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Energy from photobioreactors: Bioencapsulation of photosynthetically active molecules, organelles, and whole cells within biologically inert matrices*

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Abstract: Photosynthesis is a highly efficient solar energy transformation process. Exploiting this natural phenomenon is one way to overcome the shortage in the Earth's fuel resources. This review summarizes the work carried out in the field of photobioreactor design via the immobilization of photosynthetically active matter within biologically inert matrices and the potential biotechnological applications of the obtained hybrid materials within the domain of solar energy to chemical energy transformation. The first part deals with the design of artificial photosynthetic reaction centers (RCs) by the encapsulation of pigments, proteins, and complexes. The action of thylakoids, chloroplasts, and whole plant cells, immobilized in biocompatible supports, in the conversion of CO_2 into chemical energy, is also addressed. Finally, the latest advances in the exploitation of the bioactivity of photosynthetically active micro-organisms are explored in terms of the production of secondary metabolites and hydrogen.

Keywords: energy materials; photobioreactors; photosynthesis; bioencapsulation; bioactive molecules; organelles; whole cells; CO_2 reduction; solar energy; chemical energy.

INTRODUCTION

The immobilization of biological species within inert frameworks, yielding so-called hybrid materials, has rapidly evolved into a highly prosperous research field owing to the vision of many scientists [1–11]. There is great diversity within these hybrid materials; however, this review shall focus solely on the encapsulation of photosynthetically active components.

In nature, processes tend to be organized and highly efficient, such is the case of photosynthetic light-harvesting systems. Light harvesting is a highly evolved biological process that arises from the smart assembly of many subunits, which work in unison, harnessing solar energy to convert into chemical energy. Scientists are keen to exploit this sophisticated system for energy transformation in the quest for biomimetic devices for solar energy conversion and more eco-friendly fuels [12–14].

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Photosynthesis has several benefits; firstly, it employs solar radiation to convert carbon dioxide and water into useful carbohydrate building blocks such as cellulose, fructose, and starch. A more in-depth look at the metabolic processes of photosynthetically active cells reveals that a host of useful compounds are synthesized by living cells [15–17], yielding the opportunity to exploit photosynthetic processes in the design of novel bioreactors [18–21]. Photosynthetically active biological species also have the ability to fix carbon, in that they assimilate carbon dioxide and produce oxygen, thus they can be employed as carbon sinks [14]. This is not the only ecological advantage they possess. Many photosynthetic cells have the ability to adsorb and assimilate substances harmful to human health that are found in our water supplies, such as heavy metals, fertilizer nutrients, and herbicides [22–25].

Typically, biomolecules and living cells are fragile such that their removal from their local environment would result in denaturation, decomposition, or cell death. Moreover, the small size of individual cells poses a problem in their efficient application to processes [26]. Hence, in order to take advantage of the benefits of photosynthesis, primarily the conversion process of CO_2 into useful chemical compounds, activated by the absorption of solar energy, one must encapsulate or immobilize the biological matter within an inert support which can offer protection by providing a stable microenvironment (Fig. 1). A surrounding matrix can also offer protection from predators by physically restricting their access [27–29], and cell death can be prevented by immobilization as the formation of microcolonies increases the tolerance to toxic substances [30,31]. In addition, the presence of a matrix surrounding the cells has been shown in many cases to promote the production of secondary metabolites, though no precise mechanism has yet been found [32–34]. The host biocomposite material should ideally be mechanically and chemically resistant, inert both to its surroundings and the guest it encompasses, nontoxic, and phototransparent, and should eventually possess affinities with the cells [35]. The hybrid material should additionally prolong the viability of the entity. For instance, certain living cells, such as vegetal cells, undergo seasonal changes. Immobilization of isolated cells could extend their lifetime, permitting continuous metabolism throughout the year.

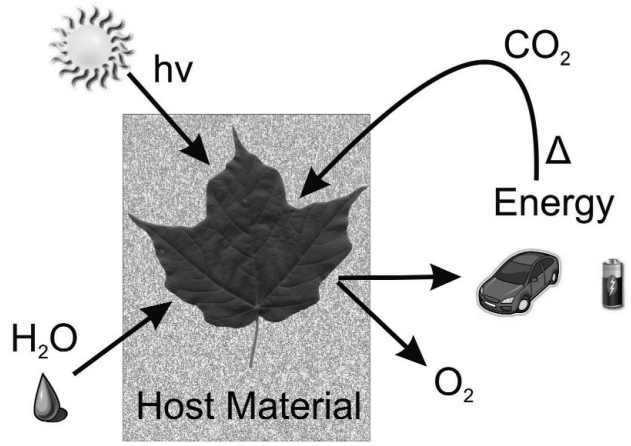


Fig. 1 Schematic representing the concept of immobilized photosynthetic material.

The hierarchical arrangement of biomolecules within a living organ contributes to an ordered nanoscale structure. The careful organization of the individual components of a biological system is crucial to the resulting efficiency of that system. The arrangement of photosynthetic pigments within a reaction center (RC) is one such example. Therefore, the re-creation of artificial, photosynthesis-based, energy conversion devices must focus attention on the nanoscale interaction of chlorophyll molecules. These are the principal photosynthetic pigments, which, for example, when complexed to proteins make up the photosystems found embedded within the thylakoid membranes of chloroplasts. The replication

of the nanoscale architectures found in photosynthetic systems has been studied by researchers interested in creating artificial chemical compounds that can efficiently transfer energy [36–38]. Another approach would be to use a host material and obtain the photosynthetic material direct from nature as opposed to artificially synthesizing light-harvesting apparatus. In terms of the immobilization of photosynthetic material, the host material must permit interactions between its guest molecules or cells in a similar fashion as to that found in nature if the efficiency is to be maintained. Nanoscale spacing in silica-based porous materials, combined with the adsorbing forces arising from the silica surfaces, may assist in the organization of supramolecules adsorbed inside the pores [39]. In fact, by constraining biological entities such as proteins and enzymes, one may prevent irreversible deformations to the structure whilst still providing sufficient room to undergo conformational changes.Moreover, silica is optically transparent within the visible region, allowing sunlight to penetrate into the core of the material [40].

The nanostructured porous systems in silica-based compounds thus lend themselves to encapsulation materials. Moreover, when immobilized, in situ cells remain in cages molded on their shape and size. The presence of many regular mesopores allows nutrients to enter and metabolites to leave, however, the cells are prevented from leaching out of the cage as the size of the mesopores is too small. There are several other benefits of porous silica materials over organic matrices such as a greater mechanical rigidity, chemical stability, and thermal stability, offering protection to the guest biological components occluded inside.

In addition, organic polymers such as polyvinyl alcohol (PVA) and polyurethane foams have been shown to be toxic to cells [41], indicating that care has to be taken in the preparation of the supporting matrices. Conversely, Ca-alginate is the most common matrix for immobilizations, since it is highly biocompatible, permeable, and transparent and does not require extreme physicochemical conditions for its preparation [42]. Nevertheless, such supports cannot be used in conjunction with nutrient media. For instance, in seawater, the substitution of Ca by Na will cause a loss of the bead structure [43]. The use of phosphate buffers can cause the onset of phosphate chelation, which also leads to a deterioration of the structure [44]. Nanostructured porous silica seems to encompass the advantages of both the natural and synthetic organic supports whilst increasing stability and minimizing toxicity.

Silica is nontoxic and biologically inert [45,46]; some bacteria even create their own silica structures [47–49].

TARGETING PHOTOBIOREACTORS VIA THE BIOENCAPSULATION OF PHOTOSYNTHETIC PIGMENTS, PROTEINS, AND COMPLEXES

Photosynthetic pigments

The basic building block of a light-harvesting system is that of the photosynthetic pigment. Chlorophyll-*a* (chl-*a*) is the most prominent photosynthetic pigment as it is common to most photosynthetic cells. Chlorophyll, a cyclic tetrapyrole, is highly conjugated, enabling it to absorb visible light. Moreover, the time taken for the energy transfer from light-harvesting antenna to RC is 10^{-10} s, which occurs with 90 % efficiency. In conjunction with chl-*a* are certain accessory pigments such as chlorophylls *b*, *c*, *d*, and *e*, carotenoids, phycocyanin, and xanthophylls. These pigments tend to absorb at different wavelengths which can be advantageous when light levels alter, such is the case for the photosynthetic organisms found below the surface of the sea.

The immobilization of such pigments is the most advanced sector within the field of photosynthetic material encapsulation. Chlorophyll and its derivatives are crucial photocatalysts in terms of solar energy conversion within the visible region of the electromagnetic spectrum. However, major difficulties arise owing to their instability toward both light and heat. Upon extraction with an organic solvent, such as acetone or benzene, the pigment swiftly loses its photostability and will rapidly decom-

pose in visible light. Thus, to exploit nature's energy conversion system effectively, researchers have turned to encapsulation.

Artificial replication of the photosynthetic apparatus has previously focused on employing surfactant solutions [50], lipid bilayers [50], liquid crystals [51], and Langmuir–Blodgett films [52] as microheterogeneous media to protect the activity of isolated pigments. Unfortunately, such immobilizing media are not suited to widespread applications in devices owing to poor mechanical strengths and thermal stabilities.

Earlier work by Inamura et al. focused on the synthesis of a chl-*a*-PVA complex film, which was subsequently deposited on a SnO_2 electrode. The optically transparent electrode was studied using an electrolyte solution which employed hydroquinone as a reducing agent, with the view to an in vitro imitation of primary photosynthetic processes [53].

Chlorophyll–protein interactions play a crucial role in the stabilization and physiological function of photosynthetic cells. Hence, several polymers were investigated, such as polyethylene glycol (PEG), bovine serum albumin (BSA), and poly(vinylpyrrolidone) (PVP), to find the most successful way of mimicking the chlorophyll–protein complex. PVA was found to swell but not dissolve in water at room temperature and thus deemed to be the most effective. It was reported that the anodic photocurrent became more negative due to chl-*a* excitation and the cathodic electrode potential more positive owing to a photochemical oxidation of the excited chl-*a* by O_2 with a subsequent electron transfer from the SnO_2 conduction band by tunnelling [52,54].

A NiO-chl-*a* hybrid optical material has recently been developed for applications as diverse as dye lasers, photocatalysis, and photosensing and also for use as a photosensitizer in photoinduced hydrogen production systems [12]. The material was synthesized via a sol-gel method. Progress in sol-gel chemistry has allowed organic chromophores to be embedded within an inorganic porous matrix formed at room temperature. Such matrices offer a rigid stability plus an increased photostability and thermal stability in comparison with their organic polymer counterparts, thus enabling the hybrid materials to exhibit novel functionalities, see Fig. 2. Thermogravimetric analysis (TGA) data on the hybrid material revealed a high thermal stability with desorption occurring at 270 °C, whereas powder X-ray diffraction and porosity measurements indicated a mesoporosity, pore size ca. 3.6 nm, within the nickel oxide hybrid material [12].

