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Development of Sustainable Solutions for Zebra Mussel Control Through Chemical Product Engineering

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Abstract The zebra mussel is an important aquatic pest that causes great damage to freshwater-dependent industries, due to biofouling. The main goal of the project discussed here is to develop improved solutions to control this species. Three approaches have been explored in an attempt to design innovative application strategies for existing biocides: (i) encapsulation of toxins; (ii) combination of toxins; (iii) investigation of the seasonal variation of the species' tolerance to toxins. In this paper, the principles behind these approaches and the major results on each topic are presented. The benefits of adopting a chemical product engineering approach in conducting this project are also discussed.
Keywords zebra mussel control, chemical product engineering and design

1 INTRODUCTION

The zebra mussel (*Dreissena polymorpha*) is currently one of the world's most important aquatic pests. Being epifaunal, having a great dispersal capacity and being able to establish highly dense colonies, this species is a powerful biofouler, causing great damage to freshwater-dependent industries [1]. The losses due to zebra mussels, including monitoring and control costs, have been estimated to be on the order of US$1 billion per year in North America alone [2].

The methods currently available for the control of this bivalve, amongst which the use of chemicals with toxic properties is the most popular, present a number of significant disadvantages, principally harmful impacts on non-target organisms [3] and expense [4].

The main goal of the project summarised here is to develop more efficient and environmentally friendly chemical solutions for zebra mussel control. Innovative application strategies and delivery methods for existing biocides rather than new toxins have been sought. Three solution concepts have been explored in this context: (i) encapsulation of toxins; (ii) combination of toxins; (iii) investigation of the seasonal variation of the species' tolerance to toxins. The unifying idea behind these three distinct approaches is "to define what to apply, how to apply and when to apply".

In this article, the principles behind each of these approaches are discussed and the main findings on each topic are summarised. The benefits of adopting a chemical product engineering perspective in conducting this project are also discussed.

2 ENCAPSULATION OF TOXINS

2.1 Principle

The concept behind the formulation of control methods based on the encapsulation of toxins is the exploitation of the great filtration capabilities of the zebra mussel (up to 100 $ml \cdot h^{-1} \cdot individual^{-1}$ [5]). As efficient filter feeders, zebra mussels should filter toxins dosed in water in particulate form, concentrating them within themselves. As a result, the lethal bulk-water concentrations of the poisons will be reduced. Furthermore, the encapsulation of toxins also represents a "Trojan horse" approach to control, with the entrapment of the biocides into a nutritious coating minimising the defensive valve closing response the mussels show in the presence of certain chemicals [6].

In addition to obvious economic benefits, an enhancement of the toxicity of molluscicides provided by their encapsulation will offer environmental advantages. If particulate poisons are designed so that they degrade before discharged into waterways, the low concentrations at which toxic materials are presented will not pose a danger to non-target organisms.

The principle of controlling zebra mussels based on the encapsulation of toxins has been demonstrated in a previous study using potassium chloride as a model poison [7, 8].

2.2 Major results

In this study the principle of toxin encapsulation has been extended by (i) coating other materials than potassium chloride and (ii) investigating the mode of action of particulate poisons.

The major economic concerns about previous formulations of particulate toxins are associated with the fact that potassium chloride, being an extremely soluble salt, is very difficult to effectively coat. In this project a water-soluble, moderately high molecular weight polymer, which has proved toxic to the zebra mussel, has been encapsulated. A powder form of the polymer has been coated with a mixture of vegetable oil and vegetable wax by a modified spray chilling process, proprietary to TasteTech Ltd (Bristol, UK). It

