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Changes in tolerance to herbicide toxicity throughout development stages of phototrophic biofilms

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ABSTRACT

Ecotoxicological experiments have been performed in laboratory-scale microcosms to investigate the sensitivity of phototrophic biofilm communities to the alachlor herbicide, in relation to the stages of phototrophic biofilm maturation (age of the phototrophic biofilms) and physical structure (intact biofilm *versus* recolonization). The phototrophic biofilms were initially cultivated on artificial supports in a prototype rotating annular bioreactor (RAB) with Taylor–Couette type flow under constant operating conditions. Biofilms were collected after 1.6 and 4.4 weeks of culture providing biofilms with different maturation levels, and then exposed to nominal initial alachlor concentration of 10 $\mu g\,L^{-1}$ in either intact or recolonized biofilms for 15 days in microcosms (mean time-weighted average concentration – TWAC of $5.52 \pm 0.74\ \mu g\,L^{-1}$).

At the end of the exposure period, alachlor effects were monitored by a combination of biomass descriptors (ash-free dry mass – AFDM, chlorophyll *a*), structural molecular fingerprinting (T-RFLP), carbon utilization spectra (Biolog) and diatom species composition. We found significant effects that in terms of AFDM, alachlor inhibited growth of the intact phototrophic biofilms. No effect of alachlor was observed on diatom composition or functional and structural properties of the bacterial community regardless of whether they were intact or recolonized. The intact three-dimensional structure of the biofilm did not appear to confer protection from the effects of alachlor. Bacterial community structure and biomass level of 4.4 weeks – intact phototrophic biofilms were significantly influenced by the biofilm maturation processes rather than alachlor exposure. The diatom communities which were largely composed of mobile and colonizer life-form populations were not affected by alachlor.

This study showed that the effect of alachlor (at initial concentration of 10 $\mu g\,L^{-1}$ or mean TWAC of $5.52 \pm 0.74\ \mu g\,L^{-1}$) is mainly limited to biomass reduction without apparent changes in the ecological succession trajectories of bacterial and diatom communities and suggested that carbon utilization spectra of the biofilm are not damaged resulting. These results confirmed the importance of considering the influence of maturation processes or community age when investigating herbicide effects. This is particularly important with regard to the use of phototrophic biofilms as bio-indicators.

1. Introduction

Pesticides are one of the most important sources of pollution for continental aquatic environments (Kreuger, 1998). By mechanisms including surface run-off, residual pesticides applied to agricultural lands migrate to surface and ground waters (*e.g.* Payraudeau et al., 2009). The intensive use of pesticides causes many disturbances in aquatic ecosystems (reviewed in DeLorenzo et al., 2001), the most important being the erosion of structural and/or functional biodiversity. In terms of ecotoxicology, the issue should not be limited to risk assessment on individual organisms but expanded to assessment at the ecosystem scale through the use of bio-indicators to assess ecosystem health. In this context, for aquatic ecosystems, phototrophic biofilms, microbial aggregates composed

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of heterotrophic micro- and meio-organisms and phototrophic micro-organisms embedded in an exopolymeric matrix, provide a particularly relevant model, based on their complex microbial community structures, spatial and temporal dynamics and variety of ecosystem services functions. These aggregates are considered as a key compartment of aquatic environments due to their contribution to most of the primary production, food resource for aquatic grazers, mineralization and element recycling processes (Battin et al., 2003), absorption (Lawrence et al., 2001) and biodegradation of chemical contaminants (Vercraene-Eairmal et al., 2010). Phototrophic biofilms are susceptible to biological and biochemical perturbation by herbicides, resulting in damage to overall ecosystem functioning. Numerous studies (Pesce et al., 2011; Guasch et al., 2012), in field, microcosm or mesocosm, have detected the sensitivity of phototrophic biofilms to herbicides depending on: the chemical (Debenest et al., 2009), the structural or functional endpoint observed (Villeneuve et al., 2011c), the trophic status (Pratt and Barreiro, 1998), the seasonal effects (Dorigo et al., 2004), the community composition (Guasch et al., 1997), the herbicide exposure level and frequency (Tlili et al., 2011), the phosphorus gradient (Tlili et al., 2010) and the current velocity (Villeneuve et al., 2011a).

To our knowledge mostly studies investigating the influence of phototrophic biofilms development stage as well thickness on its response to pollutants concern some metals (Admiraal et al., 1999; Ivorra et al., 2000; Duong et al., 2010). For organic pollutants, Guasch et al. (1997) observed that the response of phototrophic biofilms to atrazine was influenced by colonization time when experiments were carried out in winter when environmental conditions were relatively constant.

