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Cyanobacteria Encapsulation in Sepiolite-Alginate Beads

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Cyanobacteria can be encapsulated in biohybrid materials, with components such as biopolymers and clays, as in this case. With alginate (biopolymer) and sepiolite (clay that provides better biocompatibility), spheres of about 0.2 mm in diameter are created by using calcium chloride to form a structure in which the cells can be embedded.
This material, despite being transparent and resistant, encounters problems in guaranteeing the correct diffusion of the materials and sufficient photosynthetic activity.

The beads are easily synthesised with mixtures of alginate and sepiolite, and by forming the spheres with CaCl2, following a two steps syntesis.

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- Alginic acid salt (high molecular weight)
- Sepiolite
- Destilled water
- Powdered CaCl2
- Cyanobacteria in BG-11
- BG-11

- EtOH to wash in sterility
- Glasses (it could be crystal glasses or plastic glasses, it is not relevant). It is convenient to use little ones
- Balance
- Flow hood or similar to ensure sterility
- Centrifuge
- Micropipette
- Syringe with medium needle
- Volumetric flask
- Falcon

Working in sterility with cyanos, and take care at the lab as normally it is done; no extra caution are needed.

It is recommendable to use cyanobacteria at OD 0,6 or similar. With less OD, it is more difficult to have encapsulated cyano in beads.

Solutions preparation

1 **IMPORTANT:** the amounts used are the experimental ones did it experimentally, it could change.

Having different options to do the alginate: sepiolite solutions, best of all are explained.

First, produce a [M]**8 % (v/v)** suspension of alginate. It is **IMPORTANT** to mention that it is difficult to disolve it. To do it:

-Take a glass (recomendable to use a plastic glass), with a magnetic stirrer, put it on a heating plate and add 50 mL of water.

-Heat the water at aprox. **65 °C** .

-Weight 2 grams of alginc acid salt

-Add the alginic acid salt with stirring and heat. It is Important to add it slowly. Otherwise, it formed 'clots'.

-when all the alginic acid has been added, put off the heat and let it stirring overnight.

Then, to do the [M]**10 % (v/v)** sepiolite solution:

-Weight 5 grams of sepiolite (**NOTE:** take care of sepiolite, it is like dust) and put it on a **50 mL** volumetric flask

-Add water up to the mark in the flask. Move slowly and it is ready to use

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It is also necessary to have a [M]**10 % (v/v)** CaCl2 solution.

1.1 4:1 alginate: sepiolite

To produce **10 mL** of mix:

-Take **5 mL** of alginate [M]**8 % (v/v)** in a plastic bottle with stirring

-Add **1 mL** of [M]**10 % (v/v)** sepiolite and **4 mL** of water

-Stirring until it is completely uniform

NOTE: it is recommendable to take out the magnetic stirrer

1.2 2:5 alginate:sepiolite

To produce **10 mL** of mix, follow same steps as 4:1.

- **2.5 mL** alginate [M]**8 % (v/v)**
- **5 mL** sepiolite [M]**10 % (v/v)**
- **2.5 mL** of distilled water

1.3 1:5 alginate:sepiolite

To produce **10 mL** of mix, follow same steps as 4:1.

- **1.25 mL** alginate [M]**8 % (v/v)**
- **5 mL** sepiolite [M]**10 % (v/v)**
- **3.75 mL** destilled water

Cyanobacteria

2 Having the cyanobacteria culture, work in a flow hood or other methods to guarantee sterility. Take Falcon and add 2/3 of cyano in BG-11. With a pellet of only one Falcon, it is possible to produce a little bottle of beads (maybe 20/30 beads).

3 Centrifuge the cyanobacteria medium in the Falcon. At **8000 rpm, 00:10:00** 10m

4 Add **3.75 mL** of BG-11 into the Falcon to resuspend the cyano pellet.

NOTE: to make 2:5 beads, it is better to add a little bit more of BG-11, to make it easier to disolve with the alginate: sepiolite solution.

Beads 10m

5 Add 2/3 of cyano-BG-11 with 1/3 of mix alginate: sepiolite. **Note:** in the case of 2:5, it could be

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better to add more of cyano--BG-11. Mix it in the Falcon, or maybe in a bottle.

6 When it is mixed, take it with a sirynge and a needle. Having the CaCl2 solution in a bottle, add the syringe content. **NOTE:** add it at medium speed. If it is added to slowly, it will form deformed beads; and add it to quickly is not recommended.

7 Let the beads into the CaCl2 **00:10:00** Then, take out the beads and put it on BG-11. To take out, it is possible to use a strainer, or do it with a spoon. 10m

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