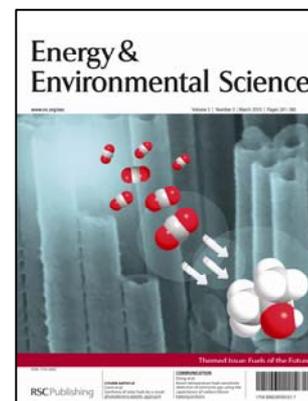


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Editorial

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Cyanobacteria immobilised in porous silica gels: exploring biocompatible synthesis routes for the development of photobioreactors†

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With the aim of designing photobioreactors (PBR) based on a smart exploitation of microalgae for the production of biofuels and metabolites of interest, this paper describes a novel approach where cyanobacteria are entrapped within highly porous silica matrices. With this concept, it would be possible to work with a constant population of organisms for a continuous (and increased) photoproduction of metabolites, in contrast to “one-shot” uses of liquid cultures. Different hybrid materials based on porous silica gels are described with a special emphasis on finding the most appropriate immobilisation conditions for prolonged cell survival. It is found that an aqueous route based on acid-exchanged sodium silicate combined with the use of silica nanoparticles as a gel-strengthening species shows the best results with a high primary production rate post immobilisation and a preservation of the photosynthetic pigments of up to 35 weeks. Oxygen production, though very low, could be evidenced up to 17 weeks after entrapment, demonstrating the suitability of using porous silica matrices in PBR design.

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† Electronic supplementary information (ESI) available: Reflectance mode UV-vis spectra of cyanobacterial strain PCC 6301 immobilised in type C (left) and type D (right) silica gels as a function of time. See DOI: 10.1039/b923859j

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Introduction

The present and future development of humanity and the growth of global economies has produced an escalating need for more energy. However, besides the effects of price fluctuations of crude oil and the consequences on global markets, the exploitation of conventional energy sources inevitably leads to increasing CO₂ concentrations in the atmosphere. Thus, new generation fuels that are not dependent on fossil fuel supplies, which can be produced continuously at a reasonable cost with minimal impact on the environment have to be developed. Cleaner energy sources such as first generation biofuels are not the best solution since their production requires arable land normally used for food crop cultivation in addition to the use of fertilisers, which have a negative impact on the environment and can affect the price of staple foods.¹ Second generation biofuels based on the exploitation of inedible food crop residues and woody biomass partially solve these issues but further research has to be carried out in order to find cost-competitive production methods with minimal effects on the environment.^{2,3} An attractive pathway towards biofuels is the exploitation of microalgae, referred to as

Broader context

This study highlights the benefits of a solid-state photobioreactor based on immobilized cyanobacteria for the dual aim of i) reducing the levels of industrially produced CO₂ pollutants and ii) conversion of CO₂ into valuable organic products, typically biofuels and high-value metabolites. The paper describes the advantages of using microalgae in CO₂ capture, as photosynthetic organisms are widely recognized as a potential solution against rising atmospheric pollution, and the use of carbon dioxide in the production of “3rd generation” biofuels. Our approach goes a step further by combining these properties with the benefits of a solid porous silica matrix, leading to hybrid photobioreactors where the algae are separated from the growth medium. In this way, it can be assumed that continuous photosynthetic production of metabolites could be achieved with easier recovery of the produced species together with conferring protection to the microalgae against contamination and harsh conditions found in industrial environments.

“3rd generation biomass” by Posten and Schaub.⁴ It is indeed well-known that microalgae are the most efficient species for CO₂ sequestration, with ten times more solar energy converted to biomass than plant cells.⁵ In fact, they were the first giant carbon sink, resulting in the evolution of a viable atmosphere on Earth. Moreover, microalgae can be produced and harvested continuously and make use of salt- and wastewaters.⁶ As a consequence of these benefits, a huge body of work is currently being carried out in order to exploit microalgae in the CO₂-neutral production of not only biofuels like biodiesel and biomethane but also hydrogen.^{7–10} A review paper by Schenk *et al.* elegantly describes the most recent advancements that have been made in this field, addressing different aspects ranging from economic considerations to the design and optimisation of large-scale cultures in open ponds or closed photobioreactors (PBR).⁶ They describe the processing of cultures to extract biodiesel and the efforts that are made to increase the photosynthetic efficiency by selecting and genetically modifying algal strains and by optimising the culture conditions.^{6–11}

