Silica sol-gel encapsulation of cyanobacteria: lessons for academic and applied research

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Abstract

Cyanobacteria inhabit nearly every ecosystem on earth, play a vital role in nutrient cycling, and are useful as model organisms for fundamental research in photosynthesis and carbon and nitrogen fixation. In addition, they are important for several established biotechnologies for producing food additives, nutritional and pharmaceutical compounds, and pigments, as well as emerging biotechnologies for biofuels and other products. Encapsulation of living cyanobacteria into a porous, silica gel matrix is a recent approach that may dramatically improve the efficiency of certain production processes by retaining the biomass within the reactor and modifying cellular metabolism in helpful ways. Although encapsulation has been explored empirically in the last two decades for a variety of cell types, many challenges remain to achieving optimal encapsulation of cyanobacteria in silica gel. Recent evidence with *Synechocystis* sp. PCC 6803, for example, suggests that several unknown or uncharacterized proteins are dramatically up-regulated as a result of encapsulation. Also, additives commonly used to ease stresses of encapsulating living cells, such as glycerol, have detrimental impacts on photosynthesis in cyanobacteria. This mini-review is intended to address the current status of research on silica sol-gel encapsulation of cyanobacteria and research areas that may further the development of this approach for biotechnology applications.

Keywords: cyanobacteria, encapsulation, silica gel, sol-gel, stress, bioproducts, bioenergy

Introduction

Cyanobacteria are diverse and ubiquitous, inhabiting virtually every terrestrial and aquatic ecosystem from the equator to the poles. They are responsible for producing much of the oxygen in the atmosphere and for fixation of substantial quantities of inorganic nitrogen and carbon. Cyanobacteria have been used extensively in basic research of photosynthesis and nitrogen fixation, as well as in biotechnology for the production of food additives, nutritional supplements, pigments, pharmaceutical compounds, and, more recently, biofuels and biosensors. Established and most emerging technologies emphasize growth, followed by harvesting biomass and extracting the compound(s) of interest (Brennan & Owende, 2010; Parmar et al., 2011). By avoiding expensive and energy-intensive steps in harvesting and extraction (Brennan & Owende, 2010; Stephens et al., 2010; Lu, 2010), a more efficient approach recently attracting research attention is to retain biomass in a photobioreactor to produce and excrete desired products like ethanol (Fu, 2008), hydrogen (Dickson et al., 2009), sucrose (Ducat et al., 2012), and others. Encapsulation (i.e., fully enclosing living cells in a three-dimensional solid or semi-solid matrix) is an approach that can hold cyanobacteria within a bioreactor system and exploit their ability to convert solar energy, carbon dioxide, and water into valuable products. *Encapsulation* is distinct from *immobilization*, the adhesion of living cells on a two-dimensional surface, partly because it constrains growth and retains biomass while immobilization allows growth and exchange of biomass.

Encapsulation of living cells in an inert, biologically compatible matrix can provide several advantages over cyanobacteria-based biotechnology approaches that rely on immobilization or suspension of cells in a liquid media. First, encapsulation prevents the loss of production cells, which may be an engineered mutant strain of proprietary value and/or environmental concern. Similarly, contaminant cells cannot penetrate and enter the matrix. In addition, because growth of encapsulated cells is physically constrained, energy normally focused on supporting cell growth may be directed instead toward production of desirable secondary metabolites (Pressi et al., 2003). Also, living cells can perform maintenance and self-repair, which can prolong metabolic activity (Yu et al., 2005). Purified enzymes, while highly efficient at specific conversions, are less stable *in vitro* than in whole cells. Maintaining enzymatic functions in living cells not only preserves activity but also avoids expensive protein extraction and purification. Maintaining live cells is especially important with cyanobacteria and other phototrophs because they have sophisticated processes for repairing damage inflicted by sunlight and reactive oxygen species produced during oxygenic photosynthesis.

Encapsulation of living cyanobacteria for biotechnology was explored in the 1980's using polyurethane (Muallem et al., 1983), and has been investigated with inorganic matrices, particularly silica sol-gel, mainly in the last few years (Rooke et al., 2008; Dickson et al., 2009; Leonard et al., 2010; Meunier, 2010; Dickson & Ely, 2011; Leonard et al., 2011; Dickson et al., 2012a). Although much empirical development has occurred, many questions remain and prevent optimal encapsulation protocols to be designed from fundamental principles. This mini-review is intended to describe recent advances in encapsulation of cyanobacteria for applications in biotechnology, to provide information about sol-gel processing important to encapsulation of cells, and to identify areas of on-going inquiry. Other recent reviews cover the topic of biological encapsulation more broadly (Avnir et al., 2006; Kandimalla et al., 2006; Depagne et al., 2011; Leonard et al., 2011).

Silica Sol-gel Encapsulation of Biological Components

Silica gels can be processed under conditions compatible with encapsulation of biological components and, unlike organic polymers, are inert, robust, and stable. Also, despite their amorphousness, the optical properties of silica gels vary little from quartz, the crystalline form of silica, making them highly transparent to visible light (Laughlin et al., 1979; Gupta, 1985; Koslowski et al., 1997). Carturan et al. (1989) first described silica gel encapsulation of living cells, using TEOS-derived alkoxide gel and *Saccharomyces cerevisiae*, which was selected for its ethanol tolerance. Since then, researchers have encapsulated proteins and enzymes (Ellerby et al., 1992; Frenkel-Mullerad & Avnir, 2005; Kriegl et al., 2003; Pierce, 2004), bacterial cells (Nassif et al., 2003; Taylor et al., 2004; Kuncova & Podrazky, 2004), cyanobacteria (Rooke et al., 2008; Dickson et al., 2009), diatoms (Gautier et al., 2006), algae (Fiedler et al., 2007; Nguyen-Ngoc & Tran-Minh, 2007a; Nguyen-Ngoc & Tran-Minh, 2007b), plant cells (Carturan et al., 1998; Pressi et al., 2003), and mammalian cells (Pope et al., 1997; Boninsegna et al., 2003; Nieto et al., 2009). Reported advantages of sol-gel encapsulation have included stabilization of purified enzymes (Frenkel-Mullerad & Avnir, 2005), such as hydrogenases (Zadvorny et al., 2010) and photosystems (Kriegl et al., 2003), and prolongation of activity of living cells (Taylor et al., 2004). However, silica gel encapsulation of living cells and other biological components is not yet understood from first principles, due both to the complexity of sol-gel chemistry and to the variety of materials that may be considered for encapsulation. Therefore, optimizing an encapsulation protocol for each new component or cell type requires an empirical approach. Many recent reviews on silica encapsulation of biological components are available (Jin & Brennan, 2002; Armon, 2000; Bergogne et al., 2000; Bhatia & Brinker, 2000; Pierre, 2004; Bottcher et al., 2004; Livage et al., 2001; Avnir et al., 2006; Gadre & Gouma, 2006; Bupta & Chadhury, 2007; Meunier et al., 2010).

