

Photosynthesis within porous silica gel: viability and activity of encapsulated cyanobacteria

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In the framework of designing novel bioreactors, the encapsulation of photosynthetic cyanobacterial strains of the genus *Synechococcus*, PCC 6301, PCC 7002 and *Cyanothece* PCC 7418, within mesoporous silica networks has been achieved *via* the acidification of aqueous colloidal silica precursors at ambient temperature. The effect of the silica matrix on the external membrane of the cells has been studied. The viability of the cells over a three month duration has been assessed using transmission electron microscopy, epifluorescence microscopy, UV-visible spectroscopy and high-performance liquid chromatography. The bioactivity of the encapsulated cyanobacteria was detected *via* the assimilation of $\text{NaH}^{14}\text{CO}_3$. Although most cells entrapped within the silica gel remain undivided, some cells continued to divide even when there was limited space. TEM studies have revealed an interaction between the silica gel and the cell membrane. HPLC studies highlight that the photoactive pigments in PCC 6301 and PCC 7002 can be preserved for up to 12 weeks whilst PCC 7418 lost its photosynthetic pigments after two weeks post-immobilisation. These results suggest that certain strains of cyanobacteria are able to photosynthesise within a hybrid gel yielding the possibility of novel photobioreactors.

Introduction

Increasingly one observes science borrowing heavily from nature, owing to the efficiency with which it performs highly complex tasks. This has led to the rapid evolution of biomimetics: the imitation of natural processes by systems engineered by man. Examples include the exploitation of echolocation, a phenomenon carried out by bats, in medical ultrasound technology¹ or the creation of nanostructures that replicate the pearlescent effect of butterfly wings, a major development in the field of photonics.² The encapsulation of biomolecules and cells within inanimate networks is one way in which we can mimic nature.

Cyanobacteria belong to a phylum of bacteria which acquire energy through photosynthesis.³ These prokaryotic organisms are photoautotrophic and are able to perform oxygenic photosynthesis similar to plant cells. Cyanobacteria produce oxygen *via* the conversion of carbon dioxide and therefore there exist potential applications in industries seeking to reduce their carbon footprint.⁴ A by-product of this conversion is that they produce complex organic compounds which can be used by man as an alternative fuel to power small devices.⁵

In almost every conceivable habitat one can find cyanobacteria: from fresh water to marine environments, damp soils to desert rocks, hot springs to the Antarctic wilderness, species of

cyanobacteria are known to exist. Thus this robust versatility lends itself to diverse uses.

There are many known biotechnological applications of cyanobacteria. For instance some strains, such as *Arthrospira* (previously known as *Spirulina*), are used as a nutrient complement and it has been proposed that they could be classified as a superfood owing to their phytonutrient content.⁶ Certain cyanobacterial strains produce the enzyme nitrogenase which is used to fix nitrogen gas into an absorbable form for plants, such as nitrates, nitrites and ammonia. This role of cyanobacteria is critical in the production of rice.⁷ Furthermore some strains produce hydrogen in a secondary photosynthetic reaction and subsequently are of interest in the search for alternative clean energy sources.^{4,8} Finally, specific secondary metabolites have drawn the attention of the medical community, for example the anti-HIV activity of cyanovarin.⁹

Generally speaking, biomolecules and live cells isolated from their superstructures are fragile and thus one must confer protection on them in order to exploit their properties and physiological functions. Their immobilisation within a host cage is an excellent way to achieve such protection and has already been realised in the design of biocatalysts,¹⁰ bioreactors,^{11,12} biosensors^{13,14} and bioartificial organs.¹⁵

There are numerous reported examples of immobilised cyanobacteria in the literature, though the support matrix is usually organic, such as poly(vinylalcohol) biosensors¹⁶ and polysulfone based biosorbents.¹⁷ There are drawbacks to organic compounds such as their stability towards thermal changes, material strength and whether the inherent carbon content is a source of food to the encapsulated living entity. Hence scientists turn to inorganic materials such as silica or titania in the search for superior alternatives. A particular example, of interest to this project, is that of hypoliths: photosynthetic organisms that shelter underneath rocks from harmful UV radiation and harsh winds in

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climatically extreme locations. The rocks, such as quartz (*i.e.* silica), are generally translucent, which allows light to penetrate. This supports the use of silica gel as an encapsulating material.¹⁸

Much work has been done by Livage and co-workers in the field of *E. coli* immobilisation within silica gel using biocompatible methods that preserve enzymatic activity.^{19–23} When immobilised in silica gel *E. coli* are no longer able to form colonies thus this offers a novel method for studying the phenomenon of quorum sensing, the ability of one bacteria to communicate with another *via* the release of signalling molecules.²⁴

Silica materials have many advantages over rival counterparts. Not only do they provide chemical stability and mechanical rigidity, they are also non-toxic and biologically inert. The biocompatibility between silica and living cells is highlighted in the case of diatoms. Silica materials can also be porous allowing small molecules to diffuse through the channels. Silica is also optically transparent allowing solar energy to penetrate, a key prerequisite for photosynthetic organisms to continue to function. More importantly it is not organic and therefore more resistant to microbial attack.^{25,26} Considering these properties and the existence of hypoliths it is envisaged that living cells, such as cyanobacteria entrapped within silica gel, could continue to be viable. This would ultimately lead to smart hybrid gels with enormous potential in many diverse biotechnological applications. The ability of a material to harness solar energy and convert it to more useful energy forms, at the same level of efficiency as photosynthesis in nature, could revolutionise the way in which we source energy. It would enable scientists to devise better solar cells with a previously unprecedented level of efficiency. A positive side effect would be the reduction in CO₂ that accompanies the process, thus effectively creating a closed-loop energy system whereby the CO₂ emitted in the combustion of the synthesised biomass could be reutilised to produce more biofuel. This would enable man to keep a stable level of atmospheric CO₂. Furthermore a photosynthetic material which produces both oxygen and edible biomass could sustain man in space,¹¹ facilitating long distance exploration as sustainability is a limiting factor in today's space age.

