

## 폐수처리장치를 위한 독성 테스트 - I

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### Toxicity Screening Tests for Wastewater Treatment Plants - I

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#### 서론

A wastewater treatment plant recently had failures in bioassay tests. There are several industries discharging wastewater to the sewer. There appears to be no consensus on what caused the toxicity.

The toxicity screening tests were performed using the Reserve Electron Transfer (RET) assay (MitoScan Co., Madison, WI) and an electrolytic respirometer with various wastewater samples taken in a textile mill and other industries over time depending on production schedules to determine the effect of various wastewater streams on inhibition to trickling filter (TF) biomass obtained from the wastewater treatment plant (WWTP) and activated sludge (AS) biomass obtained from the Nine Spring WWTP, Madison, WI.

The objectives of this study were to determine which wastewater stream may have caused the toxicity failure, to find which dyes are most toxic to ecological systems, and eventually to develop strategies for reducing the toxicity of a suspected wastewater.

#### 본론

##### **Experiment Methods**

Toxicity screening tests were performed using the MitoScan technology which utilizes submitochondrial particles (SMP) and is based on the membrane-linked enzymes associated with cellular electron transport and oxidative phosphorylation.

Mitochondria are the energy centers of the cell where oxidative metabolism takes place. The MitoScan production process allows the SMP to retain their full capabilities for electron transport and oxidative phosphorylation.

The replication of these key biochemical processes in a controlled in-vitro environment makes rapid low cost and reliable toxicity assessment possible. These biochemical reactions are virtually identical across the entire Animal Kingdom. MitoScan tests provide information about toxicant disruption that impacts higher levels of biological organization such as are usually only available from whole organism tests.

The sub-cellular process upon which Mitochondrial reactions depend include :

Protein - Membrane Interactions

Protein - Protein Interactions

Protein and Membrane Integrity

These processes are integral to all biological systems not just to mitochondria. By tracking the ability of the SMP to carry out these reactions in the presence and absence of test samples, we can assess the degree to which that sample is toxic to living systems.

Because the MitoScan SMP are fully functional biochemically and operate as a concerted complex of membrane-based enzymes, a variety of options are available to track different aspects of these vital processes. Two such bioassay protocols using MitoScan SMP are widely used.

### Reverse Electron Transfer Bioassay Protocol

The Reverse Electron Transfer assay (RET) uses only a part of the electron transport enzyme chain by adding an alternative energy source to force the electron flow in the reverse direction of normal living systems (Figure 1). This assay allows the user to focus on a different set of functions relative to the ETr assay.

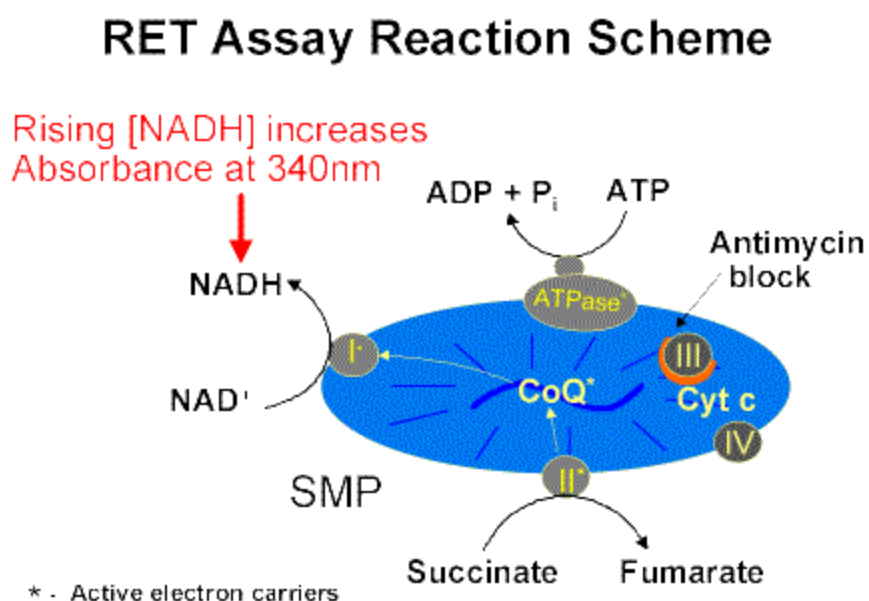


Figure 1. RET assay reaction scheme.

