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Effects of Pluronics on a Beneficial Soil Microbe Pseudomonas putida Strain KT2440

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EFFECTS OF PLURONICS ON A BENEFICIAL SOIL MICROBE *Pseudomonas putida* STRAIN KT2440

by

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Priyanka Gajjar

Outline

This report summarizes the effects of a range of Pluronics on cellular responses in bacterial and mammalian cells through a review of published findings. Effects of Pluronics on drug delivery, efflux systems, growth, and nutrition are reported for eukaryotic cells. For microbial cells, their effects on adhesion, movement, biofilm formation, energy status and secondary metabolism are discussed. Guided by findings in the literature, a series of experiments were conducted exploring the responses of a beneficial environmental soil microbe, *Pseudomonas putida* strain KT2440, to selected Pluronics. These results are presented. The report has four sections: an Introduction that covers published literature on eukaryotic and prokaryotic cells, the results of studies on *Pseudomonas putida* strain KT2440 and the overall conclusion and potential future studies. Thus, the material is presented in the following sections:

1. General Introduction
    1.1. Structural features of Pluronics
    1.2. Pluronics and mammalian cells, an overview
        1.2.1 Effects of Pluronic unimers on efflux pumps in mammalian cells
        1.2.2 Responses of other Eukaryotes
    1.3. Pluronic effects on bacteria
        1.3.1 Efflux pumps in bacterial cells
        1.3.2 Types of ATPase
        1.3.3 Synergy between Poloxamer and antibiotics in antimicrobial activity
        1.3.4 Pluronics and surface attachment/biofilm formation
2. Examination of metabolic effects of Pluronics on an environmental bacterium, *Pseudomonas putida* KT2440

1. General Introduction

1.1 Structural features of Pluronics

Pluronic block co-polymers consist of ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a linear A-B-A structure: EO_a-PO_b-EO_a as is illustrated in Figure 1 where a = number of hydrophilic EO units and b = number of hydrophobic PO units. The water solubility and properties of the copolymer vary with the changes in chain lengths (Figure 1).

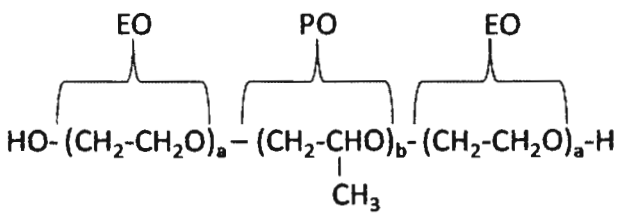


Figure 1: Pluronic molecular formula.

Pluronics, a trademark of the BASF Corp that manufactures these chemicals, are designated with a letter to define their physical form at room temperature followed by two or three digits. Letter 'L' denotes liquid, 'P' denotes paste, and 'F' denotes flake or solid. In the numerical designation, the first digit (two digits in a three-digit number) multiplied by 300, gives the approximate mass of the PO hydrophobe; and the last digit multiplied by10 gives the percentage EO content (Figure 2). For the generic term

"poloxamer", copolymers are designated with the letter "P" followed by three digits. The first two digits multiplied with 100 indicate the approximate molecular weight of the PO hydrophobe, and the last digit multiplied by 10 gives the percentage EO content.

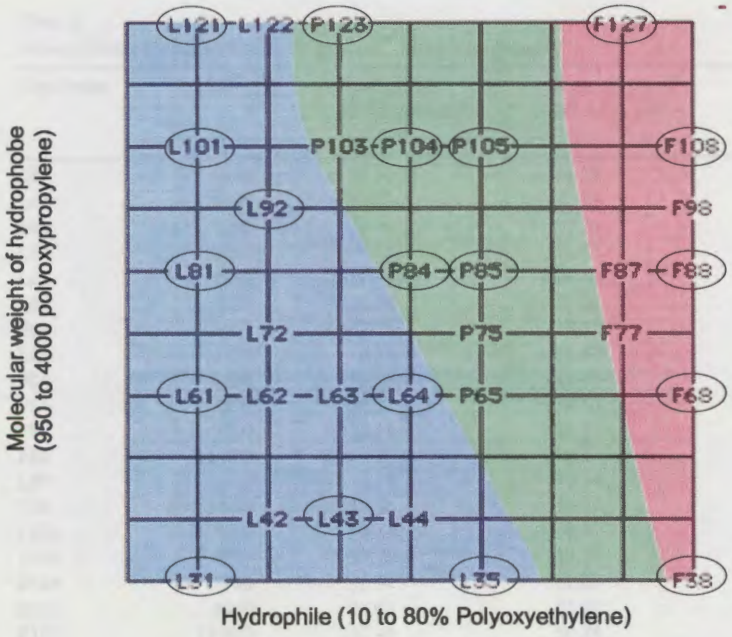


Figure 2: Compositions of EO and PO in Pluronics. The physical state of the Pluronics is indicated by the differently shaded regions in the Figure.
Source: http://www2.basf.us/performancechemical/bcperfpluronic_grid.html. Circles designate the Pluronics discussed in this report.

The Pluronics range in molecular weights (MW) from a few kD to over 14 kD (Figure 2, Table 1). The number of hydrophilic EO (a) units and lipophilic PO (b) units defines the hydrophilic-lipophilic balance (HLB) and critical micelle concentration (CMC) of individual polymer. Small variations in a and / or b may result in co-polymers with similar MWs but highly different physical properties. Depending on the Pluronic MW and a:b ratio the properties are tuned to specific applications such as wetting agents, emulsifiers, defoaming agents, and lubricants [http://wwwstage.basf.com/performancechemical/bcperfapplications.html].

Table 1 shows the physicochemical characteristics of several Pluronics.

Table 1: Physiochemical properties of selected Pluronics (Kabanov et al., 2003)

Table 1
Physicochemical characteristics of Pluronic® block copolymers

Copolymer	MW[a]	Average no of EO units (n)[b]	Average no of PO units (m)[b]	HLB[c]	Cloud point in 1% aqueous solution, °C[d]	CMC, M
L35	1900	21.59	16.38	19	73	$5.3\cdot10^{-3}$
L43	1850	12.61	22.33	12	42	$2.2\cdot10^{-3}$
L44	2200	20.00	22.76	16	65	$3.6\cdot10^{-3}$
L61	2000	4.55	31.03	3	24	$1.1\cdot10^{-4}$
L62	2500	11.36	34.48	7	32	$4.0\cdot10^{-4}$
L64	2900	26.36	30.00	15	58	$4.8\cdot10^{-4}$
F68	8400	152.73	28.97	29	>100	$4.8\cdot10^{-4}$
L81	2750	6.25	42.67	2	20	$2.3\cdot10^{-5}$
P84	4200	38.18	43.45	14	74	$7.1\cdot10^{-5}$
P85	4600	52.27	39.66	16	85	$6.5\cdot10^{-5}$
F87	7700	122.50	39.83	24	>100	$9.1\cdot10^{-5}$
F88	11 400	207.27	39.31	28	>100	$2.5\cdot10^{-4}$
L92	3650	16.59	50.34	6	26	$8.8\cdot10^{-5}$
F98	13 000	236.36	44.83	28	>100	$7.7\cdot10^{-5}$
L101	3800	8.64	58.97	1	15	$2.1\cdot10^{-6}$
P103	4950	33.75	59.74	9	86	$6.1\cdot10^{-6}$
P104	5900	53.64	61.03	13	81	$3.4\cdot10^{-6}$
P105	6500	73.86	56.03	15	91	$6.2\cdot10^{-6}$
F108	14 600	265.45	50.34	27	>100	$2.2\cdot10^{-7}$
L121	4400	10.00	68.28	1	14	$1.0\cdot10^{-6}$
P123	5750	39.20	69.40	8	90	$4.4\cdot10^{-6}$
F127	12 600	200.45	65.17	22	>100	$2.8\cdot10^{-6}$

[a] The average molecular weights provided by the manufacturer (BASF, Wyandotte, MI)
[b] The average numbers of EO and PO units were calculated using the average molecular weights
[c] HLB values of the copolymers.
[d] The cloud points were determined by the manufacturer.
[e] CMC values were determined previously using pyrene probe

HLB is an expression used to demonstrate the relationship between the hydrophilic and hydrophobic groups of a surfactant. The HLB dictates polymer amphiphilicity and the resulting surface activity. HLB values shown in Table 1 are obtained from data reported by BASF Corporation. There are many publications that discuss theoretical or experimental methods to determine HLB values. Griffin (1949) computed HLB = 20 * Mh/M; Mh = molecular mass of the hydrophilic EO region and M = molecular mass of the whole molecule. According to Davies' method (Davies, 1957), HLB can be calculated as:

HLB = 7 + Σ (hydrophilic group numbers) + Σ (lipophilic group numbers)

Though the Davies' method is easy, these calculated HLB values for polyethoxylated surfactants differ significantly with experimental data. Guo et al. (2006) addressed this limitation of Davies' method by the introduction of effective chain length for straight alkyl chain, EO chain, and PO chain in the calculation.

Pluronics self assemble into micelles in aqueous solutions above a concentration called the critical micelle concentration (CMC). Below the CMC, Pluronics exist in solution as isolated molecules known as unimers. Kabanov et al. (1995) studied Pluronics F68, F108 and P85 in aqueous solutions to determine their CMC using surface tension measurements and fluorescent probes. Light scattering and ultracentrifugation techniques were utilized to determine the size and molecular masses of micelles. Figure 3 depicts the structure of a Pluronic micelle (average diameter ranges from 15 to 35 nm, Kabanov et al., 1995) or 10 to 100 nm (Batrakova and Kabanov, 2008) with hydrophilic EO chains facing outward and hydrophobic PPO chains forming the inner boundary surface.

The hydrophobic inner core of micelles serves as a microenvironment for the incorporation of hydrophobic drugs while the hydrophilic outer shell maintains the dispersion stability of micelles (Oh et al., 2004). This characteristic of Pluronic micelles makes them an attractive candidate as a drug delivery vehicle. Micelle-drug delivery enhances the transport of low molecular substances across cell membranes (Slepnev et al., 1992) permitting easy penetration into capillaries of the target cells (Kabanov et al., 1995). Such Pluronic micelles deliver drugs across the blood brain barrier (Kabanov et al., 2003), permit oral delivery (Batrakova et al., 1998) and allow tumor specific delivery

of antineoplastic agents (Kabanov et al., 2002). Also, these drug delivery-microcontainers reduce the interaction of the drug with blood or other external cell components increasing their stability as they circulate in the blood system (Kabanov et al., 1989; Kabanov et al., 1995).

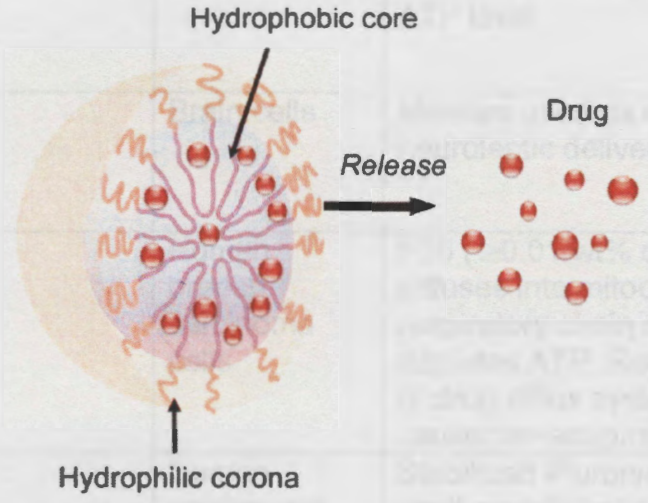


Figure 3: Pluronic block copolymer in micelle form showing encapsulation of a drug (Batrakova et al., 2008). At a concentration above CMC, these copolymers self-assemble into micelles capable of carrying hydrophobic molecules.

1.2 Pluronics and mammalian cells, an overview

Several studies focus on the interaction of Pluronics with mammalian cells (Kabanov et al., 1995, 2005; Batrakova et al., 2003; reviews: Batrakova and Kabanov, 2008; Alakhova et al., 2009). As illustrated in Table 2 the findings have a direct relationship to biomedical and pharmaceutical fields.

Table 2: Pluronics and mammalian cells

Pluronics	Type of cells	Mode of action/time of efficacy	References
F108, L35, L121 and P85	Bovine brain micro endothelial cells (BBMEC)	Inhibition of Pgp efflux system in BMECs at below CMC level of individual Pluronic; altered membrane fluidization; ATP level changes.	Batrakova et al., 2003
P85	Kidney epithelial cells	At 0.01 wt% inhibits Pgp efflux pump; inhibits ATPase activity and reduces ATP level.	Batrakova et al., 2004
P85	Brain cells	Micelles used as microcontainers for neuroleptic delivery into the brain.	Kabanov et al., 1989
P85	Human breast carcinoma cells	P85 ($\geq$0.01 wt% concentrations) diffuses into mitochondria: inhibits respiratory chain complexes I and IV, depletes ATP. Reduces the function of drug efflux system. In MDR cells cause pre-apoptotic effects.	Alakhova et al., 2009
L121	Human epidermoid carcinoma KBv cell line	Stabilized Pluronic L121 micelle (with shell crosslinks) at 0.003 wt% inhibits efflux system in MDR cells and depletes ATP.	Yang et al., 2007

The Pluronics influence drug performance in normal cell lines and more effectively in multidrug resistant (MDR) cells. Pluronics are considered as inert components that play a crucial role in protecting drugs from degradation. Pluronics also help in increasing drug exposure to tissues and their transport into cells. However, as demonstrated in Table 2, certain Pluronics do affect mammalian cell functions that contribute to drug efficacy. Altered responses include changes in membrane microviscosity, inhibition of the electron transport chain in mitochondria, reduced ATP level, impaired Pgp efflux systems, and altered respiration, (Batrakova et al., 2002; Kabanov et al., 2005; Alakhova et al., 2009).

Two modes of action must then be considered depending whether the Pluronics are present at concentrations above or below the CMC. As discussed already, above the CMC, Pluronic micelles physically encapsulate and transport drugs across cell membranes (Slepnev et al., 1992). They permit oral delivery (Batrakova et al., 1998) and will target drugs to the central nervous system (CNS) across the blood brain barrier (Kabanov et al., 2003). However Pluronics are also active below their CMC values as unimers leading to reduced ATP levels and altered drug efflux as is discussed in the next section (Batrakova et al. 2003, 2004).

1.2.1 Effects of Pluronic unimers on efflux pump in mammalian cells

Efflux pumps are major causes of anticancer drug resistance in eukaryotic cells (Batrakova et al., 2003; Fatma et al., 2006). Figure 4 illustrates a model for a mammalian cell Pgp efflux pump performing drug extrusion. If the pump is in the plasma membrane, then drug concentration is reduced in the cell interior through its function. Two efflux pumps that are ATP-driven are the Pgp transporter, found in normal cell lines, and a multidrug resistance - associated protein transporter (MRP) (Homolya et al., 2003) found in tumor cells. These pumps may normally be involved in the transport of organic anions or movement of lipid components (Homolya et al., 2003).

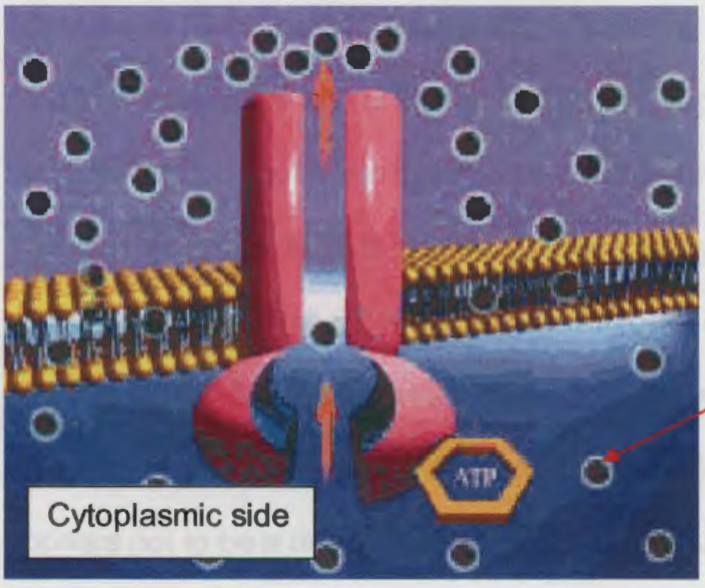


Figure 4: An ATP-dependent Pgp efflux pump extrudes drugs and other exogenous compounds from the cytoplasm (Webber and Piddoc, 2003).

Batrakova et al. (2003) using bovine brain micro endothelial cell (BBMEC) monolayers in studies with a range of Pluronics designated by circles in Figure 5, show how the reactivity of these compounds with Pgp-type efflux systems differs with their composition. To explore the effects on the drug efflux pumps, Batrakova et al. (2003) examine how Pluronics influenced the intracellular level of a dye R123 that is a substrate for a Pgp transporter in the plasma membrane. Figure 6 shows how each of four Pluronics differentially affects accumulation. In Figure 6A arrows from left to right correspond to the CMC of Pluronics L121, P85, F108, L35 used at a range of concentrations in the exposure of BBMEC cells for 60 min. Accumulation of R123 reaches a maximum level below the CMC values for each Pluronic and decreases above the CMC value. This result is consistent with earlier studies (Batrakova et al., 1998) demonstrating that Pluronic unimers (single molecular chains of the block copolymer) inhibit the Pgp efflux pump. A R123 enhancement factor (R123 levels in the

presence of Pluronic /R123 levels in the absence of Pluronic) was calculated and plotted versus the length of PO block for each copolymer (Figure 6B). Figure 6B shows that P85 and L81 both with intermediate PO chain lengths are the best inhibitors although they differ in HLB (16 and 2, respectively, Table 1). Another Pluronic with HLB similar to P85, L35 (HLB 19) has little activity nor do hydrophilic Pluronics F38 (HLB 31) and F127 (HLB 22, Table 1) that have short and long PO chain lengths, respectively. Similarly, activity is weak with Pluronic L121 (HLB 1), which has long PO chains. There appears not to be a direct correlation between HLB and affects on R123 dye accumulation in BBMEC monolayers.

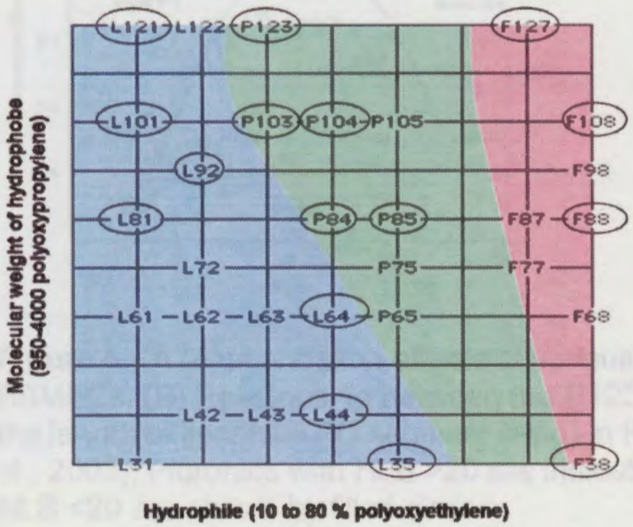


Figure 5: Pluronic grid shows the Pluronic (with circles) that are used in the study of Batrakova et al. (2003).

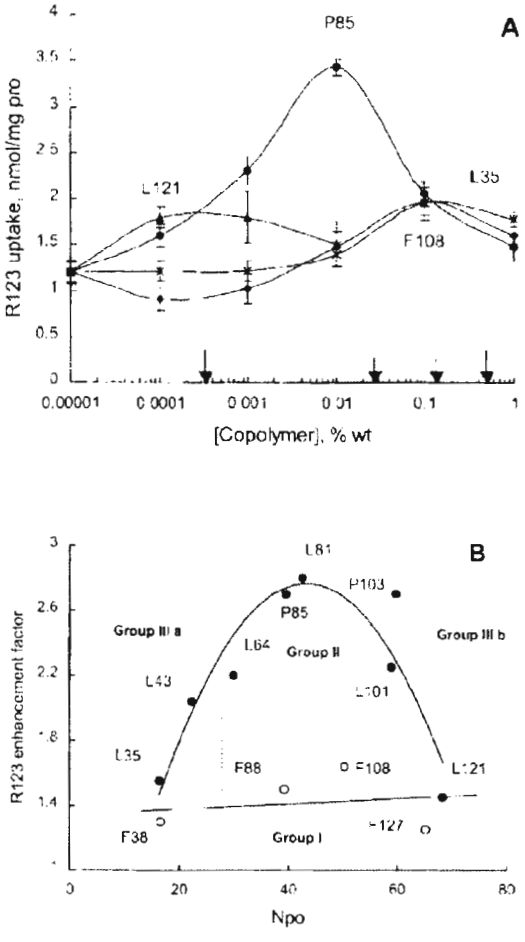


Figure 6: (A) concentration effects of various Pluronics on R123 accumulation in BBMECs. (B) Relationship between the R123 accumulation enhancement factor and the length of lipophilic PO segment (Npo) in Pluronic block copolymers (Batrakova et al., 2003). Pluronics with HLB >20 are indicated by empty circles and Pluronics with HLB <20 are shown by filled circles.

In another earlier study, Batrakova et al. (2001) proposed that membrane fluidizers abolish Pgp ATPase activity thereby inactivating Pgp-mediated drug efflux. They report (Batrakova et al., 2001) that P85 inhibition of Pgp ATPase activity occurs in the same dose range that is inhibitory to Pgp efflux in BBMEC monolayers. Batrakova et al. (2003) correlate effects of Pluronics on R123 accumulation in the cytoplasm of these cells with changes in membrane microviscosity as shown in Figure 7. All Pluronics were added at concentrations (less than CMC level) producing maximal accumulation of

R123 in BBMECs (Figure 6). The hydrophilic Pluronic F88 (39 PO units, HLB 28, Table 1) and Pluronic L35 with 16 PO units (HLB 19, Table 1) caused membrane solidification, as evidenced by an increase in the membrane microviscosity. In contrast, the most lipophilic Pluronic L121 (68 PO units, HLB 1, Table 1) and a Pluronic with an intermediate lipophilicity P85 (40 PO units, HLB 16, Table 1) decrease the membrane viscosity. With P85 these effects occur with the unimers, i.e. at concentrations below the CMC. More recent studies show that P85 unimers enter cells through lipid raft docking and calveolae formation (Sahay et al., 2010) In contrast entry of P85 micelles of 14.5 nm diameter (60 molecules of P85) enter by clathrin-mediated endocytosis (Sahay et al., 2010).

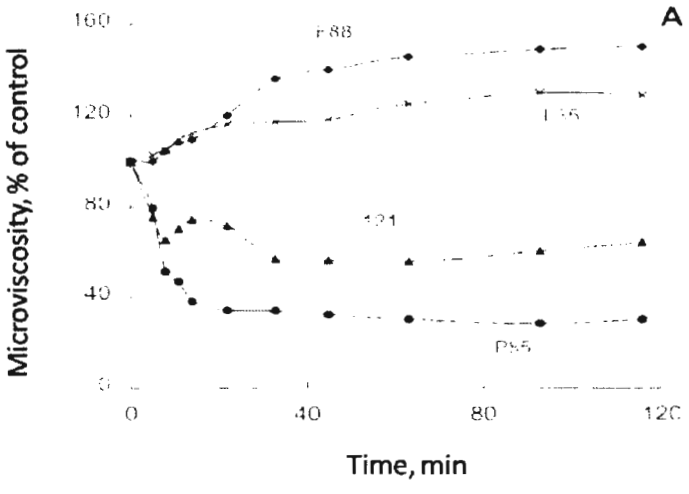


Figure 7: Kinetic effects of various Pluronics on the total membrane microviscosity in BBMEC (Batrakova et al., 2003).

The Pluronics also alter cellular ATP levels as shown in Figure 8. An ATP depletion factor (intracellular ATP in the absence of Pluronic /ATP levels in the presence of Pluronic) was calculated and plotted versus the length of PO block for each copolymer. In general, the hydrophilic Pluronics F38 (HLB 25), F88 (HLB 28), F108 (HLB 27), and F127 (HLB 22) had negligible effect on ATP levels showing that for these

Pluronics with high HLBs PO length was not a decisive factor of PO units. However there was a linear relationship with PO length for the series L35, L43, L64 and P85 where activity increased with PO chain length. Conversely for P85, L101 and L121 activity decreased with PO chain length. .

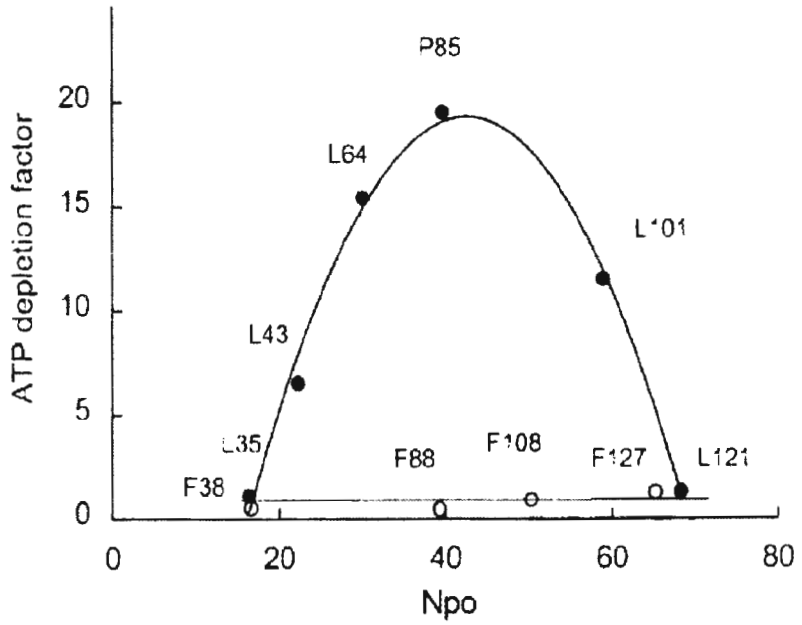


Figure 8: Relationship between the ATP depletion factor and the length of PO segments (Npo) in Pluronic block copolymer (0.01 % w/v concentration) (Batrakova et al., 2003). Pluronics with HLB >20 are indicated by empty circles and Pluronics with HLB <20 are shown by filled circles.

Confocal microscopy results (Figure 9) using FITC-labeled Pluronics (F108, L35, P85 and L121) at 0.01% w/v on BBMEC for 2 h at 37 °C show differential internalization of copolymers into the cells. Hydrophilic Pluronic F108 (HLB 27) shows poor internalization into cells (Figure 9A). Pluronic L35 (HLB 19) with short PO block and more lipophilic Pluronic P85 with intermediate PO blocks (HLB 16) accumulate throughout the cells (Figure 9B and Figure 9C). The most hydrophobic Pluronic L121 (HLB 1) localizes within endocytic compartments. These compartments are part of the

endocytic membrane transport pathway from the plasma membrane to the lysosome. During endocytosis materials are absorbed by engulfment.

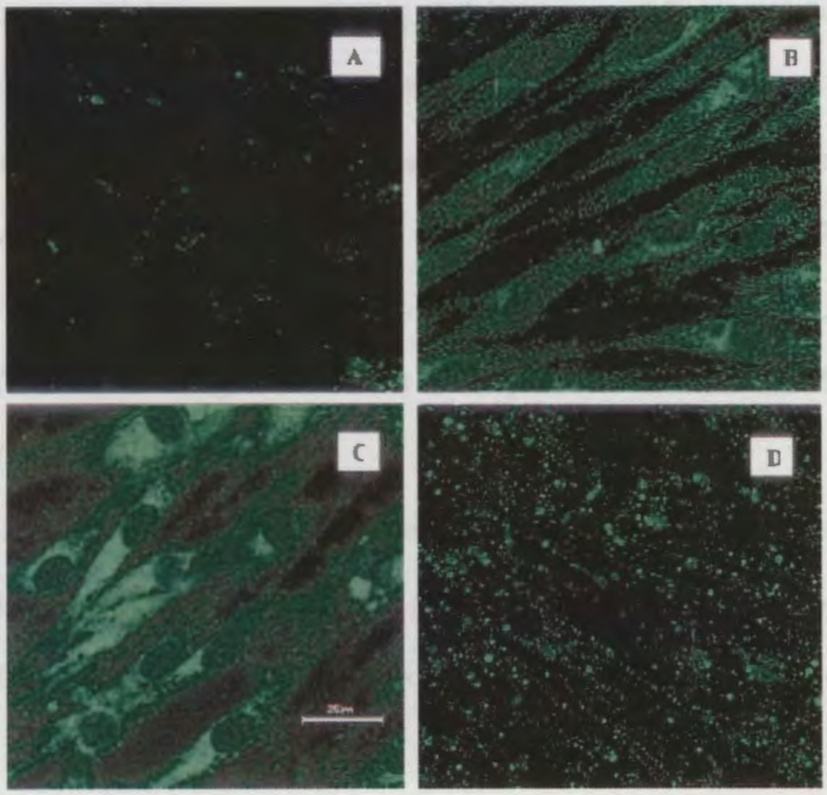


Figure 9: Localization of FITC-labeled Pluronic F108 (A), P85 (B), L35 (C) and L121 (D) using confocal microscopy (Batrakova et al., 2003).

Based on these findings the group speculates on why the composition influences Pluronic effects. Lack of internalization of hydrophilic Pluronic F108 may be the cause of its inability to affect Pgp efflux and ATP level. The extremely lipophilic copolymer L121 is sequestered in endosomes and did not change ATP level dramatically nor affect Pgp efflux. Both L35 and P85 enter the cell, but L35 marginally affects ATP levels whereas P85 is the strongest inhibitor of those tested; these two Pluronics have opposite effects on membrane fluidity decreasing and increasing respectively and they have different PO chain lengths.

Alakhova et al. (2009) continue the examination into the role of Pluronic P85. Earlier studies (Rapport et al., 2000) suggest that Pluronics may inhibit respiration in mitochondria. Alakhova et al. (2009) revisit this possibility in studies where they compared responses of MDR cells and nonresistant cells to P85. They discuss how changes in the function of MDR cells compared with normal cell lines enhance their sensitivity to the effects of P85. The distinct properties of the MDR cell lines include:

1) Mitochondria expressing high levels of mitochondrial uncoupling protein 2 (UCP2) resulting in inefficient ATP synthesis and low membrane potential (Harper et al., 2002).
2) The preferential use of fatty acids over glucose as a source of electrons entering the respiratory chain and the generation of more lactate due to fermentation.
3) Over expression of Pgp efflux pumps that are ATP dependent (Figure 10).

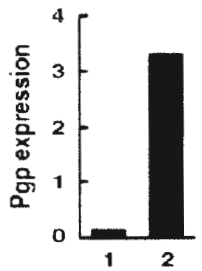


Figure 10: Western blot analysis of Pgp expression in 1) non-MDR 2) MDR cells (Alakhova et al., 2009).

Figure 11 shows how ATP levels in both cell types are altered by Pluronic P85. The half maximal effective concentrations EC_{50} of P85 for MDR and non-MDR cells are quite different with the MDR cells being sensitive to much lower concentrations. In both types of cells, ATP levels initially increased and then sharply decreased in response to increasing concentrations of Pluronic. However, increases occurred in the MDR cells

only at doses below 0.001 % wt, whereas ATP increased in the normal cells from 0.001 % to about the CMC value after which a rapid decline occurs.

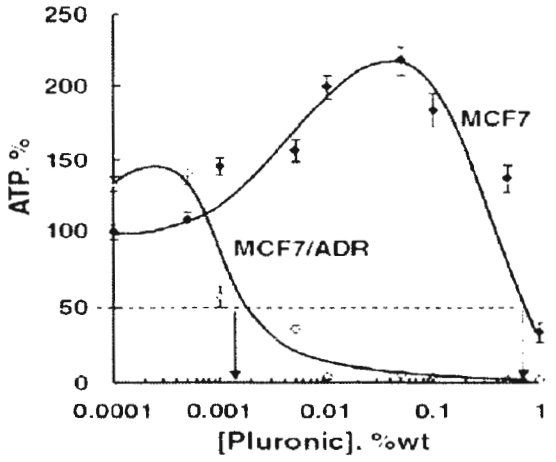


Figure 11: Effects of P85 on the intracellular ATP levels in drug-resistant (MDR) MCF7/ADR (empty diamonds) and drug sensitive MCF7 (filled diamonds) cells. EC_{ATP50} values corresponding to 50% decrease in ATP levels for each cell line are shown by arrows (Alakhova et al., 2009).

Figure 12 shows oxygen consumption following exposure of MDR and non-MDR cells to P85. In both cells types, as the Pluronic concentration increases the oxygen consumption decreases suggesting inhibition of respiration. With the MDR cells, oxygen consumption decreases rapidly with P85 above 0.001 % wt. For the normal cell line, oxygen consumption increases to a threshold at 0.01 wt. % P85 after which it decreases with Pluronic concentration. This finding suggests that changes in ATP levels in response to Pluronic exposure are due to effects on respiration.

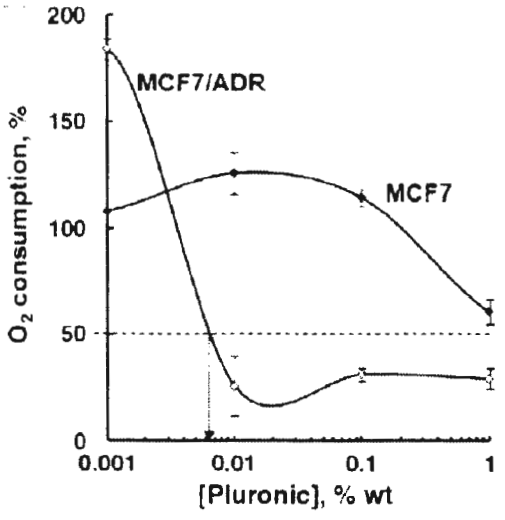


Figure 12: Effects of P85 on oxygen consumption in MCF7/ADR (MDR, empty diamonds) and MCF7 (non-MDR, filled diamonds) cells. EC_{RES50} values corresponding to 50% decrease in oxygen consumption for each cell line are shown by arrows (Alakhova et al., 2009).

To determine the components of respiration affected by Pluronic P85, Alakhova et al. (2009) further characterized the effect of P85 on mitochondrial oxidative phosphorylation in MDR and non-MDR cells. Again the dose response curves in preparations from the two cell types are distinct although for both the target sites are cytochrome c oxidase (complex IV) and NADH-ubiquinone oxidoreductase (complex I) rather than other complexes in the chain (Figure 13). In MDR cells, a dose-dependent decrease is seen for complex 1 when P85 concentrations are increased above 0.0001 % wt. With the normal cell lines, low doses (0.0001 to 0.001 wt. %) increase activity after which there is a decline. For complex IV the increasing concentrations of P85 suppress activity in MDR cells, but with normal cells there initially is an increase up to a threshold of 0.01 wt % exposure before inhibition. Membrane potential also is sensitive to P85 with dose responses differing between normal and MDR cells (Figure 13C).

Increases occur with both normal and MDR cells up to a threshold where the potential declines. Threshold points are lower for MDR (0.01 wt %) compared with 0.1 wt% for the normal cells.

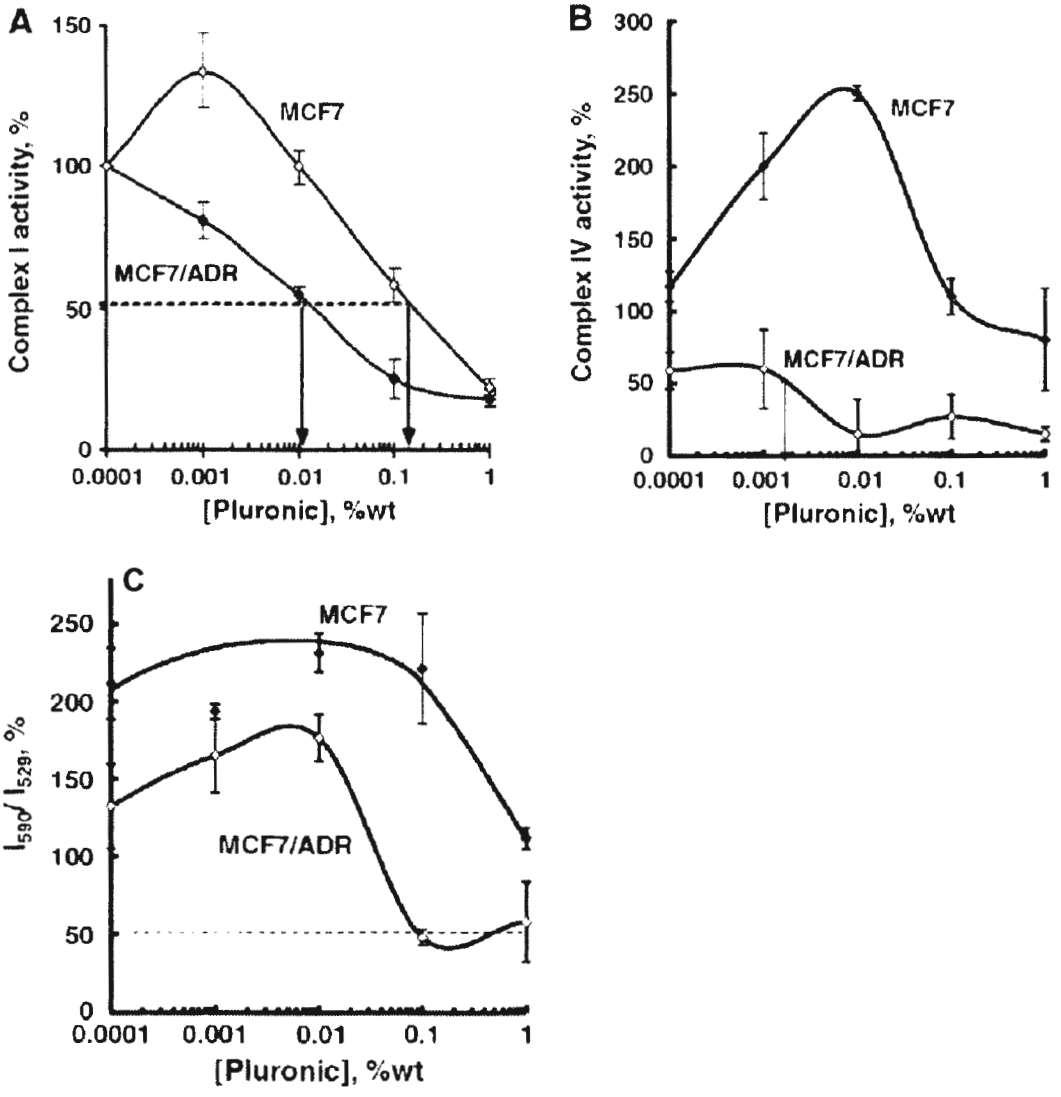


Figure 13: Effects of P85 on the activities of (A) complex I, (B) complex IV and (C) membrane potential in isolated mitochondria of MCF7/ADR (MDR, empty diamonds) and MCF7 (non-MDR, filled diamonds) cells. EC_{50} values corresponding to 50% decrease in complex I and complex IV activities for each cell lines are shown by arrows (Alakhova et al., 2009).

Pluronic-altered mitochondrial function in the MDR cells likely causes oxidative stress due to aberrant electron flow to oxygen. Enhanced production of ROS (Figure 14

A, Ott et al., 2007) and cytochrome c release (Figure 14 B) signify a pro-apoptotic condition is generated within the MDR cells. For MDR cells, the threshold level for ROS production is lower 0.01 wt% than that for cytochrome c release (0.1%). The nonresistant normal cells do not show enhanced ROS or cytochrome c release. These findings confirm that the MDR cells are ultrasensitive to P85.

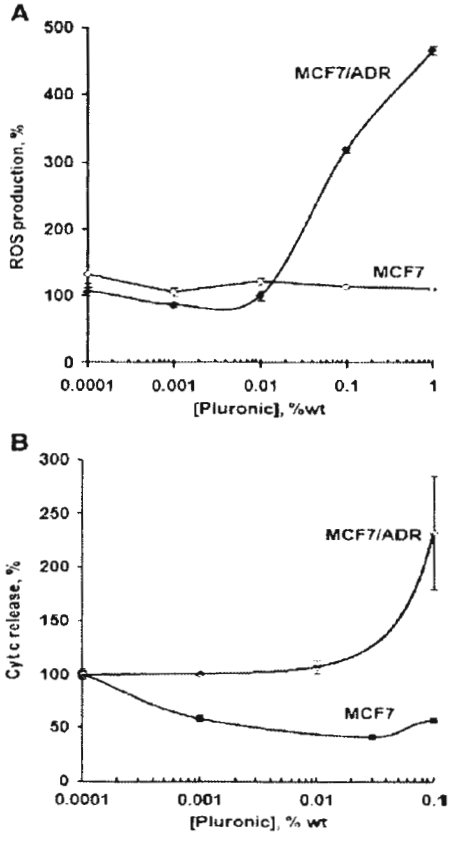


Figure 14: Effects of P85 on (A) ROS production and (B) cytochrome c release in MCF7 (filled diamonds) and MCF7/ADR (empty diamonds) cells (Alakhova et al., 2009).

In summary, in mammalian cell lines, Pluronic P85 affects metabolism at concentrations below and above CMC values. The reprogramming of cells to become multi drug resistant increases sensitivity and results in Pluronic-induced changes that are apoptotic. Generally the MDR cells respond to lower concentrations of P85 than the normal cell line. Changes in both cell types are caused by intracellular penetration of

P85 into the mitochondria to alter electron flow and membrane potential due to inhibition at complex I, which releases electron and pumps protons across the membrane while oxidizing NADH, and complex IV, which reduces oxygen. Consequently, other observed changes are reduced oxygen consumption and reduced ATP levels. An important consequence in the MDR cells is reduced efficacy of drug efflux from the cell by the over expressed Pgp efflux pumps. Both the lower ATP levels and direct inhibition of the ATPase activity of these pumps induced by P85 contribute to the reduced drug efflux activity. In the conclusion, of value to the medical field is the selective chemosensitization of MDR cells caused by Pluronics. However, even in normal cell lines higher concentrations of P85 above the CMC levels (0.1 to 1%) reduce mitochondrial function and ATP levels.

1.2.2 Responses of other eukaryotes

Other eukaryotic cells, in addition to mammalian cell lines, are affected by Pluronics. Pluronic F68 at 0.01% protects *Tetrahymena* cells against chemical and physical stress (Larsen et al., 2000). A *Tetrahymena* is a 50 μm long, rod-shaped ciliated freshwater protozoan that is used as a model for varied biomedical research as illustrated in Figure 15.

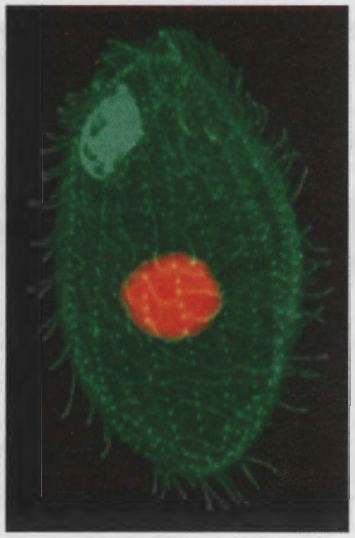


Figure 15: *Tetrahymena* cells stained with DAPI (diamidino-2-phenylindole) nuclear fluorescent stain (Credit: Jacek Gaertig, University of Georgia, Athens).

Pluronic F68 at 0.01 % below the CMC level enhances survival of *Tetrahymena* cells during starvation when cells are dispersed in HEPES buffer containing essential salts (Ca^{2+}, Mg^{2+}). Table 3 shows these data gathered over a range of temperatures. However, survival decreased when trace metals Zn^{+}, Cu^{2+}, Mn^{+}, Fe^{3+}, Co^{2+} (Eisen et al., 2006) were used; trace metals are required at low levels but above a threshold level are toxic. The authors speculate that 0.01 % w/v F68 encapsulates these metal ions promoting their uptake to levels that become toxic (Table 3). Based on the work with mammalian cells, we speculate that another possibility is that the Pluronic eliminates the efflux pump systems that would reduce toxic metal levels.

Table 3: Survival period (days) of *Tetrahymena* cells under nutrient starvation at different temperatures (Larsen et al., 2000).

	Absence of Pluronic			Presence of Pluronic (0.01% w/v)		
Treatment	36°C	28°C	16°C	36°C	28°C	16°C
None	4	8	21	17	28	35
Salts	3	5	17	16	27	43
Trace Metals	0	2	3	1	2	4

Other studies explored the effect of chemical and a physical stress imposed by growth on a rocker plate tilting up to 40° (Table 4). To measure the survival under just a chemical stress, filled tubes of cell suspensions were incubated horizontally. As compared to control cells, cells treated with 10 mM Ca^{2+} and 10 mM K^{+} show strongly reduced survival; 10 mM Na^{+} had less toxicity. Pluronic F68 at 0.01% protected cells exposed to Ca and Na, but not to K^{+}. To provide a physical stress tubes were only half-filled, and they were tilted. This combination promotes cell death but the addition of Pluronic result in 100% protection for Ca and Na exposure and prolonged survival for K^{+}. The authors speculate that Pluronic F68 encapsulates these metal ions, thereby, reducing toxicity, and also stabilizing the cell membrane against physical stress. In mammalian cells Pluronics are thought to act as channels for K transport and this may account for the differential ion effects. Ramirez and Mutharasan (1990) suggest that the protective mechanism of F68 relies on its ability to decrease membrane fluidity through direct interaction with the plasma membrane; changes in membrane fluidity correlate with changes in shear sensitivity.

Table 4: Survival under stress with and without Pluronic F68. The numbers represent % tubes with surviving cells (% survival). The number in parenthesis indicates the number of tubes (Larsen et al., 2000).

Treatment period	Without Pluronic	With Pluronic
Chemical stress (filled tubes)		
Control		
2 days	100 (13)	100 (13)
3 days	67 (28)	100 (24)
Ca^{2+} (10 mM)		
2 days	50 (8)	100 (8)
3 days	0 (10)	100 (10)
Na^{+} (10 mM)		
2 days	100 (13)	100 (9)
3 days	63 (19)	100 (9)
K^{+} (10 mM)		
2 days	44 (18)	50 (18)
3 days	13 (22)	53 (13)
Chemical and physical stress (half-filled tubes tilted)		
Control		
5 h	100 (24)	100 (15)
1 day	100 (16)	100 (16)
2 days	67 (21)	100 (20)
Ca^{2+} (10 mM)		
5 h	33 (9)	100 (9)
1 day	0 (20)	100 (20)
2 days	0 (8)	100 (8)
Na^{+} (10 mM)		
5 h	55 (11)	100 (11)
1 day	0 (19)	100 (19)
2 days	0 (18)	100 (13)
K^{+} (10 mM)		
5 h	78 (18)	100 (13)
1 day	0 (23)	100 (23)
2 days	0 (15)	33 (15)

Hydrophilic Pluronics (e.g. F68, F88, F108, etc., with 80% EO, HLB >27) protect other animal cell cultures against the physical stress of shear forces. For instance, Murhammer and Goochee (1990) find that 0.2% w/v Pluronic F68 protects *Spodoptera frugiperda* SF9 insect cells from detrimental effects associated with vortexing. At concentrations of 0.2 and 0.3%, F68, resistance towards shear stress in *Spodoptera frugiperda* SF9 cells increases 15 and 42 fold, respectively.

Pluronic F68 at concentrations below the CMC level also protects post-thaw recovery of cryopreserved plant cells enhancing the biomass production and post-thaw viability (Lowe et al., 2001). Cells, cryopreserved under liquid nitrogen at -196 °C, experience many respiratory imbalances, including ROS production upon transfer to

normal temperature. Pluronic F68 alone or in combination with an artificial oxygen carrier enhances cell viability reducing ROS levels (Lowe et al., 2001). Additionally, Cancino et al. (2001) report that Pluronic F68 (0.5 % w/v) enhances shoot regeneration in a citrus rootstock increasing fresh weight by 60% through protecting cells from environmental stress conditions and promoting uptake of nutrients.

In summary, observations in common for these studies with different types of eukaryotic cells and tissues are the protective effects of Pluronics; protection is correlated with changes in membrane properties affecting responses to physical stress and transportation of nutrients and potential toxic materials.

1.3 Pluronic effects on bacteria

1.3.1 Efflux pumps in bacterial cells

Like the mammalian cells discussed in Section 1.2, bacterial cells also utilize efflux pumps for the extrusion of toxic substances such as antibiotics and heavy metal ions outside the cell. Through these transporters, bacterial cells exhibit multidrug resistance to antibiotics causing severe problems in the medical field (Webber and Piddoc, 2003) but conversely the bacteria are useful for clean up of metal- pollution.

The bacterial efflux pumps localize in the cytoplasmic membranes, and some require ATP as a source of energy whereas others are coupled with pumping of hydrogen, sodium or potassium ions from outside the cells. Thus, these systems extrude drugs in an energy dependent manner, either by hydrolysis of ATP or by using the proton motive force (Schweizer, 2003). Illustrations of the different designs of these transporters are shown in Figure 16. LmrA from *Lactococcus lactis* is the first ABC-multidrug transporter identified in bacteria, and it shares high homology with the Pgp-

efflux pumps of mammalian cells (Vigano et al., 2000). Three other pump types use the proton motive force generated across the cell membrane rather than ATP hydrolysis. NorA probably is the best characterized efflux system in the Gram-positive bacteria. For a Gram-positive cell, only transport across the cytoplasmic membrane is required. Similarly, the AcrD system of the Gram-negative *E. coli* constitutes a cytoplasmic membrane transporter involved in the efflux of aminoglycosides into the periplasmic space. Other systems in the Gram-negative cells, such as the Mex transporter system, permit transport directly from the cytoplasm through the periplasm through the outer membrane.

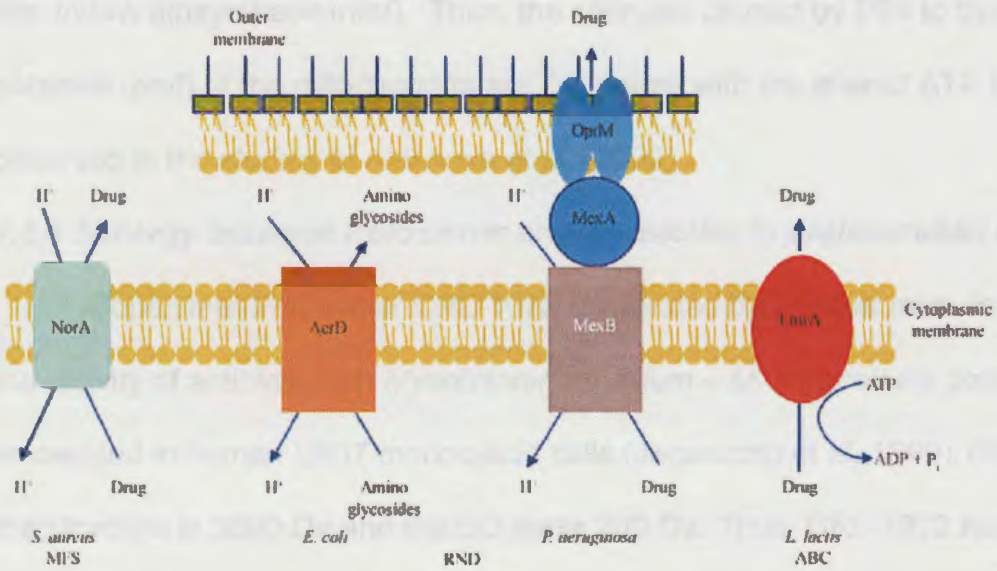


Figure 16: Schematic illustration of main types of bacterial efflux pumps shown in *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Lactobacillus lactis*. Illustrated are NorA (member of the major facilitator superfamily, MFS), AcrD and MexAB-OprM, two members of the resistance-nodulation division (RND) family and LmrA, a member of ATP binding cassette (ABC) family (Schweizer, 2003).

1.3.2 Types of ATPase

Several integral membrane protein complexes interact with ATP. Dependent on structure they are termed F, V, A, P and E type ATPases. The F and V designated

structures synthesize ATP and have greater complexity of protein subunits lodged into the membrane. These pump protons or Na^+ against the gradient across the membrane utilizing the energy to generate ATP. The other structures use the hydrolysis of ATP to transport structures such as metal ions (P-type ATPases for heavy metal efflux, Peter, 2005).

Proton motive force cannot be generated in certain conditions such as a leaky damaged membrane, presence of uncoupler, etc. In such conditions, ATP-driven proton pumping activity of ATP synthase is regulated by mechanisms that suppress activity if no proton motive force is present (Feniouk, ATP synthase website (updated 2010): http://www.atpsynthase.info/). Thus, the changes caused by P85 to the membrane potential (pmf) of the mitochondria are consistent with the altered ATP levels as observed in the studies of Alakhova et al. (2009).

1.3.3 Synergy between Poloxamer and antibiotics in antimicrobial activity

Copolymer Poloxamer CRL-1072, generated by another manufacturer, enhances the activity of antibiotics on *Mycobacterium avium – M. intracellular* complex (MAI) while embedded in human U937 monocytoid cells (Jagannath et al. 1999).The PO mass of the structure is 3500 Da and the EO mass 200 Da. Thus, CRL-1072 has similar chain lengths to Pluronic L101. A higher purity of CRL-1072 over the Pluronics is obtained by subjecting the material to supercritical fluid fractionation to remove low molecular weight materials (Jagannath et al. 1999). CRL-1072 is hydrophobic (Figure 1).

Members of the MAI complex are resistant to antibiotics by two main mechanisms: (1) the natural permeability barriers of their thick and layered cell walls and (2) mutations acquired due to sub-lethal exposure (Jagannath et al., 1999). A

minimal inhibitory concentration (MIC) of the copolymer CRL-1072 for MAI growing in U937 cells of 5.0 µg/ml is observed. However, in the presence of CRL-1072, the antibiotic clarithromycin becomes bactericidal at concentrations at which it is only bacteriostatic when used alone. Synergism with the antibiotic clarithromycin is observed at 0.125 µg/ml concentrations of CRL-1072 (Figure 17).

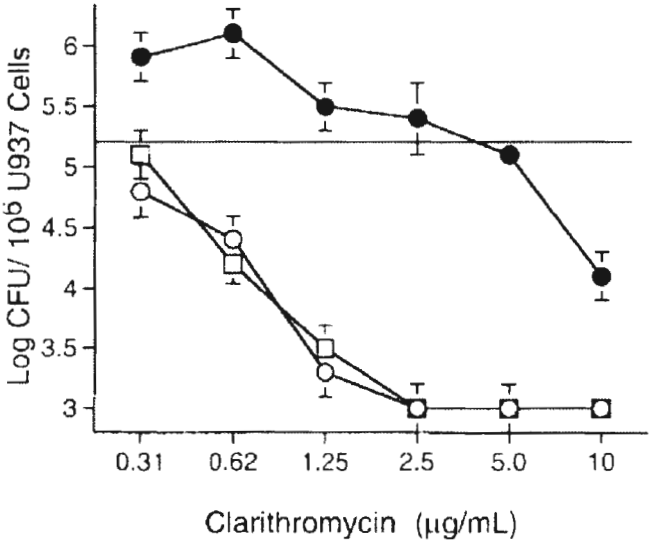


Figure 17: Effects of CRL-1072 and clarithromycin on growth of MAI in human macrophages. Multiple doses of clarithromycin were added to MAI infected U397 monocytoid cells on day 0 alone (closed circles) or with CRL-1072 at concentrations of 0.125 µg/ml (open circles) or 0.25 µg/ml (squares) (Jagannath et al., 1999).

CRL-1072 at 0.1 µg/ml similarly enhances the killing of MAI within human cells by other antibiotics (isoniazid [INH], rifampin, amikacin, streptomycin, and clindamycin) as shown in Figure 18. Thus CRl-1072 acts synergistically with antibiotics that differ widely in their modes of action. For instance INH inhibits cell wall formation, whereas rifampin and streptomycin are protein-synthesis inhibitors. This finding suggests that the Pluronic enhances the accumulation of the antibiotic rather than influencing the target of the antibiotic. As such it may enhance control of resistant strains by: 1) killing better and

limiting the chances for mutation to resistance. 2) enhancing uptake of antibiotics in cells that otherwise would be resistant.

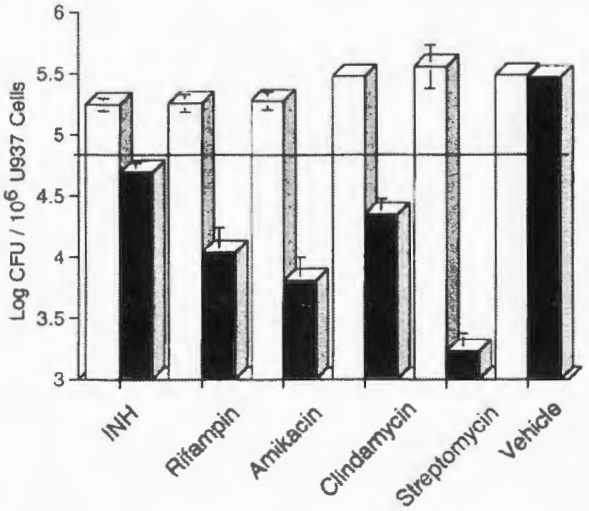


Figure 18: Effects of CRL-1072 with antimycobacterial drugs on growth of MAI in macrophages. Five antibiotics were added to MAI infected U397 monocytoid cells on day 0, each at a sub-MIC of 5 µg/ml with (black bars) or without (white bars) 0.1 µg per ml of CRL-1072 (Jagannath et al., 1999).

1.3.4 Pluronics and surface attachment/biofilm formation

Adherence of microorganisms to surfaces in an aqueous environment can initiate the formation of a biofilm. In the biofilms the cells are surrounded by a matrix of materials some of which are secreted by the microbes. Biofilms are very common in nature e. g. dental plaque. In industry, biofilms cause clogs and corrosion in the interior of pipes. In medical science, infectious bacterial biofilms are one of the most common complications associated with implanted medical devices. A serious issue is the fact that cells within a biofilm are much more resilient to the effects of antibiotics; ie the act of biofilm formation induces a resistant state although the same microbial cells in a planktonic state are sensitive. Thus, one strategy for combating biofilm infections

combines the use of antibacterial agents along with blocking of initially adhesion and the subsequent biofilm growth. Studies demonstrate that Pluronic prevents biofilm formation of bacteria on polymer surfaces (Portoles et al., 1995; Housley et al., 2009).

Studies showing altered adhesion of cells are provided in Table 5.

Table 5: Effect of Pluronics on adhesion

Pluronics	**Cells**	**Efficacy/Time of action**	**References**
Pluronic F127	*P. aeruginosa (Pa) S. aureus Reynolds S. epidermidis* M187sp8	Limits adhesion of *Pa* (92- 99%), *S. aureus* (50-70%) and *S. epidermidis* (50-70%) to hydrophilic contact lenses.	Portoles et al., 1994; Portoles et al., 1995
Pluronic F68 20% concentration	*Staphylococcus epidermidis*	Reduces adhesion to silicon wafers	Levy et al., 2004
Range of 15 Pluronics spec. L31 and F68	*Staphylococcus epidermidis* strains 900, 901 and 904	Inhibits adhesion on polystyrene. L31 affected all strains equally; F68 more effectively	Bridgett et al., 1992

Portoles et al. (1994, 1995) report that Pluronic F127 significantly inhibits (92-99%) adhesion of Gram-negative *Pseudomonas aeruginosa* to hydrophilic contact lenses whereas F127 was found to reduce adhesion by the Gram-positive isolates *Staphylococcus aureus* and *Staphylococcus epidermidis* by 50-70%. Similarly, Levy et al. (2004) find that the hydrophilic Pluronic F68 allows only 3 % adhesion of *S. epidermidis* to ventricular catheters (constructed of plastic material such as a silicone elastomer) and polystyrene surfaces. F127 blocks Gram-negative *Escherichia coli* D21 adhesion to glass surfaces (Razatos et al., 2000). Bridgett et al. (1992) reported that *S. aureus* and *S. epidermidis* adherence to polymethylmethacrylate (PMMA) surface was strongly inhibited (approximately 22% of control) by Pluronic F127. Furthermore, F127

increases the susceptibility to antibiotics (vancomycin, and gentamicin) of residual adherent staphylococci. They also observed that increasing concentrations of F127 enhance the inhibition of staphylococcal adherence. The Pluronics may function by passivation of the surface that the bacteria attach to or they may passivate the bacterial surface. This process would involve the creation of hydration layer on the cell surface, which then hinders bacterial adherence.

