Toxicity of four spill-treating agents on bacterial growth and sea urchin embryogenesis.

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Abstract

The toxicity of spill-treating agents (STAs) is a topic that needs to be assessed prior to their potential application in environmental disasters. The aim of the present work was to study the effects of four commercial STAs (CytoSol, Finasol OSR 51, Agma OSD 569 and OD4000) on the growth of marine (*Phaeobacter* sp., *Pseudomonas* sp.) and terrestrial (*Leuconostoc mesenteroides*) bacteria, and sea urchin (*Paracentrotus lividus*) embryolarval development. In general, STA did not inhibit significantly the biomass production of the tested marine bacteria. Finasol OSR 51 and OD4000 clearly inhibited the growth of *L. mesenteroides* and an accurate description of the kinetics was provided by a proposed bivariate equation. For this species, a global parameter ($EC_{50,\tau}$) was defined to summarize the set of growth kinetics. Using this parameter Finasol OSR 51 was found to be less toxic (754 μL L^{-1}) than OD4000 (129 μL L^{-1}). For the sea urchin embryo assay, the ranking of toxicity as EC_{50} (μL L^{-1}) was Agma OSD 569 (34.0) < CytoSol (26.3) <OD4000 (2.2)< Finasol OSR 51 (1.2).

Keywords: spill-treating agents; Weibull and logistic equations; bivariate model; bacteria growth kinetics; EC_{50}; sea urchin embryogenesis test.

1. Introduction

An oil spill that impacts the shoreline is a sensitive issue for the authorities due to the difficulty of cleaning, cost, ecological effects and pressure of public opinion. Spill-treating agents are chemicals developed to increase the efficiency of cleaning. Dispersants, the most commonly used spill-treating agents (STAs) group, promote the formation of droplets which implies an increase of oil exposure into the water column and a reduction of load in the water surface or the shoreline (NRC, 2005). Surface-Washing Agents or Shoreline Cleaning Agents (SCA) remove oil from solid surfaces and their use is often conditioned by the lack of effectiveness of conventional methods (water washing, bioremediation) and their approval on a case by case basis (Fingas and Fieldhouse, 2011; NOAA, 1992).

The use of dispersants in the *Torrey Canyon* oil spill (occurred in 1967) raised doubts due to their high toxicity. The composition of dispersants has changed since the 1960s in order to obtain effective STAs without the adverse effects of the first generation of dispersants (Fingas, 2011; NRC, 2005). In fact, the toxicity of shoreline washing agents is generally lower than that for dispersants (Fingas and Fieldhouse, 2011). Nevertheless, the toxicity assessment of a STA is a necessary step in most countries prior to approval for use. According to the Technical Guidance Document on Risk Assessment (European Commission, 2003) the effects assessment involves at least a battery of acute toxicity tests corresponding to different trophic levels.

The physicochemical characteristics of a dispersant influence the biodegradation rate by bacteria, although a consensus on the enhancer or inhibitory effect has not been reached (NRC, 2005). The bacterial growth is an appropriate endpoint for assessing

the toxicity of the spill-treating agents on the trophic level of decomposers at a lab scale. It also has greater ecological relevance than the assessment endpoint most common for this trophic level, namely the production of bioluminescence by *Vibrio fischeri* (ISO, 2007), but presents lower sensitivity (Gellert, 2000). The ISO 10712 (ISO, 1995) provides a practical alternative to evaluate the inhibitory effects of a toxic agent on the growth of *Pseudomonas putida*, though a more comprehensive description of the kinetics is obtained using a bivariate model which combines dose of the agent and exposure time (Rial, et al., 2011; Vázquez, et al., 2011).

Bioassays based on the success of the embryo-larval development of marine invertebrates are tools commonly used in ecotoxicology because of its simplicity, low cost and high sensitivity (His, et al., 2000). The sea urchin embryo-larval test has been the object of considerable standardization (ASTM, 2004; Beiras, et al., 2012) which facilitates its implementation, interpretation and comparison with previous results and highlights its suitability for assessing toxicity of marine water and sediments.

