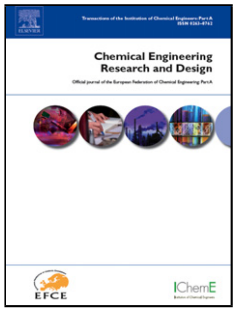

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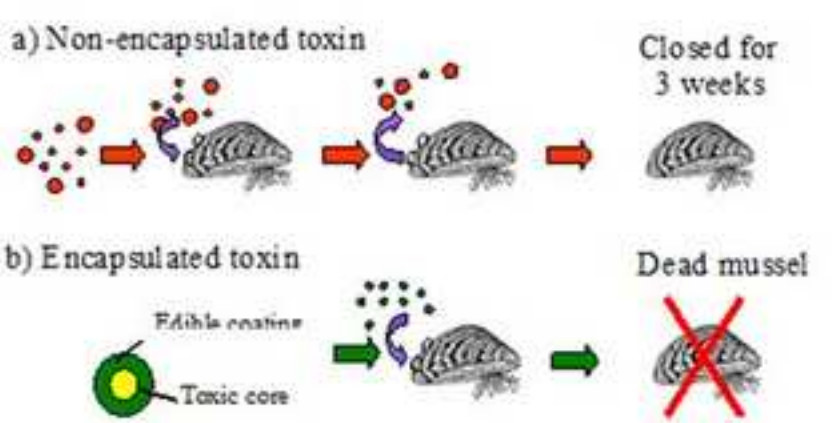
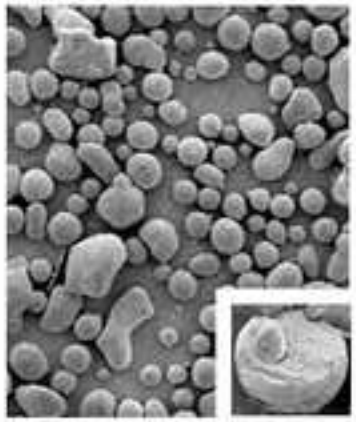
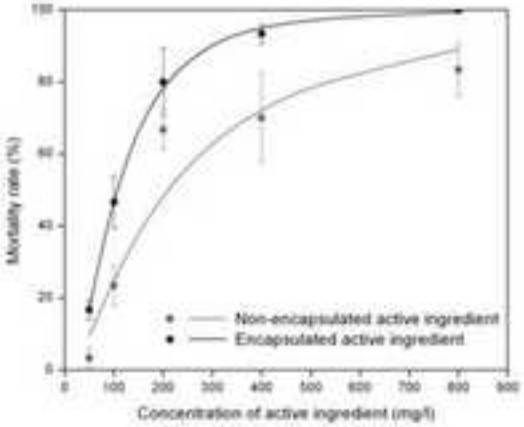
Graphical Abstract

Improved control of the biofouling zebra mussel through toxin-loaded particles: exploiting the bivalves' filtration ability and minimising their defensive responses







Preparation and evaluation of biocide-loaded particles to control the biofouling zebra mussel, *Dreissena polymorpha*

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Abstract

The freshwater zebra mussel *Dreissena polymorpha* is a powerful biofouling bivalve, which has tremendous impact on industrial facilities whose operation depends on the intensive use of freshwater, such as waterworks and power stations. The control of the pest in industrial environments remains a major challenge due to low selectivity over non-target organisms and the expense of the large quantities of biocides required. A novel delivery technique involving the encapsulation of a toxin within hundred micron-sized particles, edible for the bivalves, has been recently proposed. This strategy exploits the mussels' filtration activity and minimises their avoidance responses to certain chemicals, resulting in an increase of their susceptibility to the biocide. In the present paper, which further develops this approach, a new, promising toxin-loaded particulate formulation is presented. The effectiveness of the product as a molluscicide has been demonstrated in laboratory bioassays. Encapsulation was observed to reduce the amount of biocide required to achieve 90 % mortality in a 12-hour treatment by a factor of approximately three. The dependence of the biocide-loaded particles' molluscicidal activity on their physical characteristics is also illustrated in this paper by comparing the features of the promising formulation to those of an unsuccessful particulate product.

Key words: biofouling control, encapsulation, invasive species, spray chilling, zebra mussel

1. Introduction

Biofouling control is a major challenge in industrial facilities whose operation depends on the intensive use of water, such as drinking water treatment plants, pulp and paper mills and power stations (Jenner et al. 1998; LePage 1993). The epifaunal freshwater bivalve *Dreissena polymorpha*, commonly known as the zebra mussel, is one of the most damaging pests affecting such industries (Claudi and Mackie 1994). By attaching to and establishing dense populations on underwater structures, the species impairs performance and obstructs water flow in them. Typical problems caused by zebra mussel infestations include: pipe and equipment blockage, reduced efficiency of water cooling systems, increased corrosion, safety hazards by affecting fire protection units and disruption of plant operation associated with the need for biofouling removal (Costa et al. 2010). The economic losses caused by this invasive bivalve sum up to several billion dollars each year in the US alone (Pimentel et al. 2005).