Encapsulation of Living Cyanobacteria

Two main incentives exist for encapsulating living cells. First, they contain all enzymes, proteins, cofactors, and transport mechanisms to carry out valuable reactions that are difficult and/or costly to replicate *in vitro* or artificially. Second, by constraining growth, living cells may direct more energy toward production of secondary metabolites. Although the mechanisms for this phenomenon are not yet well understood, it has been confirmed with cells of the plant *Ajuga reptans* (Pressi et al., 2003) and with hydrogen production from *Synechocystis* sp. PCC 6803 (Dickson et al., 2009).

Figure 1 schematically depicts an encapsulated cell within a silica gel matrix. The gel physically confines encapsulated cells, keeping them contained while excluding contaminant cells. However, the gel is sufficiently porous to allow relatively rapid diffusion and transport of metabolites and waste products. The gel surface is mostly condensed, with some hydrophilic hydroxyl groups remaining, while the internal bulk of the gel is condensed silica and is also hydrophilic. Pores in the gel are usually about tens of nanometers in diameter, whereas encapsulated cells are approximately two orders of magnitude larger, at about 1-2 μm in diameter. The pore structure is often bimodal, containing fine micropores through the bulk and larger mesopores present in dilute, hydrated gels commonly used for encapsulation of biological components (Figure 2). This pore structure allows diffusion of small ions at rates approximately 25% of those in unconfined aqueous solution (Dickson et al., 2012b), suggesting that transport of metabolites, gases, and waste products can support cell activity if the system is designed to keep diffusion distances small enough (Taylor et al., 2004; Dickson et al., 2012b).

Encapsulation can stress cells in various ways, and researchers have attempted several approaches to reduce the impacts of the stresses, depending on the sensitivities of the cell of interest. *Synechocystis* sp. PCC 6803 is relatively robust, moderately halo-tolerant (Hagemann et al., 1999; Ferjani et al., 2003) and ethanol-tolerant (Dickson et al., 2012a). High sodium concentrations present during preparation of gels from aqueous precursors and ethanol evolved during hydrolysis of alkoxide gels present potential risks to *Synechocystis* sp. PCC 6803. While low levels may not be lethal, energy devoted to stress responses is energy not available for production of desired metabolites. Glycerol and PEG may help to improve biocompatibility of the encapsulation process in some cases (Conroy et al., 2000; Ferrer et al., 2003; Nassif et al., 2003), but they adversely affect cyanobacteria (Dickson et al., 2012a). Numerous other encapsulation protocols are available and which use other additives, different precursors, or organic polymers in combination with silica (for example, crown ethers (Reetz et al., 2003), organic acids (Pang et al., 2001), gelatin (Coradin et al., 2004; Ren et al., 2001; Chernev et al., 2010), alginate

(Coradin & Livage, 2003), and collagen (Armanini et al., 1999; Brasack & Bottcher, 2000)), although future work is required to determine how effective these strategies will be for encapsulating cyanobacteria to make biofuels or other desired products.

Processing of Silica Sol-Gel

Overview of Sol-Gel Processing

Sol-gel processing is a well-developed area of materials science for fabricating metal oxide gels. The gels can be formed into virtually any geometry, and then further processed into lenses, membranes, coatings, powders, and monoliths, with a variety of applications in fields ranging from optical and electrical devices to separation technologies to adsorption to biosensing. The term "sol-gel" is generic and broad, referring to various liquid chemistry routes (rather than melt extraction) for material synthesis. This approach offers flexibility in terms of available process space and materials. Many metals, transition metals, and metalloids are amenable to sol-gel processing, the most common being silicon, copper, zinc, titanium, aluminum, boron, and nickel. The two primary advantages of sol-gel processing are that it can be carried out at room temperature under mild conditions, and that it allows for the synthesis of some materials that are not possible through conventional melts because of phase separation, crystallization, or other complications (Yoldas, 1979). Also, the proportions of precursor, catalyst, and solvent (water, for biological encapsulation) may be adjusted to provide considerable latitude in tailoring gel structure (Davis et al., 1992a; Davis et al., 1992b; Jiang et al., 2006).

Sol-gel is shorthand for the two steps of sol-gel processing, which may or may not be separated temporally. The first is the preparation of a solution, or *sol*, of hydrolyzed metal monomers, small oligomers, and colloidal particles. This may be done in many ways, but the end goal is a stable or metastable solution of metal atoms covalently bound to active hydroxyl groups. For example, with silicon, the sol will be a solution of silicic acid, $Si(OH)_4$. The hydroxyl groups condense, releasing water and forming an oxygen bridge between adjacent metal atoms. Polymers and small particles form, then bridge the geometry of the solution (gelation), forming the *gel*.

While simple in concept, the chemistry of sol-gel processing is sufficiently complex that a complete theoretical description from first principles has yet to be developed, mainly because of the underlying complexity of the hydrolysis and condensation reactions. Each reaction can be described by its own rate constant, but those rate constants vary depending on the chemistry of adjacent bonds on the central metal ion. The result is that on any given silicon atom, at the next nearest neighbor, there are 1,365 distinct chemical environments requiring 199,290 rate constants for an accurate quantitative description

(Kay & Assink, 1988)! Nevertheless, sol-gel chemistry has been the subject of countless peer-reviewed publications and progress has been made toward understanding this complex chemistry, resulting in at least some reliable qualitative predictions.

Silicon is likely the most extensively studied sol-gel system, largely due to its ease of processing and biocompatibility when compared to other inorganic matrices. Publications on silica sol-gel chemistry include a treatise by Iler (1979), large sections of a seminal text by Brinker & Scherer (1990), several reviews (Klein, 1985; Brinker, 1990; McCormick, 1994; Hench, 1998), and many peer reviewed articles.

Silicon Precursors

Two general types of precursors offer distinct advantages and challenges for biological encapsulation (Table 1). The first, silicon alkoxide, has a silicon atom coordinated by ester linkages to four alkyl groups, as exemplified by tetraethoxysilane (TEOS), with a central silicon atom bound to four ethyl groups via oxygen bridges ($Si(OC_2H_5)_4$). TEOS and other alkoxide precursors are synthesized by alcoholysis of silicon tetrachloride (Brinker & Scherer, 1990). Tetramethoxysilane (TMOS) and methyltriethoxysilane (MTES), having an alkoxide precursor with one ethoxy group substituted with a methyl group, introducing a silicon–carbon bond which does not hydrolyze, may also be used. A wide variety of such substitutions are available in alkoxide precursors, collectively referred to as organically modified siloxanes, or ORMOSILs, which are discussed further below.

