Liquid-core microcapsules: small packages – big potential

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INTRODUCTION

Liquid-core microcapsules (Figure 1) can be described as miniature sized particles (usually < 1 mm in diameter) consisting of one or more liquid-cores, which are completely enveloped within a defined membrane (shell). These particles can occur naturally in nature i.e. seeds or eggs or can be manufactured from a wide range of natural and/or synthetic materials using numerous techniques. Since the 1950's liquid-core microcapsules have been applied to a diverse range of fields from chemicals to pharmaceuticals to cosmetics, textiles and printing.

Liquid-core microcapsules can take many different structural forms and are usually classified as four different types (Figure 1). Type 1: Mononuclear (sometimes referred to as simple or single core) liquid-core microcapsules: Simplest form in which a liquid-core is surrounded by a continuous membrane. The diameter of the core material and the membrane thickness can vary greatly in size with either making up 10-90% of total capsule volume. Type 2: Double/multi-membranes (walls): Usually mononuclear liquid-core microcapsules in which a second or multiple walls are added to the original capsule. In most cases these are added to modify the original stability and/or permeability of the capsules. Type 3: Polynuclear (multi-core): Consist of multiple cores in which the liquids can be similar or different, and can also have multiple membranes. Type 4: Irregular or non-spherical, most common shape found in industry, can also have multiple shells or cores.

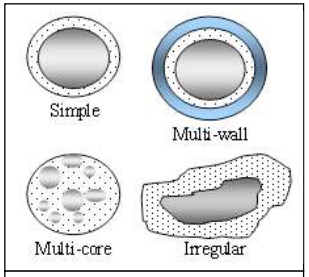

Figure 1: Different types of liquid-core microcapsules

Our research group is focused on producing Type 1 and 2 liquid-core microcapsules from a range of different membrane and core materials using the concentric jet break-up technique performed on an Inotech encapsulator. These capsules are subsequently used as a novel methodology (termed capsular perstraction) to recover significant molecules from their associated environments. In recent years our laboratory has shown how these capsules have many exploitable characteristics in biotechnological and chemical processes and have been applied successfully to areas such as water treatment (Whelehan 2009a; Wyss 2004; Wyss 2006a), enzyme technology (Wyss 2006b) and bioprocessing, and cell culture (Stark 2003; Whelehan 2009b).

This short paper will give a brief description of some of the results obtained from work performed in our laboratory.

MATERIALS AND METHODS

Production of Liquid-core microcapsules: Liquid-core microcapsules are prepared in our laboratory using the co-extrusion laminar jet break-up technique performed on an Inotech Encapsulator IE-50R (Inotech Biotechnologies, Basal Switzerland). From Figure 2: Two phases (shell and core-materials) are co-extruded through a concentric nozzle using syringe pumps. The

resulting concentric liquid jet is then broken-up into spherical droplets by the application of a set vibrational frequency, with defined amplitude to the jet. The droplets are subsequently hardened by falling into a magnetically stirred gelling bath, forming the liquid-core microcapsules. A stroboscopic lamp enables the droplets to be monitored during the break-up of the liquid-jet. An electrostatic voltage system prevents the occurrence of droplet coalescence by inducing a highly negative charge onto their surface, which enables the formation of mono-dispersed microcapsules of equal shape, size and volume to be produced.

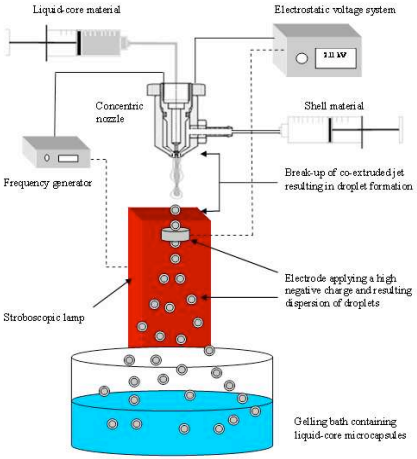

Figure 2: Schematic of vibrating concentric nozzle process used to manufacture liquid-core microcapsules

