Sol-gel process for vegetal-cells encapsulation

Hanh Nguyen-Ngoc, Canh Tran-Minh

Sol-gel process for vegetal-cells encapsulation

Hanh Nguyen-Ngoc[1] , Canh Tran-Minh[*]

Ecole Nationale Supérieure des Mines de St Etienne,
Centre Sciences des Processus Industriels et Naturels,
158 Cours Fauriel, 42023 Saint Etienne cedex 2, France

Abstract

A sol-gel process was carried out at room temperature to obtain porous silica matrixes for entrapment of vegetal cells. The process uses sodium silicate as a precursor rather than alkoxides to avoid generation of by-products that is detrimental to *Chlorella vulgaris* cells. The influence of several factors influencing the gelification was explored to optimize the encapsulation process. The technique produces a transparent matrix and is then very suitable for vegetal cells entrapment since they require light for their photosynthetic activity. Activity of the algal cells was determined by measurement of the intensity of fluorescence emission at 682 nm. This measurement also allowed to evaluate the efficiency of the immobilization technique and assess the long term activity of the encapsulated whole-cells.

Keywords: sol-gel, silica, cell entrapment, vegetal cells, *Chlorella vulgaris,* fluorescence detection

1. Introduction

Conventional glass-making processes require reaction at elevated temperatures which are not compatible with entrapment of fragile biomolecules. The sol-gel process involves the transition of a system from a molecular precursor state through the formation of nano-sized bricks during the sol colloidal phase into a solid gel phase and finally transitions into a dried ceramic material. Silica matrixes are relatively inexpensive to synthetize and have interesting

[1] University of Technology HCM, 268 Ly Thuong Kiet, Ho Chi Minh city Vietnam
[*] Corresponding author. Tel: +33 (0) 477 420033; fax: +33 (0) 477 420000, E-mail address: tran@emse.fr,

properties including optical transparency, biocompatibility and chemical inertness. The design flexibility of sol-gel technique and ease of fabrication can fulfill to create the surfaces with structural and chemical features that could be compatible with biomaterials.

Immobilization of chemicals in such inorganic structures is not restricted to organic molecules. Since the sol-gel process can be carried out in moderate conditions, the structural elements of the nano-sized sol bricks are preserved and enable the encapsulation of biomolecules [1-2] and even bacteria [3]. Biomolecules entrapped in sol-gel matrixes typically exhibit improved resistance to thermal and chemical denaturation, and increased storage and operational stability. This technique has been applied to immobilize various biological molecules (proteins, enzymes, antibodies) using the alkoxide route with tetraethyl orthosilicate (TEOS) or tetramethyl orthosilicate (TEMOS) as precursors [4], but they are not really cell compatible because the by-products ethanol or methanol are harmful for the biological species [5]. These alcohols can be removed from the acidic aqueous sol prior gelation [6] or the alkoxides can be replaced by aqueous precursors [7-8]. In this work, micro-algae cells of *Chlorella vulgaris* as an example of vegetal cells are entrapped in sol-gel matrixes using sodium silicate as a precursor to preserve the biological activity.

2. Experimental

2.1. Chemicals

Silica sources were sodium silicate solutions (purchased from Riedel-de-Haen) and colloidal silica LUDOX HS-40 (from Aldrich). Glycerol was purchased from Aldrich. All solutions were prepared with purified water (Rios Millipore system).

2.2. Cells culture

The *Chlorella vulgaris* strain (CCAP 211/12) was purchased from The Culture Collection of Algae and Protozoa at Cumbria, United Kingdom. The axenic algal strain was grown in the culture medium and under conditions described by the International Organization for Standardization (ISO 8692) [9]. The concentration of algae in suspension was determined by optical density (OD).

158 Cours Fauriel, 42023 Saint Etienne cedex 2, France

2.3. Cell entrapment

Sodium silicate (0.4M, 4ml) and LUDOX (8.5M, 4ml) were thoroughly mixed to obtain an homogeneous silica solution. A HCl, 4M solution was then added drop by drop until an appropriate pH is reached to induce the gelation process. Immediately, an algal solution containing $1.3x10^8$ cells/ml and 10% (w/w) glycerol was introduced under stirring and the resulting solution is cast in moulds or in cuvettes to produce silica matrixes containing algal cells. Gelation was performed at ambient temperature.

