Chemosphere 164 (2016) 52–58

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Environmental fate and effects of novel quorum sensing inhibitors that can control biofilm formation

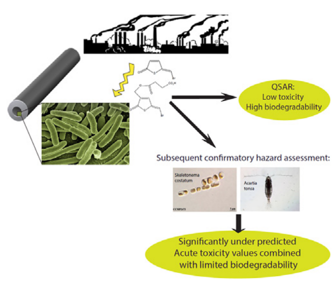
Adam Lillicrap [a,*], Ailbhe Macken [a], Aina Charlotte Wennberg [a], Merete Grung [a], Jan Thomas Rundberget [a], Lene Fredriksen [a], Anne Aamdal Scheie [b], Tore Benneche [c], Marc Anglès d'Auriac [a]

[a] Norwegian Institute for Water Research (NIVA), Oslo, Norway
[b] Department of Oral Biology, Faculty of Dentistry, University of Oslo, Oslo, Norway
[c] Department of Chemistry, Faculty of Chemistry, University of Oslo, Oslo, Norway

HIGHLIGHTS

- Thiophenones are novel quorum sensing inhibitors that can control biofilm formation.
- QSAR predictions significantly under estimated the environmental fate and effects.
- Confirmatory ecotoxicity tests are necessary when risk assessing novel compounds.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 April 2016
Received in revised form
5 August 2016
Accepted 17 August 2016

Handling Editor: Tamara S. Galloway

Keywords:
Thiophenones
Fate and effects
Quorum sensing inhibitors
Biofilms
Petroleum industry

ABSTRACT

The formation of bacterial biofilms can have negative impacts on industrial processes and are typically difficult to control. The increase of antibiotic resistance, in combination with the requirement for more environmentally focused approaches, has placed pressure on industry and the scientific community to reassess biofilm control strategies. The discovery of bacterial quorum sensing, as an important mechanism in biofilm formation, has led to the development of new substances (such as halogenated thiophenones) to inhibit the quorum sensing process. However, there are currently limited data regarding the biodegradability or ecotoxicity of these substances. To assess the environmental fate and effects of thiophenones capable of quorum sensing inhibition, candidate substances were first identified that have potentially high biodegradability and low ecotoxicity using quantitative structure activity relationships. Subsequent confirmatory hazard assessment of these substances, using a marine alga and a marine crustacean, indicated that these estimates were significantly under predicted with acute toxicity values more than three orders of magnitude lower than anticipated combined with limited biodegradability. Therefore, although these quorum sensing inhibitors appear a promising approach to control biofilms, they may also have environmental impacts on certain aquatic organisms.

1. Introduction

The control of bacterial biofilm formation (e.g. in industrial processes) is a major challenge and the development of more

* Corresponding author.
E-mail addresses: ali@niva.no (A. Lillicrap), ama@niva.no (A. Macken), acw@niva.no (A.C. Wennberg), mgr@niva.no (M. Grung), tru@niva.no (J.T. Rundberget), lfr@niva.no (L. Fredriksen), a.a.scheie@odont.uio.no (A.A. Scheie), tore.benneche@kjemi.uio.no (T. Benneche), mad@niva.no (M.A. d'Auriac).

http://dx.doi.org/10.1016/j.chemosphere.2016.08.083

effective environmentally friendly substances or better control strategies are needed whilst ideally avoiding the emergence of resistant bacterial strains. Bacterial biofilms are aggregates that develop on surfaces and are regulated by quorum sensing, a process by which bacteria communicate through regulating gene expression in response to changes in population density (Miller and Bassler, 2001). Bacteria use this process by releasing chemical signals, which in turn can control physiological processes such as virulence, competence, conjugation, antibiotic production, motility and sporulation in addition to biofilm formation (Miller and Bassler, 2001; Antunes et al., 2010). Preventing biofilm formation can be achieved by using bioactive molecules that disrupt the quorum sensing process, a phenomenon also known as quorum quenching (Kalia, 2013). The quorum sensing process operates through a wide range of signals such as oligopeptides (5–10 amino acid cyclic thiolactone), N-acyl homoserine lactones (AHLs), furanosyl borate (Autoinducer-2, AI-2), hydroxyl-palmitic acid methylester and methyl dodecanoic acid (Dong and Zhang, 2005; Kalia, 2013; McDougald et al., 2007). Several natural and synthetic molecules have been identified that can inhibit quorum sensing signalling (Kalia, 2013). One group of compounds called furanones have been documented to inhibit biofilm formation (de Nys et al., 2006; Ren and Wood, 2004). However, the toxicity of these substances to other aquatic organisms has been reported close to the concentration used to inhibit bacterial signalling (Defoirdt et al., 2012). Therefore, the use of other novel quorum sensing inhibitors, such as brominated thiophenones (sulphur analogues of brominated furanones), have recently been suggested as an alternative (Defoirdt et al., 2012). Thiophenones have already been shown to be effective in the prevention of biofilm formation (Defoirdt et al., 2012; Lönn-Stensrud et al., 2012; Witso et al., 2014). However, these novel compounds have little environmental toxicity data available to adequately assess the potential environmental impacts of these substances. For chemicals used to control biofilms to be authorised within the offshore oil industry, a Harmonised Offshore Chemical Notification Format (HOCNF) needs to be generated. This requires that any chemical, which is not on the PLONOR list (substances that Pose Little or No Risk to the Environment) needs to have a hazard and risk assessment carried out according to internationally accepted test guidelines (e.g. OECD and ISO) and in accordance with the principles of Good Laboratory Practice (GLP).