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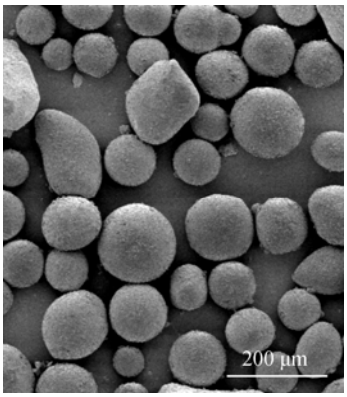
involved the dispersion of the core material, previously milled in an ultra centrifugal mill, into molten coating at 65°C. The resulted slurry was then sprayed into a cooling chamber, where heat removal occurred to solidify the atomised melt and produce the particles. These were finished by being coated with a thin layer of surfactant to aid their dispersion in water. The microstructure of the toxin-loaded formulation is elucidated in Fig. 1. Its performance has been evaluated in laboratory renewal bioassays. Adult zebra mussels were exposed to a series of concentrations of polymer dosed in both its encapsulated and uncoated forms for 12 h. The test medium was renewed every 3 h to minimise the exposure of the test organisms to toxin-depleted particles, which would hinder the estimation of the product performance under realistic flow-through conditions. The specimens were allowed to recover for 48 h in clean water before the lethal effects elicited by the different treatments were monitored. The results of the toxicity tests showed that the encapsulation of the polymer increases its toxicity by approximately 50%.



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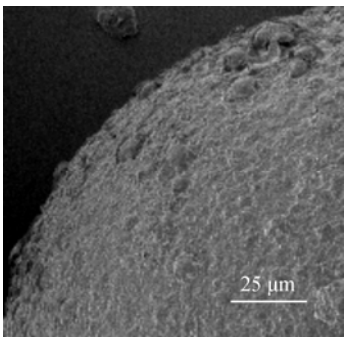

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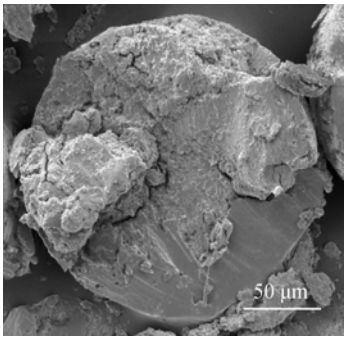

(c)

Figure 1 Scanning electron micrographs elucidating the microstructure of the toxin-loaded formulation: (a) **general external appearance of the particles;** (b) **detail of a particle's external surface;** (c) **inside of a fractured particle**

The mechanism through which encapsulated poisons exert their toxic effects on zebra mussels has not yet been fully explained. Two possible routes of toxicity are: (i) releasing of the toxic agent in the mussels' gills, or (ii) processing of the particles in the mussels' digestive system. Understanding which of these two routes constitutes the primary mode of action of the particulate biocides may provide crucial information for their design and manufacture. The degree to which toxin-loaded particles are captured, processed and ingested by zebra mussels depends on their size [9], and therefore this is expected to be an important factor determining their performance. The retardant power of the particles will also be critical for their effectiveness. Under circumstances of practical application, one of the main challenges is to guarantee that the release of the toxic agent is prevented while the particles travel through the system before being captured by the mussels. The dissolution kinetics and particle size distribution of encapsulated materials are usually related [10]. Thus understanding the mode by which particulate poisons act may assist in establishing the optimum trade-off between size and dissolution characteristics.

The effect of particle size on the performance of encapsulated toxins has been examined. Testing microcapsules of the same type, with similar release characteristics, but distinct diameters is physically impossible. This difficulty was overcome by evaluating model encapsulated materials with different particle size characteristics and poor, but similar, retardant power in a flow-through experimental facility. The fact that the dissolution kinetics of the active core from the test products was characterised by a marked initial burst, with approximately 70 % of the particles' content being released within 1.5 min of dispersion in water, minimised the difference between their retardant powers. For such quickly-releasing model materials to perform satisfactorily, they had to be tested in a flow-through experimental facility in which the particles' residence time was approximately 6 min. Only by using such an apparatus could zebra mussels be successfully treated with encapsulated toxin rather than dissolved biocide and inert, toxin-depleted microcapsules. The experimental facility consisted of a series of 4 m long open flumes with a basal width of 5 cm. River water flowed continuously through the flumes, where the test organisms were held. Two formulations of potassium chloride-loaded particles, characterized by mean diameters (by volume) of 118 and 204 μm, were tested. Four different treatments were concurrently applied. Two of them involved the dosing of each of the potassium chloride-loaded fractions at concentrations corresponding to a salt dosage of 400 $mg \cdot L^{-1}$. The third treatment consisted of the application of the same concentration of uncoated potassium chloride. The fourth was the control treatment, in which zebra mussels were not exposed to toxins. The treatments were continuously applied for 5 h. The specimens were then allowed to recover in a flow of clean water for 43 h before the mortality rates in the