Chemical treatment, involving the application of toxicants with lethal activity or compounds that impair the ability of the animals to attach to hard surfaces, is the most popular approach to control the zebra mussel in the industrial environment (Claudi and Mackie 1994). A large number of chemicals, including oxidising compounds, such as chlorine and ozone, and non-oxidising substances, such as aromatic hydrocarbons and metal salts, have been shown to have some degree of molluscicidal activity (Sprecher and Getsinger 2000). Compared to other methods, the chemical control approach tends to be cheaper as well as more versatile and flexible, because it can be easily applied in existing facilities and it allows protection of the entire system against a range of biofouling agents. In spite of its advantages, it raises significant concerns related to harmful impacts on non-target organisms and humans and the cost-efficiency of some toxins. These concerns have prompted research into improved, more environmentally

friendly solutions to control zebra mussel infestations (Costa et al. 2008a, b). In particular, the need to develop delivery techniques that allow the poisons to be targeted to *D. polymorpha* has been acknowledged.

A novel strategy for dosing molluscicides involving their encapsulation within roughly hundred micron-sized particles, embedded within a non-toxic material, edible for the bivalves, has been recently proposed (Aldridge et al. 2006; Costa et al. 2010). Microparticles have previously been applied in aquaculture to deliver nutrient supplements, antibiotics and other materials of interest to a variety of organisms, including fish, crustaceans and bivalves (Buchal and Langdon 1998; Mallo 2005). However, such an approach had not been effectively extended to the control of filter-feeding pests, especially invasive bivalves, until Aldridge and co-workers began to explore the use of particulate poisons for zebra mussel mitigation.

Two main principles are involved in the formulation of the biocide-loaded particles. Firstly, this form of toxin dosing exploits the great filtration capabilities of the bivalves. Zebra mussels are efficient filter feeders, capable of processing water at rates as high as 600 $ml.h^{-1}.individual^{-1}$ (Elliott 2005). Particle size is an important variable in the process by which the animals sort materials for ingestion from the inhalant water (Morton 1971; Ten Winkel and Davids 1982). Zebra mussels have been shown to filter algae with diameters between 0.7 and 450 μm, retaining preferentially those in the size range 5 to 35 μm (Sprung and Rose 1988). In addition, there is some evidence that the bivalves may complement this size-based selection mechanism with a chemical one that allows them to sort food based on its sensory quality (Morton 1971; Pires et al. 2004; Ten Winkel and Davids 1982). Providing that the toxic particles have suitable size and use coating materials that are edible for the zebra mussels, the animals actively filter them from the water column, concentrating them within themselves. As a result, the

lethal bulk water biocide concentrations necessary for effective mitigation are minimised. In addition to taking advantage of the bivalves' filtration capabilities, encapsulated poisons also represent a Trojan horse approach to control. The zebra mussels are able to sense some biocides in their surroundings and respond by closing their valves for hours to weeks to avoid contact with the harmful chemicals (Claudi and Mackie 1994; Sprecher and Getsinger 2000). The encapsulation of the poisons within an edible coating overcomes this defensive behaviour. By capitalising on zebra mussels' filtration activity and minimising their avoidance responses in the presence of certain toxins, particulate biocides increase the susceptibility of the bivalves to the encapsulated active ingredient, and thus provide economic, operational and environmental benefits. In the present paper, which extends and further explores the idea of mitigating zebra mussels through encapsulated molluscicidal agents, a new, promising toxin-loaded particulate formulation is presented. This formulation consists of a water soluble moderately low molecular weight polymer[2] coated with a mixture of vegetable oils and waxes by spray chilling, an efficient and low cost encapsulation technique that has been applied in the pharmaceutical and food sectors (Jacobs 2002). The active ingredient is already licensed in many countries for dosing in potable water and so is an attractive pest control agent, especially for use in the heavily regulated drinking water treatment industry. The organic coating materials used are edible for the zebra mussels and do not cause defensive valve closure behaviour in the animals (Elliott 2005). Furthermore, they are harmless, food-grade products, which should facilitate the potential licensing of the formulation for commercial application in industrial open systems that discharge

[2] The biocide-loaded formulations described in this paper are a proprietary technology of BioBullets Ltd, and for commercial reasons the nature of the active ingredient cannot be revealed. Since the paper addresses the principle of encapsulating toxins for biofouling control and the effect of the particles' physical characteristics on their effectiveness rather than the specific effects of the active ingredient itself, this information is not crucial to the paper's message. If readers would like further details of the product they should contact the authors.

effluents into surface waters. The toxin-loaded particles have been physically characterised in terms of microstructure, size distribution and retardant power, the latter described by the kinetics of active ingredient release into water. The molluscicidal performance of the formulation has been evaluated through laboratory renewal bioassays. In this paper, the influence of the toxic particles' physical characteristics on their molluscicidal activity is illustrated by comparing the features of the promising formulation to those of a less successful particulate product. The potential for application of biocide-loaded particles such as those presented here is not confined to the control of the invasive zebra mussel. This is an innovative formulation that may find use in other contexts.

2. Materials and methods

2.1. Formulation and manufacture of the biocide-loaded particles

The active ingredient is available for application in the strictly regulated drinking water treatment industry as a variety of liquid and solid products. The selection of the core material amongst such products proved to be a key effort in the development of the biocide-loaded particles. Preliminary bioassays showed that the toxicity to the zebra mussel of a wide range of active ingredient-containing products was comparable, with equivalent biocide concentrations producing similar lethal responses irrespective of the physical nature of the product or the molecular weight of the unencapsulated active ingredient. The particles' core could thus be entirely dictated by suitability for the spray chilling process. Initial encapsulation attempts using the most common liquid active ingredient-containing products were ineffective. A very viscous emulsion formed when the molten coating was mixed with the liquid active ingredient solution. This emulsion could not be properly atomised. The use of powder active ingredient-containing

products with low water-content was found to be critical for the success of the atomisation process, and hence was preferred.