In aqueous solution, alkoxide precursors can be hydrolyzed by either acid or base catalysis. Alcohol is released during hydrolysis, with TEOS releasing ethanol and TMOS releasing the more toxic methanol. Alcohol evolved during hydrolysis (which remains present through gelation) presents a significant challenge to long term viability of encapsulated cells.

The second type of precursor is a solution of sodium silicate, Na_2SiO_3, often called “water glass” and sometimes described as a solution of sodium oxide, Na_2O, and silica, SiO_2, in equilibrium. In a 40% aqueous solution, the substance forms a strongly alkaline but stable solution. Acid or base catalysis can be used to accelerate hydrolysis. However, without additional treatment, the resulting sol can have a very high concentration of sodium ions which may place osmotic stress on encapsulated components.

Hydrolysis of Alkoxide Precursors

The hydrolysis reaction of a generic alkoxide precursor, where R is an alkyl side group, is as follows:

$$\equiv \mathrm{Si} - \mathrm{O} - \mathrm{R} + \mathrm{H_2O} \leftrightarrows \equiv \mathrm{Si} - \mathrm{OH} + \mathrm{ROH}$$

The reaction is reversible, as shown, with the forward reaction being hydrolysis and the reverse reaction being esterification, which should be minimal in silicon alkoxide systems (Brinker & Scherer, 1990). In addition, once precursors begin hydrolyzing to silicic acid, condensation can occur, as follows:

$$\equiv \mathrm{Si} - \mathrm{O} - \mathrm{H} + \mathrm{H} - \mathrm{O} - \ \mathrm{Si} \equiv \leftrightarrows \equiv \mathrm{Si} - \mathrm{O} - \mathrm{Si} \equiv + \mathrm{H_2O}$$

This condensation reaction and its reverse reaction, hydrolysis, will both occur, but at low pH with acid catalysis, the hydrolysis reaction is strongly favored.

Hydrolysis is thought to proceed via nucleophilic attack of electronegative water oxygen upon a weakly electropositive silicon atom (Bechtold et al., 1968; Brinker et al., 1982; Brinker & Scherer, 1990). Compared with other metals, this reaction is slow in a silicon system because other metals are more strongly electropositive (Iler, 1979). However, the slowness of the reaction allows temporal separation between the competing hydrolysis and condensation reactions.

Without an acid catalyst, pH of an alkoxide sol may be as high as 5, further slowing the hydrolysis reaction and causing full hydrolysis to require thousands of hours (Pope & Mackenzie, 1986). A strong acid catalyst, such as hydrochloric or nitric acid, can drop the pH of the sol to below 2, the isoelectric point of silicic acid, greatly accelerating the hydrolysis reaction. Under these conditions, the rate of each subsequent hydrolysis of a silicate ester accelerates after the first; theoretically, the fourth hydrolysis of a silicate ester can occur approximately 144 times faster than the first (Assink & Kay, 1991). Also, in the presence of excess water, hydrolysis proceeds nearly to completion, leaving very few unhydrolyzed alkoxide monomers in solution (Brinker et al., 1982; Pouxviel et al., 1987). Furthermore, condensation is minimal prior to catalyzing gelation, causing formation of some small cyclic and/or short linear polymers rather than larger colloidal particles (Pouxviel et al., 1987; Kelts & Armstrong, 1989).

Sol characteristics prior to condensation are important for overall structure of the final gel. Acid catalysis of hydrolysis, in contrast to base catalysis, is superior for achieving mesoporous structure and high porosity (Brinker et al., 1984; Nair et al., 1996). With base catalysis, the sol typically has highly condensed silica particles in solution with unhydrolyzed monomers, which is undesirable for biological encapsulation because continued hydrolysis after gelation can produce cytotoxic alcohol. Importantly, the temporal separation between hydrolysis and condensation that can be achieved with acid catalysis allows for at least partial removal of alcohol generated during hydrolysis.

Hydrolysis of Aqueous Precursors

Basic catalysis is often used in systems with aqueous precursors because the precursor itself is already extremely alkaline. However, acid catalysis can also be used with aqueous precursors, as shown below:

$$Na_2SiO_3 + H_2O + 2HCl \rightarrow Si(OH)_4 + 2Na^+ + 2Cl^-$$

This reaction usually results in a highly unstable, supersaturated solution of silicic acid, often bringing the solution toward neutral pH and causing gelation (Iler, 1979). In the presence of strongly acidic ion exchange resin (e.g., Amberlite™ IR-120 H), however, this reaction will form a stable sol at an acidic pH below the isoelectric point of silicic acid. At that pH, the reaction mechanism of hydrolysis remains the same and hydrolysis should proceed virtually to completion. The ion exchange resin adsorbs sodium ions and releases protons, thus acidifying the sol and stabilizing it to avoid gelation. Adsorption of sodium ions also, very importantly, reduces ionic and osmotic stresses for encapsulation of biological components. If sodium ions are removed by ion exchange before base catalysis of gelation, protons and hydroxide ions will combine to form water and leave a much lower final salt concentration. While marine cyanobacteria may be able to tolerate high concentrations of sodium ions, this protocol may be the most promising approach for freshwater cyanobacteria.

Under comparable conditions, alkoxide and aqueous precursors undergoing acid catalysis should yield nearly identical acidic sols. However, factors such as alcohol production and its effects, the presence of ion exchange resin and sodium ions, and differences in condensation resulting from these factors all may influence the evolution of the sol and the resulting gel structure. Therefore, both should be explored as candidates for biological encapsulation. Specific protocols can be found in recent publications (Rooke et al., 2008; Dickson et al., 2009; Leonard et al., 2010; Dickson & Ely, 2011; Dickson et al., 2012a).

The preferred precursor and specific protocol for encapsulation of the species of interest typically must be determined empirically. Optimal methods will depend partially on whether the species is more sensitive to stress related to alcohol exposure (from alkoxide precursors) or stress related to salt content and larger fluctuations in pH (from aqueous precursors). Complete hydrolysis of each mole of tetraethoxysilane (TEOS), $Si(OC_2H_5)_4$, will release four moles of ethanol. Acute exposure to ethanol can have a detectable, detrimental impact on photosynthetic performance, as observed by chlorophyll fluorescence (Dickson & Ely, 2011). The primary mechanism of alcohol stress is speculated to be an attack on cellular and thylakoid membranes, disrupting membrane fluidity and the activity of

membrane-bound and membrane-associate proteins. This includes numerous proteins associated with transport, metabolism, and photosynthesis, especially the state transition and distribution of excitation energy by phycobilisomes.