In the present work, we investigated the toxic effect of four STAs on the growth of marine and terrestrial bacteria and on the sea urchin embryo-larval development. The dose-response data of bacteria and sea urchin were accurately modeled by a bivariate model and Weibull equation, respectively, in order to obtain comparative parameters of toxicity as $EC_{50,\tau}$, EC_{50} and EC_{10}.

2. Materials and methods

2.1. Microbiological methods

The bacteria used in the current study were selected because of its different habitats (marine and terrestrial) and cell wall structure (Gram-positive and Gram-negative) characteristics. Furthermore, they previously showed excellent results as target microorganisms for toxicological evaluation of organic acids (Vázquez, et al., 2011) and heavy metals (Rial et al., 2011). *Phaeobacter* sp. (Ph) was kindly provided by Dr. Lone Gram (DTU Aqua, Denmark), *Leuconostoc mesenteroides* (Lm) was supplied by Dr. B. Ray (University of Wyoming, Laramie, USA) and *Pseudomonas* sp. (Ps) (CECT 4355) was purchased to Colección Española de Cultivos Tipo (CECT, Universidad de Valencia, Spain).

Stock cultures of bacteria were kept at −80 °C in commercial MRS (Lm) and marine medium (Ph, Ps) with 25% glycerol (Cabo, et al., 2001; Vázquez, et al., 2004). Marine medium was from Difco (Becton, Dickinson and Company, MD, USA) and MRS medium from Pronadisa (Hispanlab S.A., Spain), both prepared under manufacturer specifications. Inocula and cultures were prepared according to the report by Rial et al. (2011). Toxicological assessments were performed by bacterial cultivation with orbital shaking at 200 rpm and 22 °C (Ph), 27 °C (Ps) and 30 °C (Lm). In the preliminary assessments of bacterial growth, the cultivations were studied up to 30 h. Subsequently, the two pairs (OD4000 and Finasol OSR 51 *vs.* Lm), in which the growths were affected by STAs, were conducted again extending fermentations until 40 h.

2.2. Sea urchin assay

The sea urchin embryo test was performed as described elsewhere (Saco-Álvarez, et al., 2010). Adult specimens of *Paracentrotus lividus* were dissected and the maturity of their gametes checked under the microscope. The ova were transferred to a 100

mL graduated cylinder containing sea water (5-10 ova μL^{-1}), a drop of sperm was added, and the mixture was gently shaken to facilitate fertilization. The fertilization rate was determined in quadruplicate in samples of 100 individuals, as the proportion of eggs with a fertilization membrane. Within 30 min, the fertilized eggs were transferred to vials with 10 mL of filtered sea water (FSW). Each vial received 40 eggs per mL and four replicates per concentration were performed (controls in quintuplicate). The eggs were incubated in the dark at 20 ºC for 48 h and the larvae fixed by adding 2-3 drops of 40% formalin. Maximum length of 35 individuals was measured in each vial using an inverted microscope and Leica QWIN image analysis software, version 3.4.0 (Leica Microsystems, Germany). The inhibition of growth as the increase in length was quantified according to the following expression (Rial, et al., 2010):

$$R_i = 1 - \frac{\Delta L_i}{\Delta L_0} \qquad (1)$$

where ΔL_0 and ΔL_i are the mean length increases in control and the i^{th} dose, respectively.

2.3. Chemical preparation

Three dispersants (Agma OSD 569, Finasol OSR 51 and OD4000) and a SCA (CytoSol) were selected for their effectiveness to remove fuel adhering to granite rock (Murado, et al., 2008): CytoSol (CytoCulture International, Inc., Point Richmond, California, USA), FINASOL® OSR 51 (Total Special Fluids, Paris, France), Agma OSD 569 (Agma plc, Northumberland, United Kingdom) and OD4000 (Innospec Ltd,

