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On-Line Microbial Whole Effluent Toxicity Monitoring for Industrial Wastewater

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ABSTRACT

In this study a respirometer is tested for its ability to act as an early upset warning device and whole effluent toxicity monitor for industrial discharge.
Industrial discharge water quality is commonly evaluated by comparing measured chemical concentrations to target values or regulatory limits established by governmental agencies. Unless the regulatory values are based upon empirical data, the actual effect of the discharge on aquatic systems is unknown. At the same time assessing the environmental toxicology of wastewater discharges is complicated by synergistic relationships among chemical constituents producing greater total toxicity. For example, metals may be more toxic in waters with low total hardness or more soluble at lower pH. An alternative approach that we are investigating is whole effluent toxicity testing. This study investigates the measurement of whole effluent toxicity through an on-line respirometer that measures toxicity to microorganisms comprising activated sludge. In this approach the oxygen uptake rate is monitored and used as an indicator of microbial activity or health. This study investigates the use of an online whole effluent toxicity testing system to provide early upset warning and the consistency of measured response to low pH. Repeated exposure of the microorganisms to low pH results in reduced sensitivity of the microbial population. We investigate whether this reduction in sensitivity results from physiological acclimation or changes in species composition. We identify promising applications, where, with proper calibration, respirometry based toxicity monitoring appear to be well suited for relative comparisons of whole effluent toxicity.

INTRODUCTION

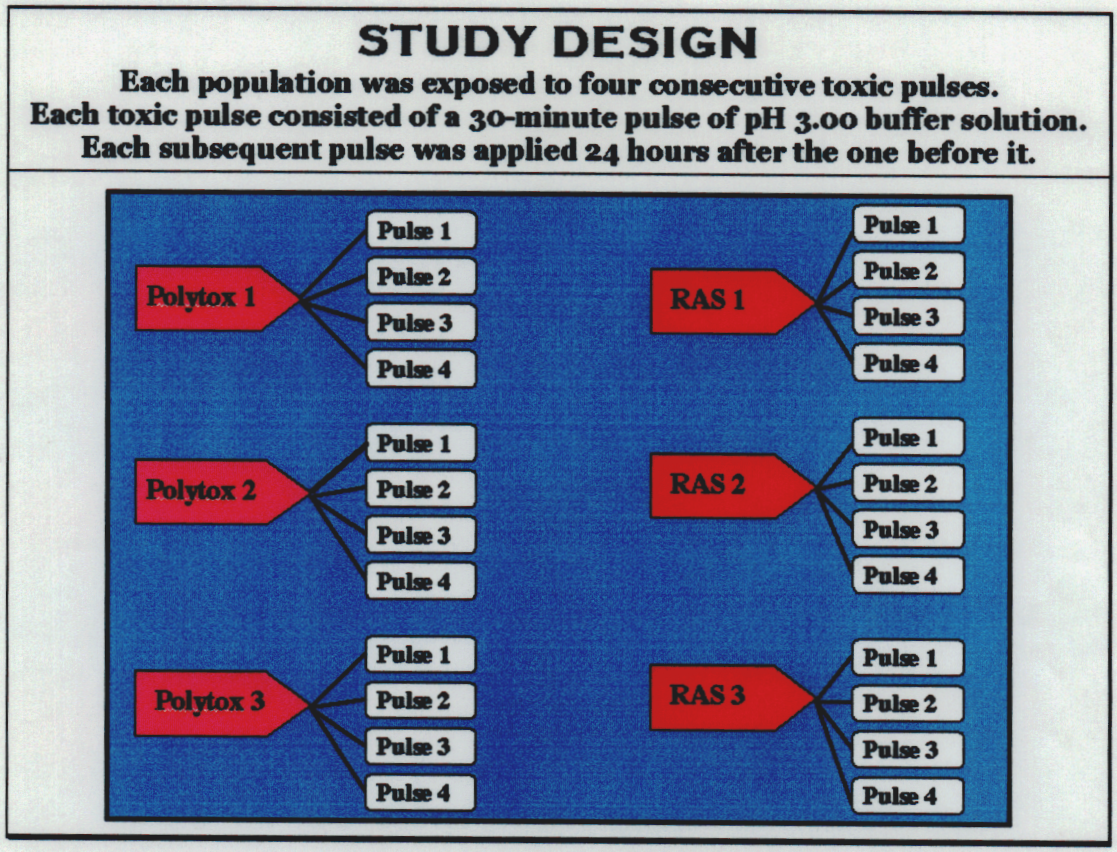
The Bioscan on-line Toxicity Monitor was chosen to study the utility and application of using a respirometer for early upset warning and as a whole effluent toxicity monitor. The Bioscan measures the effect of effluent on the oxygen demand of aerobic microorganisms. The device supports a microbial population that is representative of a wastewater treatment plant's activated sludge. The microbial population is supported in a biological filter (biofilter) that receives an aerated mixture of wastewater sample and nutrient. The nutrient assures that the microbes will have sufficient food for vigorous oxygen demand. The dissolved oxygen (DO) content of the biofilter effluent is continuously measured and recorded. A low DO reading indicates a healthy population, unaffected by the wastewater. An elevation in DO indicates that toxins present in the wastewater are impairing the respiration rate of the microbes, and could negatively impact biological treatment at the sewage treatment plant. Using the Bioscan system, studies were performed to examine rapidity and consistency of response, as well as recovery time following a toxic pulse. System response when using commercially available microbial seed (Polytox™) was compared to system response when growing an activated sludge population in the biofilter. Decreased magnitude of response was observed following repeat pulses of an individual toxin.