Based on the knowledge that historic chemicals used to control biofilms have been shown to be toxic to non-target organisms, Quantitative Structure Activity Relationships (QSARs) can be used to identify less environmentally hazardous chemicals for the same purpose. To test this hypothesis, appropriate candidate thiophenone substances, capable of quorum sensing inhibition and thus biofilm inhibition, were first identified and then assessed using QSARs. The potential candidate substances were selected for high inhibition of biofilm formation, low effect on planktonic bacteria and low cost of synthesis. Based on the results of QSAR models, the substances were ranked in order of predicted low toxicity and bioaccumulation potential combined with high biodegradability. Following the assessment of the QSAR data, two substances were selected for environmental fate and effect testing with the objective of validating the QSAR predictions. These tests included the regulatory accepted test species *Acartia tonsa* and *Skeletonema pseudocostatum*, and the determination of the potential biodegradability. All tests were performed following internationally accepted ISO standards (*A. tonsa* and *S. pseudocostatum*) and OECD test guidelines (biodegradability in seawater) and according to GLP. This paper describes the screening process and the confirmatory validation testing to determine if these new novel quorum sensing inhibitors (the brominated thiophenones) are likely to have adverse effects in the marine environment.

2. Materials and methods

2.1. Selection of chemicals

A library of 74 candidate thiophenones, potentially capable of quorum sensing inhibition, was developed by the University of Oslo. Screening for predicted lowest toxicity, highest biodegradability and lowest bioaccumulation potential was performed using the Episuite software from the US EPA (version 4.11). From these data, the substances were assigned a score to rank which substances should proceed to the biodegradability and ecotoxicity evaluations. Three of the compounds that were ranked highest (in terms of lowest scores) were T323, T310 and T349 (see Table SI 1). The predictions assumed that these substances have low fat solubility, an estimated degradation in the area of "weeks", and the estimated $LC_{50} > 10$ mg/L. The three compounds which received the highest score during ranking were all acids and subsequently, only one of these substances (T310) was chosen for testing. An additional substance (T101) that was ranked only 1 point lower, but which did not contain an acid group, was also chosen. These two substances (T101 and T310) had previously been shown to also have a high level of bioactivity and proven prevention of biofilm formation through quorum quenching (Defoirdt et al., 2012; Lönn-Stensrud et al., 2012; Witso et al., 2014). Details of these substances are shown in Table 1. The two substances were synthetized by Synthetica AS (Oslo Research Park, Gaustadalléen 21, Oslo, Norway) as previously described (Defoirdt et al., 2012; Benneche et al., 2011).

2.2. Testing for biodegradability

The biodegradability of the two thiophenones under aerobic, static exposure conditions in seawater was conducted in accordance with the OECD Guideline for Testing of Chemicals 306 (OECD, 1992) and to GLP, using the closed bottle test method. This method (OECD 306, 1992) uses dissolved oxygen consumption relative to the theoretical oxygen demand (ThOD) of the test substance as the endpoint for determining the rate of biodegradability in seawater and is a regulatory accepted method for the assessment of biodegradation in seawater (e.g. for the purposes of OSPAR for preparing a HOCNF). The seawater used for the test was collected from a depth of 60 m in the outer Oslofjord and stored in 30 L polyethylene containers. Surface seawater (2 L from 1 m depth) from the same location was added to the 60 m seawater to obtain an appropriate microbial load. The dissolved organic carbon concentration was analyzed after storage (1.0 mg C/L). The concentration of heterotrophic bacteria was also determined (3.5×10^4 CFU/L) by plating on marine agar (BD Difco 212185) and incubated for 3 days at 22 ± 2 °C. Prior to test initiation, the seawater was mixed and aged at 20 °C for seven days in the dark. On day 0, the seawater was aerated for 5 h and nutrient stock solutions (as specified in OECD 306, 1992) were added before the test media was added to the test flasks. Due to the low solubility of the test compounds, an inert support technique was used according to ISO 10634 (ISO, 2011) enabling the test substance to be added directly to the test bottles. The reference compound used to assess the viability of the indigenous bacteria was Aniline ($C_6H_5NH_2$, 99%, Mw 93.13, Sigma). A toxicity control was also included to determine if the test compounds inhibited the respiration of the microorganisms in the seawater test medium. Inhibition was calculated as the respiration in the toxicity control divided by the sum of respiration in the reference control and the test bottles.

The test bottles were incubated in a water bath in a temperature controlled room (20 ± 2 °C) and maintained in the dark. All bottles were stirred for at least 10 s twice per week and the dissolved oxygen (DO) concentrations were measured on days 0, 7, 14, 23 and