Photosynthetic algae entrapped in silica in the quest for novel bioreactors

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INTRODUCTION

Photosynthesis is a phenomenally efficient and highly sophisticated process that uses solar energy to convert CO_2 into oxygen. If this mechanism could be cost-effectively exploited it would be a step forward in mitigating the problem of increasing atmospheric CO_2 levels that are detrimental to the environment. Primary producers such as cyanobacteria can assimilate CO_2 and through a photosynthetic pathway, generate carbohydrates which could provide us with a reusable carbon source. Additionally, some of the metabolites produced are expensive pharmaceutical and fine chemical products, presenting economical alternatives to costly and cumbersome syntheses (Pulz O. 2004). Other benefits of algae include the degradation of nitrogenous and phosphorous compounds (Lodi A. 2003) and the absorption of toxic metals adding to their value in maintaining an ecologically sound environment.

Previous exploitation on the photosynthetic system has seen the encapsulation of photosynthetic pigments (Hata H. 2000), reaction centres (Kriegl J. 2003.) and chloroplasts (Hara M. et al 1999) into various inert matrices. This immobilisation process is essential in preserving light sensitive molecules and sub-components of cells. The encapsulating host material must confer stability and ideally promote the activity of the biological component found within. The work in our laboratory started with the immobilisation of thylakoids (Meunier C. 2009) but currently focuses on the entrapment of whole cells, such as algal (Rooke J. (I) 2008) or plant cells, as these are more stable than their sub-units. Previous studies have shown the possibility of immobilising cyanobacterial strains within organic matrices to target biosensors (Avramescu A. 1999) and water treatment devices (Blanco A. 1999). However there are many negative aspects to organic matrices which could hinder the development of other applications and thus a more robust material is sought.

Inorganic materials, in particular silica, perform well against external conditions owing to their high mechanical strength and chemical stability providing excellent protection to the entrapped biological entities. Silica's advantages lie in its diversity. It is a material which can be tailored to suit its application. In this case mild conditions are employed with biocompatible approaches to encapsulate living cyanobacteria. Silica is both porous and optically transparent which allows both light and nutrients to penetrate the core of the resulting biocomposite material whilst preserving activity (Rooke J. (II) 2008). It has thus been envisaged that these hybrid materials could hold promise in the design of photobioreactors acting as an artificial carbon cycle. On combustion of the biofuel harvested from such reactors, further CO_2 would be produced for the cyanobacteria to fix.

MATERIAL AND METHODS

The biocomposite gels were prepared by harvesting cyanobacterial strains PCC 6301, PCC 7002 and PCC 7418 via centrifugation. The supernatant media was discarded and the remaining pellet resuspended in LUDOX HS-40 (Aldrich) and glycerol (Ph. Grade, Merck). Glycerol is a protective agent to counter osmotic shock which could be brought about through an elevated Na^+ concentration. This mixture was added to a sodium silicate solution (Assay 25.5-28.5%, 10x

dilution, Merck) in an equal volume with respect to Ludox. Through the addition of HCl, the mixture was acidified to ca. pH 8. This not only resulted in rapid condensation and polymerization but was also similar to the pH of most cyanobacterial environments. Gels were left to age in the appropriate aqueous nutrients and kept under constant fluorescent lighting in the same conditions as the free growing strains.

Several techniques such as ^{29}Si MASNMR, transmission electron microscopy and N_2 adsorption-desorption measurements were employed to characterise the morphology of the gels both with and without cells. The activity of cells within inorganic matrices was analysed through their ability to fluoresce (i.e. UV-Vis spectroscopy, epifluorescent microscopy) and also through the uptake of ^{14}C. Further details of these techniques can be found elsewhere. (Rooke J. (I & II) 2008)

RESULTS AND DISCUSSION

The silica gels formed had a mesoporous network, meaning that the cells were firmly entrapped within the hybrid materials with no leaching possible; the size of the cells being several orders of magnitude larger than the pore openings. Table 1 shows a brief summary of these results.

	No Cells	PCC 6301
Surface area (BET) / m^2g^{-1}	33.7	62.4
Pore diameter / nm	13.2	15.2
Pore volume/ cm^3g^{-1}	0.138	0.286

Table 1 : Morphological data obtained from nitrogen adsorption-desorption measurements

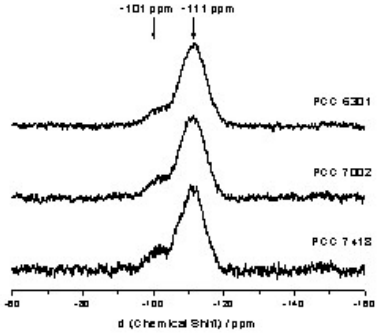

Figure 1: ^{29}Si MASNMR spectra for hybrid gels containing cyanobacteria

The table shows that the incorporation of cyanobacteria cells into silica gel alters the porosity characteristics of the material. This is a direct result of the size of the cell (μm) compared to the diameter of the pores in the blank gel. As the gel forms in-situ around the cells this enables pores of larger diameters to form. As these cavities are filled by the cells themselves, the actual volume of the porous network decreases post immobilization.

Figure 1 shows the ^{29}Si MASNMR spectra for the hybrid gels. The peak at -111 ppm is assigned to the Q_4 silicon environment, highlighting a high proportion of quaternary SiO_4 units within the gel. These results suggest most of the silicic acid formed in-situ has undergone complete condensation meaning further shrinkage of the network should not occur. This means theoretically the cell should

not be placed under any undue stress as a direct result of constriction to the framework. There is also a shoulder peak at -101 ppm revealing the presence of Q_3 units within the gel. These are from the terminal OH groups found on the surface of the silica which presents an aqueous-like environment, advantageous to cell viability.

