

EVALUATION OF MUNICIPAL WASTEWATER QUALITY AFTER DIFFERENT ADVANCED TREATMENT TECHNOLOGIES USING *IN VITRO* AND *IN VIVO* BIOASSAYS

Cornelia Kienle¹, Robert Kase¹, Christian Abegglen², Jonas Margot³, Anoy's Magnet⁴, Denis Thonney⁴, Michael Schärer⁵, Inge Werner¹

¹Swiss Centre for Applied Ecotoxicology Eawag/EPFL, Dübendorf, Switzerland, ²Process Engineering, Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf, Switzerland, ³Ecological Engineering Laboratory, Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland, ⁴Wastewater Treatment Plant of Lausanne, Switzerland, ⁵Federal Office for the Environment, Switzerland

Objectives

Conventional tertiary treatment is unable to remove a number of organic chemicals, in particular pharmaceuticals, pesticides, corrosion inhibitors, natural and synthetic hormones and other hormonally active chemicals from municipal wastewater. The goal of this study, funded by the Federal Office for the Environment of Switzerland, was to evaluate the efficiency of two advanced wastewater treatment methods: (I) ozonation followed by sand filtration (Ozonation-SF) and (II) powdered activated carbon addition followed by ultrafiltration (PAC-UF) in eliminating concentrations and biological effects of such micropollutants and potential ozonation by-products.

Materials and Methods

Experimental Design

- Four sampling campaigns were conducted.
- Samples (7-day composites) were collected before and after each treatment step.
- Concentrations of 58 selected chemicals were determined analytically.
- Bioassays were conducted using concentrated samples (*in vitro* endpoints) or native samples (whole organism bioassays).

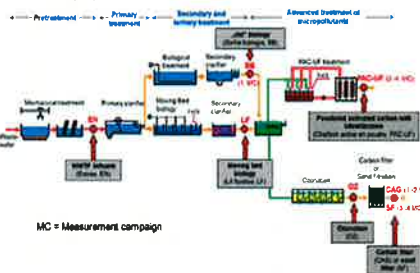


Fig. 1 Overview of the sewage treatment plant Lausanne and the different sampling points

Bioassay Selection

- In vitro Bioassays with Concentrated Samples :**
 - covered relevant modes of action, such as endocrine disruption (e.g. androgen, estrogen, and glucocorticoid receptor activation), mutagenicity, genotoxicity, and herbicidal effects.
- In vivo Bioassays:**
 - measured whole organism toxicity at different trophic levels, as well as effects based on more specific endpoints, such as vitellogenin induction and number of offspring

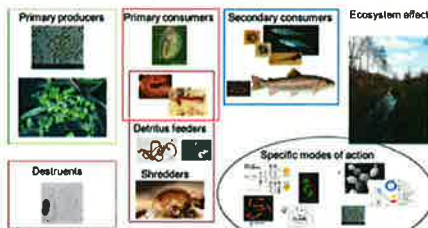


Fig. 2 Overview of test organisms and test systems used in the two pilot studies Regenadorf and Lausanne

Comparison of Bioassay Results

A **Change Index (CI)** was used to calculate the relative change in toxicity after each treatment step. The CI approach allows direct comparison of tests with and without sample enrichment.

$$CI = \frac{\text{Toxicity after treatment}}{\text{Toxicity before treatment}}$$

For toxic equivalent concentrations (TEQs):

$$CI_{TEQ} = \left(\frac{\text{Toxicity after treatment}}{\text{Toxicity before treatment}} \right) - 1$$

Change Index CI > 1 decreasing toxicity
Change Index CI ~ 1 equal toxicity (range: 0.75 < CI < 1.25)
Change Index CI < 1 increasing toxicity