This work, continuing on from the previous work which reported about the encapsulation process and material properties,²⁷ monitors the viability and activity of cyanobacteria immobilised in silica gel over a period of three months in order to assess the feasibility of a photobioreactor made from a smart hybrid gel. Hence, on the principle that it is better not to destroy nature in order to reap the benefits, this project proposes the idea of immobilising a complete single-celled photosynthetic organism within a porous silica material whilst preserving the physiological functions of the cell.²⁷ This would therefore allow the photosynthetic reactions to continue *in vivo* within the biocomposite material thus allowing the exploitation of the benefits of photosynthesis.

Experimental

Cyanobacteria cultivation

Axenic strains of *Synechococcus* sp. PCC 6301†, PCC 7002† and *Cyanothece* PCC 7418† were obtained from the Institut Pasteur

(Paris, France). Further particulars on the strains and growth conditions are detailed in previous work.²⁷

Cyanobacteria immobilisation

Silica gels were synthesised using a method adapted from the literature^{23,25} using LUDOX HS-40 (Aldrich) and a solution of sodium silicate (Assay 25.5–28.5%, 10× dilution, Merck) in equal volume ratios. Glycerol (pharmaceutical grade, Merck) was added to prevent osmotic shock from the build up of sodium ions. The cyanobacteria were harvested through centrifugation after a period of growth in fresh media, *ca.* 1 cm³ stock culture per 1 cm³ reagent mix. The supernatant was discarded and the pellet re-suspended in glycerol and LUDOX. This mixture was added to a solution of sodium silicate then acidified to *ca.* pH 8 with HCl (3 M), the mixture was shaken and left to gel in the culture tube. Gelation typically occurred within a few minutes at room temperature. This method is the same as method B, published in a previous report.²⁴

The gels were left to age in the appropriate media, which penetrated the porous network, providing nutrients to the immobilised cyanobacteria. The culture tubes were left under the same fluorescent lighting as the growing strains, with the lids left slightly open to prevent the build up of gases within the tube.

Characterisation techniques

Transmission electron microscopy was performed on a Philips Technai 10 with an accelerating voltage of 100 kV. Prior to analysis, the fixation of the cyanobacteria was carried out using the following method: the gel was cut into tiny cubes and placed in a fixative (2.5% gluteraldehyde in a 0.1 M cacodylate buffer at pH 7.4) overnight. The samples were rinsed in 0.2 M cacodylate buffer at pH 7.4 and left overnight in a 1% OsO₄ solution for post-fixation. After further rinsing in cacodylate buffer the samples were dehydrated through a graded ethanol series with a final step using propylene oxide. The samples were set in epoxy resin, LX112, then cut using an ultramicrotome. The samples were negatively stained with uranyl acetate and lead citrate to impart contrast.

Epifluorescence microscopy was performed on a Zeiss Axioskop equipped with plan Neofluar objectives and an AxioCam digital camera. A sample of the gel was sandwiched between a microscope slide and a cover slip and a drop of low fluorescence immersion oil was placed between the lens and cover slip. The autofluorescence of chlorophyll *a* was clearly visible under blue light excitation (filter set 09, 450–490 nm) and red emission (>590 nm).

UV-Visible spectroscopy was carried out on a Perkin Elmer Lambda 35 spectrometer. Datasets were collected in transmission mode for free growing cyanobacteria in a suspension culture, diluted in appropriate media (1 mL culture per cuvette), over a twelve week period. Spectra for cyanobacteria encapsulated within silica gel were collected in reflectance mode using the solid state detector over an identical time period.

Samples for pigment analysis followed a procedure described by Sarmiento *et al.*:²⁸ for suspension cultures of cyanobacteria, 2 mL of culture was filtered on GF/F glass fibre filters (Whatman, Germany), with 0.7 μm pore size. For the hybrid

† PCC—Pasteur Culture Collection

gels, a predetermined weight of gel was first crushed prior to extraction. Pigment extraction was carried out in 5 mL 90% HPLC grade acetone. After two 15 min sonications separated by an overnight period at 4 °C in the dark, extracts were filtered and stored in 2 mL amber vials in a freezer (at -25 °C). HPLC analysis was carried out using the Wright *et al.*²⁹ gradient elution method, with a Waters system comprising a PDA detector and a fluorescence detector. Calibration was made using commercial external standards (VKI, Denmark). Carotenoids not present in the standard were quantified against fucoxanthin, using as relative response, the ratio of the specific absorbance coefficients at 440 nm in methanol.³⁰

The primary production rate was determined by the ¹⁴C incorporation method.³¹ A radioactive tracer, NaH¹⁴CO₃, was added to the sodium silicate precursor and the immobilisation carried out as above. The gels were left to incubate within a culture chamber at 20 °C for 24 h with the appropriate media. The media were decanted off and added to 5 mL of 0.1 M HCl to neutralise any excess NaH¹⁴CO₃, the gels were subsequently broken down with 5 mL 0.1 M HCl with a plunger action, again to neutralise the excess bicarbonate. The samples were subsequently bubbled with air overnight, to dissipate the inorganic ¹⁴CO₂ leaving only organic ¹⁴C, assimilated and excreted by the cyanobacteria. An Instagel (Packard) scintillation cocktail was added and mixed with the aid of a vortex. Radioactivity was measured using a Beckman scintillation counter (LS 6000 SC) and the external standard method for quench correction.