2.4. Microscopy of entrapped cells

Photomicrographs of silica membranes containing algal cells were obtained from an Olympus BH2 upright microscope. With an objective lens (x100) and an eyepiece (x10), the overall magnification is x1000.

2.5. Fluorescence measurements

Fluorescence measurements were achieved with a Spex Fluorolog 2 fluorometer from Jobin-Yvon equipped with a microcomputer for data recording. The algal matrixes were illuminated with a 469-nm excitation light and the fluorescence was collected at 682 nm. Fluorescence assays were carried out in cuvettes with algal cells in solution or entrapped in a silica matrix. For long-term activity, the algal cells are stored at 4°C between measurements.

3. Results and Discussion

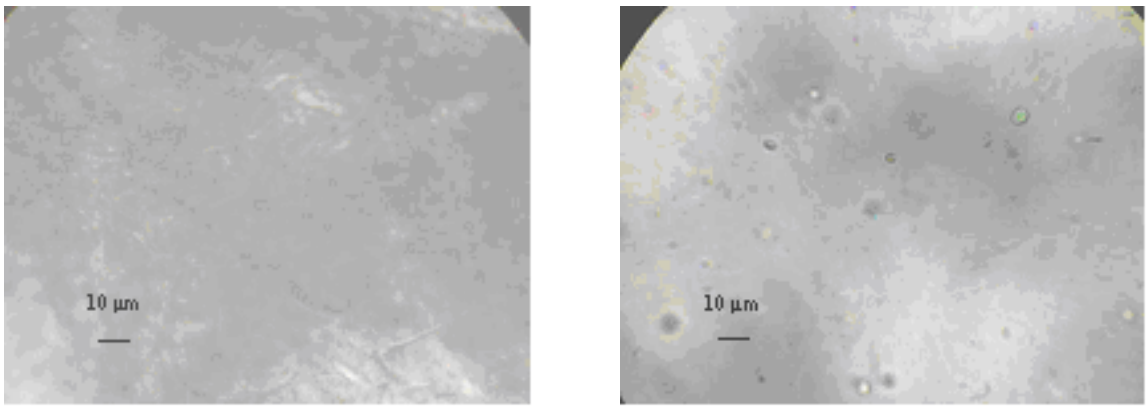
3.1 Sol-gel process to obtain active algal cells in silica matrixes

Whole-cells (bacteria, yeasts, fungi, plant tissues, mammalian tissues) are much larger than enzymes and can thus be immobilized intact using physical entrapment [10]. In the past, they have been trapped in various supports: agar or polyacrylamide gels [11], dialysis membranes [12], collagen suspensions [13], glass microfiber filter or alginate gel [15]. The susceptibility of alginate beads to phosphate chelation involve a complex interaction of cation type, concentration, and pH of phosphate solution. Gel swelling and shrinking along with deterioration of alginate structure in the presence of phosphate ions limit the use of this technique. Vegetal cells require light for their photosynthetic activity and detection, so they have been immobilized on the surface of the support [16]. Basically, immobilization of live

cells is very similar to the enzyme counterpart. Any entrapment technique in polymeric structure for enzyme immobilization [17] could be applied to whole cells. One of the problems is the mass transfer resistance imposed by the fact that the substrate has to diffuse to the reactive site and inhibitory or toxic products must be removed to the environment. Sol-gel silica matrixes enable those possibilities because they are mesoporous materials with regularly arranged, uniform mesopores that facilitate chemical transfer between the immobilized cells and the bulk solution.

