the different biocide shocks in the circuits;
- Parallel measurements between Intracellular ATP and Legionella species PCR method
- Mapping of the cooling tower process from makeup water to the cooling circuits;
- Evaluation in laboratory of the decrease of biomass with different biocides at different concentration and contact time.

300 analyses were performed during one month. All ATP measures were performed within the two hours following sampling. Analysis on several neutralized or not neutralized samples allowed verification that no interferences exist between measurements and residual biocides.

RÉSULTS

Three strategies of continuous biocide treatment were compared:

- Hypobromous acid (HOBr), initial treatment started in 2005
- Oxygenated water stabilized with silver ions (H_2O_2/Ag), tested during 3 months in effort to improve environmental impact
- Solution containing 90% of hypochlorous acid and 10% of chlorine dioxide produced by electrolysis (OVIPUR system – BIO-DES technology, finalized by Marin Développement – Davézieux France). The solution was first tested on four circuits and then generalized on all cooling towers.

Physico-chemical and microbiological quality of make-up water before treatment was strictly similar in the three case studies.

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Active biomass through ATP : GF CTF and FS

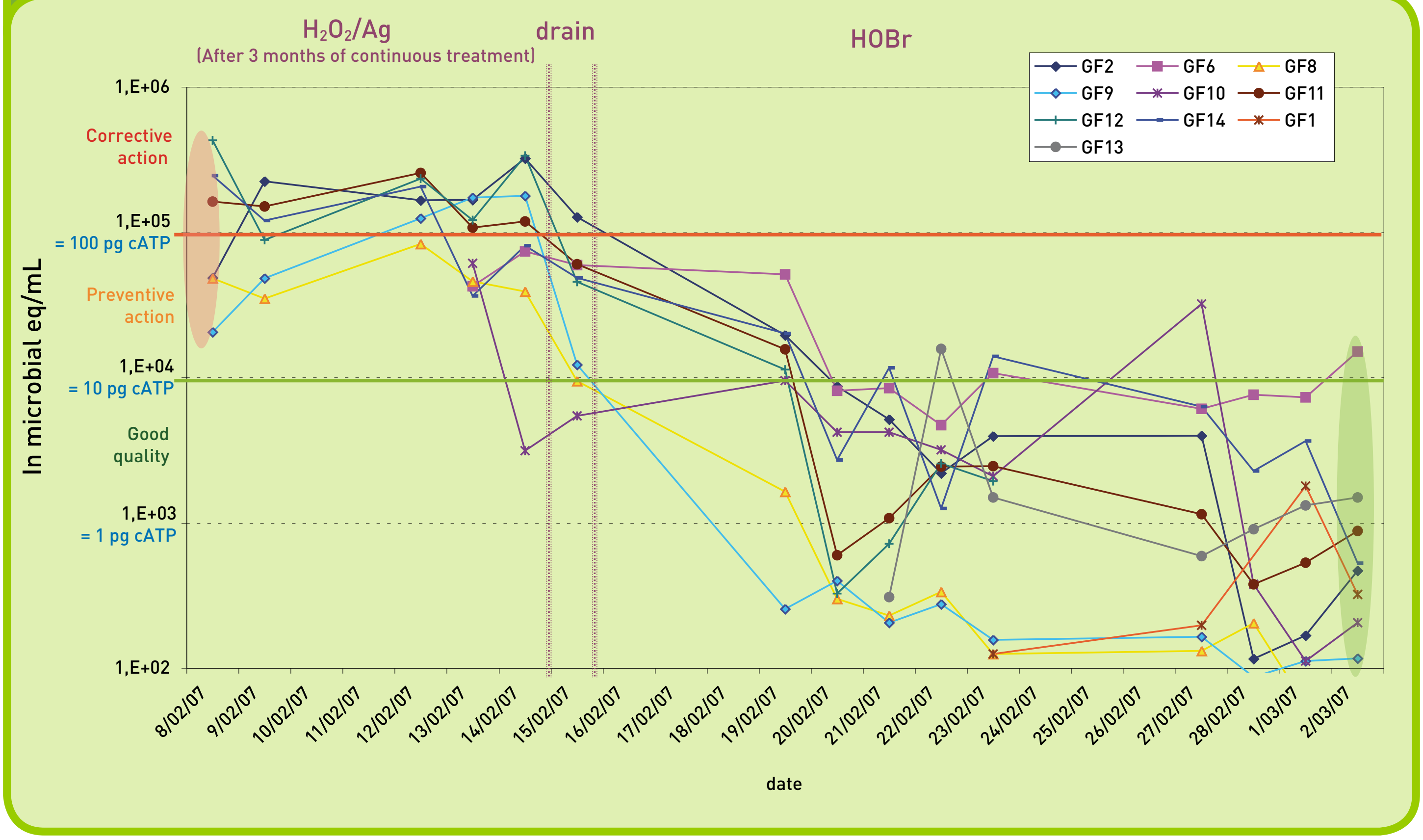

Figure 1 : Intracellular ATP follow-up on 10 cooling circuits treated successively by H2O2/Ag first, then by HOBr