Denaturing gradient gel electrophoresis (DGGE) analysis was performed to determine if this decrease in sensitivity occurred due to changes in species composition.

DISCUSSION

1) Include graphics from old poster
   -picture of bioscan w/ descriptions
   -picture of biofilter w/ arrows leading to microbes & secondary treatment

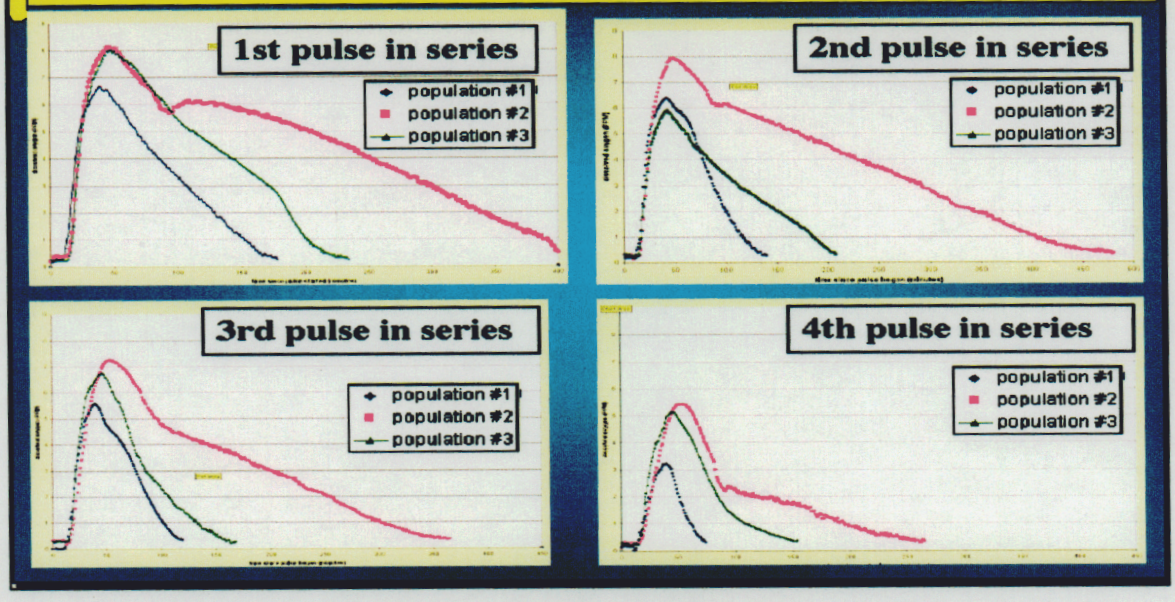
2) include picture of study design with text shown below:



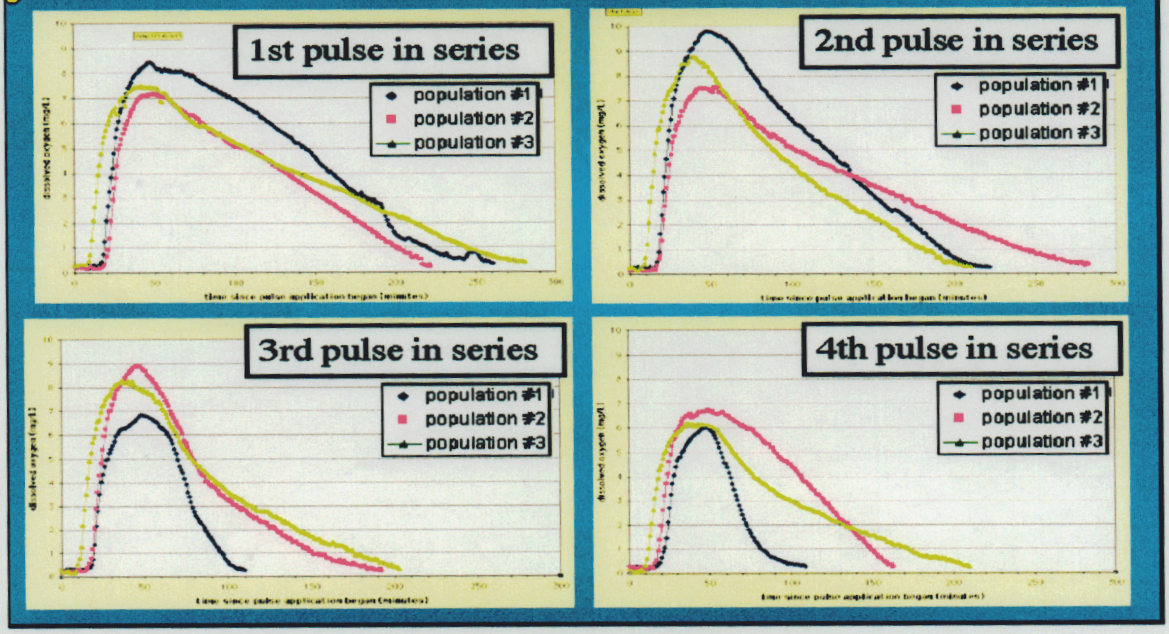
2) include all eight graphs that appear in the excel file to demonstrate lack of consistency. Lay them out in a manner similar to the one below. Text above each block should read: "Individual _____ populations responded inconsistently to the same toxic pulse."

Response Consistency

Individual Polytox populations responded differently to the same toxic pulse, indicating that response is not replicable.