147 Cheshire, United Kingdom). The products were kindly provided by the manufacturers
148 or trade representatives.
149
150 Stock solutions of the STAs were obtained by dissolving them in acetone (4-400 mL
151 of the STA per liter of acetone for the bacterial assay and 0.15-300 mL/L for the sea
152 urchin assay) and shaken before use. 10 µL of each stock solution was added to a
153 vial with 10 mL of FSW for the sea urchin bioassay and 50 µL to each 30 mL culture
154 tube with 10 mL of marine broth/MRS medium for the bacterial assay to obtain the
155 concentrations to be tested. The maximum concentration of acetone in the solvent
156 control group was 1 and 5 mL/L for the sea urchin and bacterial assays respectively.
157
158 *2.4. Mathematical equations*
159 *2.4.1. Modelling of bacterial growth inhibition*
160 The toxicodynamic evaluation of Lm growth affected by Finasol OSR 51 and OD4000
161 was performed by a bivariate equation based on the combination of Weibull function
162 as STA-concentration model modifying the most important parameters of the
163 reparameterized logistic equation used for bacteria growth description (Rial, et al.,
164 2011; Vázquez, et al., 2011):
165
166 $$X = \frac{X_{m\bullet}}{1+\exp\left[2+\frac{4v_{m\bullet}}{X_{m\bullet}}\left(\lambda_\bullet - t\right)\right]}$$; where: (2)

167 $$X_{m\bullet} = X_m\left\{1-K_x\left[1-\exp\left(-\ln 2\left(C/m_x\right)^{a_x}\right)\right]\right\}$$

168 $$v_{m\bullet} = v_m\left\{1-K_v\left[1-\exp\left(-\ln 2\left(C/m_v\right)^{a_v}\right)\right]\right\}$$

169 $$\lambda_\bullet = \lambda\left(1+b\,C\right)$$

where, v_m is the maximum growth rate, X_m is the maximum growth, λ is the lag phase and C is the STA concentration. The meanings of other symbolic notations as well as the corresponding units are summarized in Table 1. In addition, a global parameter ($EC_{50,\tau}$) was also selected for the overall description of the effects on growth. This parameter was defined as the STA concentration (in μL L^{-1}) that reduces the biomass by 50% compared to that produced by the control without agent at time (τ) (Rial, et al., 2011).

2.4.2. Sea-urchin bioassay modelling

The obtained dose–response relationship, the inhibition of sea urchin embryo development recorded as a reduction of the size increase in the treatments with the STA regarding to the solvent control, was modelled using the reparameterized Weibull model. This allowed a direct calculation of EC_{50} according to the following expression (Murado, et al., 2011; Riobó, et al., 2008):

$$R = K\left\{1-\exp\left[\ln 0.5\left(\frac{C}{EC_{50}}\right)^{a}\right]\right\} \quad (3)$$

The same data of sea urchin inhibition in function of STA concentration were also fitted to another formulation of Weibull equation in which the toxicological parameter (EC_{10}) was written in an explicit form (Murado, et al., 2011; Riobó, et al., 2008):

$$R = K\left\{1-\exp\left[\ln 0.9\left(\frac{C}{EC_{10}}\right)^{a}\right]\right\} \quad (4)$$

where, R is the response (inhibition of sea urchin length growth), K is the maximum response, C is the STA concentration (μL L^{-1}), EC_{50} is the concentration corresponding to the semi-maximum response (μL L^{-1}), EC_{10} is the concentration equivalent to 10% of the maximum response (μL L^{-1}) and a is a shape parameter related to the maximum slope of the response.

2.4.3. Numerical Methods and Statistical Analysis

The fitting procedures and parametric estimates from the experimental results were performed by minimizing the sum of quadratic differences between the observed and model-predicted values using the nonlinear least-squares (quasi-Newton) method provided by the '*Solver*' macro from Microsoft Excel spreadsheet. The confidence intervals of the best-fit values for the parametric estimates (Student's t test, $\alpha = 0.05$), consistency of the mathematical models (Fisher's F test; $p < 0.05$) and covariance and correlation matrices were calculated using the '*SolverAid*' macro available from Levie's Excellaneous website http://www.bowdoin.edu/~rdelevie/excellaneous/

3. Results and discussion

3.1. Toxicity of STA on bacterial growth

In most of the combinations of chemicals *vs* bacteria, the toxicity of STA was null or negligible. Preliminary evaluations of bacterial growth affected by a range of STA concentrations from 0 to 2000 μL L^{-1} showed non significant differences between control and maximum level tested. A representation of selected cases is displayed in Figure 1. The experimental results revealed that STA was not especially toxic for the marine bacteria (Ps and Ph) even at the maximum concentration of chemical tested.