Two biocide-loaded particulate formulations are described in this paper. One (hereinafter referred to as Formulation A), which constitutes a promising solution for zebra mussel control, incorporates one particular commercially available solid active ingredient-containing product, characterised in Table 1, as the core. In order to illustrate the dependence of the success of the encapsulated toxin on the particles' physical characteristics, the features of this formulation are compared to those of a different and less satisfactory particulate product. This (hereinafter referred to as Formulation B) incorporates as the core a different commercially available powder active ingredient-containing product, also characterised in Table 1. As shown in Table 1, toxicity tests on both unencapsulated active ingredient-containing products showed that they have similar biocidal activity for zebra mussels (on a weight for weight basis). Differences in the performance of the two toxin-loaded formulations can therefore be confidently attributed to the physical characteristics of the particles, rather than differences in the active ingredients used.

Both Formulation A and Formulation B were manufactured using a modified spray chilling process (TasteTech Ltd, Bristol, UK). They were prepared to incorporate the active ingredient-containing core material at a weight fraction of around 30 and 20 % (w/w) respectively. A food-grade mixture of vegetable oils and waxes (where hydrogenated triglycerides was the main ingredient) with no more than 10 % (w/w) impurities (including diglycerides and fatty acids) was used as coating. The core material, previously milled, was dispersed into the molten coating material under conditions of controlled shear. The resulting slurry was then sprayed through an ultrasonic atomising nozzle into a cooling chamber, where heat removal occurred to

solidify the atomised melt and produce the particles. These were finished by being coated with a thin layer of a food-grade nonionic surfactant in a fluid bed processor. Further details on the manufacturing process may be obtained from the authors.

2.2. Physical characterisation of the biocide-loaded particles

2.2.1. Microstructure examination

The microstructure of the biocide-loaded particles was analysed by Scanning Electron Microscopy (SEM) complemented by Energy Dispersive X-ray spectroscopy (EDX). Approximately 50 mg of particles was mounted on an SEM sample stub using doubled-sided carbon tape. About half of the particles were fractured using a blade so that their internal structure could be observed. The sample was then sputter coated with gold for SEM analysis or carbon for EDX scanning, and examined under a Philips XL30-FEG scanning electron microscope equipped with a LinkIsis EDX spectrometer.

2.2.2. Particle sizing

Particle size distributions were determined using a Coulter LS230 light scattering device. Ultra-pure water was employed as the dispersing medium.

2.2.3. In vitro release profile analysis

The ability of the biocide-loaded particulate formulations to suppress the release of the active ingredient was characterised by conductivity measurements. A 1-litre beaker containing 500 ml of deionised water was placed onto a hot plate stirrer thermostated at 25 °C. The beaker was covered with cling film to avoid water losses by evaporation. Once the temperature in the test container stabilised, 1 g of particles were dispersed into the water and maintained suspended by operating the magnetic stirrer at approximately

60 rpm. The conductivity of the aqueous medium was measured using a Jenway Model 4310 conductivity meter and conductivity cell. The LabVIEW platform (National Instruments (2004), LabVIEW, version 7.1. www.ni.com) was employed for real-time conductivity data acquisition. The sampling time in the measurements was 10 s. The measured conductivities were converted into active ingredient concentration values via calibration curves, previously obtained from known dilutions of the appropriate active ingredient-containing products (Table 1). The calibration curves were shown not to be affected by the presence of dispersed particles. The particles' core release tests lasted up to 24 h, but in all cases the conductivity of the aqueous medium was not observed to vary significantly after the first 5 h. The conductivity values measured at the end of the tests were used to determine the active ingredient load in the particulate formulation. The biocide dissolution kinetics was expressed in terms of the weight fraction of the particles' active ingredient content released to the aqueous medium over time.

2.3. Evaluation of the molluscicidal performance of the of the biocide-loaded particles

2.3.1. Test organisms

Zebra mussels were collected from the walls of a filter bed in a water treatment plant in London (UK) by using a paint stripper blade to carefully cut their byssus threads. Immediately after collection, the bivalves were transported to the laboratory in field water, and adult individuals with shell length in the range 20 to 30 mm were selected and thoroughly rinsed. The specimens were kept in aerated water in a temperature-controlled chamber at 19 °C (±1 °C), on a 12 h dark: 12 h light cycle. The bioassays, which were conducted in the temperature-controlled room, were initiated within one week of collection. Zebra mussels with a mean shell length of 23.5±1.9 mm (mean±SE)

and 25.2±1.8 mm (mean±SE) were used to assess the molluscicidal performance of Formulation A and Formulation B, respectively. Dechlorinated municipal water was used throughout all experiments.

