DEGRADABILITY AND SEDIMENT SORPTION OF AN ALCOHOL POLYGLYCOL ETHER SURFACTANT PUTATIVELY USEFUL FOR THE CONTROL OF RED SWAMP CRAYFISH IN RICE FIELDS

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Abstract. This work reports studies of the degradation rates of a fatty alcohol polyglycol ether non-ionic surfactant, *Genapol OXD-080*, putatively useful for the control of red swamp crayfish (*Procambarus clarkii* Girard) in rice fields under laboratory and field conditions. The influence of temperature, sediment site specificity and sorption were taken into account. The degradation kinetics of the surfactant depends on the experimental conditions: type of inocula and temperature. The distribution of this chemical in aquatic systems was also examined. *Genapol OXD*-080 was removed into the sediments readily after application, and sorption was considered the major path of removal from the water phase. Data suggest that further studies are required regarding the effects of *Genapol OXD-080* in aquatic organisms resident in rice fields, in parallel with the development of technologies related with the use of surfactants to control *P. clarkii* populations.

1. Introduction

The non-ionic surfactant *Genapol OXD-080* is polyglycol (8 glycol units) ether of a fatty alcohol (12 to 15 C) formulated by HOECHST. It has been shown to efficiently depress oxygen uptake in red swamp crayfish (*Procambarus clarkii* Girard) at concentrations as low as 0.005% (w/vol). As the surfactant interferes with *P. clarkii* respiratory exchanges (gill hematosis), a limitation of physiological and physical activities of animals occurs, yet allowing full recovery of physical activity after surfactant removal (Fonseca *et al.*, 1996). Therefore, the application of this chemical in rice fields was suggested as a way to putatively depress crayfish activities in rice fields and reduce the damages caused by crayfish to rice crops (Anastácio *et al.*, 1995; Fonseca *et al.*, 1996). This methodology is regarded as the basis for the development of cleaner and less harmful technologies (pesticide alternative), for an integrated control of crayfish populations in rice fields. Besides, it may permit harvesting crayfish as a food resource and the application of this surfactant during the early growth stage of rice plants (Anastăcio *et al.*, 1995; Fonseca *et al.*, 1996). This approach has been investigated during the EU research project (AIR 3-CT 94-2432).

The Louisiana red swamp crayfish was introduced in Europe foreseeing a double

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production of rice and crayfish. This introduction was expected to solve ecological and market problems left by the extinction of the indigenous crayfish *Austropotamobius pallipes* (Lereboullet), as consequence of a disease caused by a fungus (*Aphanomyces astaci* Schikora) (Habsburgo-Lorena, 1978; Gaudé, 1984). Nevertheless, crayfish populations increased without control invading most of the rice fields and wetlands areas. The presence of large populations of *P. clarkii* in rice fields (detected in Portugal since 1984) resulted in substantial losses of rice yield. This occurs mainly, as a consequence of damages caused in the drainage systems and increased turbidity of the water column resulting from crayfish burrowing activities (Gaudé, 1986; Adão, 1993; Anastácio and Marques, 1995). Therefore, the crayfish 'acquired' the status of pest and farmers started to use noxious xenobiotic chemicals such as pesticides (e.g. parathion, hidrosulphon and dimethoate) envisaging pest eradication. The crayfish *P. clarkii* exhibits a high resistance to toxic compounds and hence it was realised that the effectiveness of these methods was very limited and, most important, negative impact on useful species was developed (Velez, 1980; Roqueplo and Hureaux, 1989).

The non-ionic surfactant *Genapol OXD*-080 was selected as the most effective to interfere with respiratory exchanges, from a set of vegetal oils and surfactants tested, in the course of a EU research project (Fonseca *et al*., 1996). For further developing the methodologies concerning the application of the surfactant *Genapol OXD*-080 for the control of crayfish populations in rice fields, it is necessary to assess the environmental risk due to the use of surfactants. Namely, the level to which these compounds will be available to aquatic organisms must be monitored (Tolls *et al*., 1994).

