

# **Use of Total Control Biological (TCB™) kits for operating the biological activity in Waste Water Treatment Plants**

**→ APPLICATION NOTE #3 - NOVEMBER 2008**

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## Introduction

Control tests for monitoring waste water treatment plants are performed on daily basis by the operators, concerned by the obligation to respect the strict European regulation regarding waste water effluents. Biological monitoring is based on parameters such as Mixed Liquor Suspended Solids (MLSS), measuring the total solids contained in the bioreactor, and the Mixed Liquor Volatile Suspended Solids (MLVSS), measuring the total microorganisms' compound, regardless to the fact if it is dead or alive. However, only living and active biomass is important for degrading organic pollution and there is a lack today of operational tools allowing direct measurement of active biomass in the bioreactors.

Total Control Biological (TCB™) kits are a new technology developed by LuminUltra™ (Canada) for measuring living microorganisms in waste water treatment bioreactors. TCB™ kit is measuring the concentration and health of living biomass through the quantification of intracellular Adenosine Triphosphate (ATP) molecule. Intracellular ATP is the energy stock of all living cell and is degraded within minutes when released in the environment. The monitoring of ATP concentrations in waste water over the time conducts to having a good alarm system for identifying degradation of the operation conditions of the installation and the presence of toxicity in the primary influent.



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## Presentation of the technique of ATP metry

The technique of ATP-metry allows a fast measure of the concentration of the active biomass in water. This method is based on the quantification of the Adenosine TriPhosphate (ATP), which is an essential molecule in the microbial life. ATP molecule can be found in all living organisms and is indicative of their metabolic activity and the viability of the biomass present in environmental samples. The ATP is a carrier of energy situated inside the living cells. ATP manages all the biological functions, such as nutrition, maintenance and reproduction.

The principle of ATP measurement is based on a bioluminescence technique, in which one photon of light is produced in the enzymatic reaction between Luciferase enzyme and an ATP molecule. Intensity of light is measured with a luminometer. The quantity of produced light is directly proportional to the active biomass present in the sample.



**ATP measurement is directly proportional to living microbial flora: it allows quantifying rapidly the concentration, health and activity of all microorganisms contained in water or sludge sample. Final quantitative results are delivered in only minutes.**



TCB™ kit, developed by LuminUltra™, is a new generation of kits of ATP metry. This kit is specifically developed for the follow-up of the functioning of the biological wastewater treatment plants (WWTP).

### Total Control Biological (TCB™) kit allows :

- Real-time surveillance of the concentration of microbial flora (active biomass) in the bioreactors
- Early detection of operational problems and prevention of the decrease of treatment efficiency
- Surveillance and studies of the toxicity of influent water

### The advantages of these kits are:

- Real quantification in a wide range of concentrations
- Fast results, in only 15min
- Inter-comparable results (various days, various sites), in favour to the conversion of the result in picogram of ATP.
- Flexibility of use with possibility of tests on sites, portability of the machine and the reagent, the storage of the ATP samples during 7 days
- Stability of the reagents
- Reliability and reproducibility of the results with only 10 % of coefficient of variation. The precision of the measure is notably due to the quality of the reagents, allowing:
  - Measure of the totality of free extracellular ATP
  - Measure of the totality of total ATP
  - No possible inhibitions of the reaction of bioluminescence
  - Performant extraction reagents for releasing intracellular ATP
  - Calibration of the results by UltraCheck™, external calibrated and stable ATP solution

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## Total Control Biological kits and the analyzed parameters

### COMPOSITION OF THE KIT

Included in TCB™ Kits	Content
Luminase	Luciferase Enzyme Reagent
UltraCheck 1	1 ng/mL ATP Standard
UltraLyse 30	ATP Extraction Reagent
UltraLute	tATP Dilution Buffer
LumiSolve	dATP Stabilizing Buffer

The Total Control Biological (TCB™) kit contains every reagent necessary to make the analysis of 50 samples of water.

The first essential component of the kit is the Luminase, Luciferine-Luciferase enzyme complex, which will allow to measure the available ATP in the sample.

The Luminase used in LuminUltra's kit was developed to allow, in the first place, a better sensibility of the test for weak concentrations in ATP and secondly, to allow the quantification of the signal in the strong values of ATP. The TCB™ kit has a range of quantification going from 1 to 60 000 ng/ml of ATP or of 0.5 to 30 000 mg/L of dry biomass.