Figure 1 shows impact of H_2O_2/Ag and HOBr treatment on active biomass measured by ATPmetry (intracellular ATP). During treatment with oxygenated water stabilized with silver ions, active biomass concentration varied between 10^4 and 10^6 microbial equivalents/ml, whereas the residual H_2O_2 concentration varied between 1 and 200 mg/L.

A beginning of decrease of active biomass concentration was observed during the flushing step before changing treatment.

After several days of hypobromous acid treatment, active biomass decreased to 10^2 and 10^4 microbial equivalents/ml for concentrations varying between 0.5 and 10mg/L.

Process	Param.	Good Qualtiy	Preventive Action	Corrective Action
Potable Water	cATP (pg/ml)	< 0,5	0,5 to 10	> 10
Cooling Water (Non-Oxidizing Biocides	cATP (pg/ml)	<100	100 to 1.000	> 1.000
Cooling Water (Oxidizing Biocides)*	cATP (pg/ml)	<10	10 to 100	>100
Surface or Recycle water	cATP (pg/ml)	< 5	5 to 100	> 100

Including chlorine, bromine, peroxyde, etc.

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Biomass Stress Index follow-up at change of treatment

Figure 2: Biomass Stress Index follow-up at change of biocide treatment

Using the TCM™ kit, the Biomass Stress Index (BSI™) was measured. BSI is the ratio between dATP (dissolved ATP = extracellular ATP) and tATP (total ATP contained in the sample).

Figure 2 shows that Biomass Stress Index during H_2O_2/Ag treatment varied between 20% to 40%, which is a low level of stress index .The normal stress of microorganisms without any biocide treatment is estimated to be 25%. During the flushing step, the BSI increased to 60%, whereas with hypobromous acid it reached 100%.

Parameter	Good activity	Preventive activity	Corrective activity
BSI* (%)	> 75	50 to 75	< 50