2.3.2. Laboratory renewal bioassays

To assess the potential of each particulate formulation, sets of 10 zebra mussels were placed into 33 1-litre containers holding 500 ml of water. The test vessels were continuously aerated during the course of the experiment. 15 of them were set on magnetic stirrers operating at approximately 60 rpm. 2.5-centimetre magnetic fleas were located at the bottom of the containers away from the test organisms, which were distributed around the perimeter. Specimens acclimated for 48 h. Those that failed to attach to the bottom of the containers after this period were discarded. 90 to 100 % of the mussels in each vessel produced byssus threads during the acclimation period. The particulate formulation being tested was then dosed into the stirred containers so that the bivalves were exposed to 50, 100, 200, 400 and 800 mg/L of active ingredient. The biocide-containing product used as active core in the toxin-loaded particles was applied in non-stirred vessels to treat the zebra mussels with the same concentrations of the biocide dosed in its original, non-encapsulated form. The active ingredient concentrations tested were selected based on preliminary studies to produce mortality rates adequately covering the range 0 to 100 % at the end of the bioassays. Control organisms were not exposed to toxins. Each of the 11 treatments was applied in triplicate, the replications being randomly distributed within the temperature-controlled room. The zebra mussels were exposed to the treatments for 12 h. During this period, the test medium in all containers was renewed every 3 h. The renewal rate was selected taking into account the expected residence time of the toxin-loaded particles in real

systems being treated as well as the need not to disturb the mussels' filtration activity for reasonably long periods over the laboratory bioassays. The renewal of the test medium minimised the exposure of the animals to toxin-depleted particles, which would hinder the estimation of the performance of the encapsulated biocide under realistic flow-through conditions. After the treatments, the zebra mussels were allowed to recover in clean water. The mortality rates in the test containers were assessed at the end of the treatments and after 24 h and 48 h recovery periods. Failure to respond to an external tactile stimulus provoked by a blunt probe was used as the death criterion. At each mortality assessment, dead specimens were discarded. The results of the bioassays were analysed in terms of the dose-response data obtained after the 48 h recovery period.

2.4. Statistical analyses

The statistical methods for general data analysis were used as outlined by Zar (1999) and implemented in STATISTICA (StatSoft, Inc. (2003), STATISTICA (data analysis software system), version 6. www.statsoft.com). The dose-response data obtained in the toxicity tests were modelled by Probit analysis using the software StatsDirect (StatsDirect, Ltd. (1990), StatsDirect statistical software. www.statsdirect.com). A significance level of 5% was used in all statistical analyses.

3. Results

3.1. Formulation A

3.1.1. Physical characteristics

The scanning electron micrographs presented in Figures 1a and 1b elucidate the microstructure of Formulation A. Particles within a wide size range were observed. The

smaller particles tended to be spherical, while the larger ones had more irregular shapes. Active ingredient rich zones entrapped in a matrix of the coating material (rather than a core shell type structure) could be observed in fractured particles. The presence of such zones was confirmed by EDX analysis (Figure 1b).

As shown in Figure 2, the particles in this formulation followed a unimodal size distribution and had a mean diameter (by volume) of 225.1±2.4 μm (mean±SE). More than 92 % (in volume) of the particles had diameters between 0.7 and 450 μm, but only approximately 1 % fell in the size range 5 to 35 μm (Figure 2b).

The actual weight load of active ingredient in Formulation A was 25.2±0.2 % (mean±SE). The dissolution kinetics of the biocide from the formulation was characterised by a marked initial burst, with 30 % of the particles' active ingredient content being released within the first minute of dispersion in water (Figure 3). The particles were observed to still retain approximately 20 % of their toxin load after two hours in the aqueous medium, and 10 % one hour later (Figure 3).

3.1.2. Molluscicidal performance

The laboratory renewal bioassays provided clear evidence of the Formulation A potential. As illustrated by Figure 4a, the biocide dosed as active core in this formulation was significantly more effective in mitigating zebra mussels than its non-encapsulated form alone (Table 2). The estimated biocide concentration necessary to kill 50 % of the test organisms (LC_{50}) nearly halved when it was incorporated in the particulate formulation, decreasing from 210 mg/L (95 % confidence interval limits for the estimate: 162 – 273 mg/L) to 107 mg/L (95 % confidence interval limits for the estimate: 84 – 132 mg/L). The increase of the active ingredient toxicity provided by Formulation A was even more dramatic for higher lethal responses. The toxin

concentration producing 90 % mortality (LC_{90}) was found to be almost three times lower when it was applied in the particulate formulation compared to the dosing of uncoated material: 296 mg/L (95 % confidence interval limits for the estimate: 227 – 451 mg/L) and 844 mg/L (95 % confidence interval limits for the estimate: 574 – 1599 mg/L), respectively.

3.2. Formulation B

3.2.1. Physical characteristics

Figures 1c and 1d illustrate the microstructure of Formulation B. The particles have spherical shape and smooth surfaces. Analysis of fractured particles by EDX, such as that presented in Figure 1d, revealed biocide lumps randomly embedded within the coating material in a matrix type structure. Information on the size of the particles in this formulation is presented in Figure 2. Their size distribution was unimodal, and their mean diameter (by volume) was 101.4±0.1 μm (mean±SE). No more than 1 % of the volume of particles had diameters outside the 0.7 to 450 μm range, but only approximately 2 % had sizes between 5 and 35 μm (Figure 2b). The aim of producing this formulation was to obtain smaller particles than those of Formulation A, with the thought that these might be preferentially ingested by the zebra mussels.

Formulation B contained an actual biocide weight load of 21.3±0.1 % (mean±SE). The particles had poor retardant power, releasing more than 80 % of their content within the first minute of dispersion in water (Figure 3). The particulate material did not retain more than 3 % of the toxin load after two hours in the aqueous medium (Figure 3). A side effect of producing smaller particles was that their retardant power in water was very significantly reduced, presumably because the active ingredient was less effectively embedded in the thinner coating matrix. Thus in comparing Formulation B

to the effective Formulation A, both a modest decrease in particle size and a large increase in the rate of release of the active ingredient in water are compared.