Biofilm formation by an environmental pseudomonad, *Pseudomonas chlororaphis* O6, is differentially affected by Pluronics in manners altered by nutrition (Housley et al., 2009). Four Pluronics (P123, P104, F108, 25R2) varying in molecular weight and HLB have differential effects on biofilm formation although each Pluronic increased surface motility (swarming) to a similar extent. Enhancement of swarming is consistent with similar surface activity of each of the Pluronics. F108 and 25R2 significantly reduce biofilm formation in a defined minimal medium, while P104 and P123 show little effect. In a rich medium, only P104 limits biofilm formation. These nutrient effects suggest that the primary mechanism is not through surface passivation. Rather the plasticity of the mechanisms involved in biofilm formation caused by nutrition results in differential sensitivity to the Pluronics. The Pluronics also affect phenazine production in this bacterium, Pluronic 25R2 increases production, whereas P104 and P123 cause decreases and F108 has no effect (Housley et al., 2009). Because phenazine production is energy dependent, it seems that the effective Pluronics could influence the energy status of the cell.

2. Examination of metabolic effects of Pluronics on an environmental bacterium, *Pseudomonas. putida* KT2440

2.1 Metabolic changes reported through bioluminescence

The aim of this study was to examine the effects of different Pluronics on *P. putida* KT2440. Pluronics are being used widely as wetting agents, emulsifiers, defoaming agents, lubricants, cosmetics, and pharmaceuticals (Fusco et al., 2006). They have several roles in agriculture including being part of the formulations for pesticides. This varied use stems from their commercial availability over a wide range of molecular weights and hydrophilic: lipophilic ratios (HLB), chemistries that allow different properties. Thus, with the increasing use of Pluronics, it is important to understand how Pluronics might affect beneficial soil bacteria.

Although Pluronics are thought of being "inert carrier compounds" some show the ability to alter discrete cellular functions as discussed in the literature review in Section 1.2. For instance, a copolymer was bactericidal for a mycobacterium complex within human cells (Jagannath et al., 1999). This polymer also promotes killing of the mycobacterium complex by a range of antibiotics (Jagannath et al., 1999).

Selected Pluronics differentially affect biofilm formation by a soil pseudomonad *Pseudomonas chlororaphis* O6 depending on nutrition (Housley et al., 2009). Thus, Pluronics may have an impact on a process essential for survival as a root colonizer. Altered production by Pluronics of antibiotics termed phenazines in this same pseudomonad also occurs; again a process that could affect survival of the bacterium in soils (Housley et al 2009).

Pluronic studies in animal cells reveal that some are highly damaging to the activity of the electron transport chain (Alakhova et al., 2009). At concentrations above the CMC value of P85, normal animal cell lines show inactivation of the electron transport chain (at complexes I and IV), reduction in oxygen consumption to 50% of the control value, decreases in ATP levels to 20% of control and reduction by 50% of the membrane potential of isolated mitochondria.

The energy demand in aerobic bacterial cells also is met by electron transfer chains that exist in the plasma membrane and it has not been demonstrated whether the bacterial complex I and IV are sensitive. Consequently, we examined the effects of Pluronics on bacterial energy status using an engineered strain of *Pseudomonas putida* KT2440. This biosensor emits light based upon a luciferase enzyme expressed from a plasmid containing a fusion between the promoter of the gene at locus PP_0588 with the *luxAB* genes (Gajjar et al., 2009). Transcription of the *luxAB* cassette produces a message that is translated to the LuxA and LuxB subunits that constitute an active luciferase (Miller et al 1997). This luciferase requires $FMNH_2$ production and an aldehyde substrate [RCHO], such as decanal. Light is emitted in the oxidation of the aldehyde (Eq. 1) (Koga et al., 2005).

$$RCHO + FMNH_2 + O_2 \rightarrow RCOOH + FMN + H_2O + hv. \quad [1]$$

In the cell NADH reduces flavin mononucleotide [FMN], thus, generating $FMNH_2$ (Eq. 2).

$$NADH + H^+ + FMN \leftrightarrow NAD^+ + FMNH_2 \quad [2]$$

Thus, luminescence is dependent on energy level of *P. putida* cells. Previously we have shown that this strain responded with loss in light output when exposed to toxic heavy metals and metal-containing nanoparticles (Gajjar et al., 2009). With exposure to

Cu ions and Ag ions and NPs, there was a loss in light output that correlated with loss in cell culturability. Consequently in these studies In addition to examining changes in light output, we measured cell culturability after 60 min of exposure to the Pluronics to determine whether any changes in Lux activity correlated with toxicity.

Strain KT2440 is a good model for a beneficial environmental isolate. This pseudomonad has bioremediation potential and is a strong root-colonizer (Molina et al., 2006; Child et al., 2007; Ramos-Gonzalez et al., 2005). Root colonization may involve its ability to generate biofilm (Avevalo et al., 2005, Housley et al., 2009) and exhibit surface motility (Housley et al., 2009; Matilla et al., 1987; Mozes et al., 1987).

We selected for our study Pluronic 85 that has documented effects on animal lines (see Alakhova et al., 2009), plus, others that provide a range of MW and HLB as shown in Table 6.

Table 6: Physicochemical properties of Pluronics used in the bacterial biosensor study

Pluronics	**HLB**	**CMC % wt.**	**Molecular Weight**
L121	1	0.0004	4400
L101	1	0.0008	3800
L81	2	0.0063	2750
L61	3	0.0022	2000
L31	5	0.0231	1100
L92	6	0.0010	3650
P123	8	0.0025	5750
P104	13	0.0020	5900
P84	14	0.0300	4200
L64	15	0.1400	2900
P85	16	0.0300	4600
F108	27	0.0320	14600
F88	28	0.2800	11400

2.1.1 Methods

Preparation of the biosensor

Construction of the biosensor and its storage was as reported in Gajjar et al. (2009). Briefly, the cells possess a stable plasmid that bears a promoter fusion with the *luxAB* reporter cassette. The promoter of the gene at locus PP_0588 was ligated to the reporter cassette so that the biosensor is termed strain PP_0588.

Preparation of co-polymer solutions

The list of the block copolymers used in this study and their characteristics are presented in Table 6 and Figure 1. Pluronics were kindly provided by BASF Corporation, NJ, USA. Aqueous solutions of Pluronic were prepared by using five g of Pluronic in 95 ml sterilized deionized water. The highly lipophilic Pluronics (L121, L101) gave cloudy solutions at RT. The 5% Pluronic solutions (50 ml) were sterilized in an autoclave at 121 °C for 30 min followed by stirring overnight. The prepared stock solution of Pluronic was made fresh weekly to limit the problems with possible microbial contamination. For these studies, all Pluronics were used within six months of receipt from BASF.

Bioluminescence assay

Logarithmic phase cells of the biosensor were generated by reculturing from an overnight culture grown in minimal medium (MM) with shaking at 25 °C to $OD600_{nm}$=0.1. MM was prepared as described in Gajjar et al., (2009). MM contained in 1 L: 10.5 g K_2HPO_4, 4.5 g KH_2PO_4, 0.5 g sodium citrate ($2H_2O$), 1.0 g $(NH_4)_2$ SO_4, 0.25 g $MgSO_4.7H_2O$, and 2.0 g sucrose. The culture (200 ml) was centrifuged at 10,000 g for 10 min and the pelleted cells were resuspended in 200 ml sterile distilled water and

used immediately in the Lux assay. The sterile distilled water was equilibrated to 25 °C to limit changes in light output due to temperature effects. After dividing into 50 ml aliquots in 125 ml flasks, the suspensions were amended with the 5% stock solution of Pluronic to generate final concentrations of 0.1, 0.25, 0.5, 0.75 and 1 % v/v of Pluronic. For the controls cells were suspended in water. Flasks were shaken at 200 rpm and 25 C during the study. At defined times, 200 µl of the suspensions were transferred in triplicate into well plates for Lux readings. The luciferase substrate, 1% decanal in ethanol, 10 µl, was added automatically in the LMAXII Luminometer (Molecular Devices Corporation, Sunnyvale CA). Light output was recorded with a 10 sec. exposure. The first data point was collected within 1 min of mixing Pluronic and bacterial cell suspension. Samples were withdrawn from the cultures every 10 minutes up to 1 h for the Lux assay. Each treatment had three analytical replicates within a study. The whole experiment was replicated in three or more separate studies. In the Results section, data for the effects of Pluronic on Lux activity are provided for one study typical of those performed. Results of other repeated studies are shown in an Appendix. The observation that the Lux activity for the control cells at time zero means that there would be large error bars negating any trend that is observed. The % RLU at defined concentration of Pluronic relative to control was calculated as:

$$\frac{\text{RLU at defined concentration}}{\text{RLU of control}} \times 100$$

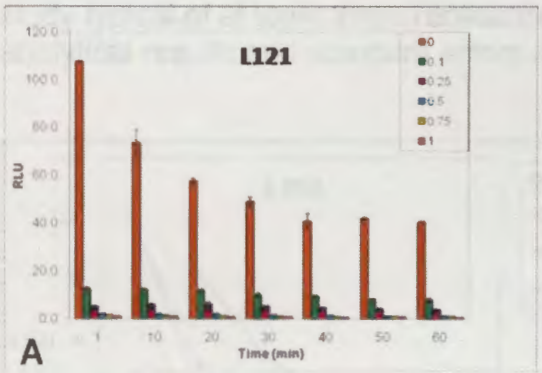
In all of these studies there was no equilibration of the Pluronic into more dilute suspension before the assays were performed.

2.1.2 Results

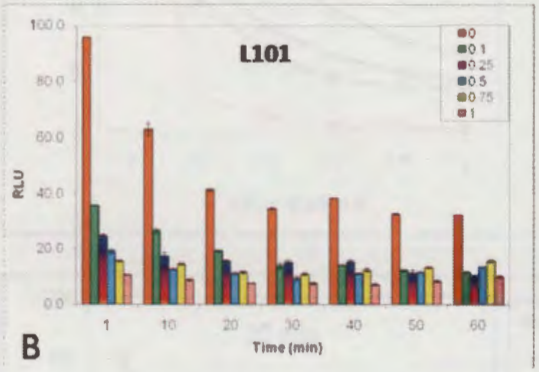
Group 1: Pluronics with strong inhibitory effect on Lux activity

Four of the Pluronics gave strong rapid inhibition of Lux activity. These Pluronics termed Group 1 included highly hydrophobic Pluronics of similar MW, L121 (HLB 1, CMC 0.0004, MW 4400), L101 (HLB 1, CMC 0.0008, MW 3800), P123 (HLB 8, CMC 0.0025, MW 5750) and P84 (HLB 14, CMC 0.0300, MW 4200). These Pluronics strongly inhibited Lux activity in a dose-dependent manner (Figures 19 and 20). Figure 19 shows the rapid change in Lux output with time at different concentrations of the Pluronics. Inhibition in treated cells compared to control at 60 min was 98.3% for L121; 87.9% for P123; 69.2% for L101 and 67.8% for P84. Figure 20 shows the % RLU relative to control for defined concentrations for Group 1 Pluronics at each 10 min interval of Lux assay.

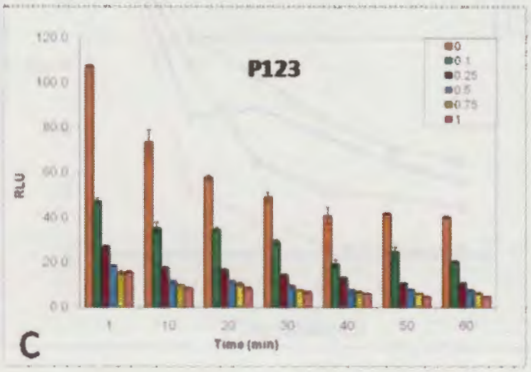
It is clear from Figure 20 that the Group 1 Pluronics show rapid, within a minute, dose-dependent inhibition in Lux response. Throughout the 60 min assay, Pluronic L121 (HLB 1) showed the highest inhibitory effect. With L101, although it has the same HLB as L121, the response curve differed with inhibition being maximum at 0.25 % dose.



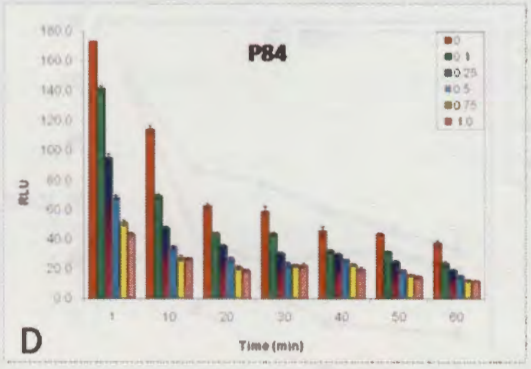
Treatments L121	Log 10 Cfu./ml	% RLU at 60 min
Control	9.4±0.0	100.0±0.9
0.1 %	11.2±1.0	19.6±1.0
0.25%	11.8±0.2	9.4±0.2
0.5%	11.5±1.7	3.2±0.1
0.75%	11.8±1.6	2.2±0.0
1.0%	9.9±0.6	1.7±0.0



Treatments L101	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.0	100.0±0.0
0.1 %	10.8±0.8	36.0±0.2
0.25%	11.1±0.6	32.3±0.5
0.5%	12.5±0.5	42.1±0.4
0.75%	11.6±1.6	48.3±1.0
1.0%	12.6±0.2	30.8±0.5



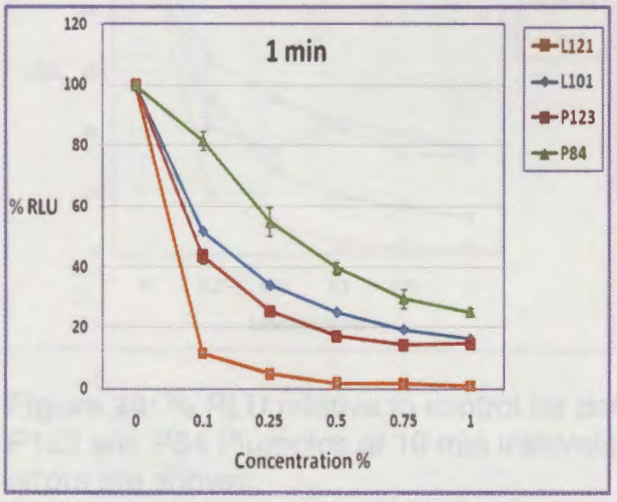
Treatments P123	Log 10 Cfu./ml	% RLU at 60 min
Control	9.0±0.6	100.0±0.9
0.1 %	10.9±2.5	50.9±0.6
0.25%	10.9±1.3	27.2±0.3
0.5%	11.3±2.2	18.8±0.4
0.75%	11.5±2.3	15.6±0.5
1.0%	11.3±0.9	12.1±0.3

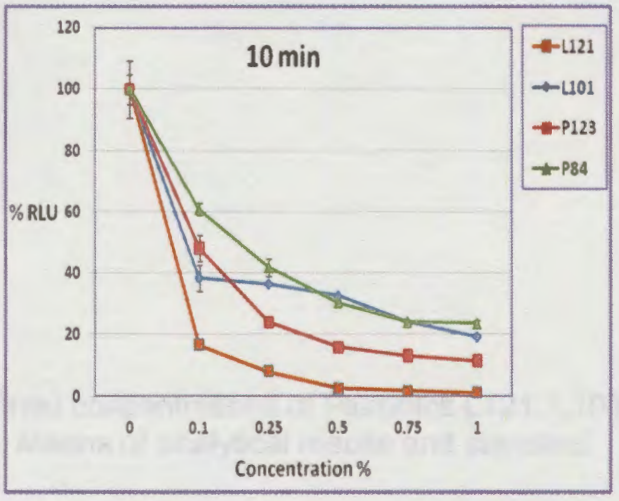


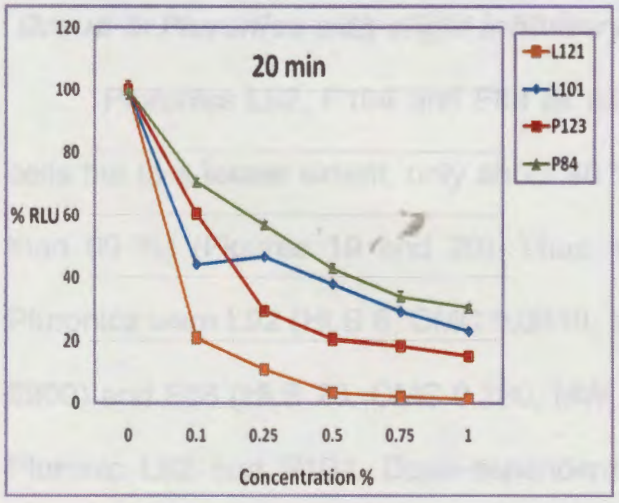
Treatments P84	Log 10 Cfu./ml	% RLU at 60 min
Control	9.3±0.5	100.0±0.9
0.1 %	10.6±0.1	63.8±1.0
0.25%	11.3±0.5	51.2±1.1
0.5%	12.3±1.6	41.3±0.6
0.75%	13.3±0.3	32.7±0.1
1.0%	13.0±0.6	32.2±0.2

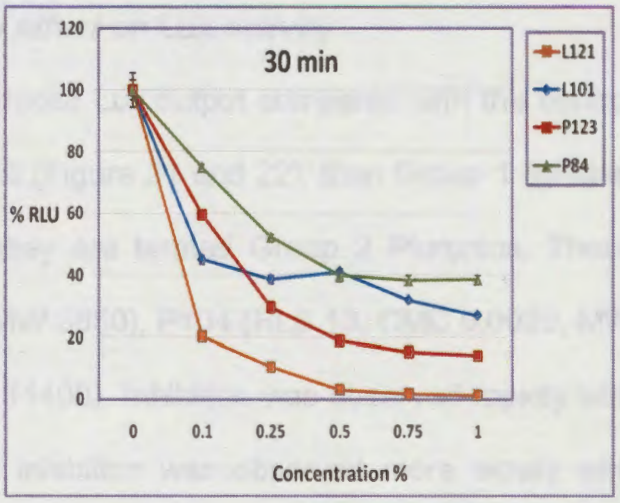
Figure 19: Lux response of *P. putida* KT2440 biosensor exposed to Pluronics L121 [A], L101 [B], P123 [C] and P84 [D] at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. Data are from one

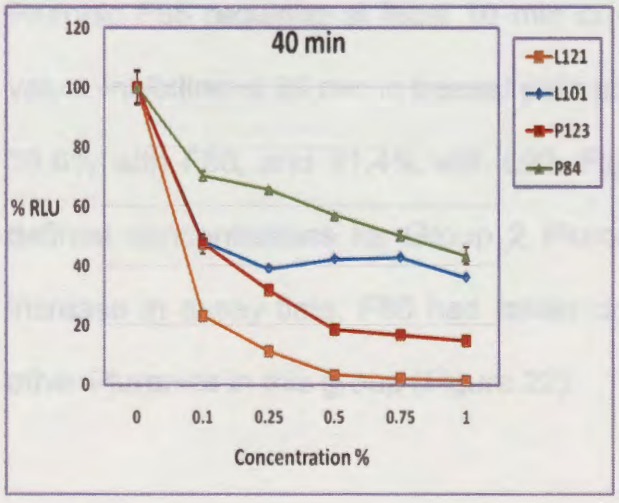
study typical of at least three replicates generated under the same conditions. Means of analytical results and standard errors are shown.

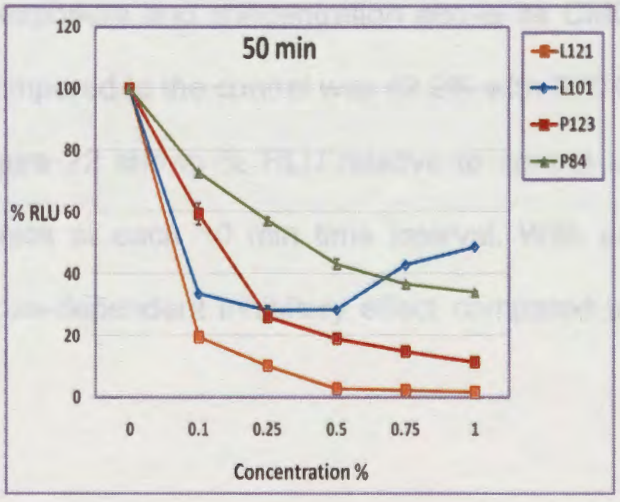












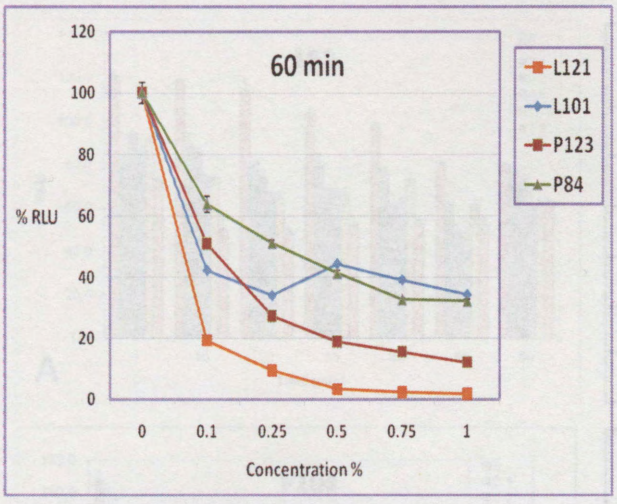
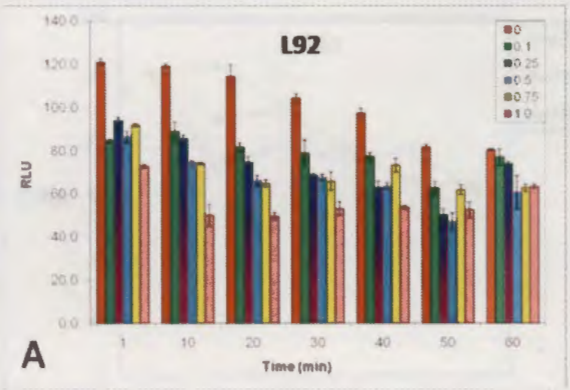


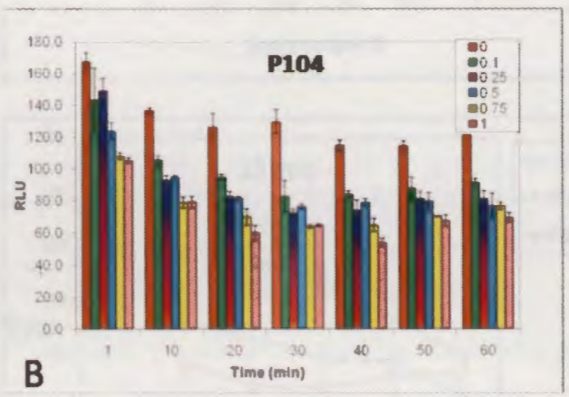
Figure 20: % RLU relative to control for defined concentrations of Pluronics L121, L101, P123 and P84 Pluronics at 10 min intervals. Means of analytical results and standard errors are shown.

Group 2: Pluronics with slight inhibitory effect on Lux activity

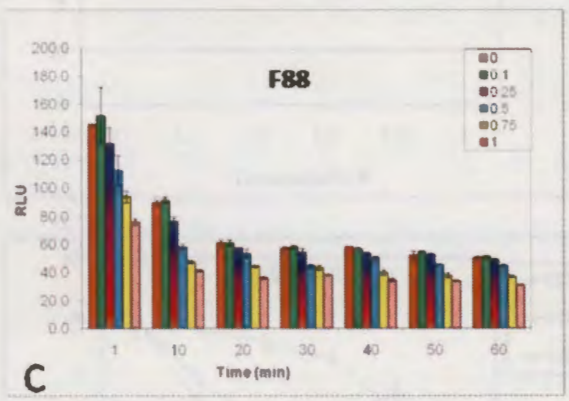
Pluronics L92, P104 and F88 all reduced Lux output compared with the control cells but to a lesser extent, only about 40 % (Figure 21 and 22), than Group 1 (greater than 60 %) (Figures 19 and 20). Thus, they are termed Group 2 Pluronics. These Pluronics were L92 (HLB 6, CMC 0.0010, MW 3650), P104 (HLB 13, CMC 0.0020, MW 5900) and F88 (HLB 28, CMC 0.280, MW 11400). Inhibition was observed rapidly with Pluronic L92 and P104. Dose-dependent inhibition was observed more slowly with Pluronic F88 requiring at least 10 min of exposure and concentration above its CMC value. Inhibition at 60 min in treated cells compared to the control was 42.9% with P104, 38.6% with F88, and 21.4% with L92. Figure 22 shows % RLU relative to control at defined concentrations for Group 2 Pluronics at each 10 min time interval. With an increase in assay time, F88 had lesser dose-dependent inhibitory effect compared to other Pluronics in this group (Figure 22).



Treatments L92	Log 10 Cfu./ml	% RLU at 60 min
Control	9.3±0.5	100.0±0.9
0.1 %	12.2±0.1	95.7±1.0
0.25%	12.7±0.5	91.8±4.9
0.5%	12.8±1.6	75.3±6.7
0.75%	12.2±0.3	78.0±6.0
1.0%	12.5±0.6	78.6±10.3

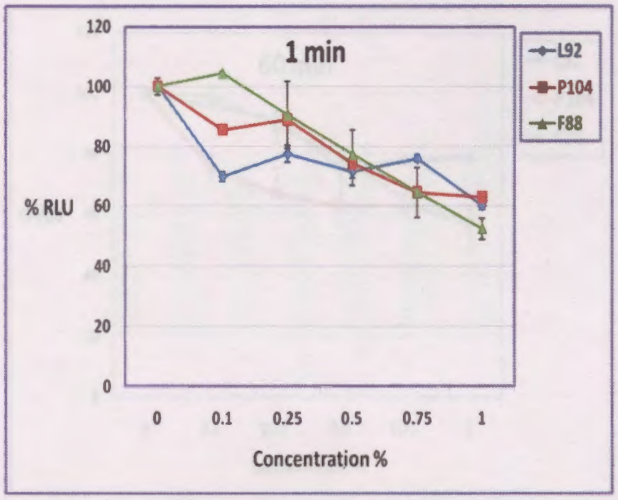


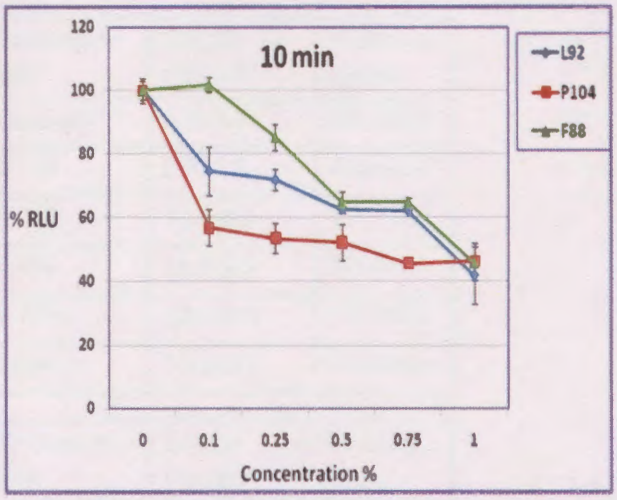
Treatments P104	Log 10 Cfu./ml	% RLU at 60 min
Control	9.4±0.6	100±0.0
0.1 %	11.3±2.3	75.2±4.3
0.25%	11.6±1.3	66.9±8.5
0.5%	12.4±0.3	63.1±13.8
0.75%	12.5±1.2	63.1±4.1
1.0%	12.3±1.3	57.1±5.0

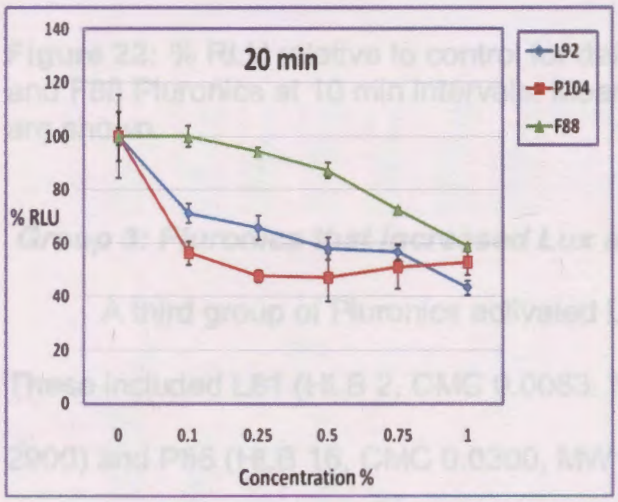


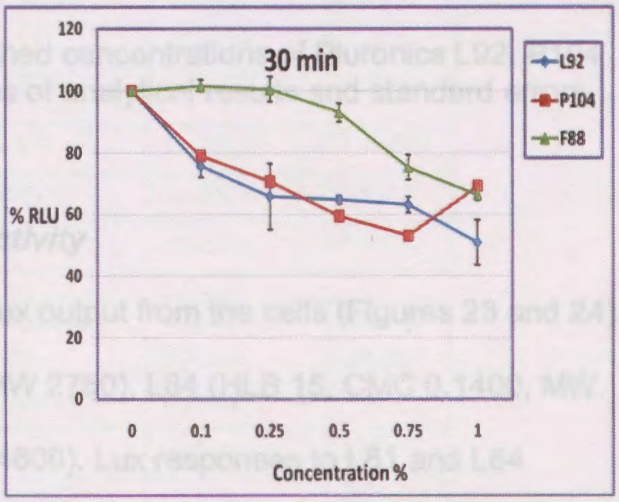
Treatments F88	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.1	100.0±1.5
0.1 %	10.5±0.2	101.4±1.1
0.25%	10.2±0.5	96.2±1.3
0.5%	12.4±0.9	87.9±1.2
0.75%	12.2±0.8	73.0±1.1
1.0%	11.8±1.0	61.4±0.5

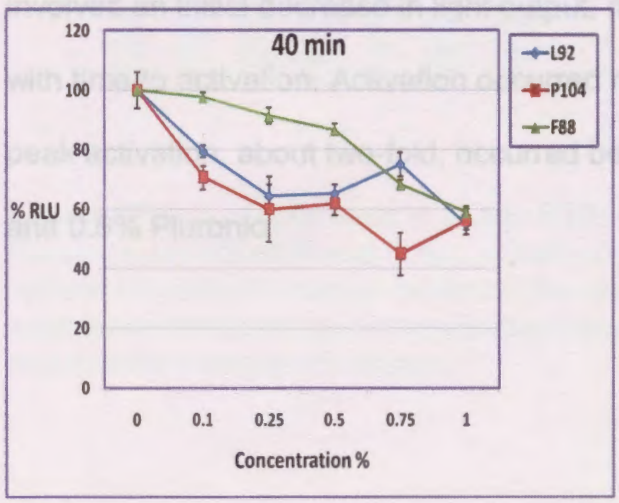
Figure 21: Lux response of *P. putida* KT2440 biosensor to L92 [A], P104 [B] and F88 [C] Pluronics at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. Data are from one study typical of at least three replicates generated under the same conditions. Means of analytical results and standard errors are shown.

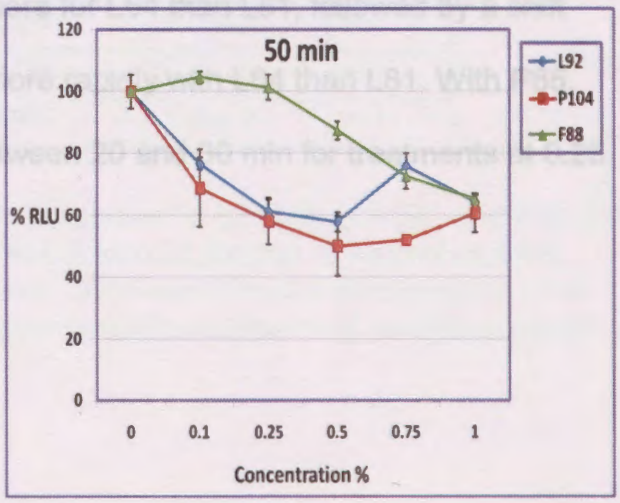

1 min
L92
P104
F88
% RLU
120
100
80
60
40
20
0
0
0.1
0.25
0.5
0.75
1
Concentration %


10 min
L92
P104
F88
% RLU
120
100
80
60
40
20
0
0
0.1
0.25
0.5
0.75
1
Concentration %


20 min
L92
P104
F88
% RLU
140
120
100
80
60
40
20
0
0
0.1
0.25
0.5
0.75
1
Concentration %


30 min
L92
P104
F88
% RLU
120
100
80
60
40
20
0
0
0.1
0.25
0.5
0.75
1
Concentration %


40 min
L92
P104
F88
% RLU
120
100
80
60
40
20
0
0
0.1
0.25
0.5
0.75
1
Concentration %


50 min
L92
P104
F88
% RLU
120
100
80
60
40
20
0
0
0.1
0.25
0.5
0.75
1
Concentration %

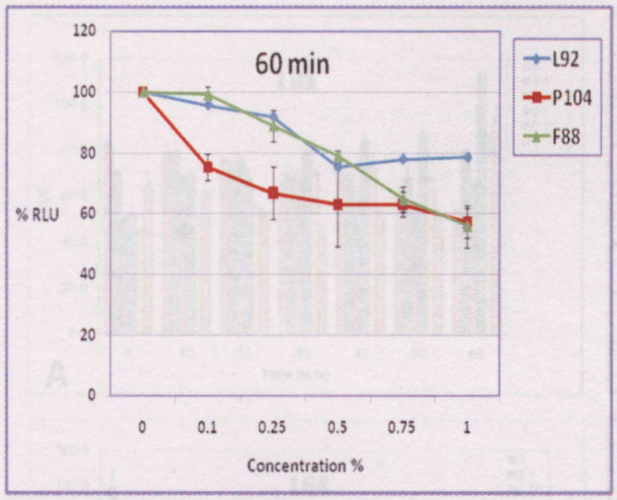
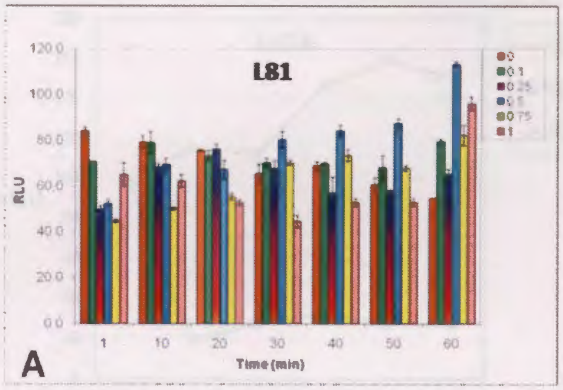


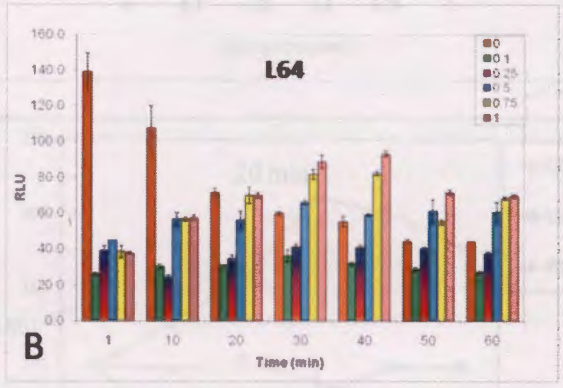
Figure 22: % RLU relative to control for defined concentrations of Pluronics L92, P104 and F88 Pluronics at 10 min intervals. Means of analytical results and standard errors are shown.

Group 3: Pluronics that increased Lux activity

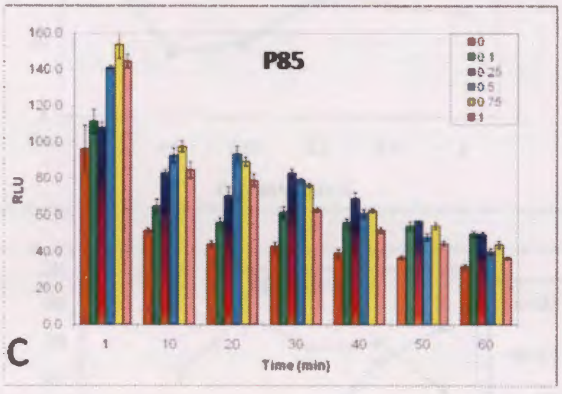
A third group of Pluronics activated Lux output from the cells (Figures 23 and 24). These included L81 (HLB 2, CMC 0.0063, MW 2750), L64 (HLB 15, CMC 0.1400, MW 2900) and P85 (HLB 16, CMC 0.0300, MW 4600). Lux responses to L81 and L64 involved an initial decrease in light output, more for L64 than L81, followed by a shift with time to activation. Activation occurred more rapidly with L64 than L81. With P85, peak activation, about two-fold, occurred between 20 and 30 min for treatments at 0.25 and 0.5% Pluronic.



Treatments L81	Log 10 Cfu./ml	% RLU at 60 min
Control	9.2±0.0	100.0±0.0
0.1 %	9.9±0.3	144.5±2.0
0.25%	13.3±0.1	118.7±3.9
0.5%	11.4±0.4	206.0±2.0
0.75%	12.8±0.0	149.8±7.8
1.0%	13.3±0.1	174.7±5.0

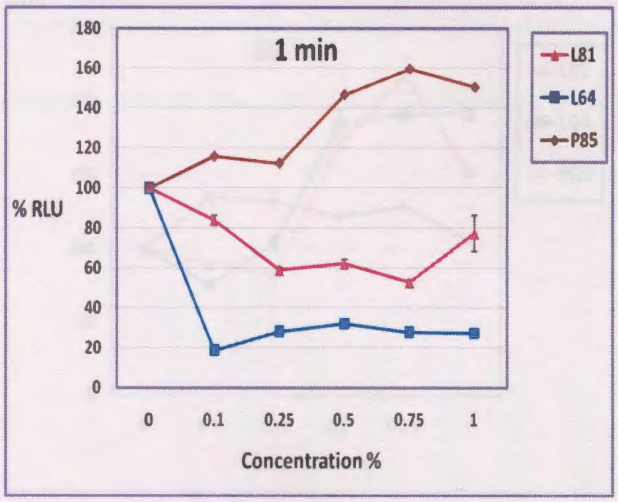


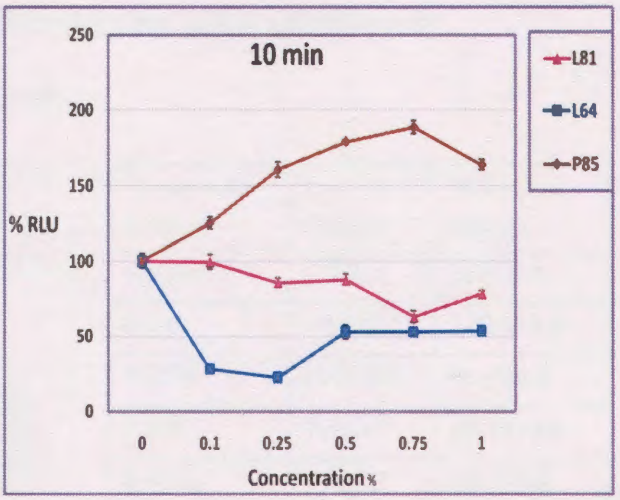
Treatments L64	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.2	100.0±0.3
0.1 %	11.0±2.3	61.2±1.4
0.25%	11.3±2.3	85.3±1.4
0.5%	11.5±1.2	137.1±10.0
0.75%	11.8±1.6	154.8±1.2
1.0%	11.2±2.1	157.9±1.8

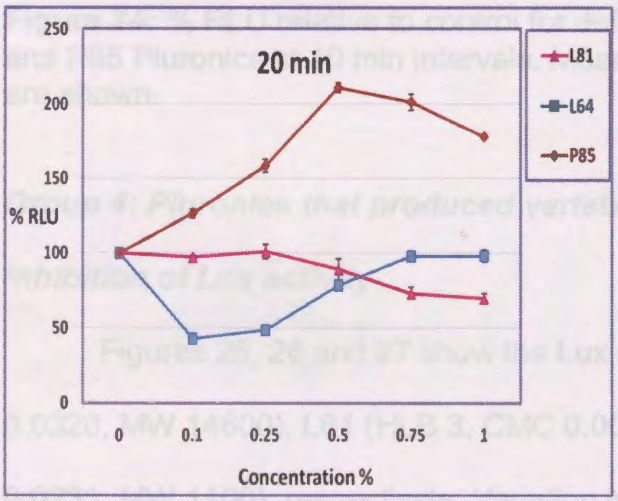


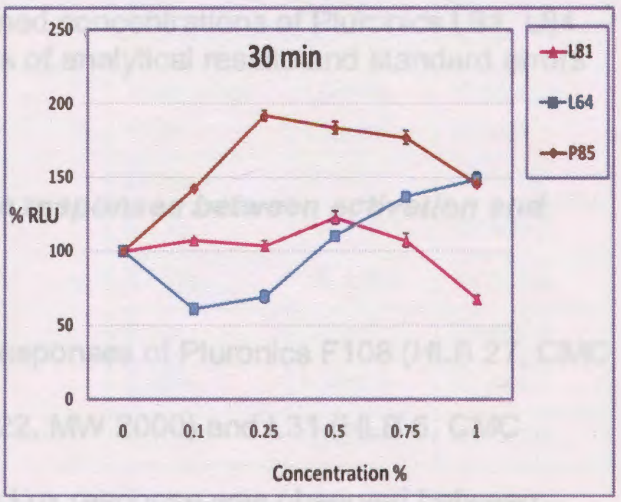
Treatments P85	Log 10 Cfu./ml	RLU at 60 min
Control	9.3±0.0	32.1
0.1 %	12.8±0.0	49.8
0.25%	13.2±0.2	49.7
0.5%	12.5±0.5	40.1
0.75%	11.7±1.6	44.1
1.0%	12.0±1.9	36.7

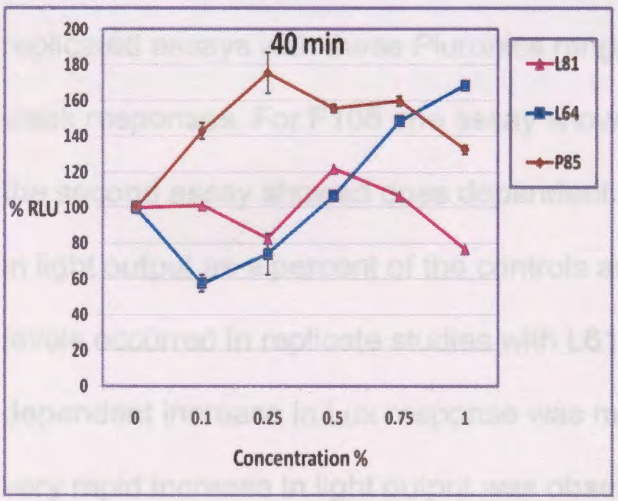
Figure 23: Lux response of *P. putida* KT2440 biosensor to L81 [A], L64 [B], and P85 [C] Pluronics at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. Data are from one study typical of at least three replicates generated under the same conditions. Means of analytical results and standard errors are shown.

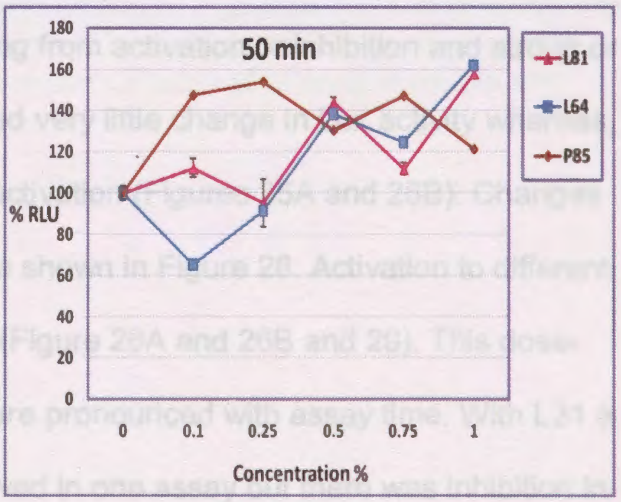

1 min
% RLU
Concentration %
L81
L64
P85


10 min
% RLU
Concentration %
L81
L64
P85


20 min
% RLU
Concentration %
L81
L64
P85


30 min
% RLU
Concentration %
L81
L64
P85


40 min
% RLU
Concentration %
L81
L64
P85


50 min
% RLU
Concentration %
L81
L64
P85

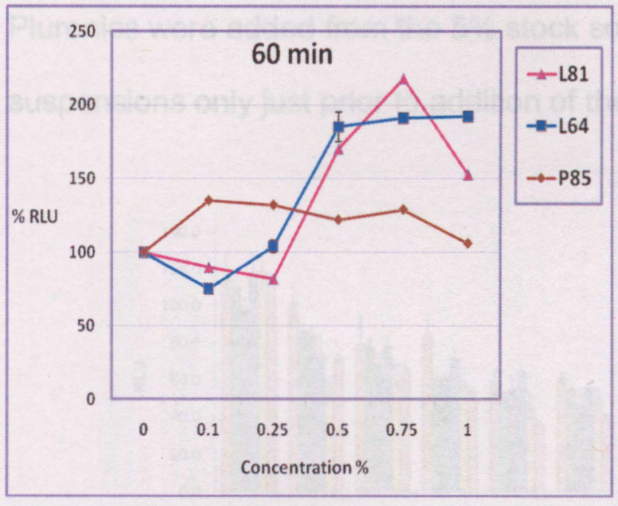
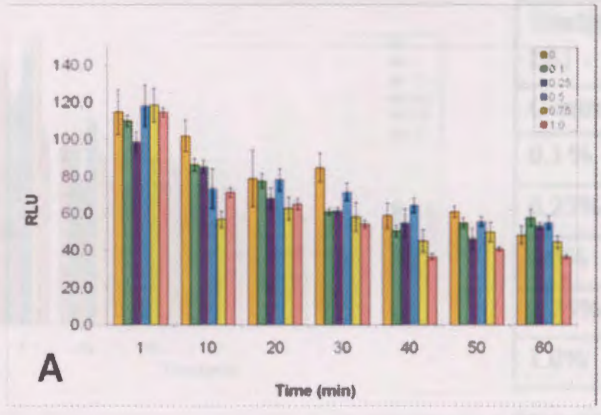


Figure 24: % RLU relative to control for defined concentrations of Pluronics L81, L64 and P85 Pluronics at 10 min intervals. Means of analytical results and standard errors are shown.

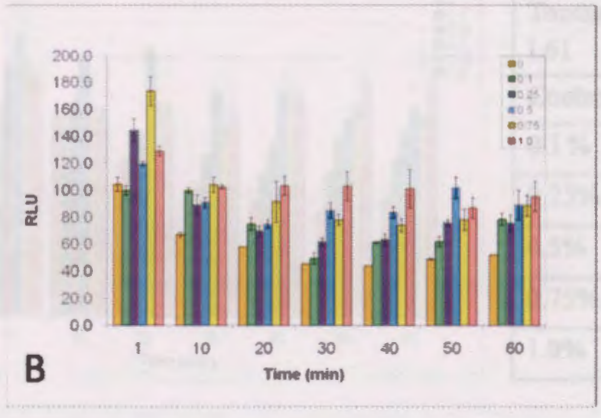
Group 4: Pluronics that produced variable responses between activation and inhibition of Lux activity

Figures 25, 26 and 27 show the Lux responses of Pluronics F108 (HLB 27, CMC 0.0320, MW 14600), L61 (HLB 3, CMC 0.0022, MW 2000) and L31 (HLB 5, CMC 0.0231, MW 1100), respectively. Variation in Lux response was observed between replicated assays with these Pluronics ranging from activation or inhibition and strong or weak responses. For F108 one assay showed very little change in Lux activity whereas, the second assay showed dose dependent activation (Figures 25A and 25B). Changes in light output as a percent of the controls are shown in Figure 28. Activation to different levels occurred in replicate studies with L61 (Figure 26A and 26B and 29). This dose-dependent increase in Lux response was more pronounced with assay time. With L31 a very rapid increase in light output was observed in one assay but there was inhibition in a second (Figure 27A and 27B and 30). The two sets of assays for the designated Pluronic were performed with only a one day interval. In each of the assays the

Pluronics were added from the 5% stock solutions to the water used for cell suspensions only just prior to addition of the cells.

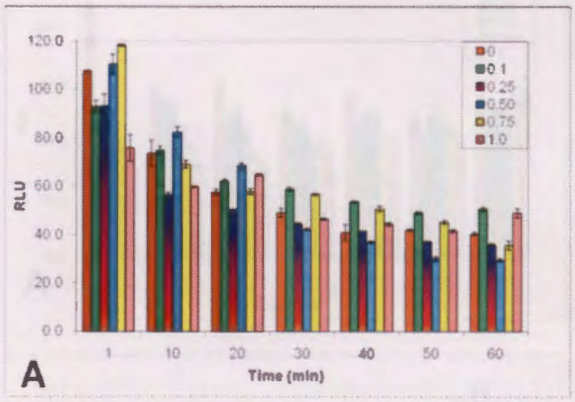


Treatments F108	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.1	100±0.9
0.1 %	10.5±0.1	119.2±1.0
0.25%	10.3±0.2	66.9±8.5
0.5%	9.5±0.1	63.1±13.8
0.75%	9.9±0.3	63.1±4.1
1.0%	11.3±0.1	57.1±5.0

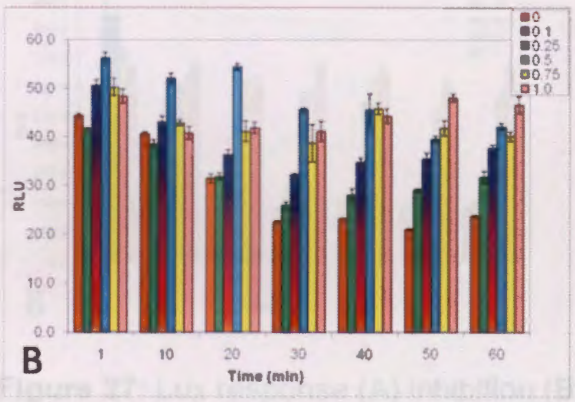


Treatments F108	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.1	100±0.0
0.1 %	10.5±0.1	78.4±0.2
0.25%	10.4±0.2	75.5±0.4
0.5%	10.0±0.1	89.0±0.6
0.75%	9.9±0.3	88.6±0.2
1.0%	11.2±0.1	95.3±5.0

Figure 25: Lux response (A) slight effect (B) activation of *P. putida* KT2440 biosensor to F108 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. Means of analytical results and standard errors are shown.

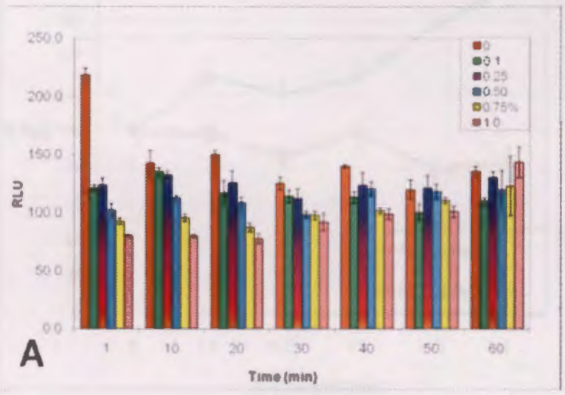


Treatments L61	Log 10 Cfu./ml	% RLU at 60 min
Control	9.4±0.1	100±0.9
0.1 %	10.0±0.1	50.5±0.1
0.25%	10.4±0.2	35.8±0.3
0.5%	10.0±0.1	29.5±0.5
0.75%	10.0±0.3	35.5±0.4
1.0%	11.9±0.1	49.0±0.2

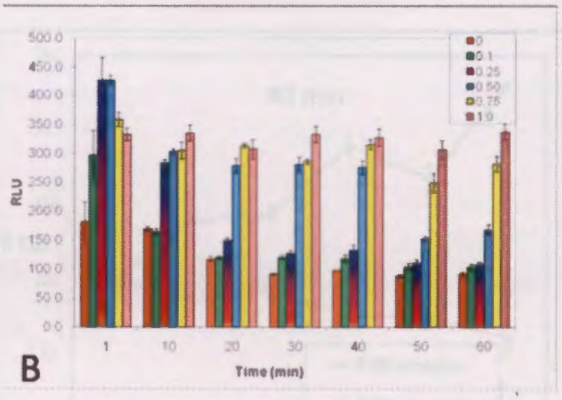


Treatments L61	Log 10 Cfu./ml	% RLU at 60 min
Control	9.1±0.1	100±0.9
0.1 %	12.0±0.1	31.8±0.1
0.25%	11.4±0.2	37.8±0.3
0.5%	9.6±0.1	42.2±0.5
0.75%	9.9±0.3	40.2±0.4
1.0%	11.0±0.1	46.7±0.2

Figure 26: Lux response (A) slight activation (B) high activation of *P. putida* KT2440 biosensor to L61 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. Means of analytical results and standard errors are shown.

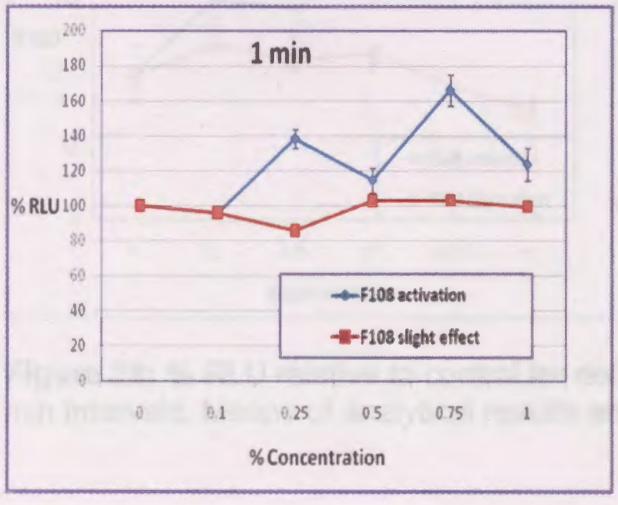


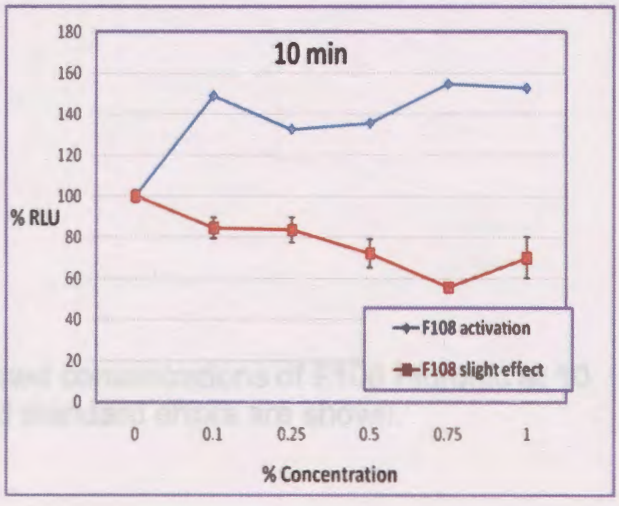
Treatments L31	Log 10 Cfu./ml	% RLU at 60 min
Control	9.1±0.1	100±0.0
0.1 %	12.0±0.1	81.2±0.2
0.25%	11.4±0.2	96.4±0.4
0.5%	9.6±0.1	88.3±0.6
0.75%	9.9±0.3	90.8±0.2
1.0%	11.0±0.1	105.8±5.0



Treatments L31	Log 10 Cfu./ml	% RLU at 60 min
Control	9.1±0.1	100±0.0
0.1 %	12.0±0.1	113.0±0.2
0.25%	11.4±0.2	117.8±0.4
0.5%	9.6±0.1	182.4±0.6
0.75%	9.9±0.3	306.8±0.2
1.0%	11.0±0.1	366.2±0.3

Figure 27: Lux response (A) inhibition (B) activation of *P. putida* KT2440 biosensor to L31 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. Means of analytical results and standard errors are shown.





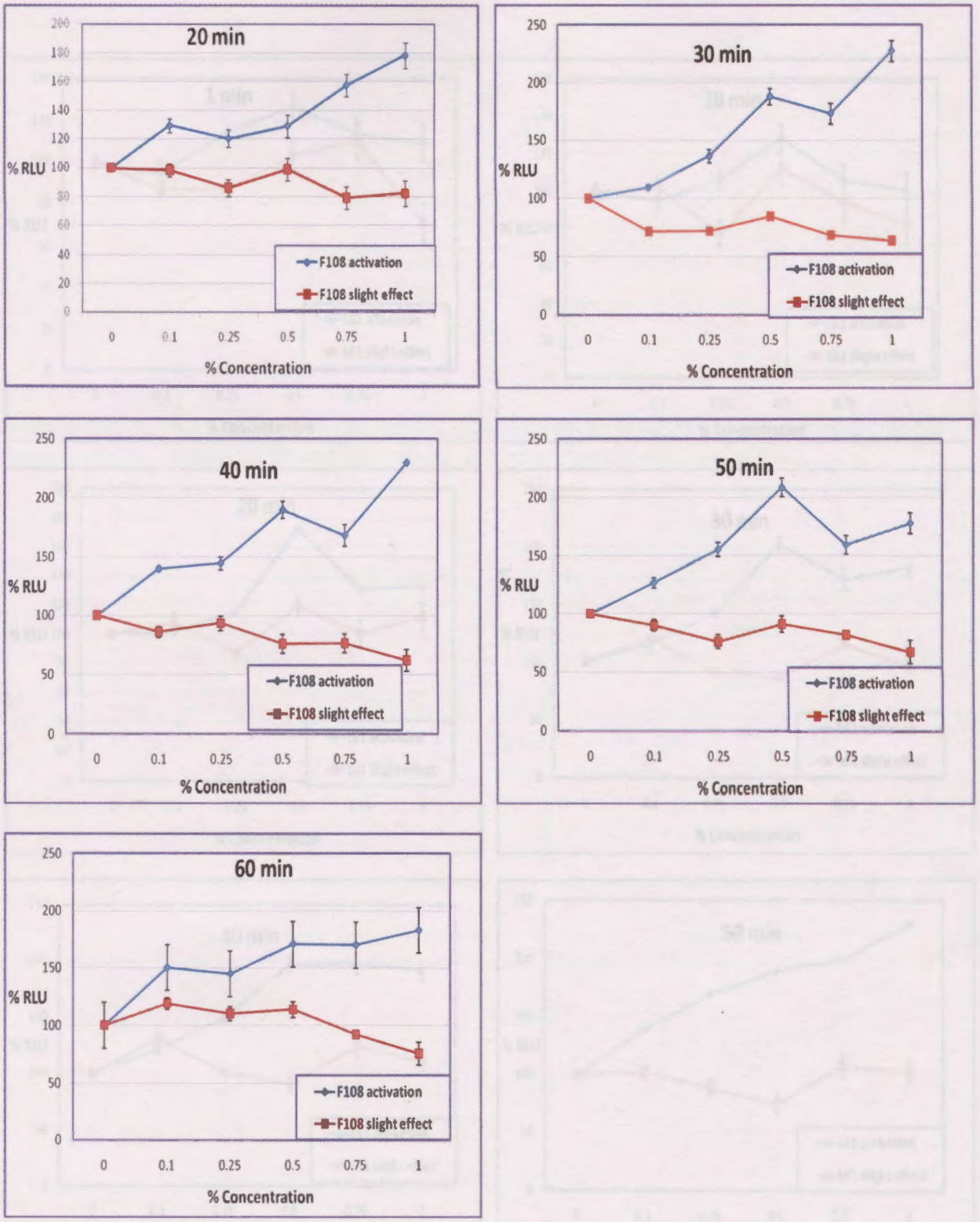
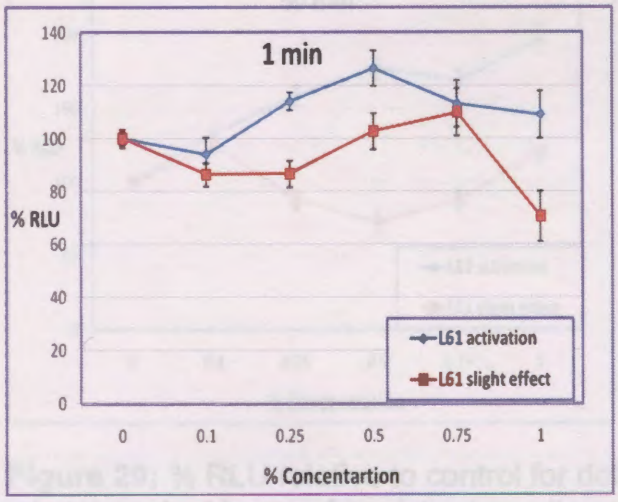
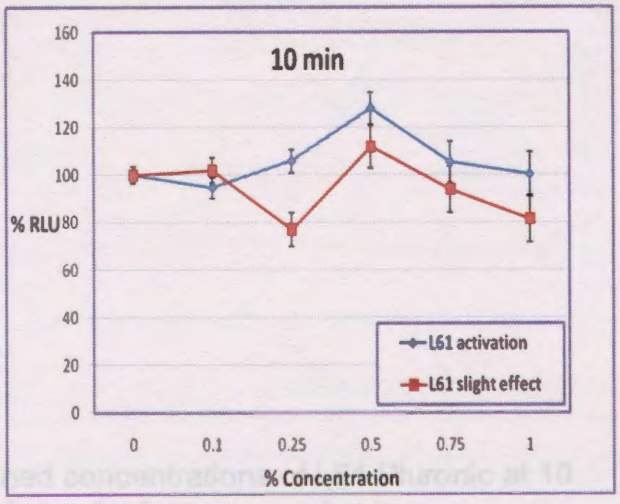
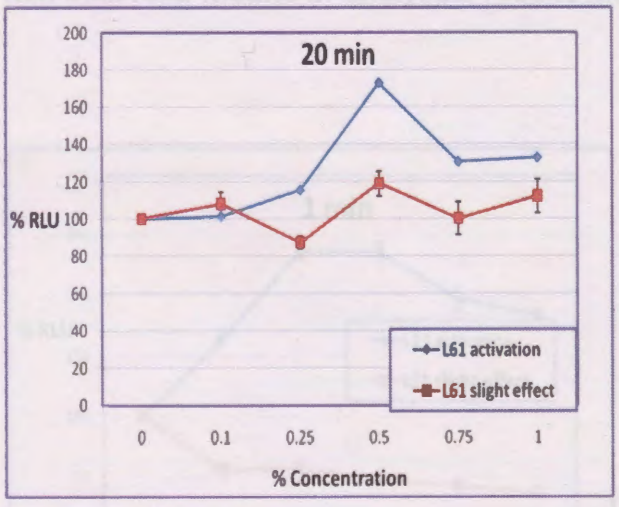
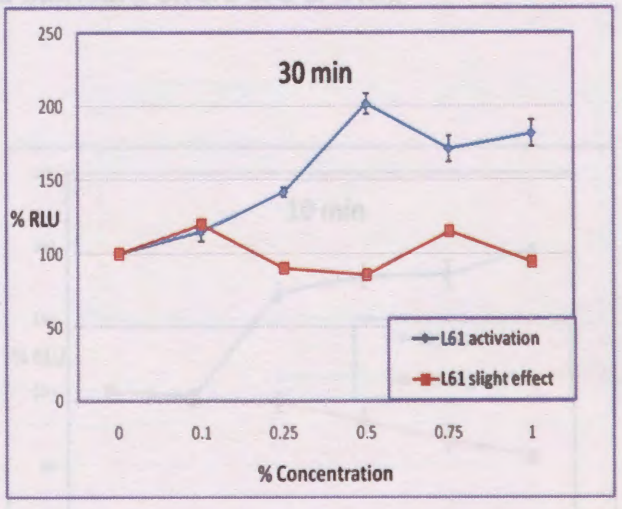


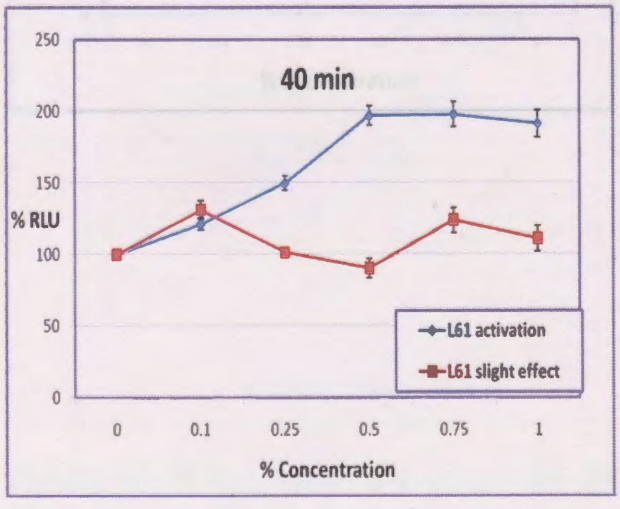
Figure 28: % RLU relative to control for defined concentrations of F108 Pluronic at 10 min intervals. Means of analytical results and standard errors are shown.

