

## **Biothane Toxicity Test**

### **Exsan**

### **J-7117**

### **June 2007**

#### **INTRODUCTION:**

The Biothane laboratory received 4 chemicals samples from Exsan in May 2007. Exsan 100 is a cleaner, Exsan Graffiti is a floor protector, Exsan 20 is a grease remover and Exsan 21 is a degreaser. The chemicals are for use in Modelo plants in Mexico. Tests were run in our laboratory to determine if these chemicals had any acute adverse effect on the activity of Biothane anaerobic bacteria. Tecnologia Intercontinental provided Biothane with the typical dosage rates for all four chemicals and these dosing rates were used in the test.

#### **METHOD:**

The toxicity testing is conducted using an anaerobic respirometer with eight - 500 milliliter cells. Two chemicals can be tested for toxicity simultaneously. Each cell is started with 5 gVSS of biomass from an operating Biothane system. At the beginning of the test, 3750 ml of reference feed solution is made and used throughout the test. The reference solution is a pH adjusted solution consisting of propionic, butyric, and acetic acids, macronutrients and micronutrients. One hundred milliliters of the reference solution is added to each cell for each test run. The cells are diluted to a final volume of 500 milliliters with tap water. The final COD concentration in each cell is approximately 5000 mg/l. The resulting F: M ratio in the tests is approximately 0.50 gCOD/gTSS/d. The cells are maintained at 35°C using a circulating water bath and are intermittently stirred.

Gas, generated by the biomass, flows from the test cell to a gas flow-measuring unit via a needle and tubing. Bubbles of a fixed volume are formed in the flow-measuring unit. The measuring unit is connected to an interface module, which counts the number of bubbles. The interface module is connected to a computer, which converts the number of bubbles to cumulative gas volume. The computer also stores the data for later processing.

Four consecutive 24-hour runs are done on each cell. After each run the cell contents are allowed to settle and 350 milliliters of supernatant are decanted off. Fresh reference solution is then added to each cell to start the next run. The total COD fed to each cell is roughly the same. The pH of the cells is checked at the beginning of each run to

ensure that it is in the optimal operating range. Four different chemical dosage rates are tested. One, two, four and eight times the average plant use rate are tested. The average test dosages are based on the expected dosage rates given to us by Tecnologia Intercontinental.

In Run 1 each cell is fed only the reference solution. The gas production in Run 1 determines the base activity of the biomass. In Runs 2 and 3 each cell is fed the reference solution along with the defined chemical dose. The gas production in Runs 2 and 3 determines the effects of the chemical on the biomass. In Run 4 each cell is fed only the reference solution. The gas production in Run 4 determines if the biomass can recover from any toxicity witnessed in Runs 2 and 3.

Table 1.1 lists the chemicals and their average usage rates as defined by Tecnologia Intercontinental.

**Table 1.1 Chemical Use**

Chemical	Type	Use (L/d)	Spec. Grav. (kg/l)	1x Ave. Conc. in WW (ppm)
Exsan 100	Cleaner	600	1.0409	104
Exsan Graffiti	Floor Protector	100	0.8822	15
Exsan 20	Grease Remover	77	0.9893	13
Exsan 21	Degreaser	77	0.9786	13

\*Assuming average WW flow of 6,000,000 lpd = 6,000 m<sup>3</sup>/d.

Table 1.2 lists the chemical dilution used and the dosing rate to each cell.

**Table 1.2 Exsan Toxicity Testing Schedule**

Chemical	Chem. Dilution Factor	Use	1x Dose	2x Dose	4x Dose	8x Dose
Exsan 100	10	ml	0.50	1.00	2.00	4.00
		ppm	104	208	416	833
Exsan Graffiti	100	ml	0.83	1.67	3.33	6.67
		ppm	15	29	59	118
Exsan 20	100	ml	0.64	1.28	2.57	5.13
		ppm	13	25	51	102
Exsan 21	100	ml	0.64	1.28	2.57	5.13
		ppm	13	25	50	100

Dose: (ml) is the amount of diluted sample to be used per cell, (ppm) is the chemical concentration per cell. (1X) = the design average use in the plant. (2X) = 2 times the average use rate, (4X) = 4 times the average use, and (8X) = 8 times the average use rate.