experimental flumes were assessed. The results of the bioassays showed that the enhancement of potassium chloride toxicity provided by the smaller particles is greater than that provided by the larger ones, which confirms the importance of size for particle performance (Table 1). The reason why smaller particles are more effective remains to be clarified—they may either be ingested and reach the mussel stomach more easily or, although acting on the gills, they may be subject to a lower level of rejection.

Table 1 Performance of potassium chloride-loaded particles with different size characteristics as determined in field-scale flow-through bioassays

Treatment	Mortality (mean±SE)/%
control	2.0±1.3
non-encapsulated toxin	1.5±0.9
encapsulated toxin-particles with a mean diameter of 118 μm	65.2±14.1
encapsulated toxin-particles with a mean diameter of 204 μm	40.1±10.8

Current research on toxin encapsulation includes (i) the scale up of the manufacture of polymer-loaded formulation; (ii) its testing at a large scale under field conditions; (iii) the generalisation of the use of encapsulated poisons to the control of other biofouling bivalves; (iv) the investigation of the influence of size on particle capture and processing.

3 COMBINATION OF TOXINS

3.1 Principle

Intuitively, the combination of toxins, with potential cumulative and synergetic effects, is a promising approach to zebra mussel control. However, this area has not been investigated extensively, and no consensus has yet been reached as to its potential. Only a small number of binary combinations of poisons have been tested, and in some cases the combined treatments seemed to actually perform worse than the toxins applied individually [11, 12].

3.2 Major results

The toxicity of several combinations of potassium chloride and poly(diallyldimethyl ammonium chloride) (polyDADMAC) has been evaluated through laboratory bioassays. These toxins have been selected as model chemicals because they have proved to be efficient against zebra mussels and they are licensed for dosage in drinking water treatment in the UK. In addition, potassium chloride, having paralytic effects on zebra mussels, seems promising for use in combined treatments because it may increase the exposure of mussels' soft tissues to other poisons. Adult zebra mussels were pre-treated with potassium chloride for 12 h and then exposed to mixtures for 30 d. The mortality in the test containers was monitored every 24 h in the first 10 d and every 48 h in the next 20 d. The medium in all containers was renewed every 48 h during the course of the experiment. The results of the bioassays showed that potassium chloride increases polyDADMAC toxicity when the latter is dosed at 2 $mg \cdot L^{-1}$, but not when it is applied at 10 $mg \cdot L^{-1}$ (Fig. 2). These results are relevant from two perspectives. First, they may have practical application in zebra mussel control because the concentrations used in the bioassays are below the regulatory dosage limits for both toxins in the UK drinking water treatment industry. Second, they show that the performance of the combination depends on the range of concentrations applied. Therefore, in exploratory studies aiming at the study of combined treatments, realistic concentrations should be used.