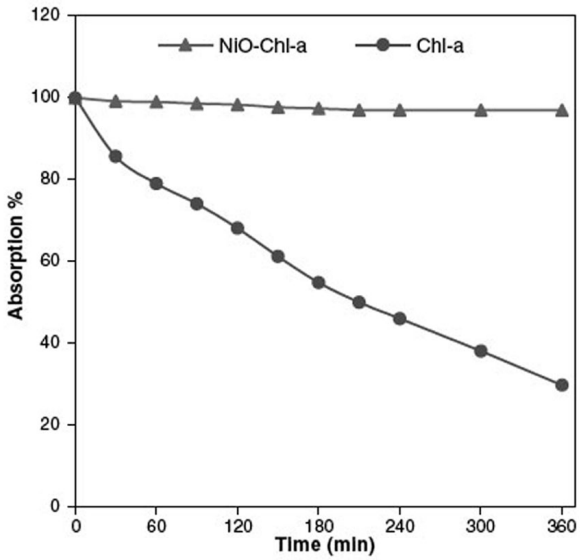
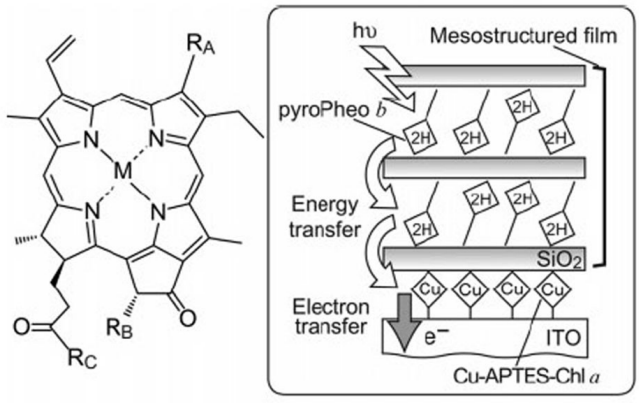


Fig. 2 Photostability of NiO-chl-*a* in *n*-hexane against illumination with visible light [12].

Furukawa et al. have synthesized layered silica/surfactant mesostructured thin films of ca. 0.36 μm thickness, approximately 100 silica layers, on surface-modified indium tin oxide (ITO) electrodes. Chlorophyllous pigments, pyroPheo *b* or Zn-pyroChl *b*, were embedded within these films to target light-harvesting devices which could generate a photocurrent (Fig. 3). The incorporation of the antennae pigments, pyroPheo *b* and Zn-pyroChl *b*, resulted in an increase in the photocurrent density when compared to films, grafted onto chl-modified ITO electrodes, which did not contain these pigments [55]. Hence, the antennae pigments enabled the system to capture more photons and in turn the device becomes more efficient (Fig. 4).



	M	R_A	R_B	R_C
Chl *a*	Mg	Me	COOMe	$OC_{20}H_{39}$
Cu-APTES-Chl *a*	Cu	Me	H	$NH(CH_2)_3Si(OEt)_3$
pyroPheo *b*	2H	CHO	H	$OC_{20}H_{39}$
Zn-pyroChl *b*	Zn	CHO	H	$OC_{20}H_{39}$

Fig. 3 Molecular structure of chlorophyllous pigments (left) and schematic representation of the energy and electron transfer in mesostructured thin films (right) [55].

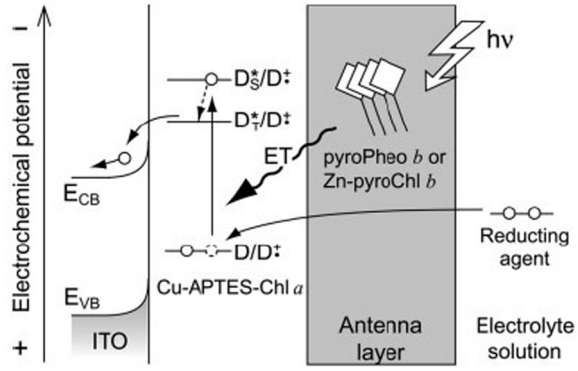


Fig. 4 Mechanism of photocurrent generation from the chl-containing mesostructured electrode. E_{CB} and E_{VB} denote the conduction and valence band edges, respectively [55].

The photostabilization of chl-*a* has been achieved via multilayer adsorption onto aluminum phyllosilicates such as hectorite [56] and bentonite [57]. These smectite-type clay minerals formed a conjugate with the chlorophyll, thereby mimicking the in vivo complex. Water was subsequently added to

form green colloidal solutions. Upon isolation, the absorption maximum of chlorophyll was shifted to shorter wavelengths; thus, conjugates were targeted that have absorption maxima which are similar to those found for living cells. The adsorption maximum of the photostable chlorophyll-bentonite conjugate was comparable to that of intact spinach leaves [57]. Furthermore, this photostability increased with increasing chlorophyll adsorption [56]. Inada found that whilst free chlorophyll was completely bleached after 10 h exposure to visible radiation, the chlorophyll-bentonite conjugate suffered no bleaching after 40 h irradiation exposure when in the presence of water. However, when in the dried powder state, the conjugates underwent bleaching almost as quickly as free chlorophyll in benzene [57]. Following on from this work and that of organic polymers, Inada et al. synthesized a smectite intercalated with PVP into which chl-*a* was adsorbed. This material was an improvement on the previous complexes as it brought the absorption maximum to within 1 nm of the value found in nature for intact spinach leaves and resulted in greater photostability [13]. In addition, experiments showed that this chl-*a*-PVP-smectite conjugate was able to photoreduce the electron carrier methyl viologen, with the subsequent evolution of hydrogen, in the presence of the electron donor 2-mercaptoethanol and hydrogenase [13]. Such artificial photosynthetic reactions are of interest for applications in water splitting and CO_2 fixation.

Mesoporous M41S materials, such as MCM-41, have large surface areas and very narrow pore size distributions, making them ideal hosts for photosynthetic pigments. The encapsulation of large molecules within M41S compounds can yield innovative composite materials that possess novel optical properties. It has been suggested that such materials could be employed in optical data storage, optical sensing, and photocatalysts [58,59]. Farzaneh and Mehraban have proposed the use of MCM-41 with polar Si–OH groups for storing chlorophyll [60]. This has stemmed from their work in separating chlorophyll and cartenoid mixtures using MCM-41. UV–vis reflectance measurements on the chl-*a* adsorbed MCM-41 material revealed a splitting of the reflectance peak consistent with delayed fluorescence of chl-*a* within a solid matrix, see Fig. 5 [59–61].

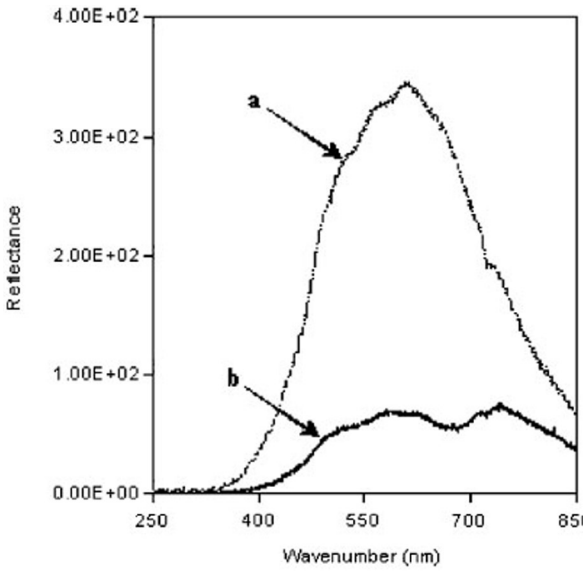


Fig. 5 UV–vis reflectances of MCM-41 (a) and chl-*a*-MCM-41 (b) [60].

Chl-*a* has also been adsorbed onto other silica materials such as folded-sheet mesoporous silica (FSM) [39,62–64]. In the case of chl-*a*-FSM conjugates, there is a nanometer-scale interaction between the adsorbed molecules which resembles the same level of interaction as in living plants. The meso-

pores of the FSM provided nanoscale spacing which aided both the interactions at the silica–chlorophyll interface and the interaction between adsorbed chlorophylls. The overall achievement was a greater photostability of the chlorophyll. Both MCM and FSM materials have honeycomb structures consisting of ordered cylindrical channels, 2–10 nm in diameter. This provides the ideal space to occlude bulky organometallic complexes, such as porphryrins [65–67] and even enzymes [68]. It has been postulated that the shift in the absorbance maximum from 665 nm, for free chl-*a* in benzene, toward 678 nm, the value observed from intact leaves, may have arisen from the formation of chlorophyll dimers (Fig. 6) [62]. The interactions between the closely spaced encapsulated chlorophyll molecules imparted greater photostability as the arrangements of chlorophyll molecules in thylakoids is similar to the chlorophyll dimers recreated in the nanospaces of FSM. Furthermore it was found that the FSM materials with pore sizes greater than 2 nm proved more efficient at stabilizing chl-*a* as they could accommodate more adsorbed chlorophyll with sufficient space to form dimers [39,63].

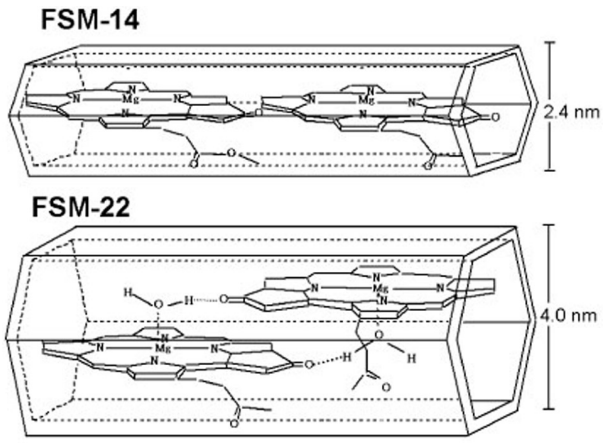


Fig. 6 Tentative arrangement in chlorophyll-FSM [63].

It was also shown that more chlorophyll can be adsorbed onto the surface of FSM materials in comparison to smectite or silica gels (Fig. 7) [62]. Greater chlorophyll adsorption would yield greater hydrogen evolution and thus an interesting development in the pursuit of devices exploiting artificial photosynthesis.

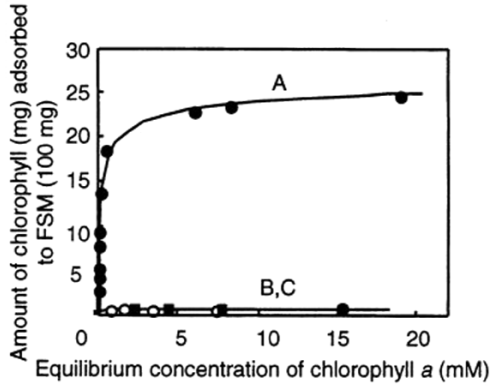


Fig. 7 Adsorption of chl-*a* to the pores of FSM powder (A), silica gel (B), and smectite (C) in benzene was spectrophotometically measured with respect to the equilibrium concentration of chl-*a* [62].