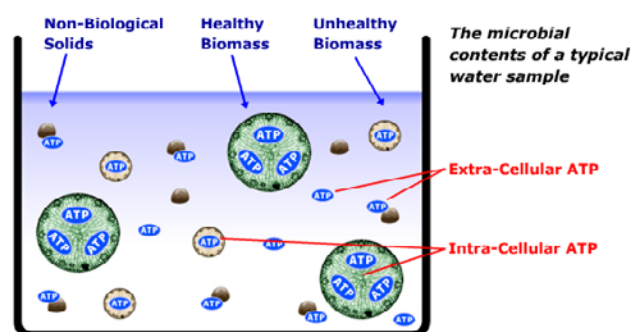
The second essential component of the kit is the UltraCheck™: a calibrated standard, containing 1 ng/ml of ATP. Ultracheck allows to calibrate the enzymatic reaction and to transform the analytical results in ng of ATP. It's only once that the results are transformed in ng of ATP that they can be compared between them. UltraCheck also allows verifying the quality of the Luminase before the essays. It is a guarantee of the reliability of results.

### PARAMETERS MEASURED WITH THE TCB™ KIT

Within a sample of water containing microorganisms, there are two types of ATP:

- Intracellular ATP (cATP) that is the ATP contained in the living biological cells. cATP is representative of the concentration of living microorganisms in the sample.
- Extracellular ATP (dATP) that is the ATP contained outside of the living biological cells. Extracellular ATP is issued from dying and dead microorganisms.

The total ATP (tATP) is the sum of the intracellular ATP and the extracellular ATP.



LuminUltra's kits are the only kits able to quantify the intracellular ATP, the extracellular ATP and the total ATP, in order to have a complete vision of the alive microorganisms and their health in a bioreactor.

#### a) Intracellular ATP – cATP

The parameter « intracellular ATP » stands for ATP contained within living cells.

To make accessible the cATP to the reaction of bioluminescence, the sample of water is submitted to a bacterial lyse, which allows to destroy cellular membranes and to release the cATP. In the range of Total Control tests, the cATP™ is calculated by difference between the dATP™ and the tATP™ ( $cATP = tATP - dATP$ ).

Surveillance of cATP allows to quantify in real-time the active living biomass and to follow its growth under optimum conditions or detect early the inhibition of activity under stressful environmental and operational conditions. cATP shows a relatively stable level in an optimized, well performing bioreactor.

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## b) Biomass Stress Index - BSI™

**BSI is representing the stress of microorganisms under degrading environmental and operational conditions. It is linked to the mortality of microorganisms.** BSI is the ratio between extracellular ATP issued from dead microorganisms and the total ATP of the water sample.

When operating conditions degrade and become unfavorable for living microorganisms in the bioreactor, their stress – eg. mortality – increases and the BSI will also increase. Follow-up of BSI allows detecting operational problems responsible for the degradation of the quality of treated water. BSI is an important parameter for the study of toxicity of influents. Higher the BSI of an influent, more it will disturb the active biomass when introduced in the bioreactor.

ATP-metry specific protocols are available for evaluating the toxicity of individual influents or chemical products that can lead to a reduction of efficiency of water treatment by inhibiting or killing all or part of the microorganisms.

## c) Ratio of Active Biomass - ABR™

**The ratio of active biomass ABR™ is a control parameter extremely important for the processes of biological treatment of waste water.**

**The parameter ABR defines the percentage of active biomass among total suspended material in the bioreactor.**

The ABR corresponds to the ratio between the concentration of alive biomass and the concentration in Total Suspended Solids (TSS):

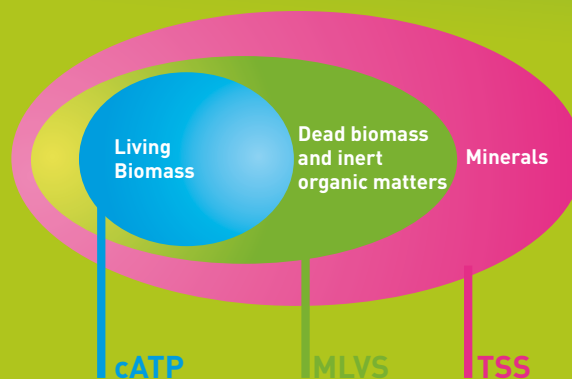
$$\text{ABR (\%)} = (\text{cATP}^{\text{TM}} \text{ (ng/mL)} * 0,5) / \text{TSS (mg/L)}$$

The availability of this parameter allows the operator to measure the activity of bio-solids contained in the bioreactor. For a large number of elements, including a reduced competition, an elimination of the expensive aerobic digestion, and an improvement of the operations of mass transfer, it is very beneficial to maximize the ABR ratio for a process of biological treatment

The improvement of this parameter can lead to a decrease of robust minerals or dead organics, bringing no profit for the functioning of the installation.