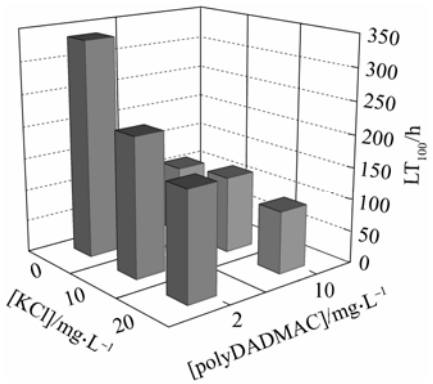

Figure 2 Time to achieve 100% mortality (LT_{100}) using different combinations of potassium chloride and polyDADMAC

The combination of potassium chloride with another organic zebra mussel poison has also been studied. This combined treatment was not more efficient than the application of the organic toxin alone. Therefore current research aims to clarify the reasons for the different performance of mixtures on the basis of potassium chloride sublethal effects and their interaction with the mode-of-action of the second toxin in the combination. Another aspect of current research on the combination of toxins is the extension of the principle to mixtures of more than two poisons and the control of larval stages.

4 SEASONAL VARIATION OF TOLERANCE TO TOXINS

4.1 Principle

The identification of a seasonal peak of increased susceptibility of the species to toxicants is a logical avenue to explore when seeking improved application strategies for existing toxins. However, this topic has not received much attention and no agreement has been reached on the effects of season on the sensitivity of natural zebra mussel populations. Only one published work has addressed this issue [13]. However, the authors of this work were interested in defining protocols for toxicological studies with zebra mussel rather than identifying the best time of the year to apply chemical treatments. For this reason, the study

covered only 5 months and it did not take into account the effect of the annual cycle of water temperature on mussels' susceptibility, which compromises the extension of the results to real scenarios. The zebra mussel was found to be more susceptible to toxins in early June. Other authors, although not explicitly addressing the effects of season on the tolerance of the species, have speculated about the optimum time to apply chemical treatments based on several aspects of the animal's biology [5, 14, 15]. Suggestions for peaks of sensitivity include the summer and autumn.

4.2 Major results

The annual profile of mussels' tolerance was recorded based on static bioassays, conducted using three reference toxins with different modes-of-action. The seasonal profiles of mussels' physical condition (expressed as the dry tissue mass of a standard mussel of 25 mm shell length) and filtration rate were also recorded. The annual cycle of water temperature was mimicked throughout the study, and therefore the effect of this variable on the mussels' response was taken into account. Although not directly monitored, the seasonal profile of zebra mussel metabolic demands was also considered, because freshly collected test organisms were used in all experiments.

The results of this study showed that the sensitivity of mussels to toxins varies significantly over the year, being higher in June/July. This peak of susceptibility coincides with an optimal combination of low values of physical condition and high values of filtration rate and water temperature. Increased water temperature should contribute to chemicals' toxicity by augmenting their reactivity and promoting uptake. Higher filtration rates should enhance the exposure of the mussels' soft tissues to toxicants.

The outcome of this study may have practical implications for the setting of protocols for conducting toxicological studies with the species, the design and timing of control treatments, and water quality management.

5 CHEMICAL PRODUCT ENGINERRING PERSPECTIVE

In recent decades, the chemical industry has undergone tremendous changes. Naturally, these changes have impacted on the chemical engineering profession. 50 years ago, the petrochemical and commodity chemicals sectors were at their height. At that time, a well-prepared graduate was expected to be thoroughly familiar with all aspects of a narrowly defined chemical engineering curriculum. Nowadays, commodity plants are highly optimised, and a number of other sectors have developed and grown in importance. Such sectors often deal with the production of what have been generically termed chemical products [16], rather than simple substances. Chemical products, which include pharmaceuticals and engineered formulations for example, differ from commodity chemicals in three main ways. First, their value is much higher than that of the raw materials. Second, their commercial success is determined by their performance rather than their price. Finally, their viability is highly dependent on time-to-market. For these reasons, the design, manufacture and marketing of chemical products require a set of skills that was not central to traditional chemical industry sectors. Flexibility, wider vision, willingness to get involved in non-traditional areas and aptitude for multidisciplinary teamwork are now as essential for professional success in chemical engineering careers as the traditional core skills of the discipline remain.