Results and discussion

There are several ways in which one can monitor the survival of photosynthetic cells. Several of these techniques exploit the electronic properties of the photosynthetic pigments such as their ability to autofluoresce and absorb radiation in the visible region, whereas the uptake of radio-labelled inorganic compounds (*i.e.* NaH¹⁴CO₃) infers the physiological function of the cell. These methods have been adapted in order to observe cell survival and biological activity within the gels.

Microscopy techniques

Transmission electron microscopy has been employed to monitor the hybrid materials over a month in order to see the interactions between the cells and their surroundings. The images shown in Fig. 1 highlight the differences between the strains and thus illustrate that certain strains adapt better to the new environment than others. The cells are rod shaped, and thus as the cells are randomly orientated within the gel some images show a cross-section along the length of the cell whilst the others are cut through the middle producing spherical images. These images also highlight that the cells are truly immobilised within the gels as the order of magnitude of cells is far greater than that of the porous network of the silica gel.

From these images one can see that the different strains interact with the host material in different ways. The images taken on the samples that were fixed after just one hour post-immobilisation, show that the cell walls are still intact. This suggests that the immobilisation process is not cytotoxic and the cells are still alive after encapsulation within the silica network.

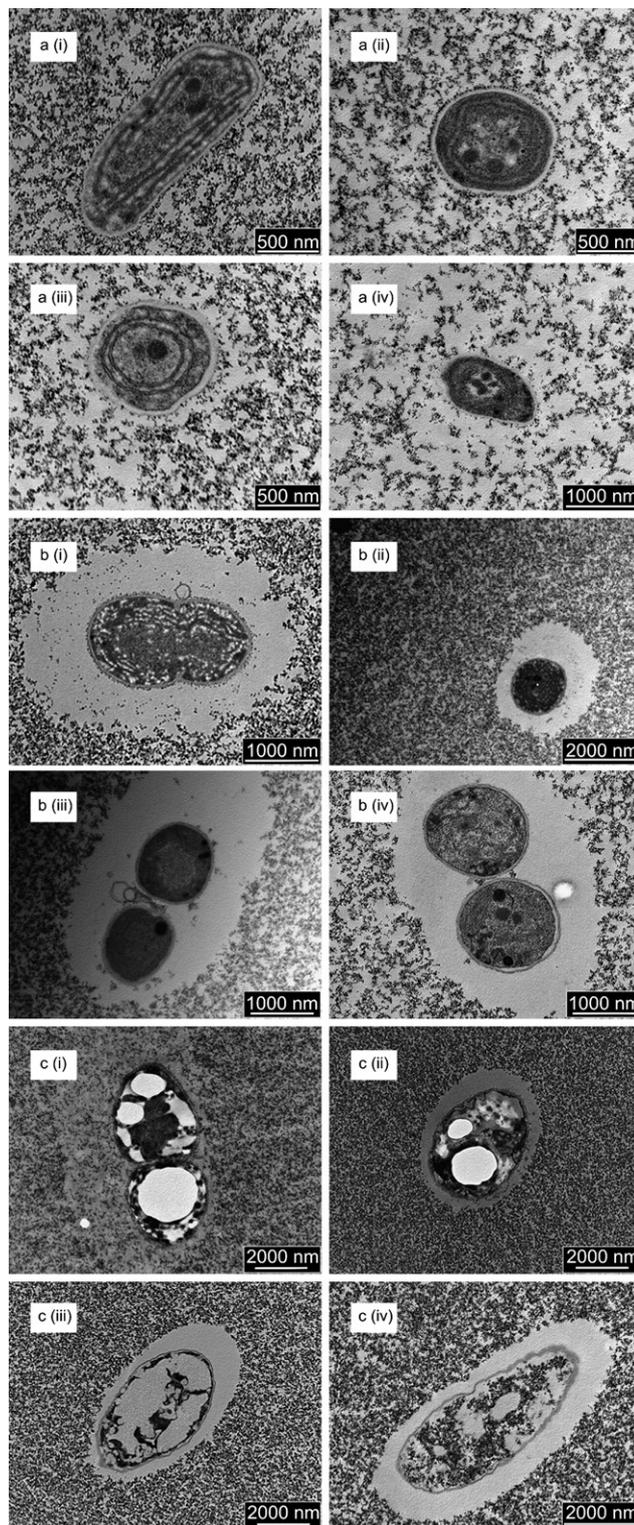


Fig. 1 Transmission electron microscopy images of cyanobacteria immobilised within silica gel after (i) one hour, (ii) one day, (iii) one week and (iv) one month, for strains (a) PCC 6301, (b) PCC 7002 and (c) PCC 7418 respectively.