In order to evaluate the degradability of this non-ionic surfactant, degradation experiments were carried out, following its application in an experimental rice field. These degradation studies of *Genapol OXD-080* consisted, initially, of laboratory tests to evaluate the compound degradability in microcosms constituted of water and sediment from the experimental rice field submitted to controlled laboratory conditions. These experiments were run for ca. one month. Afterwards, field experiments were also performed by the application of this compound in an experimental rice field, in lower Mondego region, Portugal, following its degradation *in situ*. The lower Mondego valley is located at the central region of Portugal (40° 10′ N, 8° 41′ W) consisting of approximately 15000 ha, and rice is the main agricultural crop corresponding to 60% of the valley (Ferreira, 1991). Periodically, water samples from laboratory and rice field experiments were withdrawn, and the remaining surfactant extracted by sublation. The non-ionic surfactant content of samples was determined by means of the cobalt-thiocyanate method. Results were expressed as mg of CTAS (cobalt thiocyanate active substance) or as percentage of CTAS relatively to the initial content of samples, at the beginning of experiments.

The aim of this study was to evaluate the degradation rate of this compound, assessing the influence of temperature, sediment site specificity and sorption in this process. Results will provide further data and support for predictive fate models and environmental impact assessment. These are a 'must' for the proper evaluation of the potential use of this non-ionic surfactant in the development of cleaner methodologies for crayfish control.

2. Methods

The non-ionic surfactant *Genapol OXD-080* used in this work was formulated and provided by HOECHST-Portugal*. This surfactant is a mixture of polyglycol ethers of a fatty alcohol, with the following chemical formula:

$$CH_3\text{-}(CH_2)_y\text{-}O(\text{-}CH_2CH_2O)_x\text{-}H$$

consisting of a synthetic unsaturated linear fatty alcohol with 12 to 15 carbon atoms (y value), linked by an ether bound to polyoxyethylene (x = 8).

Field experiment consisted of *Genapol OXD-080* application, at the nominal concentration of 0.005% (w/vol), in an experimental rice field of 0.7 ha, previously flooded, at the 'Seminário' farm near the Pranto river, in lower Mondego region (Portugal). The surfactant was diluted in water and dispersed over the field. This application occurred during rice germination period. An average value of 20 cm for the water column was considered in the calculations of the amount of surfactant needed to achieve a final nominal concentration of 0.005% (w/vol). This average depth of the water column in the rice fields was derived from field measurements. Disappearance of the chemical from rice field water column was followed by withdrawing water samples at 10, 24, 144, and 264 h after the application. Samples were collected into polystyrene bottles and carried to the laboratory for surfactant content estimation.

For the laboratory studies five different situations were considered: *system 1* (S.1): non-sterile sediment (3 cm column); non-sterile local water (20 cm column); aerobic; 20 °C; *system 2* (S.2): non-sterile sediment (3 cm column); non-sterile local water (20 cm column); aerobic; 4 °C; *system 3* (S.3): non-sterile sediment (3 cm); non-sterile local water (20 cm); anaerobic; 20 °C; *system 4* (S.4): sterile sediment (3 cm); sterile local water (20 cm column); anaerobic; 20 °C; *system 5* (S.5): sterile sediment (3 cm); sterile local water (20 cm); anaerobic; 4 °C. Additional flasks were filled with distilled water and left without sediment (*controls*). Temperature controlled conditions were provided transferring flasks 2 and 5 into an acclimated room, at 4 °C, and flasks 1, 3 and 4 into another room, at 20 °C. All these flasks were left standing for 24 h, at the laboratory conditions, as mentioned. The work solution of *Genapol OXD-080* was prepared by dissolving a pre-weighted amount of this non-ionic surfactant in distilled water to a final concentration of 1% (w/vol). After this period, aliquots of the *Genapol OXD-080*