The use of microalgae in biofuel production is actually based on an idea of cultivating and harvesting; biofuel precursors can be extracted from the algae prior to processing the remaining biomass into C-rich useful products. Another way of thinking would be to exploit microalgae in a non-destructive way, *i.e.* by triggering a continuous production of metabolites by CO₂ sequestration through photosynthesis, without continuously renewing the cell population or lessening their advantages. This would lead to biomimetic systems, inspired by nature, yet designed to work in a more productive and controlled way than natural systems. However, live cells are known to be quite fragile when isolated from their environment, underlining the necessity to think about protective devices. Immobilisation within a stable matrix is an excellent way to confer protection to biomolecules as already widely proven in the design of biocatalysts, bioreactors and biosensors *via* the entrapment of proteins, enzymes and whole cells.^{12–18}

Research on immobilised microalgae has been gathering momentum in the past few years, mainly with the aim of designing sensitive biosensors^{19–23} and biosorbents for harmful pollutants such as heavy metals,^{24–31} nitrates, phosphates and organic substances^{32–34} and also for improving metabolic production, as is the case in biohydrogen production.³⁵ The benefits of immobilising microalgae was recently demonstrated by Munoz *et al.* in the design of PBRs for the treatment of wastewaters that showed a higher efficiency compared to equivalent cell suspensions.³⁶ An in-depth review paper by Moreno-Garrido describes in detail the most recent advances realised in the immobilisation of microalgae.³⁷ Most of the immobilisation techniques rely on the use of organic supports like polyvinyl alcohol (PVA) or polysulfone (PSU), which certain algal strains may find toxic. More biocompatible supports include biopolymers such as Ca-alginate; however they suffer from a lack of stability over time, precluding their use in long-term devices. Thus far the most appropriate entrapment matrices appear to be porous silica materials that are non-toxic towards living cells and resist microbial attack. Since the formation conditions imply biocompatible sol-gel chemistry routes (soft conditions such as ambient temperature and physiological pH), the frameworks can be formed *in situ*, which means that the silica

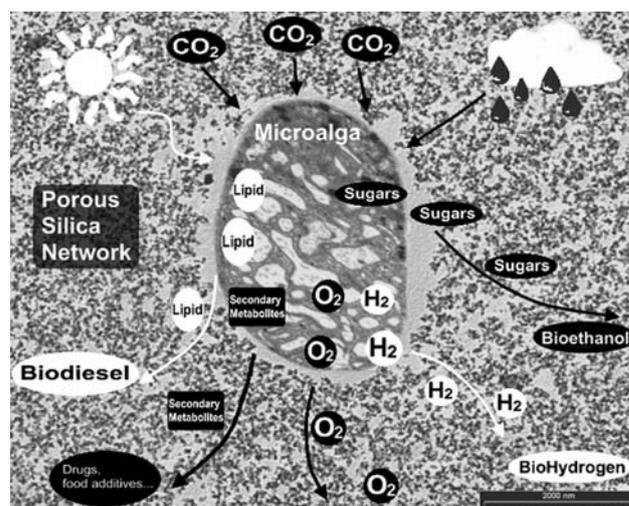


Fig. 1 Illustration highlighting the concept of a hybrid PBR based on microalgae immobilised in porous silica.

polymerises around the microorganisms, embedding them in cages moulded to their size. Moreover, silica gels inherently contain a substantial quantity of pores that enables an effective diffusion of nutrients and metabolites to and from the cells. Finally, silica is optically transparent, which is of outstanding importance for photosynthesis to continue post immobilisation.^{38,39} Livage has reported pioneering work on the use of silica as a whole-cell immobilising matrix.^{40–45} The immobilization of a series of biological species into different matrixes for photobioreactor conception has been recently reviewed.⁴⁶

The immobilisation of microalgae could bypass several problems encountered in the design of liquid-phase biofuel production devices, such as contamination by dominant competitive species that occurs in open ponds.⁶ Moreover, it is widely reported that immobilisation can lead to a concentration effect, *i.e.* more cells per unit volume compared to liquid cultures, thus potentially enhancing the natural photosynthetic yield.

The aim of this paper is to find the optimal conditions for the design of more efficient PBRs based on microalgae immobilised in porous silica gels whilst exhibiting all the advantages of these two constituents as cited above (Fig. 1). For this realisation, it is necessary to take into account many physico-chemical parameters such as temperature, pH, light cycles and intensities, porosity, nutrient availability and global mass transfer, gas exchanges *etc.* which greatly influence metabolite production and cellular activity and can be manipulated to direct the production towards the desired end-products.^{47,48} The strains were selected owing to their resistance and fast-growth to optimise the fundamental design but the acquired methodology will eventually be transferred to more specific algae dependent on the desired end-product.^{49,50} The nutrients will be based on model growth media and subsequent studies will deal with the use of natural fresh- or seawater or even wastewaters, to reduce the operating costs of the PBR.^{46,36} Concerning nutrient availability and gas flow, the surrounding matrix will possess a very high level of porosity and will also be shaped as films, hollow cylinders *etc.* to facilitate diffusion and maximise contact for the entrapped organisms inside cages moulded to their shape and size. The light levels are also very important for cellular activity, making the use

of a transparent host matrix necessary. This will be combined with an appropriate energy source such as solar, LEDs, *etc.*^{51,52} All of these requirements are believed to be fulfilled by the use of cyanobacteria immobilised within highly porous silica matrices. We describe here the most recent advances realised in the design of the above-cited photobioreactors with a special emphasis on the immobilisation conditions required for optimal survival and metabolic activity of the cyanobacteria.