Kuroda et al. have produced nanocomposite silica films which contained photosynthetic pigments and were highly transparent. This was achieved through the solubilization of chl-*a* into surfactant micelles [69]. During the drying process, it was found that a great proportion of the chl-*a* molecules were converted to pheophytin *a*, nevertheless, the immobilized pigments were more stable toward visible light than in free chl-*a* solution. Chlorophyll and pheophytin molecules consist of a hydrophilic chlorin ring and a hydrophobic phytol chain. The size of this molecule from the top of the ring to the end of the phytol chain is ca. 2.8 nm. In an aqueous surfactant solution, chl-*a* molecules are predominately found in the peripheral hydrated crowns of spherical micelles. The chlorin rings, being directed outwards to the hydrophilic area of the micelles, were exposed to the inorganic silica layer. Therefore, it is possible that these films could be used in the photosensitized charge separation between photoactive inorganic species and sensitizing pigments [69].

The light-harvesting pigment, bacteriochlorophyll, isolated from green sulfur photosynthetic bacteria, was self-assembled in aqueous solution with tetraethoxysilane and octadecyltriethoxysilane. This was followed by polycondensation of the alkoxysilanes. The resulting hybrid silicate capsules exhibited circular dichromism and visible absorption spectra similar to the natural chlorosomes, the light-harvesting structure of the green sulfur bacteria [70]. The capsules were found to be several hundred nanometers in size, comparable to the long axis of the chlorosomes. The silicate capsule was seen to offer protection from pheophytinization under acidic conditions. Natural chlorophylls have a tendency to loose their central magnesium ion under acidic conditions, thus, the silicate cage increased the stability toward acidic environments. This could be attributable to the hydrophobic microenvironments within the matrix where few water molecules and protons were present. The structures of chlorosomal self-aggregates differ greatly to other photosynthetic apparatus which feature photofunctional pigments embedded within protein networks. Such chlorosomes could be employed in the design of an artificial photofunctional nanodevice.

Light-transducing proteins

Light-transducing proteins are proteins that are able to convert light energy into electrical impulses and are typically located in the photosynthetic membrane of the chloroplast.

Within the last 15 years, new sol-gel techniques have been developed that enable the immobilization of biologically active materials in optically transparent, porous silicate glasses [71,72]. Initial techniques required the biological material to be water-soluble and resilient to the alcohol liberated during condensation of silicon alkoxides. Many interesting proteins were either lipid-soluble or denatured by alcohol; thus, studies were needed to determine whether the sol-gel method could be extended to encapsulate these proteins. Bacteriorhodopsin (bR) is one such protein. It is a membrane-bound, alcohol-sensitive, light-transducing protein found in archaea. The purple membrane of Halobacteria acts as a proton pump. Following photoexcitation, protons are transported across the protein membrane bilayer, from the cytoplasm to the extracellular surface of the membrane, thus creating an electrochemical gradient. This provides an energy source for the bacteria to use in the synthesis of ATP. This protein has a high thermal and photochemical stability, coupled with its efficiency in energy conversion, high quantum yield, and the great variation in time between formation and decay of the various intermediates in its photocycle [73]. Its photochromic properties are far superior to synthetic materials, and thus there is significant scope for the development of optical imaging and optically based ion-sensoring devices [46,73–77].

The photosynthetic bR membrane has been successfully encapsulated within a porous silica network [73,78]. Zink et al. adapted the sol-gel process such that the drying gels were washed twice daily to remove any alcohol that formed. In removing the alcohol, the protein was protected from denaturation to preserve its activity [73], which is represented in the schematic in Fig. 8. Furthermore, results showed that the global trimeric structure and the local structure of the retinal chromophore were preserved [73]. In addition, there was no adverse affect on the proton pump function of the membrane when

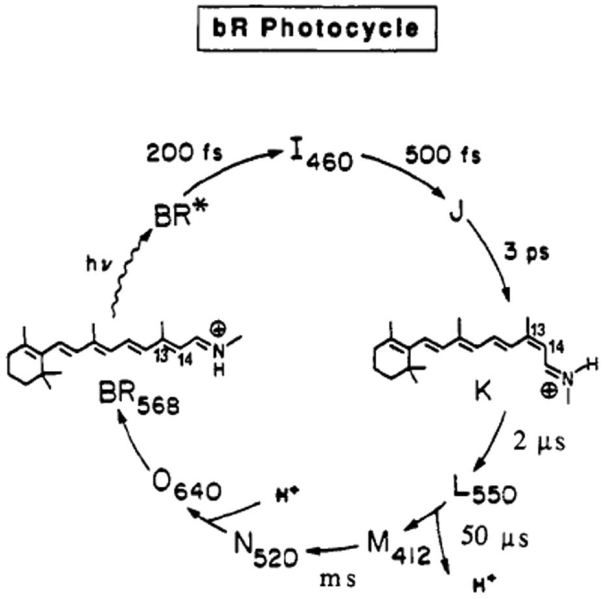


Fig. 8 Scheme of the photochemical cycle of bR. The photointermediates formed during the cycle are abbreviated by single letters. Index numbers represent the absorption maxima of intermediates. The deprotonation and reprotonation steps of the Schiff base are indicated by the release and uptake of the proton during M formation and decay processes [73].

stored at 4 °C. However, when exposed to room temperature and subsequent drying at 100 °C, the activity was lost [78]. This could be due to constraints in molecular motion arising from a contraction of the pores.

Another light-transducing protein, phycoerythrin has also been immobilized within silica gel via an alkoxide sol-gel synthesis route. Phycoerythrin along with phycocyanin and allophycocyanin are phycobiliproteins, bimolecular assemblies which are situated on the outer thylakoid membranes of marine algae. They act as light-harvesting proteins which funnel ambient light toward the photoreaction center promoting photosynthesis. These proteins are orderly stacked within the phycobilisome and can efficiently absorb low-intensity light and transfer it to the chlorophyll molecules at the RC. Absorption and fluorescence measurements revealed that the optical properties of phycoerythrin are preserved and that photodegradation was less than for free phycoerythrin, see Fig. 9. In addition, potential applications arise from the two photon-induced fluorescence phenomenon observed in both immobilized and solution phycoerythrin such as biosensing, three-dimensional biomolecular imaging, and three-dimensional optical storage [79]. A thin film of phycoerythrin immobilized in silica gel was successfully coated onto an optical fiber surface. The evanescent wave excitation from this coated fiber resulted in strong fluorescence, highlighting the possibilities for use in biosensors [80].

In contrast to phycoerythrin, phycocyanin and allophycocyanin underwent significant and irreversible conformational changes when they were immobilized within silica gel. Furthermore, they tended to aggregate, which rendered them ineffectual for optical device applications [80].

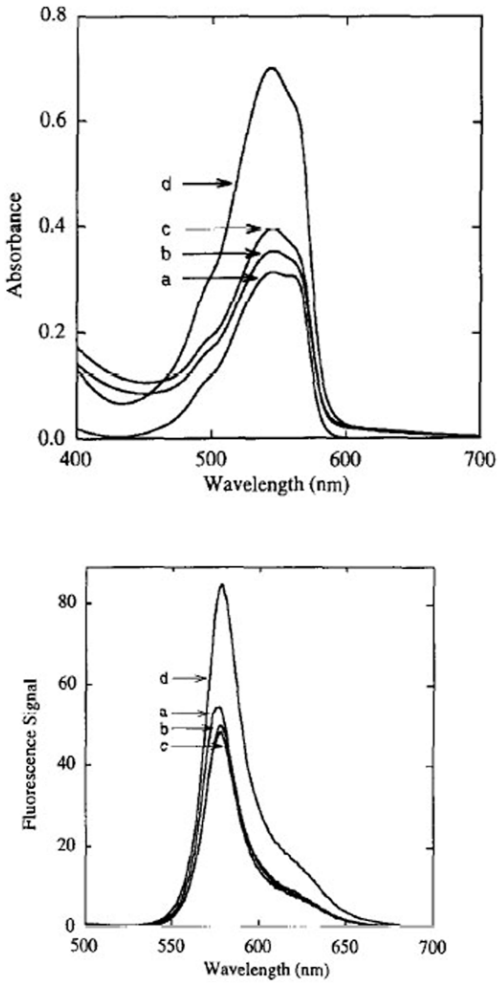


Fig. 9 Absorption spectra (top) and fluorescence spectra (bottom) of the PE in aqueous suspension and at different stages of the sol-gel process. (a) PE in buffer, (b) fresh gel, (c) aged gel, (d) dried gel [80].

Reaction centers and light-harvesting complexes

The light reactions of photosynthesis occur in what is commonly called the photosynthetic RC. They are comprised of both photosynthetic pigments and binding proteins and can be found both in green plants, i.e., photosystems I and II, and in microalgae and photosynthetic bacteria, where the RC present depends on the strain. In order to focus light inwards toward the core, the RC is surrounded by a light-harvesting complex, consisting of polypeptide chains and accessory photosynthetic pigments.

A few studies have focused on the entrapment of bigger photosynthetic structures rather than one of the individual components. One study concentrated on the immobilization of pigment-protein complexes enriched in photosystem 1 (PS1), isolated from a thermophilic cyanobacteria, in PVA films. The molecules were then orientated by stretching the films to around four times their original length [81–83]. Immobilization of the complexes avoided the tendency to aggregate into supramolecules. The study focused on the comparison of the photosystems to those of higher plants rather than an applied approach concerning potential uses of isolated photosystems. Another such study immobilized the photosynthetic RC from *Rhodobacter sphaeriodes* in PVA and trehalose glasses in order to study the

coupling between long-range electron transfer and conformational dynamics in the RCs [82,83]. These studies revealed the need for changes in conformation in order for the systems to work effectively; hence, porous materials are ideal encapsulating environments. Whilst the biological material can be permanently retained within a matrix tailored to the size of the entrapped molecule, it is not confined to the extent it cannot change conformation.