First let us consider the strain PCC 6301, Fig. 1a (i and ii). Here we see that there is an interaction between the silica and the cell wall. It appears that silica particles have assembled neatly around the outside of the cells, which is best seen in images a and b. As the cells are added before the formation of a silica network this could be an interaction between the LUDOX nanoparticles and active sites, of the same order of magnitude, on the cell wall itself. Alternatively, during the condensation step, there could be a reaction between the silicic acid and the functional groups on the cell wall generating chemical bonds between the host and the cell. In general, algal cell walls are constituted of compounds such as cellulose, glycoproteins, lipids, lipopolysaccharides, peptidoglycan, proteins and polysaccharides.³² Some photosynthetic algae have cell walls synthesised from silicic acid. Diatoms polymerise predominately orthosilicic acid within the cell and subsequently extrude the polymer to form a frustule (silica wall) which encases the cell wall.³³ However Yee *et al.* have demonstrated that within hot springs there is little affinity between aqueous silica species and the thermophilic cyanobacteria *Calothrix sp.* Furthermore they state that silica nucleation does not necessarily occur. The microalgae act as passive surfaces whereby bacterial silification is predominately controlled by inorganic mechanisms.³⁴

Even if the cyanobacterial cell wall does not nucleate silica mineral formation, it could still play an important role in the deposition of silica colloids and the aggregation of polymeric silica. Therefore it is possible that an interaction between the cell wall and extracellular silica exists. After one week one can clearly see that the silica gel around the cell has begun to break down. This is more apparent after one month where the density of gel is substantially less. Thus the gel has been re-hydrolysed, either by the cyanobacteria themselves, or owing to the presence of the aqueous media.

The images of both PCC 7002 (Fig. 1b) and PCC 7418 (Fig. 1c) entrapped in silica gel show large voids around the cell with little silica present. For PCC 7002 the images highlight that the edge of the silica network is not regular and there is a presence of several silica particles within this void. Thus this void may be present before fixing the cyanobacteria, maybe from a slight repulsion between the cell and the host material. However the edges of the voids in the images containing PCC 7418 are more regular and seem to follow the outline of the cell. They are most likely to have arisen from shrinkage of the cell upon preparation for TEM, in particular during the dehydration step with ethanol.

The images selected during the time study for PCC 7002 suggest that the cells can still divide when encapsulated within the gel even when there is a limited space, as they would do in a free suspension culture. Thus the creation of macrovoids within a mesoporous material would be an ideal environment for preserving the cells. In a favourable environment (presence of light and nutrients) cyanobacteria will produce organic compounds from photosynthesis that allow them to divide. On the contrary, if growing conditions are not favourable, cyanobacterial cells will enter into a period of dormancy. A material with macrovoids would give more space for division processes to occur.

Finally the images for PCC 7418 (Fig. 1c) show the presence of vacuoles which aids the buoyancy mechanism of the cell. They also illustrate how rapidly this strain dies. After one week most of

the cellular structure is lost and the cell wall has begun to break down. The image acquired one month after immobilisation reveals the presence of silica either within or on the surface of the cell. One cannot say for certain whether silica has been internalised as there is no way to know where the cut has been made. The information is in 2-dimensions only. It does however reveal the changes to this type of cell over a period of one month and thus cell degradation with time.

Epifluorescence microscopy, a technique frequently used in life sciences, has been performed on the immobilised cyanobacteria over the course of three months to monitor the preservation of photosynthetic apparatus in the hybrid gels. In this technique biological samples are labelled with a fluorophore which absorbs the light of illumination set at a predetermined value. This technique is advantageous as chlorophyll and other photosynthetic pigments act as fluorophores and thus autofluoresce. The images in Fig. 2 are not sharp owing to the fact that not all the cyanobacteria were in the same plane and it was difficult to achieve a uniformly flat sample of gel sandwiched between the slide and cover slip. Thus the cells in different planes contribute to the blurry unfocused parts of each image. Fig. 2 shows that even after twelve weeks there are cells that continue to autofluoresce within the hybrid gels (PCC 6301 and PCC 7002). Hence this indicates the presence of chlorophyll a within the immobilised cells and therefore suggests that they still have the ability to photosynthesise. Fig. 2 also highlights that the hybrid gels fabricated with PCC 6301 and PCC 7002 have a high concentration of cells throughout the period of monitoring. This is not the case for PCC 7418, with only one cell visible per image displayed, which is representative of all the images obtained over the course of the study. This could indicate that many cells die immediately upon immobilisation or that fewer cells were isolated during centrifugation owing to the slow growth of this strain. No fluorescing PCC 7418 cells could be detected after 6 weeks which indicates that the cells have died or entered a dormant phase and are thus not suited to sol-gel immobilisation.

UV-visible spectroscopy

Fig. 3 shows spectra from UV-Visible spectroscopy experiments collected over a period of 12 weeks for the cyanobacteria immobilised in silica gel and also, for the sake of comparison, the same strains in liquid culture suspension. For each strain there are two defined bands, one at *ca.* 680 nm attributed to chlorophyll a and another at *ca.* 630 nm ascribed to phycocyanin, an accessory blue pigment and a subunit of the phycobilisome protein structure.³⁰

In every case there is a shift to shorter wavelengths of the principal peaks of reflectance (solid state mode, gel) from the peaks shown in transmission mode (liquid cultures) thus despite an experimental error of *ca.* 5% it is highly probable that the silica is affecting the measurement and shifting the electronic transitions to higher energies.