**Biomass Stress Index*

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Active biomass GF11

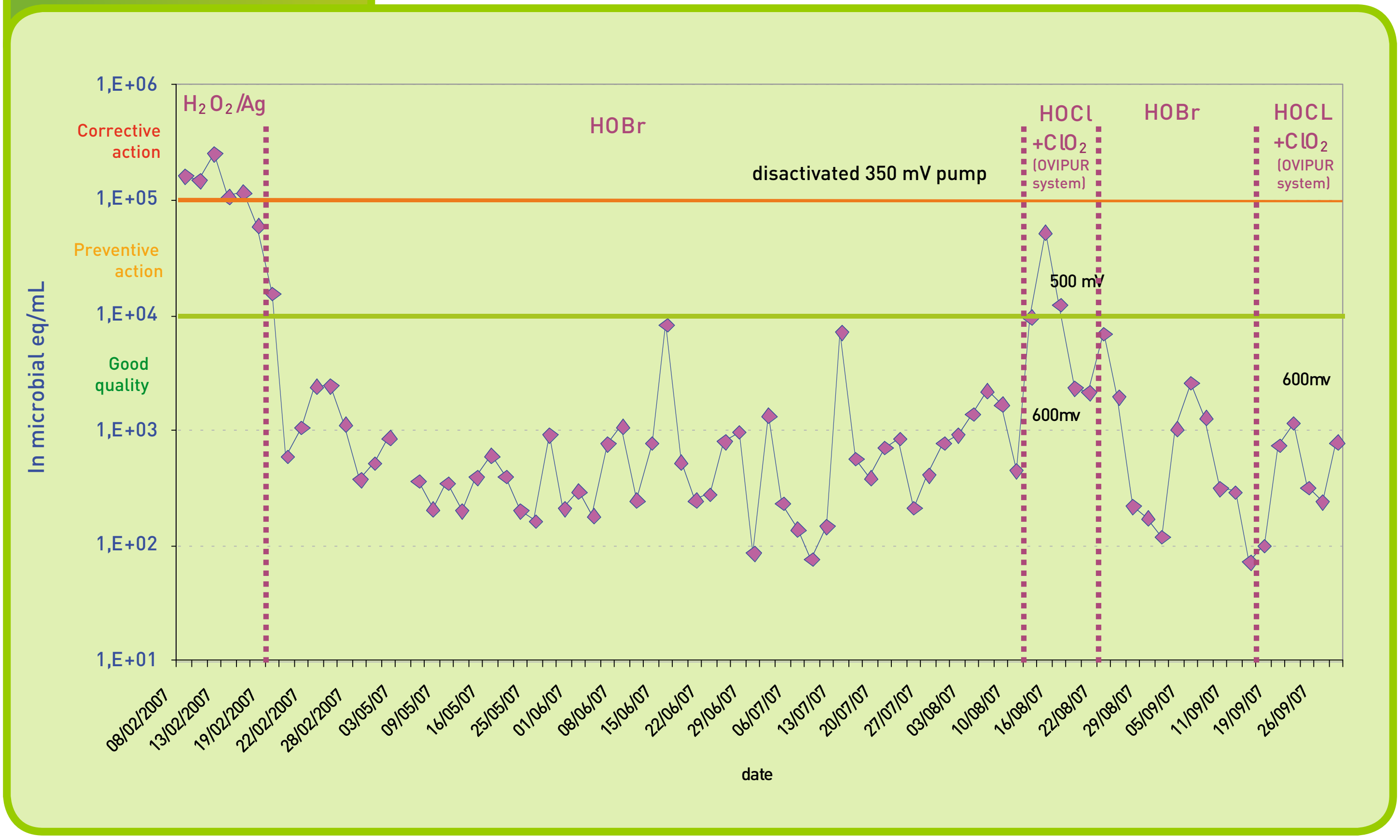

Figure 3: intracellular ATP follow-up on a circuit treated successively by H2O2/Ag first, then by HOBr and a mixture of HOCl/ClO2

ATP method was additionally used in a study aiming the implementation of local biocide injection points at each cooling circuit with direct injection of the product. The study was to determine the minimum oxidizing biocide dose required for each installation (according to its state, location, historical data, functioning mode...).
The injection of $HOCl/ClO_2$ mix obtained by electrolysis (OVIPUR system) is depending on the redox potential in each circuit.
Tests on 3 circuits (Figure 3) showed that a redox potential of 600mV is required for maintaining cATP and Legionella spp PCR values conform to guidelines.
An important increase of cATP was indeed observed during a incidental stopping of the biocide dosing pumps. cATP values then decreased rapidly after the start of the pumps.

For one of the circuits with more important risk factors, the required redox potential was fixed at 700mV based on cATP results.

