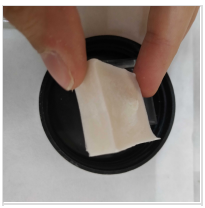

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Sepiolite - Chitosan Biocompatible Foams

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4cFuels

celiamm

The foams are biohybrid, resistant, biocompatible, biodegradable materials that allow cyanobacteria to survive inside them and produce the desired biofuel. It is a macroporous material with high porosity.

Its synthesis is relatively simple, and can be classified into three main steps: formation of the chitosan and sepiolite solutions, freeze-drying them and finally the introduction of the cells inside.

This will result in a material with many possibilities, which, despite having some disadvantages such as its low resistance to liquids after synthesis, could be a good option by applying different modifications to suit the needs of the cells.

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- Sepiolite
- Destilled water
- 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
- Syringe
- Volumetric flask
- Falcon
- Petri's boxes or similar
- Lyophilizer

Solutions preparation

1d

1 Having different options to do the chitosan: sepiolite solutions:

To make a 1:1 mix chitosan:sepiolite:

First, produce a 4% solution of chitosan(that after doing the mix will be a 2%). 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

-Weight 1,0 grams of chitosan

-Add 25 mL of CH3COOH. **24 °C**

Then, to do the 4% sepiolite solution (that after doing the mix will be a 2%):

-Weight 1,0 grams of sepiolite (**NOTE:** take care of sepiolite, it is like dust) and put it on a 25 mL volumetric flask

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

Finally, add the sepiolite solution to the chitosan solution and then stir at least overnight.

24:00:00 At least overnight

NOTE: there are two options to do the mixes. On the one hand, you can add the sepiolite solution directly into the chitosan solution without being homogenized, and then let it stir. On the other hand, you can also wait until the chitosan solution is homogeneous.

1.1 To make a **2:1 mix chitosan:sepiolite**:
Follow the steps previously written, change only the amount of sepiolite in the mix. So, to do a 2:1 mix, take the same amount of chitosan as to have a solution with the same percentage; and to make the sepilite solution, take 0,5 grams of sepiolite instead of 1,0 grams.

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1.2 To make a **3,5:1 mix chitosan:sepiolite**:
Change only the quantity of sepiolite, or also, it could be possible to make a chitosan solution with a little bit more percentage of chitosan, and modify the amount of sepiolite. It is able to do this, but it is necessary to take into acount that the chitosan is too complicated to disolve it, so, the more the amount of chitosan, the more difficult to do the mix.

Lyophilization

2 Once the different chit:sep solutions are ready, they are frozen in the freezer for at least 2d
24:00:00
Switch on the freeze-dryer and, as quickly as possible, remove the solutions from the freezer. They will be left freeze-drying for about **24:00:00** , reaching 0.116 mbar and temperatures of -80°C.

Special care must be taken when using the freeze dryer. It is necessary to close all the valves that make it up when it is connected, close the lid tightly and check the first few minutes of operation in case any error occurs.

Cyanobacteria 10m

3 Measure the OD of the cyano culture. In this case, it was 0,441 at 680 nm.
Then, Disinfect and sterilize all the materials that gonna be necessary.

4 30 ml of culture (OD 0,441) have been caught and then have put into a centrifuge Falcon. (2/3 of the tube)
Take an other Falcon and add water into it (30 ml) to have the same amount in both Falcon.

5 10m
8000 rpm, 24°C, 00:10:00
Centrifuge it

Foams with cyano

6 Having the pellet after centrifugation, add 1 ml of BG-11 in order to resuspend it.

7 Cut a piece of foam so that it fits perfectly inside a syringe. Then put this piece of foam into the syringe (previously disinfected and washed with alcohol or bleach).

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8 Insert the cyano on top of the foam in the syringe and then insert the plunger of the syringe and press down. Cyanobacteria burrow into the foam.

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