To entrap the whole cells, the usual sol-gel process is based on acid hydrolysis and condensation reactions in solution followed by immobilization of the biocomponent at neutral pH to preserve the cell activity. Polyvinylalcohol and polyvinylpyrrolidone were used in some cases to minimize cracks in the gel microstructure [18], but glycerol is preferred since they also improve the mechanical properties of the polymer [19]. Unlike methanol or ethanol, glycerol appears to have no inhibition effect on the algal cells, in terms of fluorescence emission. Glycerol at 10% (w/w) was added to the algal solution at the beginning of the immobilization process and the algal activity in the glycerol containing matrix was regularly tested by fluorescence measurements during 4 weeks. No inhibition effect was observed compared to the reference matrix without glycerol.

3.2. Microscope images of the silica support and the algae encapsulated matrix



A B

Fig. 1 : Silica support without (A) and with (B) entrapped algal cells

A characteristic feature of the sol-gel silica matrix is the relative optical transparency of the resulting insoluble support. It makes the technique very suitable for vegetal cells immobilization since they require light for their photosynthesis and activity. It also enables the algal cells entrapped inside the support to be seen with an upright microscope and compared to the algal-free matrix (Fig. 1A), particularly when the vegetal cells are illuminated and photosynthesis is triggered. Using the same algal concentration (ie. $6x10^7$ cells/ml) and pH 7, the fluorescence emission is $25\text{-}30x10^5$ a.u. with silica matrix and $10\text{-}15x10^5$ a.u. in BSA (bovine serum albumin)+glutaraldehyde. On the photograph, some algal cells exhibit higher light intensity under illumination probably due to their proximity to the surface (Fig. 1B). Of course, no algal cells are detected on the reference support .

The BET method used gave an average pore size of 10 nm in diameter which corresponds to a pore volume of 0.40 cm^3/g. Since the surface area also depends on gelation pH (178 m^2/g for pH 7), the gelation time will affect the porosity of the matrix. The fact that the cells are entrapped in the inner part of the matrix prevent them from microbial attack and leaching out. On the other hand, all the chemical and biological transformations can be continuously observed and monitored by means of optical techniques: fluorescence, luminescence or light absorption.

3.3. Effects of algal concentration and pH on gelation time

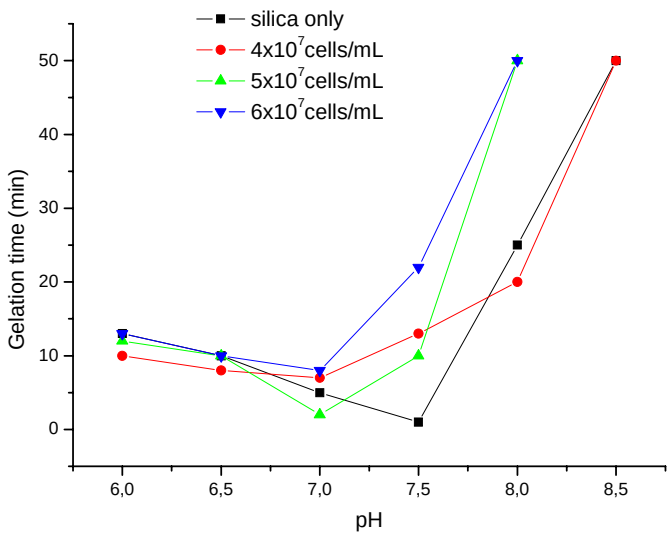


Fig. 2 : Effect of pH and algal concentration on the gelation time

An optimized polymerization pH should allow an efficient entrapment of the algal cells while preventing loss of cells activity under polymerization conditions. The effect of cells encapsulation pH on the gelation time in the silica matrix was studied for pHs ranging from 6 to 8.5 (Fig. 2). A minimum of gelation time at pH 7-7.5 is noted for different cell concentrations in silica gel. Gelation time becomes higher with increasing pH and no gel is formed beyond pH 8.5. The particles are so charged [20] that they cannot agglomerate to undergo the polymerization process. The amounts of immobilized algal cells also affect the gelation time. High algal concentration tends to increase the gelation time particularly for pH>7.

3.4. Viability of algal cells after encapsulation

The algal cells viability is determined by measuring the chlorophyll fluorescence which reflects their photosynthetic activity. The fluorescence signal also provides information on the fluorescence induction and the activity of the Photosystem II (PSII), which is the target of many herbicides.