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Active biomass through ATP : Cooling Tower process mapping

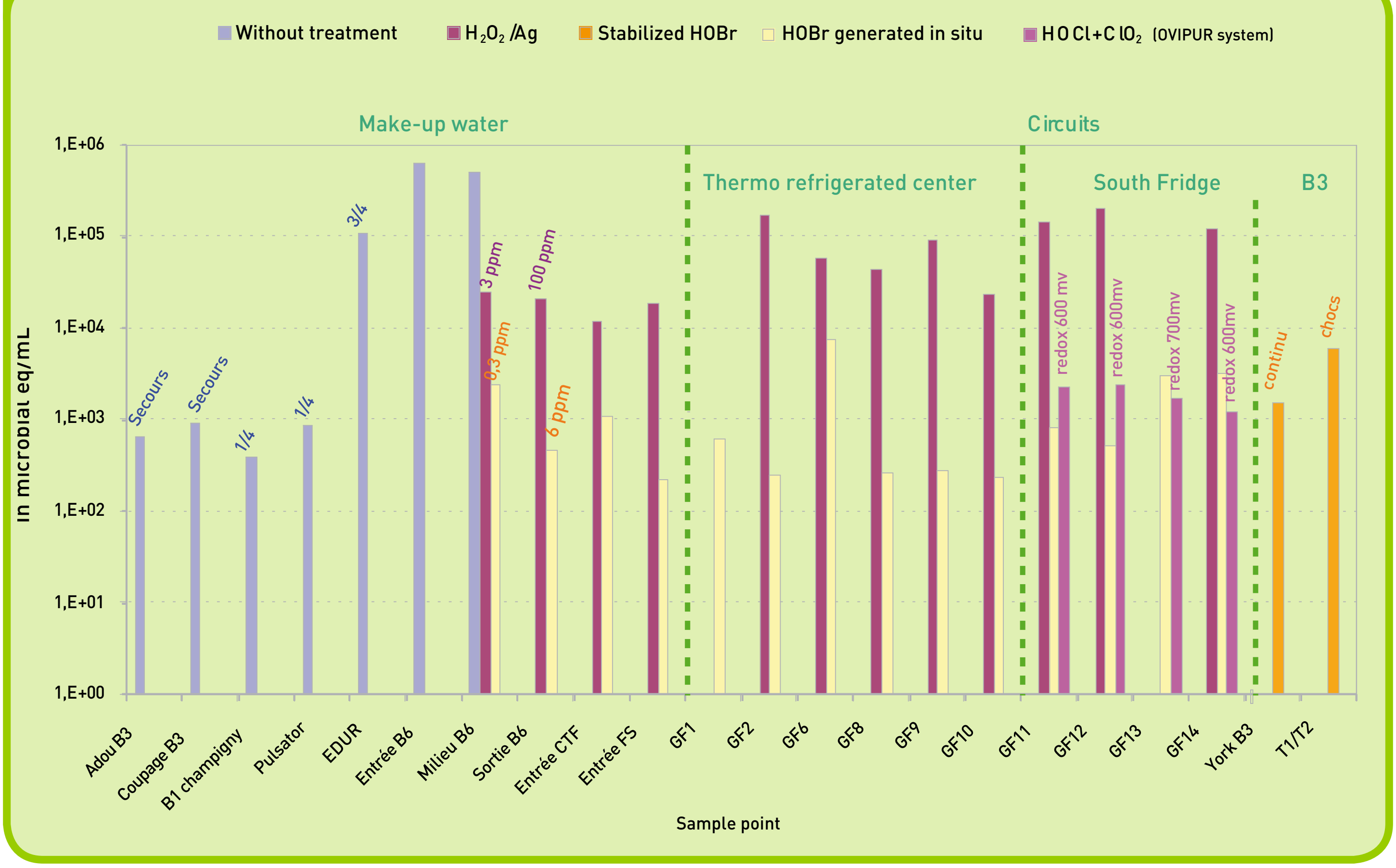

Figure 4: Cooling Tower process mapping through intracellular ATP of Altis facility

Active biomass (cATP) and Legionella spp PCR results on Altis cooling towers showed limited efficiency of H_2O_2/Ag treatment compared to HOBr treatment, even though residual concentrations were ten times higher with H_2O_2/Ag treatment. Mapping of the process using ATP metry (Figure 4) demonstrated that the initial point of make-up water treated with 100 pm H_2O_2/Ag already had higher concentration of active biomass: this treated make-up water distributed in the cooling towers was a contamination source.