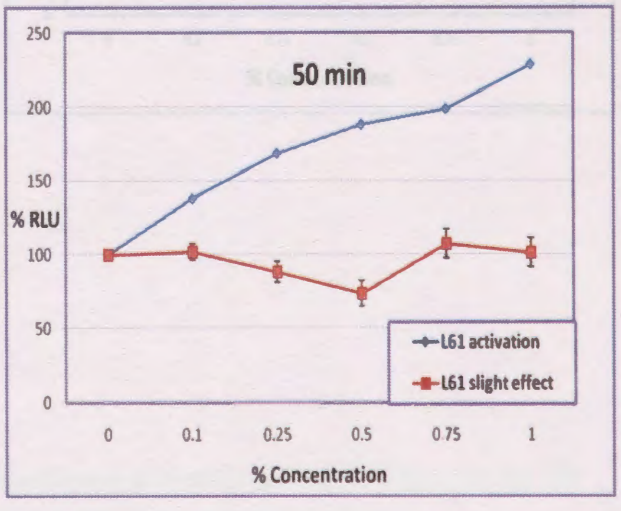

1 min
% RLU
% Concentartion
L61 activation
L61 slight effect


10 min
% RLU
% Concentration
L61 activation
L61 slight effect


20 min
% RLU
% Concentration
L61 activation
L61 slight effect


30 min
% RLU
% Concentration
L61 activation
L61 slight effect


40 min
% RLU
% Concentration
L61 activation
L61 slight effect


50 min
% RLU
% Concentration
L61 activation
L61 slight effect

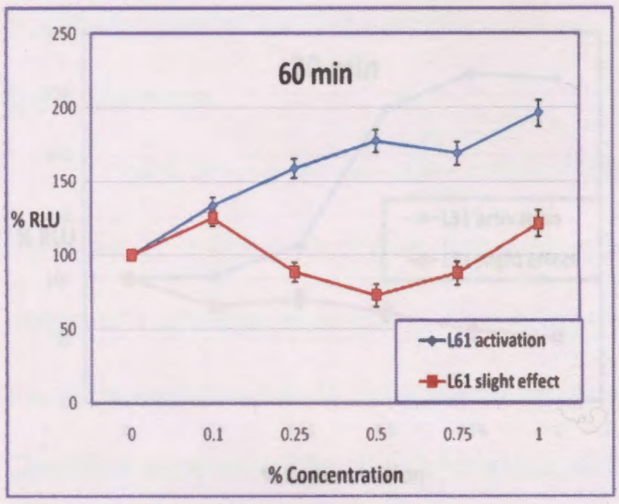
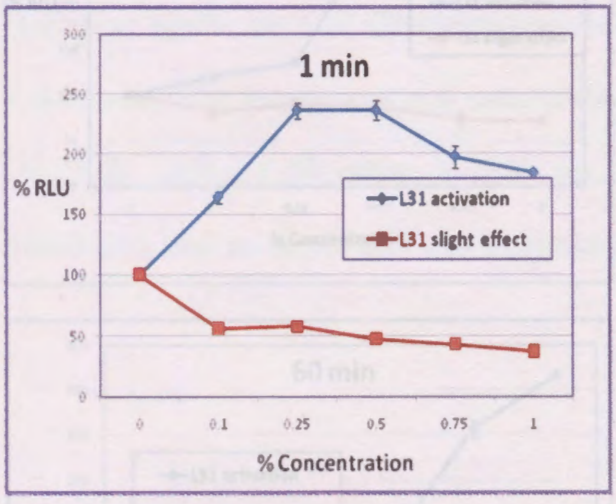
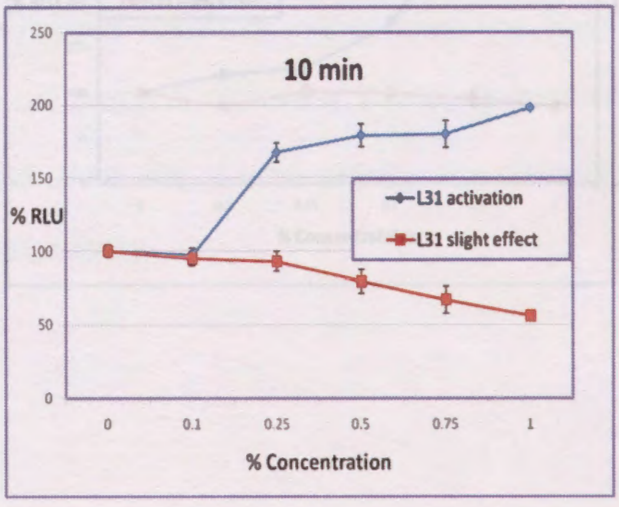


Figure 29: % RLU relative to control for defined concentrations of L61 Pluronic at 10 min intervals. Means of analytical results and standard errors are shown.





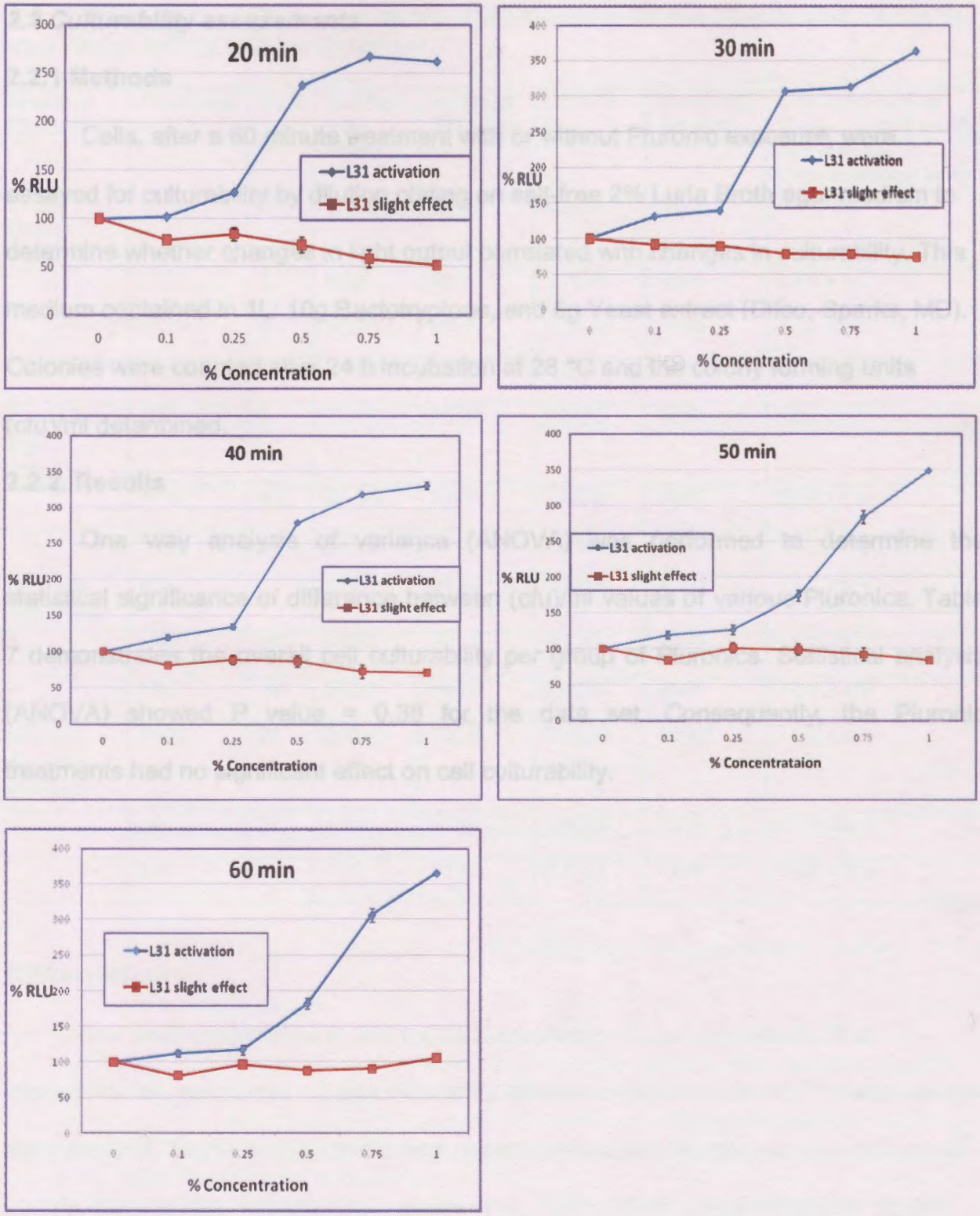


Figure 30: % RLU relative to control for defined concentrations of L31 Pluronic at 10 min intervals. Means of analytical results and standard errors are shown.

2.2 Culturability assessments

2.2.1 Methods

Cells, after a 60 minute treatment with or without Pluronic exposure, were assayed for culturability by dilution plating on salt-free 2% Luria Broth agar medium to determine whether changes in light output correlated with changes in culturability. This medium contained in 1L: 10g Bactotryptone, and 5g Yeast extract (Difco, Sparks, MD). Colonies were counted after 24 h incubation at 28 °C and the colony forming units (cfu)/ml determined.

2.2.2. Results

One way analysis of variance (ANOVA) was performed to determine the statistical significance of difference between (cfu)/ml values of various Pluronics. Table 7 demonstrates the overall cell culturability per group of Pluronics. Statistical analysis (ANOVA) showed P value = 0.38 for the data set. Consequently, the Pluronic treatments had no significant effect on cell culturability.

Table 7: Physiochemical properties and cell culturability for the Pluronics according to their Group activity with the biosensor

Group of Pluronics	Pluronics	HLB	Average EO units	Average PO units	CMC % wt.	Molecular Weight	Cell culturability (Log_{10} cfu/ml) of 1% of Pluronic concentration level
Group 1: Strong inhibitors	L121	1	10.00	68.28	0.0004	4400	9.9 ± 0.6
	L101	1	8.64	58.97	0.0008	3800	12.6 ± 0.2
	P123	8	39.20	69.40	0.0025	5750	11.3 ± 0.9
	P84	14	38.18	43.45	0.0300	4200	13.0 ± 0.6
Group 2: Slight inhibitors	L92	6	16.59	50.34	0.0010	3650	12.5 ± 0.6
	P104	13	53.64	61.03	0.0020	5900	12.3 ± 1.3
	F88	28	207.27	39.31	0.2800	11400	11.8 ± 1.0
Group 3: Activators	L81	2	6.25	42.67	0.0063	2750	13.3 ± 0.1
	L64	15	26.36	30.00	0.1400	2900	11.2 ± 2.1
	P85	16	52.27	39.66	0.0300	4600	12.0 ± 1.9
Group 4: Activators / inhibitors	F108	27	265.45	50.34	0.0320	14600	11.3 ± 0.1
	L61	3	4.55	31.03	0.0022	2000	11.9 ± 0.1
	L31	5	4.00	17.00	0.0231	1100	11.0 ± 0.1

3. Discussion

The findings provided in this report illustrate that the energy status of an environmental pseudomonad was differently affected depending on the Pluronic used in the treatment. These studies add a new dimension to other Pluronic studies performed mainly with pathogenic bacteria (Portoles et al., 1994, 1995) where effects on biofilm production were correlated with surface passivation rather than changes in microbial cell metabolism. The altered metabolism that are reported here supports the work of

Housley et al (2009) where changes in production of secondary components, phenazines, clearly due to altered metabolism, were observed with Pluronic treatments of another environmental pseudomonad. Thus, both surface events and metabolism in microbes are sensitive to Pluronics.

We correlate reduction in Lux output caused by some Pluronics with altered energy status because cellular generation of $FMNH_2$ is required as a substrate for the luciferase enzyme in the biosensor. $FMNH_2$ is generated using the reducing power of NADH produced through cellular metabolism. However, we do not know the mechanism by which the Pluronics affect their metabolic changes on Lux activity. Analogy with effects on mammalian cells would suggest that the functioning of the electron transfer chain is disturbed. Studies with both the normal and the MDR mammalian cell lines suggest a sequence of events occurs after exposure to Pluronic 85:

1) Penetration through membranes with some effect on membrane microviscosity (Batrakova et al., 2003).
2) Entrance into mitochondria to alter electron transport chain function at complex 1 and IV and levels of ATP
3) Inhibition of the ATPase activity of efflux pumps that function to remove intracellular drugs.

The mammalian cell response to P85 is highly sensitive to the concentration of the surfactant. This sensitivity is illustrated by the following findings for normal cell lines as reported by Alakhova et al. (2009):

1) ATP levels increased below CMC but above CMC decreased to 20% control level.

2) Oxygen consumption above CMC dropped to 50% control level.

3) In the Electron Transport Chain, Complex 1 activity was activated below CMC but inhibited above and complex IV increased in activity below CMC with little affect above CMC.

4) Pluronic in the range 0.1 to 1%, above the CMC, caused a 50 % decrease in membrane potential value compared with control.

The assays performed with the pseudomonad biosensor were in the range of 0.1 to 1%), and most were above the CMC value. Exceptions were for F88 (CMC 0.28% and L64 (0.13 %). It is interesting that for F88 there was no response in the bacterial cell light output at the 0.1% dose. At 0.25% (around the CMC value) and higher inhibition was observed. In all other cases presumably the Pluronics interacted predominantly as micelles rather than unimers. The pseudomonad cell has complexes I to IV and an ATP synthase associated with oxidative phosphorylation in its plasma membrane. Consequently, we suggest that Pluronics could be changing the function of the electron transport chain. Elevated Lux output seen with Group 3 Pluronics, with dose relationship effects, could relate to better coupling of oxidative metabolism. However another possibility could be that there were impurities and degradation products that were active in these Pluronics. Decreased light output caused by exposure to Group 1 and Group 2 could relate to impaired electron flow, loss of membrane potential and depletion of ATP.

Group 4 Pluronics showed variable Lux responses between replicated assays in the extent of inhibition. Our interpretation is the interaction of these Pluronics with the bacterial cells is sensitive to the experimental conditions. All of the experiments were conducted in water; thus, any degradation products affecting solution pH would have

greater effects than in buffered solutions. The experiments were performed with Pluronics preparations obtained from December 2008 to May 2009 from the manufacturer. The manufacturer suggested shelf life of the products to be between 1 and 2 years and they indicated that acids are generated upon aging. As of January 2010 several of the Pluronic stocks at 5 % were highly acidic (Table 8). These findings question whether acidity or breakdown products generated the responses. Additionally the experiments were performed without an equilibration time for the dilution of the Pluronics into the cell suspensions.

Table 8: pH of 5% Pluronic solutions as of Jan 2010 (Data courtesy of Alyssa Calder, 2010)

Date Received From Manufacturer	Pluronics	pH
December'08	L121	5.28
January'09	L101	3.47
January'09	P123	4.65
December'08	P84	6.22
January'09	L92	3.66
December'08	P104	6.51
December'08	F88	5.99
January'09	L81	3.68
November'08	L64	3.80
January'09	P85	6.10
January'09	F108	NA
January'09	L31	3.66
January'09	L61	3.88

'NA' denotes not available

Batrakova et al. (2003) and Batrakova et al. (2008) found with mammalian cells that the molecular structure and composition of Pluronic copolymers influenced their activity. Some of the variance may be due to hindered penetration into the cell. Although P85 and L35 both penetrated mammalian cells, intracellular accumulation of L121 was not observed and F108 appeared to remain on the cell surface. However, bacterial cell walls are quite different from membrane layer of mammalian cells with its plasticity. For

the Gram-negative pseudomonad there are two membrane layers. The inner layer, the plasma membrane, has a normal lipid bilayer, but the outer membrane has an outer leaflet containing the charged lipopolysaccharides. Additionally, the periplasmic space in between the two membranes has a band of extensively cross-linked polysaccharides termed the peptidoglycan layer. We do not know how these barriers to entry affect permeation of Pluronics into the cell or affect their surface interactions. Micelles of the predicted size of Pluronics, with average diameter ranges of 15 to 35 nm, (Kabanov et al., 1995), are larger than the predicted channel sizes or pores within the membrane that have a maximum diameter of less than 6 nm. Work with polyol penetration into a Gram-positive cell (thicker peptidoglycan layer but only the single plasma membrane) has a threshold cutoff of 1,200 (1.1 nm). Studies with *P. aeruginosa* suggested a smaller cutoff of 670 when oligosaccharides were used (Caulcott et al., 2006). Consequently it seems only L31 may be at the limit of permeability into the cell. Thus, we assume that the Pluronics exert their effects by surface interactions that disrupt normal membrane functions.

Table 7 lists the physiochemical properties for the Pluronics according to their Group activity with the biosensor. It is perplexing that examination does not show a clear correlation between activity type and physiochemical property. Within each Group, there are Pluronics with low HLBs and relatively higher CMC values. Neither does the estimated chain length of the Pluronics show a correlative role.

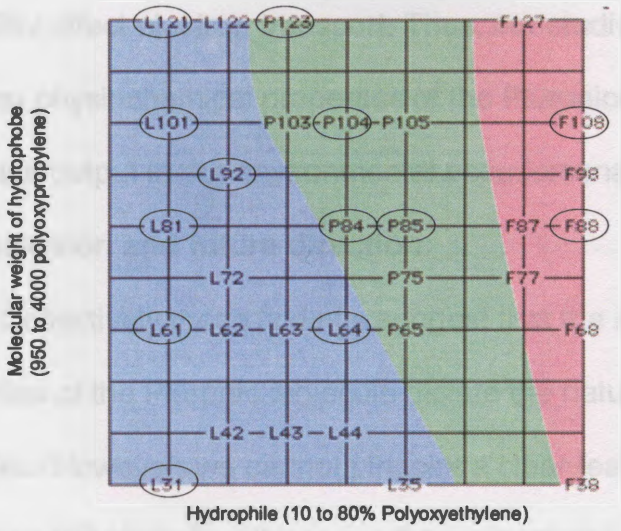


Figure 31: Pluronic grid shows the Pluronic (with circles) that are used in our study.

Although with some Pluronics there was a major decrease in light output, the cells retained normal culturability. Consequently although toxicity was displayed when sensing light output this did not lead to bacterial cell death. The concentration of Pluronic would be decreased by the serial dilution process used in the assessment of culturability performed after 60 min of contact. Reversibility of the effect of Pluronic 85 also was demonstrated by Alakhova et al. (2009). Washing the animal cells to remove Pluronic P85 caused restoration of ATP levels, of Pgp activity and drug resistance in MDR cells. Although the drug efflux pump activity was restored in about 1 h, the ATP levels remained low for at least 5 h. Thus, it appears that both the bacterial cells and mammalian cells recover from inhibitory doses of Pluronics. It is interesting that previous studies (Gajjar et al., 2009) also found that loss in Lux output was not always correlated with bacterial cell death when cells were exposed to NPs of CuO and ZnO. The active player in these responses again is uncertain because the NP suspensions contain ions, NPs of 1-5 nm size and agglomerates of greater than 300 nm (Gajjar et al., 2009). It seems possible that membrane disturbances were occurring again to

reversibly affect electron transport. Thus, our studies do not clearly reveal a correlation between physiochemical properties of the Pluronics with their effects on biosensor luciferase output in the environmental pseudomonad.

4. Conclusion and future direction

Collectively these findings suggest that the molecular structure, composition and properties of the Pluronic molecule dictate the nature of its impact on beneficial soil microbes. However, we cannot pinpoint a clear feature from the HLB, CMC values or the EO or PO chain lengths to predict their activity.

Pluronics have attracted global attraction during the last two decades for their use in medical science, consumer, industrial, and agricultural products. Thus with the increasing use of Pluronics, it is increasingly important to understand how Pluronics affect the environment. Our findings demonstrate that environmental soil microbes, such as *P. putida* KT2440, which has bioremediation potential, and the biological control agent *P chlororaphis* O6 (Gajjar et al., 2009) show changes in metabolism when contacted with certain Pluronics. Such changes could render them more susceptible to antibiotic or ROS damage and thus disrupt elemental cycles in the environment.

Additional understanding of the role of Pluronics could come from the following studies:

1) The Pluronics concentrations used in this study were above the CMCs suggesting that all effects were due to micelles. Studies should be run at lower levels to probe the role of Pluronics as unimers. Buffered solutions should be considered for the bioluminescence assays to address acidity associated with Pluronic solutions.

2) To probe the role of the outer membrane in Pluronic interactions studies could be performed with Gram-positive cells.

3) To see whether, as in animal cells, Pluronic micelles can act as vectors, studies should be run in the presence of potentially toxic materials. Antibiotics, heavy metals and nanoparticles are appropriate amendments.

Such studies may help better predict the potential environmental impacts of Pluronics on bacteria that have a role in soil processes.

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Overview

This appendix summarizes the data from all the repeated experiments including the data shown in results section 2.1.2. In the present study, effects of various Pluronics on *P. putida* KT2440 were observed using bioluminescence and cell culturability assay. We discussed earlier that the molecular structure and composition of Pluronic copolymers influenced their activity. Pluronics L121, L101, P123 and P84 showed strong inhibition in Lux response while Pluronics L81, L64 and P85 showed activation in Lux response. Pluronics L92, P104 and F88 showed slight inhibition in Lux activity. The same trend in Lux response in presence of Pluronics was observed for all the repeated studies. However, variations in RLU values were observed. This can be explained by any variation in day to day experimental conditions such as assay or room temperature, optical density of cells, age and pH of Pluronic solutions.

Methods

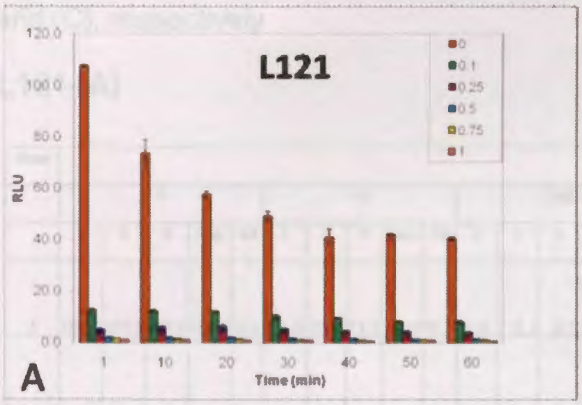
As described earlier in section 2.1.1.

Results

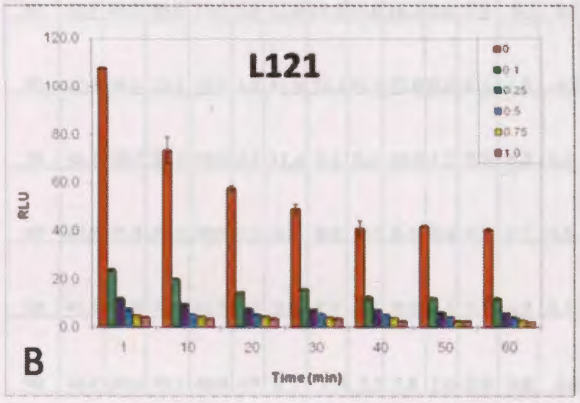
For each Pluronic, data from the repeated experiments are presented in the following manner:

- RLU vs. Time graphs at different concentrations of Pluronic
- RLU values with average and standard deviation from analytical triplicates of individual study in Tabular format
- % RLU relative to control vs. concentrations graphs at every 10 min intervals of 60 min Lux assay

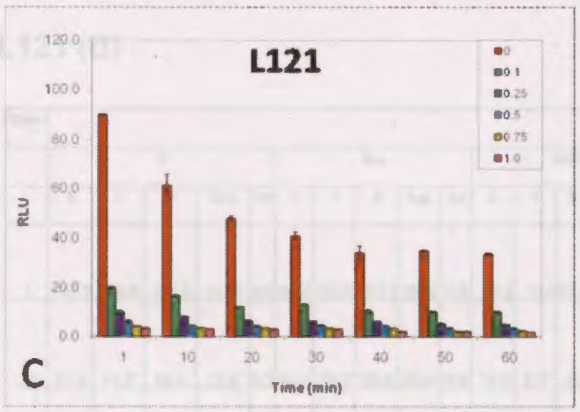
Pluronic L121: RLU vs. Time graph



Treatments L121	Log 10 Cfu./ml	% RLU at 60 min
Control	9.4±0.0	100.0±0.9
0.1 %	11.2±1.0	19.6±1.0
0.25%	11.8±0.2	9.4±0.2
0.5%	11.5±1.7	3.2±0.1
0.75%	11.8±1.6	2.2±0.0
1.0%	9.9±0.6	1.7±0.0



Treatments L121	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.1	100±0.9
0.1 %	10.5±0.1	29.7±0.8
0.25%	11.7±0.2	14.6±0.2
0.5%	10.3±0.1	10.1±0.5
0.75%	10.4±0.3	7.2±0.2
1.0%	10.4±0.1	5.7±0.1



Treatments L121	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.5	100±0.8
0.1 %	10.1±0.3	29.6±0.7
0.25%	11.1±0.4	14.5±0.1
0.5%	10.7±1.0	10.1±0.4
0.75%	10.4±0.5	7.1±0.1
1.0%	11.2±0.9	5.6±0.1

Figure A1: Lux response of *P. putida* KT2440 biosensor to L121 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 12-18-2008, (B) Study 2 performed on 1-13-2009, (C) Study 3 performed on 1-15-2009. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic L121 were performed within 2 months of its receiving from the supplier.

Table A1: Lux response (RLU values) of *P. putida* KT2440 biosensor to L121 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables L121 (A), (B) and (C), respectively

L121 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	107.1	108.0	107.9	107.7	0.5	12.9	13.1	11.7	12.6	0.7	5.0	5.1	5.5	5.2	0.2	2.2	2.1	1.7	2.0	0.3	1.6	1.8	1.5	1.7	0.1	0.8	1.0	1.0	1.0	0.1
10	67.4	84.3	69.0	73.6	9.3	12.9	12.2	11.8	12.3	0.6	5.8	5.7	6.5	6.0	0.4	2.1	1.7	2.0	1.9	0.2	1.2	1.4	1.4	1.3	0.1	1.0	0.9	0.9	0.9	0.0
20	57.3	56.1	59.7	57.7	1.8	11.4	12.5	12.1	12.0	0.5	6.4	7.3	4.9	6.2	1.2	2.1	1.9	1.6	1.9	0.2	1.4	1.2	1.1	1.2	0.2	0.8	0.7	0.8	0.8	0.1
30	45.7	49.2	52.3	49.1	3.3	11.4	9.1	9.4	10.0	1.2	5.5	5.0	5.2	5.2	0.3	1.7	1.7	1.4	1.6	0.2	0.9	0.9	0.9	0.9	0.0	0.7	0.7	0.7	0.7	0.0
40	39.8	35.8	47.1	40.9	5.7	9.6	9.8	9.0	9.5	0.4	4.6	4.7	4.5	4.6	0.1	1.5	1.5	1.3	1.4	0.1	0.9	0.8	0.9	0.9	0.0	0.7	0.6	0.5	0.6	0.1
50	41.3	42.6	41.9	41.9	0.7	8.6	7.7	8.2	8.2	0.5	4.7	4.2	4.1	4.3	0.3	1.3	1.1	1.0	1.2	0.1	1.0	0.9	1.0	1.0	0.0	0.8	0.8	0.7	0.8	0.1
60	40.7	39.3	41.1	40.4	0.9	9.1	7.4	7.2	7.9	1.0	3.9	3.9	3.6	3.8	0.2	1.3	1.2	1.3	1.3	0.1	1.0	0.9	0.9	0.9	0.0	0.7	0.6	0.6	0.7	0.0

L121 (B)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	107.1	108.0	107.9	107.7	0.5	24.4	23.9	23.1	23.8	0.6	12.3	12.4	10.9	11.9	0.8	7.5	6.9	8.2	7.5	0.7	5.2	5.1	4.9	5.1	0.2	4.0	4.2	4.4	4.2	0.2
10	67.4	84.3	69.0	73.6	9.3	19.5	20.5	20.0	20.0	0.5	10.0	9.6	9.8	9.8	0.2	5.5	5.4	5.2	5.4	0.1	4.5	4.6	4.4	4.5	0.1	3.5	3.8	3.8	3.7	0.2
20	57.3	56.1	59.7	57.7	1.8	14.5	14.8	14.6	14.6	0.1	8.6	7.0	7.3	7.6	0.9	5.0	5.2	5.8	5.4	0.4	4.5	4.6	4.6	4.5	0.1	4.1	3.8	3.3	3.7	0.4
30	45.7	49.2	52.3	49.1	3.3	16.1	16.0	15.1	15.7	0.6	7.4	7.7	7.6	7.6	0.2	5.5	5.6	5.8	5.6	0.1	4.3	3.9	4.1	4.1	0.2	3.6	4.0	3.4	3.7	0.3
40	39.8	35.8	47.1	40.9	5.7	11.8	11.1	14.0	12.3	1.6	7.4	7.5	7.4	7.4	0.1	5.3	5.5	5.3	5.4	0.1	4.1	3.9	3.8	4.0	0.1	2.1	2.8	2.7	2.6	0.4
50	41.3	42.6	41.9	41.9	0.7	12.3	12.0	12.4	12.2	0.2	5.3	6.4	6.7	6.1	0.7	4.6	4.3	4.0	4.3	0.3	2.4	3.1	2.4	2.6	0.4	2.0	2.7	2.6	2.5	0.4
60	40.7	39.3	41.1	40.4	0.9	12.4	11.0	12.5	12.0	0.8	5.9	5.8	6.1	5.9	0.2	4.1	4.6	3.6	4.1	0.5	3.0	2.7	3.0	2.9	0.2	2.2	2.4	2.3	2.3	0.1

L121 (C)

Time	Concentration %																													
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	89.3	90.0	89.9	89.7	0.4	20.3	20.0	19.2	19.8	0.5	10.2	10.3	9.1	9.9	0.7	6.2	5.7	6.8	6.3	0.6	4.3	4.2	4.1	4.2	0.1	3.3	3.5	3.7	3.5	0.2
10	58.2	70.2	57.5	61.3	7.8	16.3	17.1	16.8	16.7	0.4	8.3	8.0	8.1	8.2	0.1	4.8	4.5	4.3	4.5	0.1	3.8	3.8	3.7	3.8	0.1	2.9	3.2	3.1	3.1	0.1
20	47.8	46.8	49.7	48.1	1.5	12.1	12.3	12.1	12.2	0.1	7.2	5.8	6.1	6.4	0.7	4.2	4.3	4.9	4.5	0.3	3.7	3.8	3.8	3.8	0.1	3.4	3.2	2.7	3.1	0.3
30	38.1	41.0	43.6	40.9	2.7	13.5	13.3	12.6	13.1	0.5	6.1	6.4	6.4	6.3	0.1	4.6	4.7	4.8	4.7	0.1	3.6	3.2	3.4	3.4	0.2	3.0	3.4	2.8	3.1	0.3
40	33.2	29.8	39.3	34.1	4.8	9.9	9.2	11.6	10.2	1.2	6.1	6.3	6.2	6.2	0.1	4.4	4.6	4.4	4.5	0.1	3.4	3.3	3.2	3.3	0.1	1.8	2.4	2.2	2.1	0.3
50	34.4	35.5	34.9	34.9	0.6	10.3	10.0	10.3	10.2	0.1	4.4	5.4	5.6	5.1	0.6	3.8	3.8	3.3	3.8	0.2	2.0	2.6	2.0	2.2	0.3	1.7	2.3	2.2	2.1	0.3
60	33.9	32.8	34.2	33.7	0.8	10.4	9.2	10.4	10.0	0.7	4.9	4.8	5.1	4.9	0.1	3.5	3.8	3.0	3.4	0.4	2.5	2.2	2.5	2.4	0.1	1.8	2.0	1.9	1.9	0.1

Pluronic L121: % RLU vs. % Concentrations graphs

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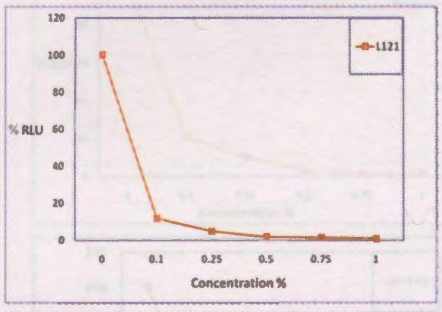
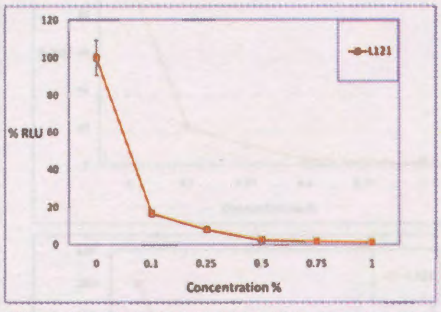
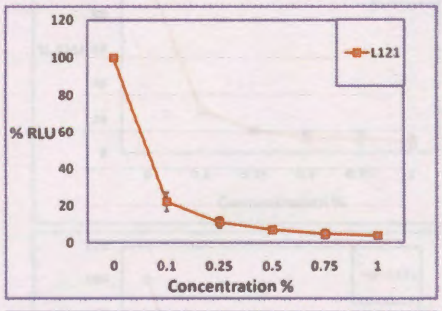
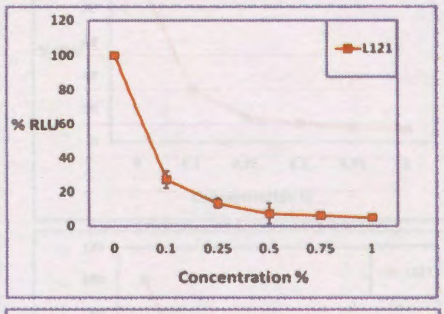
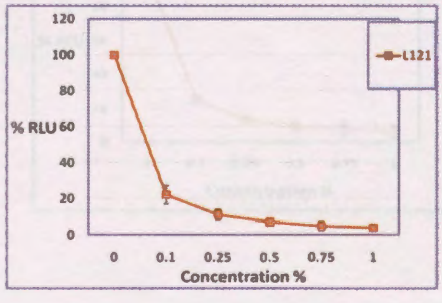
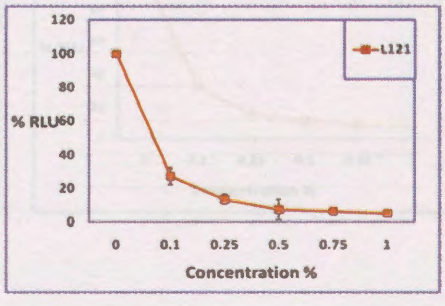
STUDY 1		
STUDY 2		
STUDY 3		

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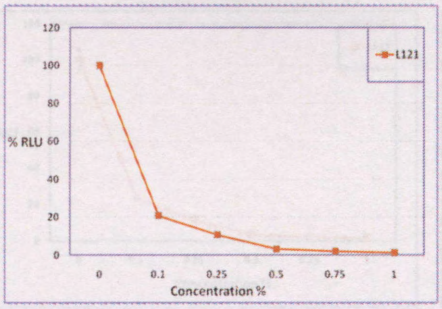
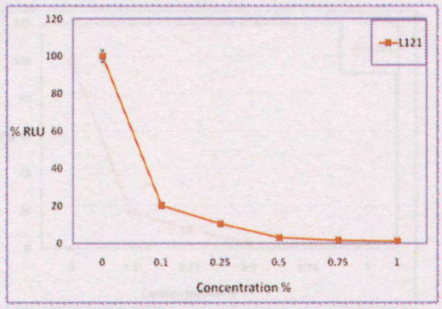
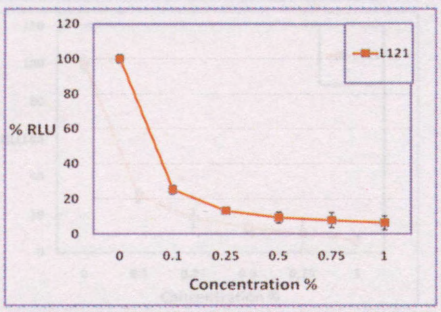
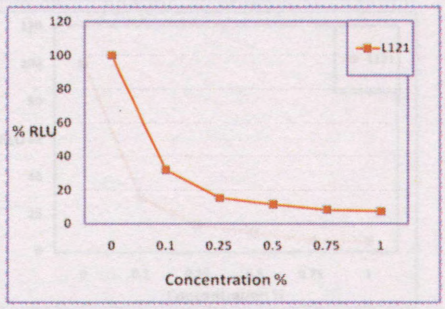
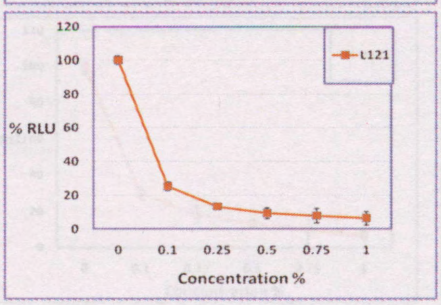
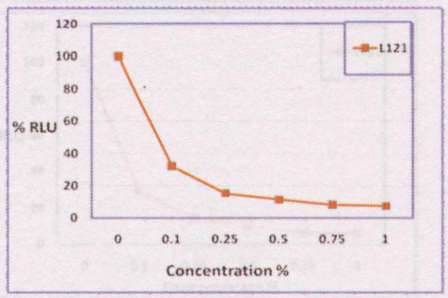
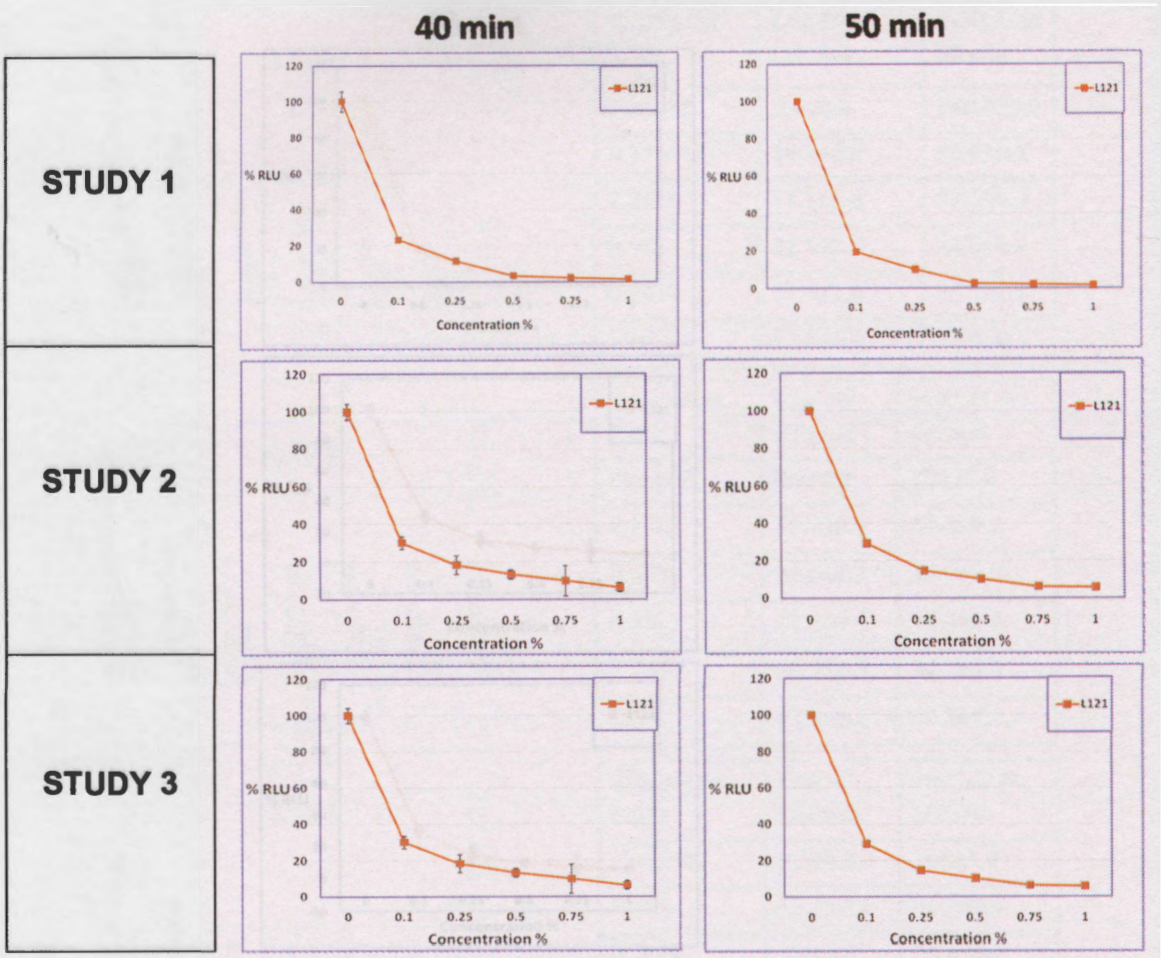
STUDY 1		
STUDY 2		
STUDY 3		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
L121
% RLU
Concentration %
0
0.1
0.25
0.5
0.75
1
20
40
60
80
100
120

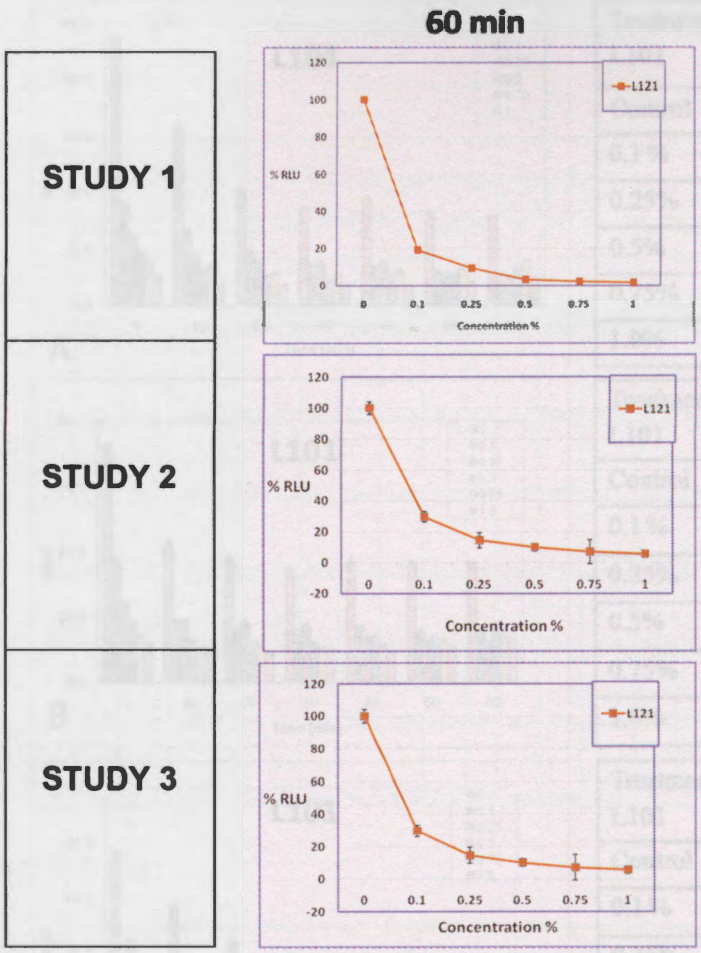
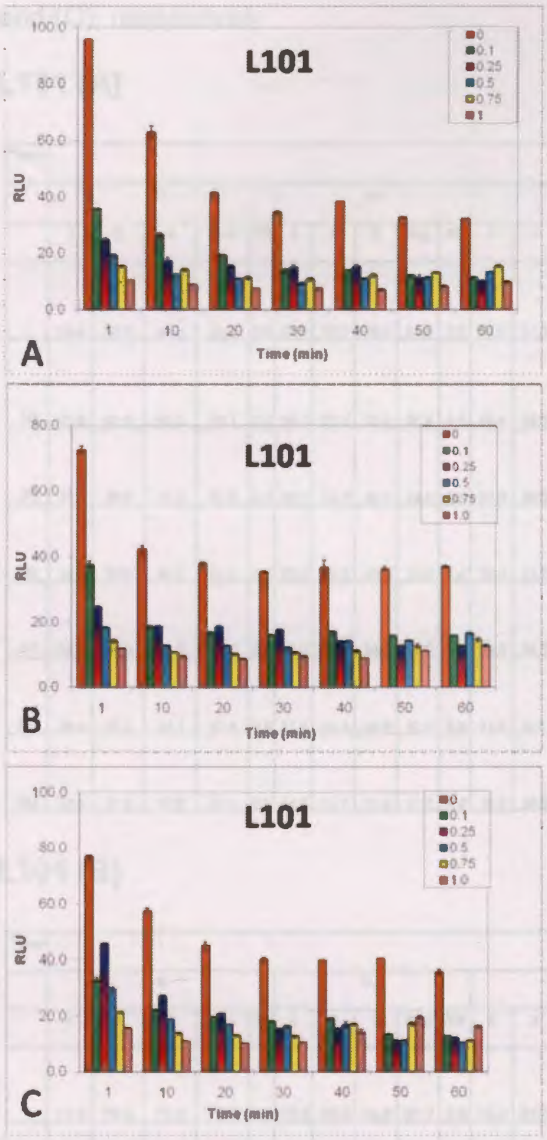


Figure A2: % RLU relative to control for defined concentrations of Pluronic L121 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

Pluronic L101: RLU vs. Time graph



Treatments L101	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.0	100.0±0.0
0.1 %	10.8±0.8	36.0±0.2
0.25%	11.1±0.6	32.3±0.5
0.5%	12.5±0.5	42.1±0.4
0.75%	11.6±1.6	48.3±1.0
1.0%	12.6±0.2	30.8±0.5

Treatments L101	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.1	100±0.0
0.1 %	10.2±0.1	42.2±0.2
0.25%	11.6±0.2	33.9±0.5
0.5%	12.2±0.4	44.4±0.4
0.75%	10.5±0.3	39.0±1.0
1.0%	12.5±0.2	34.2±0.5

Treatments L101	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.2	100±1.4
0.1 %	10.1±0.3	36.5±0.1
0.25%	9.9±0.5	34.5±0.2
0.5%	12.8±0.4	29.3±0.2
0.75%	13.0±0.5	31.4±0.2
1.0%	11.8±0.2	45.8±0.5

Figure A3: Lux response of *P. putida* KT2440 biosensor to L101 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 1-9-2009, (B) Study 2 performed on 1-8-2009, (C) Study 3 performed on 1-10-2009. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic L101 were performed within 2 months of its receiving from the supplier.

Table A2: Lux response (RLU values) of *P. putida* KT2440 biosensor to L101 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables L101 (A), (B) and (C), respectively

L101 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	95.8	95.5	94.3	95.2	0.8	35.9	32.2	35.4	34.5	2.0	24.9	24.3	26.1	25.1	0.9	18.4	19.0	20.3	19.3	1.0	15.8	15.8	15.2	15.6	0.3	10.8	10.7	10.6	10.7	0.1
10	66.8	62.9	59.6	63.1	3.6	28.0	28.3	25.9	26.8	1.1	19.5	15.5	17.5	17.5	2.0	12.6	12.5	12.9	12.7	0.2	14.9	14.6	13.9	14.5	0.5	9.3	8.3	9.1	8.9	0.5
20	41.2	42.0	41.3	41.5	0.4	20.1	19.1	19.1	19.4	0.6	14.6	16.0	16.1	15.6	0.9	10.8	11.4	11.5	11.3	0.4	10.9	11.9	12.1	11.6	0.6	7.5	7.7	7.9	7.7	0.2
30	34.1	34.9	35.2	34.7	0.6	12.4	14.1	15.3	13.9	1.4	16.3	13.8	15.9	15.3	1.4	8.4	10.0	10.0	9.5	0.9	10.3	11.6	11.1	11.0	0.7	7.2	7.7	8.3	7.8	0.5
40	38.6	38.3	33.3	36.7	3.0	13.9	14.3	14.2	14.2	0.2	14.4	16.7	15.3	15.4	1.2	10.8	11.3	11.5	11.2	0.4	12.1	13.6	11.6	12.4	1.0	6.7	7.2	7.8	7.2	0.5
50	33.0	32.5	32.1	32.6	0.5	11.9	12.3	13.0	12.4	0.5	11.9	9.4	12.7	11.3	1.7	11.8	11.4	11.9	11.7	0.2	13.7	13.3	12.3	13.1	0.7	8.0	8.5	8.9	8.5	0.4
60	32.5	31.0	31.0	31.5	0.9	11.5	11.7	11.8	11.7	0.2	10.3	10.5	10.6	10.5	0.2	13.5	13.7	13.8	13.7	0.1	15.6	15.4	16.3	15.7	0.5	9.4	9.9	10.7	10.0	0.7

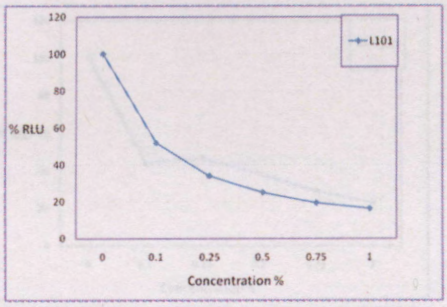
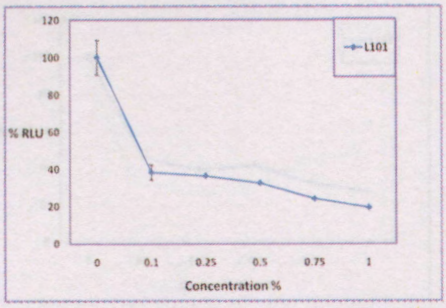
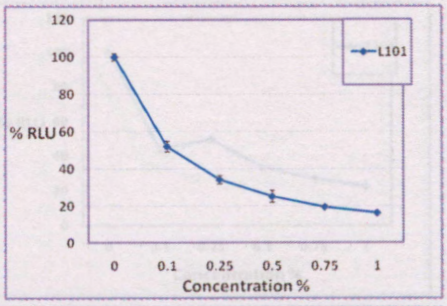
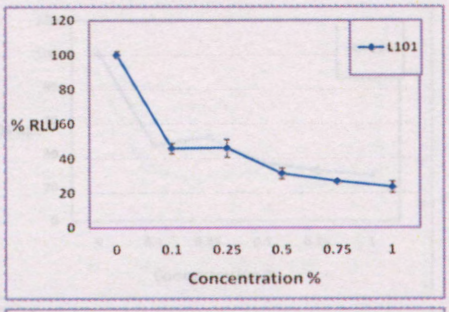
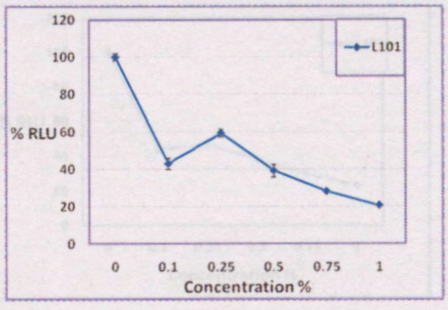
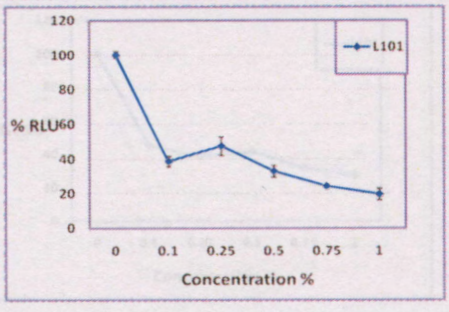
L101 (B)

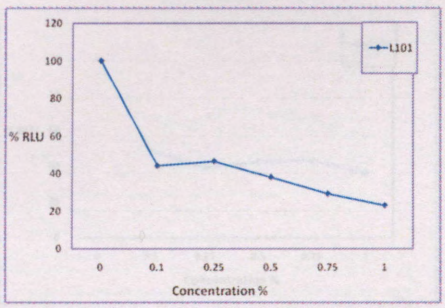
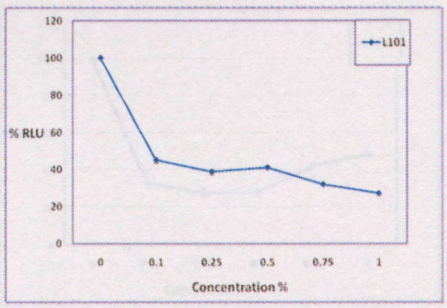
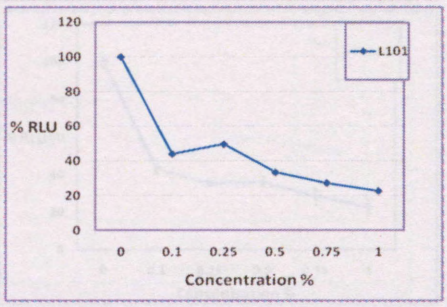
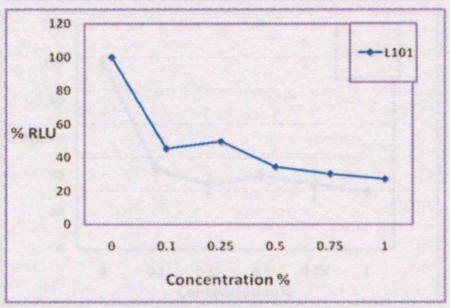
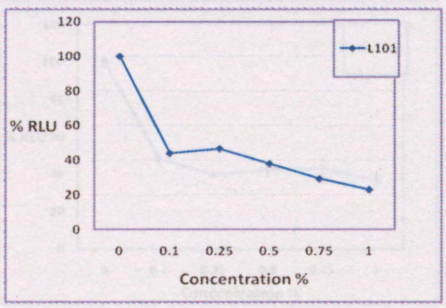
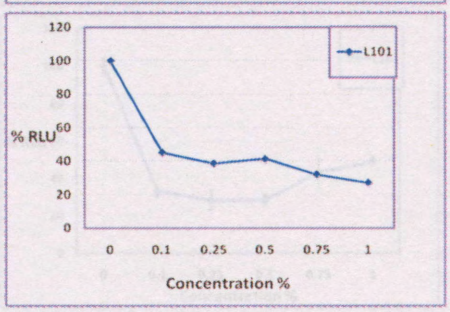
Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	74.8	70.5	72.2	72.5	2.2	39.5	36.6	38.0	37.7	2.0	25.4	24.5	24.5	24.8	0.5	18.2	18.8	17.7	18.3	0.6	15.1	14.1	13.5	14.2	0.8	11.8	11.7	12.2	11.9	0.3
10	40.8	42.3	44.2	42.4	1.7	19.2	18.6	18.3	18.7	0.5	18.5	19.1	18.7	18.8	0.3	12.9	12.7	12.6	12.8	0.2	10.7	10.8	11.2	10.9	0.3	9.8	9.4	9.5	9.6	0.2
20	37.2	38.3	38.6	38.0	0.8	17.3	16.6	16.3	16.7	0.6	18.6	19.0	19.1	18.9	0.3	12.4	12.7	13.0	12.7	0.3	10.1	10.5	10.6	10.4	0.3	8.2	8.8	9.0	8.6	0.4
30	34.9	36.1	35.4	35.5	0.6	16.0	15.9	16.3	16.1	0.2	18.0	16.8	17.9	17.6	0.7	11.8	12.0	12.6	12.2	0.4	10.2	11.1	10.8	10.7	0.5	9.1	9.9	9.8	9.6	0.4
40	39.6	38.6	32.2	36.8	4.0	17.2	16.8	17.3	17.1	0.3	13.8	14.4	14.0	14.1	0.3	13.7	14.4	14.6	14.2	0.5	10.7	11.7	11.1	11.2	0.5	9.0	9.2	9.0	9.1	0.1
50	35.4	36.4	37.5	36.4	1.1	15.9	15.7	15.4	15.7	0.3	11.9	13.1	12.8	12.6	0.6	14.4	13.9	14.6	14.3	0.4	12.5	11.6	13.8	12.6	1.1	11.0	11.3	11.5	11.2	0.3
60	37.4	37.5	36.4	37.1	0.6	15.6	15.8	16.0	15.8	0.2	13.2	12.2	12.6	12.7	0.5	17.0	16.6	16.1	16.6	0.4	15.1	15.4	13.4	14.6	1.0	12.2	12.8	13.2	12.8	0.5