Results

CI - In Vitro Bioassays

Bioassay	Substance classes (Effect parameter)	Effect Biological Treatment	Effect Ozonation	Effect Sand filtration (3.4, MC)	Effect Ozonation + Sand filtration (3.4, MC)	Effect Powdered Activated Carbon addition - UF
YES assay	Estrogens (Estradiol equivalents, ng/L)	+	+	+	+	+
ER CALUX		+	+	+	+	+
AR CALUX	Androgens (Dihydrotestosterone equivalents, ng/L)	+	+	+	+	+
GR CALUX	Glucocorticoids (Dexamethasone equivalents, ng/L)	+	+	+	+	+
PR CALUX	Progesterones (Utg-2058 equivalents, ng/L)	+	+	+	+	+
PPARγ1 CALUX	Peroxisome proliferator like acting substances (Rosiglitazone equivalents, ng/L)	+	+	+	+	+
H296R Assay	Estradiol induction	+	+	+	+	+
Green algae	Herbicides (Cruston equivalents, µg/L) (Photosynthesis inhibition) General toxicity (baseline toxic equivalent index, mg/L (toxicity inhibition))	+	+	+	+	+

↓ CI > 1 decreasing effects, ~ CI = 1 equal effects ↑ CI < 1 increasing effects var. varying results

CI - In Vivo Bioassays

Test organism	Endpoint (Toxicity parameter)	Effect Biological Treatment	Effect Ozonation	Effect Sand filtration (3.4, MC)	Effect Ozonation + Sand filtration (3.4, MC)	Effect Powdered Activated Carbon addition - UF
<i>Lumbriculus variegatus</i>	Reproduction	+	+	+	+	+
	Biomass	+	+	+	+	+
<i>Oncorhynchus mykiss</i>	Overall Survival	+	+	+	+	+
	Survival of embryos	+	+	+	+	+
	Survival of larvae and juveniles	+	+	+	+	+
	Hatching rate	+	+	+	+	+
	Swim up of hatched eggs	+	+	+	+	+
	Fresh weight of larvae at end of test	+	+	+	+	+
	Length of larvae at end of test	+	+	+	+	+
	Vitellogenin concentration	+	+	+	+	+

↓ CI > 1 decreasing effects, ~ CI = 1 equal effects ↑ CI < 1 increasing effects var. varying results

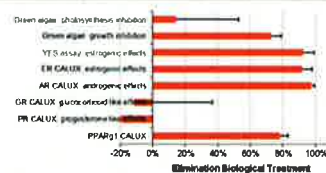
→ Both advanced treatment technologies significantly decreased concentrations of micropollutants and most biological effects by up to 100 %.

→ *In vitro* bioassays indicated a significant reduction in endocrine disruptive activity and herbicidal effects and no genotoxicity and mutagenicity after advanced treatment.

→ There was some evidence (biomass of *Lumbriculus variegatus*) of toxic effects due to the formation of reactive ozonation by-products, which were eliminated after passage of the effluent through a sand filter.

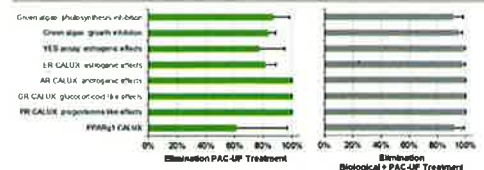
→ Tests with rainbow trout showed a significant reduction of toxicity after advanced treatment.

Elimination: Biological Treatment



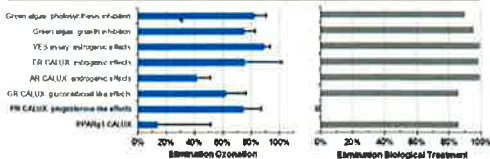
➢ Biological effects were partly eliminated.

Elimination: PAC-UF



➢ Additional 61 - 100% elimination by PAC-UF
➢ Total elimination in WWTP of 92 - 100%

Elimination: Ozonation-SF



➢ Additional 14 - 87% elimination by ozonation
➢ Total elimination in WWTP of 86 - 99%

In vitro bioassays conducted with concentrated samples are better suited than *in vivo* bioassays for the routine monitoring for biological effects, but the early lifestage bioassay with *Oncorhynchus mykiss* also demonstrated the beneficial effects of both advanced treatment technologies.

Conclusions

- Advanced treatment enabled the elimination of more than 80 % of a broad range of micropollutants and associated biological effects.
- Overall, the quality of treated effluent was significantly improved after advanced treatment, leading to improved surface water quality.

This study was conducted by the sanitation service of the city of Lausanne, mandated by the Swiss Federal Office for the Environment, with the support of the canton of Vaud.