Interview with Mr Guillouet

WHO ARE WE INTERVIEWING? (job, studies…)

Stéphane Guillouet is the Head of the Microbial engineering pole and Fermentation Advances and Microbial Engineering Team at TBI (Toulouse Biotechnology Institute).

CONTEXT (Why did we do this interview?)

To answer our problem, we had the idea to set up a co-culture between two microorganisms. In order to evaluate the feasibility of our idea, we contacted Mr. Stéphane Guillouet.

INTERVIEW (summary of the interview)

I- ELECTROSYNTHESIS

Substrate for electrosynthesis: CO2, H2 (coming from water electrolysis)

Contact: Specialist in acetogen, bioflim engineering, energy, synthesis and corrosion Contact Benjamin Erable 0534323623

II- CO-CULTURE

a) Yeast

- **Features :**

Yeast full of vitamins will also be full of proteins. We can make yeast to make proteins, so the astronaut doesn't eat GMOs.

- **Strains used :**

Saccharomyces cerevisiae grows aerobically on acetate. If we do this, the yeast won't make a lot of ethanol. It will grow better on ethanol.

- **Risks :**

The microorganism can get rid of the modifications that have been made to it by spontaneous mutation.

- **Timeline**: start by modifying saccharomyces cerevisiae
- **To deepen**:
  - To see if the yeast gets into biofilm.
  - Strain that generates vitamins: look if we don't have strains that allow us to go further in the metabolic pathways. Can we express vitamin D3 in Saccharomyce cerevisiae?
  - Can we make our yeasts and acetogens communicate?

b) Acetogen

- **Experimentation**: We can test in strict and partial anaerobic conditions.

c) How co-culture works

- **Risks** :

Productivity will probably be quite low .

- **pH Correspondence:**

Acetogen works well at a pH around 7 while yeast works best at acidic pH.

- **Nitrogen source:**
    - We can try to recycle urea from astronauts' urine. For this we need to see if acetogens and yeast are able to grow on it and degrade the urea.
    - We also need to see if we can recover the urea from the filter when they recycle the water.

III- PHOTO INDUCTION

- **Problems of photo induction :**
    - Difficult to centralize the light in the center of the reactor in large reactors, not ideal, not the most practical.
    - It is necessary to pay attention to promoter leaks (basal expression level).

- **Alternative solutions :**
    - It is necessary to look at what exists as a promoter in saccharomyces cerevisiae and to see if we can make thermo-induction in saccharomyces cerevisiae.
    - It is also important to consider if induction would not also lower the growth rate.

IV- BIOREACTOR(S) AND SPATIAL CONSTRAINTS

- **Problem of the origin of energy:** solar energy is not present everywhere.
- **Choice of the number of reactors**: One if the acetogen is GRAS (Generally Recognized As Safe) two otherwise
- **Reactor oxygenation :**
    - 1 reactor system: O2 is produced by the electrode and immediately consumed.
    - 2 reactor system: Oxygen must be supplied through a membrane, this means that a simple diffusion through the membrane must be carried out.
    - Ideally we need a reactor without gas phase. The gas must dissolve directly in the liquid phase thanks to membranes.
- **Prerequisite for the implementation of the reactor :**
    - The reactor must be super hermetic so that no drops come out of the reactor.
        - The system must be robust enough to withstand take-off and travel.

- **Recycling :**
    - The use of water is a problem because it is a limited resource but we can't do otherwise, so we have to think about recycling. How do you re-inject water and what's left in the system?
    - We can imagine centrifuging and filtering to recover the yeasts and we can reinject the medium.
- **Cleaning constraints :**
    - How are we going to disinfect the reactor? (no bleach, no CMR).
    - We must find a system to stop the experiment, clean and restart the reactor.
    - It is necessary to think about how to implement a continuous system. We are in continuous operation on the flow of yeasts, but the acetogens are in biofilm, we are in continuous operation on the liquid medium but not on the biofilm, so we risk losing productivity. We will therefore need a system for cleaning our biofilms. For the acetogen it is necessary to change the biofilm every 3 months. Acetogens may grow until they die. Some strains can be kept in a state of maintenance for a very long time.
- **Agitation systems**: if you have a biofilm, do you agitate on both sides?