Base Catalyzed Condensation & Gelation

In condensation, as shown above, two adjacent hydroxyl groups condense to form an oxygen bridge between silicon atoms, releasing a water molecule:

$$\equiv \mathrm{Si} - \mathrm{OH} + \mathrm{HO} - \mathrm{Si} \equiv \rightarrow \equiv \mathrm{Si} - \mathrm{O} - \mathrm{Si} \equiv \ + \mathrm{H_2O}$$

This reaction is thought to occur via nucleophilic attack of a deprotonated silanol upon a protonated silanol (Brinker & Sherer, 1990). Because the first pK_a of silicic acid ($Si(OH)_4$) occurs at pH 9.8, it is fully protonated at acidic pH, strongly suppressing condensation. Raising the pH increases the concentration of deprotonated silanol groups, initiating condensation. Condensation then causes a modest increase in acidity of adjacent silanol groups, causing the concentrations of protonated and deprotonated groups to be comparable at higher pH and accelerating the condensation reaction (Brinker & Sherer, 1990). Buffered media added in this step can contain live cyanobacteria and should be added in proportions to create a final pH compatible with the cell culture (e.g., approximately pH 7.2). The amount of silicic acid needed to form a stable gel can vary depending on processing protocols but can be as low as approximately 2% silica by mass (Dickson et al., 2012b). To support the encapsulated cells, the gel must remain hydrated. Therefore, post-gelation treatments such as annealing, solvent exchange, or super-critical drying would be inappropriate.

Aging

Gelation occurs when condensation brings about solid phase bridging of the entire solution geometry. Hydrolysis and condensation continue, however, and the gel ages and evolves, with structural changes, caused by processes such as Ostwald ripening, coarsening, and syneresis, persisting until monomers and particles in solution are consumed and equilibrium is achieved. Each of the processes is caused by different forces and affects the final gel structure differently.

Ostwald ripening occurs due to differences in solubility between smaller and larger particles. Solubility is higher at the surfaces of small particles because of the tight, positive curvatures of the particles (Ibach, 2006; Brinker & Sherer, 1990). This phenomenon is related to relative accessibility of each surface monomer to reaction. In tight positive curvature, surface monomers are highly exposed and accessible for hydrolysis. As curvature decreases and becomes negative, surface groups are increasingly tightly

packed, which both reduces their accessibility to hydrolysis and promotes complete condensation with adjacent surface groups. A concentration gradient results around smaller particles as they dissolve, which drives diffusion of free monomers toward larger particles, where, because the larger radius of curvature causes reduced solubility, they condense upon the surfaces of the larger particles. As small particles dissolve and large particles grow, a more uniform and stable particle size distribution is created. The amount of ripening before gelation can influence how coarse or fine gel structure becomes. In an acidic system, silica particles should be stable in the sol at a diameter of approximately 2 nm. With a base catalyzed system at pH 7 or greater, particles may be 10 nm in diameter or larger (Brinker & Sherer, 1990).

Coarsening is also driven by surface energy differences related to local curvature that result in dissolution in one area and condensation in another. While Ostwald ripening occurs with mobile particles in solution, coarsening occurs with particles that are fixed in the gel structure. Two particles in contact create an area of tight negative curvature between the two. Silica dissolved from areas of broad positive curvature tends to deposit and condense at this surface, causing necking, i.e. the thickening of connections between adjacent particles. This can strengthen and stabilize the gel, but it can also decrease surface area and hinder diffusion by obstructing pore spaces.

Upon gelation, the gel is soft and flexible, and gel contraction occurs during ongoing condensation with relatively minor capillary forces. This syneresis continues until equilibrium is achieved between coarsening, which strengthens the gel and causes an increase in bulk modulus, and internal capillary pressures (Raman et al., 1996), which, with pores on the scale of nanometers, can be very high (Brinker & Sherer, 1990). In wet gels, i.e., gels not subjected to heat treatments and complete drying, capillary forces are never fully realized and syneresis, while observable, is often minimal, generally about 5% or less. Severe syneresis, as would be expected when gels are dried, would be detrimental to encapsulated biological components.

Organically Modified Siloxanes

Organically modified siloxanes, or ORMOSILs, have an alkoxide precursor with at least one organic functional group bonded to a silicon atom. The silicon–carbon bond does not hydrolyze, so the side group remains in the final gel matrix, which can have profound effects throughout the process. A commonly used ORMOSIL, and the simplest, is methyltriethoxysilane (MTES; $SiCH_3(OC_2H_5)_3$). Increasing complexity and chemical functionality can be achieved by multiple substitutions on a single silicon or by

substitution of larger groups, including longer alkanes and branched and cyclic side groups. Hundreds of ORMOSILs are available, providing a spectrum of gel properties.

Covalently bonded side groups contribute to both steric and inductive effects during hydrolysis. Steric effects are easy to predict qualitatively, in that a larger side group will tend more to hinder or prevent nucleophilic attack, thus impeding hydrolysis. Methyl and ethyl groups, being smaller than ethoxy or other larger side groups, for example, tend to cause minimal steric interference (Brinker & Sherer, 1990). Inductive effects accelerate hydrolysis of ethoxy side groups due to differences in electron density on silicon-carbon bonds versus silicon-oxygen bonds (Brinker & Sherer, 1990). This occurs because the bond to the functional group carbon, which is less electronegative than oxygen, stabilizes charge distribution of reaction intermediates. Because hydrolysis proceeds nearly to completion in acid-catalyzed systems with excess water, inductive effects can increase the hydrolysis rate but they should not alter the final equilibrium.

After gelation, organic side groups in ORMOSIL gels create two competing effects. First, because they are hydrophobic and the interior of the gel is hydrophilic, the side groups tend to locate at the surface of the gel, thus making the surface more hydrophobic. This decreases interfacial energy between the solid and liquid phases, resulting in reduced capillary forces and syneresis. Second, organic side groups can occupy potential bridging sites and sterically hinder condensation, which can increase gel porosity and flexibility and facilitate more complete condensation. Brittle gels of pure TEOS have been seen to be only 87% condensed, while more flexible ORMOSIL gels can achieve 98% condensation (Glaser & Wilkes, 1989). The two effects of increased flexibility (i.e., decreased bulk modulus) and decreased capillary forces must be managed to tune gel structure. With too many organic side groups, ORMOSIL gel loses pore structure and becomes viscous, unable to maintain a structure. With too few organic side groups, the gel resembles brittle TEOS gel. The optimal composition of a blend of TEOS and MTES, in terms of maximizing porosity and minimizing syneresis, is about 20% MTES with 80% TEOS (Fahrenholtz et al., 1992).