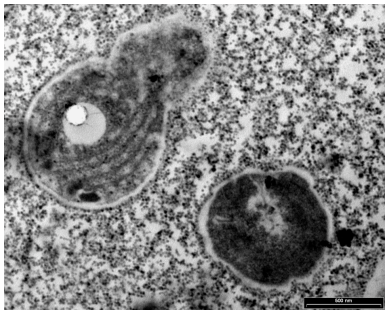

Figure 2: TEM image of PCC 6301 cells immobilised within silica gel

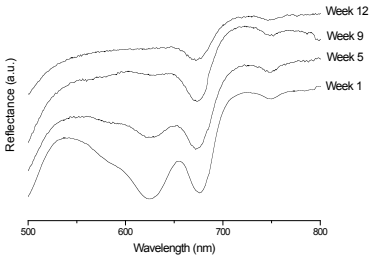

Figure 3: UV-visible spectra (diffuse reflectance mode) for immobilised cyanobacteria (PCC 6301) over 12 weeks

Figure 2 shows a TEM image which reveals the presence of integral cyanobacterial cells within silica gel, taken a day after immobilisation. TEM images have revealed that silica often has a tendancy to arrange itself around the walls of the cells, also with time there is dissolution of the silica around the cells. The reasons behind this are unclear and thus further study into the interaction between silica gel and cyanobacteria is ongoing. TEM images have shown the cell walls can remain intact 1 month after immobilization (Rooke J (II) 2008), a promising result for the long term viability of encapsulated cells.

Figure 3 shows a collection of UV-visible spectra taken over a period of three months on hybrid gels. The bands within the spectra are characteristic of the predominant photosynthetic pigment chlorophyll, ca. 680 nm, and also the blue accessory pigment phycocyanin, ca. 630 nm. The results have shown that for both PCC 6301 and PCC 7002 the pigments can be preserved for over 10 weeks. This is crucial for the activity of the cell to continue, for without these pigments the cells could no longer photosynthesise. It is indeed possible for photosynthetic cells to enter a period of dormancy, in which case there is no renewal of the photosynthetic reaction centres. Other techniques such as HPLC and epifluorescent microscopy have been exploited to confirm the presence of photosynthetic pigments over a similar timescale (Rooke J (II) 2008).

One method to test the actual activity of an immobilised cell is via the incorporation of ^{14}C. An inorganic radioactive isotope was incorporated into the precursor mixture prior to gelification. The hybrid gel was then left to assimilate the radioactive tracer as described elsewhere (Rooke J (II) 2008). In Table 2 it can be seen that the count rate is far higher for the hybrid gels than for the blank controls. This highlights how the cells have managed to incorporate the radioactive carbon into the

cell, transforming it from an inorganic carbonate source to the organic metabolites excreted or into the building blocks of the cell itself. This simple test shows how the cells can continue to photosynthesize after immobilization.

	PCC 6301	PCC 7002
Hybrid gel / ^{14}C DPM	56,487	1,022,531
Blank gel / ^{14}C DPM	12,597	126,070

Table 2 : ^{14}C incorporation in hybrid gels

CONCLUSIONS

This summary of our work highlights the potential role silica encapsulated cyanobacteria can play in the design of novel photobioreactors with important biotechnological applications. Various studies have proved that the pigment structures within the cells can remain fluorescent for at least 10 weeks and that the cell walls of the cyanobacteria can remain intact post immobilisation. Furthermore, the most promising result is that the cyanobacteria can assimilate carbon whilst immobilised. This is a real sign that photosynthesis continues within the porous, optically transparent host silica material. The next step is towards the scaling up of the immobilisation process to investigate how this biomaterial could be best employed in a bioreactor.

REFERENCES

Pulz O. et al. (2004) *Valuable products from biotechnology of microalgae.* Applied Microbiology and Biotechnology 65 635-648

Lodi A. et al. (2003) *Nitrate and phosphate removal by Spirulina platensis.* Journal of Industrial Microbiology and Biotechnology 30 656-660

Hata H. et al. (2000) *Immobilization of Photosynthetic Pigments into Silica-Surfactant Nanocomposite Films* Journal of Sol-Gel Science and Technology 19 543–547

Kriegl J. et al. (2003) *Charge Recombination and Protein Dynamics in Bacterial Photosynthetic Reaction Centers Entrapped in a Sol-Gel Matrix* Biophysical Journal 85 1851–1870

Hara M. et al (1999) *Immobilization of P450 Monooxygenase and Chloroplast for Use in Light-Driven Bioreactors* Journal of Bioscience and Bioengineering 87(6) 793-797

Meunier C. et al (2009) *Thylakoids entrapped within porous silica gel: towards living matter able to convert energy.* Journal of Materials Chemistry 19(11) 1535-1542

(I) Rooke J. et al (2008) *Targeting photobioreactors: Immobilisation of cyanobacteria within porous silica gel using biocompatible methods*. Journal of Materials Chemistry 18(12) 1333-1341

Avramescu A. et al. (1999) *Potential for use of a cyanobacterium Synechocystis sp. immobilized in poly(vinylalcohol): Application to the detection of pollutants.* Biotechnology Techniques 13, 559-562

Blanco A. et al. (1999) *Biosorption of heavy metals to immobilised Phormidium laminosum biomass*. Journal of Biotechnology 69 227-240

(II) Rooke J et al. (2008) *Photosynthesis within porous silica gel: viability and activity of encapsulated cyanobacteria.* Journal of Materials Chemistry 18(24) 2833-2841