The spectra for PCC 6301 show that the photosynthetic pigments are preserved within the gels for 12 weeks with a peak present for chlorophyll a even after 12 weeks. However the peak representing the phycocyanin cannot be detected at week 12. By comparison the spectra of the suspension culture show an increase in intensity of the pigment peaks for the first 7 weeks and

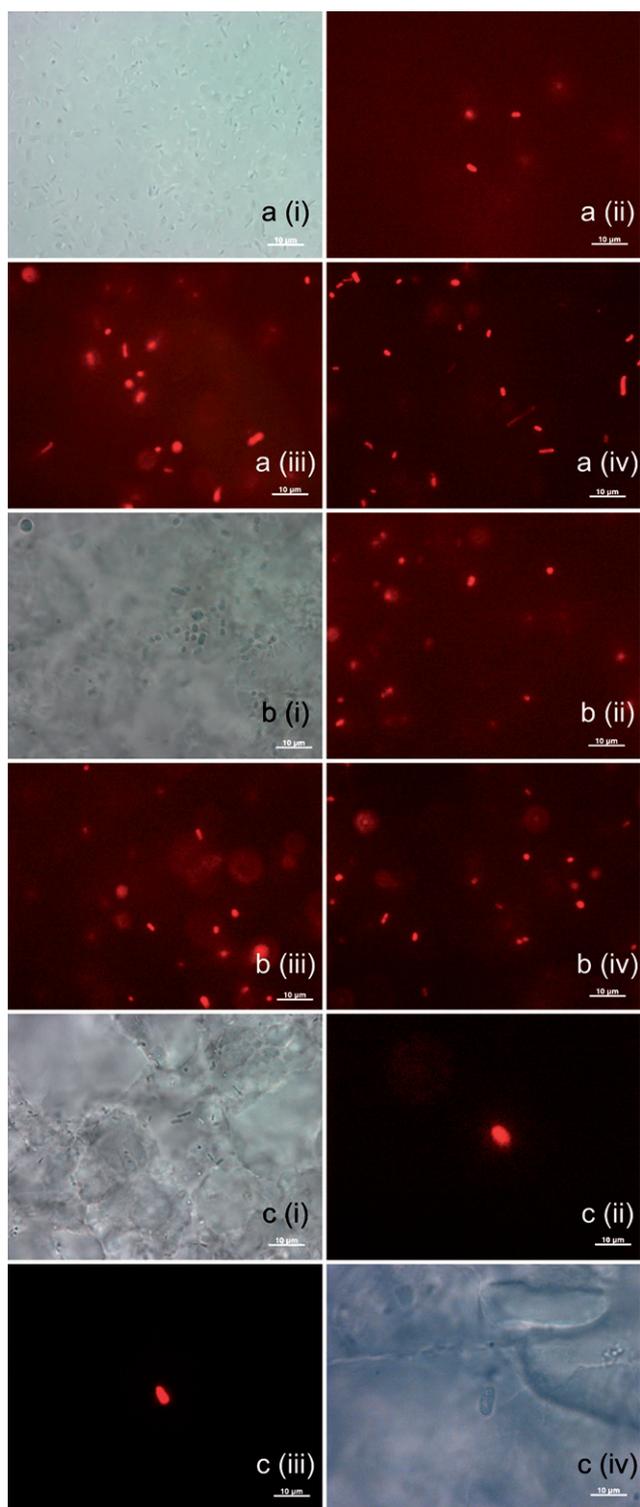


Fig. 2 Optical light microscopy images of cyanobacteria immobilised within silica gel after (i) one week; epifluorescence microscopy images of cyanobacteria immobilised within silica gel after (ii) one week, (iii) four weeks, (iv) twelve weeks [no fluorescence detected in strain PCC 7418, thus c (iv) corresponds to an optical light microscopy image], for strains (a) PCC 6301, (b) PCC 7002 and (c) PCC 7418 respectively. Scale bar represents 10 μm .

then the bands decrease again as the exponential growth phase of the cyanobacteria is complete.

UV-Visible spectra of PCC 7002 encapsulated within silica gel have shown that the photosynthetic pigments are detectable within the hybrid gels for between 6 and 7 weeks. Interestingly it seems that in contrast to PCC 6301, it is the phycocyanin which gives the more intense peak. Looking between weeks 1 and 7 one can see that the reduction in chlorophyll a is greater than that of phycocyanin over time. From the free suspension culture data it can be observed that there is no reduction in photosynthetic pigments with time and therefore this strain is suited to long term preservation.

Finally, these spectroscopy datasets also confirm the rapid degradation of PCC 7418 as seen in the TEM experiments. In fact, the pigments and presumably the cells immobilised within the gel rapidly disintegrate such that no pigments are detected beyond the second week for the hybrid material. Therefore this suggests that the cells from this strain die prematurely and are not suited to immobilisation within silica gel. The free culture suspension also displays the slow growth of this sample. Faster growth is indicated by an increase in intensity of the transition bands owing to increased chlorophyll a, and thus cell, concentration.

High performance liquid chromatography

Fig. 4 shows a series of bar charts revealing the concentrations of pigments within the hybrid gels and for the sake of comparison within liquid suspension cultures over time. The accessory pigments such as echinenone, zeaxanthin and myxoxanthophyll are identified. HPLC is a more sensitive technique with which to monitor the accessory photosynthetic pigments than UV-Vis spectroscopy, however as only acetone soluble pigments are extracted no information can be ascertained about the water soluble phycocyanin detected in the spectroscopy study.

Hybrid gel PCC 6301 highlights a massive reduction in photosynthetic pigments within the first week. This suggests there is a great loss in cell viability within this timeframe. From the second week till the end of the study the concentration levels of zeaxanthin, an accessory pigment, remain more or less the same. However the amount of chlorophyll per gram of gel fluctuates.

This study was carried out several times to find the optimal way of guarding the samples without contamination yet having a bulk quantity sufficient enough to carry out 12 extractions. In the end several samples of each hybrid gel were made up in the same culture tubes as used in the other experiments. The reasons for this were two fold. Firstly it replicates exactly the conditions used previously, thus there would be no issues over gel thickness so the same amount of light and nutrients penetrate the core of the gel. Secondly each tube represented two extractions and thus was only opened once. Previous studies in Petri dishes or in bigger vessels resulted in rapid contamination with the growth of pink coloured bacteria, which would have adversely affected the HPLC results. Hence having separate samples may account for the fluctuations in concentrations between weeks.