**ATP-metry is a performant field tool allowing management of WWTP and early detection of the degradation of quality of treated water, thus by the surveillance of a reliable parameter which is the activity and health of active, treating microorganisms.**



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## Interest of ATP-metry for the follow-up of the WWTP

### INTEREST OF THE FOLLOW-UP OF THE cATP, BSI AND ABR PARAMETERS

ATP is the measure of the energy and the activity of living biomass in a bioreactor. It is a very relevant parameter to be followed in a WWTP because it brings a direct vision of active biomass and its health. The ATP-metry allows to manage the wastewater treatment plant and to optimize its functioning by surveying the parameters cATP, BSI or ABR:

- cATP : **Maintain** stable the activate biomass in a bioreactor
- BSI : **Minimize** the toxicity of influents and the conditions of stress in the bioreactor
- ABR : **Optimize** the proportion of active solids

Indicative thresholds of the quality of active biomass in waste water installations have been established:

Process step	Parameter	Good quality	Preventive action	Corrective action
Process Influent	BSI (%)	<50	50 to 75	>75
Bioreactor	cATP (ng/ml)	Specific to process		
	BSI (%)	<30	30 to 50	>50
	ABR (%)	>25	10 to 25	<10
Process Effluent	cATP (ng/ml)	<50	50 to 250	>250

On the basis of the parameters cATP, BSI and ABR, we can pilot a wastewater treatment plant and avoid any degradation of the effluent water quality by anticipating and early detecting the drift of the functioning of the installation.

The cATP parameter is informing about the activity of living biomass, while the BSI will inform about its health or the presence of toxic influents.

A stress lower than 30 % is normal for a healthy bacterial population, but its abrupt or progressive increase and the passage over 50 % are indicative of a phenomenon disrupting the activity of microbial flora. The optimization of the ABR parameter, which must be superior to 25 %, may lead to reduction of sludge volume.

### APPLICATION FIELDS OF TCB™ KIT

TCB™ kit gives multiple possibilities of uses:

- **Monitoring the installation thanks to the ATP, which is the only true measure of living biomass and thus not subjected to interferences bound to the biodegradability of solids or other outside parameters.**
  - Map a WWTP to **characterize** its functioning
  - Evaluate the **health of active biomass** in industrial wastewater treatment.
  - Follow and **manage the functioning** of the WWTP on the basis of cATP, BSI and ABR
  - Follow the **quality of the influents** and their **toxicity**
  - Optimize the **sludge extraction**
  - Optimize the conditions of functioning of the station (nutrients, pH, oxygen)
  - **Optimize energal consumption**
  - **Reduction of operating costs**

- **Early detection of the operational drifts of an installation: use the ATP as an early alarm of operational problems.**

- **Prevent** the degradation of treated water by detecting early the problems of functioning of the station
- Study the **reaction of the biomass** to the changes of **industrial production**, quality of the toxic influents
- Detect early the problems bound to a **toxicity** of influents
- Study the **maximal concentration of toxic products** to be introduced into the bioreactor without disturbing its functioning

- **Follow the actions of improvement.**

- Follow the **restart of a WWTP bioreactor**
- Follow the **efficiency of bio-additives** (fungi) or nutriments added in the bioreactors
- Improve the quality of treated waters by optimizing the parameters of functioning
- **Decrease the quantity of produced sludge.**

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## Main use of the ATPmetry on wastewater treatment stations

### USE OF ATP FOR THE CHARACTERIZATION OF A WWTP

Data of 3 wastewater treatment plants of the pulp and paper industry were accumulated and put in parallel. These data allow comparing between them the functioning of 3 stations:

Factory	Reference factory	Factory 1	Factory 2
Period of follow-up	7 months	7 months	10 months
Number of ATP points	64	71	103
BOD (T/d)	43,9	31,6	32,1
Volume (m <sup>3</sup> )	17217	60000	44000
TSS (mg/L)	7869	4226	4221
cATP (ng/mL)	5400	2140	1718
ABR(%)	35%	25%	20.8%
F/M (Kg/kg/d)	1,04	0,49	0,85
Index of sludge volume	76	144	175
BSI (%)	3,2%	19%	31,2%

Data provided by LunimUltra™

The reference factory receives the highest BOD load and has the shortest retention time. In result, it shows the best characteristic in terms of active biomass concentration, active proportion of solids and indication of stress of the biomass.