Experimental section

Strain choice

Axenic strains of cyanobacteria *Synechococcus sp.* PCC 6301 and PCC 7002 (Pasteur Culture Collection) were obtained from the Institut Pasteur (Paris, France). Cultivation conditions have previously been outlined elsewhere.⁵³ These strains were chosen for several reasons, including their well-known growth conditions, their tolerance *a priori* to the by-products issued from gel formation and their low level of toxicity for manipulation.

Immobilisation conditions

In our previous studies, these strains were immobilised within silica gels formed using an aqueous sodium silicate precursor.^{53,54} This method has been improved by using an ion exchange resin to replace Na⁺ for H⁺ in order to avoid osmotic stress exerted on the cells. The materials in this work are based either on the previous protocol or the adapted method described below.

To prepare the H⁺-exchanged silicate (denoted as H₂SiO₃), 150 g of Amberlite IR 120, H Resin (Acros Organics) was washed with 500 cm³ HCl (pH 2) and chilled to *ca.* 0 °C together with 80 cm³ 1.5 M sodium silicate solution (Assay 25.5–28.5%, Merck). The silicate was then vigorously mixed with the resin, followed by filtration. The recovered H⁺ exchanged silicate displayed a pH of 2.3 and was kept at 4 °C.

Meanwhile, cyanobacteria were harvested from 15 mL of cell culture in the stationary phase by centrifugation (2500 rpm, 20 min). The resultant supernatant was discarded and the cells re-suspended in 3 mL of fresh medium (BG-11 for PCC 6301 and BG-11:ASN-III for PCC 7002). To this 2 mL of glycerol (40 drops, pharmaceutical grade, Merck) was added along with 3 mL of the prepared silicate solution. This was thoroughly mixed prior to the addition of KOH (125 μL, 0.2 M) which promoted the transition to the gel state. After gel formation 3 mL of fresh medium was added on top and left to penetrate the porous network.

Investigations were also carried out into the composition of the gel precursors including the omission of glycerol or the addition of 0.5, 1.0 and 2.0 mL of colloidal silica, Ludox[®] HS-40 (Aldrich). In the latter case the precursor mix becomes more basic (>pH 10) and therefore the KOH catalyst was replaced by HCl, as gel formation was sufficiently rapid around pH 7–8.

All the gels were kept under constant illumination using fluorescent strip lighting at ambient temperature and humidity. All of the gels are permanently covered by growth medium (that was topped up periodically to keep its volume constant) and no significant shrinkage was observed. As the exact amount of cells was not determined, to be able to compare the influence of gel

composition, each series of gels was prepared from the same cell culture.

Characterisation techniques

The structure and morphology of the hybrid materials were characterised using a Philips Tecnai 10 transmission electron microscope. The pigment concentration was verified using a Perkin-Elmer Lambda 35 spectrometer by taking samples periodically from the gels under sterile conditions. The activity (primary production) of the cells was determined using the ¹⁴C incorporation method and by oximetry with a Hansatech Oxy-lab Clark-type electrode. Further details on these techniques are found elsewhere.⁵⁴

Results and discussion

The most common route towards porous silica gels involves the spontaneous hydrolysis and polycondensation, under appropriate pH conditions, of alkoxy silanes. However, these reactions produce small aliphatic alcohols that are often lethal for the microorganisms. Thus, we turned towards aqueous precursors such as sodium silicate. To avoid over fast polycondensation rates the sodium silicate precursor is diluted. The gels may be strengthened with nanoparticulate silica by adding commercial colloids, such as Ludox[®], prior to gel formation.³⁸ After adjusting the pH of the silicon based precursors, the silicate, either H⁺ exchanged silicate (silicic acid) or sodium silicate, forms a porous silica network that makes a cement between the Ludox[®] nanoparticles and encompasses the cyanobacteria. The inherent porosity of the gels is very important since it provides pathways for the diffusion of an aqueous environment bearing nutrients and for the evacuation of the metabolites produced. Glycerol can be added as a protective agent to minimise the stress caused by the presence of sodium in the silica precursors (colloidal silica is stabilised with traces of Na⁺).^{44,55} For clarity Table 1 explains the encoding for the hybrid gels discussed here.