Itoh et al. have recently incorporated the light-harvesting protein LH2 into FSM, see Fig. 10 [84]. LH2 is a functional membrane protein with a cylindrical structure of 7.3 nm diameter, which is comprised of 27 bacteriochlorophyll-*a* and 9 cartenoid molecules. This complex was isolated from the thermophilic purple photosynthetic bacterium *Thermochromatium tepidum*. The FSM materials had nanometric-sized pores with a honeycomb-like hexagonal cylindrical structure inside. It is believed that the pore channels provided the proteins with similar environments to those inside the hydrophobic membrane matrix. The benefits of using light-harvesting components of thermophilic strains include the knowledge that they should be more resistant to heat. The amount of LH2 adsorbed depended upon the pore size of FSM-16. Two materials were synthesized, one with a pore diameter 2.7 nm, the other 7.9 nm. Substantially more protein complex was adsorbed into the FSM material with a slightly larger pore diameter, 7.9 nm, than that of the complex itself, 7.3 nm (1.11 mg of protein complex per mg of SiO_2 cf. 0.24 mg/mg for the smaller pore diameter FSM) [84]. The stability of the adsorbed protein complex was superior to its stability in solution; moreover, the thermal stability was increased by ca. 10 °C. According to the study, this increase in stability arose from a decrease in both the apparent activation energy and collision factor for the degradation process [83]. The interaction between the silica walls and LH2 seemed to prevent an irreversible breakdown of the ring structure and subsequent loss

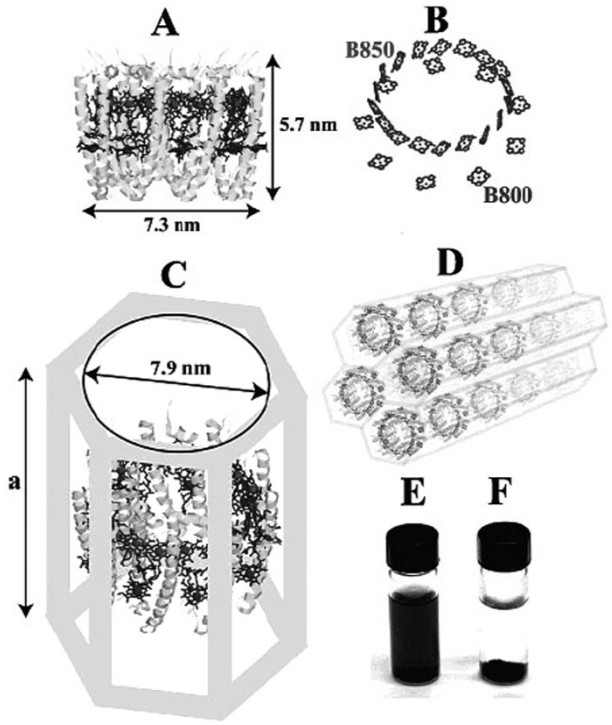


Fig. 10 Molecular structure of LH2 complex (A), arrangements of bacteriochlorophyll-*a* molecules inside a single LH2 molecule (B), schematic view of LH2-FSM7.9 conjugate (C, D), and photographs of vial tubes containing LH2 solution before (E) and after (F) addition of FSM7.9. A value for *a* in (C) was estimated to be 9.2–11.4 nm upon the binding of 1.11 mg of LH2/mg of FSM7.9 based on the results in this study [84].

of pigments. The LH2 adsorbed within FSM pores was fully active and thus still able to capture and transfer excitation energy.

BIOENCAPSULATION OF ORGANELLES AND WHOLE PLANT CELLS

Thylakoids and chloroplasts

Another way of taking advantage of solar energy is to use systems that are more complex, such as thylakoids or chloroplasts. Even though membrane fragments containing photoactive proteins like bacteriorhodopsin are able to convert light energy into chemical energy (i.e., via a transmembrane proton gradient), they are not able to use chemical energy to fix CO_2 to synthesize organic compounds like sugars. In the eukaryotic algae, bryophytes, ferns, gymnosperms, and angiosperms, which all perform oxygenic photosynthesis, possess photosynthetic membranes (thylakoids) that are enclosed within an envelope consisting of a double membrane, forming a structure called the chloroplast. This organelle performs all the primary processes (e.g., light capture and electron transport, leading to reduced nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine 5'-triphosphate (ATP) synthesis) and most of the secondary processes (e.g., the synthesis of 3-carbon phosphorylated compounds from CO_2) of photosynthesis. They also synthesize many proteins and other components, thus this biostructure has some self-sufficiency [85].

Immobilization into organic matrices

Over the years, numerous studies have been performed to develop technological applications arising from the immobilization of photosynthetic material. However, the lifetime of isolated chloroplasts and thylakoids was very short; the instability of the activity of photosystems over a long period of time was a crucial limitation [86]. In order to overcome this problem, biological structures have to be stabilized by immobilization, as a matrix can confer a suitable microenvironment to the biostructures. Several different methods like adsorption or encapsulation within matrices, including synthetic polymers (photocross-linkable resins and polyurethanes), polysaccharides (agar and alginate gels), or proteins (cross-linked albumin-glutaraldehyde, gelatin) have been successfully reported.

Adsorption of chloroplasts on a support is a simple, mild, and reversible process, permitting the reuse of both organelles and substratum. For example, chloroplasts were adsorbed onto diethylaminoethyl-cellulose to design multifunctional photobiocatalysts [87]. Cellulose-nitrate membrane filter was also used to form a thin layer of chloroplasts, opening the way to in vitro measurements of photosynthesis, by chloroplasts under conditions more closely resembling those in leaves [88].

Immobilization of active photosystems or whole chloroplasts within synthetic and biopolymers stabilizes enzymatic activities. The lifetime of immobilized systems within polyacrylamide gel, obtained by redox polymerization, was three times longer than that of free chloroplasts. Moreover, the production of 3-phosphoglyceraldehyde and other compounds by these immobilized organelles was detected [89,90]. It has been reported that radical polymerization provides an efficient method of chloroplast immobilization. The method involved finding a suitable protective agent, such as albumin or PEG, to prevent the inactivation of the chloroplast by the monomer used in the immobilization process. The use of monomers such as poly-2-hydroxyethyl acrylate, methoxypolyethyleneglycol, methacrylate, and acrylamide preserved the photochemical activity of immobilized chloroplasts at –24 °C. Furthermore, the deterioration in activity of immobilized chloroplasts occurred more gradually in comparison with that of free isolated chloroplasts. The thermostability of the chloroplasts was also greatly increased by immobilization using these monomers, especially hydrophilic monomers. However, optical microscopy has shown that numerous fragments of chloroplasts are dispersed within the swollen polymer gel (Fig. 11) [91]. Chloroplasts and thylakoids were also successfully entrapped into agar, agarose, and calcium alginate gels. The immobilization within biopolymers can preserve enzymes involved in the Hill reaction (Fig. 12) [92], which in some cases can continue to produce ATP by the addition of ascorbate [93].

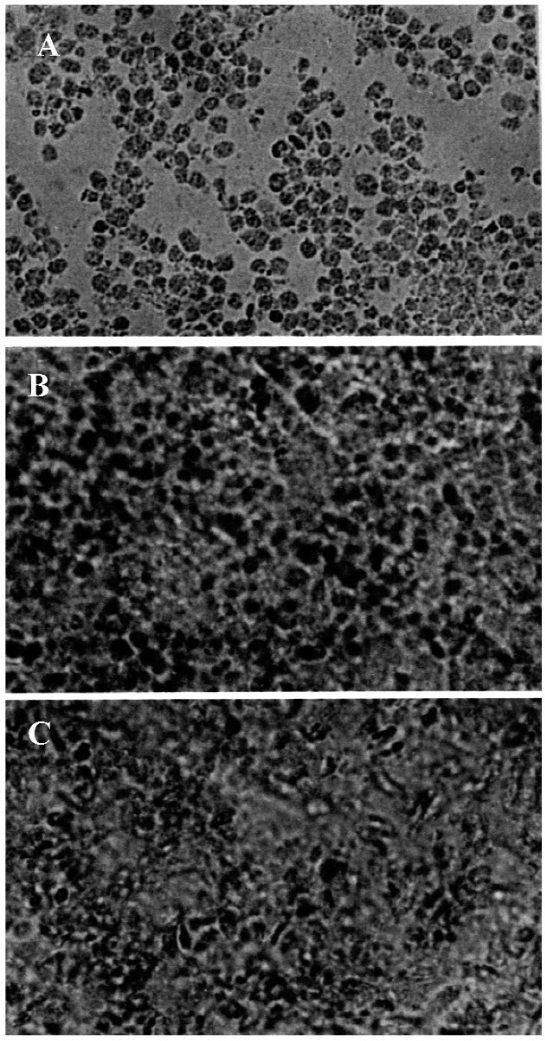


Fig. 11 Optical micrographs of intact chloroplasts in suspension (A), chloroplasts immobilized with 2-hydroxyethyl acrylate (B), and chloroplasts immobilized with methoxypolyethyleneglycol methacrylate (C) [91].

$$H_2O + A \xrightarrow{h\nu} \tfrac{1}{2} O_2 + AH_2$$

Fig. 12 The Hill reaction is formally defined as the reduction of an electron acceptor (A) by electrons and protons from water, with the evolution of oxygen, when chloroplasts are exposed to light.

Proteins like BSA cross-linked by glutaraldehyde can be used to form a matrix. This immobilization technique has proved to be the most efficient way to maintain enzymatic activity and thylakoid stacking (Fig. 13) compared to the methods mentioned previously [94,95]. The monitoring of oxygen production of thylakoids by adding an artificial electron acceptor (ferricyanide) and an uncoupling reagent (ammonium chloride) shows that the activity of photosynthetic membranes can be prolonged by eight days when they are immobilized with a cross-linked albumin polymer in contrast to isolated

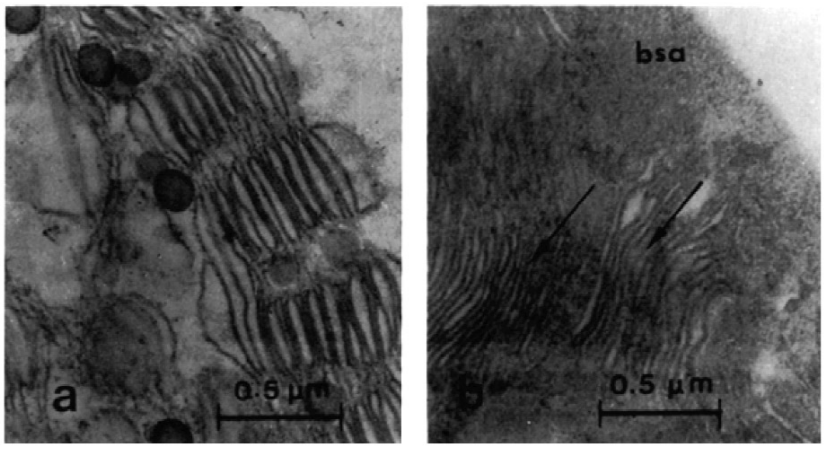


Fig. 13 TEM micrographs of (a) lettuce thylakoids in suspension and (b) immobilized thylakoids into albumin foam [94].

thylakoids stored at the same conditions (in the dark, 4 °C). BSA is known to stimulate various photoreactions in isolated chloroplasts owing to its affinity to bind fatty acids, which are responsible for inhibiting the electron flow, released during isolation, in vitro aging, or also when added exogenously [96,97]. This immobilization pathway partially preserves ATP production [98].

Immobilization into silica matrices

A novel and promising way to preserve these kinds of biosystems is to use silica precursors as reagents to form a hybrid silica gel. Silica matrices are quite inexpensive to synthesize, possess a chemical inertness, high mechanical strength and thermostability, optical transparency, tunable porosity, and are resistant to microbial attack [99]. Moreover, porous silica presents a mild preparation pathway [100] (in opposition to polymeric supports) and its silica-based structures contain water, providing a beneficial aqueous environment and physiological stability for entrapped cells. Many studies have shown that biomolecules can be entrapped into a wide range of silica matrices [101]. However, much less work has been performed with more complex systems like organelles and whole cells. In particular, we report the entrapment of thylakoids and chloroplasts into silica matrixes. By controlling ionic forces, osmotic pressure, pH and avoiding any denaturing agent like alcohols, the stabilization of the photosynthetic thylakoids was impressive. In the presence of ferricyanide, oxygen production by entrapped thylakoids (conserved at 8 °C) could be detected over a one-month period, which contributes a major advancement to the field since the activity of thylakoids within free suspensions declines very quickly and is nonexistent after three days (Fig. 14). Confocal fluorescence microscopy clearly shows the beneficial effect of a silica matrix on the structural properties of chloroplasts. Grana (stacked thylakoids) are well preserved after their encapsulation. Moreover, these organelles are better maintained and remain isolated within the support over time (Figs. 15e,f) than in suspension (Figs. 15b,c). The gel prevents the aggregation seen in the free suspension, which provokes the denaturization of organelles. The silica matrix thus unequivocally confers a real protection on such biosystems.

