Introduction of a Novel Toxicity Analyzer for Wastewater

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Introduction

Waste Water Treatment Plants (WWTP's) often have a requirement for determining the level of toxicity of incoming wastewater to the facility. Toxicants present in the incoming wastewater can have detrimental effects on the culture of microorganisms being used in the WWTP to digest or treat the waste. All WWTP's are different in their performance and mix of microbiological populations, and therefore having an instrument that can address the unique aspects of each WWTP is of great interest. Rapid determination of incoming trade waste toxicity may allow some type of remedial action to be taken, or the rejection of the waste.

The SciTOX ALPHA is a Rapid Toxicity Measurement System (RTMS); it provides a rapid means of measuring the relative toxicity of any wastewater sample by using a biosensor and electrochemical detection technique. In this technique, the same microorganisms that exist in a WWTP are utilized to measure the metabolic effect of toxins to bacteria in the WWTP. Tests are run on pure influent samples without pre-treatment.

The SciTOX ALPHA (Figure 1) is a toxicity analyzer designed specifically for use in wastewater treatment plants, and has a small footprint allowing it to fit easily in crowded facilities. It features touchscreen operation with step-by-step methodology to guide the user through the analysis. A wireless electrode minimizes any risk of entangling the sensor in wires, or loosening wire connections. There are accompanying photos and graphics to let the user know results, incubation times, and other information on the analyzer's performance.

Figure 1: The SciTOX ALPHA

Traditional assays for biological metabolism may measure oxygen respiration directly, or other metabolic affects that can require lengthy incubation of a sample. To overcome issues with extended incubation periods, sample morphology, and low oxygen consumption or solubility, the SciTOX ALPHA uses a patented analytical process and Potassium Ferricyanide as a redox mediator (electron acceptor). This technique replaces the oxygen measured as respiration. Due to the high solubility of Potassium Ferricyanide, the toxicity analysis can therefore be completed in less than 20 minutes, including all incubations when using inoculum that has been previously prepared and checked.

In this application note, the SciTOX ALPHA product design and basic analytical technique is introduced, and the toxicity of a known toxicant, 3,5 DiChloroPhenol (DCP), on microorganisms from one WWTP is assessed. The bacterial inoculum is sourced from an aerobic treatment system.

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Experimental

The bacterial inoculum is prepared from Return Activated Sludge (RAS; the sludge that is normally returned to the treatment process to keep the treatment/digestion running) collected from a WWTP (Figure 2). Approx 200 *ml* of the RAS is pre-filtered using a coarse strainer (10-mesh) to remove large particles and fibrous material. Approx 100 *ml* of the pre-filtered RAS is then slowly filtered through a fine strainer (50-mesh) to collect the sediment. This sediment is suspended in an equal volume of 375*mM* KCl buffer. This preparation is referred to as the inoculum, and is viable for 2 to 3 days. The inoculum should be refrigerated when stored, but can be left at room temperature during normal use.

Figure 2: Preparing the Inoculum

The reagent (Mediator) for the assay is 660 *mM* Potassium Ferricyanide, and is prepared by dissolving 10.865 *g* of Potassium Ferricyanide (Potassium hexacyanoferrate (III)) in 50 *ml of* DI water. This reagent is light-sensitive, so should be stored in a dark bottle.

Analysis

Three assays are run for each analysis: a control assay (using clean water) and duplicate sample assays. The three assays are run concurrently, and ALPHA provides on-screen instructions for each step. For this paper, aqueous solutions of DCP are used as sample so that a range of DCP concentrations are analysed (in duplicate). The range of DCP concentrations is 6.6 *ppm* to 440 *ppm*. At wastewater plants, any incoming trade waste (from sewage discharge or via tank truck) might be tested, or the WWTP raw influent could be checked.

In this analysis (of 3 assays), 0.4*ml* of water or DCP solution is added to the sample vial, (a 1.5 *ml* tube), and then 0.4*ml* of inoculum is added (Figure 3). The mixture is then mixed and pre-incubated at 25°C for 5 minutes. Then 80*µl* of mediator (660*mM* Potassium Ferricyanide) is added to the vial and the contents are mixed and incubated. After 10 minutes of incubation at 25°C, the amount of Ferricyanide reduced to Ferrocyanide is measured electrochemically. The measurements are made as nano-amps (nA).