In comparison the free suspension culture of PCC 6301 shows that the level of zeaxanthin remains, on average, the same as for the hybrid gel. However the decrease in chlorophyll a is less pronounced.

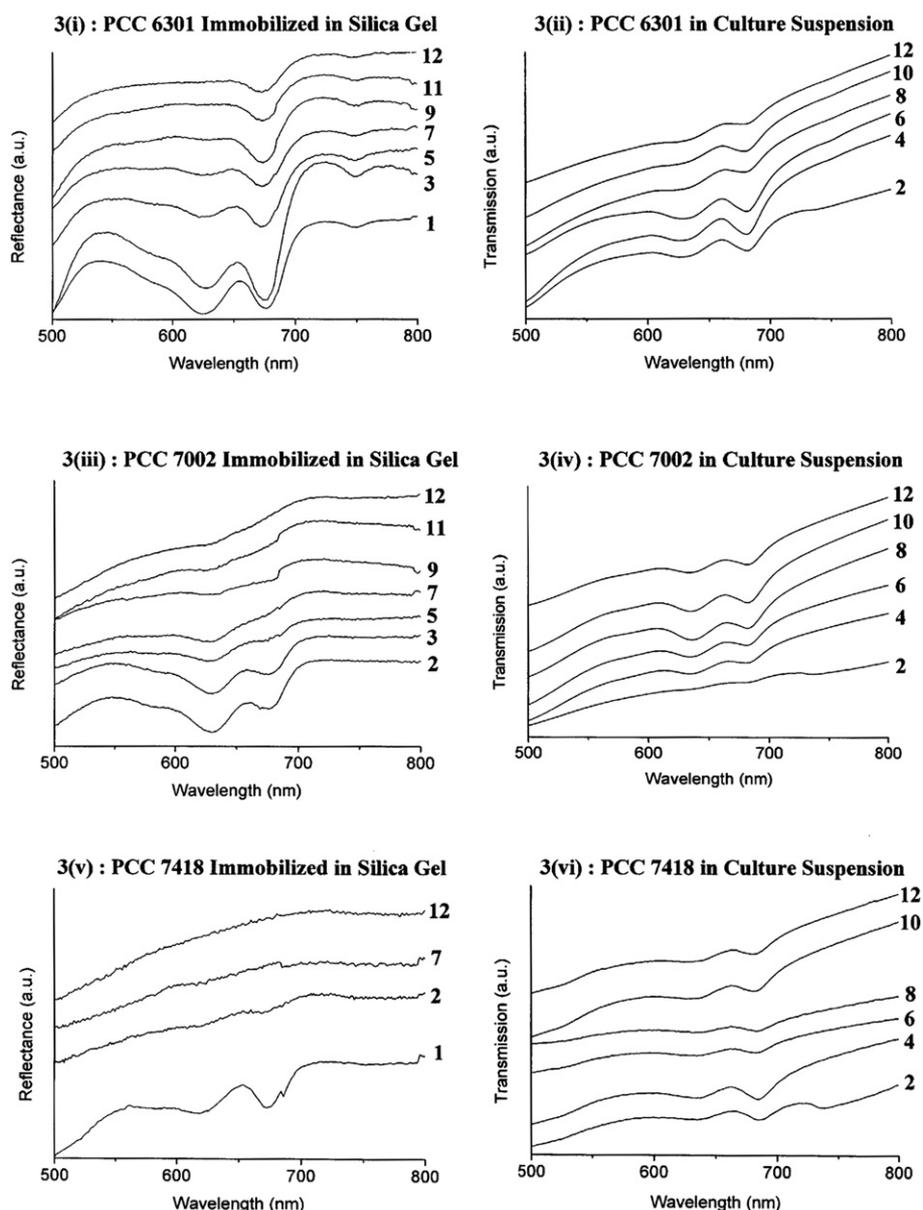


Fig. 3 UV-Visible spectra of hybrid gels and free culture suspensions measured over the course of 12 weeks, certain weeks have been omitted for clarity. Numbers to the right of each spectra represent the week.

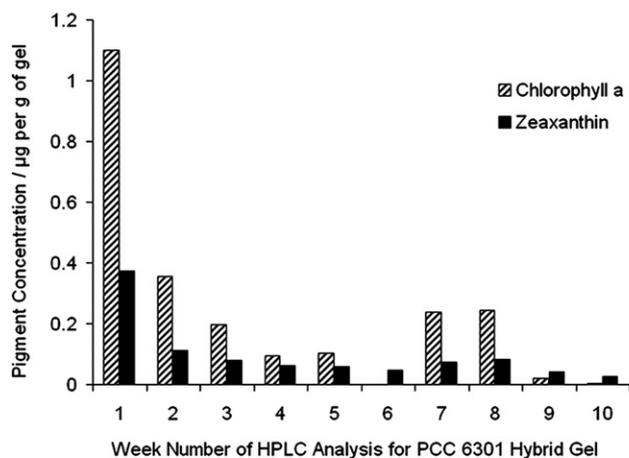
For hybrid gel PCC 7002 the concentration of accessory pigment zeaxanthin fluctuates over time whereas the chlorophyll a concentration undergoes a rapid decrease after one week and then maintains on average a level of 1.5 μg per gram of hybrid gel. In the free suspension culture the zeaxanthin levels stay the same whereas the chlorophyll a concentration decreases with time.