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work solution were added to the flasks to obtain a final nominal concentration of 0.005% (w/vol). These laboratory experiments were run for 744 h. At each sampling time (0, 72, 168, 264, 504, 672 and 744 h), water samples were collected from flasks, by withdrawing aliquots from the overlaying water column and centrifuging (1500 g_{av}, 20 min) to spin down sediments. Residual surfactant in the supernatant was extracted in Winckbold's columns by the sublation process and the non-ionic surfactant content of extracts was quantified by the cobalt-thiocyanate method (APHA, AWWA and WEF, 1992). Known quantities of pure *Genapol OXD-080* were used as standards of cobalt-thiocyanate active substance (CTAS), corresponding to a range of concentrations from 0.0100 to 0.0001% (w/vol) (Crabb and Persinger, 1964; Winckbold, 1971). Samples from the rice field experiment were treated with the same procedure. Results for each sample were expressed as the content of *Genapol OXD-080* (concentration of CTAS, % w/vol) or as percentage (%) of surfactant relatively to the initial concentration of *Genapol OXD-080*, at the beginning of the experiments. Degradation rates of *Genapol OXD-080* for each system were calculated by fitting first-order kinetics, accordingly to the linear regression model:

$$\ln \mathrm{C} = \ln \mathrm{C}_0 - k.\mathrm{t}$$

where C is the surfactant concentration, C_0 is the concentration at time zero, and, thus, ln C_0 is the ordinate intercept; k is the slope (first-order constant) and t is time. The time taken to degrade Genapol OX-080 to half of its initial concentration (half-life time, $t_{1/2}$), in each system, under different laboratory conditions, was compared. The half-life periods ($t_{1/2}$) were determined as:

$$t_{1/2} = 0.693/k.$$

For the evaluation of *Genapol OXD-080* sorption to sediments, the content of surfactant in initial samples withdrawn from flasks containing local sediment and water was compared with the content in samples collected from the control system (distilled water, without sediment) (Table II). These results were expressed as percentage of surfactant adsorbed to sediments (% of sorption).

A preliminary study was undertaken, before the more detailed degradation experiments, to provide a first approximation of the time period required to achieve half of the initial content of Genapol OX-080 due to degradation (half-life period). All containers (polystyrene or glass) used in this work were previously rinsed with distilled water and acetone to remove any trace of possible surfactant contamination.

The statistic parameter F (deviation-from-linearity 'mean squares'/within groups 'mean squares') from an analysis of variance procedure was used to test the null hypothesis of linearity. A 'wholly significant difference test' was applied to compare the slopes correspondent to the different systems. With regard to the sorption experiment, differences between variance and between means were tested with a F variance ratio test and a two-tailed t-test, respectively. (Zar, 1996). A P value ≤ 0.05

TABLE I

Degradation of the surfactant *Genapol OXD-080* using a first-order model ($\ln C = \ln C_0 - kt$)

	Systems					
	S.1	S.2	S.3	S4	S5	Rice-Field
k (day^{-1})	4.055×10^{-3}	1.791×10^{-3}	2.899×10^{-3}	1.746×10^{-3}	1.074×10^{-3}	4.5355×10^{-3}
$t_{1/2}$ (days)	> 7	> 16	> 9	> 16	> 26	> 6
Corr. Coefficient	–0.87007	–0.95564	–0.94663	–0.96309	–0.95564	–0.91451
Water	non-sterile	non-sterile	non-sterile	sterile	sterile	–
Sediment	non-sterile	non-sterile	non-sterile	sterile	sterile	–
conditions	aerobic	aerobic	anaerobic	anaerobic	anaerobic	aerobic
Temp (°C)	20	4	20	20	4	20#

k – curve slope; # average water temperature (°C) observed in the rice-field; (*P = 0.05)

was selected for determining significance of model and comparison of degradation rates.

3. Results and Discussion

Degradation curves of *Genapol OXD-080* under laboratory and rice field experimental conditions are illustrated in the plots of Figures 1 and 2. The content of surfactant in water phase relatively to its initial concentration (% initial content) is graphically depicted for the laboratory systems and rice field experiments. Laboratory experiments were designed to evaluate the degradation rate of the non-ionic surfactant *Genapol OXD-080* at two temperatures, 20 and 4 °C, and the influence of rice field inocula (sterilised or non-sterilised), over this process. Sediment sorption of *Genapol OXD-080* was also examined.

