O9-3 **Cyanobacteria immobilized in silica gels by "chimie douce": Towards a new generation of photobioreactors**

Leonard A., [#] **Rooke J.C. and Su B.L.** [*]
Laboratoire de Chimie des Matériaux Inorganiques, University of Namur, Namur, Belgium
* Supervisor # alexandre.leonard@fundp.ac.be

INTRODUCTION

The progressive depletion of fossil fuels combined with the elevated pollution levels in atmosphere lead to a double problem whose issue could be solved by the introduction of clean energy sources. After the major breakthroughs made in the development of first and second generation biofuels, the exploitation of microalgae is promised to a bright future.(Posten 2009) These organisms very efficiently assimilate carbon dioxide into higher-value hydrocarbons and lipids with sunlight as energy source. Worldwide research actually deals with these organisms in the CO_2-neutral production of not only biofuels like biodiesel and biomethane but also hydrogen.(Markov 2006, Schenk 2008) To further increase the efficiency and lower the operating costs of photobioreactors, immobilized species could potentially be used continuously and would allow for easiness of metabolite recovery since the cells are physically separated from the aqueous phase. The advantages of biostructure immobilization have already been demonstrated for a long time, with higher productivities, easy handling and facilitated product recovery. Extension of this methodology to microalgae is however still limited with only few examples of hydrogen production. Owing to the high efficiency of photosynthesis to convert solar energy and CO_2 in useful compounds, our aim is to develop photobioreactors based on immobilized photosynthetic bacteria and microalgae within silica gels. These inorganic matrices are very appropriate since their synthesis can be carried out under soft conditions, *i.e.* ambient temperature and physiological pH, they possess an elevated porosity (important for the efficient diffusion of nutrients and metabolites) and are biocompatible and optically transparent.(Nassif 2003) Such a smart combination should lead to a new generation of photobioreactors designed for the production of biofuels and other metabolites such as drugs as well as the sensing and degradation of pollutants. We present here the most recent advances realized in the immobilization of cyanobacteria, chosen as model cells, in silica gels prepared by a biocompatible pathway.

MATERIALS AND METHODS

Synechococcus sp. PCC 6301 and PCC 7002, originating from freshwater and marine environments respectively, were obtained from the Pasteur Culture Collection and grown under light/dark cycles in BG-11 and BG-11/ASN-III liquid media. Samples of cultures in the stationary phase were centrifuged, the supernatant discarded and the pellet resuspended in fresh growth medium. To reduce the concentration of released Na^+, sodium silicate was diluted 4 times and exchanged on an Amberlite® IR120 H^+ resin to form metastable silicic acid (residual Na^+: < 1 mM). The latter was added in equal volume to the cell suspension, silica nanoparticles (Ludox® HS-40) were eventually added and pH adjusted to 6.8. Hollow cylindrical gels were shaped by introduction of a rod before gelling, thereby increasing the contact surface between the immobilized cells and the added growth medium to provide the necessary nutrients for the cells to remain active.

The integrity of the photosynthetic apparatus was followed by the absorption bands of the pigments by reflectance-mode UV-vis spectroscopy (Perkin Elmer Lambda 35). Integrity of the cells was verified by direct observation of the gels by TEM (Philips Tecnai 10). Samples were fixed, dehydrated and embedded in a LX-112 resin before being cut into thin sections by an ultramicrotome. The activity of the immobilized cyanobacteria was verified by $NaH^{14}CO_3$ assimilation. After incubation, the medium was separated from the gels that were crushed, the residual inorganic bicarbonate eliminated by neutralization and nitrogen flushing, leaving only organic ^{14}C assimilated (in the gel) and excreted (in the medium) by the cells. Radioactivity was subsequently measured upon addition of a scintillation cocktail (Instagel, Perkin Elmer) (Beckman LS 6000 SC). Oxygen production measurements were carried out with a Clark-type electrode (Oxy-lab, Hansatech Instruments).

RESULTS AND DISCUSSION

UV-Vis spectra recorded over a period of 10 months show 2 characteristic absorption bands at ca. 675 and 625 nm, attributed to chlorophyll *a* and phycocyanin respectively, the spectroscopic signature of the same free cells. For PCC 6301, sharp and intense bands can be observed over a period of 14 weeks and remain detectable for up to 35 weeks though there is a gradual decrease in intensity, suggesting a progressive destruction of the cells. The presence of the bands for these durations however suggests that part of the cells remain intact and able to photosynthesize (Fig. 1). For strain PCC 7002, both the absorption bands are present until 23 weeks after immobilization. Compared to our previous results, it is thus evident that the decrease in Na^+ in the gel has a beneficial effect on the longer preservation of the pigments (12 and 9 weeks for PCC 6301 and 7002 respectively), the other synthesis parameters being similar as described previously.(Rooke 2008) The direct visualization of the cells reveals most intact cell walls and a very porous silica

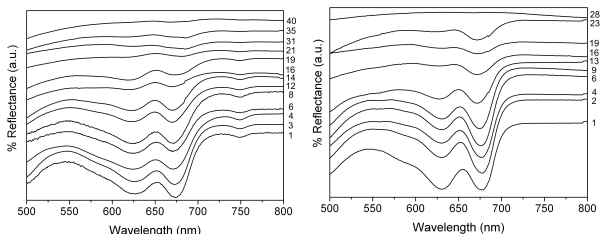

Figure 1: Reflectance-mode UV-Vis absorption spectra of PCC 6301 (left) and PCC 7002 (right) immobilized in H^+-silicate gels. Numbers on the right represent weeks.

network. As already observed with sodium silicate, PCC 6301 bacteria are located in cages moulded to their size whereas a large void is always present between the cells and the matrix for the saline strain.