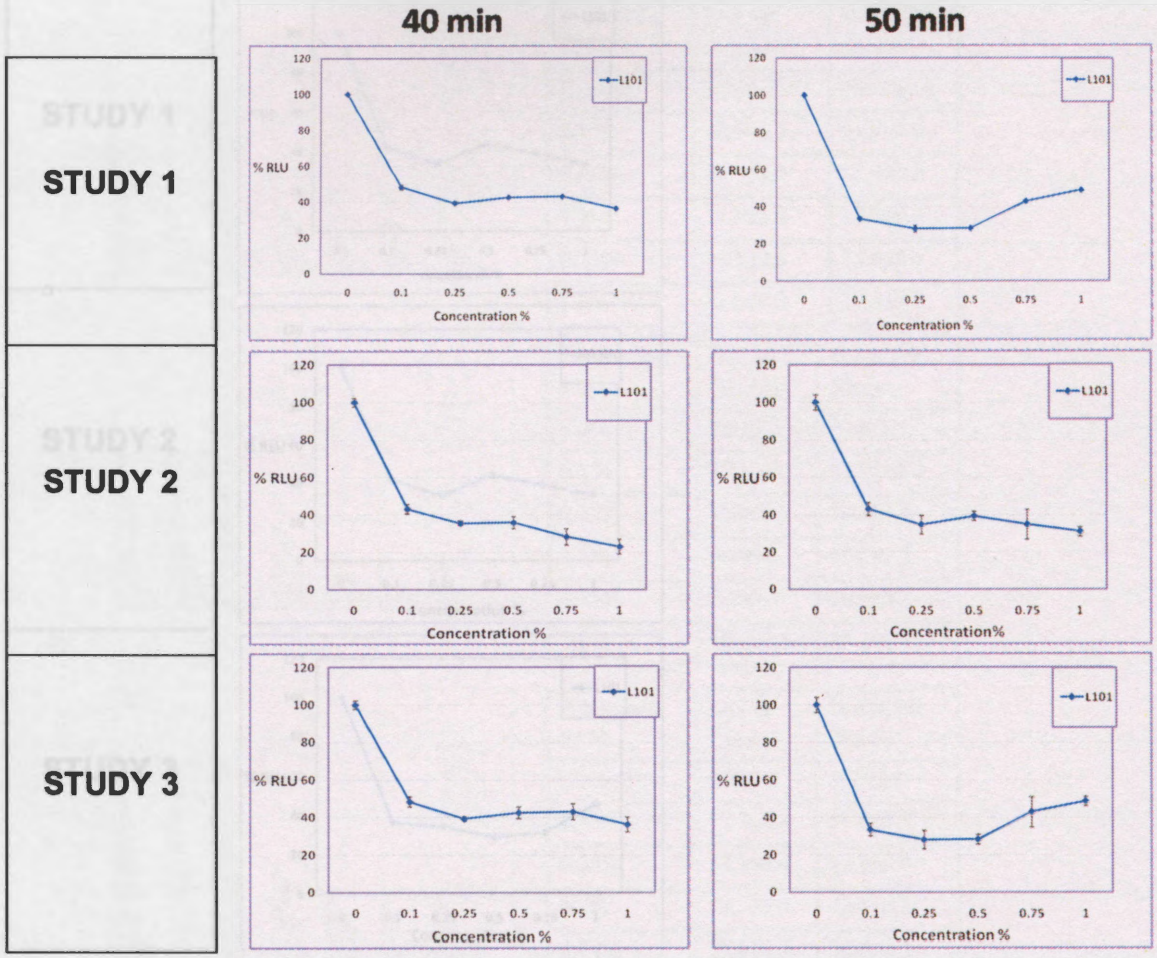
L101 (C)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	78.0	76.1	77.1	77.1	0.9	31.5	32.7	34.8	33.1	1.7	45.0	46.3	45.9	45.7	0.7	30.1	30.8	29.3	30.1	0.8	21.9	21.5	21.4	21.6	0.3	15.8	16.2	15.6	15.9	0.3
10	55.7	58.3	59.1	57.7	1.7	22.1	21.5	23.0	22.2	0.7	26.7	28.4	26.9	27.3	0.9	19.0	18.7	19.1	18.9	0.2	14.0	13.5	14.3	14.0	0.4	11.6	11.1	11.1	11.2	0.3
20	42.8	46.9	46.3	45.3	2.2	20.0	19.8	20.1	20.0	0.1	21.1	21.3	21.1	21.1	0.1	17.3	16.9	17.4	17.2	0.3	13.3	13.6	12.8	13.2	0.4	10.5	10.3	10.4	10.4	0.1
30	39.3	41.2	40.8	40.4	1.0	18.6	17.9	18.3	18.2	0.3	15.4	16.0	15.3	15.6	0.4	17.1	16.8	16.0	16.6	0.5	13.3	12.9	12.5	12.9	0.4	10.7	10.9	11.1	10.9	0.2
40	40.0	40.5	43.3	41.3	1.8	19.0	19.4	19.6	19.3	0.3	15.8	15.6	15.8	15.7	0.1	18.6	15.0	17.4	17.0	1.8	17.7	16.5	17.4	17.2	0.6	12.8	15.5	15.3	14.5	1.5
50	40.6	39.9	36.7	39.1	2.1	13.2	13.9	13.7	13.6	0.3	11.5	11.7	11.0	11.4	0.3	11.4	11.6	11.7	11.5	0.2	16.4	18.0	18.0	17.4	0.9	18.8	19.3	21.2	19.8	1.2
60	34.6	36.7	37.3	36.2	1.4	13.1	13.3	13.1	13.2	0.1	12.3	12.8	12.3	12.5	0.2	10.4	10.8	10.6	10.6	0.2	11.4	11.7	11.2	11.4	0.2	16.7	17.1	16.1	16.6	0.5

Pluronic L101:% RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
% RLU
Concentration %
L101
0
0.1
0.25
0.5
0.75
1
20
40
60
80
100
120

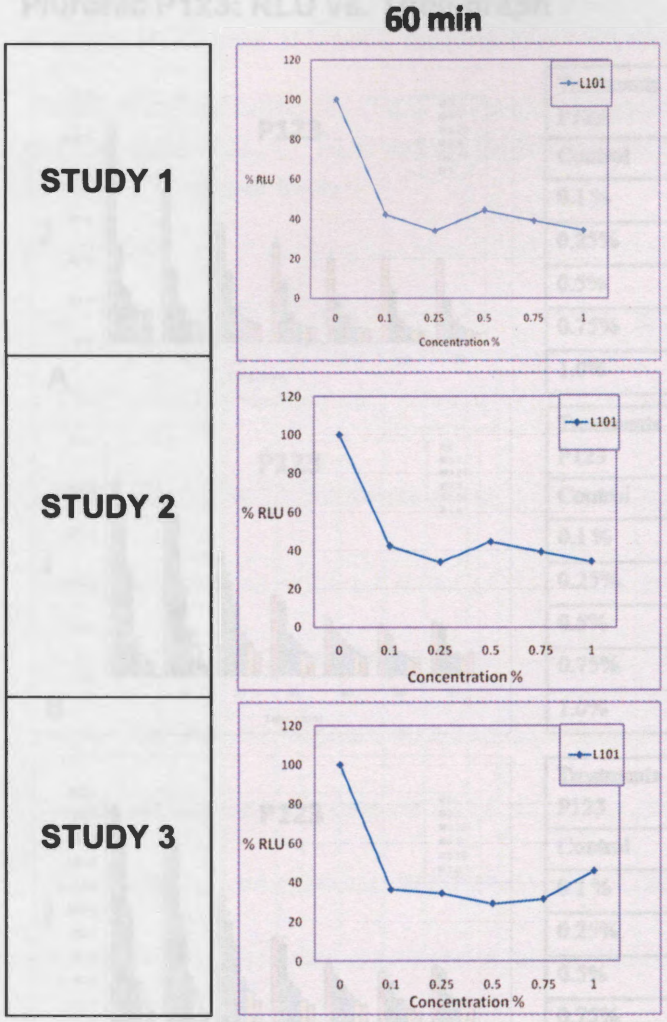
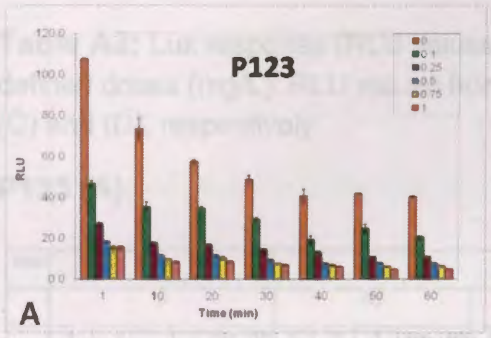
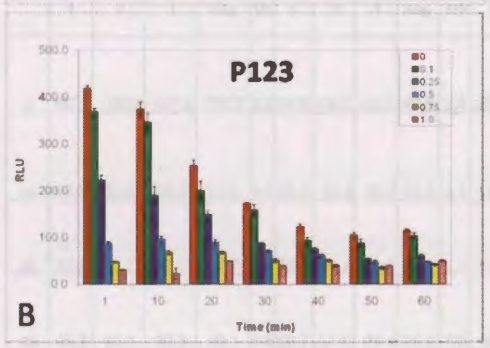


Figure A4: % RLU relative to control for defined concentrations of Pluronics L101 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

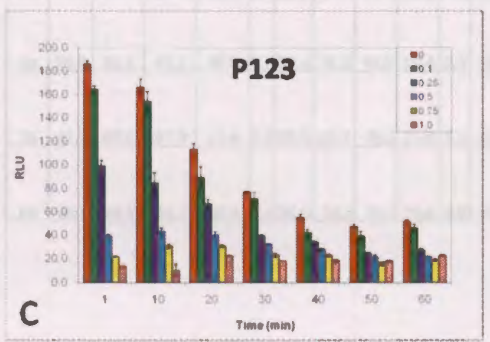
Pluronic P123: RLU vs. Time graph



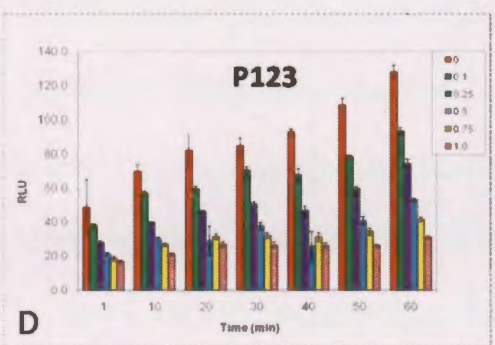
Treatments P123	Log 10 Cfu./ml	% RLU at 60 min
Control	9.0±0.6	100.0±0.9
0.1 %	10.9±2.5	50.9±0.6
0.25%	10.9±1.3	27.2±0.3
0.5%	11.3±2.2	18.8±0.4
0.75%	11.5±2.3	15.6±0.5
1.0%	11.3±0.9	12.1±0.3



Treatments P123	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.1	100±5.8
0.1 %	9.2±0.3	89.5±12.3
0.25%	10.0±0.2	52.8±5.6
0.5%	9.6±0.6	42.1±1.2
0.75%	9.9±0.3	36.6±3.5
1.0%	10.7±0.5	44.1±2.4



Treatments P123	Log 10 Cfu./ml	% RLU at 60 min
Control	9.3±0.2	100±3.9
0.1 %	12.3±0.3	89.7±8.2
0.25%	12.1±0.4	52.9±3.8
0.5%	12.7±0.4	42.0±0.8
0.75%	12.3±0.2	36.6±2.3
1.0%	12.7±0.2	44.1±1.6



Treatments P123	Log 10 Cfu./ml	% RLU at 60 min
Control	9.0±0.6	100.0±7.0
0.1 %	10.9±2.5	72.9±3.7
0.25%	10.9±1.3	58.4±4.7
0.5%	11.3±2.2	41.6±1.5
0.75%	11.5±2.3	32.5±2.1
1.0%	11.3±0.9	24.2±1.2

Figure A5: Lux response of *P. putida* KT2440 biosensor to P123 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 1-13-2009, (B) Study 2 performed on 10-8-2008, (C) Study 3 performed on 10-10-2008, (D) Study 4 performed on 7-16-2008. Means of analytical replicates and standard errors are shown for each study. Study 1

with Pluronic P123 was performed within a month of its receiving from supplier. However, age of Pluronic P123 was at least 6 months when Studies 2 and 3 were performed.

Table A3: Lux response (RLU values) of *P. putida* KT2440 biosensor to P123 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables P123 (A), (B), (C) and (D), respectively

P123 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	107.1	108.0	107.9	107.7	0.5	44.9	46.7	49.5	47.0	2.3	27.2	27.8	27.1	27.4	0.4	18.8	18.0	18.7	18.5	0.4	17.2	14.4	14.7	15.4	1.5	16.8	15.6	15.3	15.9	0.8
10	67.4	84.3	69.0	73.6	9.3	38.9	31.0	36.7	35.5	4.1	17.8	18.2	17.4	17.8	0.4	12.0	11.7	12.0	11.9	0.2	9.8	9.9	9.8	9.8	0.0	8.1	8.9	8.8	8.6	0.4
20	57.3	56.1	59.7	57.7	1.8	35.6	35.7	33.2	34.8	1.4	17.3	16.7	16.5	16.9	0.4	12.1	11.3	12.1	11.8	0.4	11.9	9.7	9.8	10.5	1.2	8.9	7.9	9.2	8.7	0.7
30	45.7	49.2	52.3	49.1	3.3	29.7	27.5	30.6	29.3	1.6	15.1	14.3	14.5	14.6	0.4	10.0	9.0	9.3	9.4	0.5	7.8	7.6	7.5	7.6	0.2	7.5	6.7	6.8	7.0	0.5
40	39.8	35.8	47.1	40.9	5.7	22.4	16.1	19.6	19.4	3.2	13.9	13.0	12.2	13.0	0.9	7.1	7.7	8.1	7.6	0.5	7.0	7.2	6.5	6.9	0.4	5.9	6.0	6.5	6.1	0.3
50	41.3	42.6	41.9	41.9	0.7	26.8	27.1	20.8	24.9	3.5	11.1	10.8	10.7	10.9	0.2	8.2	7.6	8.2	8.0	0.4	6.5	6.1	6.1	6.2	0.3	5.1	4.7	4.8	4.8	0.2
60	40.7	39.3	41.1	40.4	0.9	20.5	20.0	21.3	20.6	0.6	10.7	11.2	11.1	11.0	0.3	7.2	8.0	7.6	7.6	0.4	5.7	6.6	6.6	6.3	0.5	5.0	4.6	5.1	4.9	0.3

P123 (B)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	421.7	427.9	407.6	419.1	10.4	358.5	382.7	367.3	369.5	12.2	201.7	231.0	235.8	222.8	18.5	88.5	92.7	83.7	88.3	4.5	47.7	48.5	49.6	48.6	0.9	33.1	30.0	30.2	31.1	1.7
10	399.5	377.5	346.5	374.5	26.6	385.5	331.6	323.7	346.9	33.6	149.9	207.8	212.4	190.0	34.8	86.4	100.8	105.9	97.4	10.7	60.8	71.1	74.1	68.7	7.0	42.9	23.9	2.7	23.1	20.1
20	277.4	244.5	242.4	254.8	19.6	157.7	221.2	223.0	200.6	37.2	164.9	149.3	134.1	149.4	15.4	79.9	95.8	97.2	91.0	9.6	67.1	72.8	66.9	69.0	3.3	49.3	50.6	51.1	50.3	0.9
30	177.2	170.5	171.5	173.1	3.6	134.3	171.1	172.4	159.3	21.6	88.3	90.9	85.2	88.1	2.9	74.4	70.8	72.1	72.4	1.8	54.3	43.5	56.9	51.6	7.1	41.0	40.3	39.7	40.3	0.6
40	134.1	113.4	124.7	124.1	10.4	85.5	107.4	90.9	94.6	11.4	70.1	78.0	79.7	75.9	5.1	63.9	65.6	57.0	62.2	4.6	47.9	54.9	51.0	51.3	3.5	38.4	45.1	39.4	41.0	3.6
50	102.3	115.3	100.5	106.0	8.1	70.2	98.2	96.3	88.3	15.6	51.2	56.0	56.3	54.5	2.9	55.0	45.4	45.2	48.5	5.6	30.5	38.3	39.3	36.0	4.8	39.1	40.9	40.7	40.2	1.0
60	113.3	122.4	111.6	115.8	5.8	95.0	98.3	117.8	103.7	12.3	57.1	58.9	67.6	61.2	5.6	48.5	47.6	50.0	48.7	1.2	46.2	39.4	41.6	42.4	3.5	52.8	48.4	52.1	51.1	2.4

P123 (C)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	281.1	285.3	271.8	279.4	6.9	239.0	255.1	244.8	246.3	8.2	134.4	154.0	157.2	148.6	12.3	59.0	61.8	55.8	58.9	3.0	31.8	32.3	33.0	32.4	0.6	22.1	20.0	20.1	20.7	1.2
10	266.3	251.6	231.0	249.6	17.8	257.0	221.0	215.8	231.3	22.4	99.9	138.5	141.6	126.7	23.2	56.9	67.2	70.6	64.9	7.1	40.5	47.4	49.4	45.8	4.7	28.6	15.9	1.8	15.4	13.4
20	184.9	163.0	161.6	169.8	13.1	105.2	147.5	148.6	133.8	24.8	109.9	99.5	89.4	99.6	10.3	53.3	63.9	64.8	60.7	6.4	44.8	48.5	44.6	46.0	2.2	32.9	33.8	34.0	33.5	0.6
30	118.2	113.7	114.3	115.4	2.4	89.6	114.1	114.9	106.2	14.4	58.9	60.6	56.8	58.8	1.9	49.6	47.2	48.1	48.3	1.2	36.2	29.0	37.9	34.4	4.7	27.3	26.9	26.5	26.9	0.4
40	89.4	75.6	83.2	82.7	6.9	57.0	71.6	60.6	63.1	7.6	46.7	52.0	53.1	50.6	3.4	42.6	43.7	38.0	41.4	3.0	31.9	36.6	34.0	34.2	2.3	25.6	30.1	26.3	27.3	2.4
50	68.2	76.9	67.0	70.7	5.4	46.8	65.5	64.2	58.8	10.4	34.1	37.4	37.5	36.3	1.9	36.6	30.2	30.1	32.3	3.7	20.3	25.5	26.2	24.0	3.2	26.0	27.3	27.2	26.8	0.7
60	75.6	81.6	74.4	77.2	3.9	63.3	65.6	78.5	69.1	8.2	38.0	39.3	45.1	40.8	3.8	32.3	31.7	33.3	32.4	0.8	30.8	26.2	27.7	28.2	2.3	35.2	32.3	34.7	34.1	1.6

P123 (D)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	15.8	64.7	66.0	48.8	28.6	39.1	37.1	38.5	38.2	1.0	27.1	27.5	29.5	28.0	1.3	20.5	22.3	20.9	21.2	0.9	16.3	18.5	20.8	18.6	2.3	15.6	17.6	17.1	16.8	1.1
10	64.9	77.9	68.0	70.2	6.8	55.5	58.7	58.4	57.5	1.8	40.5	39.9	38.1	39.5	1.2	28.7	30.2	31.6	30.1	1.5	27.4	25.3	27.4	26.7	1.3	20.5	22.2	20.9	21.2	0.9
20	65.7	94.7	87.6	82.7	15.1	58.3	62.9	58.6	59.9	2.6	45.1	47.6	46.2	46.3	1.3	12.3	41.0	34.0	29.1	14.8	28.3	32.1	34.2	31.5	3.0	24.0	28.5	28.4	27.0	2.6
30	77.1	85.4	93.2	85.2	8.0	70.5	67.6	74.6	70.9	3.5	53.1	51.4	48.8	51.1	2.2	41.6	37.1	35.1	37.9	3.3	28.8	33.1	34.2	32.0	2.9	25.1	29.8	24.7	26.5	2.9
40	89.8	96.0	94.0	93.2	3.2	67.2	62.4	74.7	68.1	6.2	50.9	48.2	41.9	47.0	4.6	8.8	33.5	35.3	25.9	14.8	35.0	31.8	26.6	31.1	4.3	29.3	24.9	23.8	26.0	2.9
50	101.7	115.5	108.8	108.7	6.9	78.3	79.5	79.5	79.1	0.7	57.7	61.9	60.5	60.1	2.2	45.1	41.3	36.6	41.0	4.3	30.8	37.5	34.9	34.4	3.4	26.7	25.4	26.6	26.2	0.7
60	134.6	120.7	128.9	128.1	7.0	94.9	89.4	96.4	93.5	3.7	80.2	72.2	72.0	74.8	4.7	51.7	54.5	53.8	53.3	1.5	41.2	39.7	43.9	41.6	2.1	31.9	29.6	31.5	31.0	1.2

Pluronic P123: % RLU vs. % Concentrations graphs

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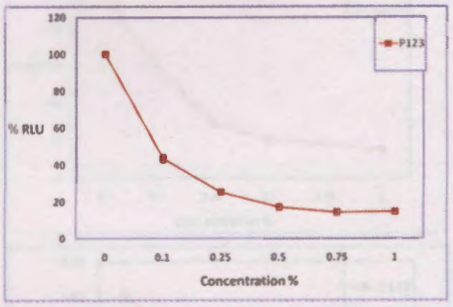
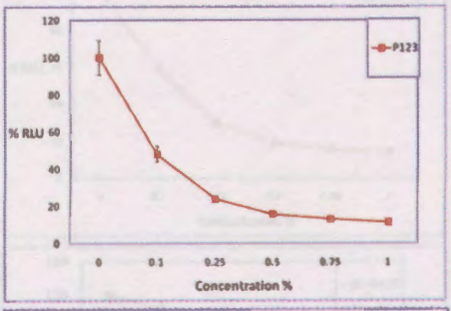
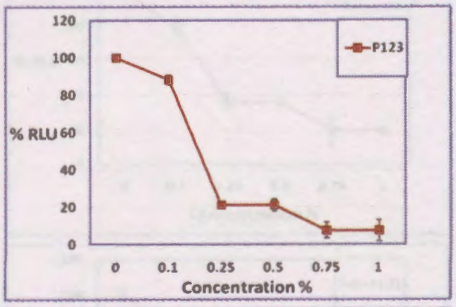
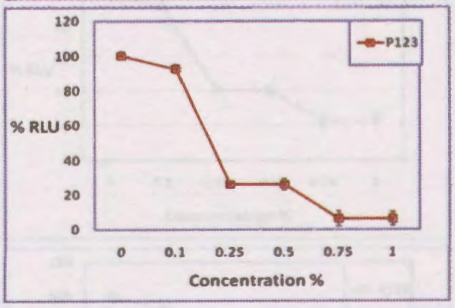
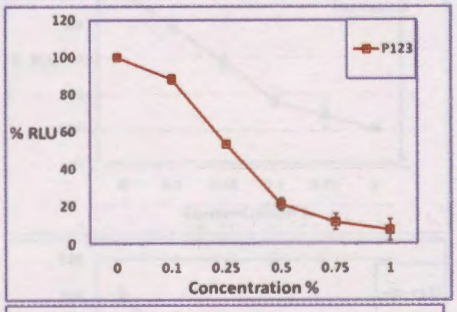
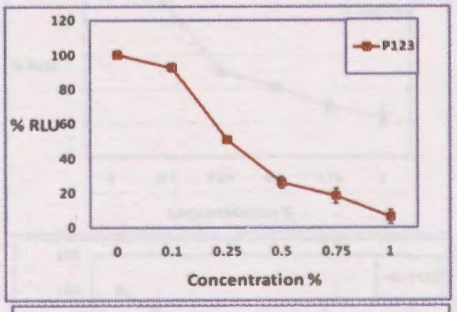
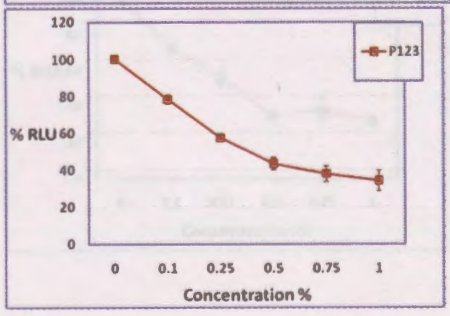
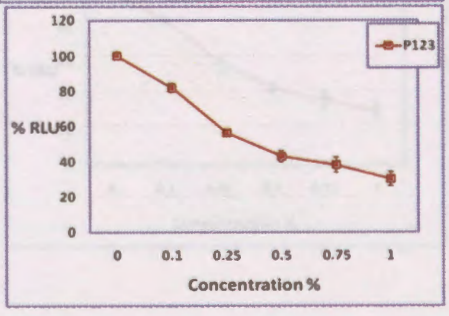
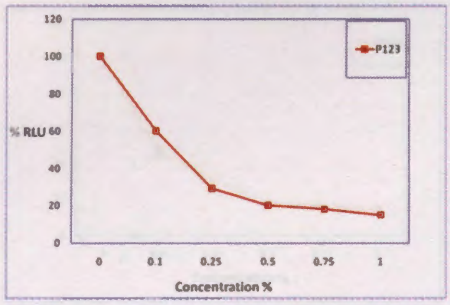
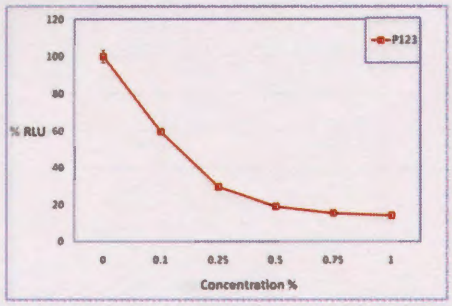
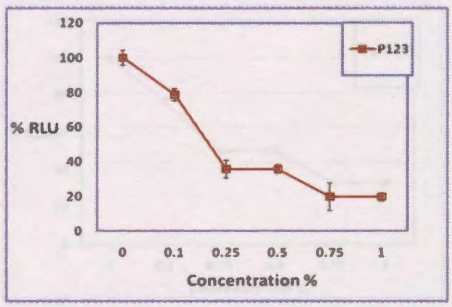
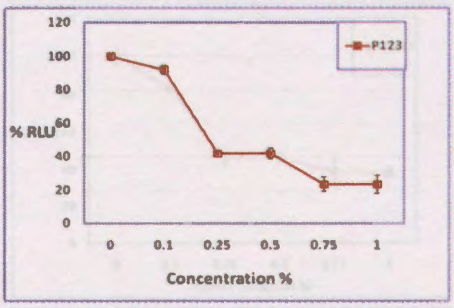
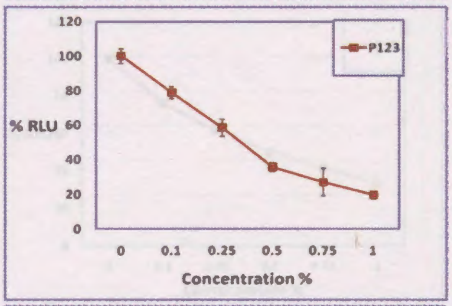
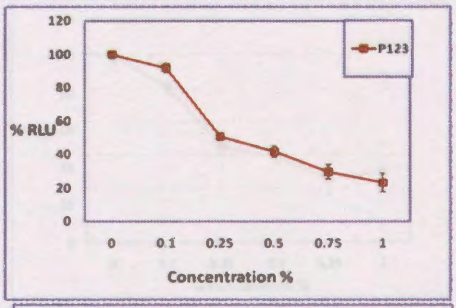
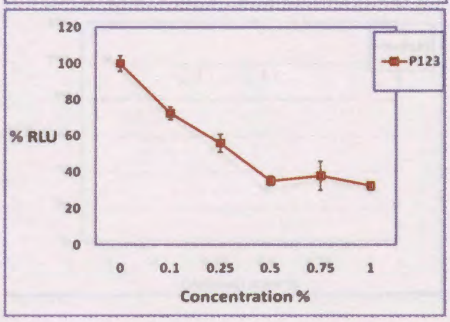
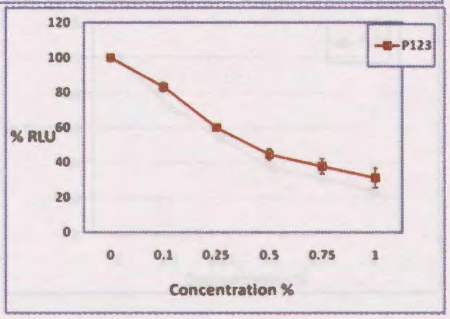
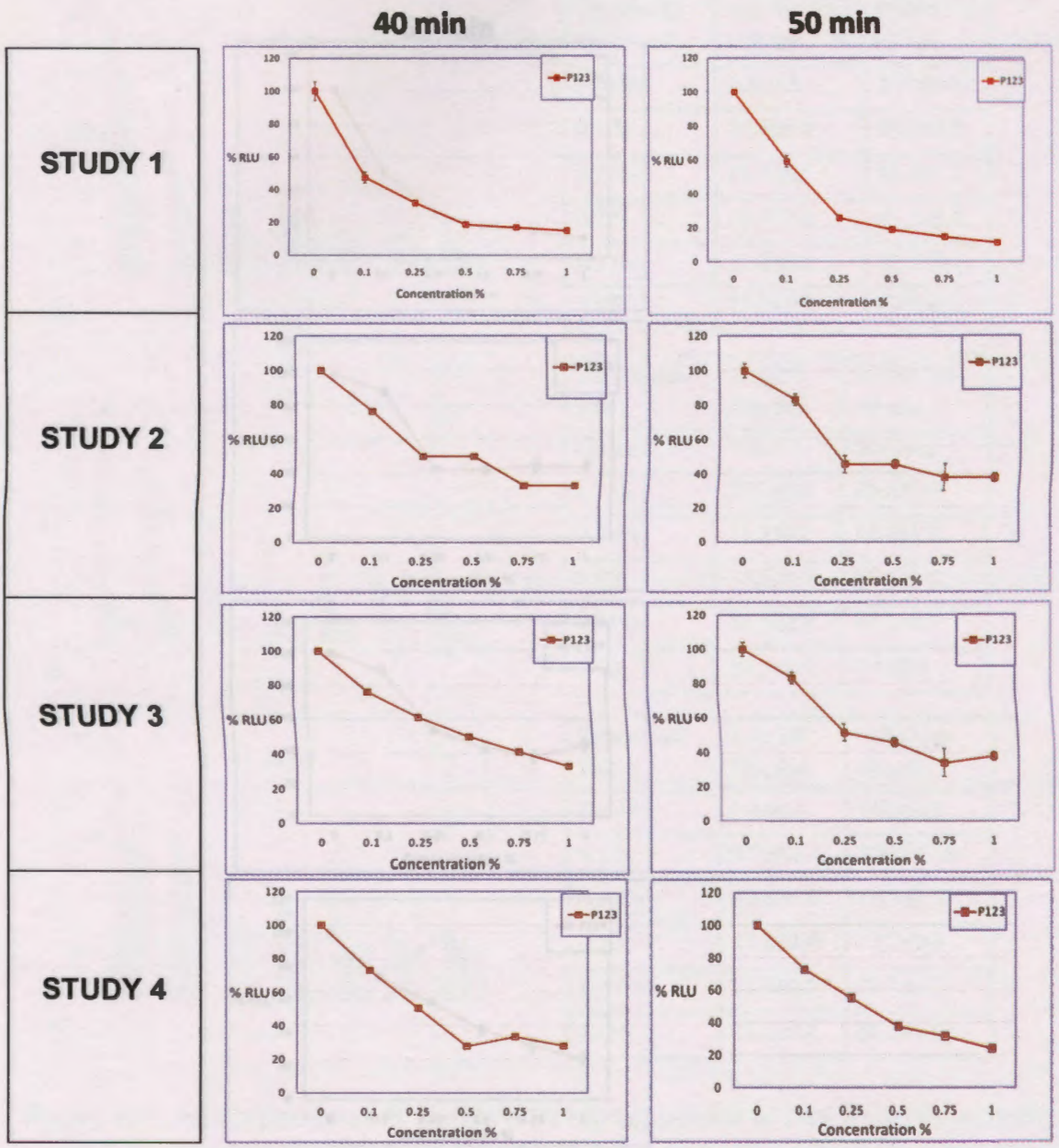
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
STUDY 4
P123
% RLU
Concentration %
0
0.1
0.25
0.5
0.75
1
20
40
60
80
100
120

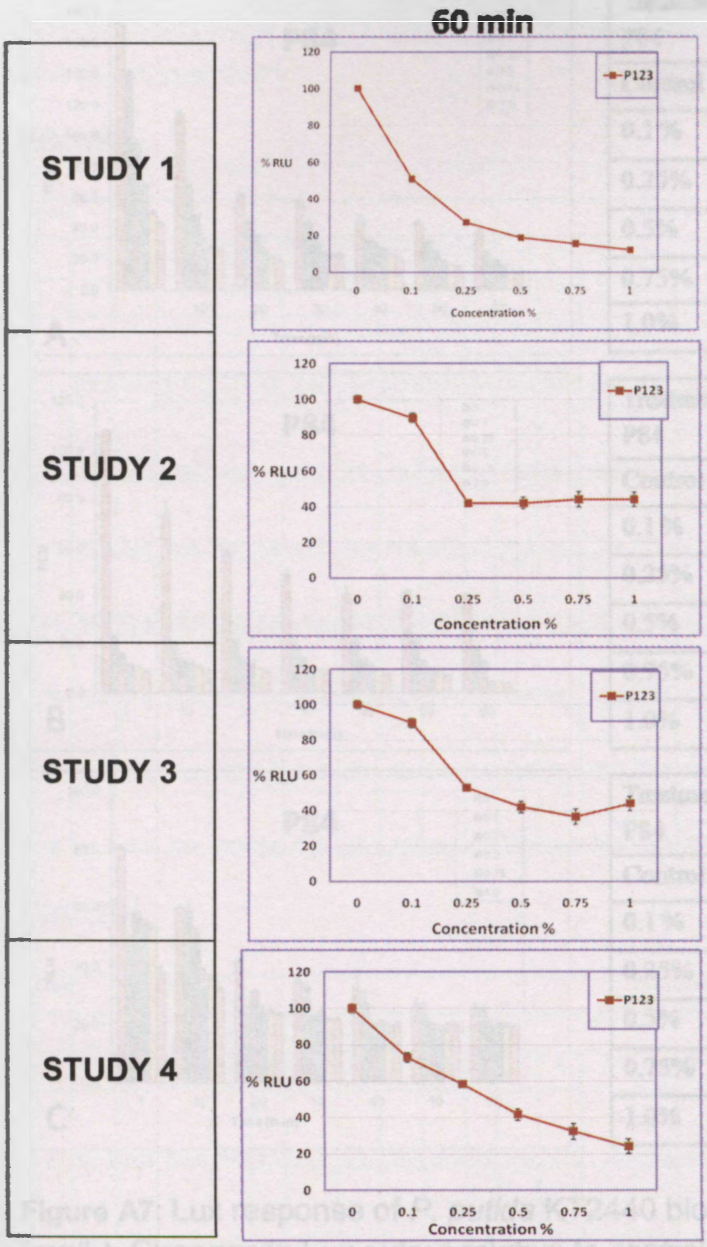
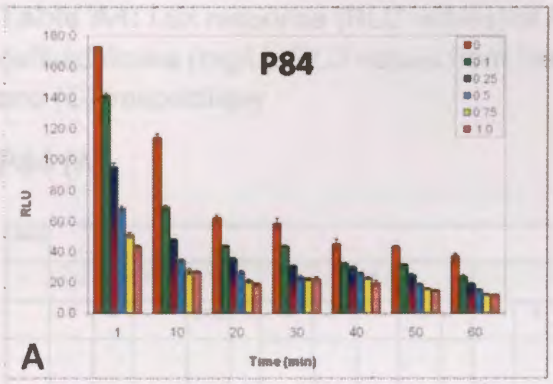
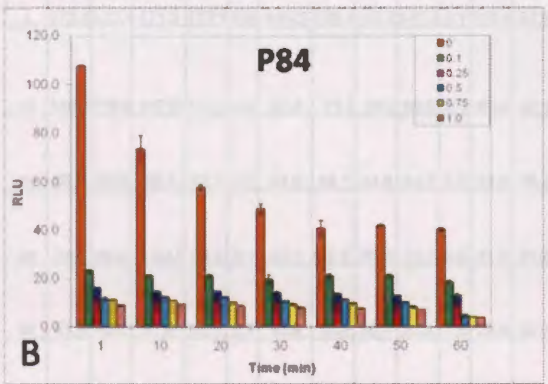


Figure A6: % RLU relative to control for defined concentrations of Pluronics P123 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

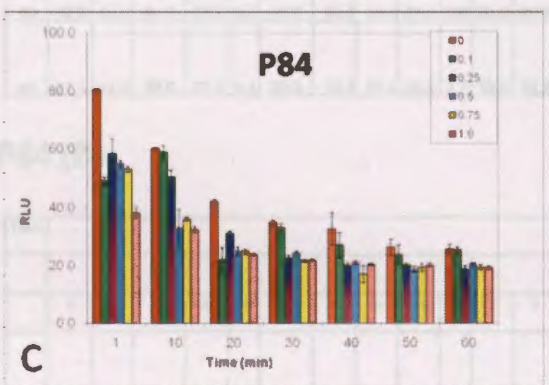
Pluronic P84: RLU vs. Time graph



Treatments P84	Log 10 Cfu./ml	% RLU at 60 min
Control	9.3±0.5	100.0±0.9
0.1 %	10.6±0.1	63.8±1.0
0.25%	11.3±0.5	51.2±1.1
0.5%	12.3±1.6	41.3±0.6
0.75%	13.3±0.3	32.7±0.1
1.0%	13.0±0.6	32.2±0.2



Treatments P84	Log 10 Cfu./ml	% RLU at 60 min
Control	9.0±0.3	100±0.9
0.1 %	10.5±0.1	46.0±1.0
0.25%	11.7±0.2	32.4±1.1
0.5%	13.5±0.7	11.4±0.6
0.75%	13.5±0.3	10.1±0.1
1.0%	12.5±0.4	9.7±0.2



Treatments P84	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.1	100±0.0
0.1 %	8.7±0.2	106.7±7.9
0.25%	10.6±0.6	66.7±2.2
0.5%	12.6±0.4	72.7±0.9
0.75%	11.3±0.5	69.1±1.5
1.0%	10.5±0.2	68.1±1.0

Figure A7: Lux response of *P. putida* KT2440 biosensor to P84 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 1-14-2009, (B) Study 2 performed on 12-23-2008, (C) Study 3 performed on 1-16-2009. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic P84 were performed within 2 months of its receiving from the supplier.

Table A4: Lux response (RLU values) of *P. putida* KT2440 biosensor to P84 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables P84 (A), (B) and (C), respectively

P84 (A)

Time	Concentration %																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	173.0	172.1	171.2	172.1	0.9	144.2	138.3	142.6	141.7	3.1	100.4	93.9	91.4	95.2	4.7	70.7	66.3	69.1	68.7	2.2	53.8	52.4	48.1	51.4	3.0	44.8	44.0	42.2	43.7	1.4
10	119.7	110.9	112.0	114.2	4.8	67.6	71.7	69.2	69.5	2.1	48.9	48.3	46.8	48.0	1.1	35.6	33.7	34.9	34.8	1.0	30.5	24.7	27.4	27.5	2.9	27.2	27.8	26.3	27.1	0.7
20	65.8	62.3	60.1	62.7	2.9	44.6	43.1	44.9	44.2	1.0	34.8	35.2	36.9	35.6	1.1	25.5	26.3	28.8	26.9	1.7	22.6	21.6	19.3	21.2	1.7	20.6	18.6	18.0	19.0	1.3
30	54.8	59.8	62.9	59.2	4.1	43.4	43.7	45.1	44.1	0.9	31.3	31.3	29.7	30.8	0.9	21.4	23.5	25.3	23.4	2.0	20.9	23.3	23.2	22.5	1.4	20.9	22.6	24.5	22.7	1.8
40	41.5	43.8	51.6	45.7	5.3	32.9	30.4	33.4	32.2	1.6	28.8	30.6	30.8	30.1	1.1	25.7	26.9	25.8	26.1	0.6	23.2	23.9	21.8	23.0	1.1	16.7	21.8	21.0	19.9	2.8
50	42.9	44.3	44.0	43.7	0.7	31.5	32.5	31.4	31.8	0.6	25.8	25.9	23.3	25.0	1.5	18.7	20.1	17.9	18.9	1.1	16.1	16.5	15.6	16.1	0.4	14.9	15.5	14.2	14.9	0.7
60	41.1	34.8	36.0	37.3	3.4	25.9	20.7	24.8	23.8	2.7	18.5	18.7	20.2	19.1	0.9	14.5	16.2	15.5	15.4	0.9	12.9	11.7	12.1	12.2	0.6	12.7	11.8	11.5	12.0	0.6

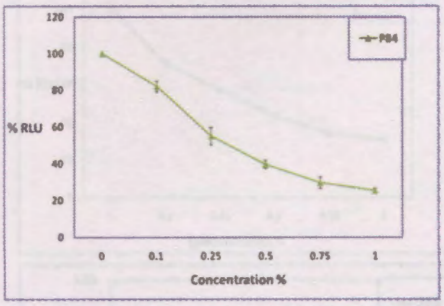
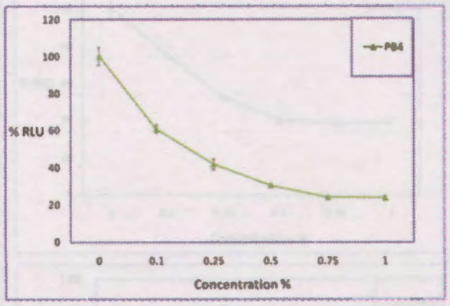
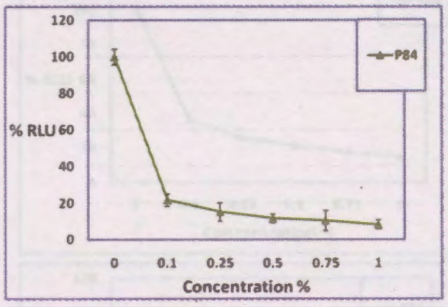
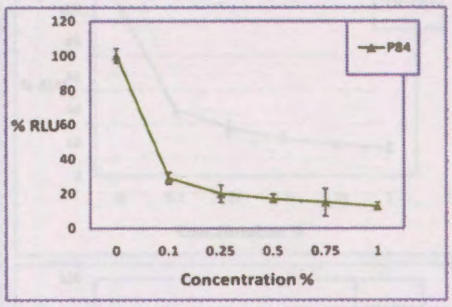
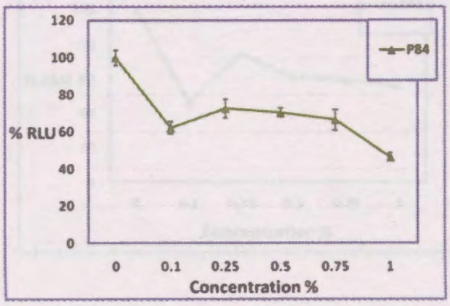
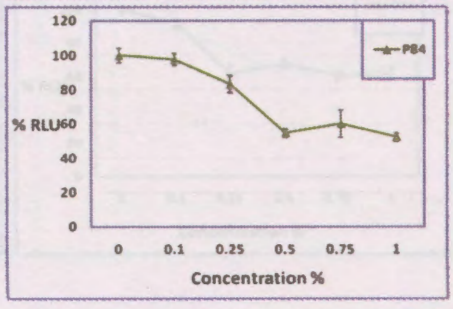
P84 (B)

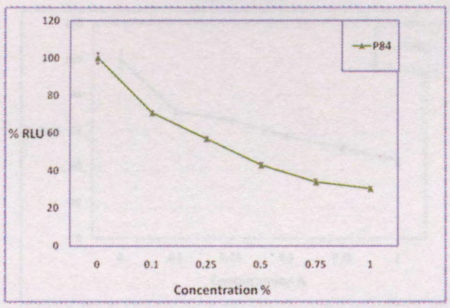
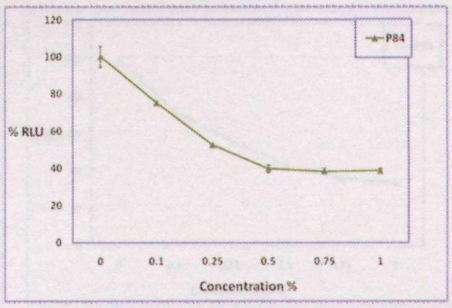
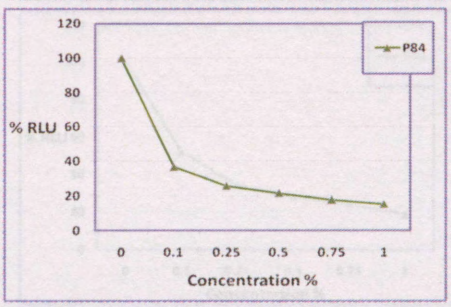
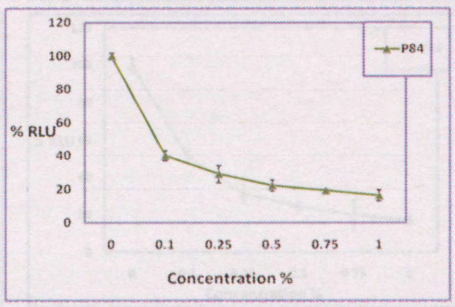
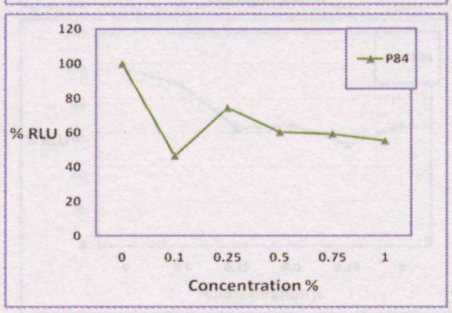
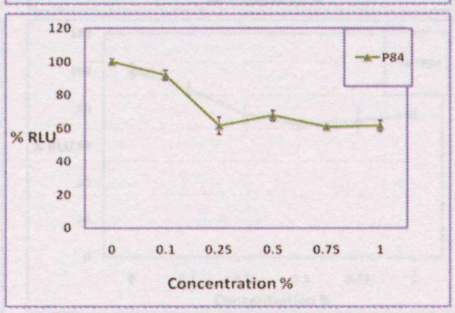
Time	Concentration %																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	107.1	108.0	107.9	107.7	0.5	22.9	22.6	24.2	23.2	0.8	16.4	16.0	16.5	16.3	0.3	12.4	12.2	10.1	11.6	1.3	11.2	11.2	11.3	11.2	0.1	9.4	8.7	8.5	8.9	0.4
10	67.4	84.3	69.0	73.6	9.3	20.7	21.0	21.7	21.2	0.5	14.7	14.3	14.9	14.6	0.3	12.8	12.4	12.1	12.4	0.4	11.0	10.7	11.0	10.9	0.2	8.9	9.3	9.6	9.3	0.3
20	57.3	56.1	59.7	57.7	1.8	21.1	22.0	20.4	21.1	0.8	14.7	15.0	14.8	14.8	0.1	12.5	12.6	12.2	12.4	0.2	10.3	10.1	10.1	10.2	0.2	8.7	8.7	8.9	8.8	0.1
30	45.7	49.2	52.3	49.1	3.3	16.1	20.4	22.5	19.7	3.3	15.0	14.0	13.7	14.2	0.7	10.5	10.9	11.1	10.8	0.3	9.1	9.4	9.6	9.4	0.3	7.9	7.5	8.4	7.9	0.5
40	39.8	35.8	47.1	40.9	5.7	19.3	22.7	21.5	21.2	1.7	13.7	14.0	13.9	13.8	0.2	11.5	11.5	11.3	11.5	0.1	9.9	10.0	9.5	9.8	0.3	7.3	7.9	7.8	7.7	0.3
50	41.3	42.6	41.9	41.9	0.7	21.1	21.2	21.8	21.4	0.3	12.9	13.3	12.9	13.0	0.2	10.2	10.5	10.3	10.3	0.2	8.4	8.7	7.8	8.3	0.4	7.2	7.2	7.2	7.2	0.0
60	40.7	39.3	41.1	40.4	0.9	18.5	19.6	17.7	18.6	1.0	12.0	14.1	13.2	13.1	1.1	4.1	4.6	5.2	4.6	0.6	4.1	4.1	4.2	4.1	0.1	4.1	3.9	3.8	3.9	0.2

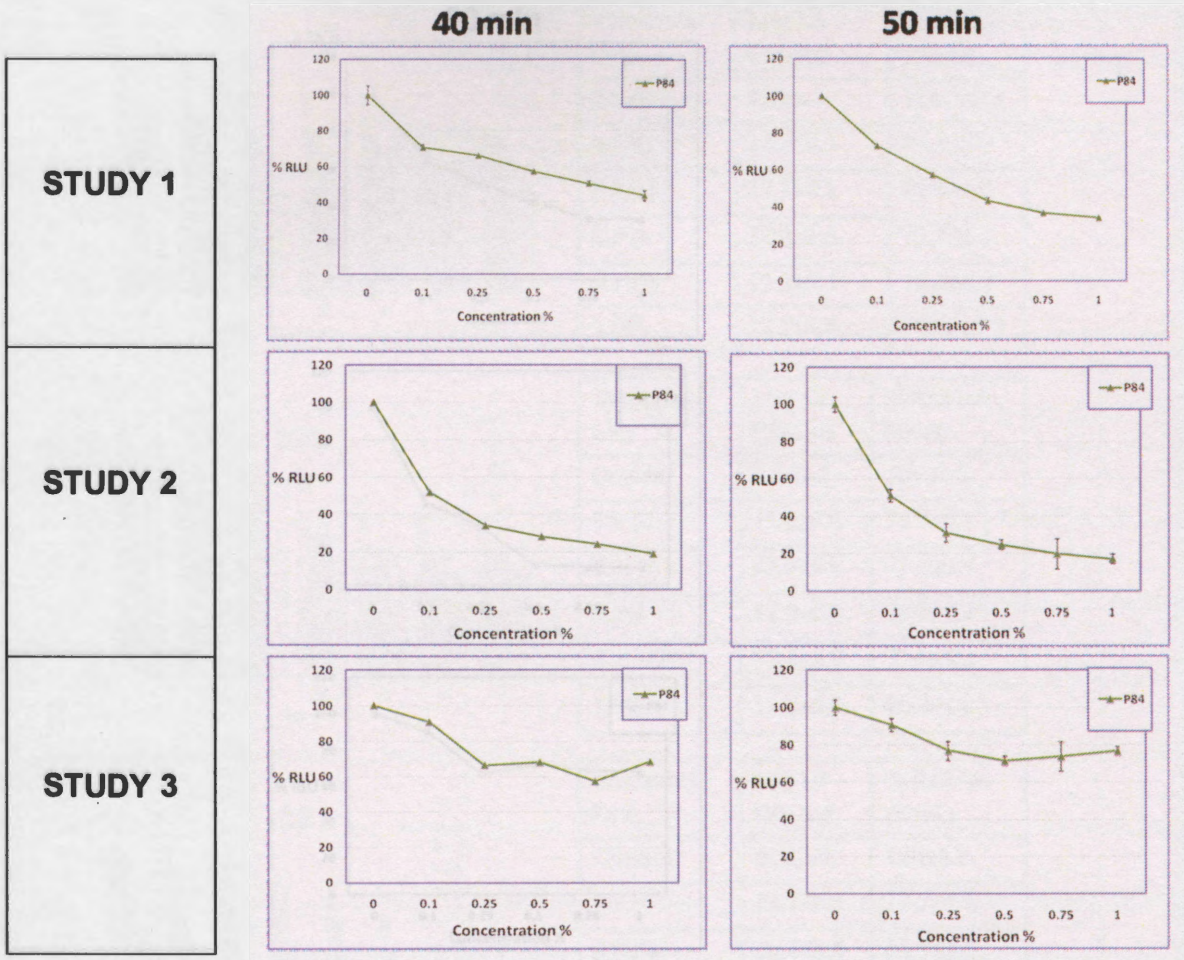
P84 (C)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	80.6	81.2	79.8	80.5	0.7	50.1	50.4	46.5	49.0	2.2	65.7	61.1	48.8	58.5	8.7	56.8	55.4	53.2	55.1	1.7	53.6	54.4	51.1	53.0	1.7	39.8	34.8	40.0	38.2	2.9
10	60.2	60.5	59.9	60.2	0.3	62.3	60.0	54.5	58.9	4.0	54.3	46.1	50.4	50.3	4.1	43.4	33.8	21.7	33.0	10.9	35.8	37.4	35.3	36.2	1.1	31.4	31.7	34.4	32.5	1.7
20	43.5	42.1	40.9	42.2	1.3	25.6	13.5	26.9	22.0	7.4	32.4	31.1	30.0	31.2	1.2	26.2	22.3	26.6	25.0	2.4	25.6	25.9	23.3	24.9	1.4	24.0	24.4	23.3	23.9	0.6
30	36.0	35.4	33.4	35.0	1.4	31.8	31.6	35.9	33.1	2.4	20.6	23.8	23.3	22.6	1.7	24.4	23.4	25.4	24.4	1.0	22.0	21.9	20.9	21.6	0.6	22.2	22.1	21.4	21.9	0.4
40	38.2	21.5	38.3	32.7	9.7	35.7	22.4	23.2	27.1	7.4	18.7	20.9	20.9	20.2	1.2	20.4	20.9	22.2	21.2	1.0	19.5	11.2	20.6	17.1	5.1	20.4	20.9	19.8	20.4	0.6
50	21.5	31.3	26.1	26.3	4.9	30.1	22.3	19.1	23.8	5.7	20.1	19.4	21.1	20.2	0.8	19.9	19.6	16.8	18.8	1.7	19.6	17.0	21.6	19.4	2.3	18.7	21.2	20.5	20.1	1.3
60	28.2	26.4	22.3	25.6	3.0	24.0	27.3	25.1	25.5	1.7	21.3	17.6	17.5	18.8	2.2	20.9	21.0	19.5	20.5	0.9	19.0	21.2	18.2	19.5	1.5	20.4	18.4	18.9	19.2	1.0

Pluronic P84: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
P84
% RLU
Concentration %
0
0.1
0.25
0.5
0.75
1
20
40
60
80
100
120

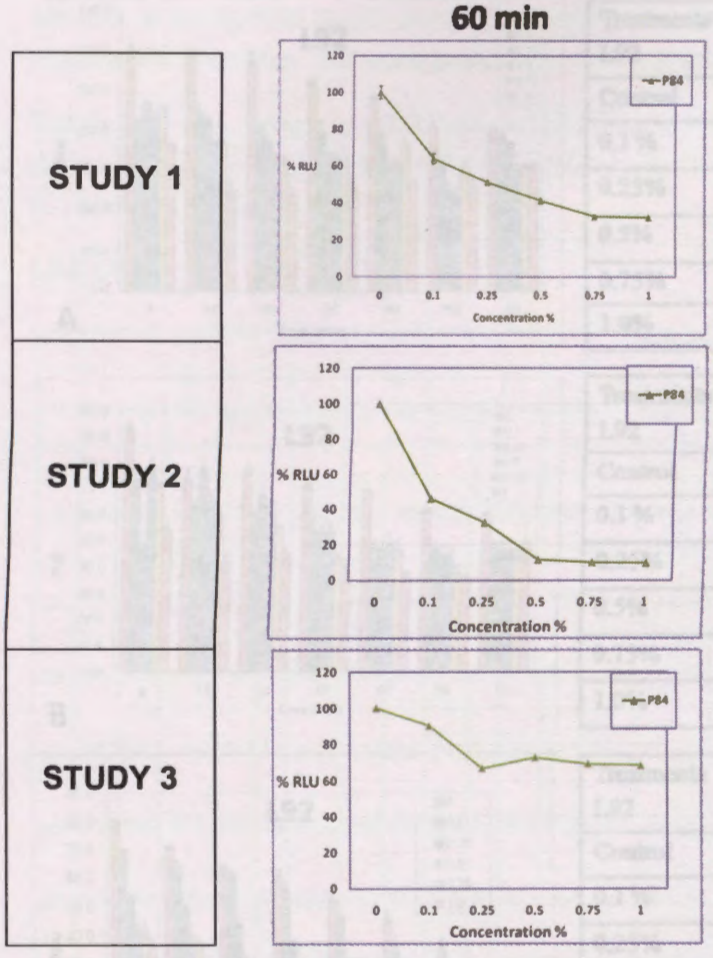
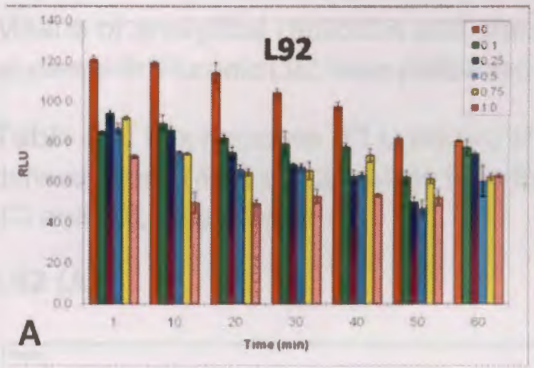
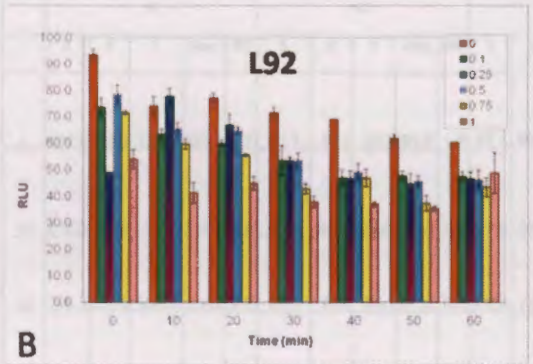


Figure A8: % RLU relative to control for defined concentrations of Pluronics P84 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

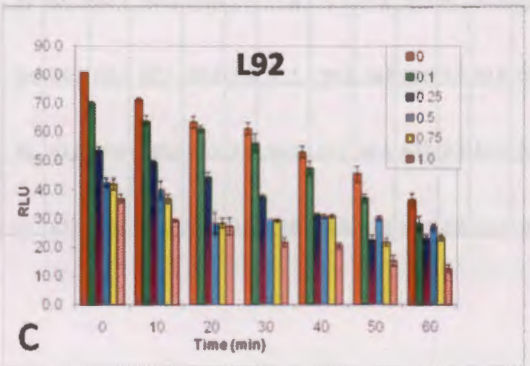
Pluronic L92: RLU vs. Time graph



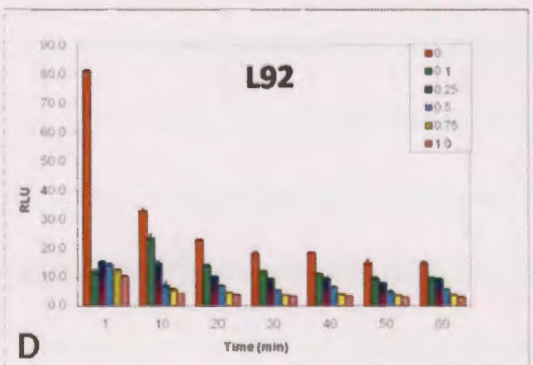
Treatments L92	Log 10 Cfu./ml	% RLU at 60 min
Control	9.3±0.5	100.0±0.9
0.1 %	12.2±0.1	95.7±1.0
0.25%	12.7±0.5	91.8±4.9
0.5%	12.8±1.6	75.3±6.7
0.75%	12.2±0.3	78.0±6.0
1.0%	12.5±0.6	78.6±10.3



Treatments L92	Log 10 Cfu./ml	% RLU at 60 min
Control	9.2±0.1	100±0.0
0.1 %	11.0±0.3	78.7±3.1
0.25%	12.7±0.9	77.0±4.9
0.5%	12.9±0.6	76.5±6.7
0.75%	12.0±0.5	71.9±6.0
1.0%	11.2±0.2	80.9±13.3



Treatments L92	Log 10 Cfu./ml	% RLU at 60 min
Control	9.2±0.8	100±3.9
0.1 %	10.7±0.2	77.7±4.1
0.25%	10.8±0.4	63.5±2.3
0.5%	13.3±0.1	74.9±1.7
0.75%	13.2±0.2	64.6±1.7
1.0%	13.1±0.3	35.1±2.2



Treatments L92	Log 10 Cfu./ml	% RLU at 60 min
Control	9.2±0.0	100±0.7
0.1 %	11.7±0.2	65.3±0.4
0.25%	10.3±0.3	62.7±0.6
0.5%	11.3±0.1	39.3±0.2
0.75%	11.2±0.2	26.7±0.1
1.0%	12.1±0.3	21.3±0.3

Figure A9: Lux response of *P. putida* KT2440 biosensor to L92 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of

treatment are shown. (A) Data from Study 1 performed on 2-25-2009, (B) Study 2 performed on 2-24-2009, (C) Study 3 performed on 2-28-2009, (D) Study 4 performed on 2-21-2009. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic L92 were performed within 2 months of its receiving from the supplier.