Additives

Many additives, including glycerol (Avnir et al., 2006; Nassif et al., 2003), polyethylene glycol (PEG) (Conroy et al., 2000; Ferrer et al., 2003), surfactants (Bagshaw et al., 1995), crown ethers (Reetz et al., 2003), gelatin (Ren et al., 2001; Coradin et al., 2004; Chernev et al., 2010), alginate (Coradin & Livage, 2003), collagen (Armanini et al., 1999; Brasack & Bottcher, 2000; Eglin et al., 2005), formamide (Hench, 1998), and organic acids (Pang et al., 2001), may be incorporated during gelation to modify gel

properties. Additives may improve biocompatibility by, for example, improving surface interactions between the gel matrix and the biological component or protecting the biological component from stresses of encapsulation. For example, glycerol can improve the viability of *E. coli* (Nassif et al., 2003), *Serratia marcescens* (Nassif et al., 2004), and *Haematococcus pluvialis* (Fiedler et al., 2007). Glycine betaine, or simply betaine, has also been used as an osmotic protectant and was superior to glycerol for *E. coli* (Perullini et al., 2011). Some additives can increase gel porosity or pore sizes, and some, such as organic polymers and surfactants, can provide a scaffold to create a more ordered structure during gelation. PEG acts as a surfactant (Iler, 1979; Conroy et al., 2000; Ferrer et al., 2003), reducing the interfacial energy between liquid and gel, leading to increased porosity through reduced syneresis and possibly improved interactions with encapsulated biological components. With cyanobacteria, however, many common additives have detrimental effects on photosynthesis. Glycerol and PEG both impair photosynthetic activity of encapsulated cyanobacteria (Meunier et al., 2010), even more so than ethanol stress (Dickson & Ely, 2011). Betaine, a potent osmotic protectant produced naturally by some halophilic cyanobacteria (Hagemann, 2011), causes less acute toxicity to *Synechocystis* sp. PCC 6803 than other commonly used additives (Dickson & Ely, 2011), so may warrant additional investigation.

Another approach, not yet fully explored, is to condition cultures to produce endogenous osmotic protectants prior to encapsulation. In addition to betaine naturally produced by some halophilic cyanobacteria, some strains, including *Synechocystis* sp. PCC 6803, produce glucosylglycerol when exposed to salt (Marin et al., 2002; Ferjani et al., 2003), which can provide a degree of halotolerance. However, conditioning cultures in this way may be time-consuming and must be highly controlled, which may decrease the overall efficiency of a system using this approach. Also, the extent to which osmotic protectants may benefit encapsulated cells is unclear because osmotic stresses appear to be secondary to other types of stress (discussed below). A better understanding is needed of encapsulation stresses and potential benefits that may be provided by osmotic protectants and other additives.

Post-Encapsulation Activity

Metabolic Changes and Long Term Viability

Cell replication, normally the main product of energy directed toward photosynthetic carbon fixation, is disrupted by encapsulation because cells are physically constrained from growing and dividing. Increased production of secondary metabolites may result, at least in the short term, because energy may be redirected from cell replication into other metabolic pathways. This effect has been confirmed with enzyme production from encapsulated plant cells (Pressi et al., 2003) and with hydrogen

production from *Synechocystis* sp. PCC 6803 (Dickson et al., 2009). In the longer term, growth constraints caused by encapsulation may lead to altered metabolism with significantly reduced activity, perhaps leading to cell senescence and death. With *Synechocystis* sp. PCC 6803, photosynthetic activity remains stable for at least eight weeks, as monitored through chlorophyll fluorescence (Dickson & Ely, 2011), yet hydrogen production tends to cease over a period of approximately two weeks. Other studies have shown similar results: stable activity for some period of time after encapsulation followed by gradual deterioration of activity and senescence. Specific metabolic changes that lead to loss of activity are unclear, but constant exposure of cyanobacteria to light can result in photoinhibition and photodamage, to which cells must respond appropriately in order to survive.

Encapsulation causes significant changes in gene expression profiles, as recently described for *Synechocystis* sp. PCC 6803 encapsulated in aqueous and alkoxide-derived silica gels (Dickson et al., 2012a). In aqueous gels, several nitrite/nitrate transporters and genes related to iron stress, particularly *isiA* and *isiB*, were up-regulated. In alkoxide-derived gels, phosphate transporters were up-regulated. These observations suggest that encapsulation may limit availability of some nutrients. Because separate studies of diffusion in gels indicated adequate transport of similarly sized and charged ions and ferric iron (Dickson et al., 2012b), this potential limited availability of some nutrients is puzzling. One hypothesis may be that some nutrients may chelate with gel precursors. Though alkoxide and aqueous precursors both yield sols composed predominantly of silicic acid at similar pH, each type of precursor contributes other dissolved constituents to the sol, some unique to the precursor. Thus the possibility that unique constituents could interact with different ions would be consistent with this hypothesis.

In alkoxide-derived gels in particular, several glycosyltransferases and unknown proteins in the same operon were significantly up-regulated (Dickson et al., 2012a), perhaps in response to ethanol stress and to help cell membranes resist ethanol effects. This could be important for using cyanobacteria in direct production of alcohols for use as biofuels (e.g., ethanol and butanol). Also, glucosylglycerolphosphate synthase (*ggpS*), important for synthesis of glucosylglycerol, was strongly *down* regulated by cells in alkoxide derived gels, indicating that *Synechocystis* sp. PCC 6803 responds differently to salt stress than to alcohol stress. Better understanding of these transcriptomic responses is needed and hopefully will assist optimization of encapsulation protocols for better long-term activity of cells.

Impacts on Photosynthesis

Even under ideal conditions, photosynthesis should be affected by encapsulation due to constraints placed on cell replication (Dickson et al., 2009; Nassif et al., 2002; Dickson et al., 2012b). This has been

confirmed by microarray analyses of gene expression profiles of *Synechocystis* sp. PCC 6803, which showed, with both alkoxide-derived and aqueous gels, that linear electron flow through photosystem II (PSII) to carbon fixation is down-regulated and cyclic electron flow around photosystem I (PSI) is up-regulated (Dickson et al., 2012a). While salt stress results in down-regulation of many genes associated with photosynthesis and respiration, encapsulation results in up-regulation of many of the same genes. For example, the rod-core linker peptide *cpcG2*, which associates with phycocyanin-containing rods associated with PSI (Kondo et al., 2007), is up-regulated in response to encapsulation in either alkoxide-derived or aqueous gels (Dickson et al., 2012a). The opposite is true with *cpcG1*, the constitutively expressed protein that links phycocyanin-containing rods to the allophycocyanin core complex to form a complete phycobilisome, which primarily associates with PSII and plays an important role in state transitions (Kondo et al., 2009).