The low level of performance of H_2O_2/Ag biocide may be due, for a part, to the capacity of some microorganisms to catalyze the dismutation of hydrogen peroxyde to dioxygen and water with the benefit of catalase enzyme. This enzyme exists in all aerobic organisms and participates, together with the peroxydase enzyme, in the defense mechanisms against toxic derivates of oxygen. This enzyme reacts at higher concentrations of peroxide than the peroxydase. Microorganisms containing these enzymes (catalase and peroxydase), including Legionella, may be therefore resistant to continuous treatments with H_2O_2.

Mapping with ATPmetry also showed that similar results were obtained with the HOBr treatment and the treatment using HOCl/ClO$_2$ produced by electrolysis (OVIPUR system). This last one has more benefits in terms of cost effectiveness, easefulness for operating and is better for environment (no bromates release).

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GF12 : legionella spp (UG/L) – active biomass (microbial eq/L)

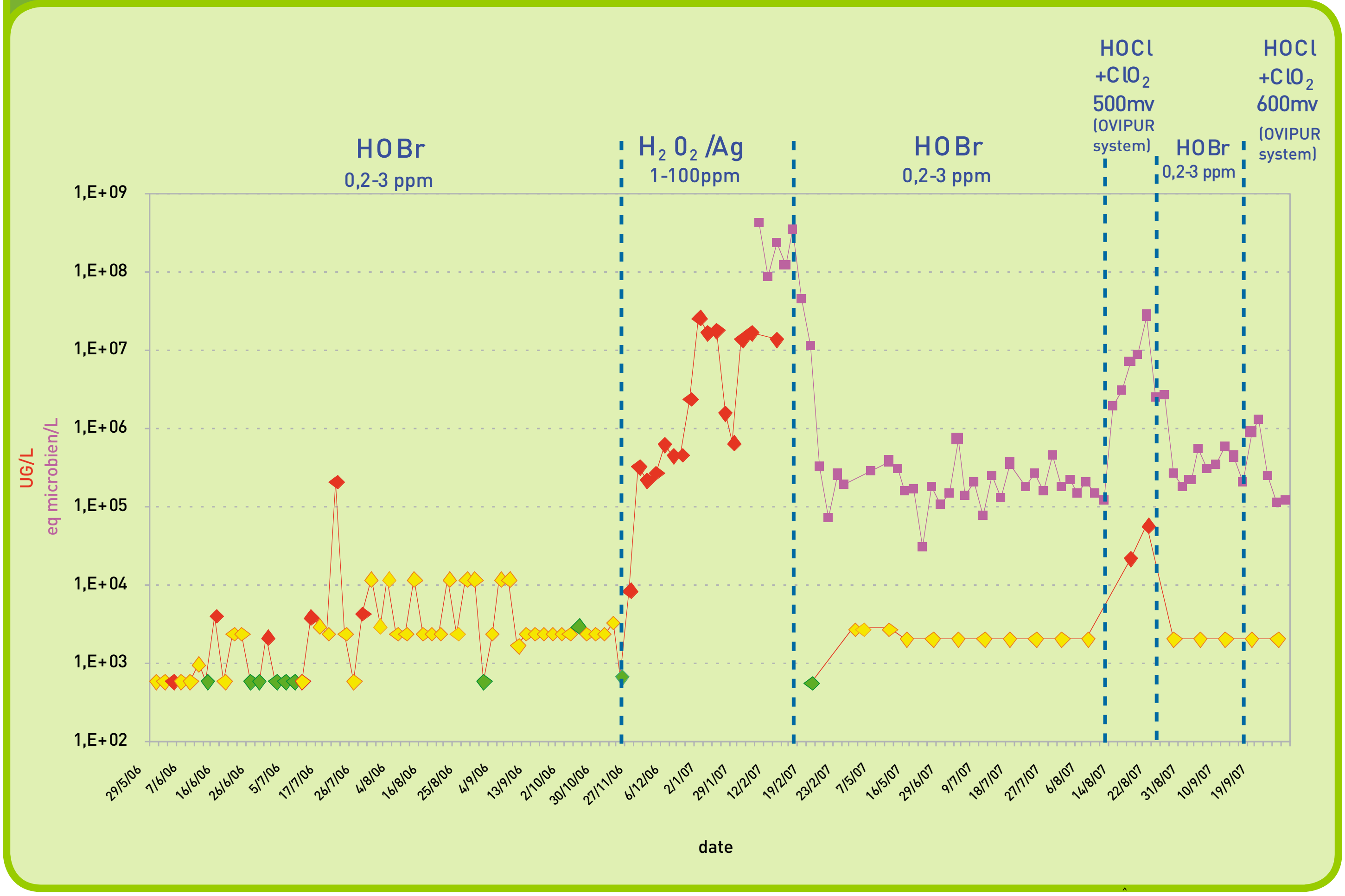

Figure 5: follow-up of intracellular ATP and of L.spp PCR on cooling circuit GF12

The measures performed in parallel by Legionella spp PCR method and cATP method (Figure 5) showed that results followed the same trend.
Curves obtained on the 10 cooling circuits during the study on the different treatments showed the same evolution of results.

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INTERPRETATION

The different measures performed on the cooling towers showed that ATP metry is a reliable and fast tool. The obtained results are in concordance with field observations, operations and knowledge that Altis has previously acquired on its networks. Results were also concordant with data on operating mode and efficiency of biocide treatments.
Guideline values and action thresholds were defined for this indicator: their day-to-day interpretation in addition to the other physico-chemical analysis allows to determine actions to undertake. The target value for the cooling towers is 10^4 microbial equivalents/ml (10pg of ATP/ml), which is representative of a microbial population under control and an efficient treatment. An alarm threshold at 10^5 microbial equivalents/ml (100pg of ATP/ml) was implemented, as indicator for a drift in the process linked to a risk of results for Legionella culture excesses. Following the implementation of ATPmetry measurements additional to the Legionella spp PCR analysis, revivable aerobic flora analyses were stopped.