**These data show that the technique ATP can characterize totally an installation and allow to compare sites between them. Data are correlated to the usual parameters followed in an installation.**

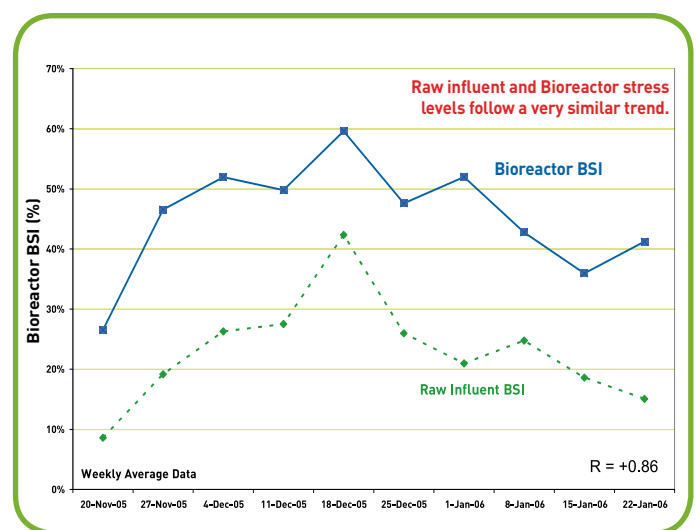


### USE OF THE ATP FOR THE STUDY OF THE TOXICITY OF WWTP INFLUENTS

The follow-up of the stress of active biomass (BSI) in the influent and in the bioreactor allows to identify the origin of dysfunction of the WWTP. The problems can be due either to toxic influents arriving in the water treatment unit or to an operational drift. **The parallel surveillance of influents and bioreactor conducts to clear distinction of actions.**

Ammonia reduction problem arose in a municipal station receiving municipal and industrial sites water. In this situation it is important to investigate why these nitrification problem arose. The influent water and the bioreactor were followed using ATP-metry over the time.

The examination of the curves of BSI allowed locating the source of disorder at the level of the influent, as BSI curves of influent and bioreactor followed exactly the same trend. Investigation then continued on the different individual influents received by the WWTP. It was discovered that an accident of abnormal heavy metals discharge occurred on one of the industrial sites. This event caused the death of an important part of nitrifying bacteria leading to a bad efficiency of water treatment.



Data provided by LunimUltra™

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If a toxicity problem linked to the influents is identified **two actions** can be implemented:

- **Perform a detailed toxicity study** in order to identify which is the individual influent or product that is causing an important dysfunctioning of the water treatment plant.
- **Identify the maximal dose of toxic influent** which can be introduced in the bioreactor without disturbing the treatment processes.

In both cases, in experimental conditions, a dose of biomass and influent water or chemical product will be mixed together. The concentration of active biomass and the stress of the biomass will be measured at different times in order to evaluate the short-term and the long-term impact of the water (or product) on the microbial flora composing the bioreactor.

Thus, it is possible to classify products and waters and implement different treatment procedures according to their impact on the active biomass of the bioreactor.

## Example of toxicity study on chemical products :

Products	cATP Active biomass concentration			BSI Biomass Stress Index		
	T0	T30	T24	T0	T30	T24
1	2554,91	644,58	261,07	15,47%	34,23%	43,51%
2	2549,12	1231,13	1531,30	16,12%	46,04%	35,23%
6	2566,15	777,30	709,10	15,64%	59,99%	56,78%
Control	2564,63	1953,93	910,50	15,61%	34,89%	46,62%

The **product 6** is presenting the **highest toxicity** to the biomass of the bioreactor. The measured biomass stress is very high at short term (30 minutes) and long term (24 hours). This product has acute and long term toxicity.

**Product 1** presents **moderate toxicity**. This product is inhibiting the activity of the biomass. There is no effect of acute stress, but the toxicity has a long term impact.

