TECHNOLOGICAL EDUCATIONAL INSTITUTE OF WEST MACEDONIA

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APPLICATION OF BIOASSAYS AND POTENTIAL NEW MOLECULAR BIOMARKERS FOR THE EVALUATION OF MUNICIPAL WASTEWATER TREATMENT PROCESSES

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INTRODUCTION

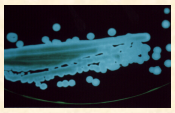
In this work, secondary treated effluents were collected from various wastewater treatment plants in the area of Thessaloniki (Northern Greece) and were assessed for their physicochemical and ecotoxicological properties using various bioassays and molecular biomarkers. The toxicity bioassays that were used included the marine photobacterium *Vibrio fischeri*, the protozoan species *Tetrahymena thermophila*, the crustacean *Daphnia magna*. Additionally the phyotoxicity of the secondary treated effluents activated sludge - fly ash mixtures was determined by utilizing 3 higher plants (one monocotyl and two dicotyls). For the molecular biomarkers, splenocytes were cultured in secondary treated effluent using RPMI – 1640 medium and their mitogenic response was assessed. Further the samples were subjected to various treatment processes including, chlorination, ozonation, ozonation - coagulation , coagulation – chlorination, chlorination - coagulation. The tertiary treated samples were also assessed for their ecotoxicological properties. The combination of physicochemical and biological assays is a promising technique in order to evaluate the efficiency of wastewater treatment techniques with parallel minimization of adverse environmental effects. The aim of this study was to examine the efficiency of various wastewater disinfection combined to coagulation treatment technologies regarding their ecotoxicity or immune cell activation reduction, with respect to their reuse potential.

MATERIALS AND METHODS

TREATMENT OF THE SAMPLES

- Ozonation
- Coagulation with 0.1 M $FeCl_3$
- Chlorination with 4 ppm chloride

BIOASSAYS



Vibrio fischeri (Microtox test)

✓Sample concentration 82 %

✓Exposure time 15 min

✓Temperature 15 °C

Daphnia magna

✓Sample concentration 100 %

✓Exposure time 24 hr

✓Temperature 20 °C

Tetrahymena thermophila

- Sample concentration 100 %
- Exposure time 24 hr
- Temperature 30 °C

Phytotoxicity

✓Species: *Lepidum sativum (L.s), Sorghum sacharatum (S.s), Alba sinapis (A.a)*

✓Solid Samples

✓Exposure time 3 days

✓Temperature 22 °C

MOLECULAR BIOMARKER

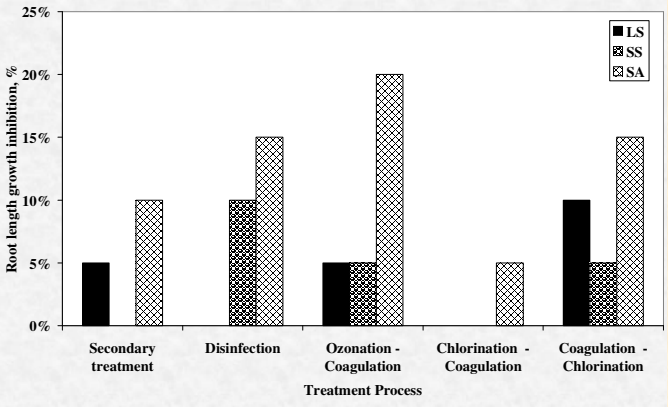
Splenocytes were cultured in autoclaved secondary/tertiary treated municipal wastewater with RPMI-1640 medium. The cytokines levels of interleukine - 1 (IL-1) and interleukine - 2 (IL-2) were evaluated by **biodetermination .**

RESULTS

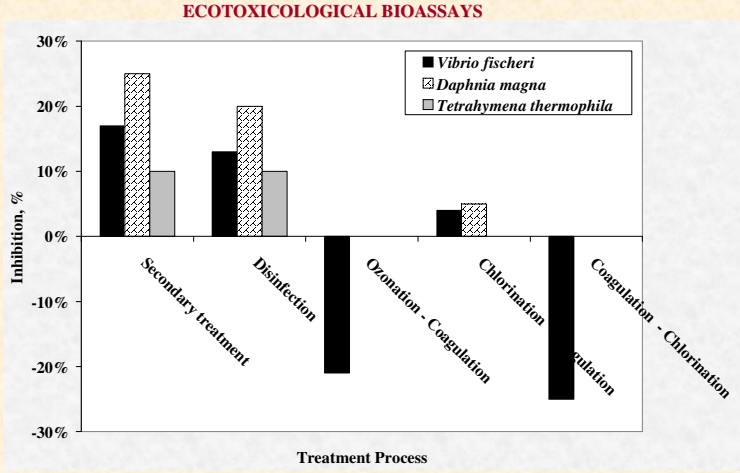
PHYSICOCHEMICAL PARAMETERS OF SECONDARY AND TREATED EFFLUENTS

	Secondary treated	Disinfection	Ozonation – Coagulation	Coagulation - Chlorination	Chlorination – Coagulation
pH	7.9	6.4	6.8	6.7	8.1
COD (mg/L)	20	20	4	14	4
BOD (mg/L)	10	-	-	-	-
NH_3-N (mg/L)	9	7	-	6	N.D.
PO_4 (mg/L)	24	24	2	4	2
SS (mg/L)	140	140	1	5	1

PHYTOTOXICITY



ECOTOXICOLOGICAL BIOASSAYS



MOLECULAR BIOMARKER

CONCLUSIONS

1. The mitogenetic responses of splenocytes during their exposure to secondary and tertiary treated effluents indicated the effects that these samples may initiate to the organisms immune system. However it should be underlined that the responses were decreased when the splenocytes were exposed to the tertiary treated samples. Thus the mitogenic response of splenocytes could be effectively used as an indicator for the assessment of wastewater treatment processes.
2. Deterioration of the microbial load by either chlorination or ozonation is not sufficient and should be followed be further treatment processes.
3. Although tertiary treated`samples exhibited very low toxicities to organismal level responses, such as inability for food uptake, bioluminescence inhibition, immobilization/mortality, seed germination (where no inhibition was observed), in the case of splenocytes a substantial proliferation of the proteins was observed.
4. Besides the fact that physicochemical parameters were within the permissible levels, the composition of the effluents was able to disturb the homeostatic mechanisms, suggesting further treatment prior to potential