Sodium silicate based hybrid gels

An in-depth study on the influence of several gel compositions based on sodium silicate as silica precursor (type A) has led, after optimisation, to hybrid gels with a high degree of cross-linking within the silica framework, as evidenced by the dominance of the Q⁴ peak in the ²⁹Si MAS NMR spectra.⁵³ Complete condensation is very important since it minimises the risk of further shrinkage and thus the stress exerted on the organisms. The presence of a minor Q³ contribution, which corresponds to surface silanols, is also advantageous for the formation of an aqueous-like environment inside the silica that contributes to the

Table 1 Encoding for hybrid gels discussed in this article

Gel	Sodium silicate	H ⁺ exchanged silicate	Ludox [®]	Glycerol
A	✓	✗	✓	✓
B	✗	✓	✓	✓
C	✗	✓	✗	✓
D	✗	✓	✗	✗
E	✗	✓	✓	✗

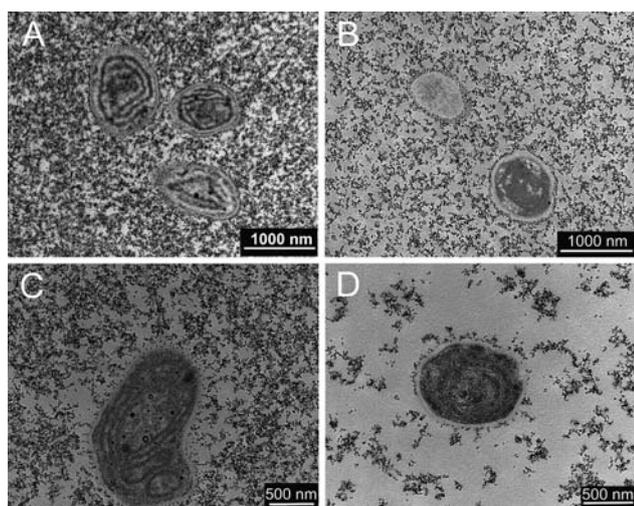


Fig. 2 Evolution of cyanobacteria PCC 6301 immobilised in type A silica gels with time: A: 1 h; B: 1 day; C: 1 week; D: 1 month.

viability of the cyanobacteria. The mesoporosity was confirmed by nitrogen adsorption-desorption measurements. Based on fluorescence and microscopy measurements, we could show that our immobilisation procedure keeps a significant proportion of the cyanobacteria intact.

Transmission electron microscopy (TEM) is a useful tool to observe the structural integrity of the encapsulated cyanobacteria and the interfacial interactions of the porous silica framework with the cell wall. Various TEM studies have been carried out on the hybrid materials over time to monitor these parameters. Fig. 2 shows a collection of images taken over the period of a month for type A hybrid materials. In all the images, the concept of entrapment is clearly evidenced, with cells isolated and present in cages moulded to their size, surrounded by a highly porous structure. Most of the cell walls are intact one hour after immobilisation, suggesting that the entrapment procedure is not cytotoxic to the microorganisms. For PCC 6301, the silica nanoparticles assemble at the outer surface of the membrane, which could be due to either positive interactions with active sites within the cell wall, to a reaction between the silicic acid intermediate and functional groups on the surface or simply to inorganic mechanisms where the bacteria act as passive surfaces.⁵⁶ The high level of porosity inherent to the silica gel is also clearly evidenced in these micrographs.

After one week (Fig. 2C), the silica gel begins to break down and an even lower density is seen after 4 weeks (Fig. 2D). Nevertheless, most of the cell walls remain intact, confirming the biocompatible character of the porous silica gels. From these observations it is evident that the gel is not stable enough to construct a PBR that can run continuously for long periods of time using type A gels as there is some re-hydrolysis, probably due to the aqueous media added to ensure nutrition of the cyanobacteria. The evolution of the gels and the cells was similar for the other two strains.

In order to determine the stability and the feasibility of the hybrid material, it is also necessary to monitor the viability and activity of the cells over time. The aim of such studies is to ascertain if the metabolism of the immobilised cells remains unaffected or, even better, is improved. Techniques such as

epifluorescence microscopy and UV-visible spectroscopy that can detect the presence of the light harvesting pigments, without which photosynthesis would not take place, can be used to monitor the potential for photosynthetic activity. For instance, epifluorescence microscopy was carried out on type A hybrid gels in order to study the preservation of the photosynthetic pigments of the cells.⁵⁴ With this technique we could measure autofluorescence for the two *Synechococcus* strains, PCC 6301 and PCC 7002, after a period of 12 weeks, which indicates the presence of chlorophyll *a* and suggests the ability of the cells to photosynthesise even after this period of immobilisation.

