Application of different reporter-gene assays for the determination of estrogen and androgen active substances of ozone treated hospital wastewater.

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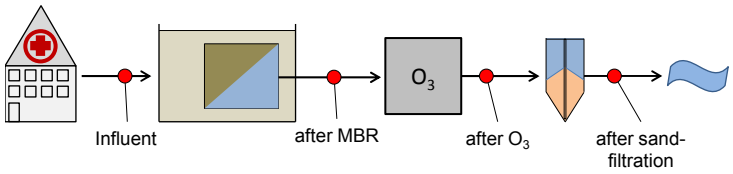
Keywords

- **Endocrine effects**
- **advanced wastewater treatment**
- **ozonation**
- **reporter-gene assays**
- **antagonistic effects**

Introduction

A good quality of water bodies is demanded e. g. by the EU Water Framework Directive, wherefore potentially harmful chemicals have to be removed. This includes endocrine disrupting chemicals, which are discharged into the environment mainly by wastewater treatment plants (WWTP). Advanced treatment with ozone successfully removes organic micropollutants but, in previous studies, an increase of estrogenic effects after the ozonation of hospital wastewater was observed. In order to investigate this effect, estrogenic and androgenic as well as anti-estrogenic and anti-androgenic activities were determined during treatment of hospital wastewater using three different effect-based reporter-gene bioassays: the estrogen and androgen receptor-mediated chemically activated luciferase gene expression assays (ER- and AR-CALUX), the Yeast Estrogen/Androgen Screen (YES/YAS) based on *Saccharomyces cerevisiae* and the *Arxula adeninivorans* Yeast Estrogen/Androgen Screen (A-YES/A-YAS).

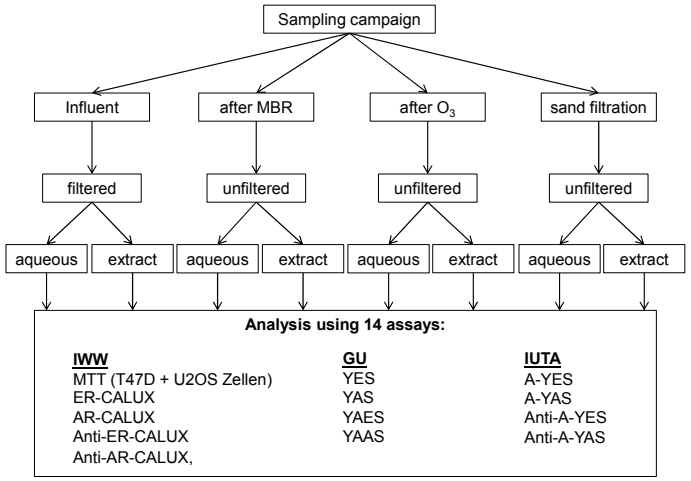
Methods



Schematic setup of the wastewater treatment plant at the Marienkrankenhaus with indicated sampling points

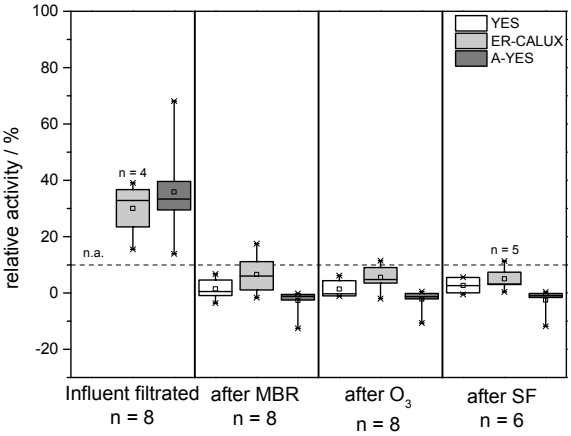
WWTP at the Marienkrankenhaus Gelsenkirchen:
- hospital wastewater without rain water
- average inflow of 200 m³ per day
- Ozone dosages: 0.20, 0.45 and 0.70 mg_{O3}/mg_{DOC}

Extraction:
- Strata XL cartridges
- Resulting enrichment factor: 20×



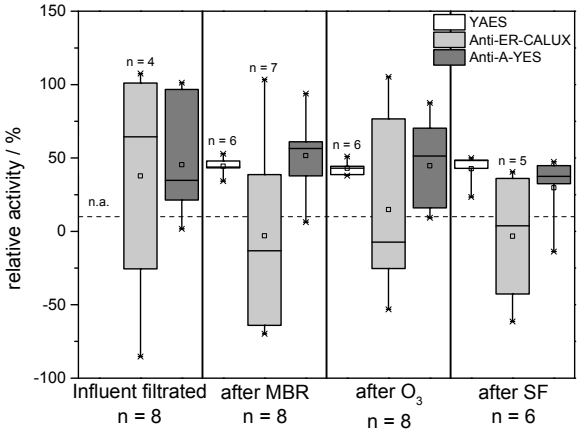
Results

Agonistic effects:
- Elimination of estrogenic and androgenic activity during MBR treatment
- Extracts showed reduction during ozonation and sand filtration
- no agonistic effects after sand filtration.



Estrogenic activity of aqueous wastewater samples (total samplings n=8). Specifications within the figure describe boxplots with a smaller dataset. Dilution factors: YES 1.6, ER-CALUX 10, A-YES 1.25. Dashed line: 10% of the maximum activity. x marks the minimum and the maximum of the dataset; □ indicates the mean activity of all tested sampling campaign, the line marks the median

Antagonistic effects:
- Low elimination of antagonistic effects
- No reduction of activity during ozonation and sand filtration
- Anti-estrogenic and anti-androgenic activity detectable after sand filtration.



Anti-estrogenic activity of aqueous wastewater samples (total samplings n=8). Specifications within the figure describe boxplots with a smaller dataset. Dilution factors: YAES 1.6, Anti-ER-CALUX 10, Anti-A-YES 1.25. Dashed line: 10% of the maximum activity. x marks the minimum and the maximum of the dataset; □ indicates the mean activity of all tested sampling campaign, the line marks the median

Comparison of assays:
- Differences among sensitivity and robustness against matrix effects
- *A. adeninivorans* assays: no cytotoxic effects, CALUX assays: dilutions necessary, *S. cerevisiae* assays: cytotoxic effects mainly in influent samples.
- Resulting classification of samples is the same for all used assay systems and the results were comparable.

Conclusions

The used receptor-gene based assays appeared to be suitable tools for the determination of endocrine disrupting substances in wastewater matrices. Although they differ among sensitivity and robustness against matrix effects the resulting classification of samples is the same for all used assay systems and the results were comparable. During wastewater treatment estrogenic and androgenic effects were removed efficiently and no effects were detectable in the final effluent. In contrast anti-estrogenic and anti-androgenic activities were only reduced partly during MBR treatment and remarkable effects could be detected in the final effluent. The persistent antagonistic effects observed in the effluent are very difficult to evaluate for their ecotoxicological relevance because so far, little attention has been paid to steroid receptor antagonists. Results of this study showed the relevance of antagonistic effects in hospital wastewater and that there is still an urgent need for a better understanding of endocrine effects and especially about antagonistic effects. In contrast to routine chemical analysis, which is not sensitive enough to detect estrogens at environmental concentrations, bioassays enable a monitoring of the proposed environmental quality standard for surface waters. An open question is how the results of the effect based assays should be classified. Therefore an effect-based trigger value should be implemented.

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