Transport Considerations

Silica gel may be thought of as similar to a glass sponge with pores large enough to allow diffusive transport of trace minerals, nutrients, and metabolites, yet small enough to prevent advection or growth of encapsulated cells. Also, the gel is rigid enough to resist swelling. Most examinations of transport in silica gels have used gel monoliths that have undergone post-gelation treatments incompatible with biological encapsulation, such as heat treatments, annealing, solvent exchange, or surface modifications (discussed in Dickson et al., 2012b). Few investigations have used hydrated gels exclusively without post-gelation treatments. However, encapsulation protocols can be optimized for cell viability and compatibility with minimal impact on diffusion rates if the diffusion distance is short enough, typically on the order of a few millimeters (Dickson et al., 2012b). Across a broad range of processing parameters using alkoxide and aqueous precursors, diffusibility depends primarily on the size of the diffusing species. Chlorophyll fluorescence data indicate that diffusion of small ions is rapid enough to support encapsulated cultures in gel monoliths up to a centimeter thick, yet the up-regulation of several nutrient stress-response genes (discussed above) in gels of similar thickness (Dickson et al., 2012a) again suggests that thinner gels, on the order of millimeters, may better support cell viability. However, as the ions increase in size, diffusion rates decrease. Silica gel can effectively encapsulate proteins, so diffusion limitations may constrain the use of this technology to applications where the product(s) of interest is small and readily diffusible.

Challenges and Future Prospects

The use of inorganic matrices, particularly silica gel, for encapsulation of cyanobacteria is a relatively new technique that is of considerable interest. Although the approach appears promising, a number of topics require further investigation to support the realization of economically viable biotechnologies:

- Develop a better understanding of stresses caused by encapsulation and metabolic responses to them, including many currently unknown or poorly described proteins;
- Determine factors that contribute to cell senescence after encapsulation;
- Examine interactions in detail that occur between surfaces of cells and the gel matrix and determine types of interactions that favor cell stability, activity, and longevity; and
- Carry out technology-specific engineering analyses to optimize gel geometry, cell culture density, and transport and production of target products.

If the above challenges can be adequately addressed, rational design and optimization of biotechnologies using encapsulated living cyanobacteria to produce valuable biofuels and bioproducts directly from sunlight and water may become practical realities.

References

Armanini L, Carturan G, Boninsegna S, dal Monte R, Muraca M (1999) SiO_2 entrapment of animal cells. part 2: protein diffusion through collagen membranes coated with SiO_2. J Mat Chem **9**:3057-3060

Armon R (2000) Sol-gel as reaction matrix for bacterial enzymatic activity. J Sol Gel Sci Technol **19**:289-292

Assink RA, Kay BD (1991) Study of sol-gel chemical reaction kinetics by NMR. Ann Rev Mat Sci **21**:491-513

Avnir D, Coradin T, Lev O, Livage J (2006) Recent bio-applications of sol-gel materials. J Mat Chem **16**:1013-1030

Bagshaw SA, Prouzet E, Pinnavaia TJ (1995) Templating of mesoporous molecular sieves by nonionic polyethylene oxide surfactants. Science **269**(5228):1242-1244

Bechtold MF, Vest RD, Plambeck Jr L (1968) Silicic acid from tetraethyl silicate hydrolysis. polymerization and properties. J Am Chem Soc **90**(17):4590-4598

Bergogne L, Fennouh S, Guyon S, Roux C, Livage J (2000) Sol-gel entrapment of enzymes. Mat Res Soc Sym Proc **628**:10.12.11-10.12.16

Bhatia RB, Brinker CJ (2000) Aqueous sol-gel process for protein encapsulation. Chem Mat **12**(8):2434-2441

Boninsegna S, Bosetti P, Carturan G, Dellagiacoma G, Monte RD, Rossi M (2003) Encapsulation of individual pancreatic islets by sol-gel SiO_2: A novel procedure for perspective cellular grafts. J Biotechnol **100**:277-286

Bottcher H, Soltmann U, Mertig M, Pompe W (2004) Biocers: Ceramics with incorporated microorganisms for biocatalytic, biosorptive and functional materials development. J Mat Chem **14**:2176-2188

Brasack I, Bottcher H (2000) Biocompatibility of modified silica-protein composite layers. J Sol Gel Sci Technol **19**:479-482

Brennan L, Owende (2010) Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev **14**(2): 557-577

Brinker CJ, Keefer KD, Schaefer DW, Ashley CS (1982) Sol-gel transition in simple silicates. J Non-Cryst Sol **48**:47-64

Brinker CJ, Keefer KD, Schaefer DW, Assink RA, Kay BD, Ashley CS (1984) Sol-gel transition in simple silicates II. J Non-Cryst Sol **63**:45-59

Brinker CJ, Scherer GW (1990) Sol-Gel Science. Academic Press. San Diego, CA

Brinker CJ. 1994. Sol-gel processing of silica. In: H. E. Bergna (Ed.) The Colloid Chemistry of Silica. American Chemical Society. Washington, D.C. pp.361-402

Carturan G, Campostrini R, Dire S, Scardi V, Alteriis ED (1989) Inorganic gels for immobilization of biocatalysts: Inclusion of invertase-active whole cells of yeast (*Saccharomyces cerevisiae*) into thin layers of SiO_2 gel deposited on glass sheets. J Mol Catal **57**: L13 - L16

Carturan G, Monte RD, Pressi G, Secondin S, Verza P (1998) Production of valuable drugs from plant cells immobilized by hybrid sol-gel SiO_2. J Sol Gel Sci Technol **13**: 273 – 276

Chernev GE, Borisova BV, Kabaivanova LV, Salvado IM (2010) Silica hybrid biomaterials containing gelatin synthesized by sol-gel method. Cent Eur J Chem **8**(4):870-876

Conroy J, Power ME, Martin J, Earp B, Hosticka B, Daitch CE, Norris PM (2000) Cells in sol-gels I: a cytocompatible route for the production of macroporous silica gels. J Sol Gel Sci Technol **18**:269-283

Coradin T, Livage J (2003) Synthesis and characterization of alginate/silica biocomposites. J Sol Gel Sci Technol **26**:1165-1168

Coradin T, Bah S, Livage J (2004) Gelatine/silicate interactions: from nanoparticles to composite gels. Coll Surf B: Biointerface **35**:53-58

Davis PJ, Brinker CJ, Smith DM (1992a) Pore structure evolution in silica-gel during aging drying. 1. temporal and thermal aging. J Non-Cryst Sol **142**(3):189-196

Davis PJ, Brinker CJ, Smith DM (1992b) Pore structure evolution in silica-gel during aging drying. 2. effect of pore fluids. J Non-Cryst Sol **142**(3):197-207

Depagne C, Roux C, Coradin T (2011) How to design cell-based biosensors using the sol‑gel process. Anal Bioanal Chem **400**(4):965‑976

Dickson DJ, Page CJ, Ely RL (2009) Photobiological hydrogen production from *Synechocystis* sp. PCC 6803 encapsulated in silica sol-gel. Int J Hyd Energy **34**(1):204-215

Dickson DJ, Ely RL (2011) Evaluation of encapsulation stress and the effect of additives on viability and photosynthetic activity of *Synechocystis* sp. PCC 6803 encapsulated in silica gel. Appl Microbiol Biotechnol **91**(6):1633-1646