In recent years, the need to update curricula in order to guarantee the competitiveness of the profession in an increasingly aggressive and diverse industrial environment has been acknowledged. The emergence of chemical product engineering as a well-established academic field has been an enduring trend in this context [16–19]. In spite of recent progress, chemical product engineering is still often not seen as a priority for research and teaching [16, 18].

The adoption of a chemical product engineering perspective was found useful to manage the complexity of the project addressed in this paper, which emerges at the interface of chemical engineering and aquatic ecology and deals with innovative chemical products.

The advantages of adopting such a perspective can be illustrated by referring to the study of the effect of particle size on the performance of encapsulated biocides (Section 2.2) for instance.

One of the main principles of chemical product engineering is the need to turn the design of chemical products into a scientific and systematised process rather than conducting it based on trial-and-error procedures. The study of the influence of size on the toxicity of biocide-loaded particles complies with this principle. It can be seen as an attempt to complete a chemical product pyramid [16] (Fig. 3), which is a key idea in chemical product engineering.

Although the size was expected to affect particle performance, a detailed study of this dependence had not previously been conducted. In previous work [7], the size specifications of encapsulated formulations had been defined intuitively, based on information on the particle size amenable for zebra mussel filtration. However, optimal efficiency of zebra mussels' filtration activity is reported for particulate materials of average size up to 30 μm [9], while microcapsules with a mean diameter (by volume) of up to 225 μm were observed to perform promisingly. In addition, the production of small microcapsules with satisfactory retardant power has proved to be very challenging.

As discussed in Section 2.2, particle size will affect the success of encapsulated toxins directly, by influencing the degree to which they are captured and processed by the zebra mussels, as well as indirectly, by dictating their retardant power. Size is also an important variable in the encapsulation process, because producing smaller particles is more demanding from the manufacturing point of view. Consequently, optimal coated biocide formulations are likely to involve a trade-off between the particle size, retardant power and manufacturing effort. Defining a property function relating the performance of the formulation to