Spectroscopy studies on the pigment molecules within type A hybrid gels carried out in reflectance mode reveal peaks present in the same regions of the spectrum as for the free species in liquid culture, with 2 defined bands at 675 and 625 nm, corresponding to chlorophyll *a* and phycocyanin, respectively.⁵⁷ In type A gels, we already reported that for PCC 6301, the peaks regularly decrease in intensity, but still remain detectable after a period of 12 weeks, suggesting the intactness of the photosynthetic apparatus.⁵⁴ The same tendency (with variable durations) is outlined for the other strains (9 weeks for PCC 7002) and consistent with the observations regarding integrity seen by microscopy. The presence of photosynthetic pigments after this period of time was further confirmed by HPLC measurements.

H⁺-exchanged silicate based hybrid gels

In order to extend the cell viability timeframe, new studies based upon the acquired knowledge were embarked upon, which saw the substitution of sodium silicate by a sodium silicate exchanged on an acid resin, the composition of the other reactants remaining the same. This brings us closer to totally biocompatible silica sols that contain no lethal by-products; the high concentration of Na⁺ ions that arise from the hydrolysis and condensation of the sodium silicate could have a detrimental effect on living cells owing to osmotic stress. The acidic form of the silica precursor that contains less than 1 mM Na⁺ was mixed with a centrifuged pellet of the same cyanobacteria resuspended in fresh media with glycerol as for the previous synthesis pathway. Different ratios of reagent composition, as well as mixing conditions have been studied. After pH adjustment with

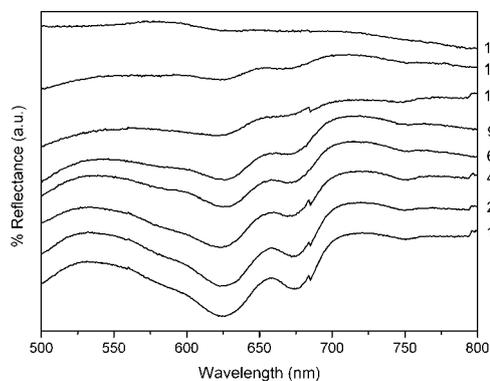


Fig. 3 Reflectance mode UV-vis spectra of cyanobacterial strain PCC 6301 immobilised in a type B silica gel as a function of time (numbers on the right correspond to weeks).

a strong base, the gel formation took about 1 h, whereas the duration could be longer in the presence of Ludox® silica nanoparticles. The idea behind this gel time control is that the cells are not instantaneously put under stress conditions from a forming silica network but given time to acclimatise to the new environment. This collection of spectra for PCC 6301 shows how the photosynthetic apparatus is preserved for almost two months by the peaks at *ca.* 675 and 625 nm before it begins to degrade at around week 13 (Fig. 3). After 19 weeks there is no detectable trace of either of the two pigments. In contrast, type A gels have lost the contribution phycocyanin makes to the spectrum by week 9.

However, one major observation is that it was often difficult to form a gel in the presence of both PCC 6301 and Ludox® (type B), with those that did gel often liquefying over the course of 1–2 months. This might mean that sodium ions, present in type A gels, which are liberated during the initial reaction with HCl play a part in the gel formation mechanism. When immobilising PCC 7002 in H⁺ exchanged silicate matrices with Ludox®, this problem seldom arises. Aside from the use of an H⁺ exchanged silicate there is also media present within the starting materials, which is a mix of salts essential to the growth of the strain, and is not present during type A gel formation. PCC 6301 being a freshwater strain has a culture medium low in sodium chloride (BG-11) in comparison with PCC 7002 which comes from a marine environment. Consequently, the media (BG-11:ASN-III = 1 : 1) is far more saline. The presence of these additional sodium ions could be the key to gel formation in the presence of silica nanoparticles.

This led us to omit Ludox® from these gels. Generally speaking, for both *Synechococcus* strains, the samples prepared in the absence of Ludox® show the best stability over time. Fig. 4 shows a time-dependent study of PCC 6301 immobilised in a type C gel (*i.e.* without silica nanoparticles). As for the previous case, both the absorption bands of the pigments characterise the intactness of the photosynthetic apparatus. The intensity of the bands decrease with time but a small contribution from both pigments can be evidenced after 35 weeks, with the sample keeping a homogeneous and deep green coloration. The same tendency could be confirmed for the immobilisations carried out on PCC 7002 with viability over 16 weeks and the clear detection of chlorophyll *a* ($\lambda \approx 675$ nm) up to 23 weeks post immobilisation