Table A5: Lux response (RLU values) of *P. putida* KT2440 biosensor to L92 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables L92 (A), (B), (C) and (D), respectively

L92 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	124.0	118.7	120.6	121.1	2.7	86.1	82.9	85.8	84.6	1.6	92.4	92.2	97.1	93.9	2.8	81.5	90.5	87.8	86.6	4.6	91.9	90.7	93.4	92.0	1.3	71.6	72.5	74.1	72.9	1.3
10	118.0	119.0	121.0	119.3	1.5	97.7	84.0	85.4	89.0	7.6	82.8	89.3	84.8	85.6	3.3	74.8	73.7	75.7	74.8	1.0	73.6	74.8	74.1	74.2	0.6	55.6	39.9	54.8	50.1	8.9
20	119.1	120.2	103.8	114.4	9.2	81.8	78.0	85.1	81.6	3.5	72.0	72.1	80.0	74.7	4.6	69.8	67.4	61.7	66.3	4.2	63.6	63.1	68.2	64.9	2.8	46.9	49.9	52.2	49.7	2.7
30	101.7	102.9	108.5	104.4	3.6	74.8	71.1	91.2	79.0	10.7	70.0	67.7	68.3	68.7	1.2	70.6	65.8	66.2	67.5	2.6	60.4	63.0	74.4	65.9	7.4	53.4	47.7	58.4	53.1	5.4
40	94.1	101.3	97.3	97.6	3.6	81.1	75.8	75.5	77.5	3.1	61.9	68.3	58.4	62.9	5.1	65.5	60.8	64.6	63.6	2.5	72.2	79.4	68.4	73.3	5.6	53.7	55.1	52.4	53.8	1.4
50	81.3	79.8	83.9	81.7	2.1	60.2	59.1	68.5	62.6	5.1	52.2	53.3	44.5	50.0	4.8	41.1	54.5	45.8	47.2	6.8	57.8	62.2	65.8	61.9	4.0	50.1	47.8	59.6	52.5	6.3
60	81.1	80.0	81.0	80.7	0.6	72.0	74.5	84.8	77.0	6.6	74.1	75.7	71.8	73.9	2.0	45.1	69.0	67.7	60.6	13.5	59.5	64.1	64.6	62.8	2.8	64.8	61.8	63.7	63.3	1.6

L92 (B)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	96.1	91.1	92.3	93.1	2.6	78.0	69.3	73.4	73.6	4.3	49.2	49.8	45.4	48.1	2.4	71.4	80.6	83.6	78.5	6.4	70.4	72.1	72.5	71.7	1.1	49.1	52.7	60.8	54.2	6.0
10	80.9	72.0	69.7	74.2	5.9	61.3	67.1	61.5	63.3	3.3	74.4	83.7	75.9	78.0	5.0	63.3	70.1	62.7	65.4	4.1	56.3	60.7	62.6	59.8	3.2	41.1	48.2	35.0	41.4	6.6
20	75.0	76.6	79.5	77.0	2.3	61.0	61.5	57.4	60.0	2.2	66.6	74.2	60.1	67.0	7.0	66.4	66.0	62.3	64.9	2.2	55.1	56.4	55.6	55.7	0.7	43.5	41.8	49.9	45.1	4.3
30	68.9	71.2	74.9	71.7	3.1	50.6	45.3	64.8	53.6	10.1	50.5	55.5	54.4	53.5	2.6	47.6	57.3	55.5	53.5	5.2	45.4	39.2	44.3	42.9	3.3	34.6	37.5	42.0	38.1	3.7
40	69.1	68.7	66.5	68.1	1.4	41.4	51.0	48.7	47.0	5.0	40.8	50.7	48.7	46.7	5.2	43.7	54.7	49.0	49.2	5.5	52.3	47.6	41.2	47.1	5.6	36.3	38.4	38.9	37.2	1.5
50	59.7	61.6	64.5	61.9	2.4	44.2	49.2	49.8	47.7	3.1	41.3	52.2	40.9	44.8	6.4	42.0	45.8	49.3	45.7	3.6	31.8	40.1	40.5	37.5	4.9	35.6	34.0	36.8	35.5	1.4
60	60.5	60.8	60.8	60.7	0.2	45.6	46.0	51.1	47.6	3.1	41.1	47.8	50.7	46.6	4.8	46.3	52.9	39.6	46.3	6.7	38.4	42.0	50.1	43.5	6.0	36.5	47.4	63.0	49.0	13.3

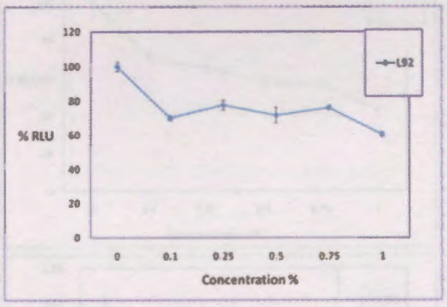
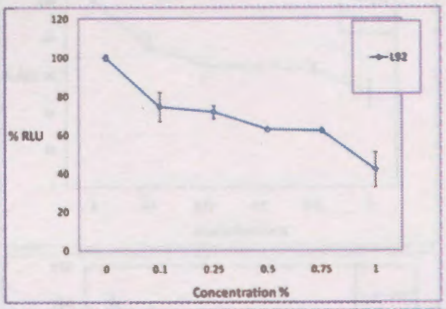
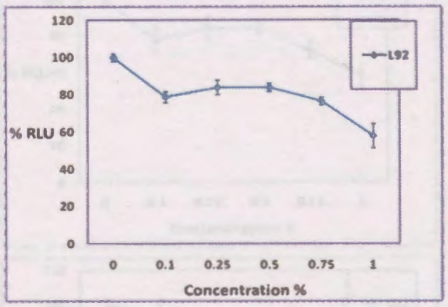
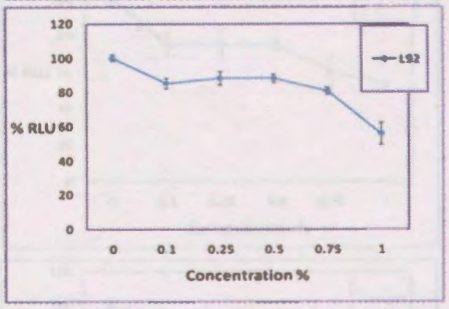
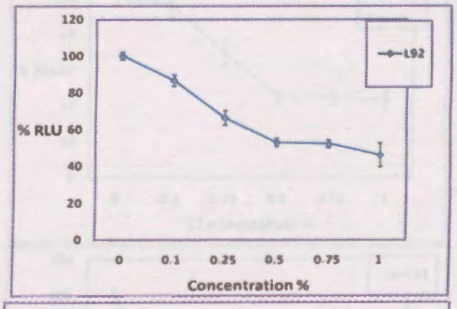
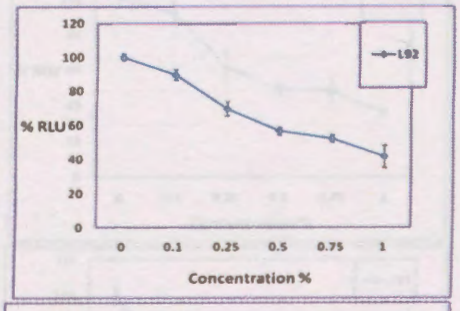
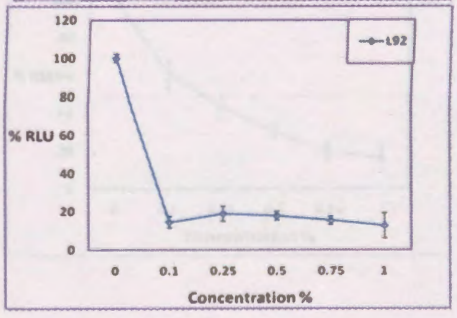
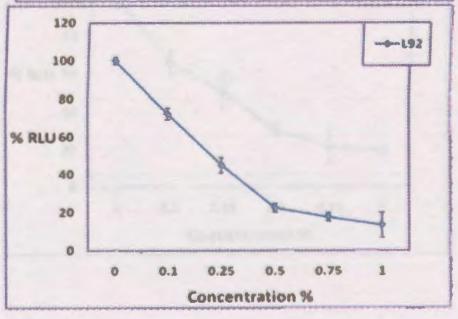
L92 (C)

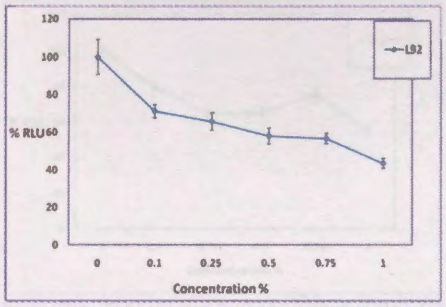
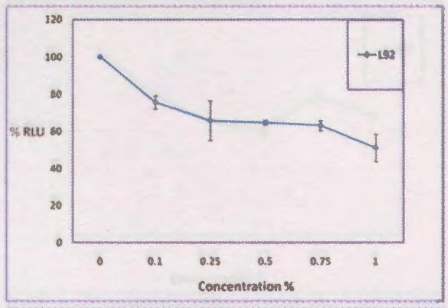
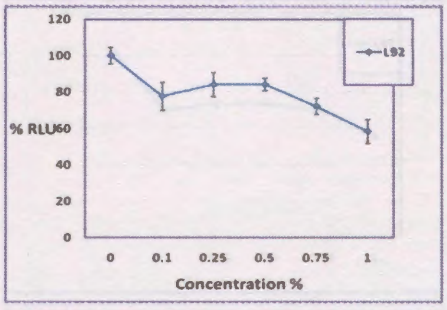
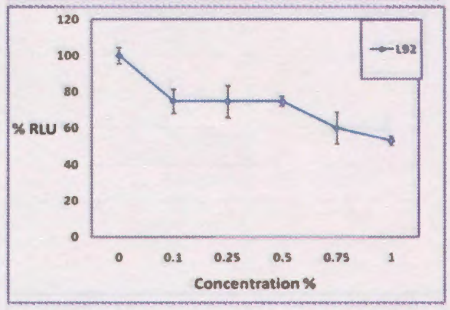
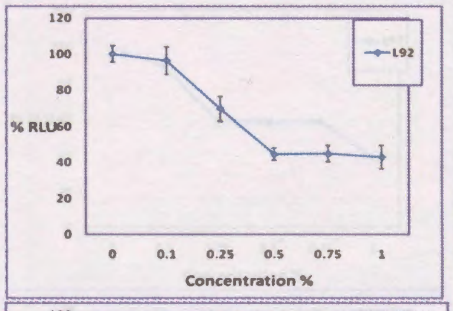
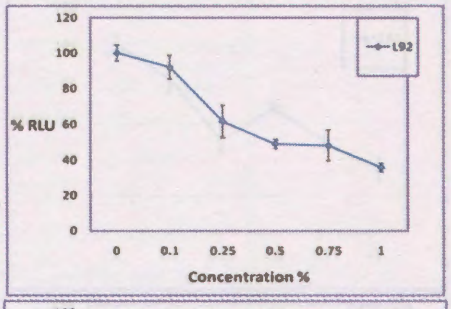
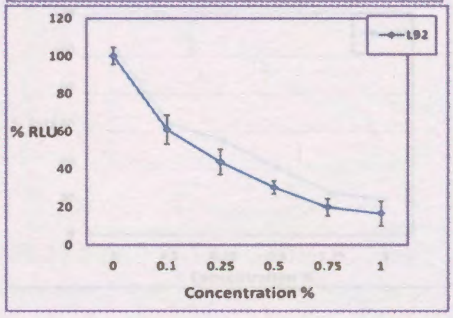
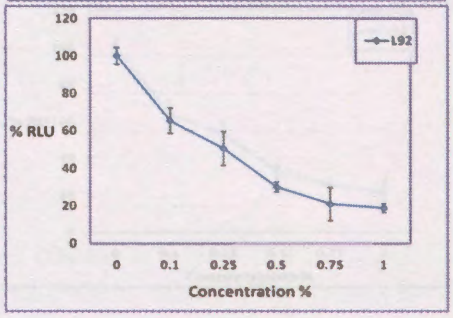
Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	81.0	81.2	80.9	81.0	0.2	69.0	71.2	70.3	70.2	1.1	54.5	55.4	51.0	53.7	2.4	41.0	44.5	43.2	42.9	1.8	44.4	40.1	40.9	41.8	2.3	39.0	35.6	36.5	37.0	1.8
10	72.3	70.5	73.2	72.0	1.4	66.0	65.7	60.2	64.0	3.2	51.0	49.1	49.0	49.7	1.1	34.5	45.1	41.4	40.3	5.4	35.7	40.1	35.7	37.1	2.6	28.9	30.0	30.1	29.7	0.7
20	65.9	61.0	64.3	63.7	2.5	60.0	60.0	63.4	61.1	2.0	45.9	45.9	41.0	44.3	2.8	33.3	31.0	21.0	28.4	6.6	29.0	31.0	25.7	28.5	2.7	30.9	30.0	21.2	27.4	5.3
30	56.9	63.4	63.2	61.2	3.7	62.1	55.5	51.2	56.3	5.5	39.0	38.0	36.4	37.8	1.3	30.0	30.0	28.8	29.6	0.7	30.0	28.9	27.6	28.8	1.2	24.3	22.1	19.1	21.8	2.6
40	56.0	54.3	49.1	53.1	3.6	45.3	44.5	52.3	47.4	4.3	31.0	32.2	31.0	31.4	0.7	30.0	31.0	32.2	31.1	1.1	30.0	31.0	32.2	31.1	1.1	22.2	21.0	18.9	20.7	1.7
50	50.0	45.9	41.0	45.6	4.5	40.0	35.5	36.7	37.4	2.3	25.7	22.4	20.0	22.7	2.8	31.0	31.2	29.0	30.4	1.2	24.4	21.2	20.1	21.9	2.3	18.9	15.4	12.2	15.5	3.3
60	41.0	36.0	33.2	36.7	3.9	33.2	27.0	25.5	28.5	4.1	21.0	25.7	23.3	23.3	2.3	25.7	29.1	27.9	27.5	1.7	22.2	23.5	25.5	23.7	1.7	15.4	12.2	11.1	12.9	2.2

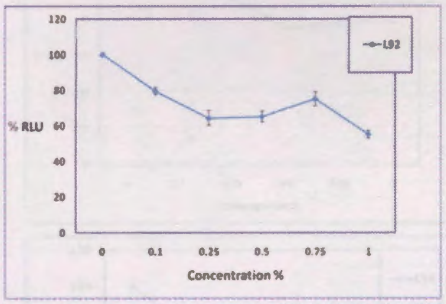
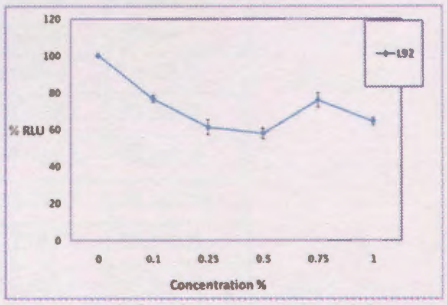
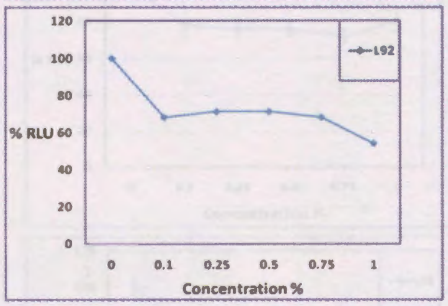
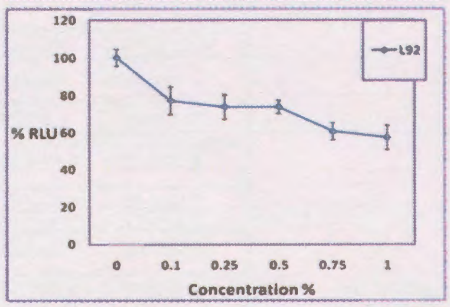
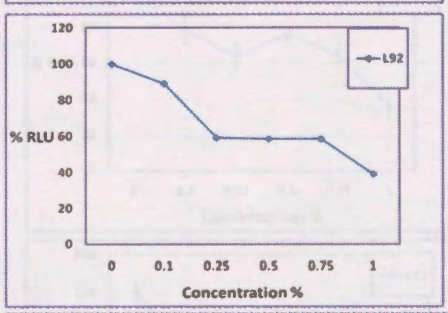
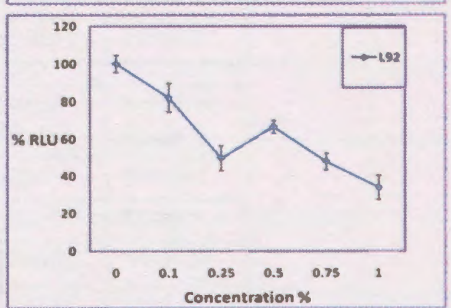
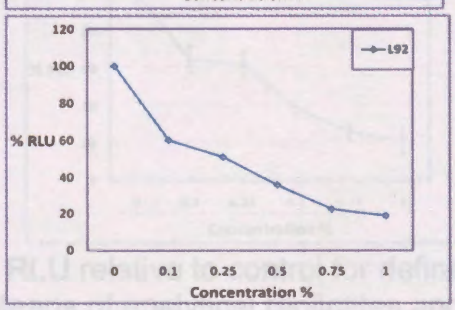
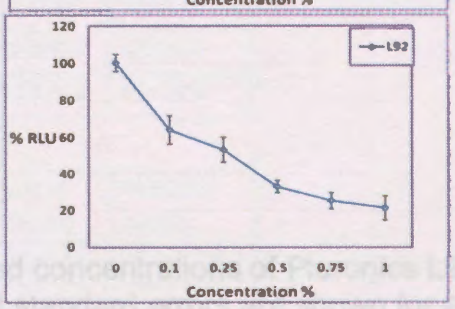
L92 (D)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	81.0	81.0	82.0	81.4	0.6	13.3	11.6	10.4	11.8	1.4	14.6	15.4	16.1	15.4	0.7	13.4	14.5	15.4	14.4	1.0	12.0	12.3	13.2	12.5	0.6	9.8	10.3	10.7	10.3	0.5
10	34.8	31.7	33.2	33.2	1.6	21.8	25.3	24.7	23.9	1.9	16.6	12.8	15.4	14.9	2.0	9.2	7.6	5.6	7.5	1.8	6.4	5.8	5.2	5.8	0.6	4.8	4.4	4.4	4.4	0.1
20	23.9	22.5	23.2	23.2	0.7	14.0	13.8	14.6	14.1	0.4	9.6	10.3	10.6	10.2	0.5	7.2	6.8	7.1	7.0	0.2	4.7	4.6	4.6	4.6	0.1	3.9	3.6	4.1	3.9	0.2
30	17.7	19.5	18.9	18.7	0.9	12.3	12.0	12.3	12.2	0.2	8.8	9.8	9.7	9.4	0.5	5.7	5.5	5.6	5.6	0.1	3.9	4.0	3.9	3.9	0.1	3.4	3.6	3.5	3.5	0.1
40	19.1	18.7	18.4	18.7	0.3	10.9	11.4	11.6	11.3	0.4	8.8	9.2	10.8	9.6	1.1	6.6	7.2	6.4	6.7	0.4	4.3	4.1	4.3	4.2	0.1	3.5	3.6	3.5	3.5	0.0
50	13.8	16.9	14.8	15.2	1.6	10.1	9.0	10.2	9.7	0.7	8.1	8.2	7.9	8.1	0.1	4.3	5.1	5.7	5.1	0.7	3.9	3.7	3.9	3.9	0.1	3.2	3.3	3.2	3.2	0.1
60	15.5	15.4	14.2	15.0	0.7	9.7	10.2	9.5	9.8	0.4	8.7	9.8	9.7	9.4	0.6	5.8	5.8	6.1	5.9	0.2	3.9	4.1	3.9	4.0	0.1	2.9	3.4	3.2	3.2	0.3

Pluronic L92: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

	40 min	50 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

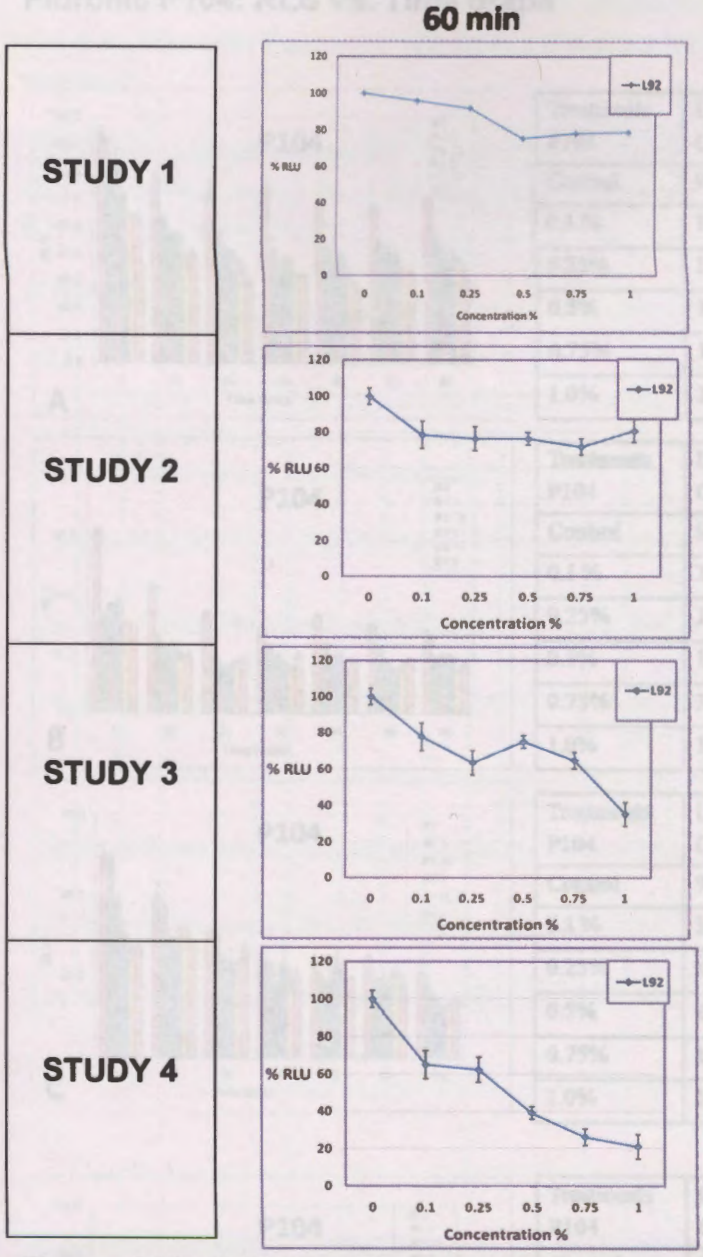
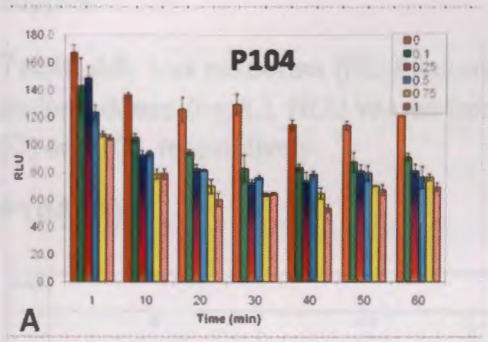
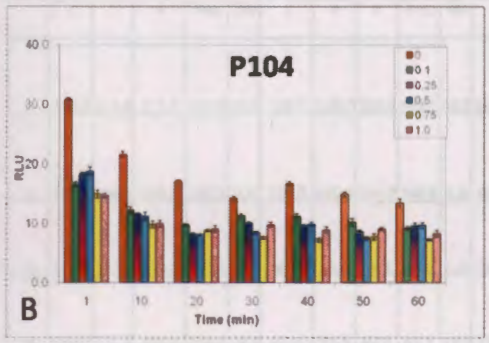


Figure A10: % RLU relative to control for defined concentrations of Pluronics L92 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

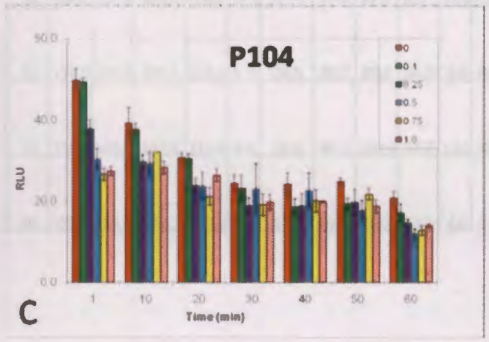
Pluronic P104: RLU vs. Time graph



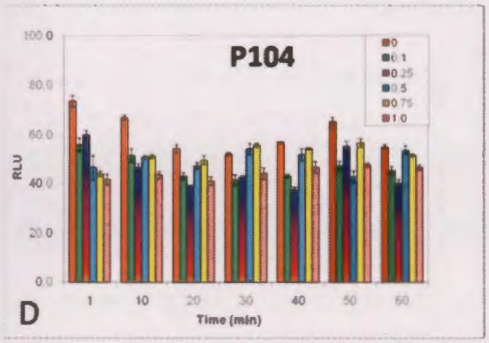
Treatments P104	Log 10 Cfu./ml	% RLU at 60 min
Control	9.4±0.6	100±0.0
0.1 %	11.3±2.3	75.2±4.3
0.25%	11.6±1.3	66.9±8.5
0.5%	12.4±0.3	63.1±13.8
0.75%	12.5±1.2	63.1±4.1
1.0%	12.3±1.3	57.1±5.0



Treatments P104	Log 10 Cfu./ml	% RLU at 60 min
Control	9.8±0.1	100±0.8
0.1 %	11.9±0.4	65.2±0.5
0.25%	12.5±0.4	68.1±0.9
0.5%	12.2±0.7	69.5±0.7
0.75%	11.7±0.4	51.8±0.3
1.0%	11.5±0.6	58.9±0.9



Treatments P104	Log 10 Cfu./ml	% RLU at 60 min
Control	9.3±0.1	100±2.7
0.1 %	10.1±0.3	82.7±3.7
0.25%	12.4±0.2	70.6±1.6
0.5%	10.6±0.6	57.7±2.3
0.75%	11.0±0.3	62.0±2.0
1.0%	12.4±0.2	67.8±0.8



Treatments P104	Log 10 Cfu./ml	% RLU at 60 min
Control	9.3±0.1	100±2.7
0.1 %	11.2±0.2	81.8±3.7
0.25%	11.7±0.2	72.4±1.6
0.5%	12.4±0.4	96.9±2.3
0.75%	12.1±0.3	93.3±2.0
1.0%	11.8±0.2	83.9±0.8

Figure A11: Lux response of *P. putida* KT2440 biosensor to P104 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 2-11-2009, (B) Study 2 performed on 1-26-2008, (C) Study 3 performed on 7-8-2008, (D) Study 4 performed on 2-12-

2009. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic P104 were performed within 2 months of its receiving from the supplier.

Table A6: Lux response (RLU values) of *P. putida* KT2440 biosensor to P104 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables P104 (A), (B), (C) and (D), respectively

P104 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	155.7	174.5	171.7	167.3	10.1	102.7	165.7	161.0	143.1	35.1	141.5	165.3	139.7	148.8	14.3	134.1	122.2	115.5	123.9	9.4	104.1	111.1	109.1	108.1	3.6	105.8	102.1	108.3	105.4	3.1
10	138.3	139.1	133.8	137.1	2.8	100.6	110.3	106.0	105.6	4.9	92.9	98.6	87.1	92.9	5.7	92.5	97.1	94.7	94.8	2.3	72.4	82.8	82.1	79.1	5.8	86.6	77.6	73.7	79.3	6.6
20	114.8	143.8	119.8	126.1	15.5	98.0	92.0	95.1	95.0	3.0	76.6	88.2	83.6	82.8	5.8	81.2	81.6	83.0	81.9	1.0	63.4	80.3	66.4	70.0	9.0	62.7	51.0	66.6	60.1	8.1
30	114.8	137.5	136.8	129.7	12.9	64.1	97.2	87.9	83.1	17.1	77.1	68.7	71.8	72.5	4.3	78.0	73.9	77.5	76.5	2.2	64.3	61.7	65.7	63.9	2.0	63.7	64.9	66.0	64.8	1.1
40	118.5	118.6	108.1	115.1	6.0	79.5	85.7	86.8	84.0	3.9	63.4	72.4	86.0	73.9	11.4	82.6	80.1	75.0	79.2	3.8	57.0	70.7	67.2	64.9	7.1	48.7	56.8	56.9	54.1	4.7
50	110.6	120.5	112.0	114.4	5.4	75.5	87.3	100.5	87.8	12.5	90.3	76.5	77.8	81.6	7.6	81.3	69.4	88.5	79.7	9.6	70.4	70.4	70.8	70.5	0.2	60.4	71.6	70.8	67.6	6.2
60	121.6	120.9	122.2	121.6	0.7	87.8	96.2	90.7	91.5	4.3	73.5	90.4	80.3	81.4	8.5	92.4	71.5	66.4	76.8	13.8	72.1	79.8	78.4	76.8	4.1	63.7	71.9	72.8	69.5	5.0

P104 (B)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	31.0	30.3	31.2	30.9	0.5	16.1	16.4	17.5	16.7	0.7	17.9	18.9	18.5	18.4	0.5	17.4	19.8	19.4	18.9	1.3	13.8	15.9	15.4	15.0	1.1	14.2	15.4	14.6	14.7	0.6
10	21.5	20.8	22.5	21.6	0.8	13.3	12.3	11.4	12.3	0.9	11.3	12.0	11.5	11.6	0.3	10.3	12.6	10.9	11.3	1.2	10.8	10.0	8.8	9.9	1.0	9.2	9.7	11.0	10.0	0.9
20	17.1	17.5	16.9	17.2	0.3	9.5	10.0	9.7	9.7	0.2	7.8	8.6	8.2	8.2	0.4	7.9	8.1	8.3	8.1	0.2	8.6	9.2	8.7	8.8	0.3	9.0	8.2	10.1	9.1	0.9
30	14.0	13.9	15.0	14.3	0.6	11.7	10.5	11.7	11.3	0.7	10.2	10.3	9.7	10.1	0.3	8.5	8.3	8.7	8.5	0.2	7.2	8.0	7.6	7.6	0.4	10.6	9.4	9.6	9.9	0.7
40	17.5	16.0	16.8	16.8	0.7	11.4	10.5	12.0	11.3	0.8	9.9	9.9	9.1	9.6	0.4	9.3	10.2	10.3	9.9	0.6	6.6	7.5	7.6	7.2	0.6	8.9	9.8	8.2	9.0	0.8
50	15.1	14.2	15.6	15.0	0.7	9.4	9.9	11.5	10.3	1.1	8.3	8.6	9.2	8.7	0.4	7.1	7.6	7.9	7.5	0.4	6.8	7.9	8.6	7.8	0.9	8.7	8.9	9.6	9.1	0.4
60	12.3	14.7	13.5	13.5	1.2	8.7	9.7	9.3	9.2	0.5	8.8	9.5	10.6	9.6	0.9	10.2	8.9	10.2	9.8	0.7	7.0	7.5	7.3	7.3	0.3	8.6	9.0	7.3	8.3	0.9

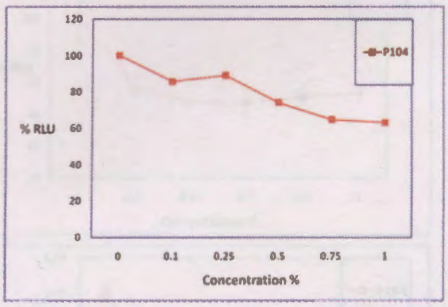
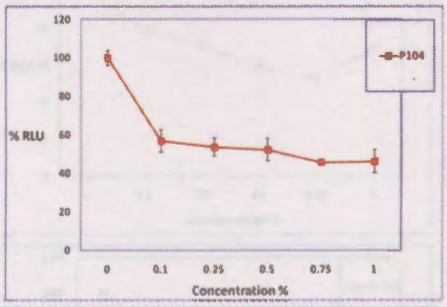
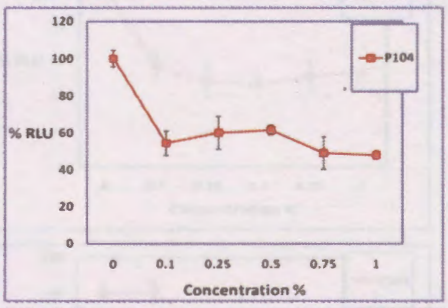
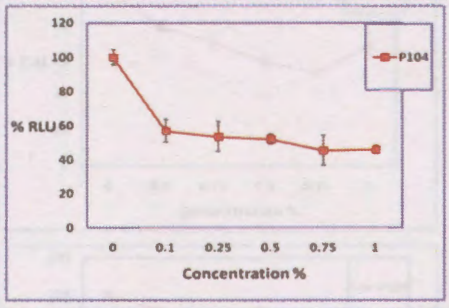
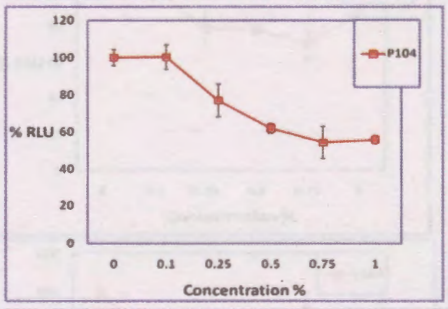
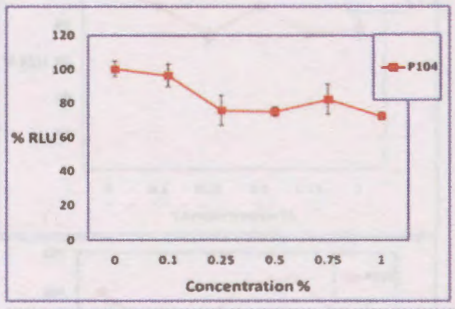
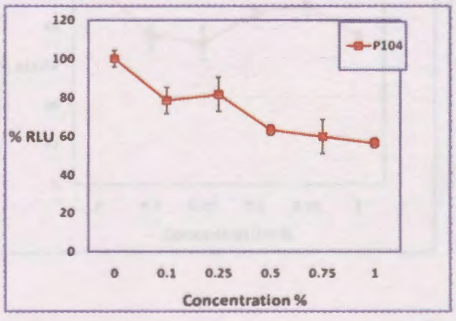
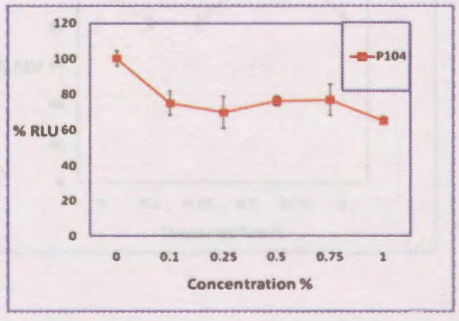
P104 (C)

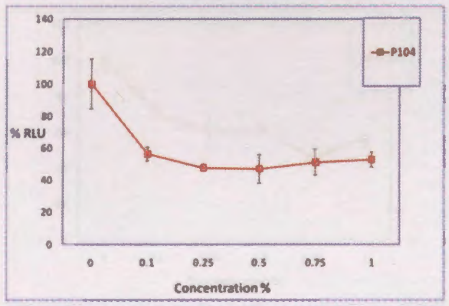
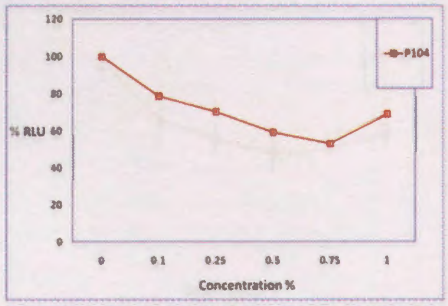
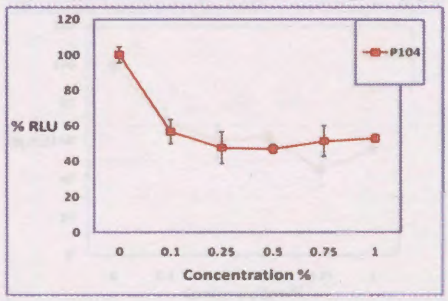
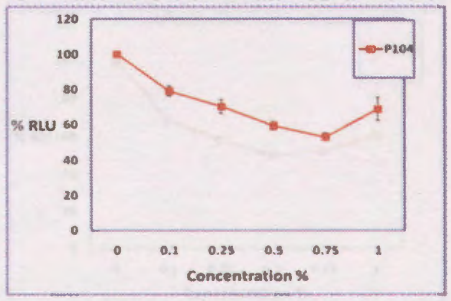
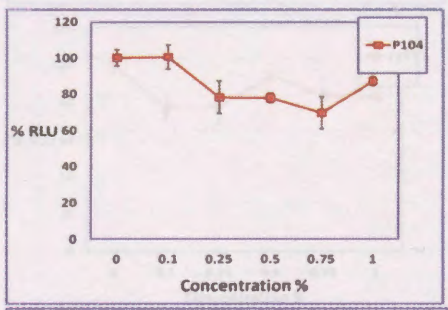
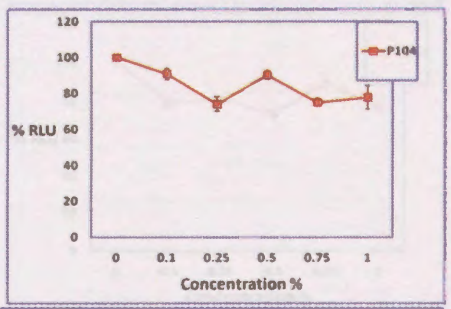
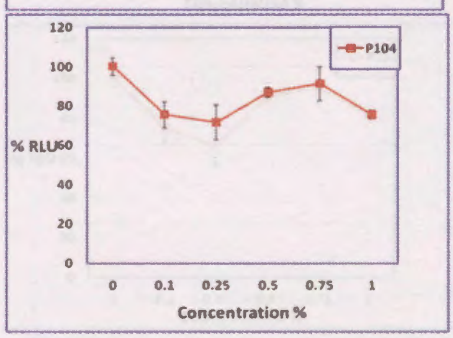
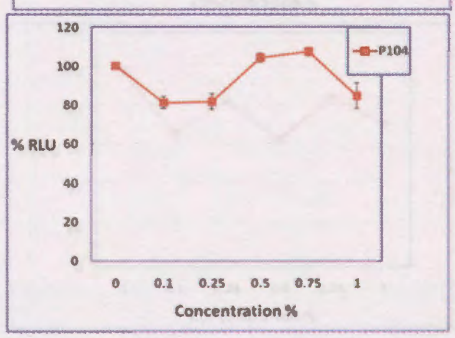
Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	50.8	54.1	44.2	49.7	5.1	49.0	47.9	51.1	49.3	1.6	42.2	35.6	35.4	37.8	3.8	25.4	33.7	31.9	30.3	4.4	23.7	29.0	27.1	26.6	2.7	27.4	29.1	25.5	27.3	1.8
10	39.2	32.2	45.9	39.1	6.8	39.9	38.0	35.0	37.6	2.5	26.5	33.0	29.3	29.6	3.2	25.5	27.3	35.0	29.3	5.0	32.1	32.1	32.0	32.1	0.1	31.4	27.4	26.1	28.3	2.8
20	28.2	32.4	31.2	30.6	2.2	31.9	31.4	28.1	30.5	2.1	23.7	27.0	20.6	23.8	3.2	18.5	22.7	29.7	23.7	5.7	16.9	24.1	22.4	21.2	3.8	29.4	23.6	26.3	26.4	2.9
30	22.2	22.0	28.9	24.4	3.9	19.9	19.9	29.6	23.1	5.6	16.3	17.6	22.8	18.9	3.4	17.2	16.2	35.6	23.0	10.9	14.2	19.9	23.2	19.1	4.6	17.6	18.5	23.4	19.8	3.1
40	28.8	24.3	19.3	24.1	4.8	14.4	19.9	21.3	18.5	3.7	12.6	21.4	22.4	18.8	5.4	31.2	17.9	18.7	22.6	7.5	15.4	20.1	24.9	20.1	4.7	20.2	20.0	19.6	19.9	0.3
50	25.4	26.0	22.9	24.8	1.7	16.8	21.6	19.8	19.4	2.4	23.8	22.0	13.2	19.7	5.7	14.9	22.3	16.4	17.9	3.9	20.4	24.5	20.3	21.7	2.4	15.7	19.5	21.1	18.8	2.8
60	23.4	21.0	18.0	20.8	2.7	18.3	13.1	20.3	17.2	3.7	13.7	16.5	13.8	14.7	1.6	11.6	9.8	14.4	12.0	2.3	13.5	14.5	10.7	12.9	2.0	14.0	13.3	14.8	14.1	0.8

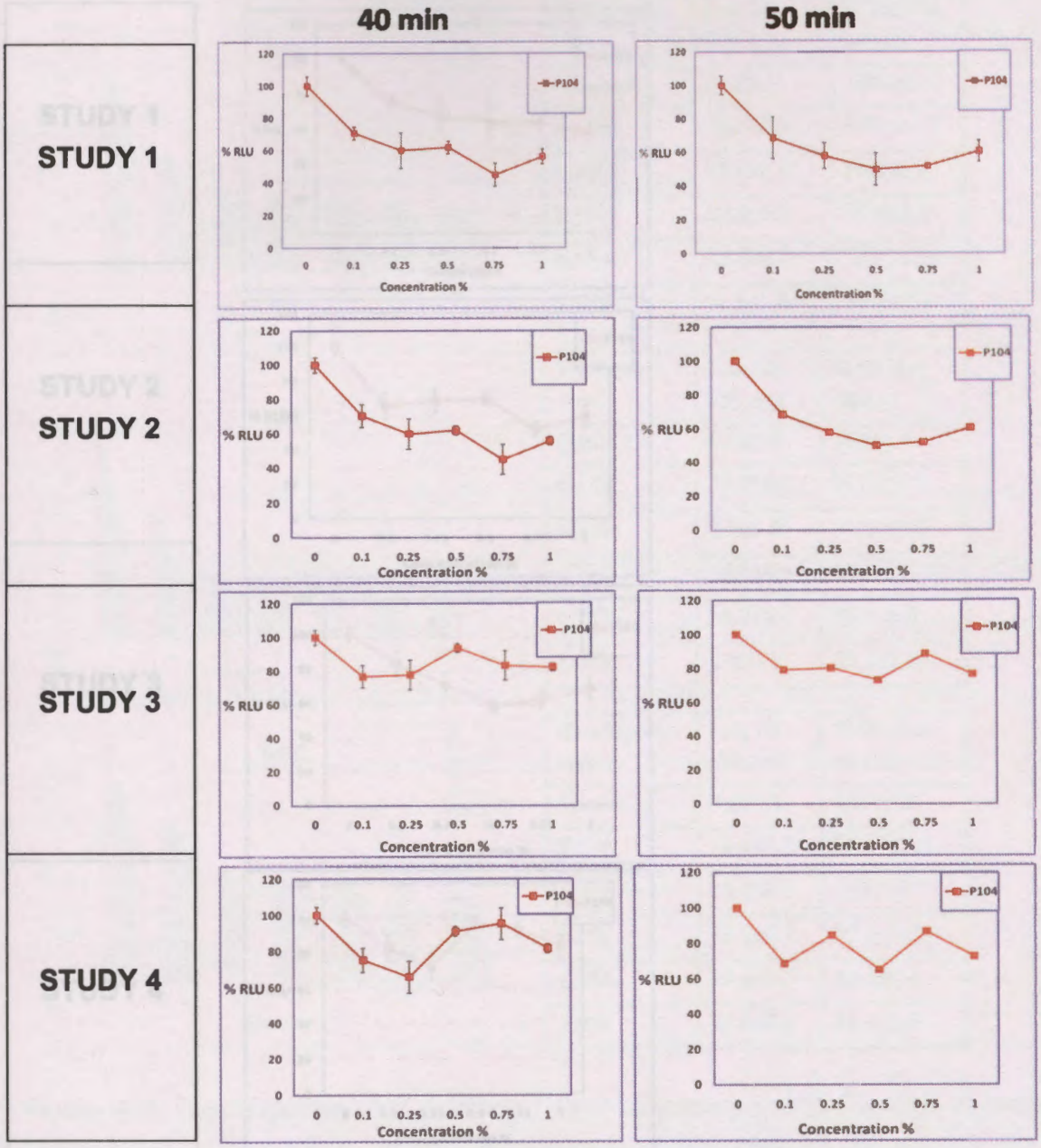
P104 (D)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	78.0	71.7	71.3	73.7	3.8	51.2	55.5	61.2	56.0	5.0	58.0	59.1	63.3	60.1	2.8	55.8	38.3	45.7	46.6	8.8	41.9	44.7	45.6	44.1	1.9	44.1	43.6	37.2	41.6	3.8
10	66.1	65.6	69.0	66.9	1.8	46.4	52.5	55.9	51.6	4.8	49.4	43.8	46.8	46.7	2.8	49.9	52.4	50.6	51.0	1.3	50.2	51.1	52.9	51.4	1.3	45.0	40.5	46.0	43.5	2.6
20	55.2	51.3	56.9	54.5	2.9	41.8	45.9	41.1	42.9	2.6	39.0	39.7	38.3	39.0	0.7	45.2	49.5	47.3	47.3	2.2	47.7	48.0	53.4	49.7	3.2	38.0	42.1	43.2	41.1	2.7
30	53.3	52.3	51.1	52.2	1.1	37.0	43.9	43.5	41.5	3.9	41.4	42.8	43.6	42.6	1.1	53.7	50.9	58.6	54.4	3.9	56.2	57.4	54.4	56.0	1.5	44.6	47.8	40.0	44.2	3.9
40	57.0	57.2	57.2	57.2	0.1	43.0	41.8	44.4	43.1	1.3	35.8	38.7	38.4	37.6	1.6	53.3	47.5	55.4	52.1	4.1	55.0	54.1	54.4	54.5	0.5	46.1	42.8	51.2	46.7	4.2
50	63.2	64.4	68.9	65.5	3.0	50.5	43.7	47.7	47.3	3.4	51.4	58.0	57.1	55.5	3.6	40.0	40.7	47.6	42.7	4.2	60.2	54.4	56.1	56.9	3.0	48.4	48.7	46.1	47.7	1.5
60	53.9	57.1	54.4	55.2	1.7	42.2	46.7	47.4	45.4	2.8	39.2	38.4	43.1	40.2	2.5	56.8	54.3	50.4	53.8	3.2	50.8	51.5	52.9	51.8	1.1	46.6	48.1	45.0	46.6	1.5

Pluroic P104: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
STUDY 4
P104
% RLU
Concentration %
0
0.1
0.25
0.5
0.75
1
20
40
60
80
100
120

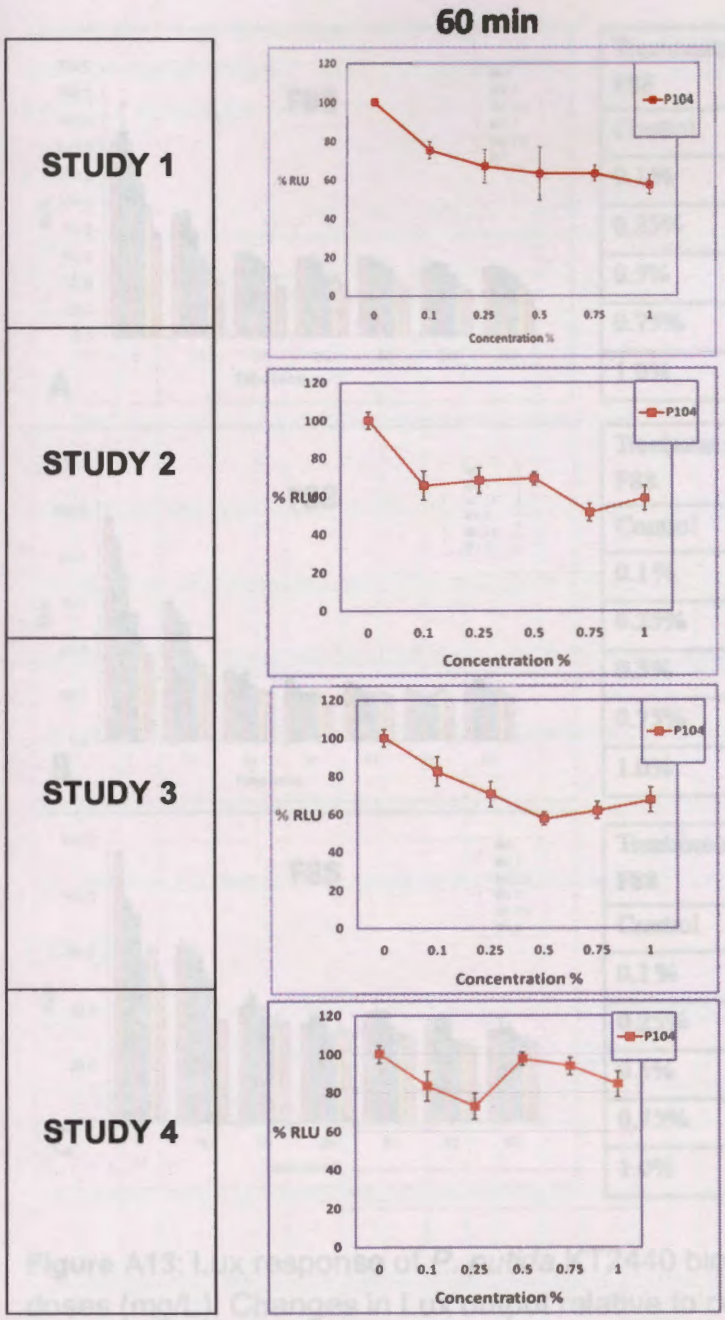
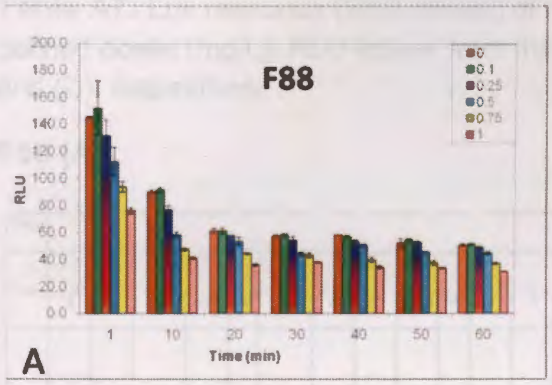
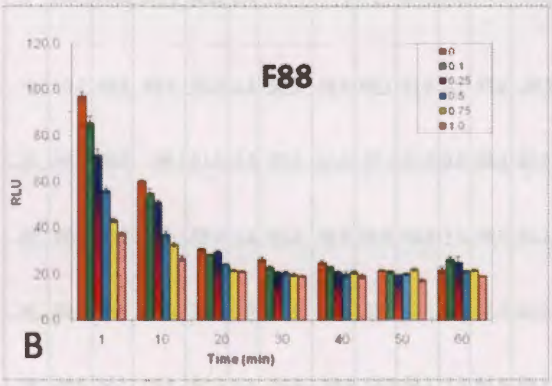


Figure A12: % RLU relative to control for defined concentrations of Pluronics P104 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

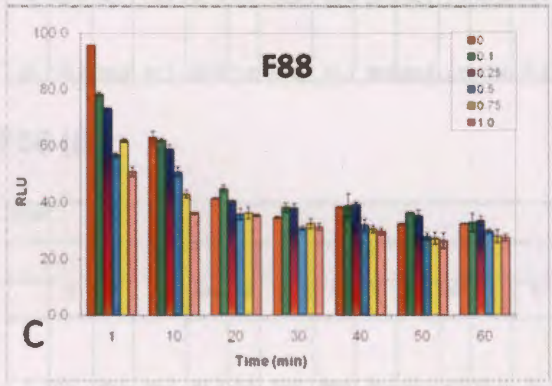
Pluronic F88: RLU vs. Time graph



Treatments F88	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.1	100.0±1.5
0.1 %	10.5±0.2	101.4±1.1
0.25%	10.2±0.5	96.2±1.3
0.5%	12.4±0.9	87.9±1.2
0.75%	12.2±0.8	73.0±1.1
1.0%	11.8±1.0	61.4±0.5



Treatments F88	Log 10 Cfu./ml	% RLU at 60 min
Control	9.0±0.8	100±0.9
0.1 %	10.7±0.3	117.1±1.1
0.25%	9.5±1.0	110.9±0.6
0.5%	10.6±0.6	92.5±1.1
0.75%	10.7±0.4	96.1±0.3
1.0%	9.7±0.9	85.1±1.0



Treatments F88	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.1	100±0.0
0.1 %	10.4±0.3	101.3±5.4
0.25%	10.6±0.4	103.2±2.7
0.5%	13.1±0.3	92.1±0.8
0.75%	12.8±0.5	86.5±3.8
1.0%	12.6±0.2	84.9±2.0

Figure A13: Lux response of *P. putida* KT2440 biosensor to F88 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 1-7-2009, (B) Study 2 performed on 1-6-2009, (C) Study 3 performed on 1-9-2009. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic F88 were performed within 2 months of its receiving from the supplier.

Table A7: Lux response (RLU values) of *P. putida* KT2440 biosensor to F88 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables F88 (A), (B) and (C), respectively

F88 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	146.1	144.9	145.3	145.5	0.6	111.1	171.9	172.7	151.9	35.3	110.5	150.2	134.3	131.7	20.0	92.2	117.0	128.3	112.5	18.5	91.0	101.3	89.5	93.9	6.4	77.1	72.7	79.7	76.5	3.6
10	93.0	89.4	88.6	90.3	2.3	90.7	89.8	95.1	91.9	2.9	82.2	76.3	72.4	77.0	4.9	61.7	59.0	55.0	58.6	3.4	47.1	46.9	49.1	47.7	1.2	41.7	40.1	42.5	41.4	1.2
20	60.5	65.0	59.1	61.5	3.1	63.5	64.0	57.1	61.5	3.9	58.2	57.8	57.9	58.0	0.2	50.4	51.2	59.5	53.7	5.0	45.0	45.6	43.1	44.6	1.3	37.5	34.8	36.4	36.2	1.3
30	58.4	57.1	59.5	58.3	1.2	59.8	58.0	58.0	58.6	1.1	58.6	50.1	55.0	54.6	4.3	43.2	44.1	46.3	44.5	1.6	40.2	44.2	45.9	43.4	2.9	38.9	37.5	38.4	38.3	0.7
40	58.5	58.5	57.6	58.2	0.5	59.0	56.5	55.9	57.1	1.6	51.2	54.6	54.8	53.5	2.0	49.5	50.6	51.9	50.7	1.2	36.9	41.6	41.5	40.0	2.7	34.3	36.7	32.6	34.6	2.0
50	46.6	54.7	55.8	52.3	5.0	54.8	56.0	53.3	54.7	1.3	51.9	54.1	52.8	53.0	1.1	45.4	45.2	46.4	45.7	0.7	39.5	39.9	34.3	37.9	3.1	32.9	34.3	34.9	34.0	1.0
60	49.7	50.0	51.9	50.5	1.2	50.2	52.1	52.3	51.5	1.1	47.4	49.4	49.9	48.9	1.3	43.4	45.7	44.9	44.7	1.2	35.9	38.1	37.3	37.1	1.1	31.1	30.7	31.6	31.2	0.5

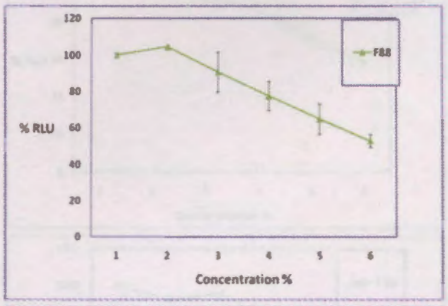
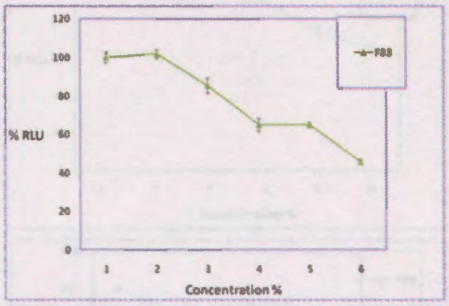
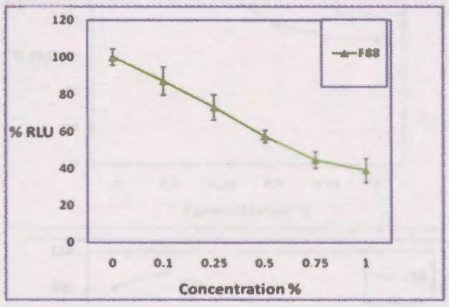
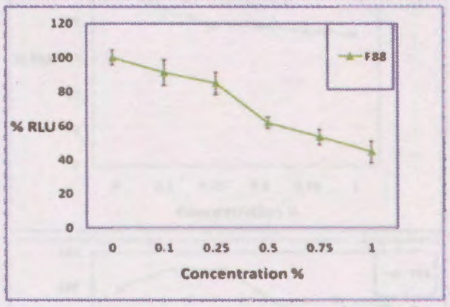
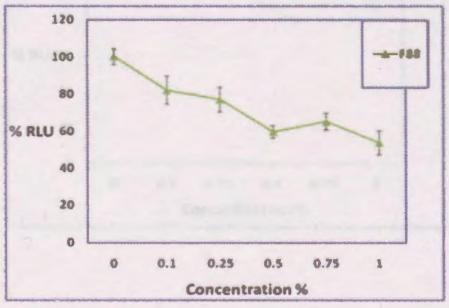
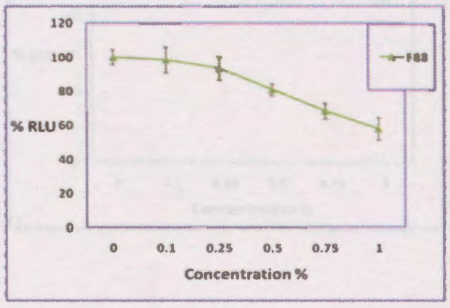
F88 (B)

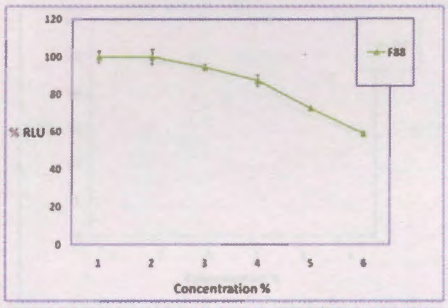
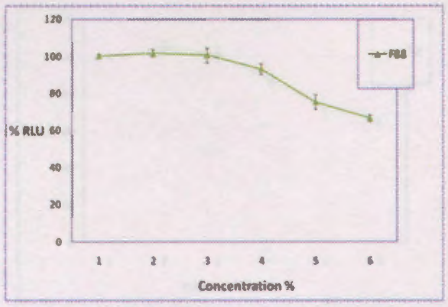
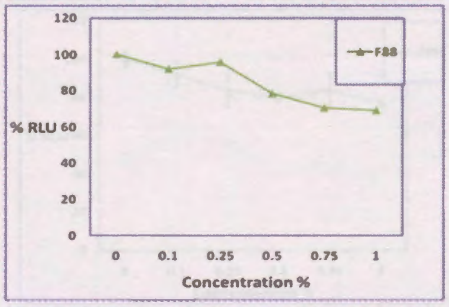
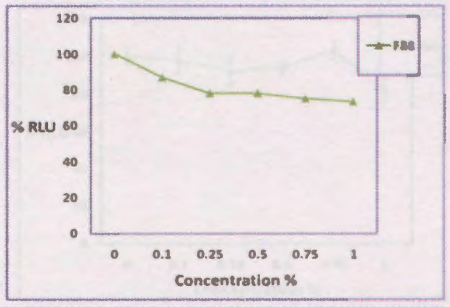
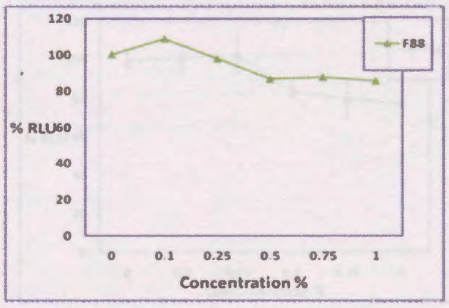
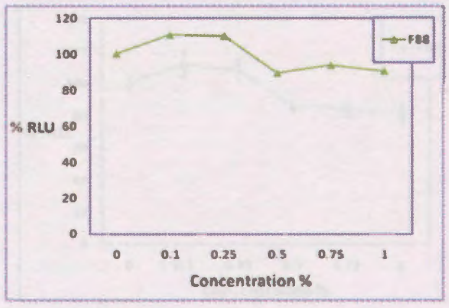
Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	100.7	95.6	95.2	97.2	3.1	80.0	88.0	89.0	85.7	4.9	68.0	70.0	77.0	71.7	4.7	54.2	56.0	58.7	56.3	2.3	42.9	45.0	42.5	43.5	1.4	39.0	37.8	38.7	37.8	1.2
10	60.2	61.2	60.2	60.5	0.6	58.0	52.0	56.0	55.3	3.1	53.0	50.0	51.1	51.4	1.5	35.0	38.5	38.9	37.5	2.1	33.1	31.6	34.4	33.1	1.4	27.0	28.9	25.4	27.1	1.8
20	31.0	30.6	31.8	31.1	0.6	29.0	28.5	28.3	28.6	0.4	28.7	31.2	29.4	29.8	1.3	24.0	25.0	24.0	24.3	0.6	22.0	21.2	22.5	21.9	0.7	21.0	21.1	22.2	21.4	0.7
30	25.0	26.7	28.4	26.7	1.7	23.2	22.1	24.2	23.2	1.1	22.1	20.1	20.2	20.8	1.1	22.1	20.0	20.3	20.8	1.1	21.1	19.8	19.1	20.0	1.0	19.8	19.9	18.9	19.5	0.6
40	26.5	24.0	25.4	25.3	1.3	24.2	22.2	23.1	23.2	1.0	22.2	21.2	19.8	21.1	1.2	20.0	22.2	18.1	20.1	2.1	22.2	21.1	20.0	21.1	1.1	21.1	18.8	18.3	19.4	1.5
50	22.0	21.0	22.2	21.7	0.6	21.1	22.1	19.8	21.0	1.2	20.0	19.7	19.4	19.7	0.3	19.4	20.0	21.0	20.1	0.8	21.0	22.2	23.2	22.1	1.1	18.8	17.4	16.4	17.5	1.2
60	21.1	24.5	20.2	21.9	2.3	26.0	29.0	25.0	26.7	2.1	29.9	24.0	22.0	25.3	4.1	21.0	22.2	21.1	21.4	0.7	22.2	21.1	22.4	21.9	0.7	19.8	19.2	19.3	19.4	0.3