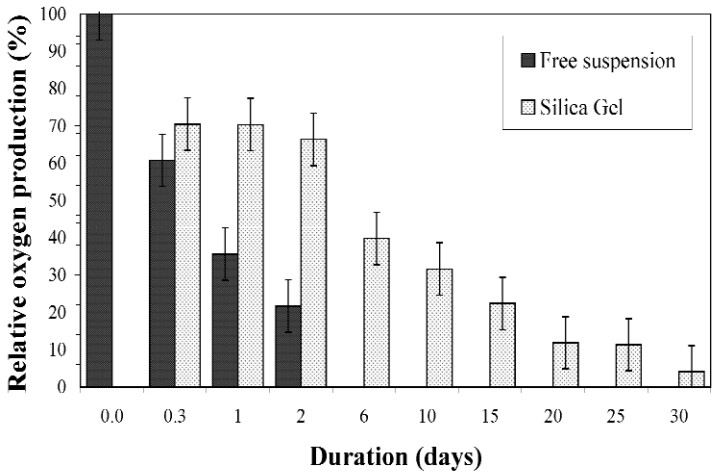


Fig. 14 Comparison of enzymatic stability of free thylakoids suspension and thylakoids entrapped within SiO_2 gels.

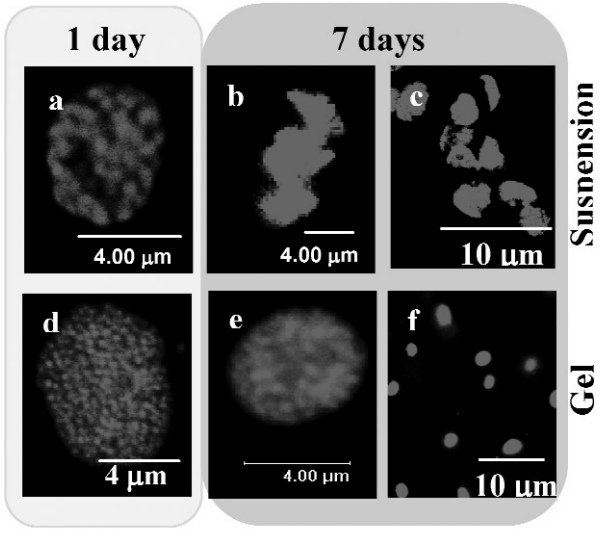


Fig. 15 Chloroplasts in suspension and silica gel analyzed by confocal fluorescence microscopy.

Potential applications in energy generation

Such immobilization techniques can be exploited to design devices suitable for photobiological solar energy conversion (biophotolysis of water, photohydrogen production, and ATP regeneration) and to construct bioreactors.

Bioreactors

A stable photoreaction system was developed using chloroplasts coupled with P450-containing microsomes. The fused enzyme between cytochrome P450A1 and the yeast NADPH-cytochrome P450, oxidoreductase, which is produced and regenerated by chloroplasts, catalyzed the oxidation of lipophilic substrates in the presence of O_2 and NADPH. Due to the instability of the chloroplasts and the microsomes, entrapment within agarose gel was necessary to allow a high conversion of lipophilic compounds. This process is not only attractive because of its ability to regenerate the costly NADPH cofactor but also because of the ease at which the products can be separated from the immobilized

catalysts (e.g., fusion enzyme-expressed microsomes and chloroplasts). Thus, this light-driven bioreactor exhibits great potential for the decomposition of various hydrophobic chemicals, including pollutants such as carcinogens [102,103].

Another promising application concerns H_2 production. Hydrogen is a renewable, non-polluting alternative energy source that has sparked a keen interest amongst researchers for its potential to replace fossil fuels. Stable biophotolytic production of hydrogen from water was first reported by P. E. Gisby and D. O. Hall [104]. Hydrogen can be produced by the illumination of an aqueous mixture of chloroplasts and hydrogenase, in the presence of an electron carrier (e.g., benzyl viologen). These biological systems have been immobilized in an active form using calcium alginate gels to overcome the instability problems previously encountered. It has also been reported that the combination and immobilization of chloroplasts with *Clostridium butyricum* cells in agar gel in the presence of benzyl viologen can result in hydrogen production. The total amount of hydrogen evolved within 6 h was reported to be ca. 41 μmol mg^{-1} chl [105]. More recently, a coupled system of *Halobacterium halobium* and chloroplasts entrapped within reverse micelles was used to enhance hydrogen production. This immobilization yielded an appreciable rate of 0.136 mmol of H_2 min^{-1} mg^{-1} (dry weight), whereas no detectable hydrogen production was observed in aqueous suspension using the same coupled system. The authors suggest that the increased rate of H_2 evolution in the reverse micelles arises from the fact that the enzymes associated with hydrogen production remain more active and stable for a longer period of time within the cellular constituents when entrapped within the reverse micelles [106].

Micropower

Thylakoids can also be exploited to convert solar energy into electrical energy for micropower applications. These photosynthetic subcellular plant structures were immobilized onto a gold electrode surface that had been functionalized by bioelectrocatalytic self-assembled monolayers (SAMs) of cystamine and pyrroloquinoline quinone (Fig. 16). In the future, this system could produce a photocurrent by direct electron transfer from thylakoids via the bio-SAMs to the gold electrode surface [107].

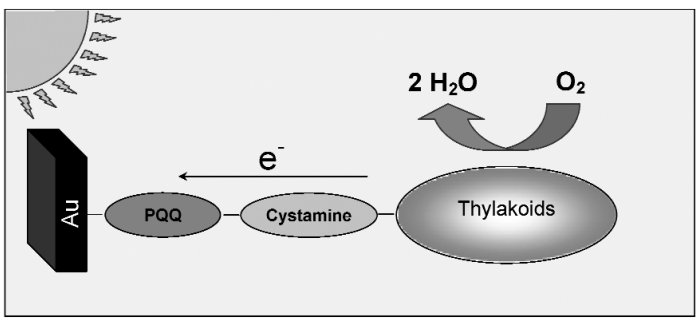


Fig. 16 Energy converter constructed from the immobilization of thylakoids onto a gold surface electrode (adapted from [107]).

Whole plant cells

The plant cell is the structural and functional unit of a plant. During these past few years, the interest for plant tissue cultures has increased considerably. Using light to drive biosynthesis, higher plants are the source of a vast array of biochemicals. Biocatalysis has emerged as an important tool in the industrial synthesis of bulk chemicals, pharmaceuticals and agrochemicals, active pharmaceuticals, and food ingredients [108,109]. The chemical diversity of the plant cell has led to the discovery of over 40 000 different structures, in which several classes serve as important pharmaceutical agents, including the anticancer agents paclitaxel (Taxol) and terpenoid-derived indole alkaloids [110]. For some of these compounds, plant cell suspension cultures can provide an interesting alternative production pathway to

chemical synthesis routes [111]. Moreover, the use of such living suspension cultures in the production of recombinant proteins is a subject of great biotechnological interest because of advantages in economy, scalability, and safety compared with traditional microbial and mammalian production systems [112,113].

Vegetal cells can also be used in phytoremediation. Some plant cells can degrade toxic compounds like polychlorinated biphenyls (PCBs), 2,6-dinitrotoluene, 2,4,6-trinitrotoluene, etc. [114–116].

Recently, cell-based biosensors have been considered for new applications in molecular diagnostics (e.g., metal ions, organic compounds, sugars, proteins). A variety of cell types, such as plant cells, have been used to fabricate different devices [117]. The advantage of this kind of system is that they can provide physiologically relevant data in response to the analyte and to measure the bioavailability of the analyte. Furthermore, through advancements in genetic engineering, cell-based systems can be designed to afford high specificity and selectivity for the analyte [118].

Immobilizations

Plant cells are very sensitive to environmental conditions and their fluctuations. Moreover, in comparison to animal cells, bacteria, and yeast, plant cells are bigger in size and very sensitive to shear stresses found in culture suspensions, which can break the fragile cell wall. Therefore, the control of extracellular factors is critical to develop stable devices or bioreactors. To overcome this problem, different methods of immobilization have been developed over the past few years. An excellent matrix should possess characteristics such as being optically transparent, inert, nontoxic, mechanically strong, and exhibiting water stability. The matrix porosity has to allow free diffusion of nutrients and metabolic products and act as a barrier against microbial contamination. The immobilization pathway should also be mild to preserve the cell viability.

Adsorption of plant cells on a substratum was one of the first methods used to control the cell environment. Different carriers were used such as polyurethane foams [119–121], nylon foams [122], fiberglass [123], and loofa sponge [124]. Polyurethane foams are quite phytotoxic, probably due to residual monomeric precursors. Washing foams with organic solvents reduced, but did not completely remove, the phytotoxic component [125]. Glass and loofa sponges seem to be nonphytotoxic and thus are a better choice as a support for immobilization. Furthermore, fiberglass was shown as the substratum having the best immobilization efficiency of *Catharanthus roseus* cells [123]. Immobilization by adsorption has the advantage of being easier than other techniques, but leaching of cells from the matrix can be detrimental for some applications.

Microencapsulation using biopolymers has been the most widely used immobilization method using alginate, agar, agarose, carrageenan, gelatin, and pectin gel as matrices because it is simple and reproducible using mild conditions [126–129].

The most popular polysaccharide used is alginate. Alginate gels are formed around the cells which remain, in principle, suspended in a normal fluid environment bounded by a hydrogel structure. The metabolic capacity of these entrapped cells has been reported to stay intact for a moderately long period of time for different plant cell species such as *Morinda citrifolia*, *C. roseus*, and *Digitalis lanata* [130]. Using PEG as a thickener, *Fragaria ananassa* has been successfully immobilized in a liquid-core alginate membrane capsule. Due to the ability of the thickener to leak through the alginate shell membrane, the core liquid is of low viscosity, enabling a good mass-transfer performance of the nutrients, and finally a 3.6-fold increase in cell weight over 9 days of cell cultivation [131]. However, the alginate immobilization method has been reported to be unsatisfactory in some cases due to the tendency of beads to rupture as cells divide and expand. Indeed, the metabolic effects by alginate itself (polyelectrolyte) and the high calcium content can reduce the efficiency of the entrapped living plant cells [123,132].

As mentioned above, encapsulation in silica gels seems to be the most promising way to create the ideal host for plant cells.

The first immobilization of plant cells in hybrid silica materials was performed by Carturan and coworkers. The immobilization process, called Biosil, was realized in three steps. First, an ordinary glass fiber fabric was coated with an organically modified gelling SiO_2 precursor solution providing a suitable support for the immobilization of plant suspension cells. Cells were then entrapped by filling the voids of the support with the cell culture suspension. The achievement of a definite immobilization was realized by subsequent treatments with SiO_2–sol, followed by a gas-phase reaction of tetraethoxysilane and diethoxymethylsilane with water adsorbed on the plant cell wall (Fig. 17). Immobilized cells maintained their viability after their immobilization. The problems of alcohols released during the condensation of silica were avoided by using a gas-phase reaction where alcohol rapidly evaporates [133].