RESULTS AND DISCUSSION

Recovery of pesticides, herbicides and pharmaceuticals from water: The presence in surface, ground and drinking (environmental) waters of commonly used medications for the treatment of human and animal diseases and pesticides/herbicides used in agriculture represents a potential health hazard. Their presence is attributed to the limitations of techniques currently used in the water industry to remove or breakdown these compounds. The feasibility of using capsular perstraction as a novel approach to remove four pesticides/herbicides and seven pharmaceuticals commonly found in environmental waters were investigated. From the results (Figure 3 and 4) it can be seen that microcapsules containing a liquid-core of either dibutyl sebacate or oleic acid are capable of rapidly extracting between 25-75% of the pesticides/herbicides and between 15-100% of the pharmaceuticals when only a 4 and 3.5% liquid volume ratio of capsules were used. Higher levels of extraction can be simply achieved by increasing the number of microcapsules used. The amount extracted in most cases is considerable higher in comparison to methods presently used for water treatment purposes.

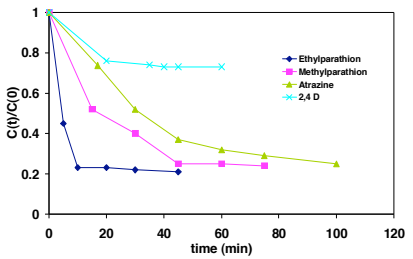

Figure 3: Capsular perstraction of herbicides and pesticides from water using microcapsules with a liquid-core of dibutyl sebacate.

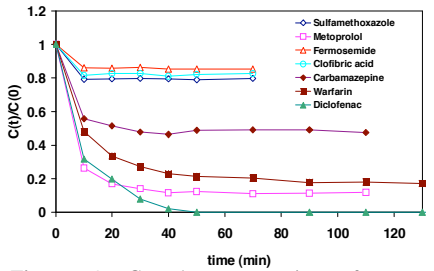

Figure 4: Capsular perstraction of seven pharmaceuticals from water using microcapsules with a liquid-core of either oleic acid or dibutyl sebacate.

Liquid-core microcapsules as reservoir for the biodegradation of the recovered organic pollutant atrazine

Although banned in the EU, atrazine is one of the most widely applied pesticides worldwide and represents a real contamination problem of ground and surface water. Bioremediation of this pesticide by the bacteria *Pseudomonas* sp. strain ADP can be successfully performed in pure aqueous systems, with a specific growth rate of 0.0575 h^{-1} utilizing 100 mg/L of atrazine as the sole nitrogen source. However, growth-rate inhibition is observed at higher pesticide concentrations. Liquid-core microcapsules have been shown to efficiently extract atrazine from water. These capsules were then used as a reservoir for the controlled delivery of the atrazine at low concentrations to allow for bacterial growth. Maximal growth rates were found to be constant for different pesticide concentrations (100, 200 and 400 mg/L, Figure 5) with values of around 0.06 h^{-1} being obtained. Compared to pure aqueous and two-phase systems, the use of liquid-core microcapsules enables biodegradation of higher quantities of atrazine (Table 1). This system also offers the advantage of the reduction of pollutant and organic phase toxicity, allowing higher concentrations of the pesticides to be degraded compared to pure aqueous systems.

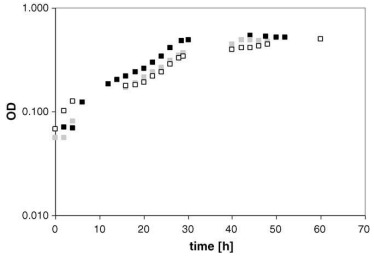

Figure 5: Growth (OD) of *Pseudomonas* sp. strain ADP using atrazine as the nitrogen source. Atrazine is contained within 12 ml of liquid-core microcapsules. Black, white and gray squares represent an initial atrazine concentration of 100, 200 and 400 mg/L respectively.

Initial conc of atrazine in the culture (mg/L)	Final conc of atrazine in the culture (mg/L)	Degradation (mg/L)	% Degradation
100*	4	96	96
200*	19	181	90.5
400*	104	296	74

*Contained within 12 ml of microcapsules

Table 1: Quantity of atrazine degraded by *Pseudomonas* sp. species ADP using liquid-core microcapsules as a reservoir for the controlled deliver of the atrazine to the culture.

