Novel synthesis route of silica based networks for immobilization of a hydrogen producing strain of *Chlamydomonas reinhardtii*

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INTRODUCTION AND OBJECTIVE

Today, sol-gel materials are mostly alkoxides or sodium silicate derived (Brinker&Scherer, 1990, Hench&West, 1990, Hench, 2007). Due to their transparency for visible light (Latthe, 2010, Medda, 2009), the hydro- and xerogels are ideal candidates for the immobilization of photosynthetically active biomaterial like *Chlamydomonas reinhardtii*.

However, there are some serious disadvantages: Alkoxide precursors like tetraethylorthosilicate (TEOS) or tetramethylorthosilicate (TMOS) show a relatively low reactivity, so that acids or bases are used for catalysing the hydrolysis and condensation. Besides, organic solvents are needed to reduce the gelation time (e.g. Latthe, 2010, Medda, 2009, Hench & West, 1990, Brinker & Scherer, 1990). These additives and the gelation by-products methanol or ethanol are known to be toxic to cells. In case of the sodium silicate based gels, high concentrations of acids and salts limit the use for immobilizing cells (e.g. Brinker & Scherer, 1990, Hench, 1990, Coradin, 2007, Nassif, 2003).

To overcome these disadvantages, we used the precursor tetra(*n*-propylamino)silane $Si(NHPr)_4$, because of its higher reactivity compared to the alkoxysilanes. Therefore, no additional organic solvent is needed, promising a novel approach for the entrapment of *C. reinhardtii* for biological hydrogen production.

MATERIALS AND METHODS

Gel synthesis

The aminosilane precursor was mixed with distilled water and stirred at room temperature forming an emulsion. The resulting particulate silica sol was then gelled by partial evaporation of the liquid phase. After gelation the aging process started, leading to glass-like xerogels.

Optical measurements

The transparency was measured using the standard DIN method EN ISO 7027. For this purpose, the gels were formed in open cuvettes und analysed with an UV-Vis spectroscopy. Absorption values below 0.06 indicate transparent hydro- and xerogels.

Cell viability

To investigate the cell viability, three methods were utilised: The morphology was studied via microscopy, while the maximum quantum yield of Photosystem II - a value for the photosynthetic activity - was measured with a Photosynthesis Yield Analyzer (Maxwell & Johnson, 2000). Furthermore, the oxygen consumption and production to assess respiration and photosynthesis were analyzed with an Oxygraph system (Beckmann, 2009).

RESULTS AND DISCUSSION

Gelation time and pH value

The gelation time of an aminosilane concentration of 25 % amounts to 3 days at an evaporation of 33 % of the liquid phase. Hence, the synthesis procedure was modified: After 6 h sol formation it was poured into glass petri dishes. Due to a constant airflow, the gelation occurred after 1 h 14 min by evaporation with a constant airflow to 30 % of the original volume. After further 27 min, a glass-like xerogel was formed. Because of the cleavage product *n*-propylamine, the pH increased from 7 to 12 during the hydrolysis. Due to the amine evaporation during the gel formation, the pH dropped to 10. When the pH of the sol was adjusted to pH 7, the gelation time was reduced to 10 min (figure 1).

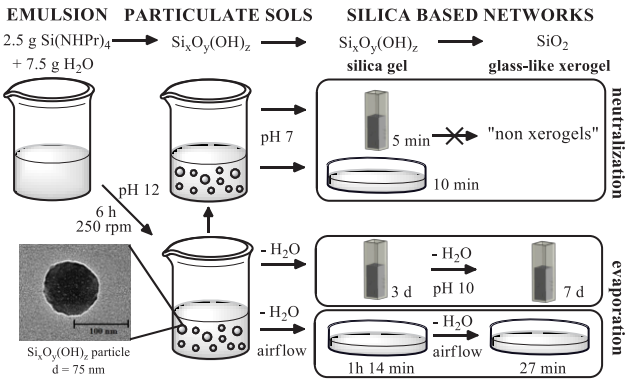

Fig. 1 : Schematic route of the synthesis used for the preparation of aminosilane-derived silica based networks from particulate silica sols.