The model toxic molecule used in the present work was the alachlor herbicide [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl) acetamide] which is extensively used as a pre-emergence chloroacetanilide herbicide applied to corn and soybeans. This molecule is detected worldwide in surface waters, at variable concentrations from less than 1 $\mu g\,L^{-1}$ with peaks of several tens of $\mu g\,L^{-1}$ (Taghavi et al., 2010; Abrantes et al., 2010). Chloroacetanilide herbicides are known to inhibit the elongation of very long chain fatty acids in plants and algae (Böger et al., 2000) resulting in impaired cell development (Junghans et al., 2003; Valloton et al., 2008). To our knowledge, the toxicity of alachlor has mainly been assessed using single-species acute toxicity tests on green algae (Fairchild et al., 1997), cyanobacteria (Singh and Datta, 2005), bacteria and protozoa (Bonnet et al., 2007). A few studies showed the response of phototrophic biofilm communities to alachlor (Spawn et al., 1997; Carder and Hoagland, 1998; Pesce et al., 2011). For concentrations lower than 5 $\mu g\,L^{-1}$ of alachlor, biomass growth (based on chlorophyll *a* and ash-free dry mass (AFDM)) were inhibited and for concentrations up to 30 $\mu g\,L^{-1}$ algal species composition was affected.

The main objectives of this project were to assess the sensitivity of phototrophic biofilm communities to alachlor, with regard to biofilm age and physical structure (intact biofilm *versus* regrown from resuspension biofilm). Firstly, phototrophic biofilms were cultivated in a prototype rotating annular bioreactor (RAB) with Taylor–Couette type flow, specifically intended for the cultivation and investigation of phototrophic biofilms and operating under constant conditions, and then used for ecotoxicological experimentation after 1.6 and 4.4 weeks of development. Ecotoxicological experiments were performed in microcosms with intact or recolonized biofilms exposed to 10 $\mu g\,L^{-1}$ of alachlor. At the end of the exposure period, the response of the communities of biofilms was assessed by a multimetric approach including both structural and functional descriptors.

2. Material and methods

2.1. Phototrophic biofilm production

Phototrophic biofilms were produced in a laboratory prototype of a rotating annular bioreactor (RAB) with Taylor–Couette type flow, as described in details by Paule et al. (2011). Initially, the bioreactor was run in batch culture mode for a seeding period to allow the micro-organisms to become attached before the continuous culture mode started. During the seeding phases, the bioreactor ran in closed recirculation, connected to an aquarium (10 L) where the inoculum obtained by a resuspension of natural biofilms from various river stone was incubated (Paule et al., 2011). Two seeding phases were separated by a 24-h period where the RAB operated in continuous culture mode. The biofilm culture was investigated under controlled turbulent flowing conditions for 8 weeks. The bioreactor was continuously fed with a synthetic culture medium (the inlet throughput was 26 mL min^{-1}) which consisted of tap water supplemented with nitrate, phosphate and silicate (average concentrations during the culture in the RAB water exit: NO_3^--N = 4.2 mg L^{-1}, PO_4^{3-}-P = 0.356 mg L^{-1}, SiO_2 = 10.9 mg L^{-1}, conductivity = 368 $\mu S\,cm^{-1}$, pH = 7.1, dissolved organic carbon – DOC = 1.1 mg L^{-1}).

The inside of the RAB was illuminated by fluorescent lamps including cool daylight (Osram L15W/865 Luminux, Germany) and fluora (Osram L15W/77, Germany) tubes in equal proportions, with light/dark periods of 16 h/8 h and average recorded values were 180 ± 10 $\mu mol\,s^{-1}\,m^{-2}$.

After 1.6 and 4.4 weeks of development in the RAB (experimental conditions called “1.6 weeks” and “4.4 weeks”, respectively), a sampling of 9 colonized plates was carried out. Among these 9 plates from each sampling time, 6 plates were used directly for the microcosm study (experimental condition called “Plates with intact biofilm”). The last set of 3 plates was scraped with a microscope slide previously treated with 95% alcohol to ensure no trace of DNA. The biofilm from each plate was suspended in 90 mL of filter sterilized tap water (0.2 μm pore size filter, cellulose acetate membrane, Whatman) and homogenized (tissue homogenizer at 13,500 rpm, Ultra Turrax, T25). Each biofilm suspension homogenate was subdivided into aliquots, a first 45 mL subsample for the analyses of biomass descriptors (AFDM and chlorophyll *a*), algal abundance, T-RFLP and carbon source utilization assays (Biolog), and a second 45 mL subsample as inoculum source for the microcosm study (experimental condition called “recolonized biofilm”).

2.2. Microcosm setup

The impact of alachlor on the phototrophic microbial biofilm communities, was assessed in microcosms containing either sampling plates from RAB, with an intact three-dimensional structured biofilm, or inoculated with a suspension of biofilm from a sampling plate. The microcosm system consisted of glass beakers of 500 mL (VWR) previously autoclaved. Moreover, the beakers used for the experimental conditions “recolonized biofilm” contained clean glass slides treated with 95% alcohol allowing to study the impact of alachlor on the development of a new biofilm. Each beaker was initially filled with 300 mL of a sterile synthetic culture medium similar to the culture medium feeding the RAB but with 2× concentrations of each nutrient to avoid nutrient limitation (NO_3^--N = 8 mg L^{-1}, PO_4^{3-}-P = 0.6 mg L^{-1}, SiO_2 = 30 mg L^{-1}). Some beakers were treated with a nominal initial concentration of 10 $\mu g\,L^{-1}$ of alachlor, and others untreated were kept as controls. Alachlor purchased from Sigma–Aldrich (purity 99%) was dissolved in acetone (analytic quality, VWR) to make a stock alachlor solution. Aliquots of this stock solution were then added to treated microcosms to