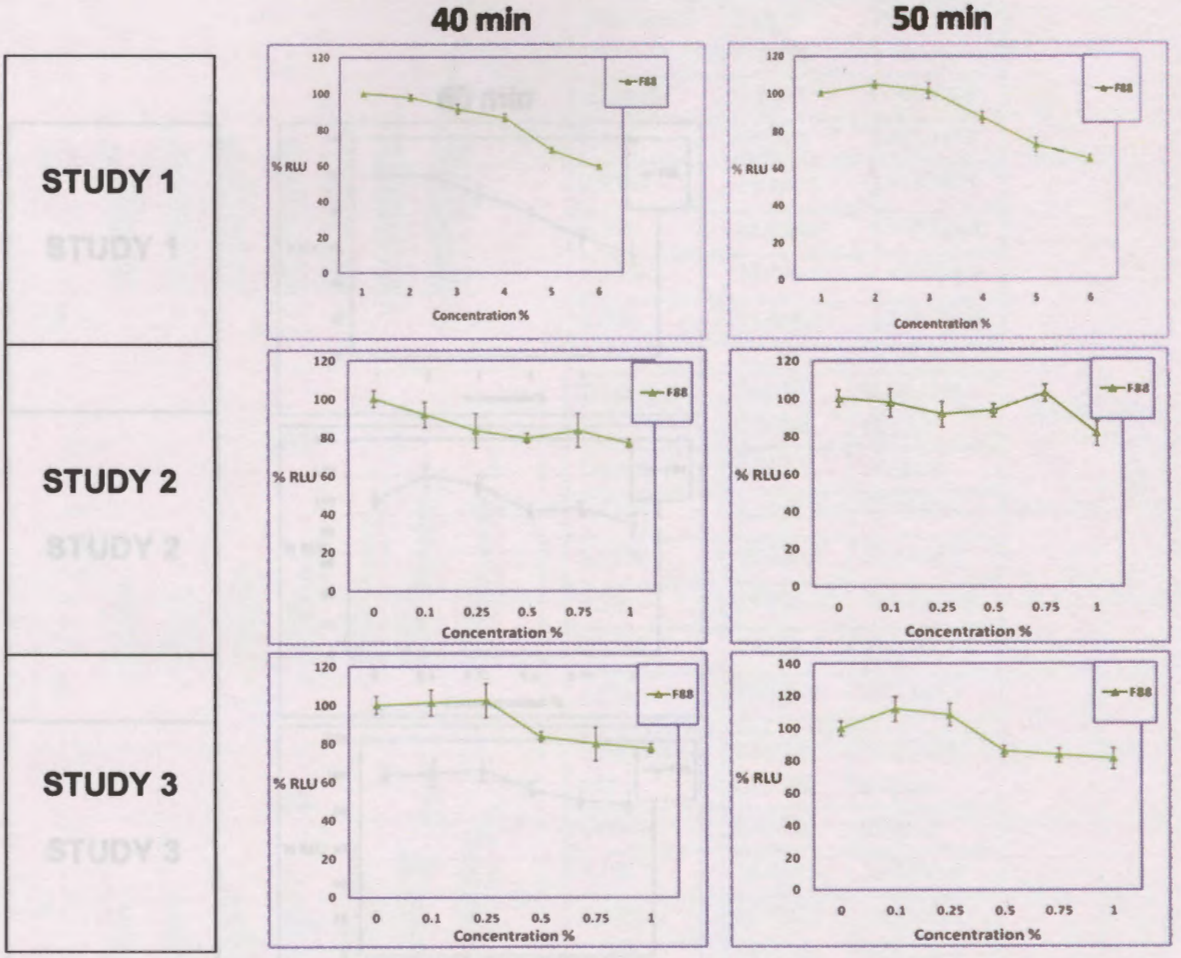
F88 (C)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	85.8	95.6	9.8	67.1	49.6	77.0	79.3	78.8	78.4	1.2	73.7	73.5	73.3	73.5	0.2	56.9	57.9	55.7	56.8	1.1	62.4	61.0	62.7	62.0	0.9	49.6	49.4	54.4	51.1	2.8
10	66.8	62.9	59.6	63.1	3.6	62.0	61.0	63.2	62.1	1.1	57.0	61.8	58.4	59.1	2.5	54.0	51.0	48.0	51.0	3.0	42.0	41.8	45.7	43.2	2.2	36.1	38.0	37.2	36.5	0.8
20	41.2	42.0	41.3	41.5	0.4	45.0	47.2	43.0	45.1	2.1	41.2	40.0	40.4	40.5	0.6	36.7	39.0	32.2	36.0	3.4	32.3	38.9	38.0	36.4	3.6	34.7	36.3	35.6	35.6	0.8
30	34.1	34.9	35.2	34.7	0.6	39.6	35.3	40.2	38.4	2.7	35.7	38.9	40.1	38.2	2.3	29.9	31.0	32.2	31.0	1.2	32.2	35.6	29.8	32.5	2.9	32.2	28.8	33.1	31.4	2.3
40	38.6	38.3	38.6	38.5	0.1	30.6	43.4	43.0	39.0	7.3	38.7	41.4	38.3	39.5	1.7	29.9	30.5	36.2	32.2	3.5	29.0	33.3	30.0	30.8	2.3	32.2	28.4	29.0	29.9	2.0
50	33.0	32.5	32.2	32.6	0.4	36.3	36.8	36.8	36.6	0.3	35.5	32.5	38.7	35.5	3.1	28.9	25.5	29.9	28.1	2.3	30.0	28.8	23.3	27.4	3.6	28.8	29.9	21.2	26.6	4.7
60	32.5	32.2	32.5	32.4	0.2	29.3	39.2	30.3	33.0	5.4	31.3	36.5	32.9	33.6	2.7	30.0	28.9	31.0	30.0	1.1	31.6	24.0	28.8	28.1	3.8	25.6	27.7	29.6	27.6	2.0

Pluronic F88: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
% RLU
Concentration %
F88

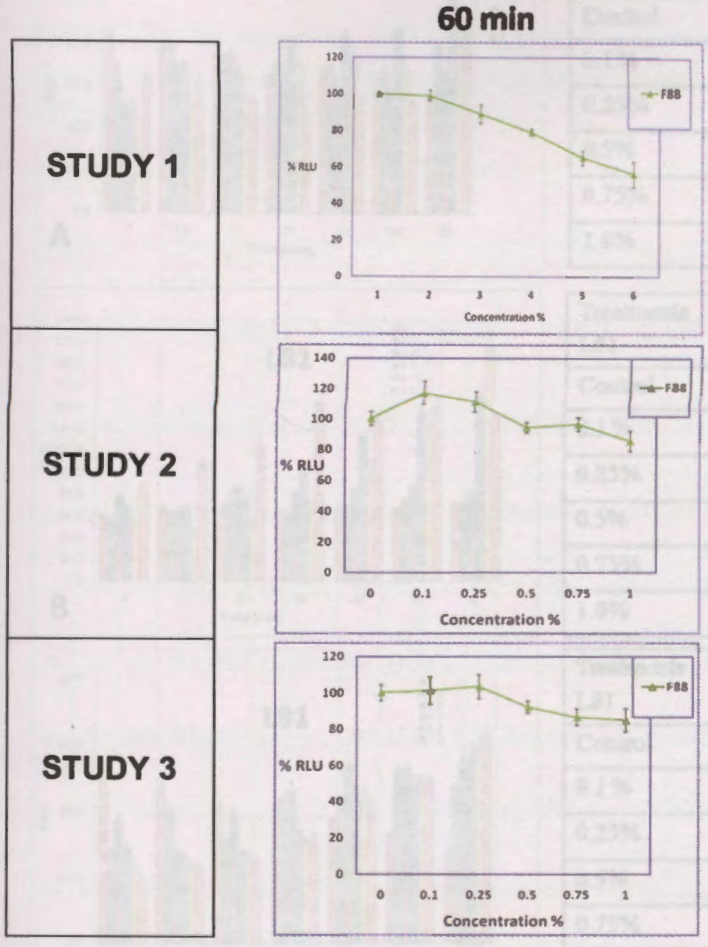
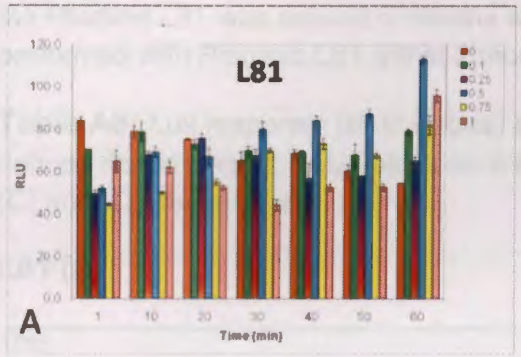
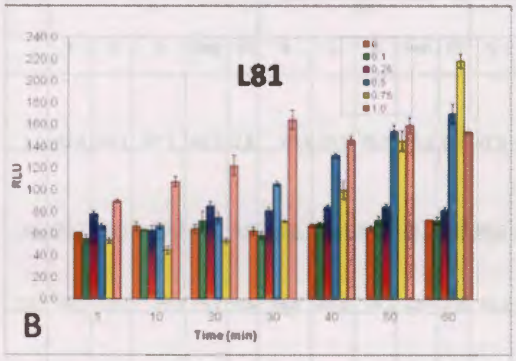


Figure A14: % RLU relative to control for defined concentrations of Pluronics F88 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

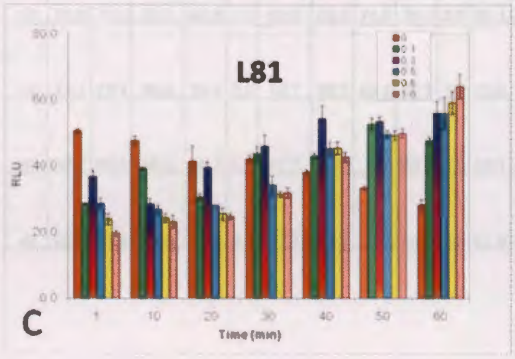
Pluronic L81: RLU vs. Time graph



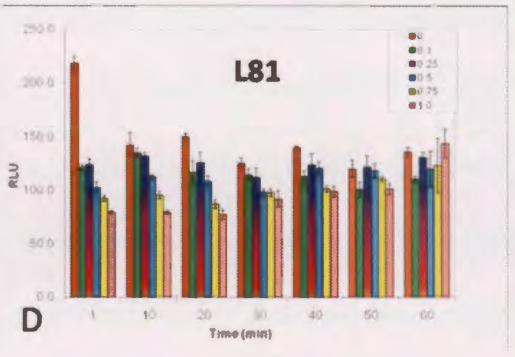
Treatments L81	Log 10 Cfu./ml	% RLU at 60 min
Control	9.2±0.0	100.0±0.0
0.1 %	9.9±0.3	144.5±2.0
0.25%	13.3±0.1	118.7±3.9
0.5%	11.4±0.4	206.0±2.0
0.75%	12.8±0.0	149.8±7.8
1.0%	11.6±0.2	174.7±5.0



Treatments L81	Log 10 Cfu./ml	% RLU at 60 min
Control	9.7±0.5	100±0.0
0.1 %	11.4±0.2	98.7±5.9
0.25%	12.7±0.3	112.3±4.9
0.5%	13.3±0.6	233.1±15.4
0.75%	13.4±0.5	299.1±11.1
1.0%	11.8±0.2	209.8±1.3



Treatments L81	Log 10 Cfu./ml	% RLU at 60 min
Control	9.7±0.5	100±3.5
0.1 %	11.4±0.2	169.7±1.9
0.25%	12.7±0.3	199.3±6.2
0.5%	13.3±0.6	199.1±8.4
0.75%	13.4±0.5	210.6±6.0
1.0%	11.8±0.2	228.5±6.2



Treatments L81	Log 10 Cfu./ml	% RLU at 60 min
Control	9.7±0.5	100±7.3
0.1 %	11.4±0.2	81.2±4.9
0.25%	12.7±0.4	96.4±7.7
0.5%	12.7±0.1	88.3±28.6
0.75%	11.5±0.3	90.8±44.0
1.0%	11.8±0.2	105.8±23.0

Figure A15: Lux response of *P. putida* KT2440 biosensor to L81 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 5-15-2009, (B) Study 2

performed on 5-14-2009, (C) Study 3 performed on 5-18-2009, (D) Study 4 performed on 2-20-2009. Means of analytical replicates and standard errors are shown for each study. Age of the Pluronic L81 was around 5 months when Studies 1, 2 and 3 were performed. Study 4 was performed with Pluronic L81 within 2 months of its receiving from the supplier.

Table A8: Lux response (RLU values) of *P. putida* KT2440 biosensor to L81 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables L81 (A), (B), (C) and (D), respectively

L81 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	81.4	84.1	87.1	84.2	2.9	70.7	70.2	70.3	70.4	0.3	47.0	50.5	52.0	49.8	2.6	49.8	54.4	52.9	52.4	2.3	45.8	44.1	44.2	44.7	1.0	66.1	74.1	65.2	65.2	9.0
10	74.7	84.5	78.8	79.4	4.9	69.9	83.4	84.2	79.2	8.0	68.4	66.0	70.7	68.3	2.4	72.1	72.2	64.7	69.7	4.3	49.3	49.8	51.3	50.2	1.1	58.8	65.2	65.1	62.4	4.8
20	76.2	75.7	75.2	75.7	0.5	74.8	69.3	76.2	73.4	3.6	80.2	73.4	75.6	76.4	3.5	65.3	61.5	75.4	67.4	7.2	53.2	57.9	54.8	55.3	2.4	51.1	52.4	54.8	52.8	1.9
30	56.8	70.2	69.5	65.5	7.6	67.5	69.5	74.0	70.4	3.3	62.1	72.0	69.8	68.0	5.2	75.3	87.1	78.9	80.4	6.0	68.5	71.9	70.6	70.3	1.7	43.5	49.7	40.7	44.6	4.6
40	71.1	72.2	66.9	70.1	2.8	70.7	70.1	68.4	69.7	1.2	43.0	64.9	63.4	57.1	12.2	78.8	86.7	87.0	84.2	4.7	78.5	71.9	70.2	73.5	4.4	50.4	56.3	52.3	53.0	3.0
50	57.2	58.3	66.6	60.7	5.1	57.2	74.8	72.2	68.1	9.5	59.1	57.6	56.9	57.8	1.1	83.4	90.3	88.7	87.5	3.6	66.6	66.9	70.3	67.9	2.1	50.6	62.4	56.1	53.0	2.8
60	55.0	55.1	54.9	55.0	0.1	77.7	81.7	79.3	79.5	2.0	62.5	68.1	68.1	66.2	3.2	112.1	111.9	114.7	112.9	1.5	89.7	83.1	74.3	82.4	7.8	97.5	100.3	90.6	96.1	5.0

L81 (B)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	60.9	60.9	60.9	60.9	0.0	47.2	58.9	59.5	55.2	6.9	76.6	83.7	75.0	78.4	4.6	62.1	72.1	67.6	67.3	5.0	49.8	58.7	54.3	54.2	4.5	92.9	88.8	88.5	90.1	2.4
10	74.9	64.9	61.0	66.9	7.2	64.2	62.8	64.6	63.8	0.9	61.3	56.7	72.0	63.3	7.9	63.0	66.8	72.2	67.3	4.6	39.2	51.2	45.0	45.1	6.0	98.6	114.1	110.7	107.8	8.1
20	72.4	63.1	59.0	64.8	6.9	54.3	82.0	80.2	72.2	15.5	83.6	79.9	93.3	85.6	6.9	66.8	83.9	74.5	75.1	8.5	50.8	52.2	58.3	53.8	4.0	104.3	132.7	131.4	122.8	16.0
30	70.3	59.9	59.2	63.1	6.2	53.9	65.7	55.8	58.5	6.4	76.2	87.4	80.3	81.3	5.7	102.9	109.6	105.6	106.0	3.4	72.6	71.9	70.5	71.7	1.0	169.1	147.3	177.4	164.6	15.5
40	68.9	69.8	65.5	68.0	2.3	70.1	65.1	71.9	69.1	3.5	81.1	89.1	83.4	84.5	4.1	135.8	130.6	129.8	132.1	3.2	108.2	107.8	83.5	99.8	14.1	145.5	152.6	140.5	146.2	6.1
50	65.1	61.9	69.3	65.5	3.7	64.8	75.3	77.7	72.5	7.0	80.2	88.1	86.6	85.0	4.2	163.6	144.9	154.6	154.4	9.4	133.2	139.2	164.0	145.5	16.4	146.4	171.3	161.6	159.8	12.5
60	73.0	73.1	73.0	73.0	0.0	67.9	78.9	69.7	72.1	5.8	84.2	76.5	85.5	82.1	4.9	154.7	170.5	185.6	170.3	15.4	205.7	226.1	223.7	218.5	11.1	153.4	154.5	151.9	153.3	1.3

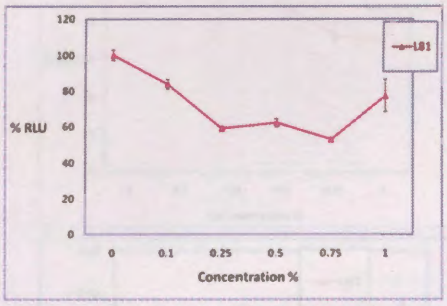
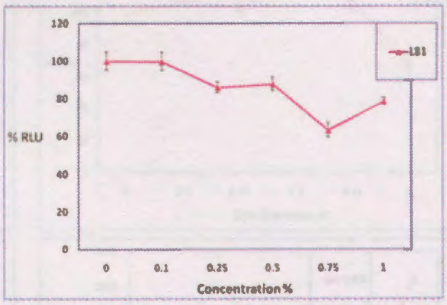
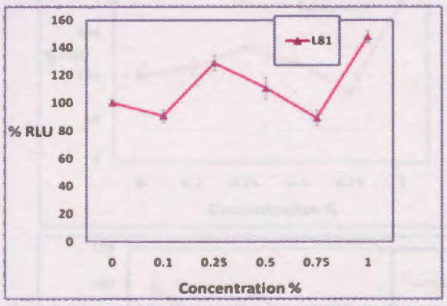
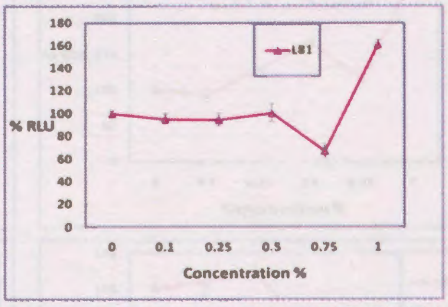
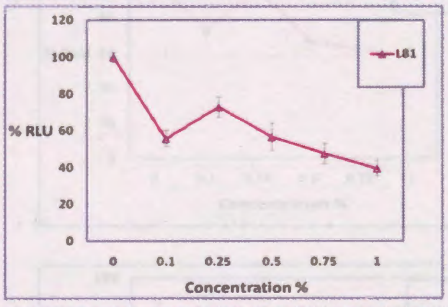
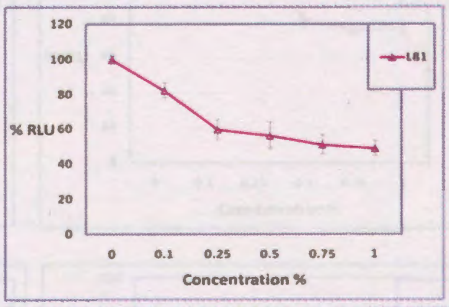
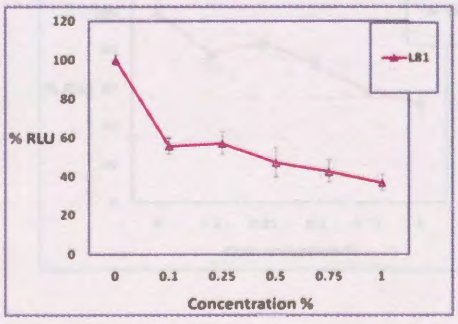
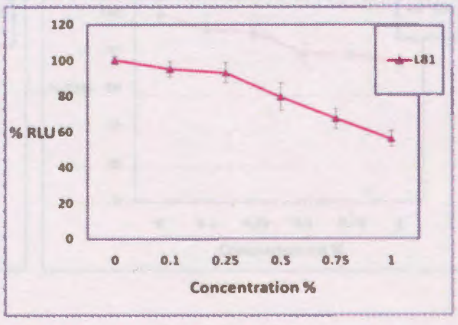
L81 (C)

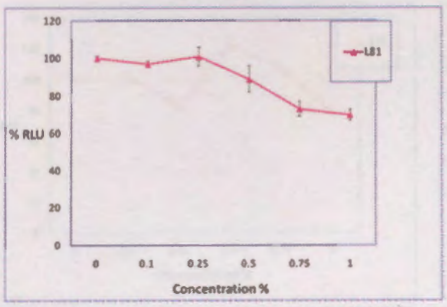
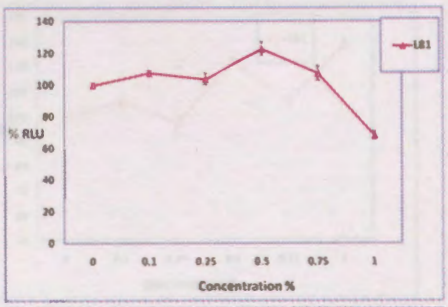
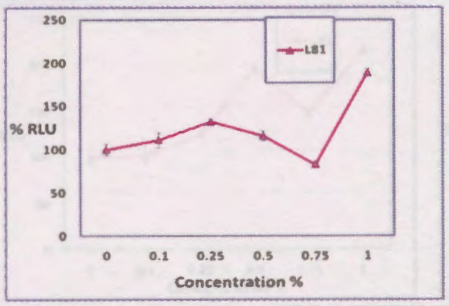
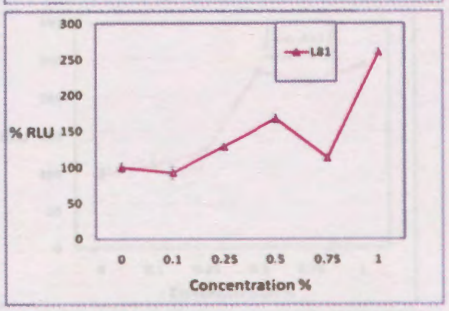
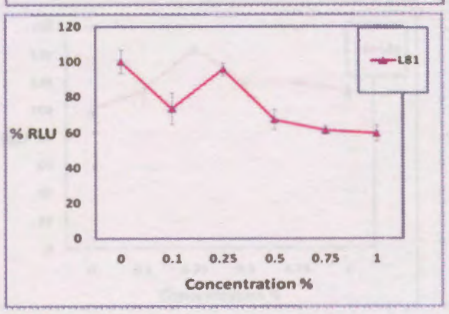
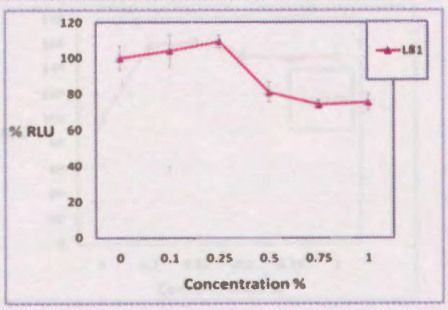
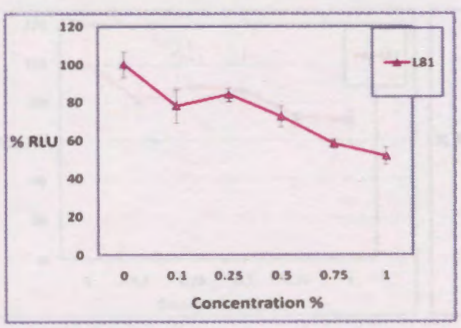
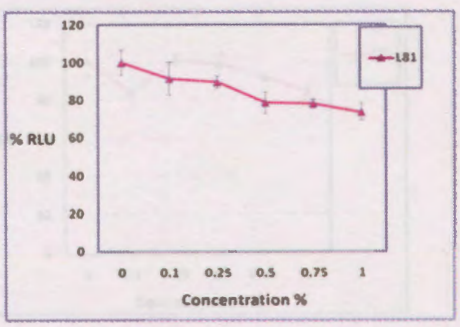
Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	50.3	52.0	50.0	50.8	1.1	28.1	28.1	28.9	28.4	0.5	33.4	37.9	39.4	36.9	3.1	25.2	29.4	31.1	28.6	3.0	22.2	22.9	26.9	24.0	2.6	20.9	18.4	20.5	19.9	1.3
10	48.2	45.1	49.8	47.7	2.4	39.1	38.6	39.9	39.2	0.7	25.3	30.3	29.9	28.5	2.8	25.7	25.6	28.9	26.8	1.9	23.6	26.5	22.9	24.3	1.9	24.2	20.0	25.7	23.3	2.9
20	42.8	33.1	48.6	41.5	7.9	28.4	31.8	31.3	30.5	1.8	39.4	37.0	42.6	39.7	2.8	27.7	28.1	28.1	28.0	0.2	23.4	28.8	24.4	25.5	2.9	24.0	24.1	26.2	24.8	1.2
30	42.3	43.9	40.5	42.2	1.7	39.9	45.5	46.4	43.9	3.5	40.9	45.5	52.1	46.2	5.6	39.0	29.5	34.2	34.2	4.7	31.7	32.6	29.9	31.4	1.4	32.4	34.1	29.2	31.9	2.5
40	38.9	36.8	38.9	38.2	1.2	43.4	44.2	41.8	43.1	1.2	48.3	61.5	53.4	54.4	6.6	44.4	49.0	42.5	45.3	3.4	46.7	41.8	48.0	45.5	3.3	42.4	40.6	45.3	42.7	2.4
50	34.3	32.2	33.3	33.3	1.0	56.1	52.6	49.4	52.7	3.4	51.7	56.2	52.9	53.6	2.3	50.3	47.7	51.2	49.7	1.8	50.4	46.4	50.9	49.3	2.5	52.4	48.1	49.2	49.9	2.2
60	31.2	27.8	25.4	28.1	2.9	46.9	46.4	50.0	47.8	1.9	50.1	55.7	62.5	56.1	6.2	61.9	46.4	59.7	56.0	8.4	58.1	65.7	54.0	59.3	6.0	57.4	65.7	69.5	64.2	6.2

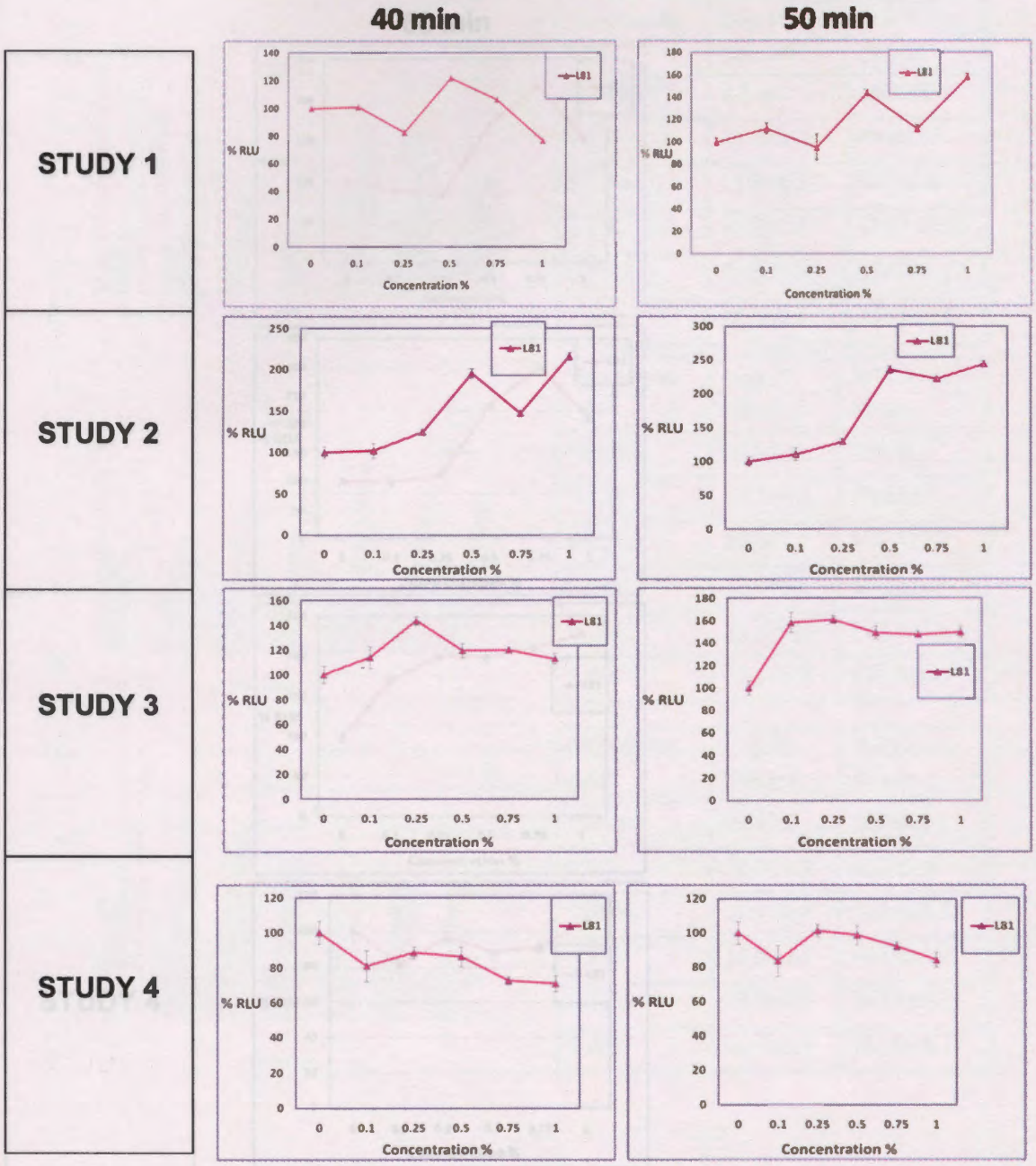
L81 (D)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	206.1	225.9	223.3	218.4	10.8	125.8	116.5	122.2	121.5	4.7	113.4	128.5	131.4	124.4	9.6	91.7	106.5	109.8	102.7	9.7	95.2	95.8	87.7	92.9	4.5	82.0	78.1	80.4	80.2	1.9
10	161.2	122.8	144.4	142.8	19.3	137.5	140.2	128.8	135.6	5.9	126.9	133.5	137.7	132.7	5.5	112.3	111.2	116.8	113.4	2.9	90.3	101.3	96.4	96.0	5.5	78.2	79.1	82.8	80.0	2.5
20	144.5	156.4	149.7	150.2	6.0	95.6	129.0	127.0	117.2	18.8	107.6	140.4	130.7	126.2	16.9	101.6	117.8	108.0	109.1	8.2	94.7	81.7	86.5	87.6	6.6	75.8	86.1	72.0	78.0	7.3
30	118.2	123.0	135.6	125.6	8.9	104.2	117.3	123.1	114.9	9.7	95.1	120.0	122.7	112.6	15.2	95.2	104.2	97.2	98.9	4.8	96.9	92.7	104.8	98.1	6.1	79.9	105.8	91.7	92.5	12.9
40	137.8	142.4	140.7	140.3	2.3	108.3	108.5	123.8	113.5	8.9	108.1	122.0	142.9	124.3	17.5	109.1	124.9	129.3	121.1	10.6	97.6	106.6	101.9	102.0	4.5	92.4	108.2	97.7	99.4	8.1
50	109.2	115.9	136.1	120.4	14.0	101.5	112.9	88.1	100.8	12.4	101.9	136.9	127.0	121.9	18.1	130.3	107.3	118.8	118.8	11.5	114.8	111.9	106.1	110.9	4.4	91.7	107.5	104.9	101.4	8.5
60	127.7	141.7	138.6	136.0	7.3	104.7	113.7	112.8	110.4	4.9	122.2	135.9	135.2	131.1	7.7	91.0	121.0	148.2	120.1	28.6	97.3	98.9	174.3	123.5	44.0	145.1	166.2	120.3	143.9	23.0

Pluronic L81: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
STUDY 4
% RLU
Concentration %
L81
0
0.1
0.25
0.5
0.75
1

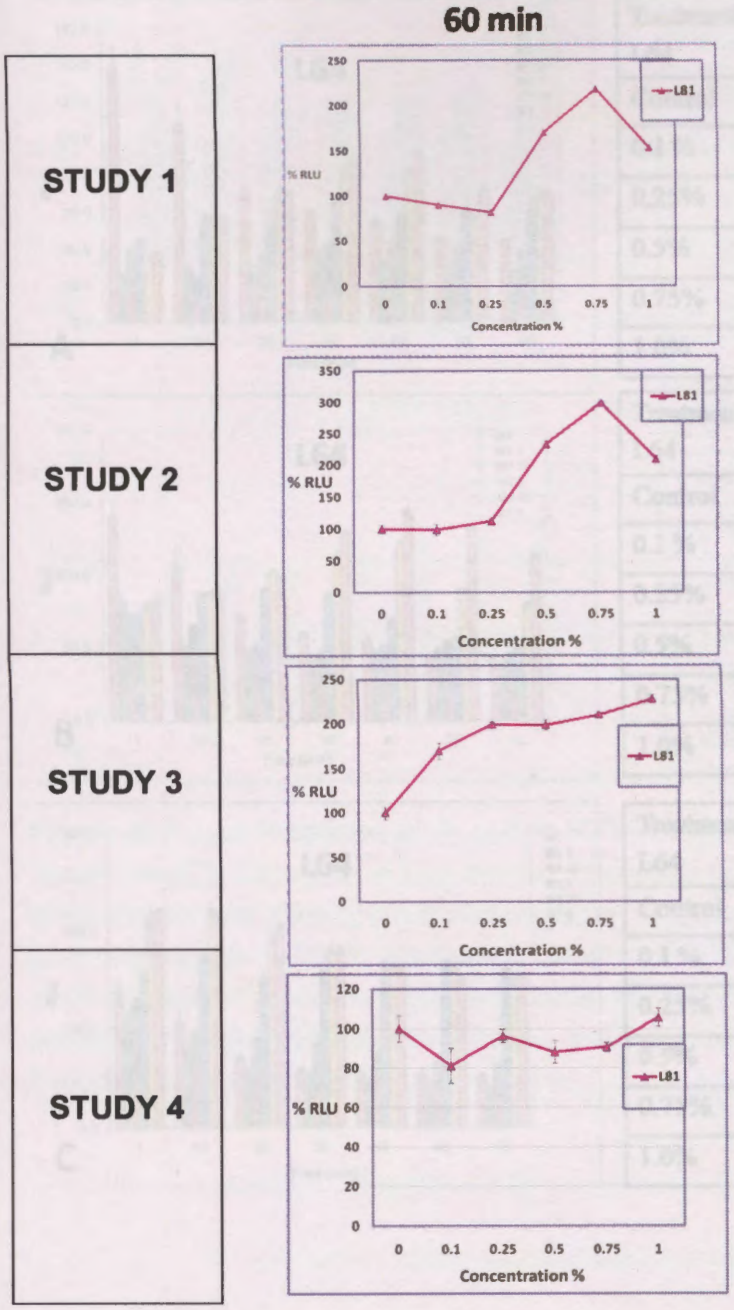
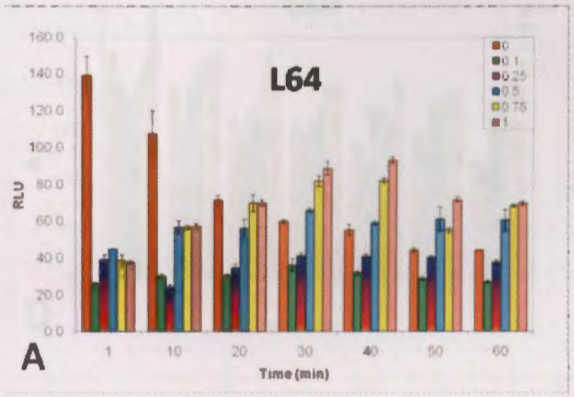
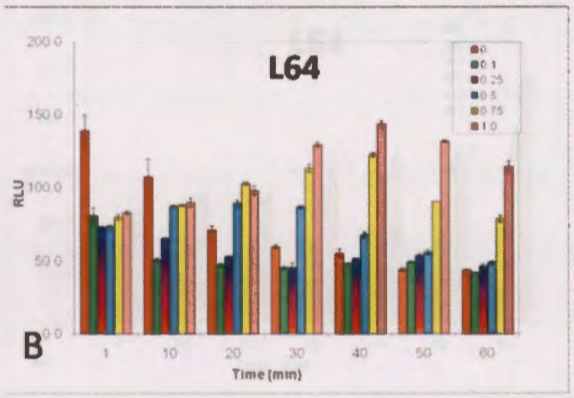


Figure A16: % RLU relative to control for defined concentrations of Pluronics L81 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

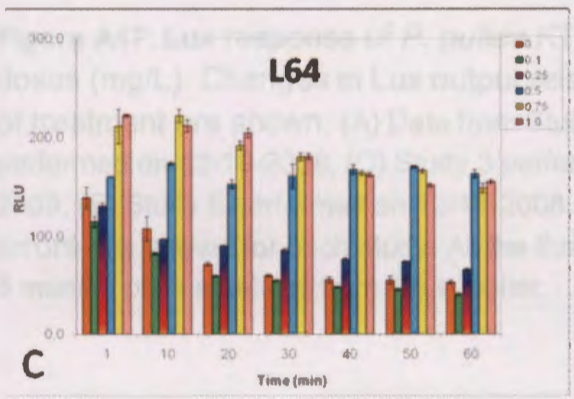
Pluronic L64: RLU vs. Time graph



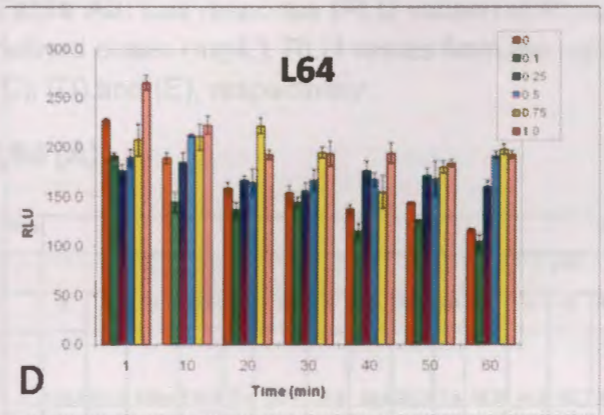
Treatments L64	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.2	100.0±0.3
0.1 %	11.0±2.3	61.2±1.4
0.25%	11.3±2.3	85.3±1.4
0.5%	11.5±1.2	137.1±10.0
0.75%	11.8±1.6	154.8±1.2
1.0%	11.5±0.4	157.9±1.8



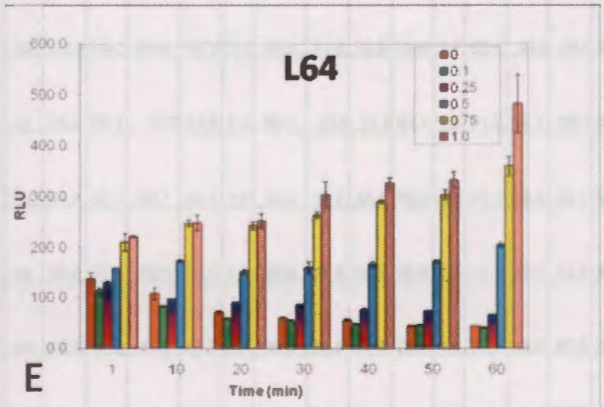
Treatments L64	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.3	100±0.3
0.1 %	13.3±0.3	95.0±0.6
0.25%	12.3±1.5	105.4±2.2
0.5%	12.5±0.6	112.2±1.5
0.75%	12..0±0.9	178.7±4.0
1.0%	12.5±0.2	259.9±5.9



Treatments L64	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.2	100±0.3
0.1 %	12.6±0.3	92.1±2.5
0.25%	12.9±0.6	149.1±2.5
0.5%	13.0±0.6	465.6±6.9
0.75%	12.9±0.5	742.1±2.5
1.0%	12.9±0.3	733.9±21.7



Treatments L64	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.5	100.0±2.6
0.1 %	11.0±2.3	89.3±12.6
0.25%	11.3±1.8	137.5±10.4
0.5%	12.5±1.5	164.6±6.7
0.75%	11.8±1.0	169.8±9.2
1.0%	10.5±0.4	165.4±5.9



Treatments L64	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.5	100.0±0.3
0.1 %	12.0±2.3	92.1±2.5
0.25%	11.3±1.8	149.1±2.5
0.5%	1215±1.5	465.6±6.9
0.75%	10.8±1.0	814.3±32.6
1.0%	10.5±0.4	1090.0±98.3

Figure A17: Lux response of *P. putida* KT2440 biosensor to L64 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 12-9-2008, (B) Study 2 performed on 12-19-2008, (C) Study 3 performed on 2-2-2009, (D) Study 4 performed on 2-20-2009, (E) Study 5 performed on 12-11-2008. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic L64 were performed within 3 months of its receiving from the supplier.

Table A9: Lux response (RLU values) of *P. putida* KT2440 biosensor to L64 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables L64 (A), (B), (C), (D) and (E), respectively

L64 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	119.8	141.4	156.4	139.2	18.4	24.9	26.8	26.9	26.2	1.1	35.3	44.5	37.7	39.2	4.7	44.8	43.1	44.3	44.1	0.9	32.2	42.0	42.0	38.7	5.6	36.6	37.8	39.3	37.9	1.3
10	123.4	116.1	83.4	107.6	21.3	28.5	31.8	31.0	30.4	1.7	22.4	23.8	26.5	24.3	2.1	49.7	61.1	59.8	56.9	6.2	55.5	56.6	58.4	56.8	1.5	56.7	55.6	60.0	57.5	2.3
20	76.3	68.2	70.2	71.6	4.2	30.7	29.8	31.7	30.7	1.0	31.3	38.1	35.1	34.8	3.4	60.5	45.8	62.0	56.1	8.9	60.8	73.9	75.3	70.0	8.0	67.1	72.5	70.6	70.1	2.7
30	61.9	58.1	59.7	59.9	1.9	32.4	33.7	43.1	36.4	5.9	41.1	39.1	44.1	41.5	2.5	63.9	66.7	67.5	66.0	1.9	77.1	87.0	81.6	81.9	5.0	81.6	93.9	91.0	88.8	6.4
40	59.2	57.3	49.6	55.4	5.1	34.0	31.8	30.3	32.0	1.9	41.4	39.0	42.6	41.0	1.8	59.7	57.5	60.2	59.1	1.4	82.9	79.8	84.2	82.3	2.3	90.8	92.9	96.3	93.3	2.8
50	46.5	44.0	42.3	44.3	2.1	29.0	30.8	27.0	28.9	1.9	39.1	39.6	42.5	40.4	1.8	50.0	72.9	60.8	61.2	11.5	52.7	55.5	57.4	55.2	2.4	74.4	70.9	69.9	71.7	2.3
60	44.4	43.9	44.3	44.2	0.3	27.8	28.1	25.6	27.2	1.4	37.6	36.4	39.1	37.7	1.4	49.0	67.1	65.7	60.6	10.0	68.4	67.2	69.6	68.4	1.2	70.9	67.7	70.7	69.8	1.8

L64 (B)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	119.8	141.4	156.4	139.2	18.4	87.4	71.3	85.1	81.2	8.7	72.6	73.5	73.7	73.2	0.6	72.1	73.6	74.8	73.5	1.4	76.5	81.8	81.9	80.1	3.1	81.0	84.7	83.2	83.0	1.8
10	123.4	116.1	83.4	107.6	21.3	49.4	52.0	52.0	51.1	1.5	63.6	66.8	65.7	65.4	1.6	87.1	88.9	86.4	87.4	1.3	88.0	87.4	89.1	88.2	0.8	90.9	94.7	84.7	90.1	5.0
20	76.3	68.2	70.2	71.6	4.2	46.8	47.2	49.2	47.7	1.3	52.3	52.5	54.4	53.1	1.1	86.2	92.8	90.2	89.7	3.3	100.7	104.7	103.9	103.1	2.1	95.4	95.1	104.1	98.2	5.1
30	61.9	58.1	59.7	59.9	1.9	43.6	46.5	46.5	45.6	1.7	40.2	48.3	48.9	45.8	4.9	84.9	87.3	88.6	87.0	1.9	109.5	113.2	118.3	113.7	4.4	128.2	133.0	127.9	129.7	2.9
40	59.2	57.3	49.6	55.4	5.1	48.6	49.1	48.0	48.6	0.5	51.2	52.8	51.0	51.6	1.0	64.5	70.3	69.5	68.1	3.1	120.3	125.4	122.6	122.7	2.5	139.7	142.7	148.4	143.6	4.4
50	46.5	44.0	42.3	44.3	2.1	49.5	48.8	50.4	49.6	0.8	55.7	54.2	51.9	53.9	1.9	55.0	54.6	58.9	56.2	2.4	90.6	90.6	90.5	90.6	0.0	132.5	132.6	130.4	131.8	1.3
60	44.4	43.9	44.3	44.2	0.3	41.9	42.7	41.5	42.0	0.6	44.3	46.8	48.7	46.6	2.2	48.5	48.6	50.7	49.2	1.2	74.9	79.1	82.9	79.0	4.0	110.6	121.6	112.5	114.9	5.9

L64 (C)

Time	Concentration %																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	119.8	141.4	156.4	139.2	18.4	104.6	117.8	121.4	114.6	8.8	125.0	134.9	133.9	131.2	5.5	160.2	160.3	161.1	160.6	0.5	177.0	224.2	232.8	211.3	30.1	221.3	220.7	225.0	222.4	2.3
10	123.4	116.1	83.4	107.6	21.3	84.1	83.7	80.8	82.8	1.8	97.6	97.2	97.0	97.3	0.3	172.4	172.2	177.7	174.1	3.1	210.0	223.2	232.3	221.8	11.2	222.1	200.9	213.3	212.1	10.7
20	76.3	68.2	70.2	71.6	4.2	60.5	60.6	57.9	59.7	1.5	89.5	91.8	90.2	90.5	1.2	148.1	161.3	150.7	153.4	7.0	200.9	188.7	187.7	192.4	7.3	213.2	200.9	199.9	204.7	7.4
30	61.9	58.1	59.7	59.9	1.9	53.0	55.3	55.9	54.7	1.5	83.5	86.2	88.4	86.0	2.5	137.7	170.0	175.9	161.2	20.6	188.2	178.2	176.3	180.9	6.4	180.0	187.2	176.5	181.2	5.5
40	59.2	57.3	49.6	55.4	5.1	49.2	48.0	47.6	48.3	0.8	76.2	73.6	80.0	76.6	3.3	159.5	174.5	170.7	168.2	7.8	167.2	165.5	159.9	164.2	3.8	166.5	161.1	159.9	162.5	3.5
50	60.1	59.89	50.1	55.1	7.1	45.4	47.2	46.1	45.9	1.2	72.7	75.0	75.3	74.3	1.4	172.4	171.9	167.7	170.7	2.6	168.8	162.2	167.6	166.2	3.5	150.0	149.9	155.4	151.8	3.1
60	55.5	54.3	49.0	52.9	3.5	38.1	41.1	43.0	40.7	2.5	63.0	67.3	67.3	65.9	2.5	171.2	158.8	160.9	163.6	6.7	156.2	150.2	140.0	148.8	8.2	166.6	167.7	162.2	155.4	3.0

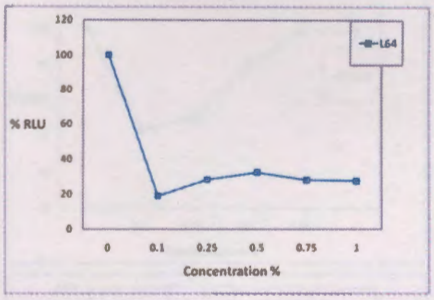
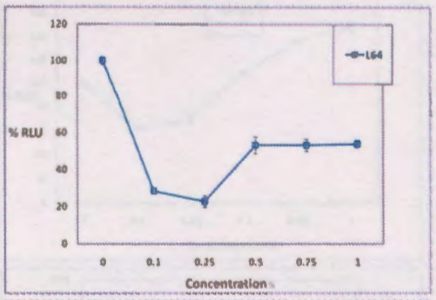
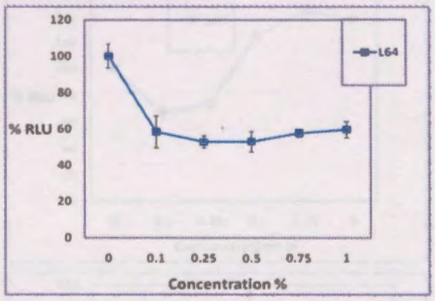
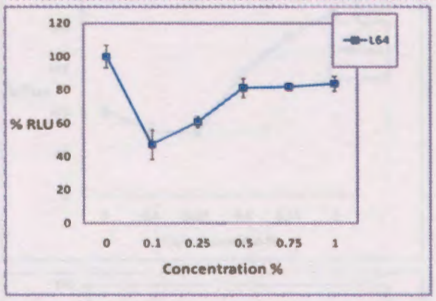
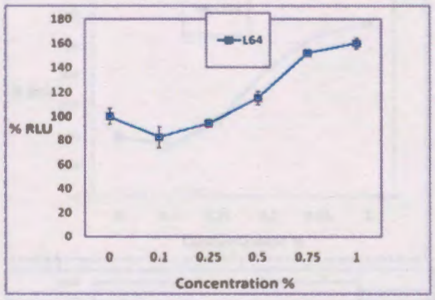
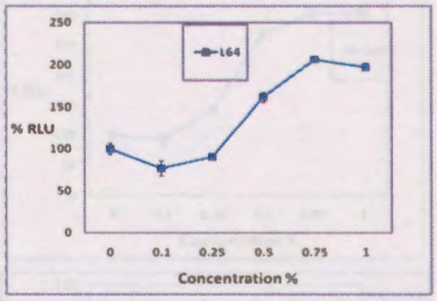
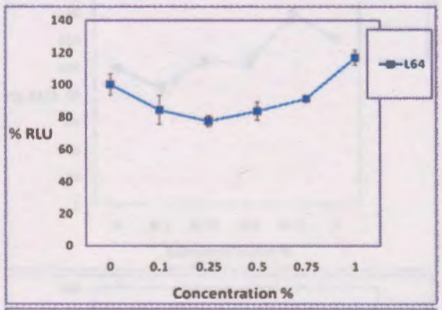
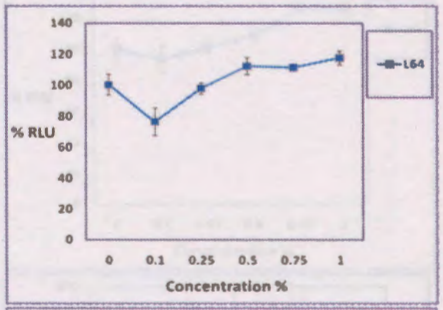
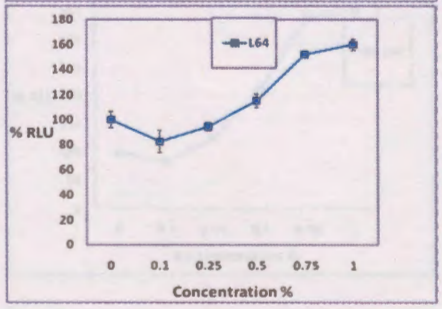
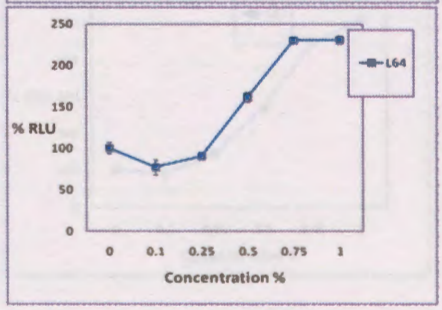
L64 (D)

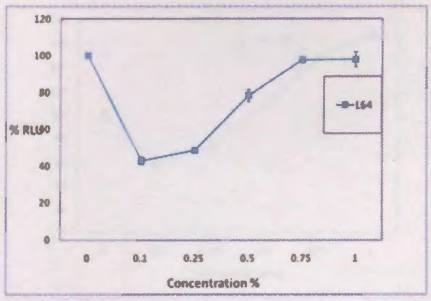
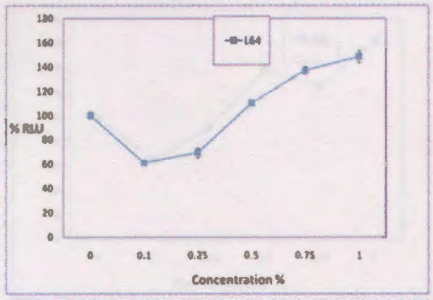
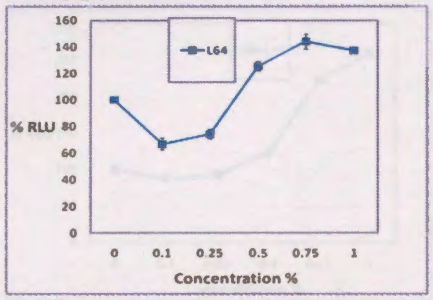
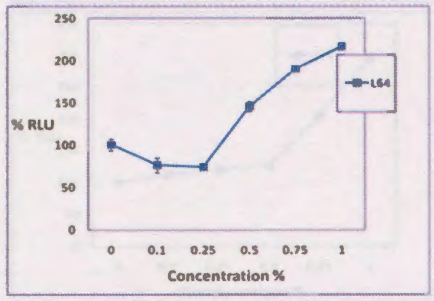
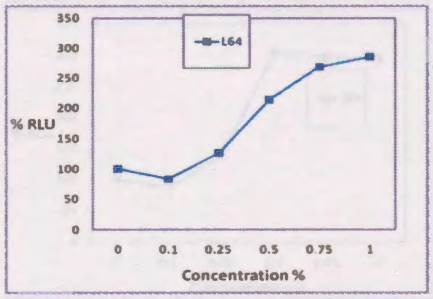
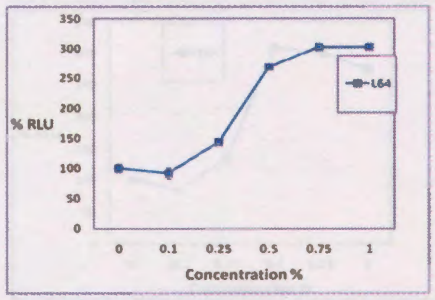
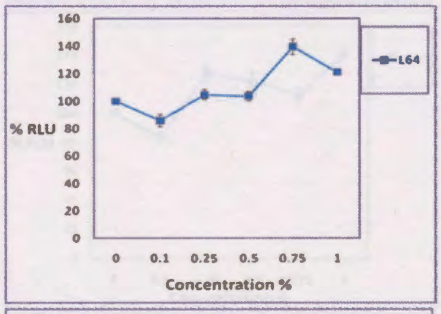
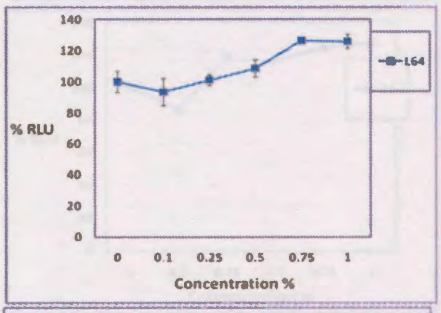
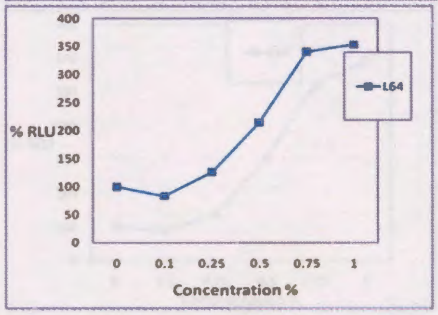
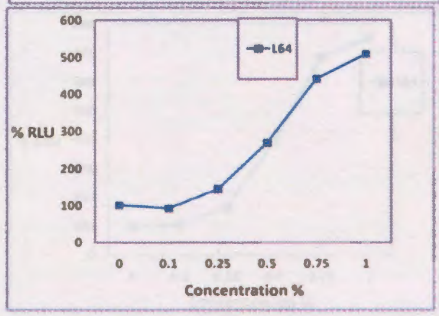
Time	Concentration %																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	224.7	227.0	231.2	227.6	3.3	185.4	197.7	191.9	191.7	6.1	188.6	172.4	167.3	176.1	11.1	181.8	181.2	207.2	190.1	14.9	184.9	199.5	239.0	207.8	28.0	251.0	272.2	275.0	266.1	13.1
10	186.5	200.1	182.1	189.5	9.4	129.3	139.3	164.4	144.3	18.1	168.3	183.7	203.0	185.0	17.4	213.6	209.3	214.2	212.4	2.7	184.3	221.3	226.4	210.7	23.0	205.4	226.2	236.3	222.6	15.8
20	149.0	168.0	161.1	159.4	9.6	122.4	139.3	148.5	136.7	13.2	158.4	169.0	173.6	167.0	7.8	142.2	188.4	165.1	165.2	23.1	206.3	227.3	233.1	222.3	14.1	185.6	191.8	201.7	193.0	8.1
30	153.8	167.1	142.4	154.4	12.4	133.5	146.0	153.3	144.2	10.0	152.7	171.5	144.3	156.2	13.9	147.6	172.5	182.7	167.6	18.1	194.4	185.9	205.1	195.1	9.6	219.0	184.2	179.1	194.1	21.7
40	130.2	140.5	144.0	138.3	7.2	122.8	124.5	98.5	115.3	14.5	157.2	177.3	193.8	176.1	18.4	177.4	155.4	171.5	168.1	11.4	122.9	163.9	179.0	155.3	29.1	190.8	178.8	214.1	194.6	17.9
50	145.8	143.7	144.6	144.7	1.0	122.7	127.1	126.8	125.5	2.4	158.1	184.2	172.5	171.6	13.0	176.8	134.4	194.6	168.6	31.0	170.1	191.1	180.2	180.5	10.5	178.9	190.6	183.8	184.4	5.9
60	113.8	118.8	117.6	116.8	2.6	113.8	90.0	109.2	104.3	12.6	170.8	150.0	161.0	160.6	10.4	198.8	185.2	192.8	192.2	6.7	192.5	193.6	208.9	198.3	9.2	188.0	199.5	192.1	193.2	5.9