Gram-negative bacteria are generally less sensitive to surfactants than gram-positive (Volkering, et al., 1995), and this coincides with the greater toxicity obtained for *L. mesenteroides*, gram-positive, compared to *Pseudomonas* sp. and *Phaeobacter* sp., both gram-negative.

In any case, the specific maximum growth rate (μ_m, calculated in the exponential phase) was significantly inhibited by the increase of STA concentration (data not shown). Curiously, high concentrations of CytoSol and Finasol OSR 51 increased the biomass obtained from Ph and Ps, respectively, in comparison with the control without STA.

Only two combinations, Lm in presence of OD4000 and Finasol OSR 51, defined a complete experimental surface of growth inhibition (Figure 2). In both cases, equation (1) successfully and consistently modelled the experimental growths with statistical significance for the parameters summarized in Table 2. OD4000 was more toxic than Finasol OSR 51 in terms of $EC_{50,\tau}$ value comparison. The concentrations of both STA affected linearly the lag phase growth of Lm and the effect on maximum biomass was significantly described by a sigmoid relationship. Nonetheless, the effect on maximum growth rate was only significant in the case of OD4000.

The modification of the maximum biomass by different chemicals, including surfactants, has been reported in a great variety of bacterial growths (Rial, et al., 2011; Vázquez, et al., 2011). For instance, Lima et al. (2011) observed that sodium dodecyl sulfate caused a decrease of the maximum growth at 4 g L^{-1} for three bacterial strains.

The effect of compounds on the maximum growth rate and lag phase parameters was less common. For instance, cadmium and formic acid separately induced sigmoid changes on both variables when the biomass production of Lm was evaluated (Rial, et al., 2011; Vázquez, et al., 2011). In many other fermentations, the supplementation of culture media with cobalt or nickel as well as lactic, butyric or propionic acid did not lead to significant variations on the lag period with the concentration increase of the mentioned compounds. For surfactants, Bramwell and Laha (2000) also found that the duration of the lag phase in the mineralization of phenanthrene by *Pseudomonas aeruginosa* was dependent on surfactant concentration, which may be associated with a dose-dependent mortality of the inoculum and subsequent growth recovery (Willumsen, et al., 1998).

The inhibitory effects of STAs on growth obtained herein cannot be directly extrapolated to the evolution of a bacterial community in a dispersant application because: a) the action of a STA on an oil slick represents an increased exposure to hydrocarbons in the water column and the simultaneous exposure to the used STA, b) the strains composing a bacterial community may have different sensitivity and c) the concentration and type of substrate of marine broth and seawater with oil are different. With regard to the second point, Hamdan and Fulmer (2011) found that Corexit EC9500A produced a reduction in production and viability for some strains (*Acinetobacter*, *Marinobacter*) of a bacterial community, although these effects occurred at concentrations (1-10 g L^{-1}) much higher than the maximum concentration of dispersant measured at sea (13 mg L^{-1}) (George-Ares and Clark, 2000). Finally, Song and Bielefeldt (2012) reported that inhibitory effects of four nonionic surfactants on *Sphingomonas chlorophenolicum* growth were more marked in a medium with pentachlorophenol as substrate than with glucose.

3.2. Toxicity of STA on sea urchin embryogenesis

The exposure of sea urchin embryos to the STAs caused an inhibition of growth according to a sigmoid shape. The growth inhibition was accurately described by model (3) for the three tested STAs (Figure 3). Adjusted coefficients of determination were in all cases higher than 0.995 and the robustness of the equation was assured in all cases (p<0.001) (Table 3). Additionally, EC_{50} and EC_{10} parameters were calculated using equations (3) and (4) and, based on these parameters, Finasol OSR 51 was found to be the most toxic STA followed by OD4000, CytoSol and Agma OSD 569. Those latter two STAs showed EC_{50} values one order of magnitude higher than Finasol OSR 51 and OD4000 (Table 3). Similar results were observed attending to the EC_{10} values. The EC_{50} is classified as: moderately toxic (1-10 ppm) for Finasol OSR 51 and OD4000, and slightly toxic (10-100 ppm) for CytoSol and Agma OSD 569 in accordance with the Ecotoxicity Categories for Aquatic Organisms (USEPA, 2013) (Table 3).