Dickson DJ, Luterra MD, Ely RL (2012a) Transcriptomic responses of *Synechocystis* sp. PCC 6803 encapsulated in silica gel. Appl Microbiol. Biotechnol **96**(1):183-196

Dickson DJ, Lassetter B, Glassy B, Page CJ, Yokochi AFT, Ely RL (2012b) Diffusion of dissolved ions from wet silica sol-gel monoliths: implications for biological encapsulation. Coll Surf B: Biointerfaces. In Press (published online 10 Sept 2012)

Ducat DC, Avelar-Rivas JA, Way JC, Silver PA (2012) Rerouting carbon flux to enhance photosynthetic productivity. Appl Environ Microbiol 78(8):2660-2668

Eglin D, Mosser G, Giraud-Guille M-M, Livage J, Coradin T (2005) Type I collagen, a versatile liquid crystal biological template for silica structuration from nano- to microscopic scales. Soft Matter **1**:129-131

Ellerby LM, Nashida CR, Nashida F, Yamanaka SA, Dunn B, Valentine JS, Zink JI (1992) Encapsulation of proteins in transparent porous silicate glasses prepared by the sol-gel method. Science **255**(5048):1113-1115

Fahrenholtz WG, Smith DM, Hua D (1992) Formation of microporous silica gels from a modified silicon alkoxide. I. base-catalyzed gels. J Non-Cryst Sol **144**:45-52

Ferjani A, Mustardy L, Sulpice R, Marin K, Suzuki I, Hagemann M, Murata N (2003) Glucosylglycerol, a compatible solute, sustains cell division under salt stress. Plant Physiol **131**(4):1628-1637

Ferrer ML, Yuste L, Rojo F, Monte FD (2003) Biocompatible sol-gel route for encapsulation of living bacteria in organically modified silica matrices. Chem Mat **15**(19):3614-3618

Fiedler D, Hager U, Franke H, Soltmann U, Bottcher H (2007) Algae biocers: astaxanthin formation in sol-gel immobilised living microalgae. J Mat Chem **17**(3): 261-266

1 Frenkel-Mullerad H, Avnir D (2005) Sol-gel materials as efficient enzyme protectors: preserving the
2 activity of phosphatases under extreme pH conditions. J Am Chem Soc **127**:8077–8081

3 Fu P (2008) Genome-scale modeling of *Synechocystis* sp. PCC 6803 and prediction of pathway insertion. J
4 Chem Technol Biotechnol **84**:473‑483

5 Gadre SY, Gouma PI (2006) Biodoped ceramics: synthesis, properties, and applications. J Am Ceram Soc
6 **89**(10): 2987-3002

7 Gautier C, Livage J, Coradin T, Lopez PJ (2006) Sol-gel encapsulation extends diatom viability and reveals
8 their silica dissolution capability. Chem Comm **44**: 4611-4613

9 Glaser RH, Wilkes GL (1989) Solid-state ^{29}Si NMR of TEOS-based multifunctional sol-gel materials. J Non-
10 Cryst Sol **113**:73-87

11 Gupta RP (1985) Electronic structure of crystalline and amorphous silicon dioxide. Phys Rev B **32**(12):
12 8278 – 8292

13 Gupta R, Chaudhury NK (2007) Entrapment of biomolecules in sol-gel matrix for applications in
14 biosensors: problems and future prospects. Biosens Bioelect **22**(11):2387-2399

15 Hagemann M, Jeanjean R, Fulda S, Havaux M, Joset F, Erdmann N (1999) Flavodoxin accumulation
16 contributes to enhanced cyclic electron flow around photosystem I in salt-stressed cells of
17 *Synechocystis* sp. strain PCC 6803. Physiol Plant **105**(4):670-678

18 Hagemann M (2011) Molecular biology of cyanobacterial salt acclimation. FEMS Microbiol Rev **35**(1):87-
19 123

20 Hench LL (1998) Sol-Gel Silica: Properties, Processing and Technology Transfer. Noyes Publications.
21 Westwood, NJ

22 Ibach H (2006) Physics of Surfaces and Interfaces. Springer-Verlag. Berlin

23 Iler RK (1979) The Chemistry of Silica. Wiley. New York

24 Jiang HM, Zheng Z, Li ZM, Wang, XL (2006) Effects of temperature and solvent on the hydrolysis of
25 alkoxysilane under alkaline conditions. Ind Eng Chem Res **45**(25):8617-8622

26 Jin W, Brennan JD (2002) Properties and applications of proteins encapsulated within sol-gel derived
27 materials. Anal Chim Acta **461**:1-36.

28 Kandimalla V, Tripathi V, Ju H (2006) Immobilization of biomolecules in sol-gels: Biological and analytical
29 applications. Crit Rev Anal Chem **36**(2):73-106

30 Kay BD, Assink RA (1988) Sol-gel kinetics: II. chemical speciation modeling. J Non-Cryst Sol **104**(1):112-
31 122

32 Kelts LW, Armstrong NJ (1989) A silicon-29 NMR study of the structural intermediates in low pH sol-gel
33 reactions. J Mat Res **4**(2):423-433

34 Klein LC (1985) Sol-gel processing of silicates. Ann Rev Mat Sci **15**:227-248

35 Kondo K, Ochiai Y, Katayama M, Ikeuchi M (2007) The membrane-associated CpcG2-phycobilisome in
36 *Synechocystis*: a new photosystem I antenna. Plant Physiol **144**(2):1200-1210

37 Kondo K, Mullineaux CW, Ikeuchi M (2009) Distinct roles of CpcG1-phycobilisome and CpcG2-
38 phycobilisome in state transitions in a cyanobacterium *Synechocystis* sp. PCC 6803. Photosyn Res
39 **99**:217-225

Koslowski T, Kob W, Vollmayr K (1997) Numerical study of the electronic structure of amorphous silica. Phys Rev B **56**(15):9469-9476

Kriegl JM, Forster FK, Nienhaus GU (2003) Charge recombination and protein dynamics in bacterial photosynthetic reaction centers entrapped in a sol-gel matrix. Biophys J **85**:1851-1870

Kuncova G, Podrazky O (2004) Monitoring of the viability of cells immobilized by sol-gel process. J Sol Gel Sci Technol **31**:335-342

Laughlin RB, Joannopoulos JD, Chadi DJ (1979) Bulk electronic structure of SiO_2. Phys Rev B **20**(12):52285237

Leonard A, Rooke JC, Meunier CF, Sarmento H, Descy J-P, Su B-L (2010) Cyanobacteria immobilised in porous silica gels: exploring biocompatible synthesis routes for the development of photobioreactors. Energ Environ Sci **3**(3):370‑377

Leonard A, Dandoy P, Danloy E, Leroux G, Meunier CF, Rooke JC, Su B-L (2011) Whole-cell based hybrid materials for green energy production, environmental remediation and smart cell-therapy. Chem Soc Rev **40**(2):860‑885