Transparency

For the application to photosynthetically active biomaterials, the gels should be as transparent as possible for visible light. The transparency is dependent on the precursor concentration: In case of pH 10, transparent gels are formed with precursor concentrations between 5 and 25 %. Above 25 %, the transparency decreased significantly. In contrast, at pH 7, transparency is achieved with concentrations up to 15 %, above this threshold the transparency decreased (figure 2).

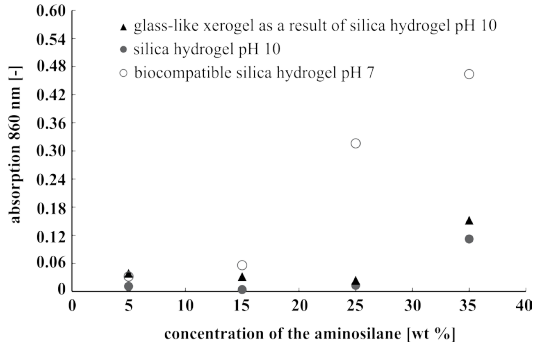

Fig. 2 : Influence of the aminosilane precursor concentration on the silica based networks transparency.

Silica network The resulting glass-like xerogels were studied with ^{29}Si and ^{13}C solid state nuclear magnetic resonance (NMR) spectroscopy. ^{29}Si solid state NMR spectra indicated a three-dimensional silica based network (chemical shift -109.7 ppm, fully condensed Q^4 units and chemical shift -99.4 ppm, Q^3 units) (Williams, 1984). The ^{13}C solid state NMR spectra showed that the gels contain *n*-propyl groups (chemical shift 11.6 ppm, 22.0 ppm and 24.0 ppm), which were assigned to *n*-propylamine entrapped inside the network and remaining Si-NHPr moieties.

***Immobilization of* C. reinhardtii** Between free and in the silica network immobilized cells no morphological differences could be detected with microscopy (figure 3 A, B).

Concerning the photosynthetic activity, a negative impact was detected: free cells showed a maximal quantum yield of 0.39, where 2 h after immobilization the value dropped to 0.13. Nevertheless, after storage for 2 h in the dark, overlaid with 3 mL of fresh tris acetate phosphate media, the maximum quantum yield increased to 0.43, indicating a regeneration of the photosynthetic activity.

The respiratory consumption in the dark dropped from -8.04 nmol O_2 mL^{-1} min^{-1} in case of the free cells to -1.18 nmol O_2 mL^{-1} min^{-1} when measured 2 h after immobilization (figure 3C).

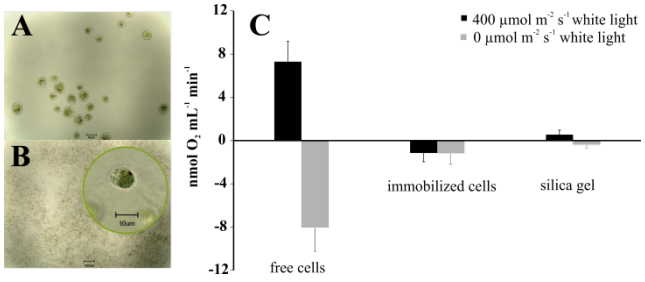

Fig. 3 : Microscopic analysis of free cells (A), immobilized cells (B) and analysis of the oxygen consumption and production (C).

These measurements indicate that the sol-gel procedure is biocompatible within limits: the *C. reinhardtii* cells are viable after immobilization, a negative impact of the procedure can be seen but a regeneration seems feasible. One cause could be the by-product *n*-propylamine which is reported to have herbicidal action (Abu-Qare, 2002, Sandusky, 1983).

CONCLUSIONS

We reported that the novel sol-gel synthesis via the aminosilane $Si(NHPr)_4$ provides an easy procedure at a mild temperature of 25°C and a short gelation time. Besides, it leads to highly transparent hydrogels and glass-like xerogels which allow an entrapment of viable *C. reinhardtii* cells in an aqueous solution. For the biological hydrogen production the toxic effect of the by-product *n*-propylamine has to be overcome. Therefore, other precursors based e.g. on less toxic methylamine or sodium silicate will be investigated. Nevertheless, the presented aminosilane-derived silica based networks are suitable for coating, entrapment and/or stabilization of many other sensitive (bio)molecules and related optical and photochemical materials.

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