3.2.2. Molluscicidal performance

The laboratory renewal bioassays showed that Formulation B resulted in little enhancement of the biocide toxicity (Figure 4b). The lethal effects produced by the active ingredient were not observed to depend significantly on the dosage form as it was incorporated in this formulation (Table 2). The estimated median lethal concentration (LC_{50}) of the toxin delivered via Formulation B was slightly lower than, but not significantly different to, that of the uncoated active ingredient: 185 mg/L (95 % confidence interval limits for the estimate: 136 – 248 mg/L) and 234 mg/L (95 % confidence interval limits for the estimate: 184 – 302 mg/l), respectively. Presumably the unfavourable influence of faster toxin release from the Formulation B particles is more than outweighing the expected favourable effect of using smaller particles, resulting in much worse biocidal performance than was observed for the Formulation A particles.

4. Discussion

4.1. Physical characteristics and molluscicidal performance of the biocide-loaded particles

The active ingredient used in this study, details of which cannot be revealed for commercial reasons (see section 1), has been previously demonstrated to be an effective toxin for controlling zebra mussel infestations in industrial environments. Being licensed for dosing in potable water in many countries, it is a particularly attractive biocide for application in the drinking water treatment industry. However, the practical

use of the compound as a pest control agent in this industry is limited by the need to maintain low treatment dosages, due to regulatory and operational constraints. Moreover, the selection of this molluscicidal agent for use in mitigation programmes often conflicts with its relatively high cost per unit volume. For these reasons, strategies to reduce the active ingredient dosage required to achieve zebra mussel mortality are desirable. The aim of the work underlying this paper was to develop polymer-loaded particles as one such strategy.

The development of a manufacturing method to make satisfactory particulate products was a long and challenging process, over which several possible formulations were designed, produced, physically characterised and tested for their molluscicidal performance. Most formulations did not provide a significant increase of the susceptibility of zebra mussels to the biocide, but eventually a promising particulate material, which has been described here (Formulation A), was achieved (Figures 1a and 1b). The molluscicidal performance of the different biocide-loaded formulations can be explained on the basis of their physical characteristics. To illustrate such an analysis, the features of one of the unsatisfactory particulate formulations tested over the development process (Formulation B; Figures 1c and 1d) have also been presented in this paper.

The median lethal concentrations obtained for the biocide applied as both the original, uncoated products (sections 3.1.2 and 3.2.2) are consistent with the toxicity data reported in other works (Waller et al. 1993). Furthermore, they are not significantly different from each other (sections 3.1.2 and 3.2.2), which is in accordance with preliminary studies that showed that zebra mussels are equally susceptible to the toxin in both active ingredient-containing products (section 2.1; Table 1).

Both Formulation A and Formulation B proved to be lethal to the bivalves (Figure 4). Neither the formulations' coating material nor the nonionic surfactant applied on the surface of the particles to aid dispersion in water is toxic to the zebra mussels when dosed at the concentrations and for the exposure periods used in the laboratory renewal bioassays (Elliott 2005; R. Costa, unpublished data). The lethal effects caused by the biocide-loaded formulations must thus have been due only to the action of the toxin. The encapsulation of the active ingredient in Formulation A was observed to significantly increase its toxicity to the zebra mussels over the 12-hour laboratory renewal treatments (section 3.1.2; Figure 4a). The molluscicidal performance of this formulation still has to be confirmed under realistic conditions of mussel density, water quality, hydrodynamics and toxin dosages, but based on the results presented here it seems to be a promising approach for controlling the pest in freshwater-dependent industries. Whilst the encapsulated biocide will be more expensive than the uncoated active ingredient, the potential of the toxin-loaded particles should not be judged only in terms of the cost of the material, but also considering other economic aspects, such as the expenditure on downstream effluent decontamination processes, as well as environmental and operational benefits (section 4.2). Besides, in the treatment of low residence time flow-through systems, where the continuous replenishment of the particulate product is assured, its performance is expected to be even higher than that estimated based on the renewal (semi-static) bioassays.

In contrast with Formulation A, Formulation B was not found to significantly enhance the biocide action (section 3.2.2; Figure 4b). This can be explained on the basis of the different physical characteristics of the two formulations. More than 90 % of the particles in both formulations had diameters between 0.7 and 450 μm (Figure 2), matching the size characteristics of the materials typically captured by the zebra

mussels from the water column (Sprung and Rose 1988). Also, on average, only 1.5 % of the particles fell in the size range 5 to 35 μm (Figure 2), meaning that the large majority of the particles in both formulations must have been captured, processed in the gills but not actually ingested by the animals (Sprung and Rose 1988). The particles in Formulation A were considerably larger than those in Formulation B (Figure 2). Consistent with this difference, Formulation A was observed to have a much greater retardant power in water than Formulation B (Figure 3), even though the molecular weight of the polymer encapsulated in the first is slightly lower than that encapsulated in the second (Table 1), which could favour leakage if the particles had identical physical properties (Buchal and Langdon 1998). Both original active ingredient-containing products were milled to powders with similar size characteristics prior to encapsulation (section 2.1). Therefore the average coating thickness was lower in the smaller particles of Formulation B. Furthermore, the surface-to-volume ratio was higher in these particles. For these reasons, their ability to suppress the release of the encapsulated biocide was inferior to that of the larger particles in Formulation A. In view of the release profile of the toxin from Formulation B (section 3.2.1; Figure 3), the zebra mussels treated with this formulation were exposed to dissolved biocide and non-toxic, nearly exhausted particles rather than encapsulated biocide for most of the duration of each 3-hour replenishment period in the renewal bioassays. Consequently, the particulate formulation exerted a negligible enhancement of the toxin activity.