This high sensitivity of embryo-larval tests with molluscs or echinoderms to dispersants had been previously reported (George-Ares and Clark, 2000; Rial, et al., 2010). In fact, the EC_{50} values for Finasol OSR 51 (1.2 µL L^{-1}) and OD4000 (2.2 µL L^{-1}) were similar to those reported with *Crasosstrea gigas* (EC_{50}/48 h: 3.1 ppm) and *Haliotis rufescens* (EC_{50}/48 h: 1.6-2.2 ppm) for Corexit 9527 (Clark, et al., 2001; Singer, et al., 1990); but lower than those obtained by *Chaetoceros tenuissimus* (EC_{50}/72 h: 21 mg L^{-1}), *Calanipeda aquaedulcis* (LC_{50}/48 h: 49 mg L^{-1}) and *Pontogammarus maeoticus* (LC_{50}/48 h: 64 mg L^{-1}) for Finasol OSR 51 (Abbasova, et al., 2005). In a previous article, the toxicity of CytoSol was assessed using sea urchin as target organism (EC_{50}/48 h: 26.3 µL L^{-1}, Table 3). Furthermore, the efficacy of this

296 STA for eliminating *Prestige* oil from impregnated rocky coastal and toxicological
297 evaluation of CytoSol and oil runoff mixtures by mysid and mussel bioassays was
298 also studied (Rial, et al., 2010).
299
300 Dispersants available today are expected to be much less toxic to rainbow trout
301 (LC_{50}/96 h: 200-500 mg L^{-1}) than dispersants formulations prior to 1970 (5-50 mg L^{-1})
302 (Fingas, 2011; NRC, 2005). Some authors (Falk-Petersen and Lonning, 1984;
303 Lonning and Falk-Petersen, 1979) tested 17 dispersants using the sea urchin
304 *Stronglylocentrotus droebachiensis* and reported greater toxicity for the so-called
305 dispersant concentrates. Fertilization was affected by the dispersant concentrates at
306 1-10 ppm and embryonic development at 10-100 ppm. The toxicity observed for
307 embryo-larval test with *P. lividus* (Table 3) stresses that the new generation of
308 dispersants has similar or even higher toxicity for sea urchin embryos to those
309 reported by the latter authors. This does not support the assumption that dispersants
310 are nowadays less toxic and indicates that sensitivity to dispersants showed
311 remarkable differences between the species and life stages of the toxicity test
312 chosen.
313
314 The toxicity of a STA is determined by its constituents. Kirby et al. (2011) studied the
315 toxicity on *Tisbe battagliai* of eight usual constituents of dispersants and reformulated
316 dispersants from these constituents and concluded that: a) anionic surfactant dioctyl
317 sodium sulfosuccinate was the most toxic component, and b) small changes in the
318 contents of two components of low or no toxicity, hydrotreated light distillate (CAS
319 No. 64742-47-8) and monopropylene glycol produced significant increases in toxicity
320 of the reformulated dispersant. The two most toxic STAs were those containing
321 dioctyl sodium sulfosuccinate (according to the material safety data sheet OD4000

15-30% and Finasol OSR 51 <10%), although these ratios do not serve to explain the EC_{50} values obtained in the present work (Table 3). The solvent of both dispersants was a hydrotreated light distillate whose content was <70% and 30-60% for Finasol OSR 51 and OD4000 respectively, so observed toxicity was probably conditioned by the solvent or other constituents not reported on the material safety data sheet. On the basis of their toxicity, Agma OSD 569 and CytoSol are more recommendable for utilization in environmental applications than Finasol OSR51 and OD4000. The action of a spill-treating agent on an oil slick involves an increased exposure to hydrocarbons in the water column and the simultaneous exposure to the used STA, so the toxicity of the mixture of oil and the STA should also be assessed before considering its use.

