The Application of Microcapsules in Bioconversion Reactions

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Introduction

Microencapsulation has been employed over many years in agriculture, environment, food, cosmetics, pharmacy, medical and numerous other fields. The basic rationale for using this technique is to separate or sequester an ingredient, compound, or cells from an environment in order to either protect, facilitate delivery or removal of a compound. Some examples of encapsulation include microbial cells to perform denitrification (Song et al 2005), animal cells to provide allo- or xeno- transplants/ bioartificial organs (Lim and Sun, 1980; Orive et al., 2003), organic phases in order to supply hydrophobic drugs or in cosmetic applications (Sukhorokov et al., 2005; Sun et al. 2000) and so on. In addition there has been a considerable literature concerning the immobilization of enzymes for use in washing powders and softeners (Michael, 1992), but in particular to carry out bioconversion reactions, since the enzymes may be stabilized, have increased activity or even an altered substrate range due to being immobilized. In the latter case this may be due to the increased solubility of the substrate and/ or product in the core of the capsules compared with the environment (Wyss et al., 2005).
In the present work, encapsulation will be explored with respect to two new techniques termed capsular perstraction (Wyss et al., 2004a, 2004b, 2006a) and reactive capsular perstraction (Wyss et al., 2005; 2006b). In particular the work will explore the environmental application for the recovery of hydrophophobic organic pollutants (HOPs) from water (perstraction) and the simultaneous extraction and enzymatic conversion of substrates (reactive perstraction).

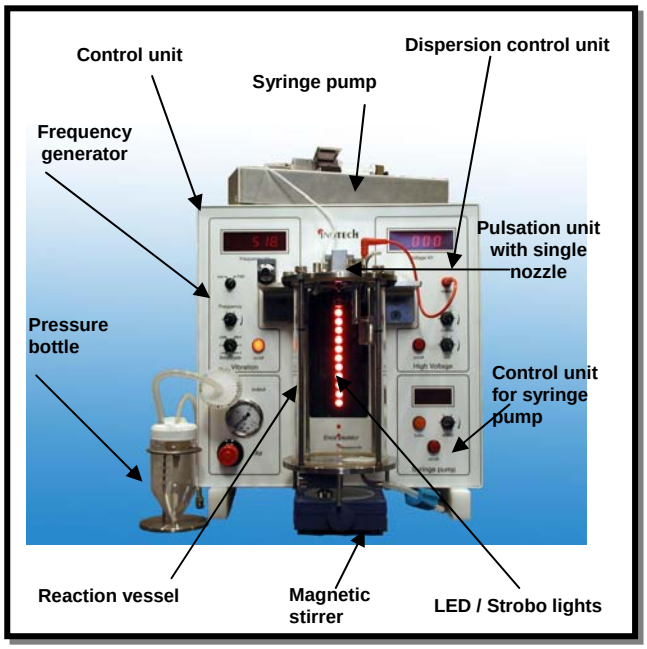
Materials and methods

Liquid-core microcapsules with a diameter of between 500-800 μm were prepared using the vibrating nozzle extrusion system (Inotech Encapsulation, Basel, Switzerland) with the core phase being an oil (dibutyl sebacate, DBS) and the outer phase a copolymer of alginate and acrylamide. A detailed description of the methods has been reported elsewhere (Wyss et al., 2004a, 2004b, 2005, 2006a, 2006b).

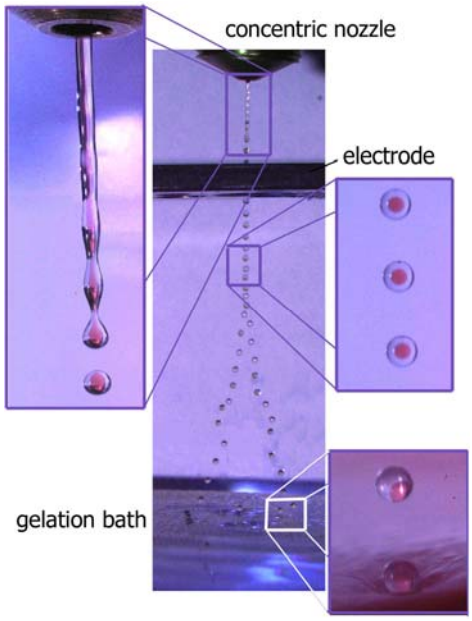
Perstraction: Application in bioremediation