In situ degradability of *Genapol OXD-080* was assessed after its application in an experimental rice field. In order to achieve realistic conditions, the application of *Genapol OXD-080* was performed during the period of rice sowing and germination.

The degradation rate of this chemical was evaluated by comparing its half-life ($t_{1/2}$), in systems under different laboratory conditions and in the experimental rice field. Differences of only few hours or perhaps days were considered of little environmental significance (Cripe *et al.*, 1987). In laboratory conditions, biodegradation (non-sterilised sediment and water) and autodegradation (sterilised sediment and water) occurred with slower rates at the temperature of 4 °C. Half-life of *Genapol OXD-080*, under these conditions, was approximately 2.3 times longer at 4 °C than the corresponding value obtained at 20 °C, for biodegradation (Figure 2: system 1 and system 2 representing biodegradation at 20 and 4 °C, respectively), and 1.6

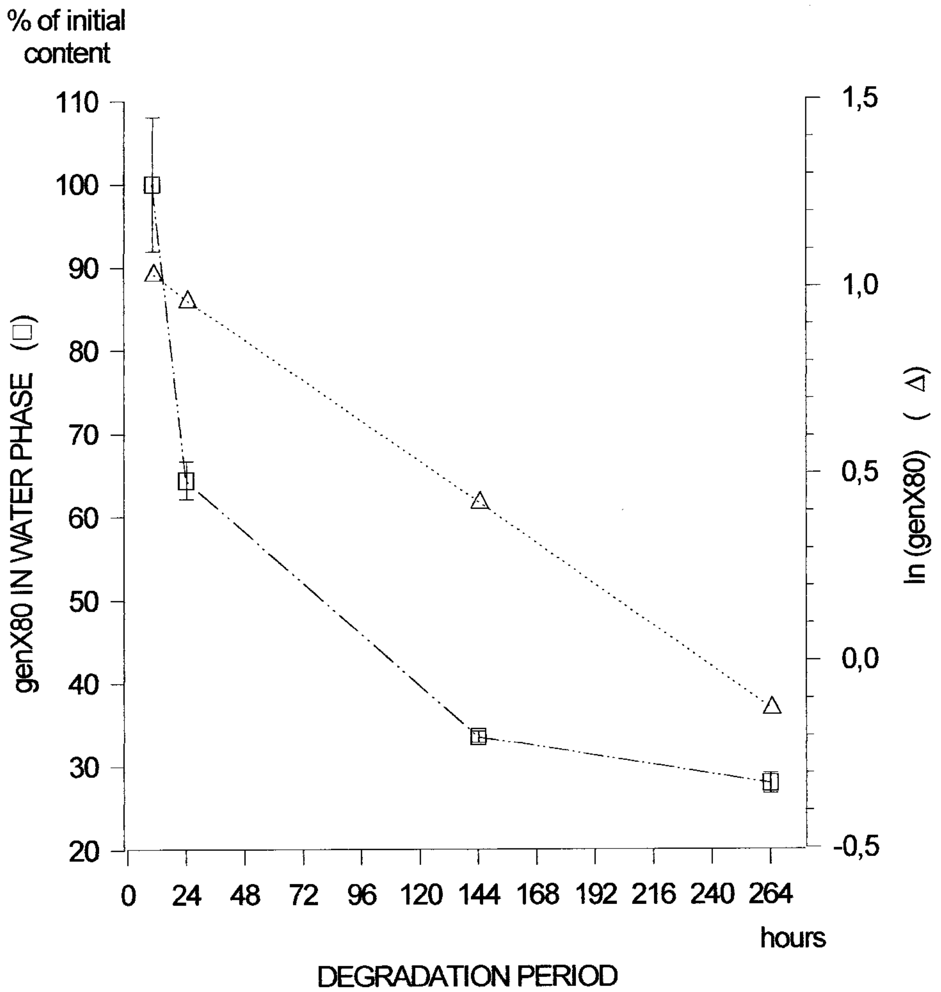

Figure 1. Degradation of the non ionic surfactant *Genapol OXD-080* (genX80) in the experimental rice field and correspondent linear model: $\ln C = \ln C_0 - k.t$. Vertical bars meaning the mean confidence interval, $\alpha = 0.05$; n = 4.

times longer at 4 °C than at 20 °C for autodegradation (Figure 2: system 4 and system 5 representing autodegradation at 20 and 4 °C, respectively).