One last aspect deserves commenting on, in connection with the particles' physical characteristics. For biocide-loaded particles to exert their toxic effects, they have to be captured from the water column by the animals and, if not ingested, at least processed in the gills prior to being rejected as pseudofaeces. The degree to which particulate materials are filtered and retained by zebra mussels depend on their size (Sprung and

Rose 1988), and therefore this is believed to be an important factor determining the biocide-loaded particles' performance. Moreover, as discussed above, size is also critical for the spray-chilled particles' retardant power, which is decisive for their effectiveness because they have to be capable of suppressing toxin leakage while travelling through the water system before being captured by the mussels. In addition to directly and indirectly affecting the toxicity of encapsulated biocides, the particle size distribution is also an important variable from the manufacturing process point of view. For instance, the effort that has to be invested in milling the active core material prior to coating depends on the final particles' target size. Consequently, optimal commercial biocide-loaded formulations will require a trade-off between the particles' size characteristics, their retardant power and the manufacturing effort. It is likely that the toxicity of encapsulated formulations could be enhanced by increasing their retardant power in water and decreasing their mean particle size (particularly if the optimal range of 5 to 35 μm could be obtained): manufacturing constraints currently limit the ability to achieve these enhancements.

4.2. Benefits provided by encapsulated biocides for zebra mussel control

Encapsulated biocides for controlling bifouling zebra mussels are expected to provide economic and operational advantages as well as environmental benefits, which are discussed in this section.

By exploiting zebra mussels' filtration activity and overcoming their avoidance behaviour, particulate formulations augment the animals' susceptibility to the biocides. Effective control would thus be achieved through reduced treatment dosages and durations as compared to the direct application of the non-encapsulated toxin. This

results in obvious economic advantages, not only by diminishing the expenditure on toxins, but also by reducing the cost of downstream effluent decontamination processes. Biocide-loaded formulations offer the possibility of being tailored for specific applications to optimise their performance. For example, particles' robustness may be augmented to face situations of high turbulence or their retardant power may be adjusted according to the residence time in the system being treated. Additionally, the increase of the biocides' toxicity through encapsulation may open the opportunity of using substances whose application as control agents would not be viable if they were used in their uncoated form. This may be beneficial for example in cases in which the use of a toxin is limited by the moderate sensitivity of zebra mussels to that compound. Further operational advantages of encapsulated poisons include the fact that they promote reduced disruption of plant operation, related to shorter treatment durations; and they do not present storage or handling issues, in contrast to other biocides, such as chlorine dioxide.

Toxin-loaded formulations, such as those discussed in this paper, may incorporate harmless food-grade coating materials and may be designed so that they degrade before discharge into natural bodies of water. Combined with the fact that the particles provide an actual reduction of the total toxic load applied, this guarantees more environmentally friendly pest control.

The structure of the particulate biocides may also be exploited to perform selective mitigation in situations where effluent decontamination is not feasible and the survival of non-target organisms is of concern, such as in fisheries. In such situations, the particles can be used to target the toxin delivery and achieve control using low bulk water toxin levels, harmful only to filter feeders. The potential impacts of particles'

application on non-target filter feeders could be overcome by tailoring them to preferentially target zebra mussels, for example by particle size manipulation.

5. Conclusions

In this paper the encapsulation of biocides has been discussed as a novel, cost-effective and environmentally friendly approach to control the biofouling zebra mussel, a damaging pest in freshwater-dependent industries. The general economic, operational and ecological benefits of the method were analysed. A promising particulate formulation containing a water soluble moderately low molecular weight active polymer, licensed for use in the drinking water treatment industry, was presented. The potential of this formulation has been demonstrated in laboratory renewal bioassays. The dependence of the toxic particles' molluscicidal performance on their physical characteristics, namely particle size distribution and retardant power, was also discussed.

The encapsulation of biocides has so far been explored in the context of zebra mussel control. However, this approach may be applicable in the mitigation of other aquatic nuisances, including other bivalves, such as the blue mussel *Mytilus edulis*, and other suspension feeders, such as sea squirts, sponges, bryozoans and the hydroid *Cordylophora caspia*. Beyond the specific toxin-loaded formulation discussed here, the encapsulated biocide technology may be modified, refined and optimised to manage other biofouling species. This may be achieved in a number of ways, including (i) the incorporation in the particles of species-specific toxins and coatings, (ii) the production of particles that are size-specific to the organisms, and (iii) the increase of the particles' selectivity for use in natural environments, for example by exploiting the valve-closing

response of non-target bivalves and using a coating material that is sensed by them but not by the nuisance species.

While achieving small biocide-loaded particles in the hundred micron size range that are able to retain a substantial amount of the active ingredient as they are dispersed and transported in a flow-through water system is not straightforward, it is certainly a challenge worth undertaking for the improved control of filter-feeding biofouling species.

Acknowledgements

Biocide-loaded particles for controlling aquatic invertebrate filter feeding organisms (commercially known as BioBullets) are protected under international patents (EP 1251741B, CA 2396938, US-2003-0140862-A1). Financial support from the Portuguese Foundation for Science and Technology (PhD scholarship SFRH/BD/18731/2004) is gratefully acknowledged. The authors would like to thank the support from TasteTech Ltd.