Perstraction or pertraction, is defined as a membrane process in which a hydrophilic membrane separates an aqueous from a non- polar phase. In the case of bioremediation, perstraction was carried out at the sub- millimetre scale with microcapsules, in which the membrane was a hydrogel composed of an alginate/ acrylamide copolymer surrounding a core (non- polar phase) (Figure 2). Due to the small size (external diameter 496 μm), monodispersity, extremely high surface area to volume ratio, a high log Kow (6.2 for atrazine) and the stability of the liquid-core microcapsules, it was possible to extract atrazine, 2,4-D, methylparathion and ethylparathion at extremely high rates with a minimum of capsules and without the formation of stable emulsions. The next question arises as to how to dispose of the microcapsules containing atrazine or other HOP, and this was addressed by inoculation of the microcapsule suspension with Pseudomonas sp.strain ADP. As shown in Figure 3 not only was the atrazine within the microcapsules completely broken down, but the growth rate of the bacterium remained high compared to similar cultures carried out in the absence of extraction or others using DBS directly in the bioreactor in a form of liquid-liquid

extraction. If the DBS was added directly to the culture rather than encapsulated it was shown to be highly toxic to the bacteria, as was atrazine if added to the cultures at levels found in the core of microcapsules. Thus encapsulation of DBS resulted in a protection of the bacterial cells from both the non-polar solvent and atrazine (Figure 4).

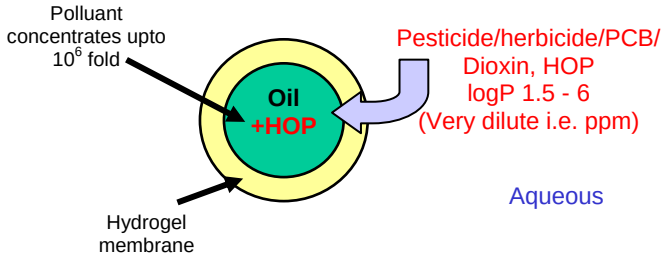


(a)

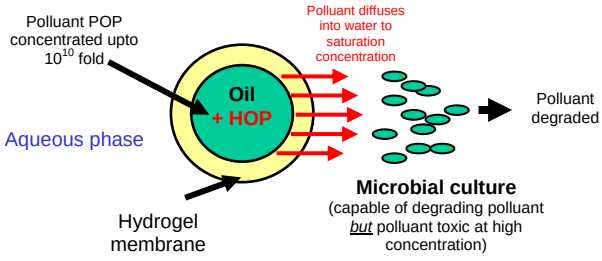


(b)

Figure 1 (a) Vibrating nozzle encapsulation device (b) Close-up of concentric nozzle: inner nozzle extrudes oil, outer nozzle extrudes polymer comprising hydrogel membrane e.g. alginate. Note dispersion of falling microcapsules from vertical due to applied electrostatic charge.



(a)



(b)

Figure 2 Use of capsular perstraction in bioremediation (a) recovery of a hydrophobic organic pollutant (POP) from a dilute aqueous environment and (b) inoculation of the microcapsule suspension with bacteria to degrade the POPs.

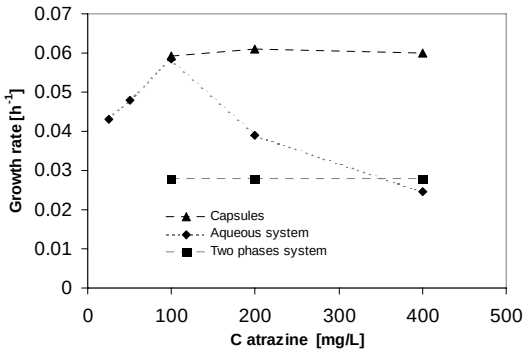


Figure 3: Growth rates (μ) for the growth of *Pseudomonas* sp. strain ADP on 1 g/L sodium citrate as a function of the total atrazine concentration in the system using a pure aqueous phase (diamonds), a two phase system with dibutyl sebacate as organic phase (squares) and a capsular system with dibutyl sebacate encapsulated (triangles).