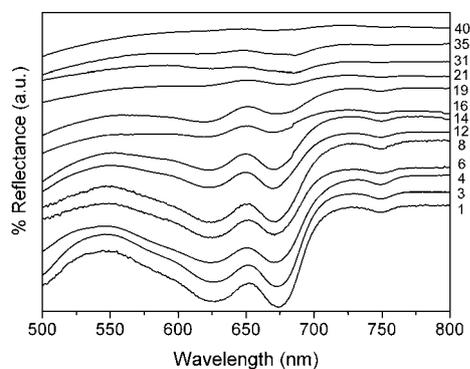


Fig. 4 Reflectance mode UV-vis spectra of cyanobacterial strain PCC 6301 immobilised in a type C silica gel as a function of time (numbers on the right correspond to weeks).

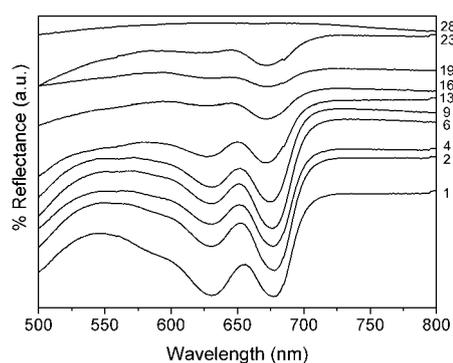


Fig. 5 Reflectance mode UV-vis spectra of cyanobacterial strain PCC 7002 immobilised in a type C silica gel as a function of time (numbers on the right correspond to weeks).

(Fig. 5). These results are very encouraging as they clearly show the beneficial effect of lowering the quantity of Na⁺ ions on the survival of the living cells, pushing our actual limit beyond 35 weeks. This improvement is most notable for strain PCC 7002, which requires a greater concentration of nutrients (marine environment). When immobilised in a type A gel the two pigments are preserved for between 6 and 7 weeks as opposed to 16.⁵⁴

One major disadvantage in using the pigments to study the hybrid gels is the fact that without special thermostatically controlled rooms the temperature varies significantly over the year. Coupled with this is the problem of light levels which can significantly alter depending on where the gel is placed in relation to the fluorescent lighting. These two key parameters modify the pigments present in the gels as the cells rapidly adapt to changes in both light and temperature. When the temperature increases growth is more rapid and when the light levels vary, the cells change their ratio of pigments either to send out better light harvesting pigments or protective pigments dependent on whether light levels are low or high, respectively. Hence, a better measure of viability is to measure the actual photosynthetic activity of cyanobacterial cells. This can be achieved *via* the ¹⁴C incorporation method. Here, a radio-labelled NaH¹⁴CO₃ tracer was added to the precursors before immobilisation and the amount of organic ¹⁴C assimilated was measured after a 24 h incubation and elimination of the excess inorganic ¹⁴C. As can be seen from Table 2, the primary production of the hybrid type C gel is significantly higher than the blank control samples (same gels without algae), showing that the photosynthetic process remained functional upon immobilisation since the DPM

Table 2 Inorganic carbon uptake by cyanobacteria immobilised in type C porous silica gels. The tracer was added either during gel formation or on top after hardening. In each case the result is an average of three samples. DPM stands for depletions per minute

Sample	DPM ¹⁴ C (Gel formation)	DPM ¹⁴ C (On top)
H ₂ SiO ₃ PCC 6301	145 363	393 961
H ₂ SiO ₃ blank	3636	1831
H ₂ SiO ₃ PCC 7002	101 072	322 711
H ₂ SiO ₃ blank	386	420

(depletions per minute, average of 3 samples) corresponds exclusively to organic ^{14}C .

To ascertain that the recorded data did not correspond to any residual activity of cells that could occur at the early stages post-immobilisation, we conducted parallel experiments where the tracer was added on top after formation of the gels. As can be seen from Table 2, the DPM are again much higher for the hybrid materials than for the blank samples, proving the ability of the cyanobacteria to photosynthesise after the immobilisation process. These results also prove the diffusion ability of the porous silica matrix, which is important for nutrient distribution and metabolite evacuation. Work is currently in progress in order to follow in more detail the behaviour of the strains in the H^+ exchanged silica gels, with a particular focus on the cell environment, by microscopy observations.