### 결과 및 결론

Toxicity screen tests were performed with filtered samples taken from each run using RET and ETr assays. In RET and ETr assays, the time ranges selected for linear regression were from 8 to 22 minutes and from 0 to 10 minutes, respectively. A least-squares fit was conducted to determine the rate of absorbance change with time. The calculation of inhibition is as follows:

$$\% \text{ Inhibition} = \left( 1 - \frac{\text{Sample slope}}{\text{Control slope}} \right) \times 100 \quad (1)$$

The results of ETr and RET assays are shown in Table 1. The  $R^2$  values obtained during curve fitting were all above 0.92 and mostly above 0.99. In order to get the inhibition values, the slope of each sample was calculated using eq. 1. Absorbance changes over time during RET assays is shown in Figures 1.

As far as the untreated samples are concerned, HLD/W showed the lowest inhibition

among wastewater samples tested with both ETr and RET assays. However, the difference of both values was large because the sensitivities of both assays might be different. DS had the second lowest inhibition, followed by H/W, ES/W, and M/W from both ETr and RET assays.

Domestic sewage also has different inhibition in both assays. These results are not intended to show the absolute inhibition, but relative inhibition values with respect to a wastewater sample.

Table 1. Slope of RET and ETr assays,  $R^2$ , and % inhibition

Title		RET assay slope	$R^2$	Inhibition (%)	ETr assay slope	$R^2$	Inhibition (%)
	AS	0.0264	0.9980	Control	-0.0520	0.9930	Control
	DS	0.0065	0.9954	75.5	-0.0040	0.9755	92.3
	M/W	0.0018	0.9469	93.2	-0.0012	0.9691	97.8
	ES/W	0.0037	0.9889	85.9	-0.0037	0.9889	92.9
	HLD/W	0.0263	0.9953	0.2	-0.0271	0.9989	48.3
	H/W	0.0041	0.9846	84.6	-0.0022	0.9361	95.9
Run #	1-1 (TF - DS)	0.0248	0.9971	6.1	-0.0410	0.9977	21.8
	1-2 (TF - DS)	0.0243	0.9964	7.9	-0.0487	0.9961	7.0
	1-3 (AS DS)	0.0283	0.9971	-7.3	-0.0501	0.9968	4.5
	1-4 (TF M/W)	0.0156	0.9943	40.8	-0.0093	0.9977	82.3
	1-5 (AS M/W)	0.0029	0.9146	89.1	-0.0128	0.9822	75.5
	1-6(TF - ES/W)	0.0196	0.9933	25.6	-0.0505	0.9205	3.7
	1-7(TF-HLD/W)	0.0238	0.9944	9.8	-0.0423	0.9986	19.4
	1-8 (TF H/W)	0.0215	0.9948	18.7	-0.0392	0.9950	25.3

Runs 1-1 and 1-2 are duplicates. The % inhibition values obtained from RET assay were almost identical while those obtained from the ETr assays were somewhat different. After 12 hours of treatment at 20C, DS with AS biomass had the lowest % inhibition from the RET assay, followed by DS with TF, HLD/W with TF, H/W with TF, M/W with TF, and M/W with AS. AS biomass reduced more toxicity from DS than TF biomass while acclimated TF biomass reduced significantly more toxicity than AS biomass, indicating the importance of acclimation. M/W was most toxic and resistant to biodegradation among six wastewater samples.

There was a strong relationship between the RET assay and oxygen uptake rate. Therefore, the toxicity of a wastewater of interest to biological treatment can be assessed using the RET assay. The textile mill wastewater was found to be most toxic to TF biomass among the wastewater samples tested. Other industrial wastewater does not appear to contribute to the toxic effect on TF biomass.

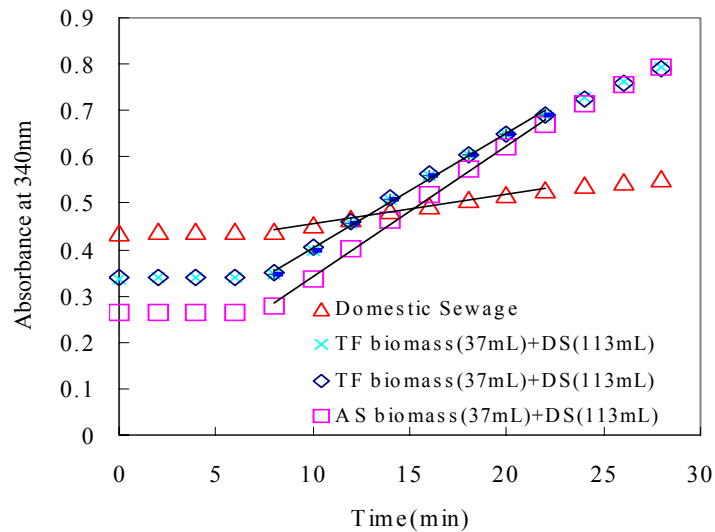


Figure 1. RET test result of the toxicity reduction of domestic sewage by TF and AS biomass

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