## **RESULTS:**

The results are presented in four tables and four graphs. The total gas production and gas production rate of Run 1 are used as a Reference throughout the toxicity test. If, in Run 2 or 3, the total gas production or gas production rate of a cell decreases by more than 10% of its Reference, the chemical is said to be inhibitory to the anaerobic bacteria at the dosage of the effected run.

The results of the test are presented below in Tables 2.1a, 2.1b, 2.2a, and 2.2b.

**Exsan 100:** As the gas production and gas production rates were all greater than the reference run, Exsan 100 is not inhibitory or toxic to the anaerobic bacteria at the tested dosage rates.

**Exsan Graffiti:** As the gas production rate increased in each run, Exsan Graffiti is not inhibitory or toxic to the anaerobic bacteria at the tested dosage rates.

**Exsan 20:** As the gas production rate increased in each run and the gas production did not decrease by more than 10% of the Reference, Exsan 20 is not inhibitory or toxic to the anaerobic bacteria at the tested dosage rates.

**Exsan 21:** As the gas production rate increased or did not decrease significantly in each run, Exsan 21 is not inhibitory or toxic to the anaerobic bacteria at the tested dosage rates. We believe that the lower total gas production in Run 4 at the 2X and 8X concentrations was an anomaly and is not an indication of toxicity.

**Table 2.1a Total Gas Production (ml) For Exsan 100 & Exsan Graffiti**

	Chemical Con.	Run 1 Run 1 Reference	Run 2 Run 2 Test Conc.	Run 3 Run 3 Test Conc.	Run 4 Run 4 Reference
Cell 1	1x Exsan 100	789	863	898	911
Cell 2	2x Exsan 100	802	856	870	856
Cell 3	4x Exsan 100	752	852	845	843
Cell 4	8x Exsan 100	773	840	870	876
Cell 5	1x Exsan Graffiti	783	820	820	837
Cell 6	2x Exsan Graffiti	803	863	874	869
Cell 7	4x Exsan Graffiti	780	827	842	845
Cell 8	8x Exsan Graffiti	623	818	720	835

**Table 2.1b Total Gas Production (ml) For Exsan 20 & Exsan 21**

	Chemical Con.	Run 1 Run 1 Reference	Run 2 Run 2 Test Conc.	Run 3 Run 3 Test Conc.	Run 4 Run 4 Reference
Cell 1	1x Exsan 20	773	820	746	814
Cell 2	2x Exsan 20	793	847	834	825
Cell 3	4x Exsan 20	795	842	837	833
Cell 4	8x Exsan 20	773	819	820	840
Cell 5	1x Exsan 21	775	837	823	813
Cell 6	2x Exsan 21	788	847	795	684
Cell 7	4x Exsan 21	787	825	835	832
Cell 8	8x Exsan 21	797	827	814	709

**Table 2.2a Maximum Gas Production Rate (ml/hr) For Exsan 100 & Exsan Graffiti**

	Chemical Con.	Run 1 Run 1 Reference	Run 2 Run 2 Test Conc.	Run 3 Run 3 Test Conc.	Run 4 Run 4 Reference
Cell 1	1x Exsan 100	51	57	62	64
Cell 2	2x Exsan 100	53	59	60	59
Cell 3	4x Exsan 100	52	57	57	58
Cell 4	8x Exsan 100	50	57	63	62
Cell 5	1x Exsan Graffiti	50	55	56	57
Cell 6	2x Exsan Graffiti	52	58	61	62
Cell 7	4x Exsan Graffiti	51	56	59	59
Cell 8	8x Exsan Graffiti	38	55	47	57

**Table 2.2b Maximum Gas Production Rate (ml/hr) For Exsan 20 & Exsan 21**

	Chemical Con.	Run 1 Run 1 Reference	Run 2 Run 2 Test Conc.	Run 3 Run 3 Test Conc.	Run 4 Run 4 Reference
Cell 1	1x Exsan 20	48	55	55	57
Cell 2	2x Exsan 20	52	58	58	60
Cell 3	4x Exsan 20	51	57	58	59
Cell 4	8x Exsan 20	49	56	59	61
Cell 5	1x Exsan 21	50	57	57	57
Cell 6	2x Exsan 21	51	58	57	56
Cell 7	4x Exsan 21	51	58	58	60
Cell 8	8x Exsan 21	52	59	58	59

### **CONCLUSIONS:**

The above Tables and attached graphs indicate that Exsan 100, Exsan Graffiti, Exsan 20 and Exsan 21 were non-toxic or inhibitory at the tested dosage rates. A summary of the test results is given below in Table 3.1.