Livage J, Coradin T, Roux C (2001) Encapsulation of biomolecules in silica gels. J Phys: Condens Mat **13**:R673-R691

Lu X (2010) A perspective: Photosynthetic production of fatty acid-based biofuels in genetically engineered cyanobacteria. Biotechnol Adv **28**(6): 742-746

Marin K, Huckauf J, Fulda S, Hagemann M (2002) Salt-dependent expression of glucosylglycerol-phosphate synthase, involved in osmolyte synthesis in the cyanobacterium *Synechocystis* sp. Strain PCC 6803. J Bacteriol **84**(11):2870-2877

McCormick A (1994) Recent progress in the study of the kinetics of sol-gel SiO_2 synthesis reactions. In: YA Attia, ed. Sol-Gel Processing and Applications. Plenum Press. New York pp.3-16

Meunier CF, Dandoy P, Su BL (2010) Encapsulation of cells within silica matrixes: towards a new advance in the conception of living hybrid materials. J Colloid Interface Sci **342**: 211-224

Muallem A, Bruce D, Hall DO (1983) Photoproduction of H-2 and NADPH2 by polyurethane-immobilized cyanobacteria. Biotechnol Lett **5**(6):365-368

Nair BN, Elferink WJ, Keizer K, Verweij H (1996) Sol-gel synthesis and characterization of microporous silica membranes I: SAXS study on the growth of polymeric structures. J Colloid Interface Sci **178**(2):565-570

Nassif N (2002) Living bacteria in silica gels. Nature Materials **1**:42-44

Nassif N, Roux Cc, Coradin T, Rager M-N, Bouvet OMM, Livage J (2003) A sol-gel matrix to preserve the viability of encapsulated bacteria. J Mat Chem **13**: 203-208

Nassif N, Roux C, Coradin T, Bouvet OMM, Livage J. (2004) Bacteria quorum sensing in silica matrices. J Mat Chem **14**:2264-2268

Nguyen-Ngoc H, Tran-Minh C (2007a) Sol-gel process for vegetal cell encapsulation. Mat Sci Eng: C **27**(4):607-611.

Nguyen-Ngoc H, Tran-Minh C (2007b) Fluorescent biosensor using whole cells in an inorganic translucent matrix. Anal Chim Acta **583**(1): 161-165

1 Nieto A, Areva S, Wilson T, Viitala R, Vallet-Regi M (2009) Cell viability in a wet silica gel. Acta
2 Biomaterialia **5**(9): 3478-3487

3 Pang JB, Qiu KY, Wei Y (2001) Preparation of mesoporous silica materials with non-surfactant hydroxy-
4 carboxylic acid compounds as templates via sol-gel process. J Non-Cryst Solids **283**(2-3):101-108

5 Parmer A, Singh NK, Pandey A, Gnansounou E, Madamwar D (2011) Cyanobacteria and microalgae: A
6 positive prospect for biofuels. Bioresource Technol **102**(22): 10163-10172

7 Perullini M, Amoura M, Roux C, Coradin T, Livage J, Japas ML, Jobbagy M, Bilmes SA (2011) Improving
8 silica matrices for encapsulation of Escherichia coli using osmoprotectors. J Mat Chem **21**(12):4546-
9 4552

10 Pierre AC (2004) The sol-gel encapsulation of enzymes. Biocat Biotrans **22**(3):145-170

11 Pope EJA, Mackenzie JD (1986) Sol-gel processing of silica II: the role of the catalyst. J Non-Cryst Sol
12 **87**:185-198

13 Pope EJA, Braun K, Peterson CM (1997) Bioartificial organs I: Silica gel encapsulated pancreatic islets for
14 the treatment of diabetes mellitus. J Sol Gel Sci Technol **8**: 635 – 639

15 Pouxviel JC, Boilot JP, Beloeil JC, Lallemand JY (1987) NMR study of the sol-gel Polymerization. J Non-
16 Cryst Sol **89**:345-360

17 Pressi, G, Toso, RD Monte RD (2003) Production of enzymes by plant cells immobilized by sol-gel silica. J
18 Sol Gel Sci Technol **26**: 1189 – 1193

19 Raman NK, Wallace S, Brinker CJ (1996) Shrinkage and microstructural development during drying of
20 organically modified silica xerogels. Mat Res Soc Symp Proc **435**:6

21 Reetz MT, Tielmann P, Wiesenhofer W, Konen W, Zonta A (2003) Second generation sol-gel
22 encapsulated lipases: robust heterogeneous biocatalysts. Adv Synth Catal **345**:717 -728

23 Ren L, Tsuru K, Hayakawa S, Osaka A (2001) Synthesis and characterization of gelatin-siloxane hybrids
24 derived through sol-gel procedure. J Sol Gel Sci Technol **21**:115-121

25 Rooke JC, Leonard A, Su BL (2008) Targeting photobioreactors: Immobilisation of cyanobacteria within
26 porous silica gel using biocompatible methods. J Mat Chem **18**: 1333-1341

27 Stephens E, Ross IL, Mussgnug JH, Wagner LD, Borowitzka MA, Posten C, Kruse O, Hankamer B (2010)
28 Future prospects of microalgal biofuel production systems. Trends Plant Sci **15**(10): 554-564

29 Taylor A, Finnie KS, Bartlett JR, Holden PJ (2004) Encapsulation of viable aerobic microorganisms in silica
30 gels. J Sol Gel Sci Technol **32**: 223-228

31 Yoldas BE (1979) Monolithic glass formation by chemical polymerization. J Mat Sci **14**(8):1843-1849

32 Yu, D, Volponi, J, Chhabra, S, Brinker, CJ, Mulchandani, A, Singh AK (2005) Aqueous sol-gel encapsulation
33 of genetically engineered *Moraxella* spp. cells for the detection of organophosphates. Biosens
34 Bioelect **20**(7): 1433-1437

35 Zadvorny OA, Barrows AM, Zorin NA, Peters JW, Elgren TE (2010) High level of hydrogen production
36 activity achieved for hydrogenase encapsulated in sol–gel material doped with carbon nanotubes. J
37 Mat Chem **20**:1065-1067

Figures

Figure 1. Schematic representation of a cyanobacterial cell encapsulated in silica gel (not to scale). The gel encloses the cell completely within a microporous bulk. The mesopores are large enough to allow diffusion of minerals and nutrients but small enough to contain the encapsulated cell. With alkoxide or aqueous precursors, the surface of the gel is likely composed of hydrophilic condensed silica with some uncondensed hydroxyl functional groups.

Figure 2. TEM images of an alkoxide-derived gel containing cells of *Synechocystis* sp. PCC 6803. (From Dickson et al., 2012b.)

Tables

Table 1. Comparison of advantages, challenges and other factors for alkoxide and aqueous sol-gel precursors.