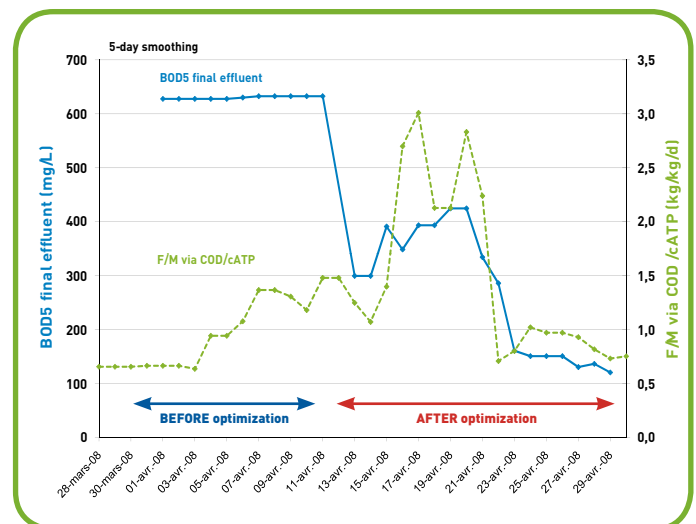
**Product 2** has a very **low toxicity** for the active biomass of the bioreactor. A slight acute stress is detected at 30 minutes, but the long term stress is comparable to the controls.

## CALCULATION OF THE F/M RATIO BY USING THE cATP PARAMETER

Given that the cATP correlates well to the COD and the BOD5 of the influent, it is a very good predictive and control parameter. It allows the calculation of F/M ratio as monitoring tool.

F/M Ratio (kg/kg/d)	Values
<b>COD/cATP</b>	<b>1,24 ± 1,18</b>
<b>BOD5/ATP</b>	<b>0,43 ± 0,14</b>

F/M thresholds based on cATP can be determined. Target values in F/M ratio via the cATP help optimizing the values of BOD5 in treated water:



As the BOD5 is an available parameter in 5 days of interval, the COD can be used for the calculation of F/M ratio. When the F/M ratio is on its optimum (below 1 in the example), the BOD5 in treated water is correct.

## Why to use the cATP for the calculation of F/M ratio?

The cATP is a precise indicator of living biomass. Change of cATP can indicate the capacity of the installation to treat the effluent and to prevent the drift on the treated water. This parameter supplies an alarm and the possibility of adapting quickly the concentration of active biomass to correct the situation.

The new generation of ATP-metry technology commercialized by Aqua-tools, together with the trainings programs and scientific conferences organized by the company, made that the company has rapidly become the N°1 reference on biological risk management in France. Among our customers we count Ondeo Industrial Solutions (Suez Group), Veolia, EDF, Arcelor Mittal and many others.

Aqua-tools is the exclusive distributor in Europe and MEA of Luminultra™ company (Canada)

## **Aqua-tools is specialized in:**

- Commercialization of ATP-metry kits for **fast quantification of microorganisms**
- **Audits and expertise** in control of microbial water quality in water installation
- Training programs on **analysis and monitoring of microbial risks** in sanitary and cooling tower water

The new generation of ATP-metry kits commercialized by Aqua-tools is based on **bioluminescence** and allows measurements in less than 10 minutes on the field for a higher reactivity for implementing preventive and corrective action.

## **The kits are implemented in the following types of installations:**

- **Cooling towers**
- **Sanitary water**
- **Production & distribution of potable water**
- **Process water**
- **Pure and ultra-pure water**
- **Biofilm**
- **Metalworking fluids**
- **Oil & Gaz**
- **Cataphoresis baths**
- **Surface treatment**
- **Activated sludge in water treatment bioreactors**
- **Reuse water**
- **Other...**

## Other Application Notes available :

**#1**  
**Water testing**  
Use of Quench-Gone™ Aqueous Kits as a tool to set up Risk Analysis and Routine Surveillance Programs for Sanitary Water and Cooling Tower Systems in Hospitals, Spa

**#2**  
**ATP-metry technology for Autocontrol of Cooling Circuits**



**aqua-tools.com**

Domaine de la Brissette  
36 rue de La Falaise  
F- 78126 Aulnay-sur-Mauldre (Yvelines)  
+33 1 30 95 79 50  
contact@aqua-tools.com  
www.aqua-tools.com