This test only measures the acute toxic effects of the chemical on the anaerobic bacteria, and it does not measure the chronic effects of the chemical on the bacteria.

**Table 3.1 Toxicity Test Summary**

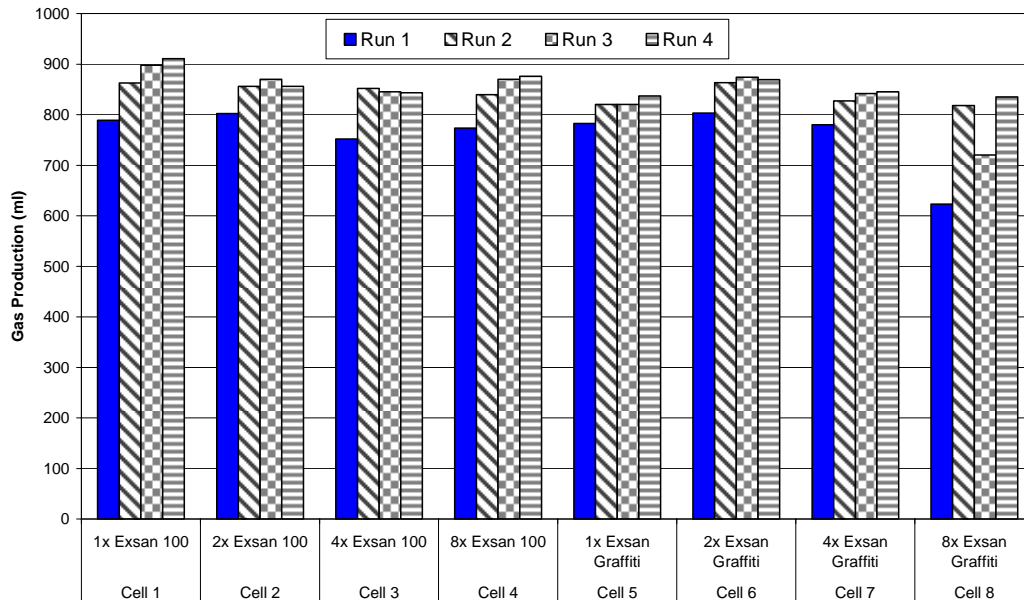
<b>Exsan 100</b>	<b>1x</b>	<b>2x</b>	<b>4x</b>	<b>8x</b>
<b>Chemical Conc.</b>	104 ppm (600 L/d)	208 ppm (1200 L/d)	416 ppm (2400 L/d)	833 ppm (4800 L/d)
<b>Toxicity Finding</b>	Not Toxic	Not Toxic	Not Toxic	Not Toxic

<b>Exsan Graffiti</b>	<b>1x</b>	<b>2x</b>	<b>4x</b>	<b>8x</b>
<b>Chemical Conc.</b>	15 ppm (100 L/d)	29 ppm (200 L/d)	59 ppm (400 L/d)	118 ppm (800 L/d)
<b>Toxicity Finding</b>	Not Toxic	Not Toxic	Not Toxic	Not Toxic