Capsular perstraction of geldanamycin from culture environments

The benzoquinone ansamycin geldanamycin (GA) is a naturally occurring antibiotic which is produced as a secondary metabolite by *Streptomyces (S.) hygroscopicus* var. *geldanus*. Significant interest in the molecule increased upon the discovery of its novel antitumor properties, which enables the drug to effectively treat many different tumor types. Previously it has been shown that the accumulation of GA in its culture environment can result in the rapid decline of antibiotic concentrations (Figure 6). To prevent this decrease in production titers, it was decided to investigate the feasibility of using liquid-core microcapsules as a novel approach to recover GA from culture environments, hence preventing the occurrence of this degradation. Initially experiments were undertaken that involved the addition of a known amount of pure GA to the culture broth (cells were removed by filtration using 0.2 µm filters) containing spent media and by-products produced

by the cells. Figure 7 highlights the results of this experiment and displays the degradation of GA in the culture environment over the time period outlined. Additional experiments (results not shown) allowed the conclusion to be contrived that by-products produced by the cell are responsible for the fall-off in production titers. To overcome this problem, microcapsules containing a liquid-core of oleic acid were added to the culture broth (cells were again removed) after the addition of the known amount of GA, to establish if the capsules could remove the GA and prevent its degradation. Figure 7 also displays how the microcapsules added to the broth absorbed most of the GA present and, thus prevented it from degrading. The added GA was extracted selectively and rapidly from the broth (results not shown), which resulted in no break-down of the GA, since it was protected from the action of compounds that were not extracted into the liquid-core of the microcapsules.

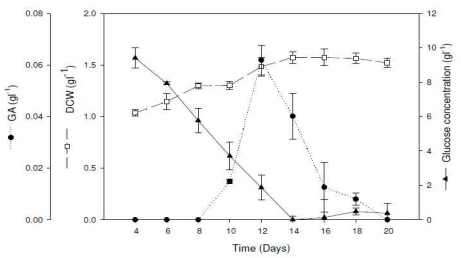

Figure 6: Fermentation profile of GA production by *S hygroscopicus* var. *geldanus* showing the decline in GA concentrations after 12 days.

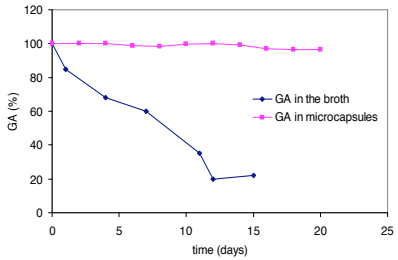

Figure 7: Graph displaying the degradation of GA in fermentation broth over a 16 day period and using liquid-core microcapsules to recover GA from the broth to alleviate the problem.

CONCLUSION

This short paper has clearly shown the potential that exists for applying liquid-core microcapsules as a successful extraction methodology in biotechnological and chemical processes. Future work will focus on improving the characteristics of the liquid-core microcapsules as well as improving the production process for capsules, which will subsequently enable larger volumes to be produced.

REFERENCES

Stark D. et al. (2003) *Novel type of in situ extraction: Use of solvent containing microcapsules for the bioconversion of 2-phenylethanol from L-phenylalanine by Saccharomyces cereviaiae*. Biotechnology and Bioengineering 83 (4) 376-385

Whelehan M. et al. (2009a) *Removal of pharmaceuticals from water: Using liquid-core microcapsules as a novel approach*. Water Research (submitted)

Whelehan M. et al. (2009b) *Capsular perstraction as a novel methodology for the recovery of geldanamycin from culture media*. Biotechnology and Bioengineering (submitted)

Wyss A. et al. (2004) *A novel approach for the extraction of herbicides and pesticides from water using liquid-core microcapsules*. Biotechnology and Bioengineering 87 (6) 734-742

Wyss A. et al. (2006a) *Microencapsulated organic phase for enhanced bioremediation of hydrophobic organic pollutants*. Enzyme and Microbial Technology 40 (1) 25-31

Wyss A et al. (2006b) *A novel reactive perstraction system based on liquid-core microcapsules applied to lipase-catalyzed biotransformations*. Biotechnology and Bioengineering 93 (1) 28-39