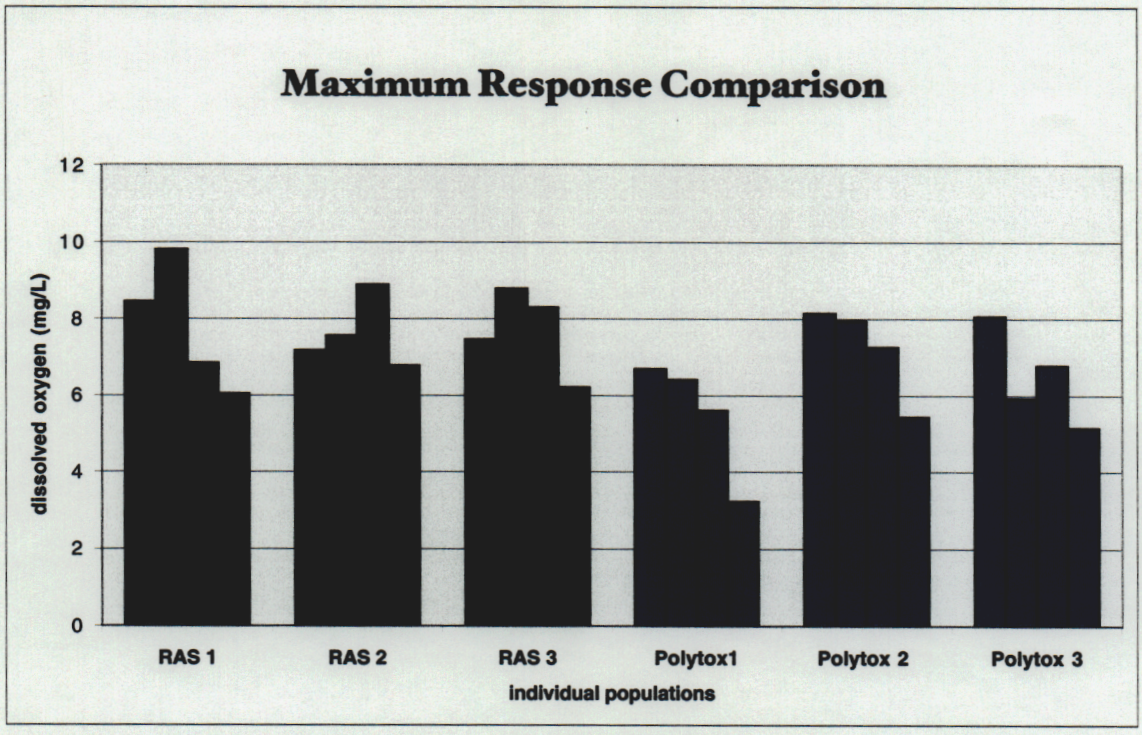


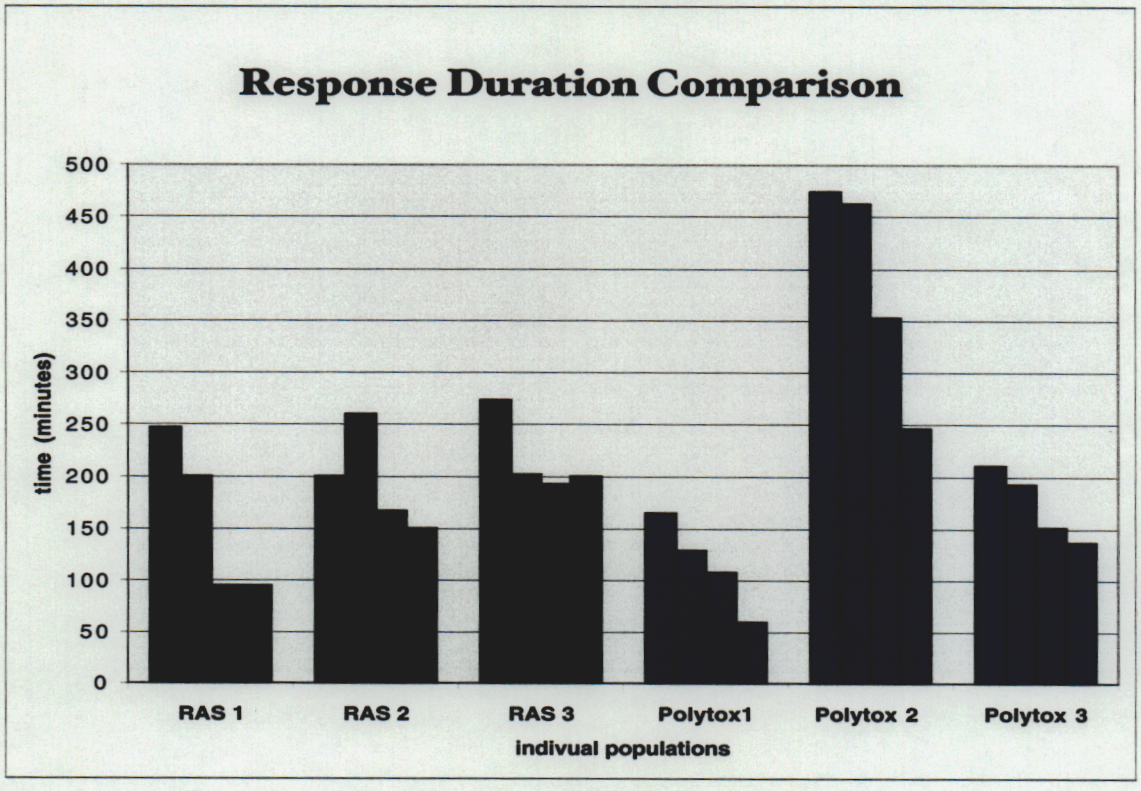
RAS populations also responded differently to the same toxic pulse, further indicating that responses are not replicable.