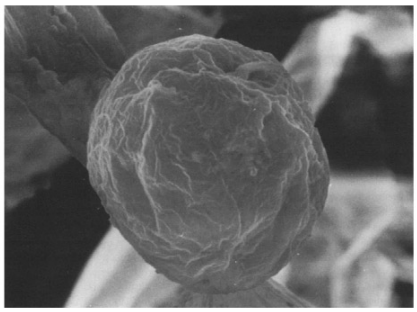


Fig. 17 SEM micrograph of an immobilized *C. roseus* cell: The cell surface is wrinkled by discontinuous silica deposits (× 2100 magnification) [133].

Other studies have been performed in which plant cells where encapsulated in silica monoliths. The silica matrix is formed using the sol-gel method. The precursors usually used are alkoxysilanes or sodium silicate. By controlling the hydrolysis and condensation reactions of these precursors in water, silica was polymerized directly around the cells, which acted as a template, finally forming a stable hybrid silica matrix [134]. However, alcohol or sodium ions released during these reactions are severely detrimental for plant cell viability. Thus, this method could only work if the by-products of polymerization are removed before adding cells or if they can rapidly diffuse outside the matrix, such is the case with thin silica films [135]. In order to overcome this drawback, an aqueous sol-gel process has been developed by our team which released no more than a few mmol of sodium ions during the entire process. This method allows the preservation of the plasma membrane, substructures (Fig. 18), and enzymatic activity of the plant cells (*Arabidopsis thaliana*) encapsulated.

Recently, BY2 tobacco cells have been immobilized in a way that allows cells to grow in cavities created inside a silica matrix (Fig. 19). This immobilization process has been realized in two steps. Initially, cells were immobilized in calcium alginate beads, which were subsequently trapped in the inorganic matrix, avoiding any harmful contact of the cells with the silica precursors. When the polymerization was complete, the alginate was liquefied and the cells remained confined within macrocavities containing a liquid medium inside a solid silica monolith. The resulting hybrid device had good mechanical stability and proved to be an effective barrier against biological contamination, suggesting that it could be employed for long-term plant entrapment applications [136].

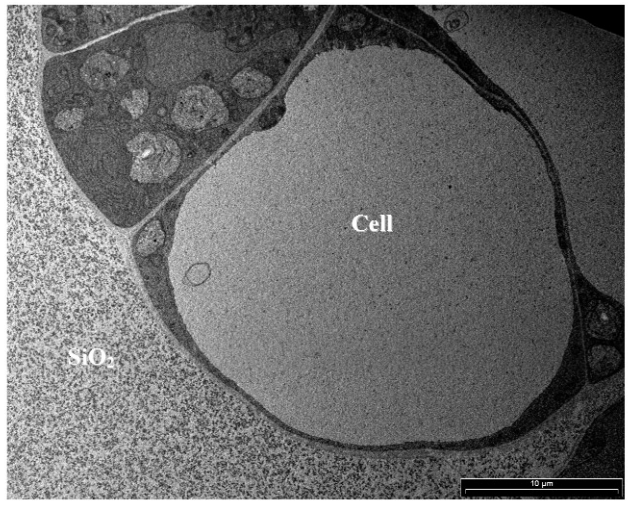


Fig. 18 TEM micrograph of *A. thaliana* (LMM-1) plant cell trapped within silica gel.

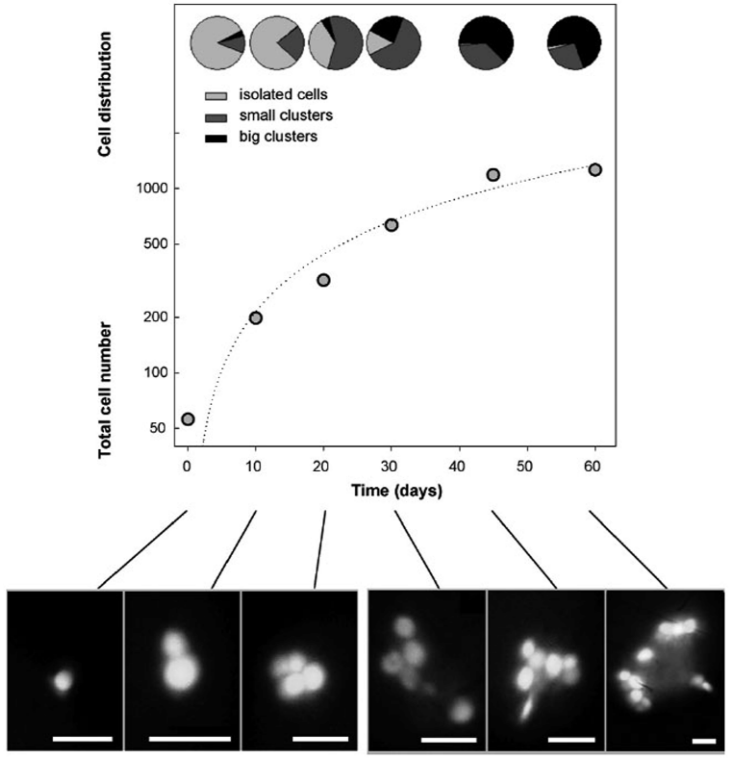


Fig. 19 BY2 Tobacco cell proliferation inside the inorganic host. (Top) Total cell content of a single cavity (15 µl) sampled after 0, 10, 20, 30, 45, and 60 days of culture inside the silica matrix (logarithmic scale). Pie charts show the distribution of cells: Isolated cells (light gray), cells forming small clusters of 2–8 cells (dark gray), and cells forming big clusters of more than 8 cells (black). (Bottom) Representative fluorescence microscopy images corresponding to each sampling time. Scale bars represent 50 µm [136].

Interests of immobilization in bioreactor design

Despite the advantages to using plant cells as micro-bioreactors to produce interesting compounds like drugs and recombinant proteins, there are drawbacks. Plant cells have a slow growth rate, a low shear resistance, and the tendency to aggregate. Therefore, the immobilization of plant cell suspension cultures has a number of advantages. In general, the immobilization matrix provides protection and stabilization for the cells from harsh environmental conditions such as shear forces, pH, temperature fluctuations, organic solvents, and toxins. Furthermore, immobilization provides a high cell density [137]. Additional advantages of immobilizing these biocatalysts are: increase in cell-line stability, ease with which the culture environment can be regulated, stimulation of secondary metabolism above the level occurring in isolated suspended cells, and the possibility of removing inhibitory biochemicals from the system by using a continuous process. All these properties allow product-oriented optimization of the environment, reduction of cultivation periods, and, thus, an increase in productivity [127].

Immobilized plant cells have been used for single and multistep biotransformations of foreign substrates to synthesize desired products as well as for the de novo biosynthesis of secondary metabolites [138,139].

Adsorption of *Papaver somniferum* cells on a polymeric resin elicited with chitin has been shown to be unsuitable for the production of secondary metabolites (sanginarine). Chitin is an elicitor (e.g., an active polysaccharide which gives a signal triggering the formation of secondary metabolites) which was used to stimulate the product formation. Indeed, the production was 3 to 14 times lower than obtained with control cultures. This poor performance of immobilized cultures has principally been explained by the limitations in the diffusion of nutrients.

Cruciata glabra cells have been encapsulated in pectin/chitosan coacervate capsules. The immobilization process enhanced hydrogen peroxide synthesis up to 533 % followed by an improved synthesis of anthraquinone of 556 %. This result can be explained by taking into account that pectin and chitosan are elicitors [140]. Based on this immobilized system, a semi-continuous bioprocess for anthraquinone production in a three-phase system has been achieved. This system allows a continuous removal of metabolites from the solid phase (immobilized cells) by addition of a secondary liquid phase (Miglyol), which has been found to stimulate the biosynthetic capacity leading to an overproduction of anthraquinone. In situ removal of metabolites has many advantages; it avoids feedback inhibition of metabolic enzymes and intracellular product degradation, as well as the inhibition of membrane transport. Thus, immobilization could be a valuable tool to improve the effectiveness of plant cell production processes [141].

C. roseus cells, entrapped in agarose or alginate, have been permeabilized (e.g., increased permeability of plasmic membranes) with dimethylsulfoxide in a way that permits the cells to remain alive. This process allows the release of high-level intracellular products (ajmalicine isomers). Moreover, it permits the possibility of reusing the biocatalyst [142]. The same species has also been encapsulated in a porous layer of SiO_2, supported by polyester fiber using the Biosil process. Not only were the viability of the cells and alkaloid (valuable drugs) production maintained after encapsulation, but the bioreactor productivity was increased by two orders of magnitude with respect to that of free cells [143].

Gossypium arboreum cells have been immobilized to design a bioreactor able to produce gossypol, an antifungal agent. Permeabilization, elicitation, and immobilization provide tools for a continuous process, increasing the productivity by over 20-fold, cf. a batch culture (Fig. 20). Simultaneous use of permeabilization and elicitation was possible in continuous operation only with an immobilized plant cell reactor [144].

Immobilization of *Nicotiana tabacum* and *Solanum chrysotrichum* plant cells within calcium alginate gel beads can also be used to produce anti-inflammatory (scopolin) and antifungal (spirostanol saponin) compounds, respectively, in a higher amount than for a free suspension culture [145,146].

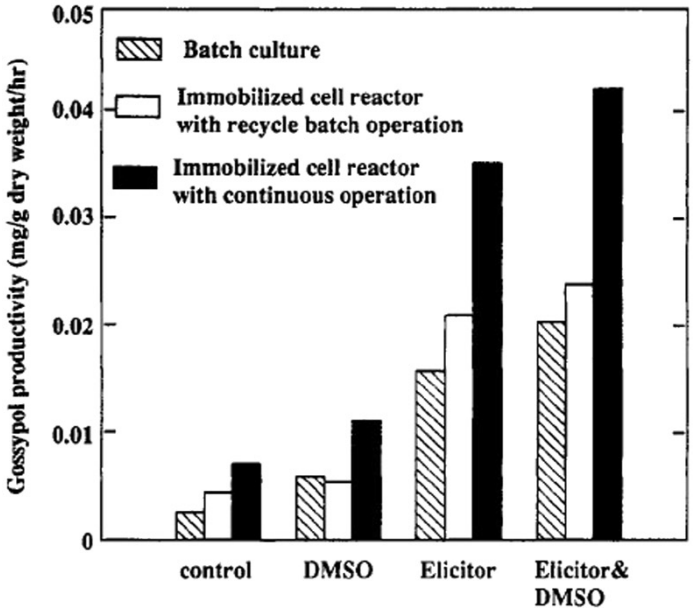


Fig. 20 Comparison of productivity in different operational systems with various treatments. Three different operational systems (batch culture, immobilized plant cell reactor with recycling batch operation, and immobilized plant cell reactor with continuous operation) were tested [144].

More recently, plant cells have been immobilized to determine the effects of encapsulation on the production of recombinant human proteins by *Nicotiana tabacum* cells. Alginate, agar, and carrageenan were investigated as potential immobilization matrices. Experiments have shown that cell encapsulation in alginate increased the production of human granulocyte-macrophage colony-stimulating factor (GM-CSF) in tobacco cells by approximately 50 % (Fig. 21). The most likely cause for this increase is the altered growth conditions within the alginate beads resulting in a prolonged exponential growth phase. Indeed, if the exponential phase is prolonged, the cells can produce protein for a longer time [147].