The influence of rice field inocula (local water and sediment) over the degradation rate of *Genapol OXD-080* was estimated by comparing results from systems with non-sterilised local sediment and water and systems with sterilised components. When non-sterilised medium was used (biodegradation), the degradation rate of *Genapol OXD-080* was 2.3 times faster than with sterilised sediment and water (autodegradation) (Figure 2: systems 1 representing biodegradation and system 2 autodegradation).

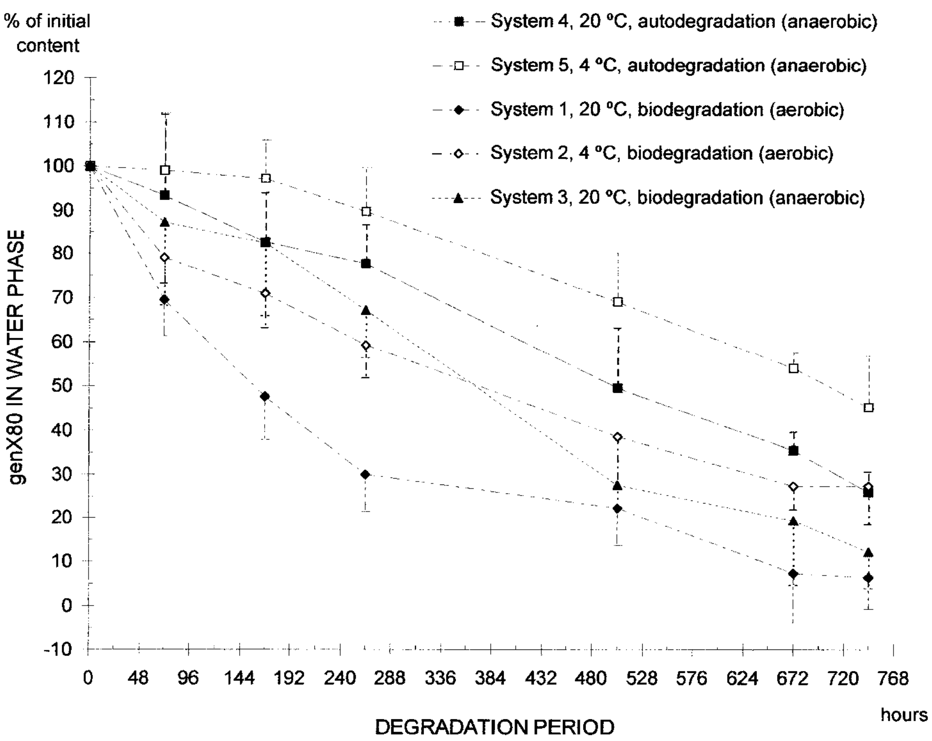

Figure 2. Degradation of the non ionic surfactant *Genapol OXD-080* (genX80) in experimental systems, under different laboratory conditions. Vertical bars meaning the mean confidence interval, $\alpha = 0.05$; n = 4.

The content of *Genapol OXD-080* in samples withdrawn from systems with local sediment and water was compared to controls (experimental media containing only distilled water and the applied surfactant), at the beginning of experiments. From these comparisons, the sorption level of *Genapol OXD-080* to sediments was estimated as *ca.* 35% ($0.002 < P < 0.005$) of the total amount applied at the beginning of tests, considering simple static microcosms constituted of local water (3 l) and sediment (300 g) (Table II).