ACCLIMATION TO TOXINS

Both RAS and Polytox populations appear to acclimate to toxins, as measured by the decrease in maximum response and response duration over the course of a series of four pulses. This decrease is more systematic for Polytox than for RAS populations.





DENATURING GRADIENT GEL ELECTROPHORESIS

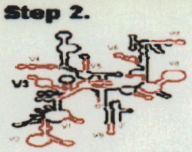
DGGE Analysis can determine if a change in population species composition is occurring by providing qualitative information about the prominent organisms in a microbial population.

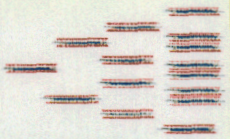
DGGE: The Procedure

Step 1.

Total DNA is extracted and purified

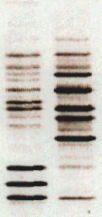
Step 2.





16S gene are targeted with specific primers and amplified

Step 3.



DGGE gel profile

Step 4.

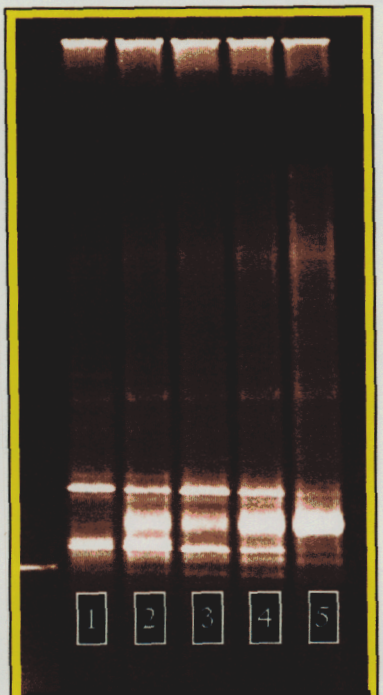
GCCAGCCGTGTTTACAGA...
GCCTTAAGCAGGCCTTCG...
GCCAGCCGTGTTTACAGA...
GCCGGCTCTAGCTTCCGT...

Prominent gel bands are excised and sequenced

Step 5.

Acinetobacter sp.
Geobacter sp.
Unknown sp X.
Methanosacina sp.

Phylogenetic analysis identifies organisms



DGGE bands from samples of RAS population

DGGE: The Analysis

- All samples were taken from one RAS population within the biofilter. A background sample (1) was taken before the population was subjected to any pulses. One sample was taken 15 hours after each pulse was run (samples 2-5).
- Different species have different DNA sequences, which migrate as bands to different heights on the gradient gel.
- Changes in the relative number of each specie are seen as a change in light intensity of that specie's characteristic band.
- A new specie appeared as a predominant member of the microbial population between samples 1 and 2. By sample number 5, following the final pulse application, this specie was the dominant member of the community.

Conclusion

This respirometer provided rapid response to low pH pulses (10-17minutes). In terms of maximum response and duration of response, inconsistencies exist in system response to an individual toxic pulse. Duration of response to a 30-minute pulse of pH 3.00 buffer solution ranged from 59-474 minutes for Polytox and 95-273 minutes for RAS. The magnitude and duration of response tends to decrease as a population is exposed to multiple toxic pulses. DGGE analysis indicates that this decrease in response is due to changes in the species composition of the population.

Potential Applications

Given our results, online respirometers like the Bioscan have the potential to be applied for:

- early upset warning of contaminants in industrial waste streams
- whole effluent toxicity microcosm indicative of waste stream effects on sanitary sewer biological treatment
- relative toxicity comparisons of various wastewater discharges