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Figures

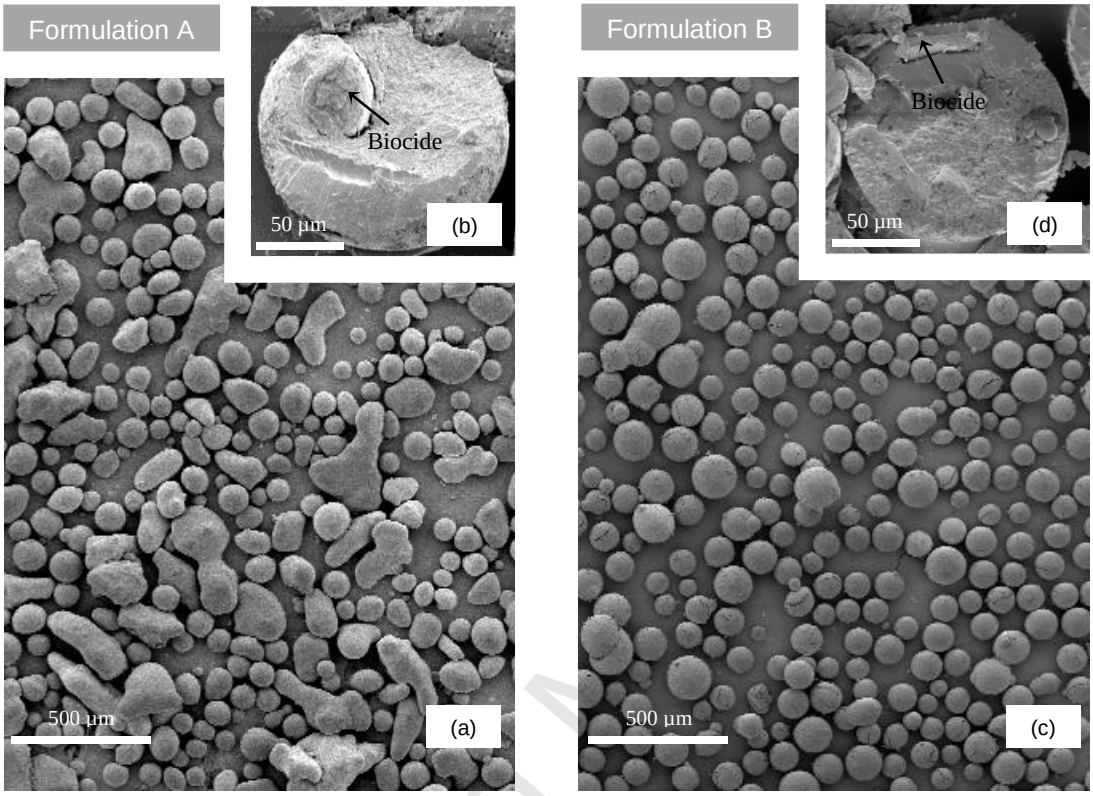
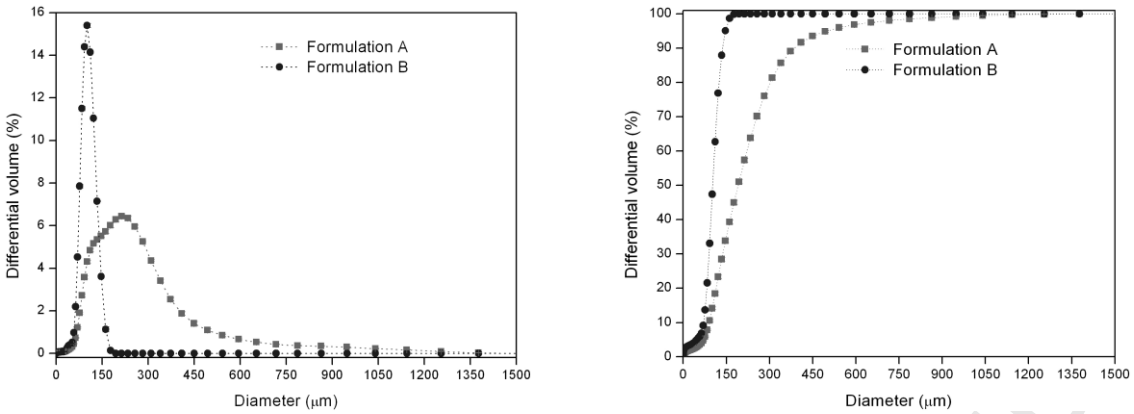


Figure 1 – Scanning electron micrographs elucidating the microstructure of Formulation A and Formulation B: (a), (c) general external appearance of the particles; (b), (d) inside of fractured particles.



(a) (b)

Figure 2 – Size characteristics of the particles in Formulation A and Formulation B: (a) differential volume (±SE) particle size distribution; (b) cumulative volume (±SE) particle size distribution.