The non-ionic surfactant *Genapol OXD-080* adsorbed to sediments was biodegraded considerably faster as compared to autodegradation. This has occurred presumably through a pathway involving oxidation of terminal primary alcohol followed by depolimerisation (aerobic) and/or through conversion into ethylene glycol and then fermented to acetate and alcohol (anaerobic) (Holt *et al.*, 1991), promoted by micro-organisms. This finding highlights the importance of selecting local sediment and water from the field as the source of local inocula for degradation studies. A site specific degradation test is preferred since it provides better information to afford protection of a particular environment (Cripe *et al.*, 1987). It is foreseen that one of the degradation pathways produces ethylene glycol. When

TABLE II

Sorption of *Genapol OXD-080* to sediments

	Systems		
	Control		Exp. system[a]
Mean Conc. (μg 100 ml^{-1})	7.50[a]		4.85[b]
STDEV.	0.8485		0.6602
N	4		4
* *F* – test (Variance ratio test)		1.285	
* *t* – test		4.976	

[a] 300 g of sediment/3 l of water;
[b] genX80 in water samples;
(* P = 0.05)

released into water, this chemical is expected to readily biodegrade and to have a half-life between 1 and 10 days. Additionally, it is not expected to evaporate and bioaccumulate significantly. Nevertheless, the ethylene alcohol is a toxic chemical when introduced into the environment, with LC50/96-hr values for fish over 100 mg l^{-1}. Therefore, future work should be developed to assess the environmental consequences of *Genapol OXD-080* degradation products.

Regarding the rice field experiment, results indicated a high removal rate of *Genapol OXD-080* from the water column, as consequence of local degradation. The first-order linear model of *Genapol OXD-080* degradation yields a half-life period of ca. 6 days.

The average temperature in rice fields, in Baixo Mondego, Portugal, during rice sowing and germination period is 20 °C (Anastácio, 1993). Nevertheless, since the degradation rate at 4 °C is considerably decreased (laboratory data), it is expected that temperature will strongly condition the exposition period to the compound of organisms in rice fields.

The surfactant content in the water phase rapidly decreased during 24 h after its application to about 63% of the initial content. Large amounts of suspended materials in rice field water were noticed, suggesting the possibility of significant transfer of the applied surfactant to the suspended material in the water column, following the application. This is in accordance with the high sorption percentage of *Genapol OXD-080* to sediments as estimated in the laboratory tests. Therefore, the approach described here of developing cleaner methodologies alternative to pesticides for the integrated control of *P. clarkii* populations (Fonseca *et al.*, 1996), should take into account this drawback to achieve effective concentrations of the surfactant. These findings also emphasise the importance of undertaking pertinent studies to supply reliable data for the risk assessment of these chemicals in rice fields. Determination of the conditions under which a material is bioavailable and identification of key routes and/or barriers to exposure are mandatory in the context of the risk assessment process (Pittinger *et al.*, 1989).

The *Genapol OXD-080* is less toxic than pesticides (e.g. parathion) commonly used by farmers to control crayfish in rice fields (Cabral, 1996) and the data collected from the present work, clearly establishes that this non-ionic surfactant is readily degraded both in laboratory and rice field conditions. From the putative use of *Genapol OXD-080* in crayfish population control a lesser bioaccumulation should be expected in comparison with pesticides to control this pest in rice fields (Brown and Avault, 1975; Baker, 1975). Data also indicate that *Genapol OXD-080* is rapidly removed from liquid phase by sorption to the sediments, stressing the importance of the distribution of surfactants, due to adsorption to particulate materials, to account for the effects in organisms of the water column (Lewis *et al.*, 1983). This reinforces the need for further studies of *Genapol OXD-080* effects on aquatic organisms present in rice fields, since surfactants are readily taken up through the gills (Tolls *et al.*, 1994).

4. Conclusions

The non-ionic surfactant *Genapol OXD-080* putatively useful in the development of cleaner non-harmful methodologies for *P. clarkii* population control in rice fields, alternative to pesticide usage, was readily degraded in laboratory assays and rice field experiments. Rice field local inocula and temperature strongly influenced degradability. The distribution of the surfactant in the aquatic systems significantly biased towards the sediments, and sorption was putatively the major process of removal from the water phase. Removal was also dependent on the activities of micro-organisms present either in the aqueous phase or the sediments. Data suggests further studies to assess the environmental effects of *Genapol OXD-080* and its degradation products (namely ethylene alcohol) on aquatic organisms present in rice fields.

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