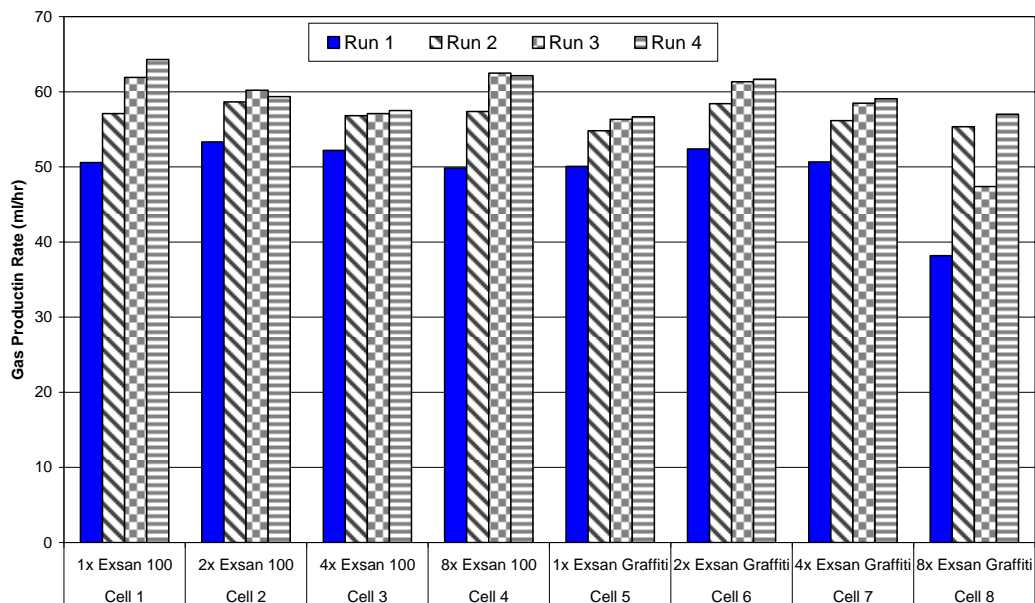
<b>Exsan 20</b>	<b>1x</b>	<b>2x</b>	<b>4x</b>	<b>8x</b>
<b>Chemical Conc.</b>	13 ppm (77 L/d)	25 ppm (154 L/d)	51 ppm (308 L/d)	102 ppm (616 L/d)
<b>Toxicity Finding</b>	Not Toxic	Not Toxic	Not Toxic	Not Toxic

<b>Exsan 21</b>	<b>1x</b>	<b>2x</b>	<b>4x</b>	<b>8x</b>
<b>Chemical Conc.</b>	13 ppm (77 L/d)	25 ppm (154 L/d)	50 ppm (308 L/d)	100 ppm (616 L/d)
<b>Toxicity Finding</b>	Not Toxic	Not Toxic	Not Toxic	Not Toxic

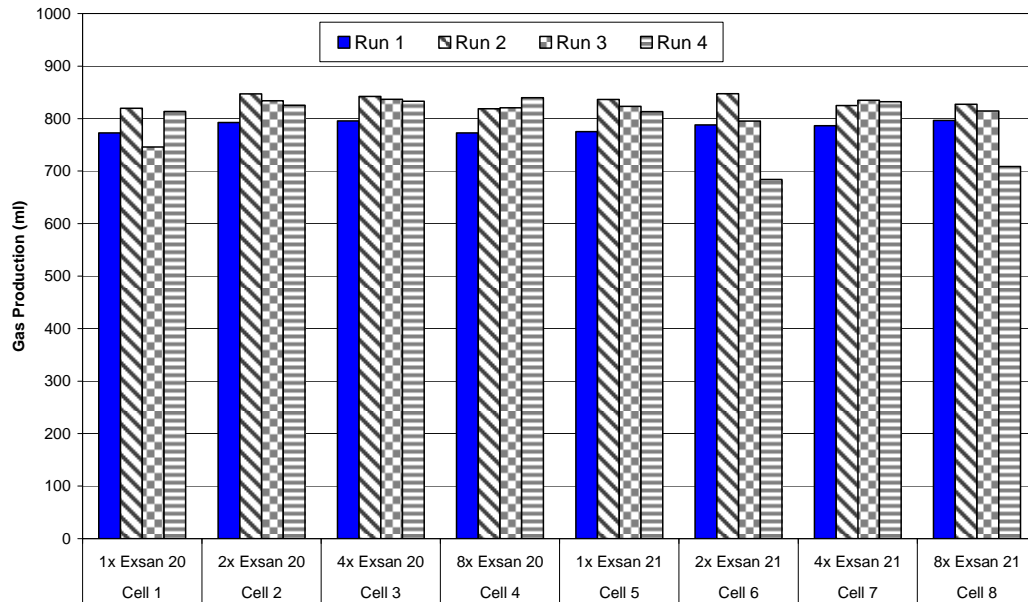
**Exsan Toxicity Test (July 2007)**  
**Exsan 100 & Exsan Graffiti**  
**Gas Production**



**Exsan Toxicity Test (July 2007)**  
**Exsan 100 & Exsan Graffiti**  
**Gas Production Rate**



**Exsan Toxicity Test July 2007**  
**Exsan 20 & Exsan 21**  
**Gas Production**



**Exsan Toxicity Test July 2007**  
**Exsan 20 & Exsan 21**  
**Gas Production Rate**