To monitor the ability of the cells to photosynthesize when immobilized, the assimilation of inorganic $^{14}CO_2$ was measured by adding the tracer during the gel formation. As can be seen from table 1, the signal of organic ^{14}C is much higher in each case compared to the same blank samples (without added cyanobacteria). Moreover, significant organic ^{14}C could also be detected in the supernatant medium, suggesting some excretion by the cells (data not shown). To make sure that this activity does not result from residual photosynthesis during immobilization, a second set of experiments were carried out by adding the same radiotracer on top of the gels, after hardening. Again, significant amounts of organic ^{14}C unambiguously confirm the ability of the cells to photosynthesize after immobilization and additionally prove that the porosity of the matrix is appropriate for nutrients diffusion.

Table 1. ^{14}C incorporation in the hybrid gels with the tracer added during gel formation (A) and on top after hardening (B).

Sample	DPM ^{14}C (A)	DPM ^{14}C (B)
H_2SiO_3 PCC 6301	145363	393961
H_2SiO_3 Blank	3636	1831
H_2SiO_3 PCC 7002	101072	322711
H_2SiO_3 Blank	386	420
H_2SiO_3-Ludox® PCC 7002	202395	250439
H_2SiO_3-Ludox® Blank	530	358

Unfortunately, these gels remain quite fragile, and a long-term utilization requires their reinforcement, for instance by adding silica nanoparticles. Based on previous works, we chose Ludox® HS40 as colloids. In the case of PCC 6301, a reliquefaction of gels was observed few hours after hardening, independently of the quantities used. This was also the case for identical blank gels, suggesting a destabilization in presence of the BG-11 growth medium. In contrast, gels made with PCC 7002 become very firm, long-lasting and easy to put into desired shapes (like hollow cylinders). Detailed investigations have revealed that the best composition is Ludox:Silicate 1:3 (v/v), too a low quantity leading to soft gels whereas higher amounts bring an increase in Na^+ ions, thus osmotic stress. A comparison of UV-vis spectra for gels made with Ludox clearly demonstrates the beneficial effect of nanoparticles addition since the band remain much better defined over time (Fig. 2). ^{14}C incorporation measurements were carried out on a comparative basis, with the same stock solution of radiotracer added in equal volume on top of the gels made with and without silica nanoparticles.

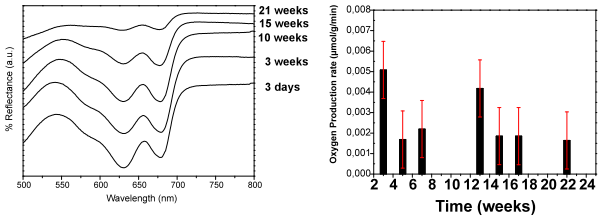

Figure 2: Reflectance-mode UV-Vis absorption spectra of PCC 7002 immobilized in H^+-silicate-Ludox® gels (left) and corresponding Oxygen production as a function of time (right).

The signal corresponding to organic ^{14}C is in each case higher for the hybrid gels than for the blank gels. As can be seen on figure 2, continued oxygen production is detected for hybrid gels after 22 weeks immobilization, which is consistent with the intactness of the photosynthetic apparatus as suggested from spectroscopic measurements. The SiO_2 nanoparticles stabilized gels thus confer a better resistance towards shrinkage upon ageing, preserving the integrity of the cells.

CONCLUSIONS

Cyanobacteria can be successfully immobilized within porous silica gels via a biocompatible route. Metabolic activity has been demonstrated for more than 20 weeks, opening the way towards the development of a new generation of photobioreactors for the production of energetic resources as well as other metabolites of interest by assimilation of carbon dioxide and utilization of solar energy.

REFERENCES

- Nassif N. et al. (2003) *Viability of bacteria in hybrid aqueous silica gels*. Journal of Sol-Gel Science and Technology 26 1141-1144.
- Markov S.A. et al. (2006) *Photostimulation of H_2 production in the green alga* Chlamydomonas reinhardtii *upon photoinhibition of its O_2-evolving system.* International Journal of Hydrogen Energy 31 1314-1317.
- Posten C. et al. (2009) *Microalgae and terrestrial biomass as source for fuels-A process view.* Journal of Biotechnology 142 (1) 64-69.
- Rooke J et al. (2008) *Photosynthesis within porous silica gel: viability and activity of encapsulated cyanobacteria.* Journal of Materials Chemistry 18(24) 2833-2841.
- Schenk P.M. et al. (2008) *Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production.* Bioenergy Research 1 (1) 20-43.