4. Conclusions

No toxic effect of STA was recorded on the growth of marine bacteria selected in the present study (Ph and Ps). Finasol OSR 51 and OD4000 significantly inhibited the lactic acid bacteria growth at the highest concentrations tested, descending such inhibition according with the bivariate equation defined in (2). The respective global $EC_{50,\tau}$ values obtained were 129 and 754 $\mu L\ L^{-1}$ highlighting the greater toxicity of OD4000 in comparison to Finasol OSR 51. A higher toxicity in terms of EC_{50} or EC_{10} values was found for both Finasol OSR 51 and OD4000 in the sea urchin bioassay. Those concentrations were much lower than caused by CytoSol and Agma OSD 569. Given these results, CytoSol and Agma OSD 569 would be the most indicated STAs to be applied on environmental oil spill due to their *in vitro* less toxicity.

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454

FIGURE CAPTIONS

Figure 1: Examples of STA effects on selected bacterial kinetics. ○: control; ●: 100 µL L^{-1}; ◆: 1000 µL L^{-1}; ✖: 2000 µL L^{-1}. Error bars are confidence intervals.

Figure 2: Effect of OD4000 and Finasol OSR 51 concentrations on Lm growth kinetics (points) and fittings to equation (1) (surface). Confidence intervals were in all cases less than 10% of the experimental mean value and omitted for clarity.

Figure 3: Inhibition of sea urchin larval growth by Finasol OSR 51 (left, ◇), OD4000 (center, □) and AGMA OSD 569 (right, O). Experimental values (points) were fitted to the Weibull equation (3) (line). Error bars are confidence intervals.

TABLE CAPTIONS

Table 1: Symbolic notations used and corresponding units

Table 2: Parametric estimates and confidence intervals (α=0.05) from the equation (2) applied to the Lm growth data influenced by Finasol OSR 51 and OD4000 concentrations. Statistical values of adjusted coefficient of multiple determination (R^2_{adj}) and *p*-values from Fisher's F-test (α=0.05) are also summarized. NS: non-significant.

Table 3: Growth inhibition of sea urchin larvae by STA. Parametric estimates and confidence intervals (α=0.05) obtained by fitting experimental data to the equations (3) and (4). Statistical values of adjusted coefficient of multiple determination (R^2_{adj}) and *p*-values from Fisher's F-test (α=0.05) are also shown.

Figure 1

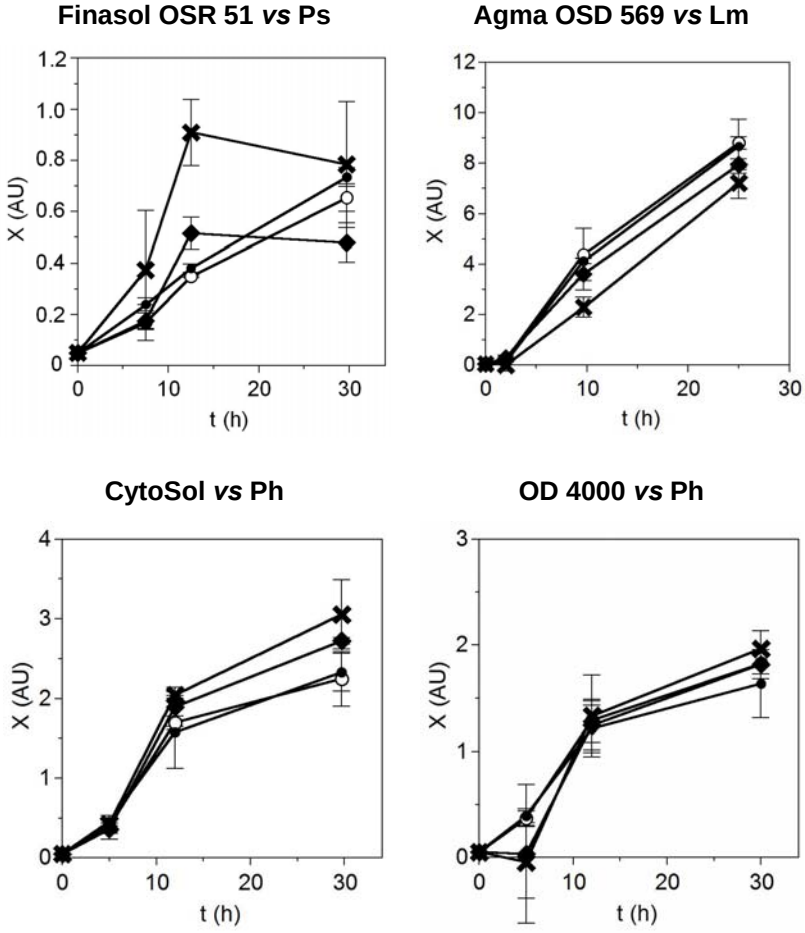
Finasol OSR 51 *vs* Ps
Agma OSD 569 *vs* Lm
CytoSol *vs* Ph
OD 4000 *vs* Ph
X (AU)
t (h)