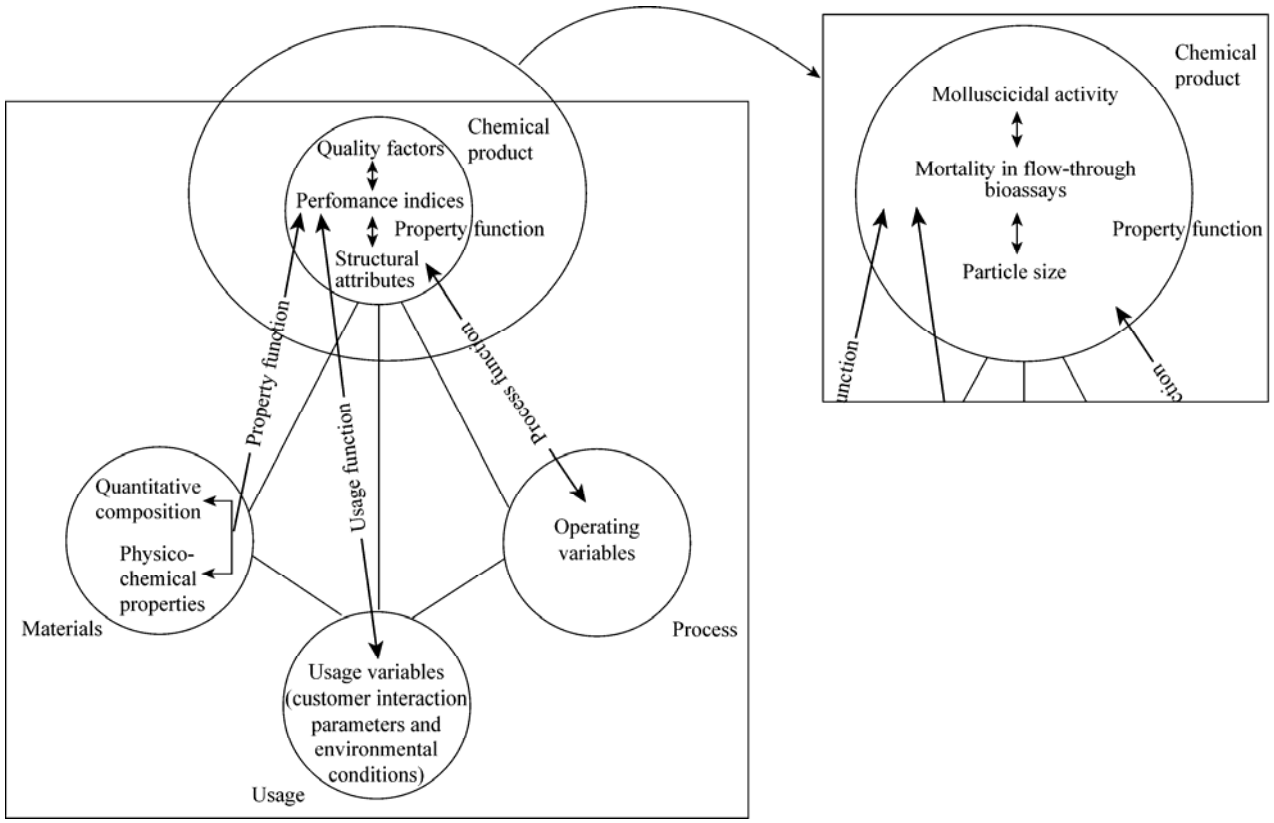

Figure 3 Completing a chemical product pyramid for particulate poisons

particle size (Fig. 3) is a crucial step in efficiently establishing this trade-off. This property function will have to be integrated with a process function relating particle size to manufacturing conditions and another property function relating the retardant power of the formulation to particle size and product performance.

6 CONCLUDING REMARKS

This paper summarises the major results of a project aimed at the development of improved solutions for zebra mussel control. In this project, three different approaches have been explored in order to design innovative application strategies for existing toxins. These approaches require very different design emphases with regard to the chemical product pyramid shown in Fig. 3. The development of encapsulated toxins requires a careful choice of materials and processing techniques in order to develop effective particles. In contrast, the combination of toxins requires negligible processing and is reliant on the quantitative composition of the chemical mixture. Finally, the identification of the seasonal peak of tolerance to toxins relates solely to the usage function in the chemical product pyramid.

Each of the approaches summarised in this paper has its own benefits and problems (Table 2). However, because the techniques are so different, they are not mutually exclusive and could be used together in an integrated control strategy. For instance, in the future it may be possible to produce particles that release

Table 2 Benefits and problems associated with the three approaches to zebra mussel control

Approach	Benefits	Problems
encapsulation of toxins	The greatest potential to reduce toxin requirements Specific to zebra mussels because of their filtration abilities Great flexibility: particles can contain different chemicals and can be altered to control different filter feeders.	Particle refinement is a difficult and time-consuming process because of the many variables that need to be considered (such as size, contents, retardant power, mussels' retention rate, *etc.*). Encapsulation can be an expensive process.
combination of toxins	Uses pre-existing control chemicals with minimal alteration Requires very little processing	The application of multiple chemicals may have additional legislative requirements. Combined chemicals may have unknown effects on non-target organisms.
investigation of the seasonal variation of the species' tolerance to toxins	Can be used for pre-existing control chemicals with no alteration Relatively easy to implement as it mainly requires monitoring of mussels at infested sites Easily complements other techniques	A largely pro-active approach Mussels at different sites may differ in their susceptibility. In some facilities it may be impossible to apply chemical treatment at certain times of the year due to constraints of consumer demand.

multiple toxins during their transit through the mussel, and which could be dosed at a time of the year when the mussels are at their lowest tolerance.

In conclusion, the three strategies outlined in this paper may each have an important impact on the future of zebra mussel control, and it is clear that the adoption of a chemical product engineering perspective is useful in managing the complexity of the problem.

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