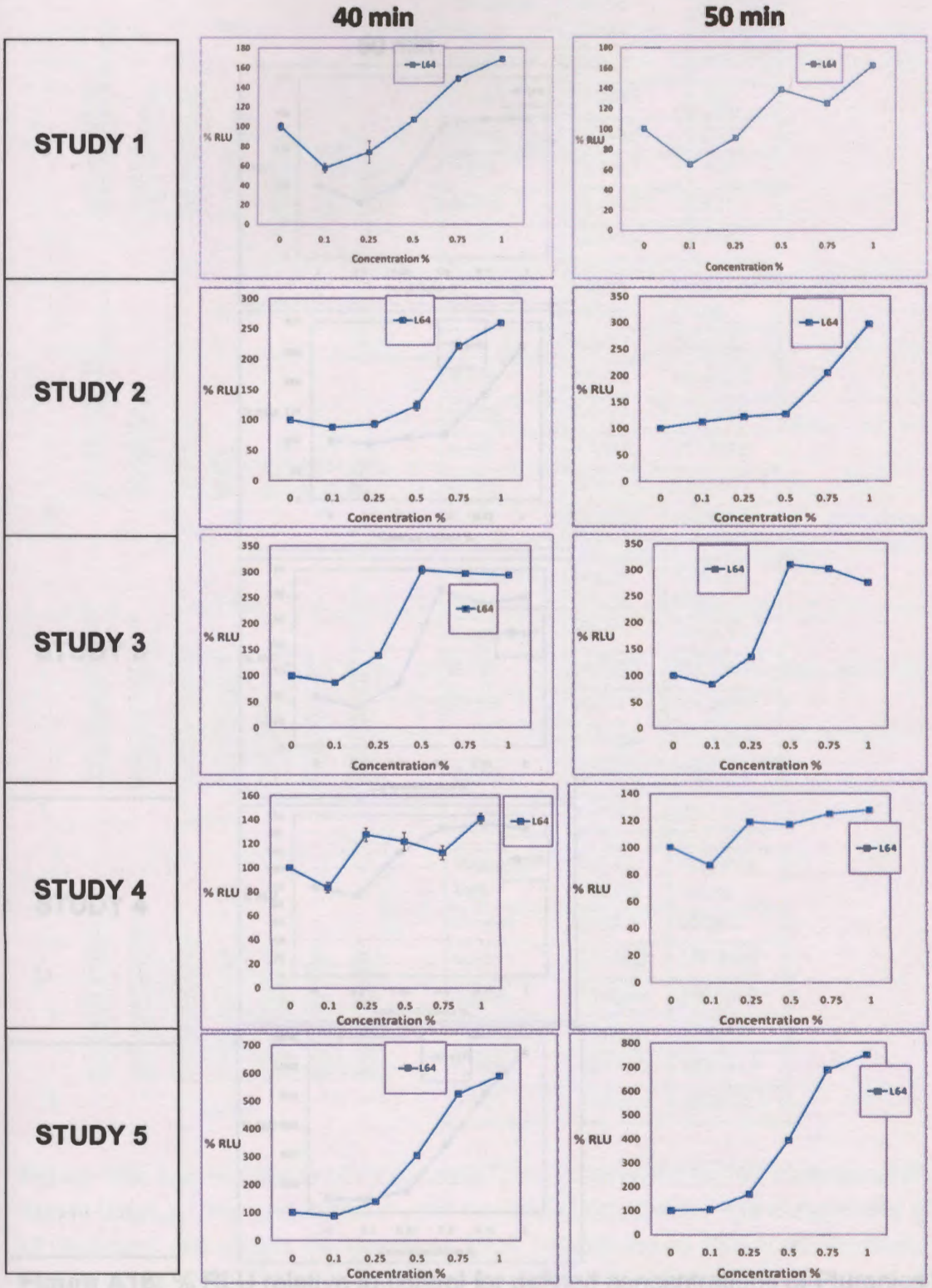
L64 (E)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	119.8	141.4	156.4	139.2	18.4	104.6	117.8	121.4	114.6	8.8	125.0	134.9	133.9	131.2	5.5	160.2	160.9	159.8	160.3	0.6	177.0	224.2	232.8	211.3	30.1	221.3	220.7	225.0	222.4	2.3
10	123.4	116.1	83.4	107.6	21.3	84.1	83.7	80.8	82.8	1.8	97.6	97.2	97.0	97.3	0.3	172.4	172.2	177.7	174.1	3.1	238.2	245.9	259.3	247.8	10.7	222.1	274.7	248.4	248.4	26.3
20	76.3	68.2	70.2	71.6	4.2	60.5	60.6	57.9	59.7	1.5	89.5	91.8	90.2	90.5	1.2	148.1	161.3	150.7	153.4	7.0	229.4	252.3	250.2	244.0	12.7	225.8	268.4	264.9	253.0	23.6
30	61.9	58.1	59.7	59.9	1.9	53.0	55.3	55.9	54.7	1.5	83.5	86.2	88.4	86.0	2.5	137.7	170.0	175.9	161.2	20.6	253.1	267.7	273.5	264.7	10.5	304.6	262.5	347.4	304.8	42.4
40	59.2	57.3	49.6	55.4	5.1	49.2	48.0	47.6	48.3	0.8	76.2	73.6	80.0	76.6	3.3	158.5	174.5	170.7	168.2	7.8	284.4	293.1	296.2	291.3	6.1	305.7	339.7	334.4	326.6	18.3
50	46.5	44.0	42.3	44.3	2.1	45.4	47.2	45.1	45.9	1.2	72.7	75.0	75.3	74.3	1.4	172.4	171.9	178.2	174.2	3.5	288.0	314.6	311.4	304.7	14.5	308.3	362.0	327.6	332.6	27.2
60	44.4	43.9	44.3	44.2	0.3	38.1	41.1	43.0	40.7	2.5	63.0	67.3	67.3	65.9	2.5	203.9	200.1	213.4	205.8	6.9	326.8	361.0	392.0	359.9	32.6	408.8	443.9	583.8	482.2	88.3

Pluronic L64: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		
STUDY 5		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		
STUDY 5		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
STUDY 4
STUDY 5
% RLU
Concentration %
0 0.1 0.25 0.5 0.75 1
L64

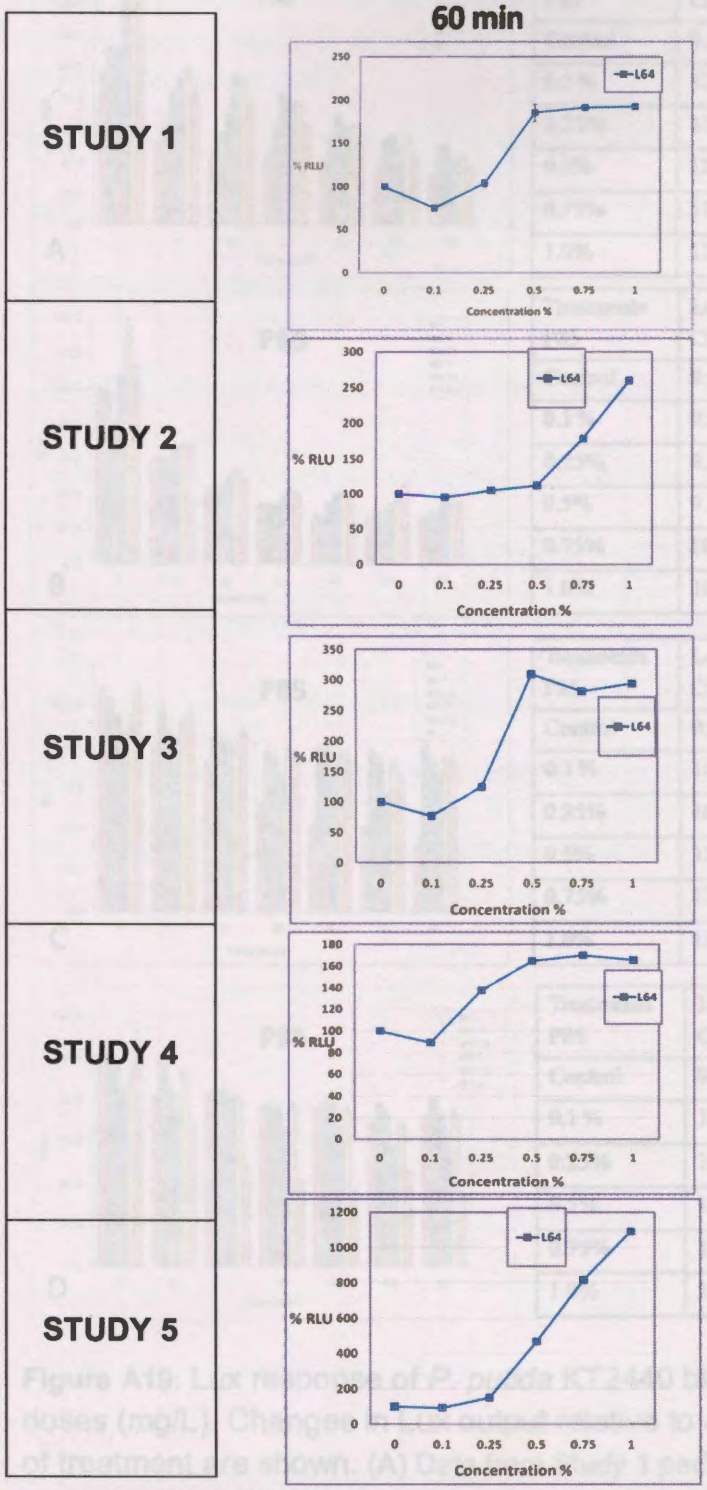
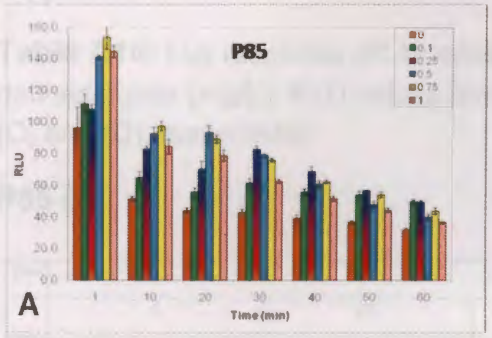
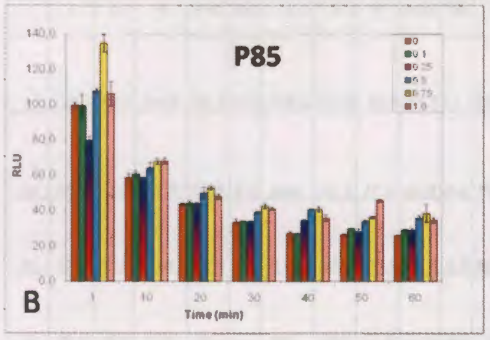


Figure A18: % RLU relative to control for defined concentrations of Pluronics L64 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

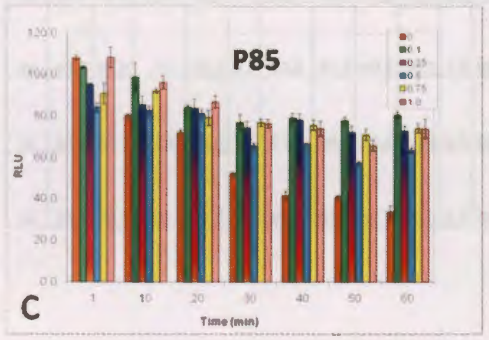
Pluronic P85: RLU vs. Time Graph



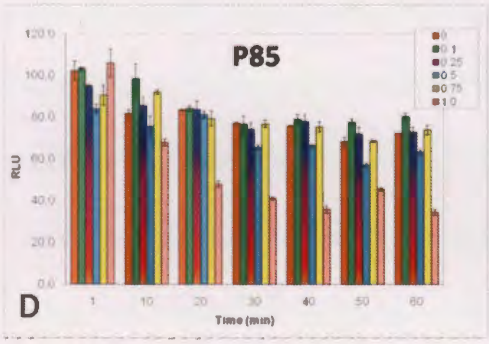
Treatments P85	Log 10 Cfu/ml	RLU at 60 min
Control	9.3±0.0	32.1
0.1 %	12.8±0.0	49.8
0.25%	13.2±0.2	49.7
0.5%	12.5±0.5	40.1
0.75%	11.7±1.6	44.1
1.0%	11.5±0.2	36.7



Treatments P85	Log 10 Cfu/ml	% RLU at 60 min
Control	9.5±0.1	100±0.3
0.1 %	9.5±1.1	111.4±1.2
0.25%	9.5±1.4	111.1±2.0
0.5%	9.5±0.6	137.9±2.0
0.75%	10.6±1.2	147.1±8.2
1.0%	10.6±1.3	132.9±2.3



Treatments P85	Log 10 Cfu/ml	% RLU at 60 min
Control	9.5±0.1	100±4.7
0.1 %	10.3±1.1	238.8±2.6
0.25%	10.6±1.4	216.7±240
0.5%	11.1±0.6	188.6±2.0
0.75%	12.1±1.2	219.6±3.8
1.0%	11.8±1.3	219.3±7.9



Treatments P85	Log 10 Cfu/ml	% RLU at 60 min
Control	9.5±1.0	100±0.1
0.1 %	11.3±1.2	111.0±2.6
0.25%	11.6±1.0	100.6±4.0
0.5%	12.1±1.0	87.6±2.0
0.75%	12.1±1.2	102.1±3.8
1.0%	10.8±1.4	47.8±2.3

Figure A19: Lux response of *P. putida* KT2440 biosensor to P85 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 12-8-2008, (B) Study 2 performed on 12-10-2008, (C) Study 3 performed on 12-20-2008, (D) Study 4 performed on 12-18-2008. Means of analytical replicates and standard errors are shown for each study. All

the three studies with Pluronic P85 were performed within a month of its receiving from the supplier.

Table A10: Lux response (RLU values) of *P. putida* KT2440 biosensor to P85 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables P85 (A), (B), (C) and (D), respectively

P85 (A)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	120.3	75.1	94.0	96.4	22.7	118.1	117.8	98.7	111.6	11.1	110.8	111.3	102.5	108.2	4.9	142.5	142.1	139.4	141.3	1.7	144.5	163.0	155.4	154.3	9.3	150.1	137.4	147.0	144.8	6.6
10	53.2	52.9	49.2	51.8	2.2	60.0	62.3	72.3	64.9	6.5	80.1	84.0	85.7	83.2	2.8	86.2	100.7	81.4	92.8	7.3	92.6	102.6	98.0	97.7	5.0	80.4	80.2	93.9	84.8	7.9
20	41.3	47.6	44.3	44.4	3.1	51.1	59.7	57.8	56.2	4.5	62.7	79.7	68.7	70.4	8.6	84.7	97.3	99.0	93.7	7.8	93.1	90.3	84.9	89.4	4.2	72.3	82.1	82.7	79.0	5.8
30	46.5	39.2	43.8	43.2	3.7	56.7	60.8	67.3	61.6	5.3	78.8	86.2	83.9	83.0	3.8	77.7	80.2	80.0	79.3	1.4	74.5	78.3	76.4	76.4	1.9	63.5	61.0	64.5	63.0	1.8
40	41.3	41.5	35.1	39.3	3.6	53.8	54.5	60.3	56.2	3.6	69.0	74.4	63.4	69.0	5.5	64.1	57.4	61.8	61.1	3.4	62.4	64.6	61.0	62.7	1.8	49.2	52.9	54.1	52.1	2.6
50	35.0	38.1	37.6	36.9	1.7	51.3	53.0	58.8	54.4	3.9	56.8	55.9	57.8	56.8	1.0	44.9	47.5	52.0	48.1	3.8	55.7	50.9	56.3	54.3	2.9	42.8	44.5	47.0	44.8	2.1
60	30.1	33.5	32.8	32.1	1.8	46.6	51.9	51.1	49.8	2.8	50.0	47.3	51.7	49.7	2.2	38.4	40.7	43.0	40.1	3.4	42.7	41.7	47.9	44.1	3.3	36.4	37.8	36.0	36.7	1.0

P85 (B)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	100.8	101.2	96.3	99.4	2.7	93.1	112.2	92.7	99.3	11.1	74.5	82.9	81.1	79.5	4.4	109.5	105.1	108.3	107.6	2.3	128.7	144.3	130.7	134.6	8.5	107.7	93.6	117.2	106.1	11.9
10	63.3	56.4	56.4	58.7	4.0	62.5	62.6	57.2	60.8	3.1	59.0	58.9	58.4	58.7	0.3	68.3	58.2	66.1	64.2	5.3	70.8	65.2	67.4	67.8	2.8	68.8	65.0	70.3	68.0	2.7
20	44.6	42.7	44.1	43.8	1.0	45.6	42.0	45.4	44.4	2.0	42.8	44.2	45.1	44.0	1.2	51.7	54.4	44.1	50.1	5.4	55.1	51.8	51.7	52.9	1.9	49.6	45.2	49.5	48.1	2.5
30	30.4	35.4	35.2	33.7	2.8	33.8	34.0	34.6	34.1	0.4	34.0	34.5	33.3	33.9	0.6	40.2	38.6	39.4	39.4	0.8	40.2	44.1	43.4	42.6	2.1	42.6	40.6	40.4	41.2	1.2
40	26.6	29.3	25.6	27.2	1.9	26.9	27.5	26.8	27.1	0.4	33.4	35.4	34.9	34.5	1.0	40.9	40.9	41.2	41.0	0.2	38.7	43.6	40.6	41.0	2.5	35.7	33.3	39.1	36.0	2.9
50	27.0	25.3	26.9	26.4	1.0	29.5	29.8	30.5	29.9	0.5	28.5	25.7	31.0	28.4	2.6	35.2	32.3	34.5	34.0	1.5	35.7	37.5	36.2	36.4	0.9	46.6	44.5	46.2	45.8	1.1
60	25.9	26.5	25.9	26.1	0.3	30.4	28.4	28.3	29.1	1.2	30.9	26.9	29.4	29.0	2.0	34.6	38.3	35.0	36.0	2.0	29.5	45.5	40.2	38.4	8.2	32.5	37.2	34.5	34.7	2.3

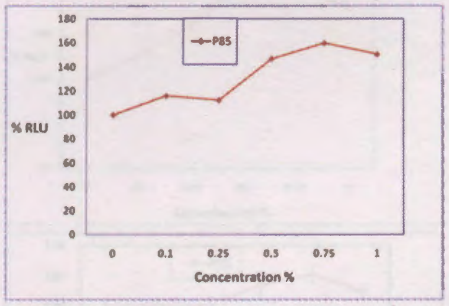
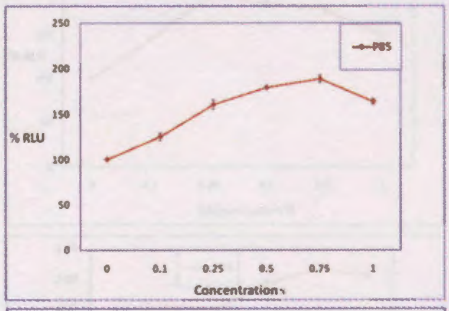
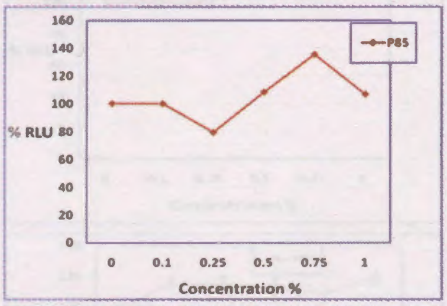
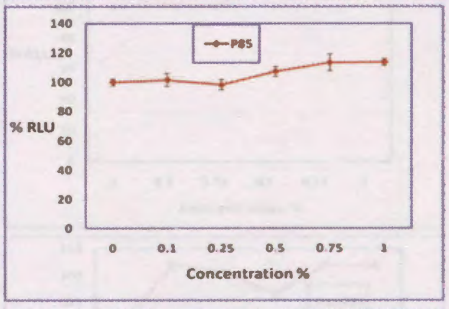
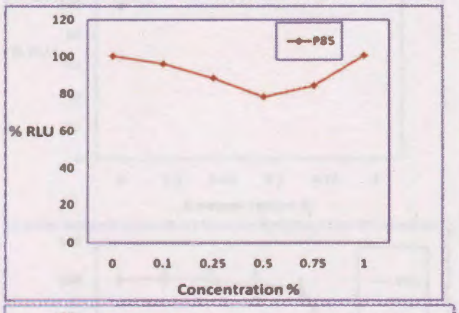
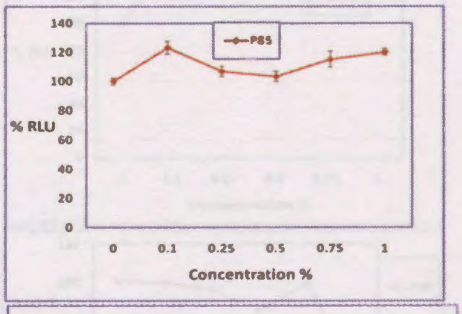
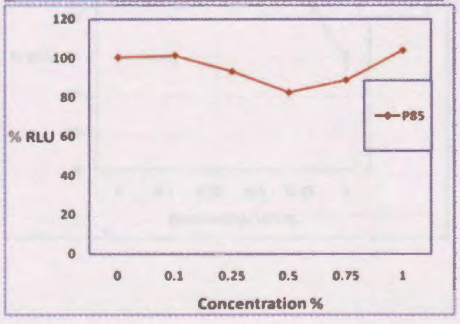
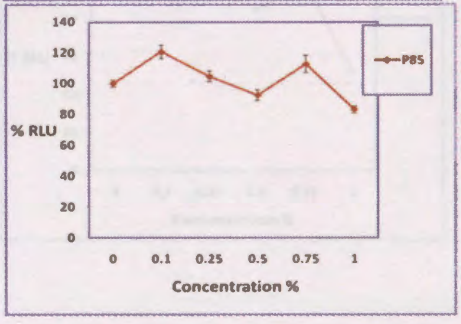
P85 (C)

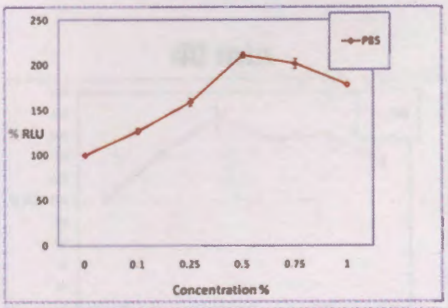
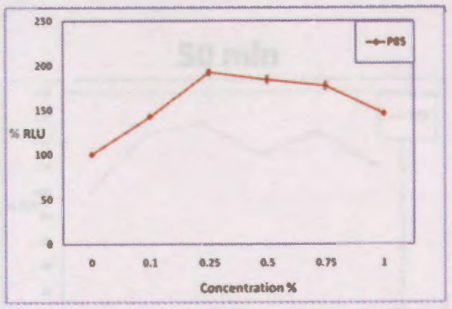
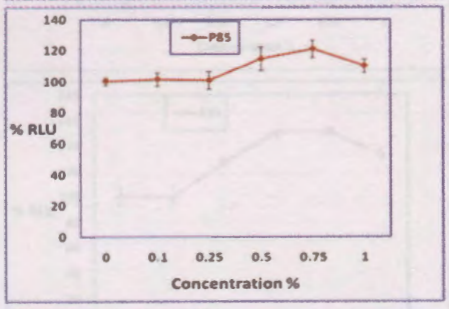
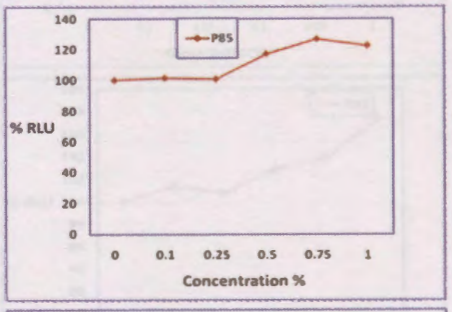
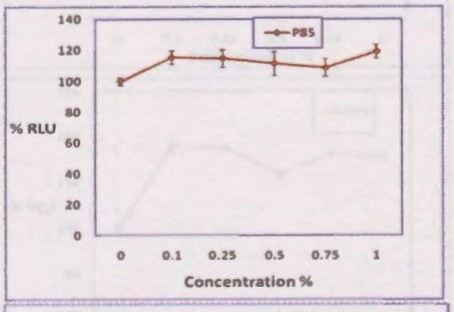
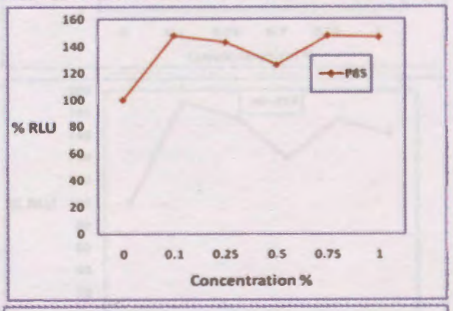
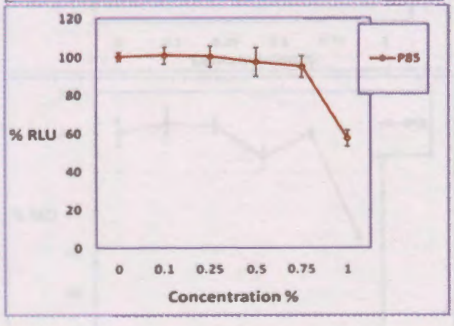
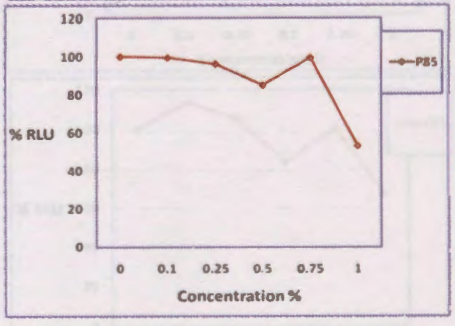
Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	105.8	110.0	108.1	108.0	2.1	104.5	102.7	103.0	103.4	1.0	95.0	94.3	96.0	95.1	0.8	86.0	80.0	86.5	84.2	3.6	82.0	90.8	99.1	90.6	8.6	107.7	100.3	117.2	108.4	8.5
10	81.4	79.1	80.0	80.2	1.2	86.4	99.5	110.3	98.7	12.0	92.3	86.0	78.4	85.5	7.0	84.2	85.0	79.0	82.7	3.3	92.3	90.2	94.0	92.2	1.9	99.0	100.0	90.0	96.3	5.5
20	74.7	71.2	70.1	72.0	2.4	81.9	85.1	85.6	84.2	2.0	76.6	83.3	91.3	83.7	7.3	77.9	82.7	83.4	81.3	3.0	86.3	77.7	73.9	79.3	6.4	89.9	89.9	81.0	86.9	5.1
30	52.2	50.9	53.4	52.2	1.3	69.9	83.2	77.7	76.9	6.7	77.0	68.0	78.2	74.4	5.6	63.5	67.0	66.9	65.8	2.0	79.1	73.5	78.2	76.9	3.0	77.9	72.3	79.0	76.4	3.6
40	45.4	40.0	39.8	41.7	3.2	80.2	82.3	75.1	79.2	3.7	73.8	77.5	83.5	78.2	4.9	67.3	65.4	66.6	66.4	1.0	72.7	73.8	80.2	75.6	4.1	72.1	81.0	69.0	74.0	6.2
50	41.0	42.2	40.0	41.1	1.1	80.3	74.7	78.3	77.8	2.9	74.8	66.2	75.6	72.2	5.2	58.4	55.1	58.1	57.2	1.8	67.9	68.8	76.2	71.0	4.6	69.8	66.5	61.0	65.8	4.5
60	39.0	32.2	30.0	33.7	4.7	82.4	81.6	77.6	80.6	2.6	71.8	69.7	77.5	73.0	4.0	61.3	64.7	64.8	63.6	2.0	78.2	73.4	70.7	74.1	3.8	81.0	75.3	65.5	73.9	7.9

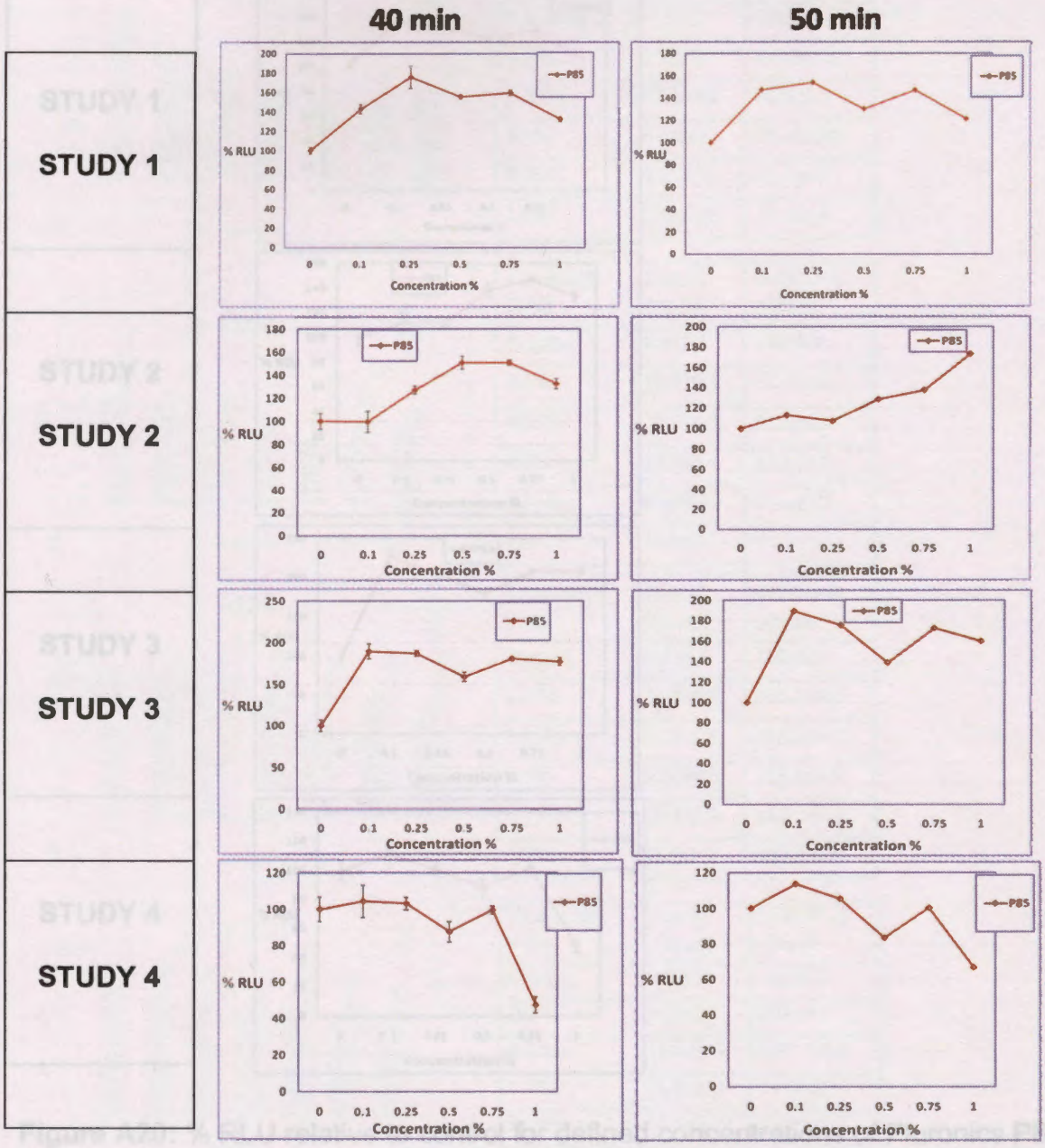
P85 (D)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	105.8	93.0	108.1	102.3	8.2	104.5	102.7	103.0	103.4	1.0	95.0	94.3	96.0	95.1	0.8	86.0	80.0	86.5	84.2	3.6	82.0	90.8	99.1	90.6	8.6	107.7	93.5	117.2	106.1	11.9
10	81.4	79.1	85.2	81.9	3.1	86.4	99.5	110.3	98.7	12.0	92.3	86.0	78.4	85.5	7.0	68.6	85.0	73.4	75.7	8.4	92.3	90.2	94.0	92.2	1.9	68.8	65.0	70.3	68.0	2.7
20	83.2	83.3	84.1	83.5	0.5	81.9	85.1	85.6	84.2	2.0	76.6	83.3	91.3	83.7	7.3	77.9	82.7	83.4	81.3	3.0	86.3	77.7	73.9	79.3	6.4	49.6	45.2	49.5	48.1	2.5
30	77.5	78.1	76.4	77.3	0.8	69.9	83.2	77.7	76.9	6.7	77.0	68.0	78.2	74.4	5.6	63.5	67.0	66.9	65.8	2.0	79.1	73.5	78.2	76.9	3.0	42.6	40.6	40.4	41.2	1.2
40	76.0	76.2	75.5	75.9	0.4	80.2	82.3	75.1	79.2	3.7	73.8	77.5	83.5	78.2	4.9	67.3	65.4	66.6	66.4	1.0	72.7	73.8	80.2	75.6	4.1	35.7	33.3	39.1	36.0	2.9
50	64.6	71.4	69.3	68.5	3.5	80.3	74.7	78.3	77.8	2.9	74.8	66.2	75.6	72.2	5.2	58.4	55.1	58.1	57.2	1.8	67.9	68.8	69.4	68.7	0.8	46.6	44.5	46.2	45.8	1.1
60	72.7	72.6	72.5	72.6	0.1	82.4	81.6	77.6	80.6	2.6	71.8	69.7	77.5	73.0	4.0	61.3	64.7	64.8	63.6	2.0	78.2	73.4	70.7	74.1	3.8	32.5	37.2	34.5	34.7	2.3

Pluronic P85: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
STUDY 4
% RLU
Concentration %
PBS
0
0.1
0.25
0.5
0.75
1

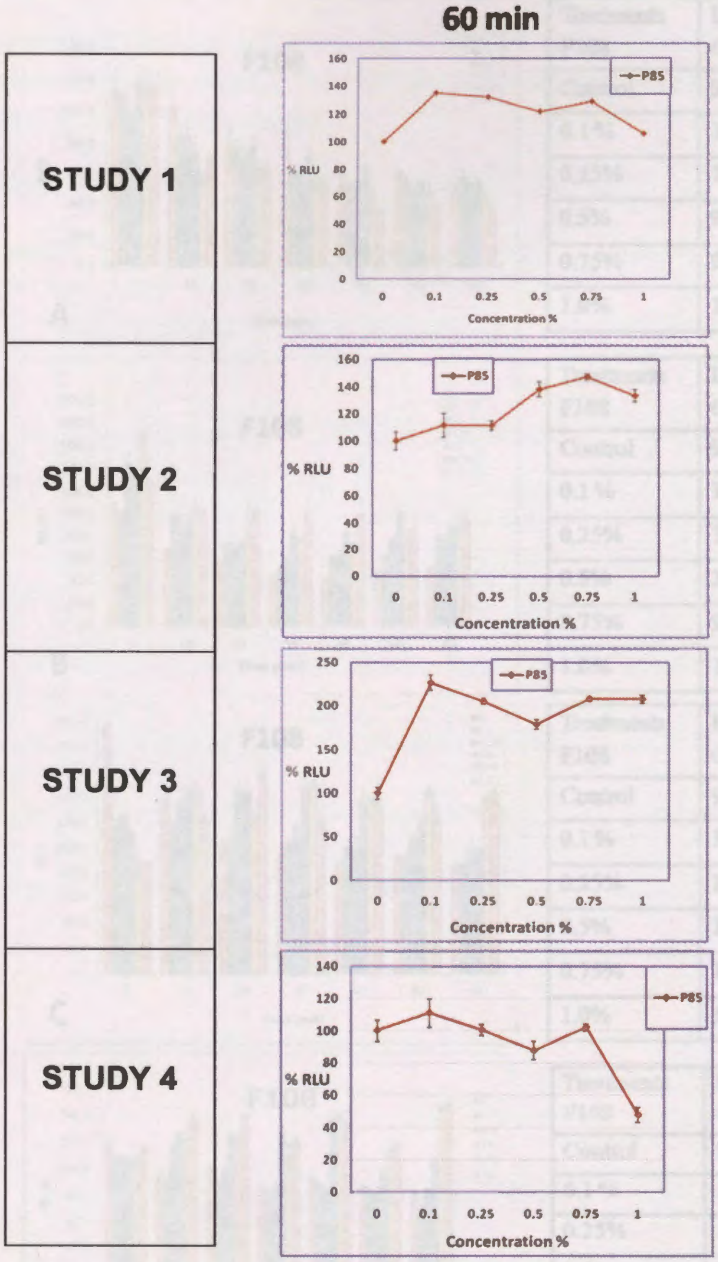
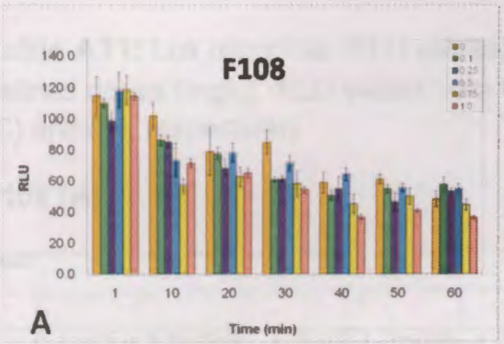
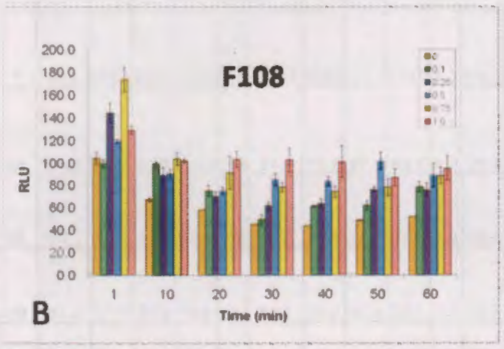


Figure A20: % RLU relative to control for defined concentrations of Pluronics P85 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

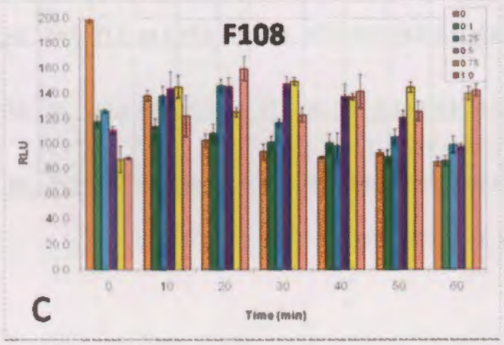
Pluronic F108: RLU vs. Time Graph



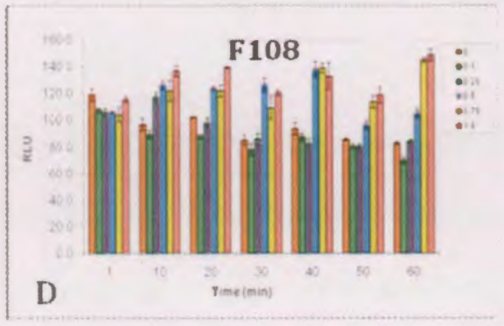
Treatments F108	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.1	100±0.9
0.1 %	10.5±0.1	119.2±1.0
0.25%	10.3±0.2	66.9±8.5
0.5%	9.5±0.1	63.1±13.8
0.75%	9.9±0.3	63.1±4.1
1.0%	10.4±1.2	57.1±5.0



Treatments F108	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.1	100±0.0
0.1 %	10.5±0.1	78.4±0.2
0.25%	10.4±0.2	75.5±0.4
0.5%	10.0±0.1	89.0±0.6
0.75%	9.9±0.3	88.6±0.2
1.0%	11.2±0.1	95.3±5.0



Treatments F108	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.1	100±5.6
0.1 %	11.5±0.1	101.2±6.5
0.25%	11.4±0.3	116.2±11.1
0.5%	10.0±0.1	114.5±3.8
0.75%	10.2±0.2	163.7±9.5
1.0%	11.2±0.1	166.5±8.5



Treatments F108	Log 10 Cfu./ml	% RLU at 60 min
Control	9.7±0.4	100±2.1
0.1 %	11.5±0.1	842±4.2
0.25%	11.4±0.3	101.7±2.0
0.5%	11.0±0.4	127.1±5.3
0.75%	11.2±0.2	175.0±2.7
1.0%	11.2±0.1	179.6±7.3

Figure A21: Lux response of *P. putida* KT2440 biosensor to F108 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min of treatment are shown. (A) Data from Study 1 performed on 6-30-2008, (B) Study 2 performed on 1-28-2009, (C) Study 3 performed on 2-6-2009, (D) Study 4 performed on 2-12-2009. Means of analytical replicates and standard errors are shown for each study. All the

three studies with Pluronic F108 were performed within 2 months of its receiving from the supplier.

Table A11: Lux response (RLU values) of *P. putida* KT2440 biosensor to F108 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables F108 (A), (B), (C) and (D), respectively

F108 (A)

Time	Concentration%																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	91.5	132.6	120.2	114.8	21.1	104.9	109.2	115.6	109.9	5.3	104.3	103.3	87.3	98.5	9.7	117.2	99.1	137.8	118.0	19.4	100.5	124.0	130.6	118.3	15.8	110.5	118.6	114.2	114.4	4.1
10	110.1	112.5	91.3	104.6	11.6	91.4	79.7	87.7	86.2	6.0	79.0	91.4	85.1	85.2	6.2	81.5	52.4	86.6	73.5	18.5	65.0	55.4	49.8	56.8	7.7	67.2	70.7	76.3	71.4	4.6
20	102.2	84.5	50.0	78.9	26.5	77.7	84.4	70.7	77.6	6.9	69.2	57.2	77.7	68.0	10.3	68.1	89.2	77.3	78.2	10.5	50.0	68.7	69.2	62.6	11.0	63.8	60.4	70.7	65.0	5.2
30	97.2	87.0	70.2	84.8	13.8	57.4	62.2	62.6	60.7	2.0	58.5	65.2	59.6	61.1	3.6	65.9	68.0	81.4	71.8	8.4	71.1	43.8	59.7	58.2	13.7	52.1	52.0	58.5	54.2	3.7
40	56.5	71.6	48.3	58.8	11.8	47.6	47.7	56.5	50.6	5.1	46.8	70.5	47.6	54.9	13.5	67.3	69.5	56.5	64.4	7.0	35.3	43.4	56.5	45.0	10.7	33.6	36.7	39.2	36.5	2.8
50	66.1	61.2	55.5	60.9	5.3	57.4	49.6	57.6	54.9	4.6	36.7	45.5	56.7	46.3	10.0	52.4	53.8	60.4	55.5	4.3	39.9	52.0	53.2	50.0	9.3	41.3	42.3	38.8	40.8	1.8
60	44.7	42.2	57.9	48.3	8.4	51.8	66.7	54.2	57.6	8.0	50.1	56.2	53.4	53.2	3.0	48.3	59.8	57.1	55.0	6.0	45.0	50.3	38.2	44.5	6.1	34.7	36.6	38.2	36.5	1.7

F108 (B)

Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	100.1	115.2	98.0	104.4	9.4	106.5	94.8	97.0	99.7	6.0	137.8	133.8	162.1	144.6	15.3	118.8	122.7	117.8	119.8	2.6	175.2	154.5	191.1	173.6	18.4	130.0	122.5	134.9	129.2	6.2
10	69.3	63.6	68.8	67.2	3.1	102.9	101.2	95.9	100.0	3.6	74.7	93.8	98.6	89.0	12.7	89.4	85.4	98.1	91.0	6.5	95.3	114.5	102.1	103.9	9.7	100.0	102.1	105.3	102.5	2.7
20	59.5	57.5	58.0	58.3	1.0	82.5	76.2	66.3	75.0	8.1	63.5	68.9	77.1	69.8	6.9	79.3	76.4	69.2	74.9	5.2	61.4	105.3	107.5	91.4	26.0	95.1	97.8	117.2	103.4	12.1
30	44.3	44.7	45.3	44.8	0.5	43.7	58.1	46.8	49.5	7.6	56.2	65.9	63.0	61.7	5.0	73.7	91.9	89.4	85.0	9.9	70.7	84.8	78.9	78.2	7.1	87.2	97.5	124.1	102.9	19.0
40	42.9	44.0	43.2	43.4	0.6	62.5	60.9	60.9	61.4	0.9	55.1	65.2	69.9	63.4	7.6	80.8	78.4	91.7	83.6	7.1	70.4	83.6	68.3	74.1	8.3	76.0	101.5	125.9	101.1	24.9
50	48.4	49.4	48.5	48.8	0.5	56.0	60.3	69.1	61.8	6.6	77.6	79.3	70.8	75.9	4.5	89.1	101.3	115.8	102.1	13.4	90.1	80.1	63.4	77.9	13.5	91.6	72.5	96.9	87.0	12.8
60	52.1	52.3	51.2	51.9	0.6	74.6	73.3	87.3	78.4	7.7	65.2	75.4	85.9	75.5	10.3	67.9	94.0	105.0	89.0	19.0	85.4	76.8	103.6	88.6	13.7	83.9	84.5	117.5	95.3	19.3

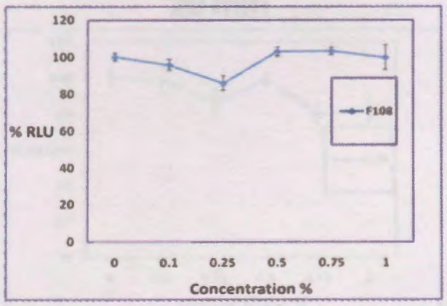
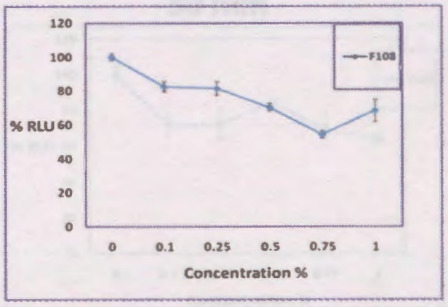
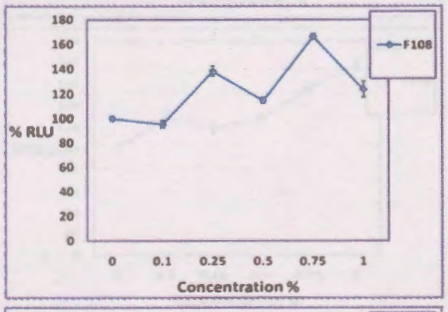
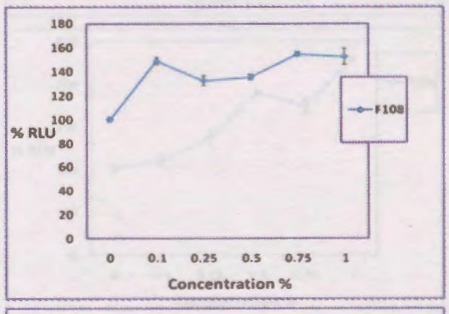
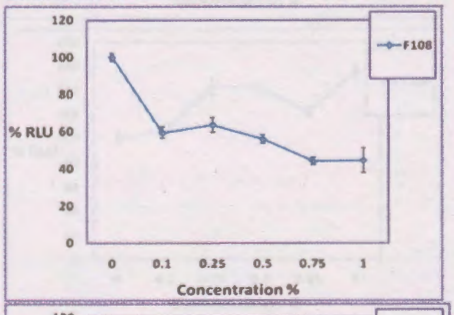
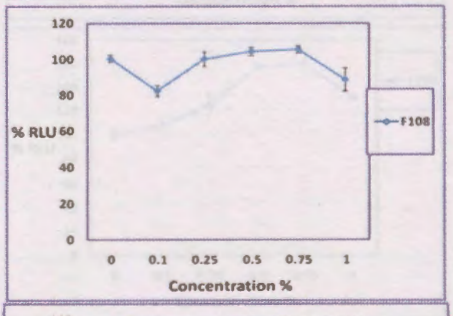
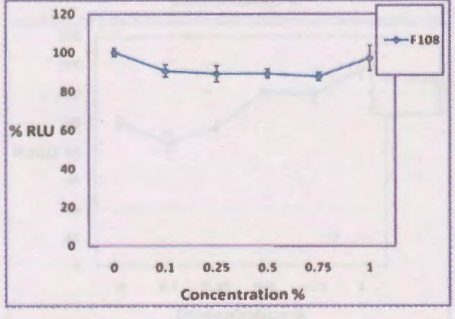
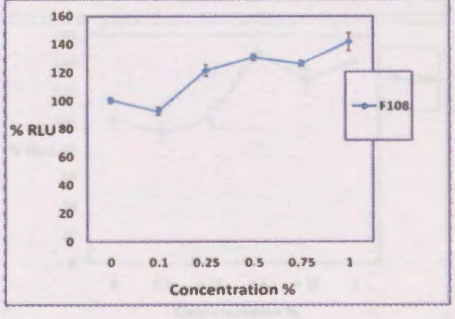
F108 (C)

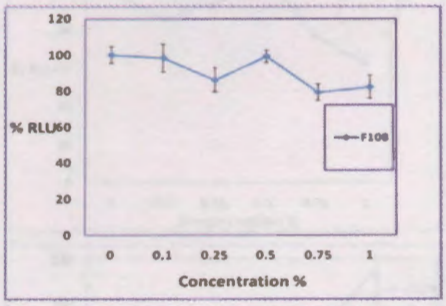
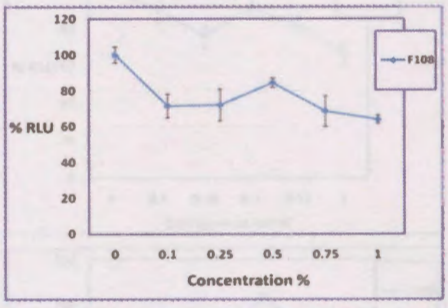
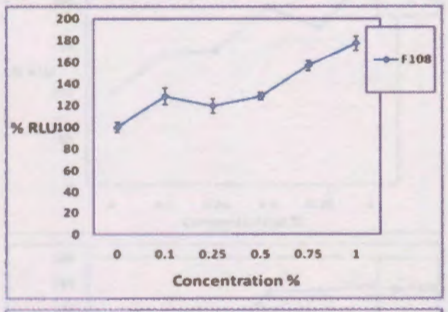
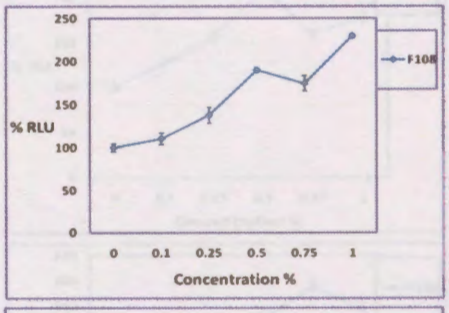
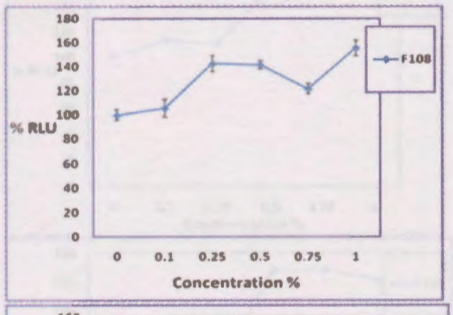
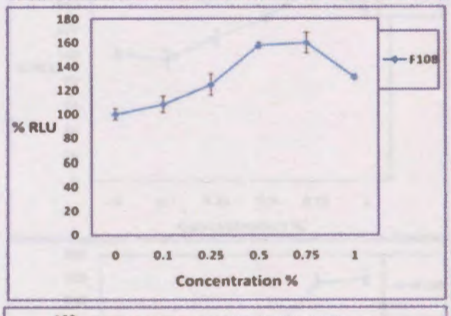
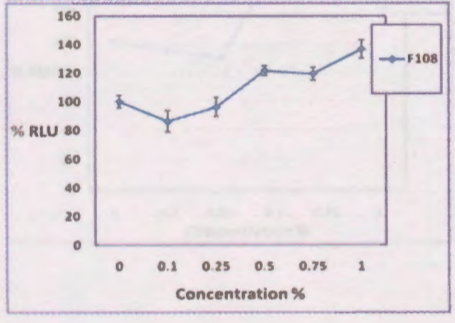
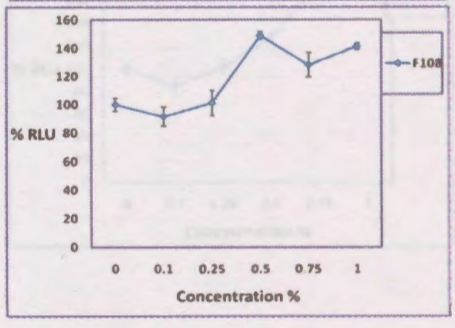
Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	203.2	196.3	196.2	198.6	4.0	119.8	124.4	109.2	117.8	7.8	126.3	126.2	123.8	126.1	2.2	112.2	114.8	105.1	110.7	5.0	76.3	108.6	77.2	87.3	18.4	90.1	87.5	87.0	88.2	1.7
10	130.1	142.9	141.5	138.2	7.0	100.4	118.9	121.2	113.5	11.4	149.3	123.6	141.3	138.0	13.1	119.0	146.5	165.6	143.7	23.4	162.8	140.9	132.4	145.3	15.7	108.5	103.3	154.4	122.1	28.2
20	105.3	92.5	110.2	102.7	9.2	97.7	106.2	121.6	108.5	12.1	144.5	155.7	139.1	146.4	8.4	148.7	155.1	132.5	145.4	11.6	124.0	119.2	131.8	125.0	6.3	174.3	163.1	141.4	159.6	16.8
30	100.2	81.2	99.8	93.7	10.8	96.6	97.4	110.5	101.5	7.8	114.8	113.9	121.9	116.9	4.4	139.1	158.3	145.4	147.6	9.8	149.4	154.8	144.5	149.6	5.2	113.3	122.9	132.6	122.9	9.6
40	87.1	89.9	89.9	89.0	1.6	100.5	112.9	88.8	100.7	12.0	77.8	108.5	108.6	98.3	17.7	118.5	149.8	144.1	137.5	16.7	141.9	135.3	134.5	137.2	4.1	127.0	129.5	168.0	141.5	23.0
50	88.6	94.2	95.7	92.8	3.8	78.4	91.9	97.9	89.4	10.0	111.2	111.4	93.4	105.3	10.3	125.5	126.7	109.8	120.7	9.5	136.6	150.5	147.5	144.9	7.3	121.7	114.6	140.6	125.7	13.4
60	85.5	91.0	79.7	85.4	5.6	91.4	88.6	79.0	86.4	6.5	106.9	104.3	86.8	99.2	11.1	95.8	102.2	95.3	97.8	3.8	130.2	149.2	139.9	139.8	9.5	132.9	144.3	149.4	142.2	8.5

F108 (D)

Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	127.2	117.9	111.6	118.9	7.8	105.7	109.9	107.4	107.7	2.2	104.8	101.8	110.9	105.8	4.7	105.2	105.8	106.8	105.9	0.8	94.9	104.3	113.4	104.2	9.3	118.9	115.9	111.7	115.5	3.6
10	88.0	100.1	102.3	96.8	7.7	90.3	84.0	93.8	89.4	5.0	116.6	123.1	111.7	117.1	5.7	124.1	132.2	122.8	126.4	5.1	116.2	112.6	137.1	122.0	13.3	130.6	135.6	144.7	137.0	7.1
20	102.6	101.2	102.1	102.0	0.7	86.1	87.1	90.1	87.7	2.1	92.5	104.7	96.8	98.0	6.2	121.7	124.5	125.8	124.0	2.1	113.7	122.1	129.7	121.8	8.0	138.3	140.7	139.7	139.6	1.2
30	78.7	91.8	85.0	85.2	6.5	86.2	73.1	74.9	78.1	7.1	84.4	80.4	93.8	86.2	6.9	122.7	136.6	121.5	126.9	8.4	100.1	101.1	126.5	109.2	14.9	118.4	124.3	118.5	120.4	3.4
40	86.9	91.2	102.4	93.5	8.0	92.0	87.7	83.2	87.6	4.4	75.5	85.4	87.0	82.6	6.2	127.7	140.3	147.3	138.4	9.9	133.1	144.9	138.9	139.0	5.9	151.4	130.4	117.1	133.0	17.3
50	84.2	86.7	85.8	85.6	1.3	82.9	77.0	81.3	80.4	3.1	78.7	84.0	78.2	80.3	3.2	98.5	90.8	100.7	96.7	5.2	122.7	111.6	107.2	113.8	8.0	107.0	125.8	123.2	118.6	10.2
60	85.2	81.6	81.6	82.8	2.1	73.8	70.1	65.2	69.7	4.3	85.4	81.9	85.2	84.2	2.0	105.4	110.4	99.8	105.2	5.3	142.7	144.0	147.9	144.9	2.7	156.9	143.1	146.1	148.7	7.3

Pluronic F108: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

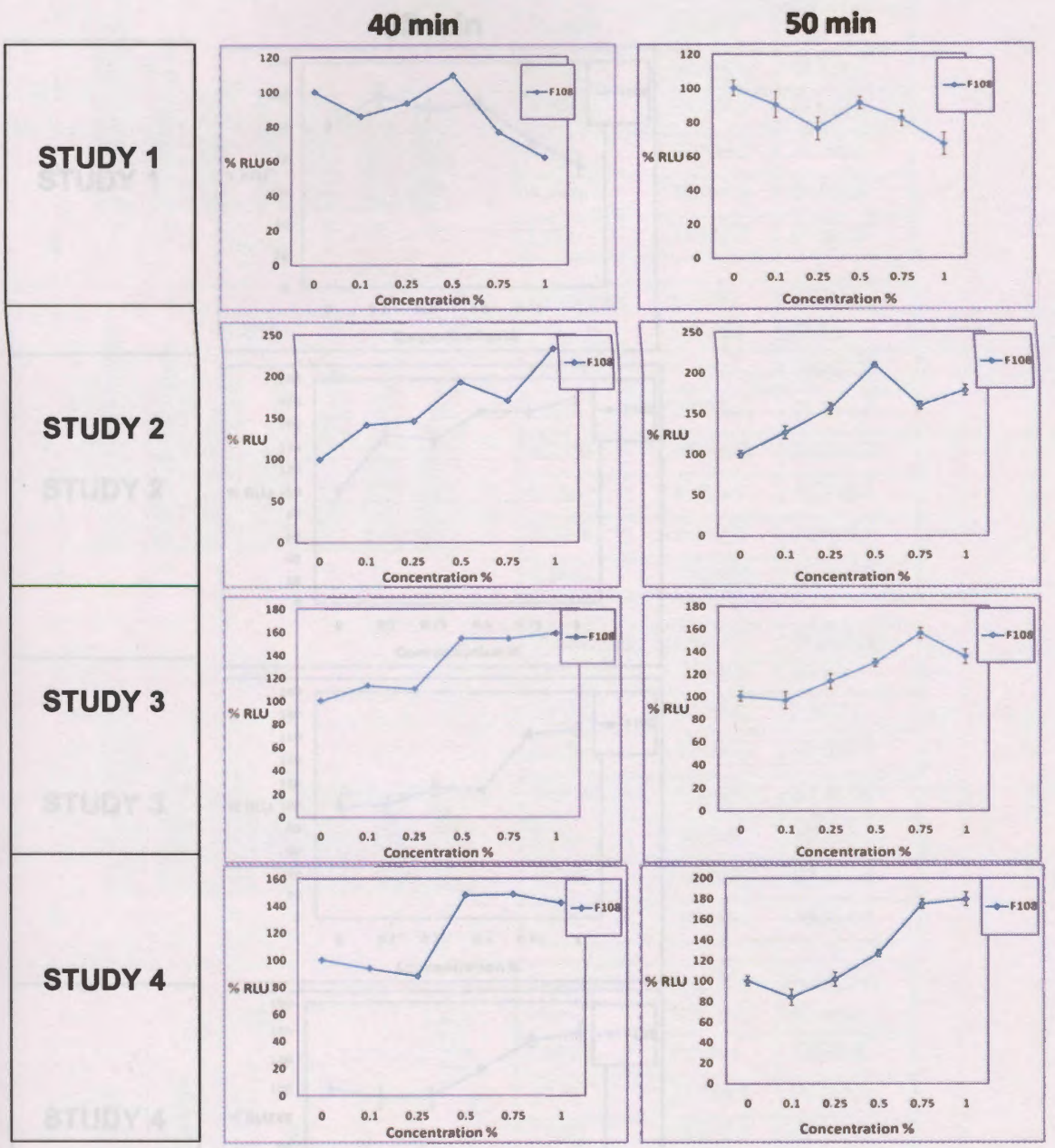


Figure A22: % RLU relative to control for defined concentrations of Pluronics F108 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

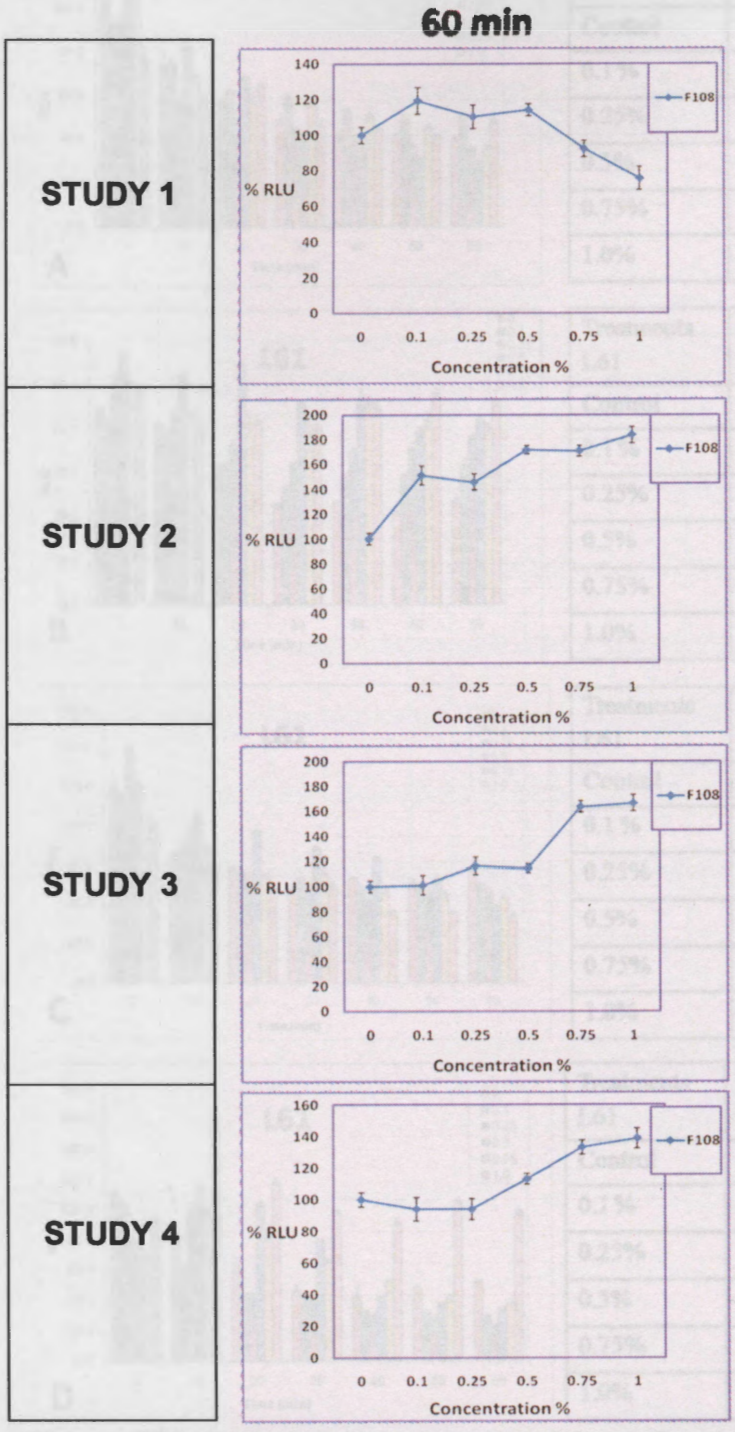
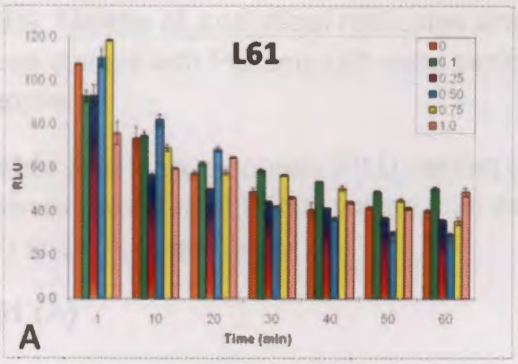
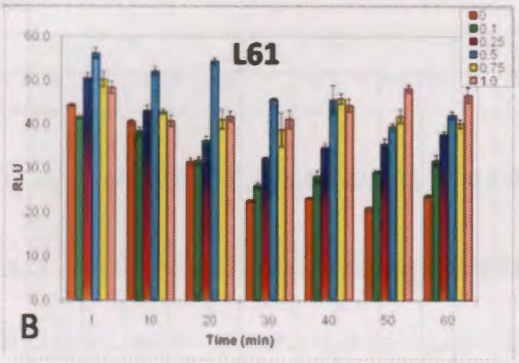


Figure A22: % RLU relative to control for defined concentrations of Pluronics F108 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

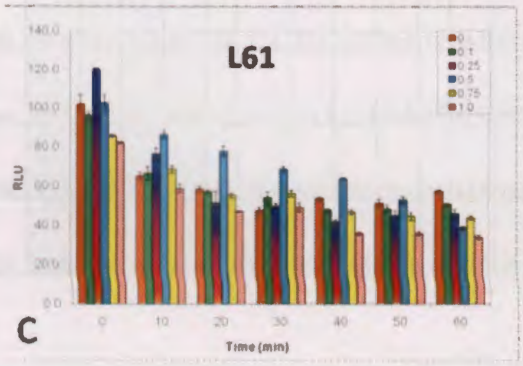
Pluronic L61: RLU vs. Time Graph



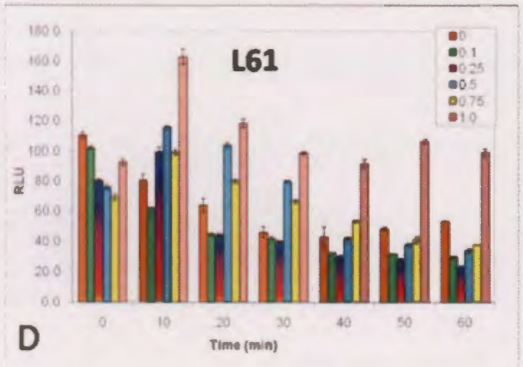
Treatments L61	Log 10 Cfu./ml	% RLU at 60 min
Control	9.4±0.1	100±0.9
0.1 %	10.0±0.1	50.5±0.1
0.25%	10.4±0.2	35.8±0.3
0.5%	10.0±0.1	29.5±0.5
0.75%	10.0±0.3	35.5±0.4
1.0%	11.9±0.1	49.0±0.2



Treatments L61	Log 10 Cfu./ml	% RLU at 60 min
Control	9.1±0.1	100±0.9
0.1 %	12.0±0.1	31.8±0.1
0.25%	11.4±0.2	37.8±0.3
0.5%	9.6±0.1	42.2±0.5
0.75%	9.9±0.3	40.2±0.4
1.0%	11.0±0.1	46.7±0.2



Treatments L61	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.4	100±1.5
0.1 %	11.0±0.3	88.5±0.2
0.25%	11.4±0.2	80.8±3.6
0.5%	10.9±0.1	68.2±1.1
0.75%	10.7±0.3	77.3±1.8
1.0%	11.0±0.1	59.8±1.7



Treatments L61	Log 10 Cfu./ml	% RLU at 60 min
Control	9.5±0.4	100±0.6
0.1 %	11.0±0.3	56.1±0.9
0.25%	10.4±0.2	44.7±0.1
0.5%	11.9±0.3	64.5±1.4
0.75%	11.7±0.3	71.3±0.2
1.0%	11.0±0.2	185.8±4.6

Figure A23: Lux response of *P. putida* KT2440 biosensor to L61 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min

of treatment are shown. (A) Data from Study 1 performed on 1-15-2009, (B) Study 2 performed on 1-23-2009, (C) Study 3 performed on 12-17-2008, (D) Study 4 performed on 1-12-2009. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic L61 were performed within 2 months of its receiving from the supplier.