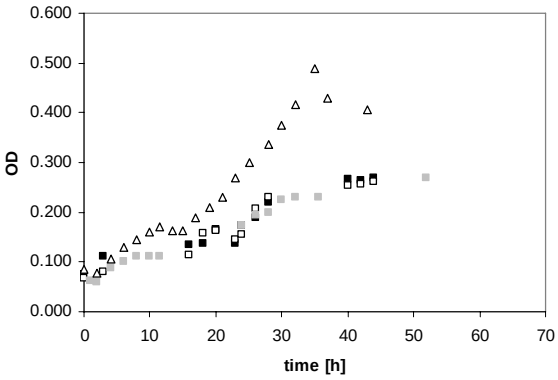
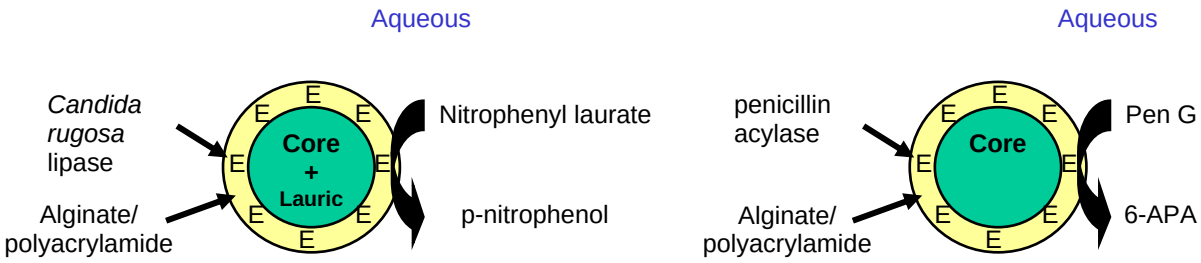


Figure 4: Optical density (OD) as a function of time for the growth of *Pseudomonas* sp. strain ADP in a two-phase culture composed of 100 ml of medium with 1 g/L sodium citrate and 2 mL of dibutyl sebacate with different concentrations of dissolved atrazine. Closed squares, white squares and gray squares represent respectively a total concentration of atrazine, C_T, of 400 mg/L, 100 mg/L and 200 mg/L. Open triangles represent the optical density in a pure aqueous system with C_T atrazine of 100 mg/L.

Reactive perstraction: Applications in enzyme-catalysed bioconversions

In a further development of the liquid-core technique enzymes have been either adsorbed at the interface of the liquid-core and hydrogel membrane of microcapsules (lipase from *Candida rugosa*) or covalently bound to the alginate matrix (Figure 5a and 5b). In the case of lipase the natural substrate is nitrophenyl laurate which is relatively soluble in water (low Log Kow) and therefore does not accumulate to any appreciable extent within the microcapsule core. However, the action of the enzyme liberates lauric acid which has a relatively high Log Kow and therefore accumulates within the core, while p-nitrophenol is very soluble in the aqueous phase. Using the adsorbed lipase (Figure 5a) it was possible to both extend the enzyme half-life, increase the rate of reaction compared with a purely aqueous phase system, and to recover one of the products thereby preventing the reverse reaction and enabling complete hydrolysis of nitrophenyl laurate.



(a) (b)

Figure 5 Reactive perstraction using (a) adsorbed lipase or (b) covalently bound penicillin acylase

Similarly the standard industrial method for the production of semi-synthetic penicillins involves the microbial production of Penicillin G (PenG) followed by enzyme hydrolysis using the enzyme penicillin acylase to form 6-amino-penicillanic acid (6-APA) and phenyl acetic acid (PAA). The same enzyme is then used to add a synthetic side chain to the 6-APA to form the desired semi-synthetic product. Unfortunately the process is complicated and has relatively low yields, due to the

competitive inhibition of the acylase enzyme by both the PAA hydrolyzed from PenG, and the side chain which it is desired to add. One way of overcoming this successfully was to immobilize the acylase onto liquid-core microcapsules (Figure 5b) and extract the PAA selectively into the non-polar DBS core. As a result a complete conversion of PenG into 6-APA could be achieved at a high rate, which could then be recovered and the semi-synthetic form produced at 100% conversion.

Conclusions

Microencapsulation has been successfully applied to create membrane separation processes for use with both whole cell and enzyme bioconversions. Thus perstraction may be used to separate any relatively hydrophobic compound from an aqueous environment. An example of this is water treatment, but equally the method may be applied to the recovery of flavour and aroma compounds from fermentation processes or other systems, particularly where the compound to be extracted is toxic to the environment or to the organisms, cells or enzymes producing it. Reactive perstraction is essentially the same principle, with the added combination of the immobilization of the cells or enzymes to the capsules hydrogel membrane. In this way the enzyme can be in an optimum environment for the activity and the substrates may be supplied from within the capsule core or from the extracapsular phase. In either case both a simultaneous reaction and separation is obtained to overcome product inhibition or competition for the enzyme active site.

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