CONCLUSION

ATP measurement brings several benefits for an industrial site. First, this is an instantaneous indicator of the level of microorganisms, that can be used as a tool for regular follow-up of the process. The quantification of biomass using ATPmetry is the only real indicator of the total flora, as viable cultivable and viable non cultivable bacteria present in the installations are both quantified with this method. Additionally, analyses are performed in laboratory on -site and take 10min per sample. At last, the cost of this measure is low compared to other available analytical measures.

The purposes for the follow-up of an installation, using ATP metry are numerous:

• **Autocontrol**
→ Reaction time: results in 10min (compared to 10 days per culture and 48h per PCR).
→ Anticipation of water quality derivation

• **Verification of effectiveness of biocide treatment** on viable cultivable and viable non cultivable micro-organisms, complementary to Legionella spp PCR.
→ Improvement of efficiency of treatments
→ Optimization of biocide concentration
→ Validation of disinfection effectiveness

• **Identification of pollutions** and zones of bacterial proliferation
• **Cost effectiveness,** optimize budget for analysis, using the most discerning indicators to reduce the budget for chemical treatments.

The use of this ATPmetry method on Altis Semiconductor site allowed the qualification of a new treatment (production of HOCl and ClO_2 by electrolyze) on a group of cooling towers. This treatment will afterwards be generalized on the entire site leading to important reduction of chemical treatments (6T/year) and improvement of environmental impact by eliminating bromates.

Measurement of intracellular ATP allowed furthermore validation of biocide concentration and contact time needed for shock disinfection treatments; this conforted Altis in their decision to stop some biocide treatments (isothialozone, H_2O_2/Ag).
ATPmetry meets the current requirements of industry: reliability, reactivity, low cost.
In a continuous effort to increase productivity, the possibility to perform on line ATP measurement is an essential optimization axis to meet industrial requirements.

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