Figure 2

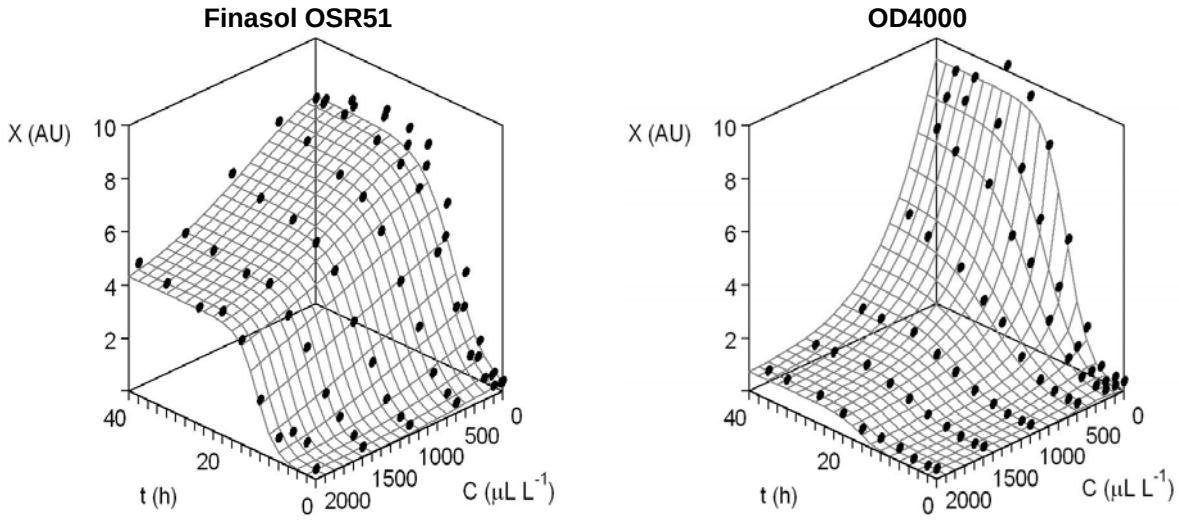
Finasol OSR51
OD4000
X (AU)
10
8
6
4
2
40
20
0
t (h)
0
500
1000
1500
2000
C (µL L-1)