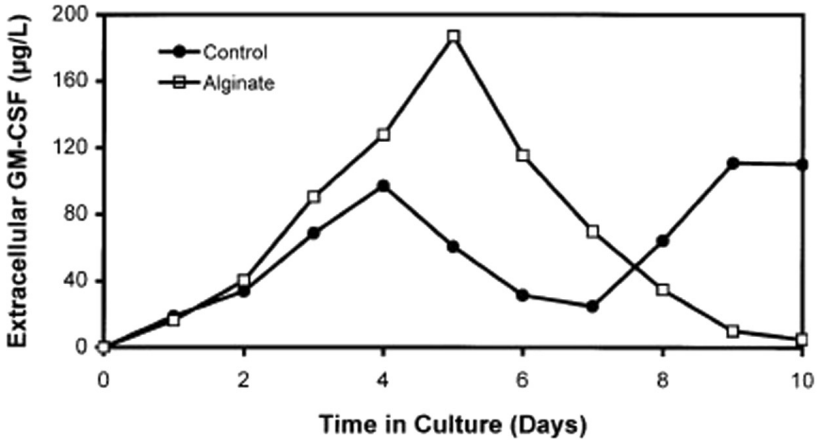


Fig. 21 GM-CSF production by 4-mm alginate beads and cell suspension [147].

Finally, *Ajuga reptans* cells have also been immobilized in a sol-gel SiO_2 membrane (Biosil process). The immobilization clearly modifies the metabolic activity of cells, resulting in a 40-fold increase in invertase production with respect to free cells. The interpretation given by the authors may be that immobilized cells cannot proliferate, thus, they cause a metabolic change, increasing invertase production [148].

BIOENCAPSULATION OF PHOTOSYNTHETIC MICRO-ORGANISMS

Cyanobacteria and green algae are photosynthetic micro-organisms that play a key role in aquatic ecosystems. In fact, around 40 % of the total photosynthesis on Earth is performed by these microalgae [149]. Their uses within biotechnological fields have significantly increased over the last few years, and they can be found in many diverse applications such as in food, cosmetics, aquaculture, pharmaceutical industries, and water purification. Scientists are also endeavoring to use cyanobacteria to combat the wide-scale pollution problems facing our planet. Microalgae are known to have the capacity to adsorb heavy metals, to metabolize nutrients like nitrates and phosphates, and to transform organic pollutants into less toxic compounds. "Photosynthetic wastewater treatment" is a key field in the search for new bioremediation techniques [35]. The cultivation of algae in wastewaters combines the advantages of removing pollutants from the wastewater and simultaneously producing an algal biomass that can be subsequently used for metabolite production, food additives, or for the synthesis of biogas and biofuels, which can then be used as an alternative energy source. In addition, photosynthetic oxygen production could replace the mechanical aeration of sewage. Their capacity to adsorb heavy metals has also been widely proven and is currently under investigation for cleaning contaminated waters or soils [150–156]. This adsorption capacity can be exploited further by preconcentrating trace heavy-metals for sensing purposes [157–159]. The removal and sensing of noxious compounds has been extended to nitrates, phosphates, and organic pollutants, sometimes by using genetically modified microorganisms for the simplification of measurements [26,44,160–167].

In addition to water treatment, the photosynthetic cycle performed by cyanobacteria could help combat air pollution by reducing the ever-rising levels of CO_2 in the atmosphere and converting it to oxygen. Photosynthesis could also be exploited for the production of hydrogen or secondary metabolites, which are of significant importance in the fabrication of new "green" fuels [168–171].

The next section describes the efforts that have been made to use *immobilized* photosynthetic microalgae in biotechnology. In particular, we will tackle the advances realized in the field of metabolites and energy production.

Use of immobilized microalgae in the production of metabolites

Microalgae also have potential applications in biosynthesis and biocatalysis, especially for antibiotics, pharmacologically active compounds, and human food additives [172]. A recent review article outlines the huge potential of microalgae in the production of useful metabolites [173]. One of the first reports about successful bioproduction by immobilized cyanobacteria was given by Stewart in 1982 [174]. The aim of this research was to get a sustained ammonia production from immobilized, nitrogen-fixing, cyanobacteria *Anabaena*. Immobilization in this case was performed in order to achieve high continuous rates of ammonia production, by using the cyanobacteria in a continuous flow reactor at high dilution, without needing to wash out the organisms.

Continuous production of astaxanthin, a fat-soluble carotinoid pigment used as a food coloring or as a dye in the cosmetics industry, has been investigated by the immobilization of living microalgae within silica [175]. In this exploratory study, the presence of the porous transparent silica matrix should account for a continuous dye production without needing to destroy the cells for its extraction, in comparison with the existing systems based on free cell cultures.

Generally, from the literature, it is not clear whether immobilization alters the productivity of these organites. Indeed, evidence of both an increase and a decrease in metabolism can be found [35]. For instance, propanediol can be obtained from the reduction of hydroxyacetone in the presence of immobilized *Dunaliella parva*, and no difference was noticed with the same reaction carried out in the presence of a suspension of free cells [176]. A better performance for alginate-immobilized algae was noticed by Tripathi et al. in the biotransformation of phenylpropanoid compounds to vanilla flavor metabolites [177]. Conversely, Wikström showed that the formation of α-keto acids from amino acids using agarose-immobilized algae was less efficient than a free cell suspension. This has been related to a restriction in the diffusion of oxygen to the interior of the immobilized biocatalyst [178].

Despite these contradictions, most of the metabolic activities seem to be enhanced when immobilized species are used, probably due to a concentration effect of cells in close proximity coupled with the protective effect of the matrix. However, improved biocatalytic activities could be attained by combining high dispersions of cells with more porous supports leading to better diffusion properties. For instance, one should consider nanoporous silica as a promising encapsulating material for photosynthetic microalgae or, more general, for living biocatalysts.

Photobioproduction of hydrogen by immobilized microalgae

With the depletion of fossil fuel reserves and the exponential increase in energy demand, many researchers are currently exploring numerous potential new energy sources that could satisfactorily replace CO_2-emitting fossil fuels. Hydrogen is one of the most promising candidates of the future for a clean energy source. Moreover, hydrogen is also a valuable reactant in the production of fine chemicals, food, and electronic devices. Photosynthetic green algae as well as nitrogen- and non-nitrogen-fixing cyanobacteria are natural hydrogen producers under certain conditions [179]. These organisms use CO_2 and water in the production of hydrogen during photosynthetic processes (bio-photolysis of water). Hydrogen production by microalgae involves the hydrogenase enzyme, which is activated when the electron pressure inside the cells is too high, occurring, for instance, during oxygen deprivation [180–182].

The main advantage of this pathway, in comparison to the hydrogen production by photofermentation, relies on the fact that the photosynthetic organisms only use water as a renewable resource and that CO_2 is consumed, thus reducing air pollution. However, the oxygen generated to some extent inhibits the hydrogenase enzyme. Though this drawback can partially be circumvented by using sulfur-deprived conditions, the total hydrogen production remains quite low. Nevertheless, biophotolysis of water by photosynthetic organisms is a pathway that should be further investigated with a view to increasing the efficiency and the concentration of micro-organisms. As for the bioremediation strains, immobilization onto solid supports could provide an improved environment which favors the optimal operation conditions of these organisms [183]. Indeed, the separation of cells from the aqueous media solution between the cycles of sulfur deprivation and sulfur re-addition is rendered easier as no long centrifugation step is required, thus no additional energy is expended. Moreover, the immobilization should achieve a higher biomass concentration without restricting the cells if the appropriate supports are used [184]. Thus, porous silica is an ideal candidate with much potential that should be studied in detail.

Immobilized cells for solar energy conversion devices have also been used in the biophotoproduction of hydrogen peroxide, a clean and efficient fuel for rocket propulsion [185]. The authors tested whole microalgae and extracted thylakoid vesicles, both immobilized in Ca-alginate. Despite the lower productivity in the immobilized state, these systems present a higher stability (especially for the thylakoid membranes) and easier manipulation in comparison to free organites, making them very suitable for solar energy conversion and storage.

Porous silica as a highly promising matrix for cyanobacteria immobilization

In our team, we are searching for biocompatible synthesis pathways for cyanobacteria immobilization in silica gel with cell encapsulation occurring in situ [186]. Aqueous silica precursors, viz. sodium silicate and Ludox, avoid the use of reagents that liberate cytotoxic by-products such as the aliphatic alcohols MeOH and EtOH from tetramethoxysilane (TMOS) and tetraethyl orthosilicate (TEOS) (typical alkoxysilanes), respectively. Neutralization and condensation reactions can be controlled such that a silica gel can be formed within minutes, thus minimizing the stress on the living cells. The addition of a nanoparticle colloid such as Ludox lends itself to strengthening the material, as upon aging the surface hydroxyls on the nanoparticles condense, forming siloxane bonds. It is this coalescence and interbonding that yields a stronger gel. Glycerol was added to the reagents as a protective agent and osmoregulator to counteract the increase in sodium ion concentration from the aqueous silicate precursors, sodium silicate, and Ludox.

These hybrid gels have been found to remain green over several months, indicating the preservation of the photosynthetic apparatus. The transmission electron microscopy (TEM) image in Fig. 22 highlights that the cell wall can remain intact following an immobilization reaction. The gels themselves are mesoporous, with pore sizes on the nanometric scale, and thus the cyanobacteria have in effect acted as a template whilst the gels form around the cells themselves.

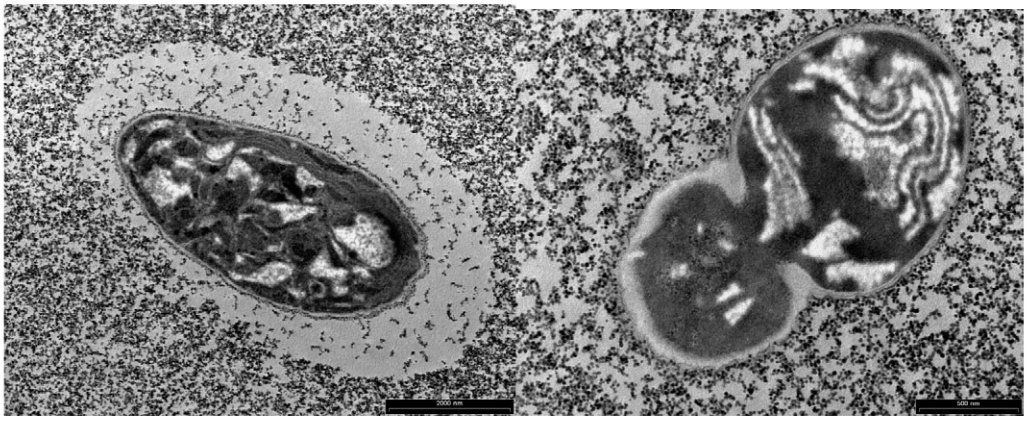


Fig. 22 TEM images of *S. cyanobacteria* immobilized within mesoporous silica gel.