Influence of glycerol in H^+ -exchanged silicate based hybrid gels

As these results without Ludox[®] hold much promise, we then turned to the other variable within the starting precursor mix, the glycerol. This was originally added as an osmoregulator to counteract the concentration gradient set up as Na^+ is liberated during type A gel formation. However, when using an H^+ exchanged silica, glycerol becomes redundant, especially when Ludox[®] is omitted (Ludox[®] HS-40 uses sodium as a stabilising agent for the colloid) as all sodium is effectively eliminated from the precursors. Although cyanobacteria grow photoautotrophically, it has been noted that *Synechococcus* can grow heterotrophically in the presence of glycerol (see ref. 58 and references therein). In fact high concentrations of exogenous glycerol inhibit the growth of cyanobacteria and thus a period of adaptation to glycerol is necessary for glycerol-dependent PCC 7002 growth. Xu *et al.* have speculated that the inhibitory effect is due to toxic products, such as methylglyoxal, produced during cellular glycerol metabolism. Methylglyoxal is an electrophile known to react with macromolecules such as DNA and proteins.

Initial results that cover a period of 17 weeks suggest that glycerol does not affect the preservation of the photosynthetic apparatus and may in fact have a negative effect. Although this is an ongoing study, we could see that the pigments are preserved for at least 17 weeks but that the ratio in the intensities of the two absorption bands changes upon using glycerol, as a result of the adaptation of the cells (chl *a* - accessory pigment ratio) to their new environment, especially in the case of strain PCC 6301 (data available as ESI[†]).

Strengthening H^+ -exchanged silicate based hybrid gels

Type D gels, synthesised from H^+ exchanged silicate alone (without glycerol) are prone to re-hydrolysis and subsequently are not as firm as other examples. This therefore led to the final combination of precursors, the addition of Ludox[®] as a strengthening agent, to gels without glycerol, or type E gels. In the case of PCC 6301, the problem of incompatibilities between the strain and Ludox[®] arose again with the gels liquefied after <24 h. The reasons behind this are not understood and this is currently under investigation as it was not originally a problem in type A gels. However, since this phenomenon also occurs for blank gels devoid of cyanobacteria, the reasons behind

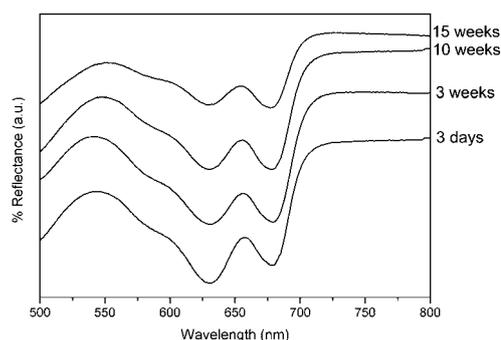


Fig. 6 Reflectance mode UV-vis spectra of cyanobacterial strain PCC 7002 immobilised in a type E silica gel as a function of time.

liquefaction must lie with interactions between Ludox[®] and constituents of the media. In contrast to this, type E gels made with strain PCC 7002 were very firm and could even be moulded into a particular shape. In this case several gels were made containing either 0.5, 1 or 2 mL of Ludox[®] for 3 mL of H^+ exchanged silica. Preliminary UV-vis spectroscopy studies indicate the presence of pigments after 15 weeks (Fig. 6). If we compare these spectra with those corresponding to Ludox[®]-free samples, it becomes evident that the addition of nanoparticles has a beneficial effect on the viability of the cells since the intensity of the signal is stronger after 15 weeks, with the band corresponding to phycocyanin in particular being well-resolved (Fig. 5 and 6).

The survival and activity of the cells was also followed by primary production measurements that involve the assimilation of $^{14}\text{CO}_2$ by the cyanobacteria. Table 3 compares the ^{14}C uptake after 24 h incubation for both Type D and E gels and also shows the corresponding depletions recorded from the growth medium, that would originate from free cells. From these results, one can see that the average depletions per minute (DPM) of ^{14}C are higher for the hybrid gels and growth medium in comparison to the same corresponding blank samples. This shows that the metabolic activity of the immobilised cyanobacteria is maintained since the higher DPM corresponds to the organic ^{14}C .

Moreover, as both sets of gels were made from the same stock precursor, we can assume that the concentration of the radioactive hydrocarbonate is the same in each gel. This enables us to make a comparison between the activities of both gel sets. From this it can then be established that the activity of gel type E is greater than that of gel type D, demonstrating again the benefits of adding the silica nanoparticles for gel reinforcement. Again, in order to verify the diffusion abilities of the matrices, a parallel set of experiments were carried out by adding the radio-tracer either

Table 3 Inorganic carbon uptake by cyanobacteria immobilised in type D and E porous silica gels. In each, case the result is an average of three samples. DPM stands for depletions per minute

Sample	DPM ^{14}C (hybrid gel)	DPM ^{14}C (medium)
H_2SiO_3 -PCC 7002 (D)	862 267	3697
H_2SiO_3 -Blank (D)	2152	1250
H_2SiO_3 -Ludox [®] -PCC 7002 (E)	1 942 019	11 982
H_2SiO_3 -Ludox [®] -Blank (E)	9016	3867