Finally the bar chart showing the levels of pigment concentrations in PCC 7418 hybrid gel confirms the fact that the cyanobacteria are not preserved within this gel, with little pigment present after the second week. Equally, the graph revealing the concentration of pigments within a free suspension culture of PCC 7418 shows that this culture is slow to grow. Hence the level of chlorophyll a is less than in the other two strains, thus confirming the results obtained by UV-visible

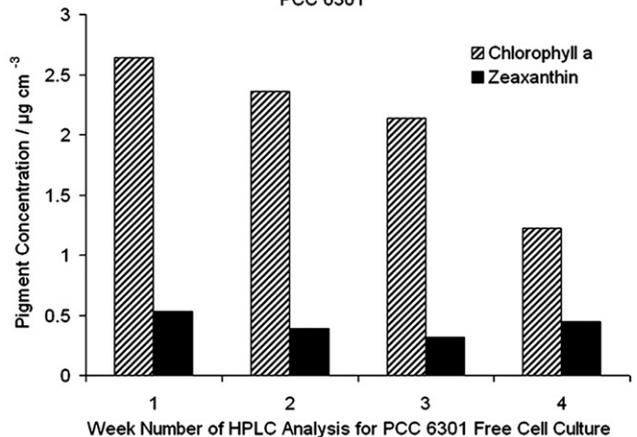
spectroscopy. The reasons behind the non-viability of this strain could stem from the immobilisation procedure.²⁷ A previous study revealed that the direct inoculation of this cyanobacterial strain into the sodium silicate precursor caused an immediate destabilisation of the sol owing to the increased salinity of the medium. This resulted in a precipitation of silica aggregates before polymerisation, thus gel formation could not take place. Therefore glycerol had been added prior to encapsulation, which may have appeared to have initially preserved the cells but potentially the process may have caused the sudden death of a great proportion of the cells.

However, the HPLC results do reveal that photosynthetic pigments are present up to 12 weeks post-encapsulation for PCC 7002 and 10 weeks after immobilisation for PCC 6301. These results hold promise for encapsulating a physiologically

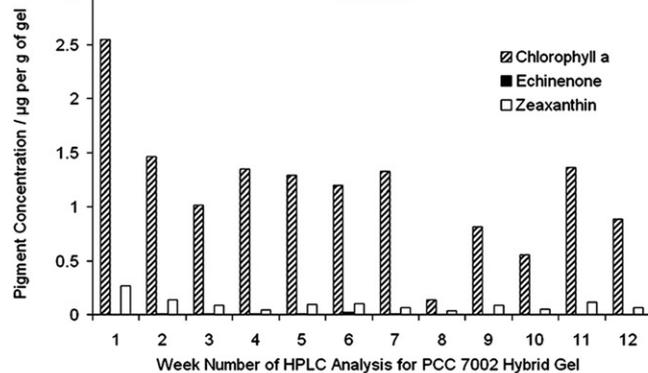
Chlorophyll a and Zeaxanthin Concentration in Hybrid Gel with PCC 6301



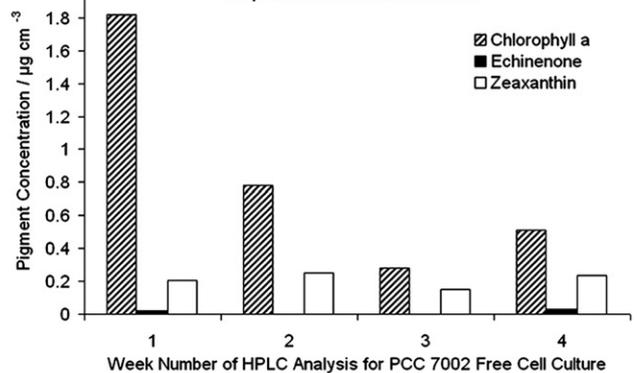
Chlorophyll a and Zeaxanthin Concentration in a Free Suspension Culture of PCC 6301



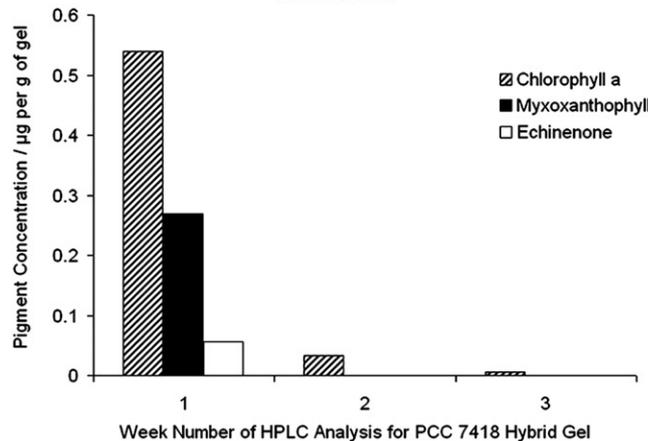
Chlorophyll a, Echinone and Zeaxanthin Concentration in Hybrid Gel with PCC 7002



Chlorophyll a, Echinone and Zeaxanthin Concentration in a Free Suspension Culture of PCC 7002



Chlorophyll a, Myxoxanthophyll and Echinone Concentration in Hybrid Gel with PCC 7418



Chlorophyll a and Echinone Concentration in a Free Suspension Culture of PCC 7418