Figure 3

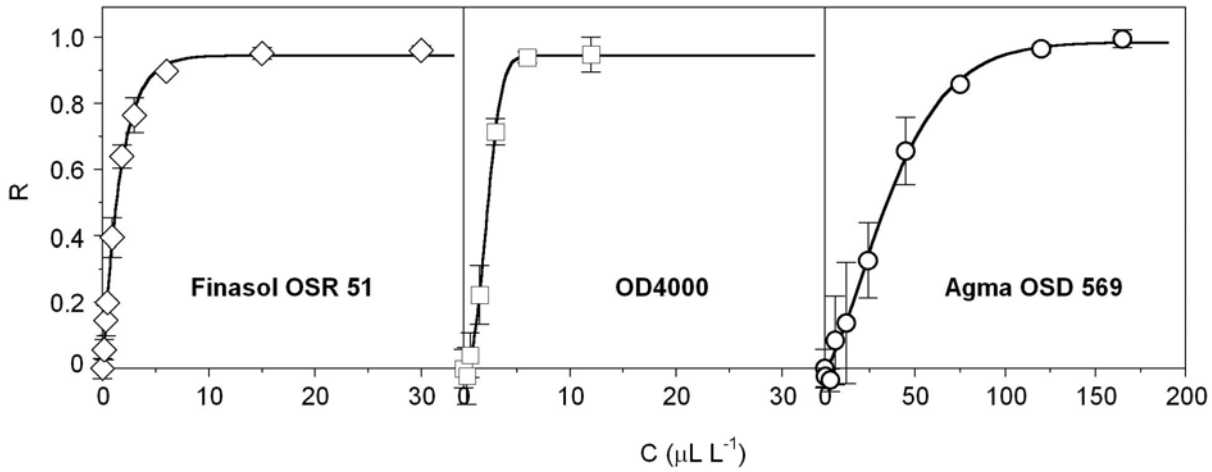
R
1.0
0.8
0.6
0.4
0.2
0
Finasol OSR 51
OD4000
Agma OSD 569
0
10
20
30
0
10
20
30
0
50
100
150
200
C (μL L-1)

Table 1

Growth dynamics measured by optical density	
X :	Growth measured as absorbance at 700 nm. Units: absorbance units (AU)
t :	Time. Units: h
X_m :	Maximum bacterial load. Units: absorbance units (AU)
v_m :	Maximum growth rate. Units: AU h^{-1}
λ :	Lag phase. Units: h
$X_{m_\bullet}$:	Maximum bacterial load affected by STA. Units: absorbance units (AU)
$v_{m_\bullet}$:	Maximum growth rate affected by STA. Units: AU h^{-1}
$\lambda_\bullet$:	Lag phase affected by STA. Units: h
Concentration effects on growth dynamic	
C :	Concentration of STA. Units: μL L^{-1}
K_x :	Maximum response affecting on X_m. Dimensionless
m_x :	Concentration corresponding to the semi-maximum response affecting on X_m. Units: μL L^{-1}
a_x :	Shape parameter affecting on X_m. Dimensionless
K_v :	Maximum response affecting on v_m. Dimensionless
m_v :	Concentration corresponding to the semi-maximum response affecting on v_m. Units: μL L^{-1}
a_v :	Shape parameter affecting on v_m. Dimensionless
b:	Slope corresponding to the semi-maximum response affecting on λ. Units: L μL^{-1}

Table 2

Parameters		Finasol OSR51	OD4000
growth model	X_m (AU)	7.81±0.32	9.20±0.21
	v_m (AU h^{-1})	0.58±0.07	1.03±0.11
	λ (h)	3.72±0.94	7.68±0.52
effect on X_m	K_x	0.50±0.25	0.93±0.03
	m_x (µL L^{-1})	959±497	372±36
	a_x	1.57±1.05	1.18±0.13
effect on v_m	K_v	NS	0.91±0.07
	m_v (µL L^{-1})	NS	113±22
	a_v	NS	0.77±0.16
effect on λ	b (L µL^{-1})	0.003±0.001	0.003±0.001
	$EC_{50,T}$ (µL L^{-1})	754	129
	p-value	<0.001	<0.001
	R^2_{adj}	0.975	0.995

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681
682 **Table 3**
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	STAs			
	CytoSol	**Finasol OSR51**	**Agma OSD569**	**OD4000**
K	0.76±0.03*	0.94±0.03	0.98±0.05	0.94±0.03
EC_{50} (μL L^{-1})	26.3±3.1*	1.2±0.1	34.0±3.9	2.2±0.1
a	1.01±0.12*	1.02±0.10	1.44±0.25	2.37±0.35
EC_{10} (μL L^{-1})	4.1 ±0.9	0.2±0.1	9.3±2.3	1.0±0.1
R^2_{adj}	0.998*	0.997	0.995	0.999
***p*-value**	<0.001*	<0.001	<0.001	<0.001

* Results previously reported by Murado et al. (2011).

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