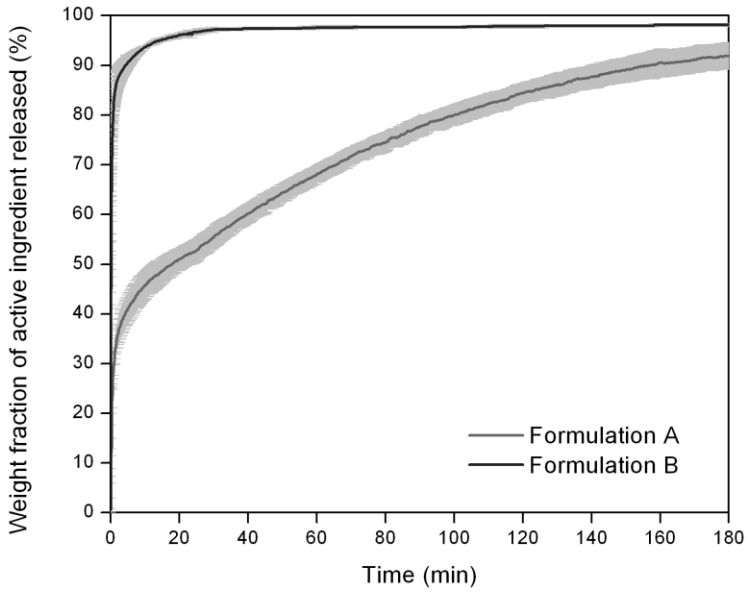
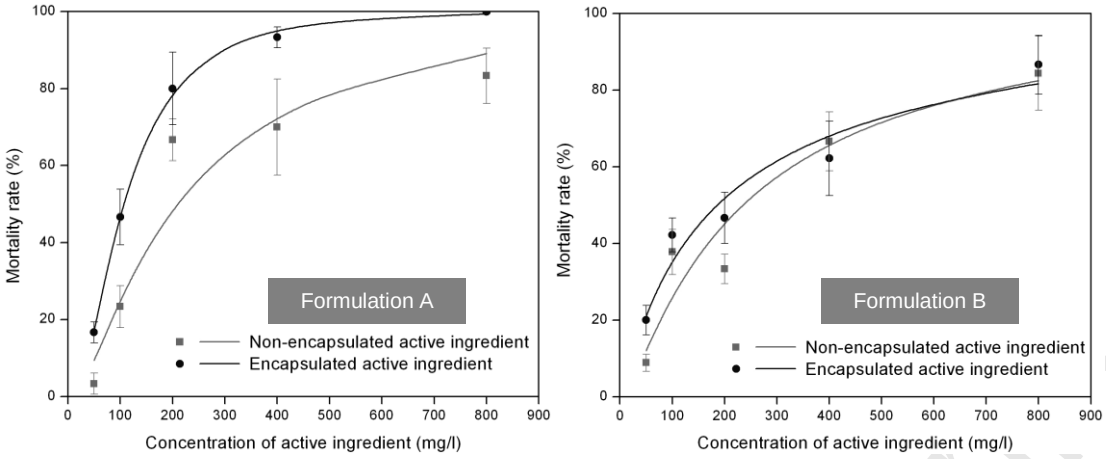


Figure 3 – Release profile of the active ingredient from Formulation A and Formulation B into water. Mean (±SE) values of the weight fraction of biocide released from 1 g of particles dispersed in 500 ml of deionised water at 25 °C are presented in the graph. The experimental toxin dissolution kinetics was determined by conductometrical technique integrated with LabVIEW platform for real-time data acquisition. The sampling time in the measurements was 10 s. See section 2.2.3 for further details.



(a) (b)

Figure 4 – Dose-response data obtained through renewal bioassays to describe the sensitivity of zebra mussels to the biocide dosed in its original form and as active core in: (a) Formulation A; (b) Formulation B. In the toxicity tests, the specimens were exposed to the biocide for 12 h and then allowed to recover in clean water for 48 h (see section 2.3.2 for further details). In the graph, the points refer to the experimental mortality data (mean±SE) and the lines represent dose-response models estimated by Probit analysis ($p < 0.01$).

Tables

Table 1 – Physical characteristics and toxicity to the zebra mussel of the powder active ingredient-containing products used as core material in Formulation A and Formulation B.

Active ingredient-containing product	Volume mean (±SE) particle diameter (originally)[a] (μm)	Active ingredient molecular weight[b]	Median lethal concentration of unencapsulated active ingredient after 48 h of exposure[c] (mg/l)	
			Estimate	95 % confidence interval
Product encapsulated in **Formulation A**	200.6±4.7	Low	28.2	20.2 – 39.4
Product encapsulated in **Formulation B**	43.0±1.2	Low medium	23.9	15.9 – 35.0

[a] A Coulter LS230 light scattering device was employed to determine the particle size distributions of the powders. Acetone was used as the dispersing medium.

[b] Only qualitative rather than quantitative information on the polymer molecular weight in the different active ingredient-containing products was provided by the supplier.

[c] The laboratory bioassays were run at 20 °C. Adult zebra mussels with shell length of 25.3±1.6 mm (mean±SE) were used as test organisms

Table 2 – Results of two-factor ANOVA for the investigation of the effects of biocide concentration and dosage form (coated *vs* uncoated) and the interaction of these factors on the mortality produced in the bioassays assessing the performance of Formulation A and Formulation B. Mortality data were arcsine transformed before analysis.

Source of variation	F	df	p
Formulation A			
Biocide concentration	28.665	4	< 0.001
Biocide dosage form	14.993	1	0.001
Interaction biocide concentration * biocide dosage form	0.095	4	0.983
Formulation B			
Biocide concentration	25.839	4	< 0.001
Biocide dosage form	1.055	1	0.317
Interaction biocide concentration * biocide dosage form	0.456	4	0.767