In order to prolong the life of the encapsulated cells, aqueous media containing the essential nutrients is added to the preformed gels. This liquid phase, which percolates through the porous network, decelerates the aging process of the silica gel, thus minimizing any constraints on the cells arising from shrinkage effects. NMR studies have shown that these silica gels possess some Q^3 $SiO_3(OH)$ species arising from surface silanols. This presents an aqueous environment to the exterior (i.e., in the cavities where the cells are found) and in turn aids to prolong their viability.

Spectroscopy studies have revealed that the pigment molecules within the cells are still physiologically viable, with peaks present in the same regions of the spectrum as for the cyanobacteria cells in liquid culture suspension, see Fig. 23.

These results reveal the real possibility of immobilizing living photosynthetic micro-organisms within porous silica networks in order to target novel hybrid materials, capable of photosynthesis, and ultimately, for use in solar energy capture and subsequent energy transformation devices.

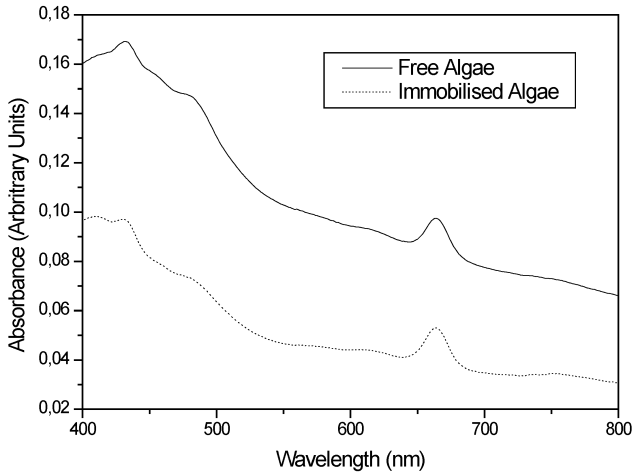


Fig. 23 UV–vis spectra of the acetone soluble pigments extracted from *S. cyanobacteria* in liquid suspension culture three weeks after transfer into fresh media (solid) and immobilized in silica gel three weeks after immobilization (dotted).

Immobilized diatoms

Diatoms are unicellular yellow or brown algae that have a unique external silica sheath that surrounds the cell. As for all vegetal cells, diatoms are photosynthetic micro-organisms that harness solar energy. Currently, scientists are keen to exploit the regular silica structures of diatoms for use in nanotechnologies. Furthermore, diatoms can be used for their metabolites such as antibiotics, anti-tumoral substances, fatty acids, and lipids that could be used in new biodiesels.

There is a scarce amount of work reported on the immobilization of diatoms, whereas their skeleton is widely used as a support for enzymes or other cells. This feature, however, highlights the benefits of employing a porous silica network as an immobilization support for biomaterials and living species.

As metabolite producers, diatoms were immobilized in tubular agar gel layers by Rossignol et al. The aim of this study was to compare two photobioreactors, free and immobilized diatom cells in the continuous production of the hydrosoluble pigment marennine, a natural dye used in the food and cosmetic industries but which also possesses potential anticancer properties [187]. Though the yield in exocellular marennine was higher in the reactor containing the free cells, the specific productivity calculated with respect to the overall cell number was more significant in the reactor with the immobilized cells, showing the high potential of immobilization [188]. Moreover, the maintenance and recovery of the metabolite are simpler in this case. In order to enhance the productivity of the photobioreactor containing immobilized diatoms, the authors propose to favor diffusion of the nutrients and marennine by reducing the gel thickness and to modify the geometry of the reactor to increase the surface/volume ratio, improving nutrients and light transfer. Regarding these considerations, we believe that the use of nanostructured silica as an immobilizing support could help in enhancing these photobioreactors as it provides a substantial amount of nanoporosity and can account for a high dispersion of the cells combined with the transparency required for correct illumination.

Lopez et al. have recently reported the encapsulation of diatoms, such as *Cylindrotheca fusiformis*, *Thalassiosira weissflogii*, and *Phaeodactylum tricornutum*, within silica gel via an aqueous colloidal sol-gel route [189]. Their results have shown the ability of diatoms to dissolve the silica in the near vicinity of a live cell. This was not the case in the encapsulation of dead cells, suggesting that physiological and protein activities of diatoms may have played a role in the dissolution process. TEM studies have revealed a network of secreted fibers inside the silica matrix, highlighting that cellular bio-

synthesis continues after immobilization, however, it has thus far been problematical to extract these polysaccharides from the gel. This is the first reported case of immobilized cells modifying the silica support. They believe that this holds promise in terms of engineering new scaffold materials whereby the entrapped organisms themselves tune the mechanical properties and porosity of their support.

This strongly suggests that diatoms should be seen as very promising candidates both for bioremediation and bioproduction of metabolites. To enhance the performances of these microalgae, immobilization is a powerful tool that needs to be investigated in more detail, especially regarding the use of porous nanostructured silica matrices.

PHOTOBIOREACTORS OBTAINED BY COMBINED SYSTEMS

By combining one or more different organisms within a support, one can increase the efficiency of the hybrid material or indeed target a multifunctional material. Madamwar et al. immobilized *Phormidium valderianum* (cyanobacteria), *H. halobium* (archaea), and *E. coli* (non-photosynthetic bacteria) in a PVA-alginate film in a combined system. By controlling an intermittent flow of nitrogen, the system yielded a continuous and stable hydrogen output for longer than four months [190]. Each component had a role within the system: The cyanobacteria acted as a nitrogen fixer and ultimately produced hydrogen with the archaea extruding protons and the hydrogenase present in *E. coli*, catalyzing the reaction. Working in unison, the three organisms increased the hydrogen production of the cyanobacteria by several folds.

Another combined system has been targeted to support life in space via a series of interconnected bioreactors, viz. MELISSA Micro Ecological Life Support System Alternative. The loop consists of four bioreactors and a higher plant chamber and has been analyzed up to a pilot-scale level for both the individual components and the system as a whole in a continuous fashion [18]. The set-up involved the fermentation of human waste by the thermophilic anaerobic bacteria. The following compartment converted the volatile fatty acids produced into biomass using *Rhodospirillum rubrum*, an anaerobic photosynthetic bacterium. The ammonium ions were subsequently transformed by immobilized *Nitrobacter* and *Nitrosomonas* bacteria present in soil to more facilely assimilated nitrate ions and fed into the last two compartments simultaneously. One compartment contained the cyanobacteria *Spirulina platensis* and the other higher plants. Both compartments generated oxygen and food sources for the crew from CO_2 produced by the crew and the other compartments, with light being the energy source. Such complex systems may benefit from enhanced immobilization techniques, and thus it is imperative that scientists strive to perfect the encapsulation of living organisms within suitable supports as their applications are far reaching and could yield limitless technological advances.

CONCLUSIONS AND OUTLOOK

This review has clearly shown that immobilization is a powerful tool for the exploitation of natural biologic species in the aim of developing photobioreactors. In particular, the smart combination of biological molecules, supramolecules, and living cells with artificial supports has proved to lead to systems with enhanced performances compared to isolated, non-encapsulated biological matter. The immobilization and adsorption of photosynthetic material has progressed rapidly over the past decade with the availability of novel encapsulation techniques. Previously, the use of organic polymers dominated the field, but with the advent of soft inorganic chemistry techniques, scientists have been looking toward silica-based supports. Silica offers many advantages over organic-based polymers, being optically transparent, thermally and mechanically stable, resistant to chemical and microbial attack, as well as structurally very versatile. Silica materials can be engineered to meet the needs of the material they encapsulate. For instance, by altering reaction conditions, the pore size can be tailored, or by changing the reaction pathway, protective agents can be added to the precursors to preserve cell metabolism, even the surface of the silica can be modified to be more biocompatible. Highly porous materials also allow for

the retention of water which stops the biological species from drying out. Furthermore, it facilitates the diffusion of nutrients and metabolites throughout the network of pores.

The hybrid materials summarized in this review have highlighted the diverse applications in which immobilized photosynthetic structures can be employed. It is envisaged that new technologies in the field of biosensors, optical devices, and bioreactors could be targeted as well as novel methods in biocatalysis, bioremediation, and water photolysis.

Indeed, there is no doubt that the endeavors to design novel devices in the domain of energy production as well as smart molecules has to encompass the combined use of both chemistry and biology. In particular, it has become evident that natural molecules have a superior performance over their synthetic counterparts; for instance, chlorophylls are one of the best energy harvesters, whereas enzymes yield the most efficient catalysts. Hence, it is important to take advantage of the natural compounds and organisms available to humans for the development of new technologies and to take advantage of the chemist's know-how to improve their efficiency further still. As it has been demonstrated throughout this review paper, the immobilization of natural molecules, supramolecules, and organisms is a very promising way to develop new devices that combine all the benefits of nature with the protection, concentration effect, and easy handling that synthetic supports can confer. We believe that very efficient energy conversion technologies should be based on the entrapment of natural photosynthetic molecules and organisms within stable, optically transparent, and biocompatible porous silica supports, giving rise to a new generation of photobioreactors. Indeed, solar energy harvesting via pigments, proteins, complexes, thylakoids, chloroplasts, plant cells, and cyanobacteria is a very important phenomenon that can be exploited to transform sunlight into a useful energy. In addition, the photosynthetic cycle can be used with the aim of metabolizing CO_2, thus providing energy and reducing pollution. Our team actually focuses on this exciting thematic by searching for new pathways to devise photosynthetic bioreactors. We immobilize thylakoids, chloroplasts, whole plant cells, and blue–green algae within porous silica, which seem to be the most appropriate support.

Regarding the immobilization process, our first objective is to build the silica matrix in situ, in the presence of the biological species. Silica is prepared via the well-known "chimie douce" concept (i.e., under mild synthesis conditions), which is an advantage considering the fragility of the biological matter and thus enables the preservation of its integrity. Our aim is to optimize this in situ immobilization by using different existing silica sources and by synthesizing new ones which liberate harmless byproducts. These fundamental studies will allow the determination of the ideal preparation conditions of the porous inorganic matrix to achieve optimum encapsulation with maximum survival of the biological species. In addition, other inorganic supports are also being tested, going beyond the employment of silica alone. Our second target is to exploit synthesis strategies of hierarchically porous silica (and other oxides) and to subsequently immobilize post-synthesis. The main advantage of this pathway is a higher flexibility in the synthesis conditions of the matrix and the possibility, for algae, for instance, to grow inside the matrix. Detailed studies are, however, currently been carried out in order to determine the optimum immobilization conditions to achieve a high concentration of immobilized species without leaching.

The first results of our work clearly demonstrate the total biocompatibility of the silica networks as growth occurs and bioactivity can be measured over long periods of time after immobilization. The following step is to determine the activity of the new photobioreactors regarding light harvesting, energy conversion, CO_2 fixing and oxygen, hydrogen, and other metabolites production in order to come up with solutions to the high demands for energy in our modern society and to fulfill the more stringent environmental regulations. We indeed believe that this research is essential for creating a new generation of materials that are not only inspired by but also exploit nature in the design of new energy-efficient and energy conversion systems.

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