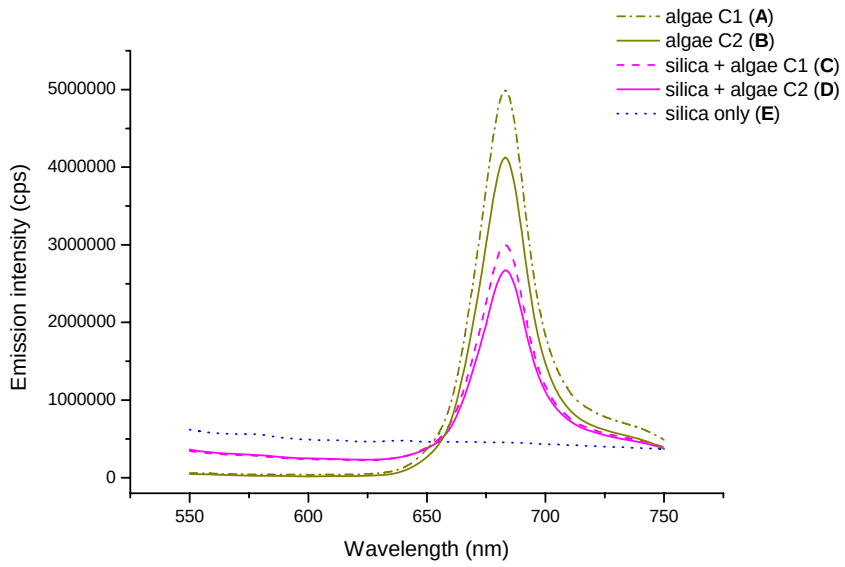


Fig. 3 Viability of algal cells in terms of fluorescence emission at 682 nm when algal cells are in solution (A and B) or entrapped (C and D) in a silica matrix, compared to a reference (E) silica support without any cells (algal concentration C_1=13x10^7 cells/ml, C_2=6x10^7 cells/ml)

Fig. 3 shows that the presence of algal cells can be detected by fluorescence emission at 682 nm. The emission intensity is lower when the cells are entrapped in the silica matrix than

in solution. This results from the partial absorption of light by the insoluble support. No fluorescence at 682 nm was emitted with the algal-free matrix. In any case, emission intensity is reduced with decreasing cell concentrations.

