



Colonized beads as inoculum for marine biodegradability assessment: Application to Linear Alkylbenzene Sulfonate

Aourell Mauffret^{a,b,*}, André Rottiers^a, Thomas Federle^c, David C. Gillan^d, Miriam Hampel^e, Julian Blasco^b, Ali Temara^a

^a The Procter & Gamble Company, Brussels, Belgium

^b Andalusia Institute of Marine Science, Puerto Real, Spain

^c The Procter & Gamble Company, Cincinnati, USA

^d Université Libre de Bruxelles (ULB), Bruxelles, Belgique

^e Institute of Aquaculture, University of Stirling, Stirling, UK

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ABSTRACT

An innovative biodegradation test system was developed in order to fill the current gap for cost effective and environmentally relevant tools to assess marine biodegradability. Glass beads were colonized by a biofilm in an open flow-through system of seawater with continuous pre-exposure to Linear Alkylbenzene Sulfonate (LAS) (20 µg/L). Thereafter, such colonized beads were added as inoculum in different test systems. [¹⁴C]-LAS (5–100 µg/L) was added and primary and ultimate biodegradation were assessed. The bacterial density collected on the beads (10⁹ bact./mL beads) was ca. 3 orders of magnitude higher than the typical seawater content. The LAS mineralization lag phase duration decreased from 55 to <1 days and the mineralization extent increased from 53 to 90% as the colonized beads volume increased from 10 to 275 mL. This is the first demonstration of marine bacteria's ability to mineralize LAS. On the opposite, less than 13% LAS was mineralized in seawater only. The colonized beads possibly enhanced the probability to encounter the full degraders' consortium in a low volume of seawater (100 mL).

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1. Introduction

Although marine biodegradability is a key end-point for persistence assessment under REACH, i.e., the latest chemical management piece of legislation in the European Union, there is limited attempt to standardize a marine bacterial inoculum. The OECD guideline (OECD306, 1992), for instance, recommends to add in closed units a sample of seawater as test medium with no addition of a specific inoculum. However, high variability with risk of false negative is associated to such procedure, as it is thereafter illustrated by the biodegradability of the surfactant Linear Alkylbenzene Sulfonate (LAS) (Shimp, 1989; Takada and Ogura, 1992; Terzic et al., 1992; Sales et al., 1999; Vives-Rego et al., 2000; León et al., 2004).

In 2003, 2.9 million tons of LAS were consumed in the world, mostly in detergents (Hauthal, 2004). Up to 99% of LAS is removed in wastewater treatment plants, mainly due to biodegradation (70 to 98%) and to a lesser extent to sorption or precipitation (2 to 20%) (Giger et al., 1989; León et al., 2006). Consequently, LAS concentrations in estuarine and coastal waters receiving treated domestic and industrial waste waters are typically below 10 µg/L (Temara et al.,

2001; León et al., 2002). Higher concentrations (up to 10 mg/L) have been measured in coastal waters close to untreated discharges (León et al., 2001; Temara et al., 2001; León et al., 2002). Several studies reported LAS primary biodegradation half life ranging from 2 to 21 days according to testing conditions, 6–9 days being the most frequently reported values (Terzic et al., 1992; Sales et al., 1999; Vives-Rego et al., 2000; León et al., 2004). Tests have been run so far using either i. estuarine (Shimp, 1989; Takada and Ogura, 1992; Terzic et al., 1992) or coastal waters (Terzic et al., 1992; Sales et al., 1999; Vives-Rego et al., 2000; León et al., 2004), ii. sediment (Shimp, 1989; Sales et al., 1999) or iii. water collected from an untreated marine discharge (León et al., 2004). In contrast, in offshore seawater (Terzic et al., 1992) and in pristine estuarine waters (Shimp, 1989), less than 10% of LAS was degraded within 14 days and 45 days, respectively. Several factors can potentially affect LAS biodegradation including LAS test concentration (Swisher, 1987; Perales et al., 1999), isomer and homologue composition (Terzic et al., 1992; León et al., 2004), temperature (Takada and Ogura, 1992; Terzic et al., 1992; Sales et al., 1999) and salinity (Takada and Ogura, 1992; Sales et al., 1999). However, the inoculum density and acclimation is likely to be a key parameter and probably the most difficult to standardize (Shimp, 1989; Courtes et al., 1995; Pagga, 1997; León et al., 2004).

A standardized inoculum should represent the large biodegradation capability of the marine environment. Large test volumes (Ingerslev

* Corresponding author. Andalusia Institute of Marine Science, Puerto Real, Spain. Tel.: +33 6 70 83 14 98; fax: +34 956834701.

E-mail address: aourell.mauffret@icman.csic.es (A. Mauffret).