Table A12: Lux response (RLU values) of *P. putida* KT2440 biosensor to L61 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables L61 (A), (B), (C) and (D), respectively

L61 (A)

Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	107.1	108.0	107.9	107.7	0.5	97.9	90.1	90.9	93.0	4.3	102.9	90.2	86.8	93.3	8.5	102.7	115.5	113.6	110.6	6.9	117.8	119.4	118.1	118.4	0.8	71.3	86.9	69.8	76.0	9.5
10	67.4	84.3	69.0	73.6	9.3	75.8	77.5	71.6	75.0	3.0	57.6	54.8	57.4	56.6	1.6	80.1	86.7	80.5	82.4	3.7	67.0	72.1	68.5	69.2	2.6	59.5	60.4	60.0	60.0	0.4
20	57.3	56.1	59.7	57.7	1.8	61.4	61.8	63.5	62.3	1.2	50.5	50.7	49.9	50.4	0.4	68.7	70.5	66.8	68.7	1.8	58.7	55.6	59.5	57.9	2.1	65.6	64.4	64.4	64.8	0.7
30	45.7	49.2	52.3	49.1	3.3	57.6	59.1	60.0	58.9	1.2	44.1	45.5	43.7	44.4	0.9	42.4	41.4	42.8	42.2	0.7	56.1	57.1	57.1	56.7	0.6	45.6	46.9	46.9	46.5	0.8
40	39.8	35.8	47.1	40.9	5.7	54.0	54.0	52.8	53.6	0.7	41.7	41.7	41.2	41.5	0.3	36.8	38.0	36.1	37.0	0.9	49.5	52.8	49.7	50.7	1.8	43.9	45.5	43.5	44.3	1.1
50	41.3	42.6	41.9	41.9	0.7	48.0	49.5	49.7	49.1	0.9	37.4	38.4	37.4	37.7	0.6	30.9	28.9	31.2	30.1	1.6	46.3	45.4	43.6	45.1	1.4	42.0	40.3	42.1	41.5	1.0
60	40.7	39.3	41.1	40.4	0.9	51.5	49.5	50.5	50.5	1.0	35.0	36.7	35.5	35.7	0.9	28.7	30.3	29.9	29.6	0.8	37.7	33.3	35.5	35.5	2.2	46.7	51.3	48.6	48.9	2.3

L61 (B)

Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	45.1	44.5	44.1	44.5	0.5	41.6	41.9	41.0	41.5	0.5	48.9	50.6	52.6	50.7	1.9	54.1	57.4	57.4	56.3	1.9	50.9	53.0	47.0	50.3	3.0	47.4	46.8	51.2	48.5	2.4
10	40.2	40.8	41.4	40.8	0.6	38.4	37.0	40.3	38.6	1.6	43.5	45.1	40.9	43.2	2.1	50.8	51.8	54.2	52.2	1.7	44.2	42.8	42.1	43.0	1.1	40.8	38.7	43.1	40.9	2.2
20	33.1	31.5	29.9	31.5	1.6	31.4	33.3	31.2	31.9	1.1	37.0	37.7	34.4	36.4	1.7	55.1	55.2	53.2	54.5	1.1	42.1	44.3	37.2	41.2	3.7	41.4	44.1	40.1	41.9	2.0
30	22.5	22.5	23.0	22.7	0.3	25.7	27.2	25.3	26.1	1.0	32.1	31.7	32.8	32.2	0.5	46.2	45.7	45.4	45.8	0.4	31.2	42.0	43.4	38.9	6.7	38.8	43.7	40.8	41.1	2.5
40	23.4	22.8	23.1	23.1	0.3	30.3	28.0	26.2	28.2	2.1	34.8	33.3	36.4	34.8	1.6	39.3	50.2	47.6	45.7	5.7	44.8	48.2	44.8	45.9	2.0	41.8	44.3	47.1	44.4	2.7
50	20.7	21.4	21.1	21.1	0.3	28.9	29.7	28.8	29.1	0.5	33.3	37.6	35.9	35.6	2.1	38.8	39.5	40.7	39.7	0.9	39.0	44.1	42.7	41.9	2.6	46.7	48.6	49.2	48.2	1.3
60	24.0	24.1	23.2	23.8	0.5	30.3	30.8	34.2	31.8	2.1	36.5	38.8	38.1	37.8	1.2	43.6	41.3	41.6	42.2	1.2	41.5	40.8	38.3	40.2	1.7	43.2	48.3	48.5	46.7	3.0

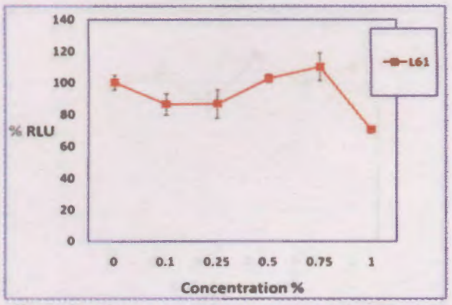
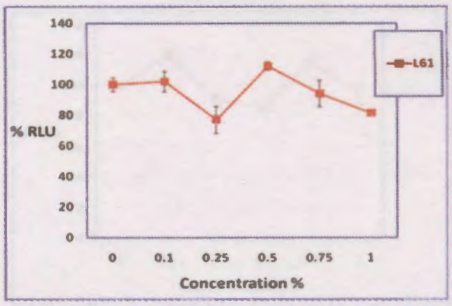
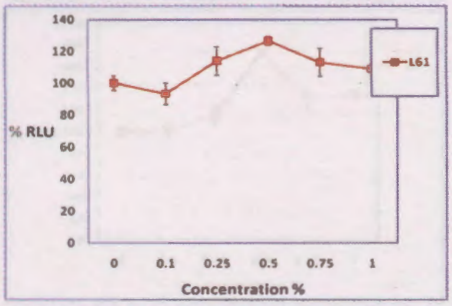
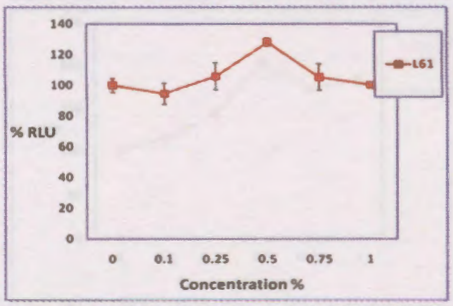
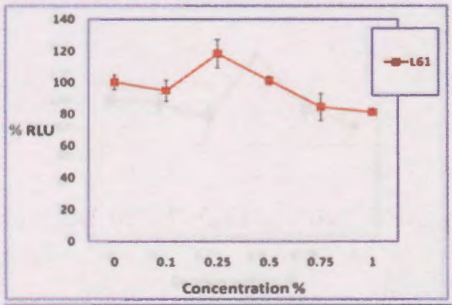
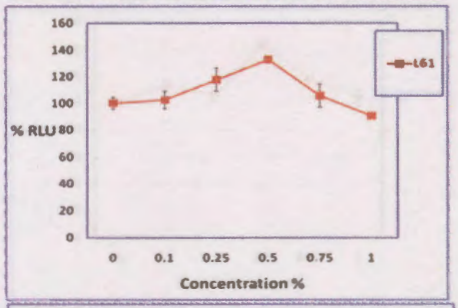
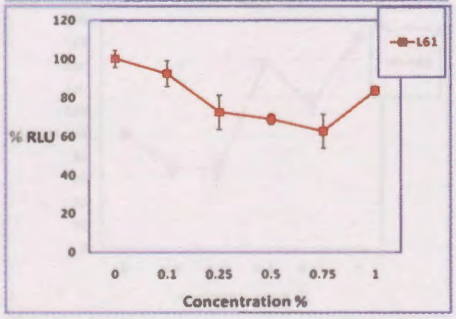
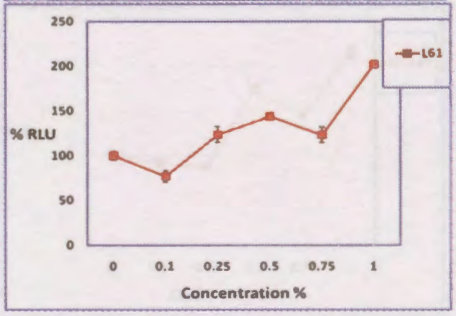
L61 (C)

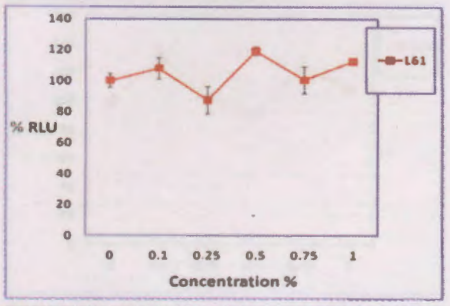
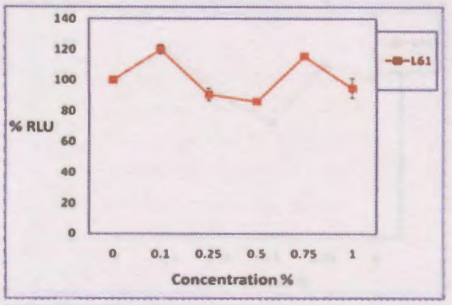
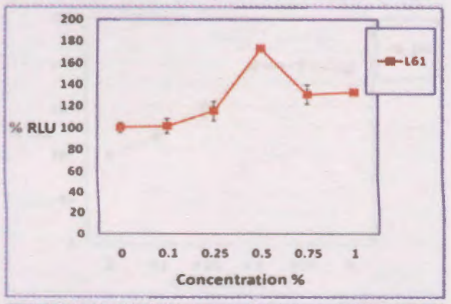
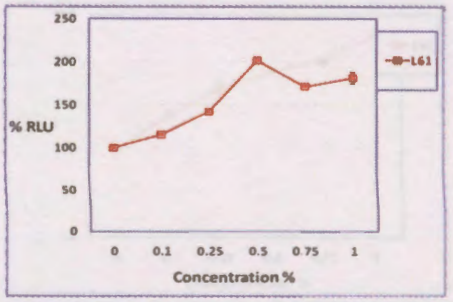
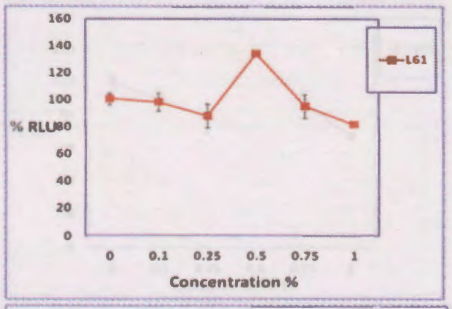
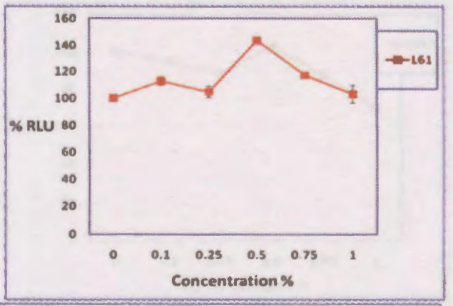
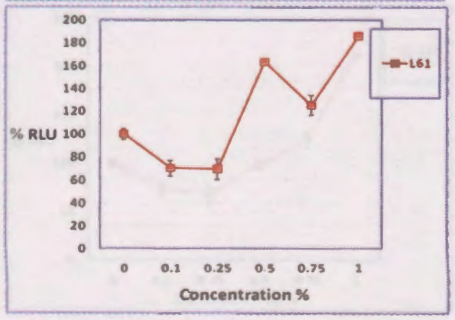
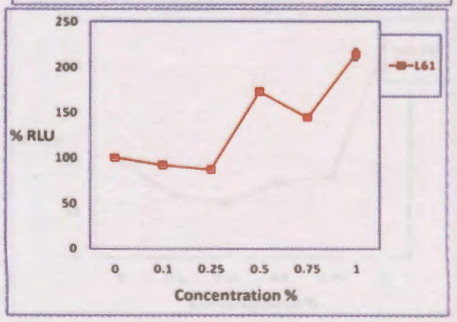
Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	107.1	106.5	91.5	101.7	8.8	96.7	93.9	97.9	96.2	2.0	120.3	119.2	120.9	120.1	0.9	95.5	106.8	105.7	102.6	6.2	85.8	84.9	86.4	85.7	0.8	82.5	81.5	82.6	82.2	0.6
10	68.9	63.6	62.3	65.0	3.5	65.8	61.2	72.4	66.4	5.6	79.5	79.4	70.3	76.4	5.3	87.5	88.7	82.9	86.4	3.0	65.3	69.2	71.0	68.5	2.9	54.6	62.4	58.8	58.6	3.9
20	61.4	57.3	56.3	58.3	2.7	56.1	59.1	55.7	57.0	1.9	40.1	50.9	61.9	51.0	10.9	76.8	83.0	74.1	78.0	4.5	56.9	53.2	55.2	55.1	1.8	47.1	47.6	47.2	47.3	0.3
30	45.6	50.7	47.3	47.9	2.6	50.6	51.2	60.1	53.9	5.3	51.6	49.8	48.8	50.1	1.6	66.0	70.2	70.0	68.7	2.4	53.6	59.5	55.1	56.1	3.0	45.1	51.7	51.1	49.3	3.7
40	52.7	54.6	53.3	53.5	1.0	46.9	49.2	47.9	48.0	1.1	41.1	40.5	43.9	41.8	1.8	63.1	63.6	64.4	63.7	0.7	44.7	47.8	48.3	46.9	1.9	36.3	37.3	34.7	36.1	1.3
50	51.7	47.7	54.2	51.2	3.3	46.2	47.0	52.2	48.5	3.2	41.2	45.5	49.0	45.2	3.9	52.3	50.3	56.1	52.9	3.0	42.0	48.3	44.2	44.8	3.2	35.3	35.0	38.0	36.1	1.6
60	57.6	58.4	55.5	57.2	1.5	50.4	50.7	50.8	50.6	0.2	43.5	50.2	44.8	46.2	3.6	37.9	40.0	39.1	39.0	1.1	46.3	43.6	42.8	44.2	1.8	32.3	34.8	35.5	34.2	1.7

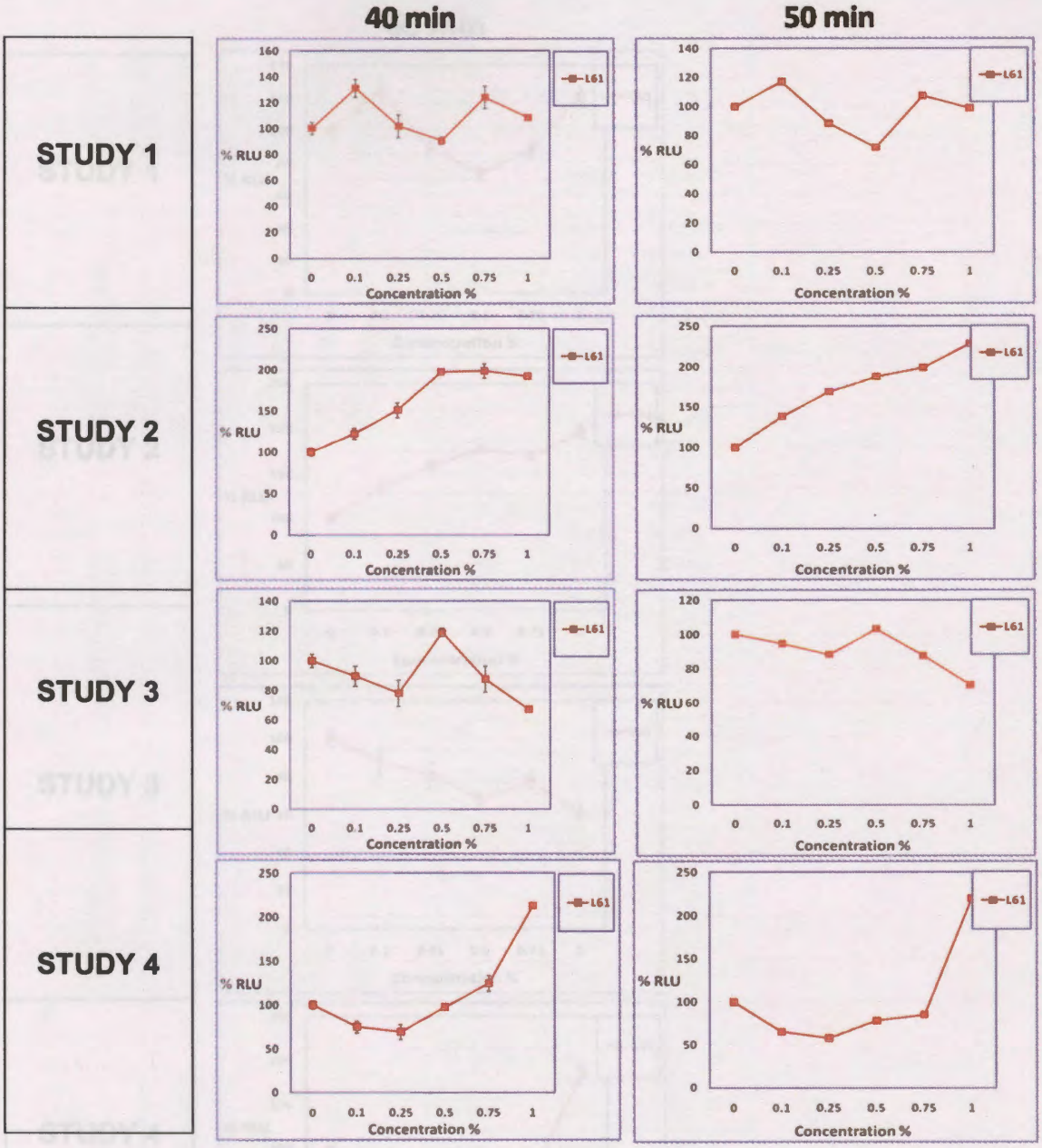
L61 (D)

Time	Concentration %																										
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	108.4	109.8	114.8	111.0	3.4	100.7	103.3	103.4	102.5	1.5	81.2	78.4	81.3	80.3	1.6	77.4	78.4	73.2	76.4	2.8	73.0	66.8	68.6	69.4	3.2	90.6	96.8	90.6	92.6	3.6
10	72.0	84.7	84.9	80.5	7.4	61.8	60.8	62.8	61.8	1.0	104.5	93.1	100.9	99.5	5.8	115.0	115.8	117.4	116.1	1.2	102.6	96.6	98.9	99.4	3.1	165.0	153.0	171.2	163.1	9.2
20	54.7	68.1	69.1	64.0	8.1	44.9	46.3	43.6	44.9	1.4	43.2	44.2	46.0	44.5	1.4	102.3	106.6	104.4	104.4	2.2	77.6	81.7	81.1	80.1	2.2	113.0	123.0	120.0	118.7	5.1
30	39.3	47.5	51.7	46.2	6.3	40.5	43.3	43.3	42.3	1.6	41.1	39.8	39.5	40.1	0.8	80.8	80.3	78.6	79.9	1.2	65.8	65.7	63.5	66.7	1.6	100.5	97.3	98.7	98.8	1.6
40	33.2	40.1	56.1	43.1	11.8	32.8	32.2	32.1	32.3	0.4	31.4	29.9	28.3	29.9	1.6	43.3	42.5	40.6	42.1	1.4	52.6	54.2	54.2	53.7	0.9	86.7	91.7	96.8	91.8	5.1
50	47.5	49.6		48.5	1.5	32.1	31.5	31.9	31.8	0.3	29.0	27.0	28.9	28.3	1.1	39.4	38.1	36.8	38.1	1.3	39.3	39.3	45.7	41.4	3.7	107.8	103.8	108.6	106.7	2.6
60	54.0	53.2	52.8	53.3	0.6	30.5	30.3	28.8	29.9	0.9	23.8	23.8	23.9	23.8	0.1	35.5	34.9	32.7	34.4	1.4	38.0	38.2	37.8	38.0	0.2	102.9	94.0	100.5	99.1	4.6

Pluronic L61: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		


40 min
50 min
STUDY 1
STUDY 2
STUDY 3
STUDY 4
% RLU
Concentration %
0
0.1
0.25
0.5
0.75
1
L61

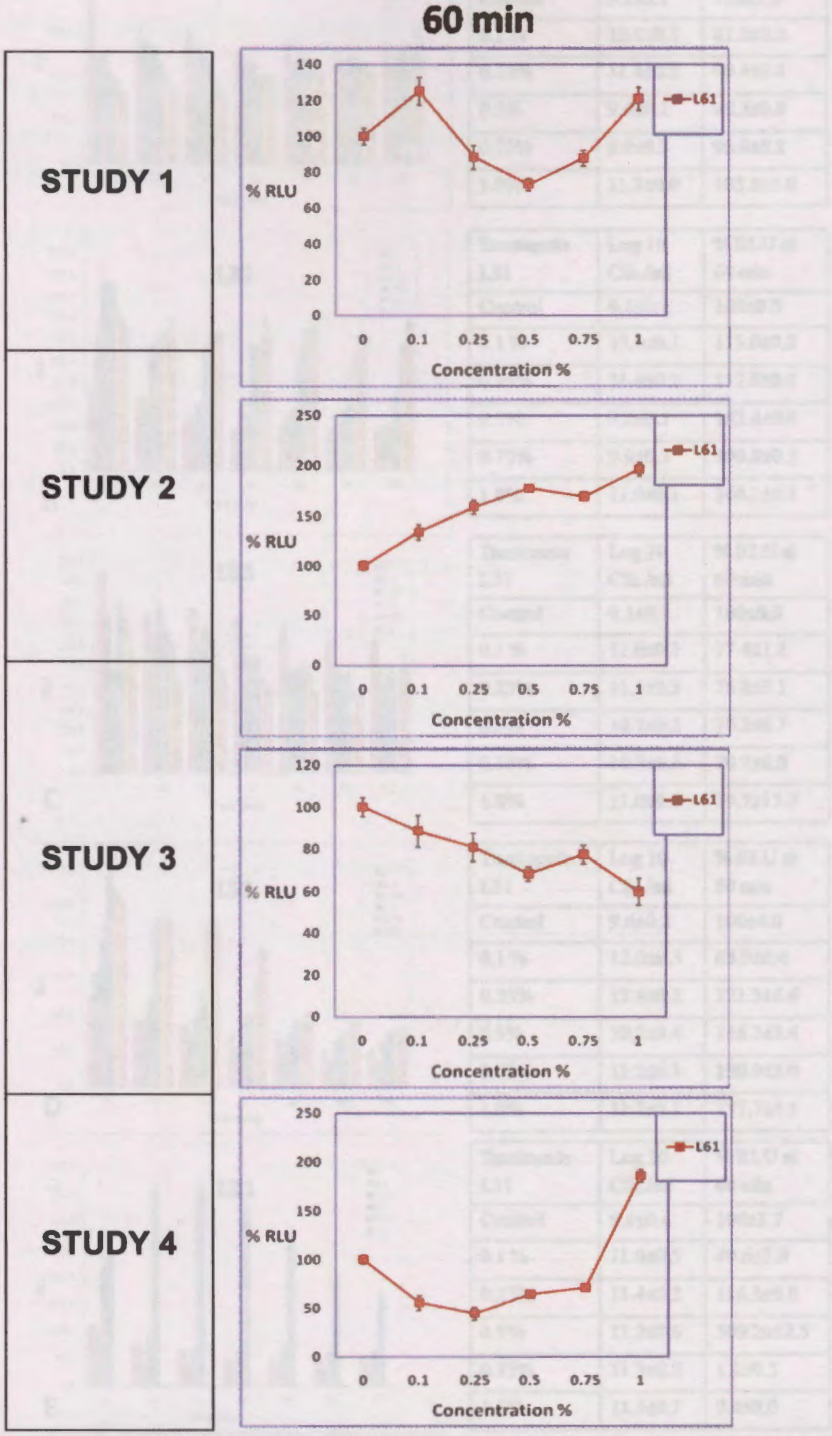
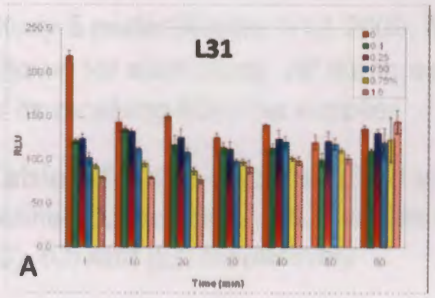
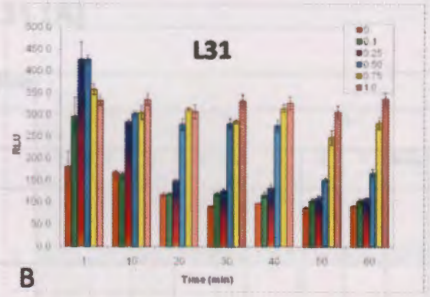


Figure A24: % RLU relative to control for defined concentrations of Pluronics L61 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.

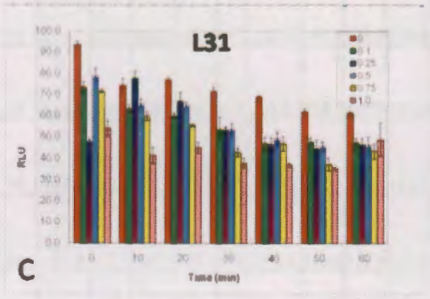
Pluronic L31: RLU vs. Time Graph



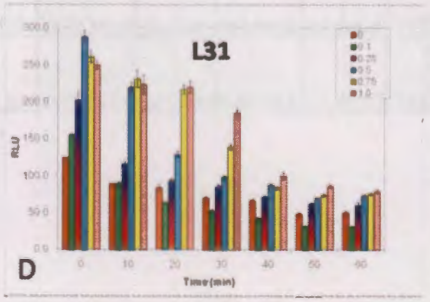
Treatments L31	Log 10 Cfu./ml	% RLU at 60 min
Control	9.1±0.1	100±0.0
0.1 %	12.0±0.1	81.2±0.2
0.25%	11.4±0.2	96.4±0.4
0.5%	9.6±0.1	88.3±0.6
0.75%	9.9±0.3	90.8±0.2
1.0%	11.3±0.0	105.8±5.0



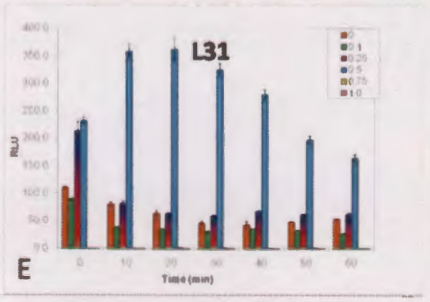
Treatments L31	Log 10 Cfu./ml	% RLU at 60 min
Control	9.1±0.1	100±0.0
0.1 %	12.0±0.1	113.0±0.2
0.25%	11.4±0.2	117.8±0.4
0.5%	9.6±0.1	182.4±0.6
0.75%	9.9±0.3	306.8±0.2
1.0%	11.0±0.1	366.2±0.3



Treatments L31	Log 10 Cfu./ml	% RLU at 60 min
Control	9.1±0.1	100±0.0
0.1 %	11.0±0.1	77.4±1.2
0.25%	11.7±0.3	75.8±3.1
0.5%	10.3±0.2	75.3±6.7
0.75%	10.5±0.4	70.7±6.0
1.0%	11.0±0.1	79.7±13.3



Treatments L31	Log 10 Cfu./ml	% RLU at 60 min
Control	9.6±0.2	100±4.0
0.1 %	12.0±0.3	63.0±0.4
0.25%	12.4±0.2	121.3±6.4
0.5%	10.2±0.4	146.3±3.4
0.75%	11.2±0.3	150.9±3.0
1.0%	11.3±0.1	157.7±4.1



Treatments L31	Log 10 Cfu./ml	% RLU at 60 min
Control	9.4±0.4	100±1.7
0.1 %	11.0±0.5	49.6±2.0
0.25%	11.4±0.2	116.3±6.0
0.5%	11.2±0.6	309.2±12.5
0.75%	11.2±0.2	1.1±0.5
1.0%	11.3±0.1	0.4±0.0

Figure A25: Lux response of *P. putida* KT2440 biosensor to L31 Pluronic at defined doses (mg/L). Changes in Lux output relative to control and cell culturability after 60 min

of treatment are shown. (A) Data from Study 1 performed on 3-4-2009, (B) Study 2 performed on 1-15-2009, (C) Study 3 performed on 2-25-2009, (D) Study 4 performed on 12-16-2008, (E) Study 5 performed on 1-12-2009. Means of analytical replicates and standard errors are shown for each study. All the three studies with Pluronic L31 were performed within 3 months of its receiving from the supplier.

Table A13: Lux response (RLU values) of *P. putida* KT2440 biosensor to L31 Pluronic at defined doses (mg/L). RLU values from the replicate studies are shown in tables L31 (A), (B), (C), (D) and (E), respectively

L31 (A)

Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	206.1	225.9	223.3	218.4	10.8	125.8	116.5	122.2	121.5	4.7	113.4	128.5	131.4	124.4	9.6	91.7	106.5	109.8	102.7	9.7	95.2	95.8	87.7	92.9	4.5	82.0	78.1	80.4	80.2	1.9
10	161.2	122.8	144.4	142.8	19.3	137.5	140.2	128.8	135.5	5.9	126.9	133.5	137.7	132.7	5.5	112.3	111.2	116.8	113.4	2.9	90.3	101.3	96.4	96.0	5.5	78.2	79.1	82.8	80.0	2.5
20	144.5	156.4	149.7	150.2	6.0	95.6	129.0	127.0	117.2	18.8	107.6	140.4	130.7	126.2	16.9	101.6	117.8	103.0	109.1	8.2	94.7	81.7	86.5	87.6	6.6	75.8	86.1	72.0	78.0	7.3
30	118.2	123.0	135.6	125.6	8.9	104.2	117.3	123.1	114.9	9.7	95.1	120.0	122.7	112.6	15.2	95.2	104.2	97.2	98.9	4.8	96.9	92.7	104.8	98.1	6.1	79.9	105.8	91.7	92.5	12.9
40	137.8	142.4	140.7	140.3	2.3	108.3	108.5	123.8	113.5	8.9	108.1	122.0	142.9	124.3	17.5	109.1	124.9	129.3	121.1	10.6	97.6	106.6	101.9	102.0	4.5	92.4	108.2	97.7	99.4	8.1
50	109.2	115.9	136.1	120.4	14.0	101.5	112.9	88.1	100.8	12.4	101.9	136.9	127.0	121.9	18.1	130.3	107.3	118.8	118.8	11.5	114.8	111.9	106.1	110.9	4.4	91.7	107.5	104.9	101.4	8.5
60	127.7	141.7	138.6	136.0	7.3	104.7	113.7	112.8	10.4	4.9	122.2	135.9	135.3	131.1	7.7	91.0	121.0	148.2	120.1	28.6	97.3	98.9	174.3	123.5	44.0	145.1	166.2	120.3	143.9	23.0

L31 (B)

Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	138.4	224.1	208.2	190.2	45.6	211.7	341.1	338.0	296.9	73.8	352.6	483.4	447.1	427.7	67.5	437.3	412.0	433.9	427.8	13.7	339.6	381.3	353.8	358.2	21.2	327.0	353.9	318.0	332.9	18.7
10	174.0	165.2	170.3	169.9	4.4	153.7	167.6	171.8	164.4	9.5	272.3	288.6	290.8	283.9	10.1	296.6	301.9	312.4	303.6	8.0	282.2	333.1	299.8	305.0	25.8	313.2	333.0	360.8	335.7	23.9
20	125.9	116.5	110.8	117.7	7.6	125.3	119.0	116.3	120.2	4.6	145.0	158.5	144.9	149.5	7.8	255.2	283.8	297.8	278.9	21.7	321.2	313.1	308.3	314.2	6.6	337.1	306.1	280.5	307.9	28.3
30	94.5	89.1	91.8	91.8	2.7	115.1	126.0	120.3	120.5	5.4	130.3	119.9	133.2	127.8	7.0	257.5	299.4	286.7	281.2	21.5	279.1	293.1	287.8	286.7	7.1	309.0	332.5	357.9	333.1	24.5
40	99.1	99.2	99.4	99.2	0.2	127.6	120.3	107.2	118.4	10.3	118.7	133.3	147.9	133.3	14.6	252.8	287.3	288.5	276.2	20.3	332.8	304.1	310.9	315.9	15.0	317.9	308.3	357.0	327.8	25.8
50	89.2	83.9	91.2	88.1	3.8	103.4	114.0	96.1	104.5	9.0	121.2	109.6	101.6	110.9	9.8	147.7	151.7	159.5	153.0	6.0	268.5	262.9	217.0	249.5	28.2	276.2	318.9	325.4	306.9	26.7
60	94.2	94.1	87.6	92.0	3.8	103.8	112.7	95.5	104.0	8.6	106.2	103.8	115.2	108.4	6.0	181.9	170.7	150.8	167.8	15.7	302.6	257.4	287.0	282.3	22.9	335.4	362.1	313.2	336.9	24.5

L31 (C)

Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	96.1	91.1	93.3	93.5	2.5	78.0	69.3	73.3	73.5	4.3	49.2	48.5	45.5	47.7	2.0	71.4	80.6	83.9	78.5	6.4	70.4	72.1	72.5	71.7	1.1	49.1	52.7	60.8	54.2	6.0
10	80.9	72.0	69.7	74.2	5.9	61.3	67.1	61.5	63.3	3.3	74.4	83.7	75.9	78.0	5.0	63.3	70.1	62.7	65.4	4.1	56.3	60.7	62.6	59.8	3.2	41.1	48.2	35.0	41.4	6.6
20	75.0	76.5	79.5	77.0	2.3	61.0	61.5	57.4	60.0	2.2	66.6	74.2	60.1	67.0	7.0	66.4	66.0	62.3	64.9	2.2	55.1	56.4	55.6	55.7	0.7	43.5	41.8	49.9	45.1	4.3
30	68.9	71.2	74.9	71.7	3.1	50.6	45.3	64.8	53.6	10.1	50.5	55.5	54.4	53.5	2.6	47.6	57.3	55.5	53.5	5.2	45.4	39.2	44.3	42.9	3.3	34.6	37.5	42.0	38.1	3.7
40	69.1	70.2	67.2	68.8	1.5	41.4	51.0	48.7	47.0	5.0	40.8	50.7	48.7	46.7	5.2	43.7	54.7	49.0	49.2	5.5	52.3	47.6	41.2	47.1	5.6	36.3	36.4	38.9	37.2	1.5
50	59.7	61.6	64.5	61.9	2.4	44.2	49.2	49.8	47.7	3.1	41.3	52.2	40.9	44.8	6.4	42.0	45.6	49.3	45.7	3.6	31.8	40.1	40.6	37.5	4.9	35.6	34.0	36.8	35.5	1.4
60	60.5	62.9	61.1	61.5	1.2	45.6	46.0	51.1	47.6	3.1	41.1	47.8	50.7	46.6	4.9	46.3	52.9	39.6	46.3	6.7	38.4	42.0	50.1	43.5	6.0	36.5	47.4	63.0	49.0	13.3

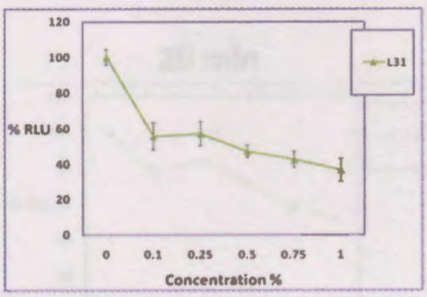
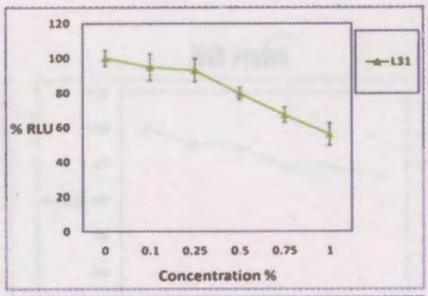
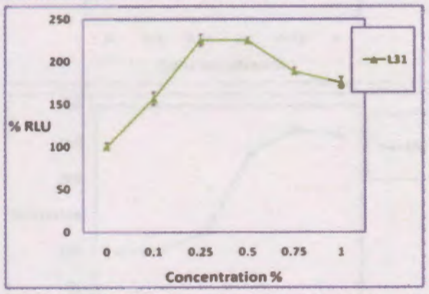
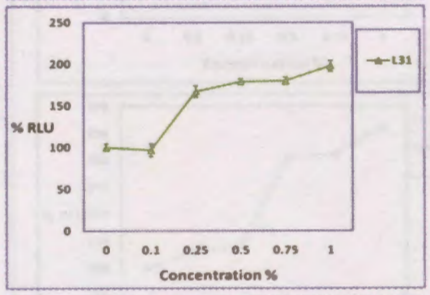
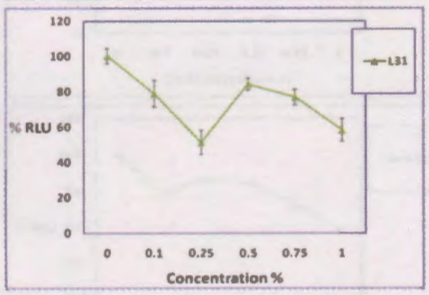
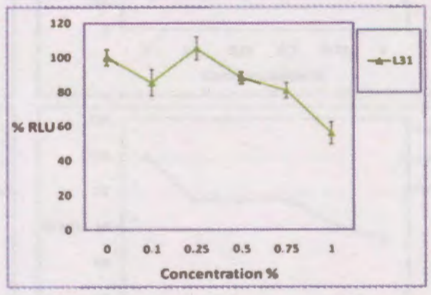
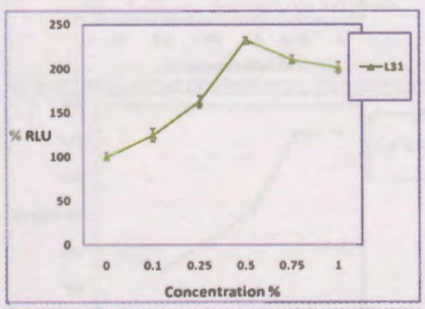
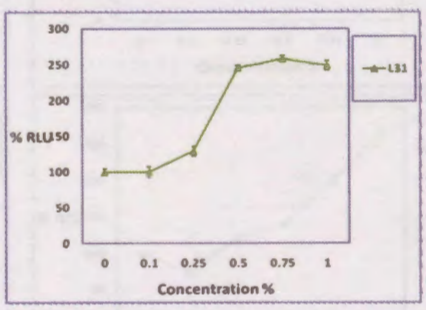
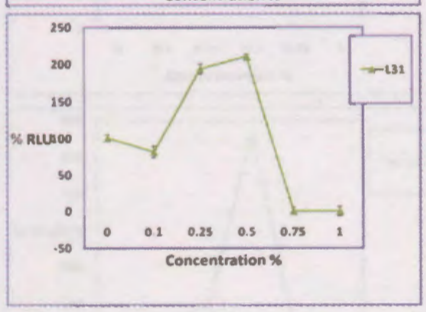
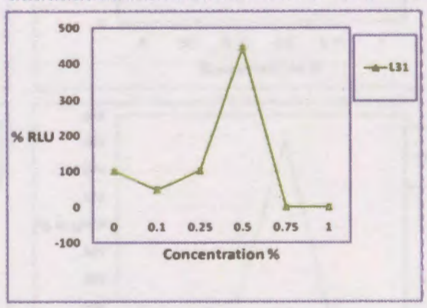
L31 (D)

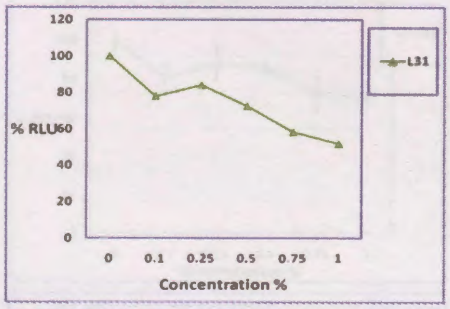
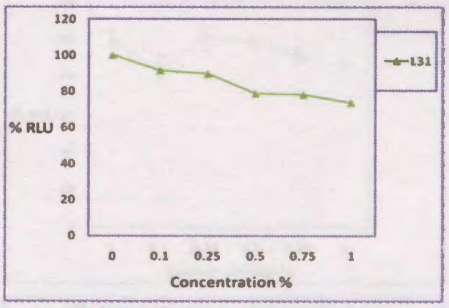
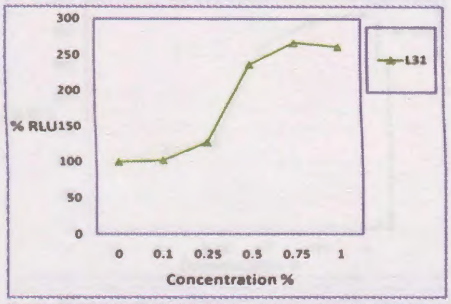
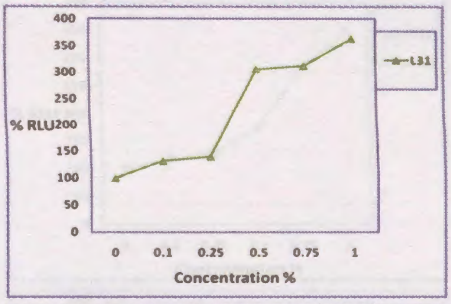
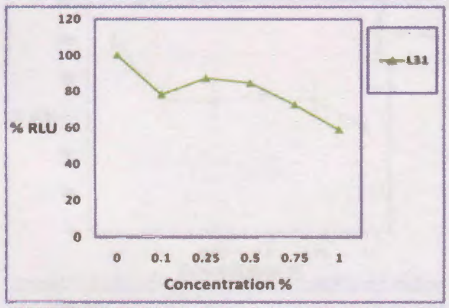
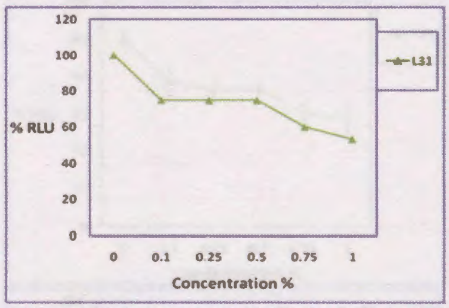
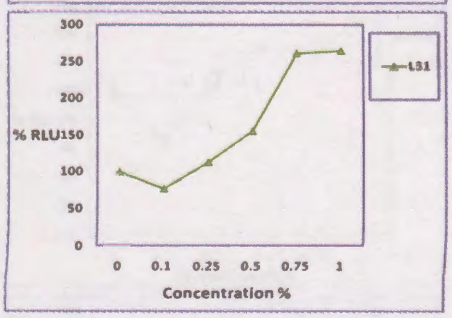
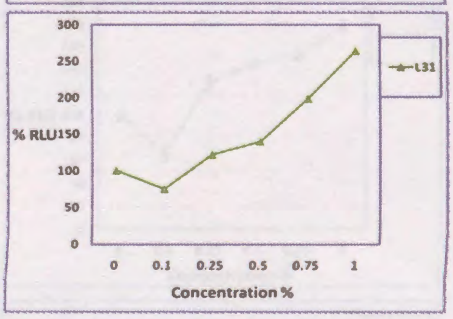
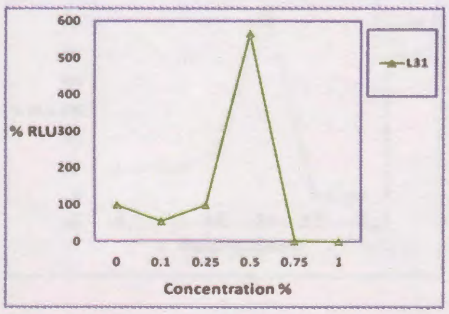
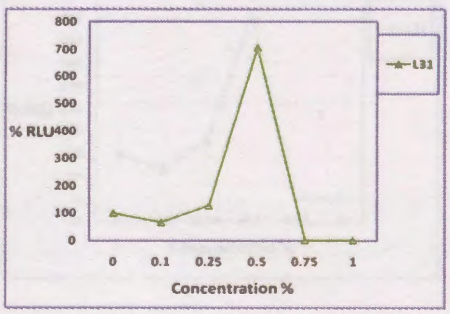
Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	123.8	125.3	125.2	124.8	0.9	155.6	156.3	155.3	155.8	0.5	212.5	216.1	179.8	202.8	20.0	301.5	284.7	281.8	289.3	10.7	266.0	247.2	271.9	261.7	12.9	253.5	258.4	239.8	250.6	9.6
10	90.2	89.2	90.2	89.9	0.6	85.9	90.3	92.8	89.7	3.5	112.0	113.0	124.3	116.4	6.8	220.2	230.6	212.0	220.9	9.3	216.0	226.9	253.6	232.1	19.3	202.9	222.9	245.8	223.9	21.4
20	89.5	82.2	78.4	83.3	5.7	64.2	64.0	64.2	64.1	0.1	97.2	90.0	94.3	93.8	3.6	136.9	125.5	125.5	129.3	6.6	228.7	212.6	211.3	217.5	9.7	234.3	220.9	205.3	220.2	14.5
30	72.9	66.0	72.3	70.4	3.3	49.9	54.8	53.4	52.7	2.5	91.8	80.7	84.4	85.6	5.7	97.2	103.6	95.5	98.7	4.3	134.1	141.7	144.2	140.0	5.3	177.7	189.6	190.7	186.0	7.2
40	65.9	70.6	64.9	67.1	3.0	42.6	44.1	40.6	42.4	1.8	68.1	73.3	74.5	72.0	3.4	87.5	85.7	91.9	88.4	3.2	82.0	88.3	82.1	84.1	3.6	91.8	107.2	102.6	100.5	7.9
50	52.6	47.2	46.1	48.6	3.5	32.1	33.1	31.3	32.1	0.9	61.6	62.0	64.9	62.8	1.8	71.5	70.7	70.5	70.9	0.6	77.9	72.9	72.0	74.3	3.2	85.9	90.4	84.3	86.9	3.2
60	46.2	50.5	54.1	50.3	4.0	31.4	32.1	31.5	31.7	0.4	53.7	63.8	65.4	61.0	6.4	69.8	75.1	76.0	73.6	3.4	77.5	72.4	77.7	75.9	3.0	76.4	77.6	84.0	79.3	4.1

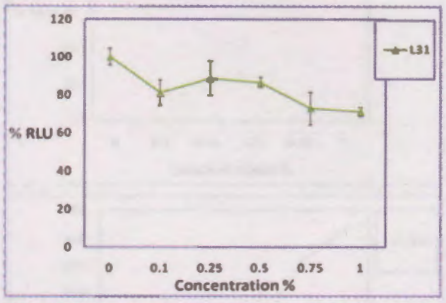
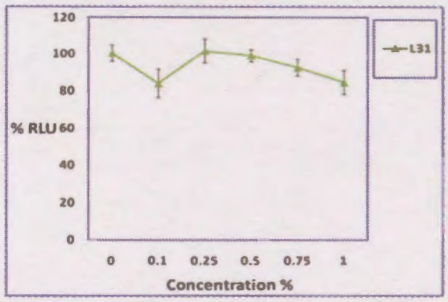
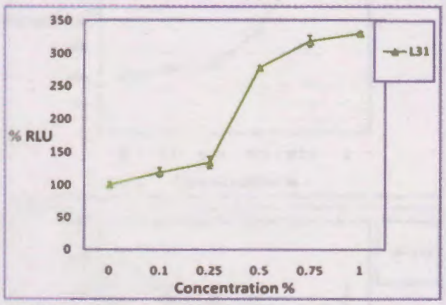
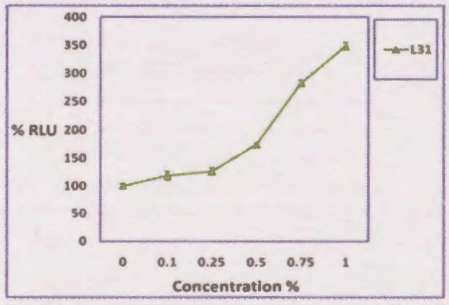
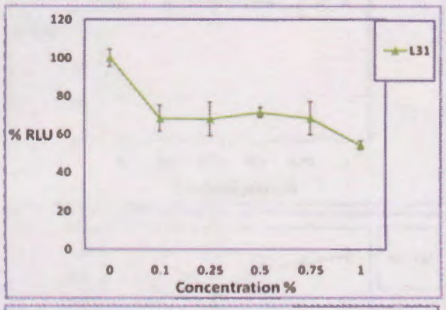
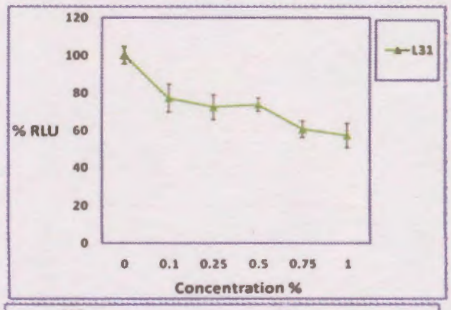
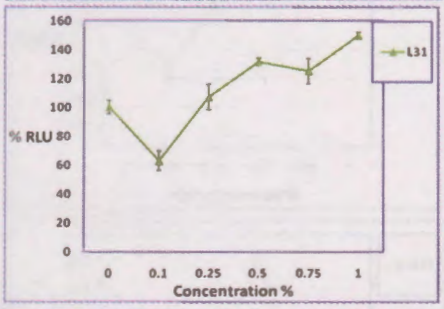
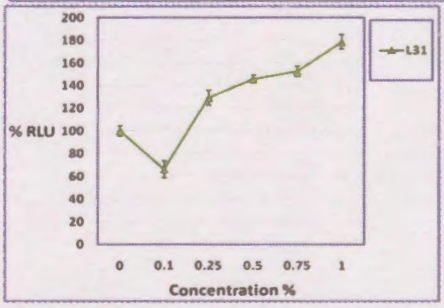
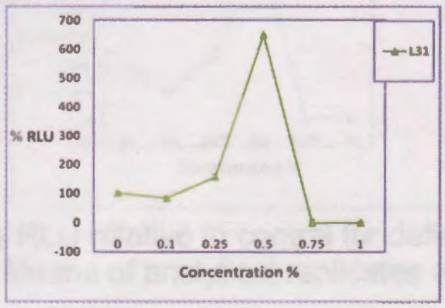
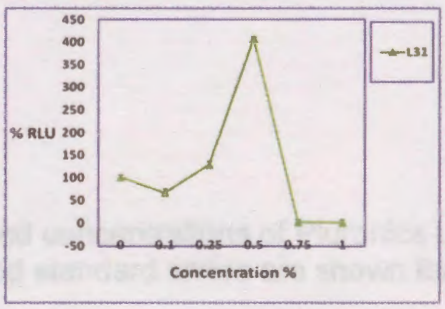
L31 (E)

Time	Concentration%																									
	0					0.1					0.25					0.5					0.75					1.0				
	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD	1	2	3	Avg.	SD
1	108.4	109.8	114.8	111.0	3.4	90.9	91.7	87.6	90.1	2.2	191.1	205.1	247.2	214.5	29.2	240.0	238.1	220.6	232.9	10.7	0.7	0.2	0.2	0.4	0.3	0.2	0.2	0.3	0.2	0.0
10	72.0	84.7	84.9	80.5	7.4	36.7	39.5	39.4	38.5	1.5	90.6	78.3	77.7	82.2	7.3	374.8	368.5	334.7	359.4	21.6	0.7	0.7	0.7	0.7	0.0	0.2	0.2	0.2	0.2	0.0
20	54.7	68.1	69.1	64.0	8.1	35.9	35.6	35.1	35.5	0.4	64.9	63.3	63.3	63.8	1.0	323.6	370.4	394.5	362.9	36.1	2.1	0.8	0.8	1.3	0.8	0.2	0.2	0.2	0.2	0.0
30	39.3	47.5	51.7	46.2	6.3	28.7	31.1	30.0	30.6	1.7	61.4	56.0	59.6	59.0	2.8	306.2	344.6	326.9	325.9	19.2	2.1	0.2	0.1	0.8	1.1	0.2	0.2	0.3	0.2	0.0
40	33.2	40.1	56.1	43.1	11.8	35.9	34.6	35.8	35.4	0.7	64.8	72.2	65.0	67.3	4.3	262.8	291.5	286.5	280.3	15.4	0.2	0.2	0.2	0.2	0.0	0.3	0.3	0.3	0.3	0.0
50	47.5	49.6	48.5	48.5	1.0	33.0	32.5	31.3	32.3	0.9	61.6	63.8	61.5	62.3	1.3	189.4	190.6	213.4	197.8	13.5	2.0	0.4	0.3	0.9	1.0	0.2	0.3	0.3	0.3	0.0
60	52.1	55.3	52.8	53.4	1.7	24.7	26.1	28.7	26.5	2.0	55.4	63.7	67.0	62.1	6.0	158.8	156.9	179.5	165.1	12.5	1.3	0.3	0.3	0.6	0.5	0.2	0.2	0.2	0.2	0.0

Pluronic L31: % RLU vs. % Concentrations graphs

	1 min	10 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		
STUDY 5		

	20 min	30 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		
STUDY 5		

	40 min	50 min
STUDY 1		
STUDY 2		
STUDY 3		
STUDY 4		
STUDY 5		

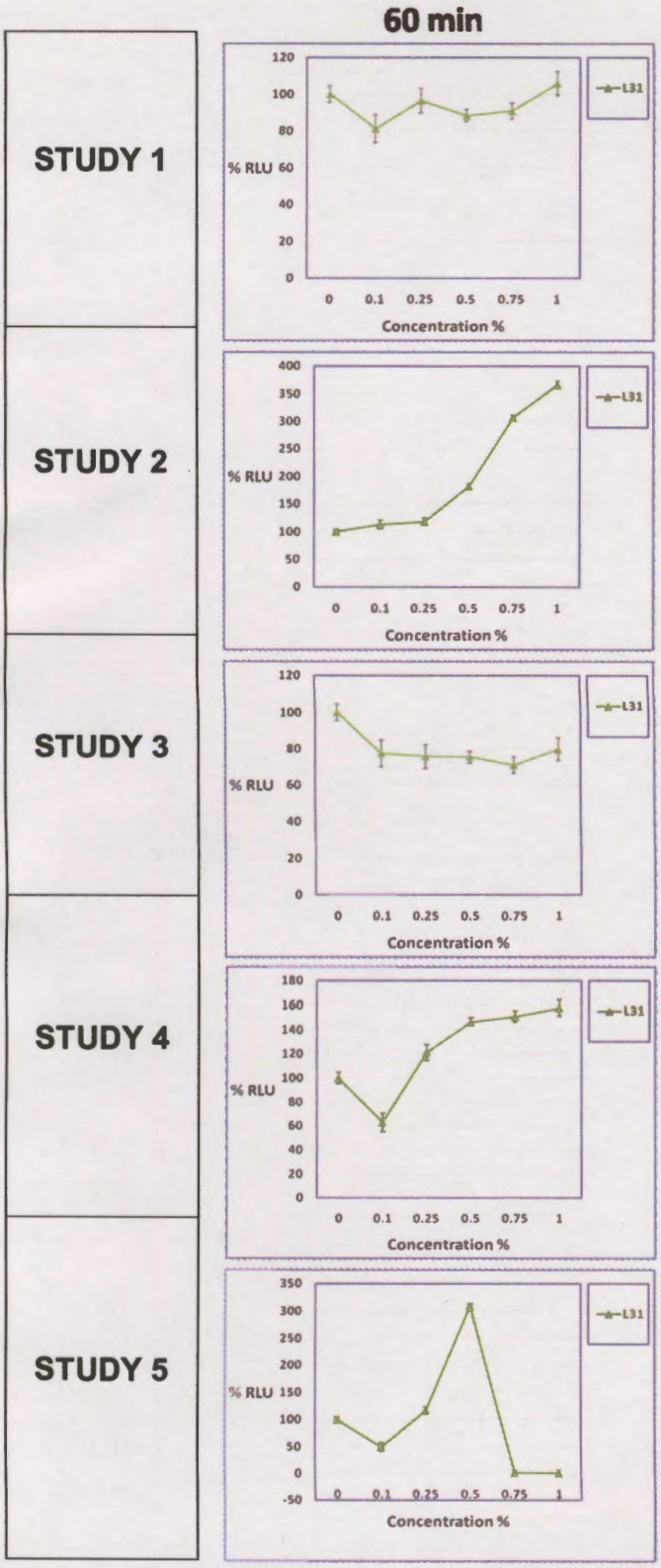


Figure A26: % RLU relative to control for defined concentrations of Pluronics L31 at 10 min intervals. Means of analytical replicates and standard errors are shown for each study.