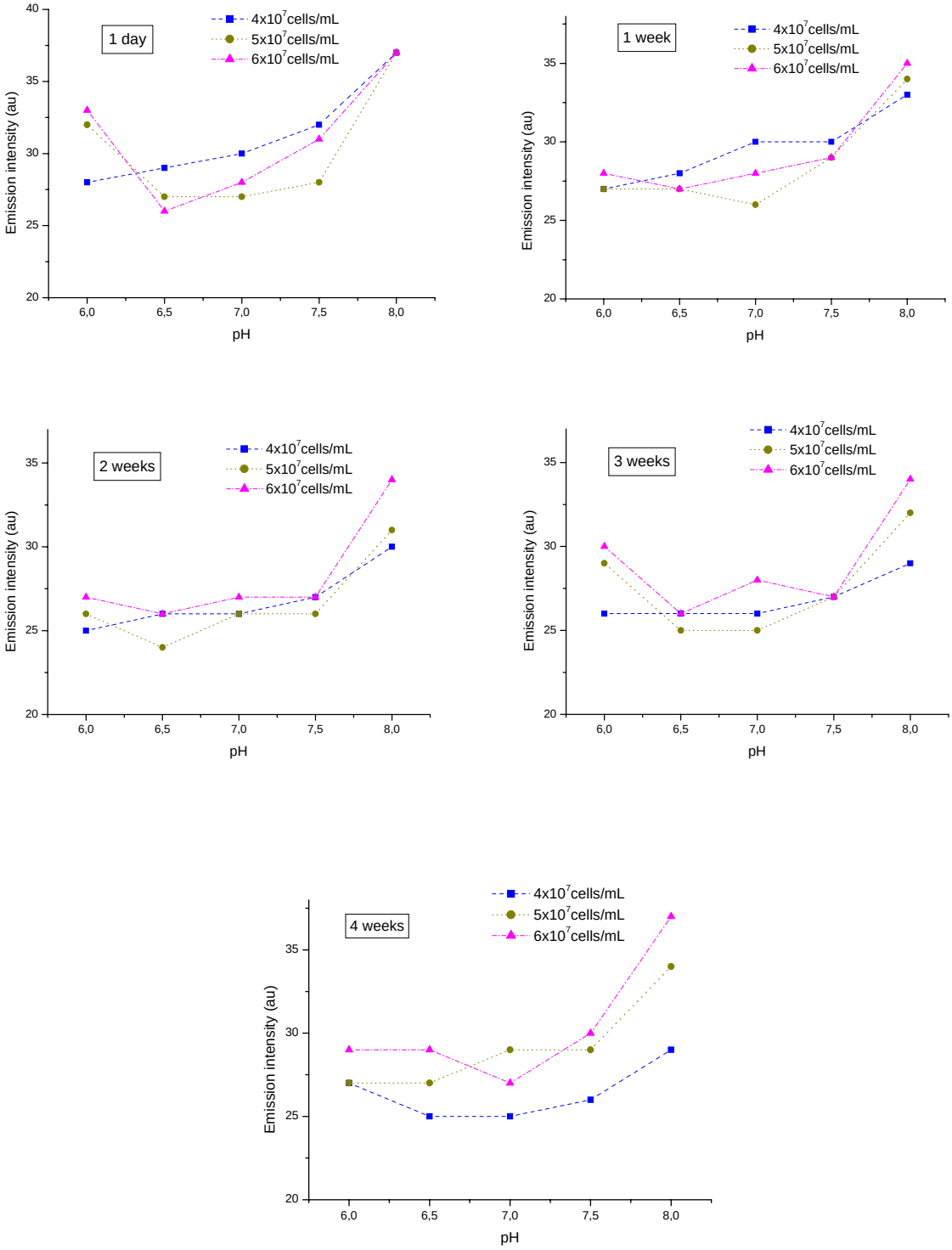


Fig. 4 Long term activity of encapsulated algal cells
as a function of pH for various cells concentrations

3.5. Effect of pH on algal cell long-term activity

The activity of algal cells depends on the working pH of the sample solution. The effect of pH on the long-term behaviour of the *Chlorella vulgaris* immobilized in a silica matrix was studied with 0.2 M phosphate buffer solution. The pH of the solution ranges from 6 to 8.
The cells activity expressed in terms of fluorescence emission is shown in Fig. 4. Although there are fluctuations in the values, a general tendency can be observed from those various curves. Fluorescence intensity increases with high pH values particularly at pH 8. Fluorescence intensity is higher at pH 8 because the activity of PSII system is affected at this pH, which induces a greater fluorescence. The same effect is observed with anti PSII herbicides. High cells concentrations slightly give rise to a high fluorescence emission for fresh cells but a decrease in fluorescence can be noted after 4 weeks: it could be attributed to the fluorescence quenching by a higher number of aging cells and their metabolites. On the whole, the activity of *Chlorella vulgaris* remains stable during the 4 week period of assays.

3.5. Application to herbicides detection

The principle of herbicides detection is based on the effect of herbicides on algal fluorescence emission. The presence of anti PSII herbicides (diuron, atrazine, simazine and isoproturon) increases the fluorescence emission. Optical biosensors based on fluorescence detection require a translucent matrix in which a fluorescent bioreceptor can be immobilized and placed in front of the tip of an optical fiber. The optical fiber is used to send the excitation radiation to the algal cells and convey the fluorescence radiation up to a fluorometer [14], to generate a significant signal. The optical transparency of the algal sol-gel silica matrix would allow its application to algal biosensors.