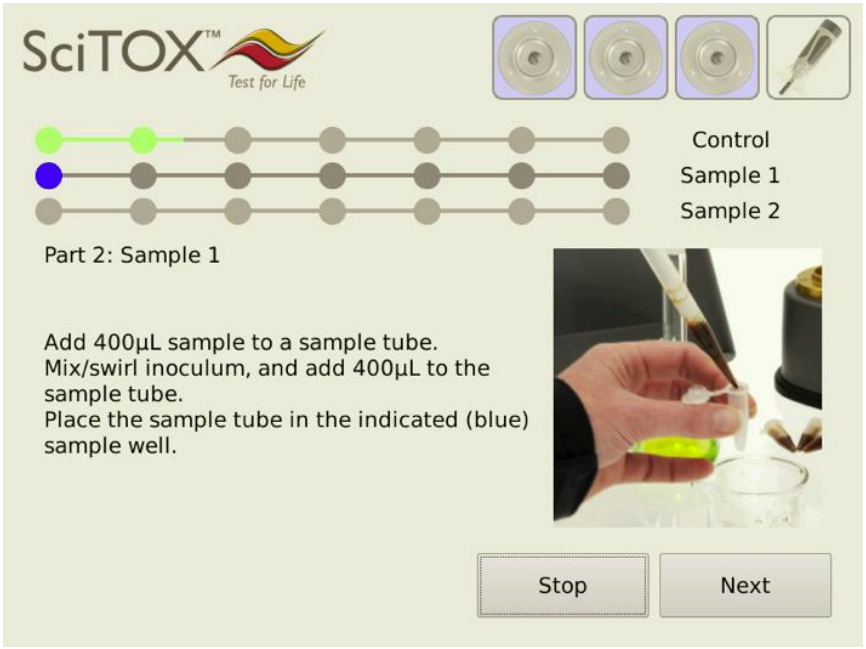

Figure 3: Operating Instructions, Preparing Sample 1

For each analysis (of 3 assays), the nA measurements of the sample duplicates are averaged, and compared to the nA measurement for the control (water). Results (Figure 4) are expressed as Biological Potential Units (BPU) and Metabolic Inhibition Quotient (MIQ), and are calculated as follows:

$BPU = 100 \times (nA_{Sample} / nA_{Control})$

$MIQ = 100 - BPU$

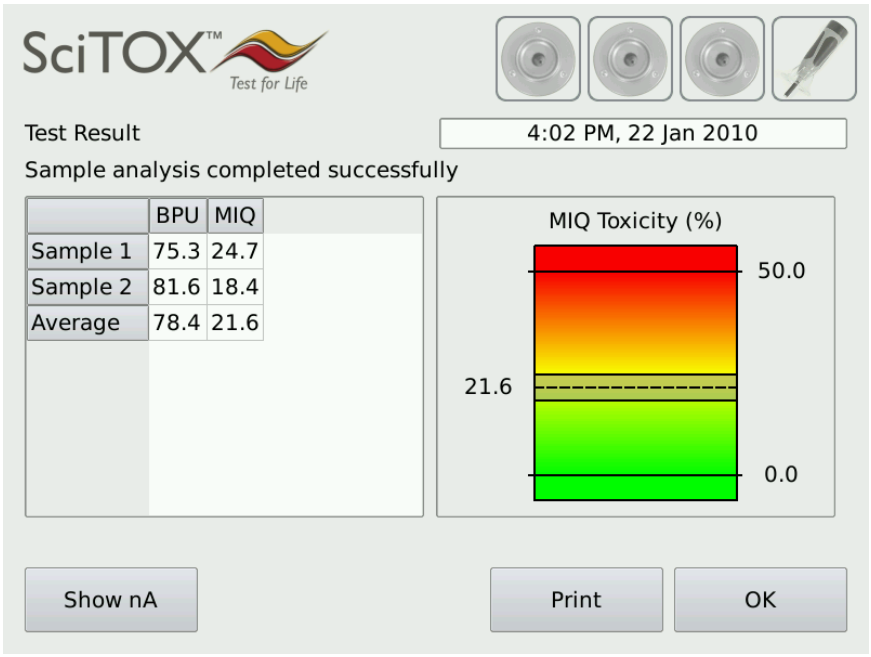

Figure 4: Results with MIQ Graph

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The BPU is a measure of relative activity of the test sample (compared to the control) and MIQ is a measure of toxicity. On the SciTOX ALPHA, the MIQ are graphed so that green represents minimal toxicity, yellow indicates increasing toxicity, and red indicates what would constitute a maximum toxicity or even lethality to the wastewater treatment plant.

Results & Discussion

Analyses were carried out on 10 concentrations of DCP. The MIQ was calculated for each analysis, and the results plotted (figure 5).

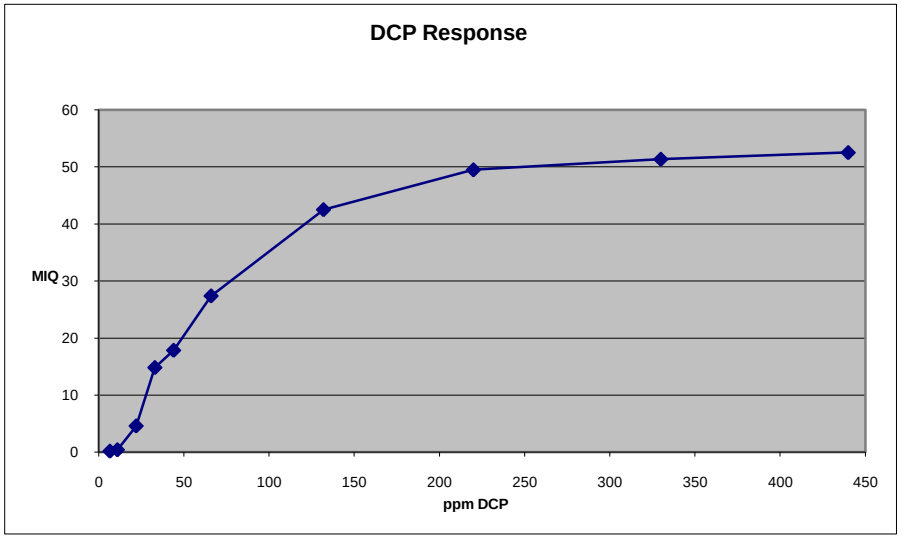

Figure 5: Metabolic Inhibition vs. Concentration of DCP

The resulting plot is a typical DCP response curve for inoculum prepared from activated sludge of WWTP. There is limited response below a threshold (approx 20*ppm* in this case), followed by a steady response to increasing DCP concentrations. This response then tails off as the DCP concentration exceeds approx 250*ppm*. The MIQ values obtained do not increase significantly beyond this maximum of approx 50 MIQ.

Other preparations of inoculum produced response curves of similar shapes. However, the threshold DCP concentration, upper limit of DCP response, and maximum MIQ obtained would vary depending on the state of treatment at the WWTP. Characteristics such as Sludge Age, types and mix of bacteria and protozoa, and sludge characterization can all vary results.

Conclusions

The SciTOX ALPHA measured the response of a preparation of WWTP microorganisms to a well-known toxicant, 3,5 DiChloroPhenol. The response curves obtained for DCP using the SciTOX ALPHA are consistent over a range of inoculum preparations.

While DCP response curves may not necessarily be of interest to operators of plants, the SciTOX ALPHA can be used to measure the toxicity of aqueous solutions that are to be processed in a WWTP. Analysis can be for organic or inorganic toxins that may be present. This is done in a one-off, 20-minute analysis for each sample of wastewater.

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The value of this technique is that the SciTOX ALPHA uses the very same microorganisms as those present in the plant (and susceptible to toxins) to measure the toxicity of the wastewater to be processed. Therefore, the analysis is specific to the individual wastewater treatment plant. Analysis can be applied to incoming trade waste, raw influent, or other samples from within the waste treatment system.

Acknowledgements

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