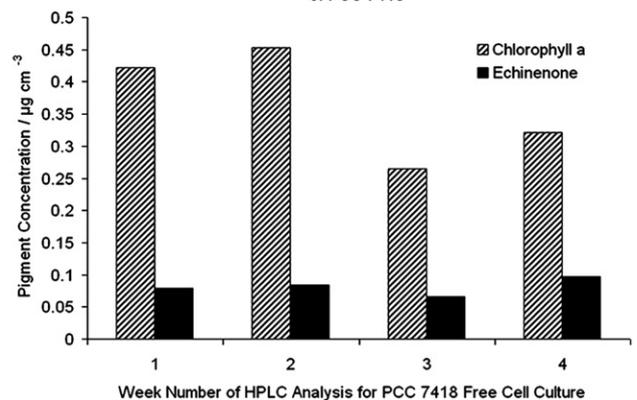


Fig. 4 Bar charts showing the concentrations of photosynthetic pigments with time for both hybrid gels and free suspension cultures.

functioning cell within a host silica material. Some of the HPLC extractions also show the presence of chlorophyll degradation products, corresponding to molecules that have detached the carbon back-bone from the porphyrin ring or that have lost the central magnesium atom from this ring. Although these results could show how the photosynthetic pigments are degraded with time one cannot say for certain whether this degradation occurs *in vivo* when the cells are within the gel, or during the extraction

process due to excess temperature or light. Thus these results have not been interpreted.

Incorporation of radio-labelled carbon

The results from the uptake of radio-labelled $\text{NaH}^{14}\text{CO}_3$ in Table 1 show that for PCC 6301 and PCC 7002 the average depletions per minute (DPM) of ^{14}C are greater for both the

Table 1 Primary production data from cyanobacteria cells immobilised in silica gel, in each case the result is an average of three samples. DPM stands for depletions per minute

	PCC 6301	PCC 7002	PCC 7418
Hybrid gel/ ¹⁴ C DPM	56 487	1 022 531	248 007
Blank gel/ ¹⁴ C DPM	12 597	126 070	544 735
Hybrid media/ ¹⁴ C DPM	7727	126 061	40 409
Blank media/ ¹⁴ C DPM	983	80 604	83 474

hybrid gel and the media than for the blank samples. This indeed gives a clear indication that the cyanobacteria still continue to photosynthesise whilst immobilised within the gel as a higher count rate is attributed to assimilated and excreted ¹⁴C. Any excess ¹⁴C from the original tracer is removed through acidification prior to further analysis. The results for PCC 7418 confirm that the physiological functions of the cell were not preserved upon immobilisation as the DPM rate is not higher than that of the blanks.

Without an in-depth study on the physiology and ecology of the cyanobacteria strains chosen it would be difficult to pinpoint exactly the reasons behind the different survival rates of the encapsulated strains. Strain PCC 7418 originates from the genus *Cyanothece*, which was isolated from a microbial mat. Microorganisms located in microbial mats are embedded in a thick layer of exopolysaccharides which provide protection from, for instance, desiccation, grazing and toxic substances.³⁵ In general, when organisms develop special abilities for specific environments it renders them extremely dependent on these conditions, thus it is likely that cells isolated from this kind of microcosm would be more sensitive to the external stresses of immobilisation. In contrast the other strains are both free-living *Synechococcus* and as such thrive in oligotrophic oceans and lakes, in rather harsh conditions of high light levels and few nutrients. Hence these strains might be more adapted to survive immobilisation and continue to photosynthesise whilst encapsulated within the inorganic framework.

Conclusions

Neutralisation and condensation reactions to form a silica gel can be controlled to occur within minutes at room temperature by the acidification of a dilute sodium silicate solution. Furthermore the addition of LUDOX, a colloidal suspension of nanoparticulate silica, reinforces the gel. On aging the hydroxyl groups on the surface of the colloidal particles condense to form siloxane bonds resulting in coalescence, interbonding and ultimately a stronger gel.

Cyanobacteria possess an elaborate, highly organised system of pigments and protein membranes which comprise the reaction centre responsible for photosynthesis. These results have shown that it is possible to encapsulate certain cyanobacterial cells within inert porous silica frameworks whilst maintaining the physiological function of the cells. TEM images have shown that the cell membranes can remain intact post-immobilisation indicating that the cells survive the stress encapsulation may pose.

In general, photosynthetic pigments are highly unstable molecules, requiring low temperatures (4 °C) and low light levels during manipulation. However, by leaving the organism intact,

the immobilisation reactions can be carried out at room temperature. HPLC results have shown that the photosynthetic pigments, the essential apparatus of the light harvesting mechanism, can be preserved for at least twelve weeks in certain strains of unicellular cyanobacteria, viz. PCC 6301 and PCC 7002. Spectroscopy and epifluorescence microscopy have confirmed that these pigment molecules are still active after 12 weeks. Finally the uptake of radio-labelled ¹⁴C has shown that metabolism performed by the cells is possible inside the hybrid gels.

Such hybrid materials could play an important role in the development of photobioreactors or closed energy loops. The benefits of photosynthesis lie with nature's efficiency coupled with the fixation of carbon dioxide during the reaction. Hence, the carbon dioxide produced on combustion of the biofuel could be reused resulting in a cleaner fuel and a reduction in atmospheric pollution and ultimately bringing metabolites and alternative fuels to the market at cheaper costs.

These hybrid gel systems now require optimisation in order to increase efficiency, by prolonging the life and bioactivity of the cells further still. It is believed improvements can be made through investigations into the most productive strains as these results have highlighted that not all strains are suited to encapsulation, with PCC 7418 displaying no activity after two weeks.

Furthermore, the form the gels take could play an important role in the optimisation process. Targeting thin film structures, owing to the increase in surface area, could potentially yield even better results. It would also facilitate the exchange of nutrients and recuperation of metabolites.

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