4. Conclusion

In this study, the possibility of using a sol-gel method to immobilize *Chlorella vulgaris* has been investigated. The use of this vegetal cells entrapment technique is motivated by the optical transparency and chemical inertness of the support. We have shown that aqueous precursors prevents the loss of algal activity during the polymerization process. This

technique also enables the use of this algae containing matrix in phosphate environment, which is damaging for alginate gel [21] . The measurement of fluorescence emission at 682 nm provides an easy method to control the activity of algal cells and optimize the various immobilization parameters. Association of algal silica matrixes with optical fibers is under way to construct a optical biosensor for determination of pollutants. This sol-gel technique can be extended to other micro-organisms immobilizations for the design and construction of whole-cell biosensors.

Acknowledgments

This work has been financially supported by Région Rhône-Alpes (MIRA fellowships and Thématiques program) and by CNRS (ACI program). The authors appreciate Dr. Claude Durrieu, Dr. Martine Naessens and Prof. Jean- Marc Chovelon for fruitful discussions and Anne-Marie Danna for experimental assistance.

References

[1] B. Dunn, J.M. Miller, B.C. Dave, J.S. Valentine, J.I. Zink, Acta Mater. 46 (1998) 737.

[2] J. Livage, T. Coradin, C. Roux, J. Phys.: Condens. Matter 13 (2001) R673.

[3] S. Fennouh, S. Guyon, C. Jourdat, J. Livage, C.R.Acad.Sci. Paris Ser. IIc (1999) 625.

[4] W. Lee, K.S. Park, Y.W. Kim, W.H. Lee, J.W. Choi, Biosens. Bioelectron. 20 (2005) 2292.

[5] A. Coiffier, T. Coradin, C. Roux, O.M. Bouvet, J. Livage, J. Mater. Chem. 11 (2001) 2039.

[6] J.F.T. Conroy, M.E. Power, J.Martin, B. Earp, B. Hosticka, C.E. Daitch, P. M. Norris, J. Sol-gel Sci. Technol.18 (2000) 269.

[7] R.B. Bhatia, C.J. Brinker, Chem. Mater. 12 (2000) 2434.

[8] D.Yu, J. Volponi, S. Chhabra, C. J. Brinker, A. Mulchandani, A.K. Singh, Biosens. Bioelectron. 20 (2005) 1433.

[9] ISO 8692, International Organization for Standardization (1989), Water quality : fresh water growth inhibition test with *Scenedesmus subspicatus* and S*elenastrum capricornutum*.

[10] S. Fukui, A. Tanaka, Annual Review of Microbiology 36 (1982) 145.

[11] T. Matsunaga, I. Karube, S. Suzuki, Anal. Chim. Acta 99 (1978) 233.

[12] G.A. Rechnitz., R.K. Kobos., S.J. Riechel, C.R. Gebauer, Anal. Chim. Acta 94 (1977) 357.

[13] I. Karube, T. Matsunaga, S. Mitsuda, S. Suzuki, Biotech. Bioeng. 19 (1977) 1535.

[14] M. Naessens, J.C. Leclerc, C. Tran-Minh, Ecotoxicol. Environ. Safe. 46 (2000) 181.

[15] D.O. Fesenko, T.V. Nasedkina, A.V. Chudinov, D.V. Prokopenko, R.A. Yurasov, A.S. Zasedatelev, Molec. Biol. 39 (2005) 84.

[16] C. Vedrine, J.C. Leclerc, C. Durrieu., C. Tran-Minh, Biosens. Bioelectron. 18 (2003) 457.

[17] S. Fabiano, C. Tran-Minh, B. Piro, L.A. Dang, M.C. Pham, O. Vittori, Mater. Sci. Eng. C 21 (2002) 61.

[18] Y.D. Kim, C.B. Park, D.S. Clark, Biotechnol. Bioeng. 73 (2001) 331.

[19] N. Nassif, C. Roux, T. Coradin, M.N. Rager, O.M.M. Bouvet, J. Livage, J. Mater. Chem. 13 (2003) 203.

[20] R.K. Iler, Polymerisation of silica, In: The chemistry of silica: solubility, polymerisation, colloid and surface properties, and biochemistry of silica, Wiley, New York, 1979, p. 174.

[21] A. L. Dainty, K. H. Goulding, P. K. Robinson, I. Simpkins, M. D. Trevan, Biotechnol. Bioeng. 28 (1986) 210.