Table 4 Inorganic carbon uptake by cyanobacteria immobilised in type E porous silica gels. The tracer was added either during gel formation or on top after hardening. In each case the result is an average of three samples. DPM stands for depletions per minute

Sample	DPM ¹⁴ C (Gel formation)	DPM ¹⁴ C (On top)
H ₂ SiO ₃ -PCC 7002 (D)	101 072	322 711
H ₂ SiO ₃ -blank (D)	86	420
H ₂ SiO ₃ -Ludox [®] -PCC 7002 (E)	202 395	250 439
H ₂ SiO ₃ -Ludox [®] -blank (E)	530	358

in the media used for gel formation or on top of the gels post-synthesis, the concentration of bicarbonate being the same in both cases (24 h incubation). From Table 4, one can see that in each case, the concentration of produced organic species containing radioactive carbon is much higher in the hybrids than in the blank samples, confirming the ability of the matrix to bring the nutrients to the cyanobacteria for absorption.

The state of the cells immobilised in type E gels was also monitored by oximetry using a Clark-type electrode. Preliminary results give a net oxygen production of 0.89, 0.63 and 0.39 $\mu\text{mol g}^{-1} \text{h}^{-1}$ after 7, 14 and 22 days within the gel and a measurement carried out on a gel of the same type gave a value of 0.031 $\mu\text{mol g}^{-1} \text{h}^{-1}$ after 17 weeks. However, these results have to be reproduced from the same batch of gels with the same culture and identical stock solutions to give reliable results, but these preliminary data are very encouraging, demonstrating the photosynthetic ability of immobilised cyanobacteria over quite long durations.

Now that the encapsulation procedure in abiotic silica has proved to be a success for “model” cyanobacteria, the concept is currently being extended to other strains such as *Chlorella* and *Botryococcus braunii*. *Chlorella* shows a very high photosynthetic activity, comparable to high energy crops such as sugar cane whereas *B. Braunii* is a dominant species in algaculture owing to the high levels of hydrocarbons it produces. Extracellular oils can easily be extracted from these organisms and are interesting precursors towards the production of octane, kerosene and diesel.

Conclusions

This work has tried to shed some light on the potential of employing immobilised cyanobacteria in photobioreactors design to produce biofuels and biogases, decrease atmospheric CO₂ and produce high-value metabolites. The results clearly show that it is possible to encapsulate cyanobacteria inside porous silica gels whilst maintaining the integrity of their membranes and the function of the photosynthetic apparatus, thus the ability to photosynthesise. The mild formation conditions of silica gels, as well as their inherent porosity, full biocompatibility and optical transparency, seem to make these matrices ideal candidates for applications in novel PBRs and, furthermore, allow the problems encountered in liquid-phase systems to be bypassed.

Our most recent achievements in this novel field of research have demonstrated that photosynthetic pigments remain detectable up to 35 weeks after immobilisation, revealing the advantages of acid-exchanged silicate as a framework-building

precursor, in contrast to previously reported data. Substantial inorganic carbon uptake has also been shown, together with the possible diffusion of nutrients within the silica matrices. Furthermore, oxygen production could be evidenced up to 17 weeks post-immobilisation for Ludox[®]-strengthened hybrid gels with cyanobacteria PCC 7002. As a consequence, the most promising synthesis routes towards hybrid photobioreactors involve the use of an acid-exchanged aqueous silicate together with silica nanoparticles for direct entrapment of cyanobacteria. That way, the Na⁺ concentration remains low making the use of osmoprotectants such as glycerol unnecessary.

Studies are ongoing in order to investigate in detail the interactions of the cells with the silica frameworks by direct microscopy observations and frequent medium renewal on top of the gels is carried out to ensure that the cyanobacteria do not suffer from nutrient deficiency. Further research will also be undertaken to quantify the number of immobilised cells in order to have a better understanding of the photosynthetic yield of the material.

These hybrid systems now require optimisation to increase viability even further and for photosynthetic activity to match the performances of liquid systems. In particular, we want to diversify our set up by adapting the entrapment to other microalgal strains, with the aim of being able to propose hybrid systems on demand, *i.e.* dependent on the final desired application. In particular, we will extend the immobilisation route to highly productive strains in order to be able to demonstrate the photosynthetic ability by dosing of metabolites. Regarding our proposal for bioreactor design, we will also focus on the fabrication of both free-standing monoliths and thin film hybrid gels that could cover the inner surface of tubular vessels, facilitating the circulation of nutrients and the recovery of the produced metabolites.

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