PBR 1250L Inoculation

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Healthy Cultures
The PBR is only as good as what you put in it. If you inoculate it with a contaminated culture, the PBR will be contaminated. There are "tricks" you can do to postpone an inevitable crash, but you cannot prevent it. Taking time up-front following sterile technique and our recommendations will pay you back in longevity and productivity of the culture.

When growing algae it is important to prevent contamination by taking as many steps possible to diminish the chance of contamination. Working with microbes is a numbers game: every time you open a culture to the world you introduce a small chance it gets contaminated. Every time you handle a culture it should be done in a way that minimizes the risk of contamination. You can use tools like laminar flow hoods, transfer hoods, flame, UV light, alcohol and many others to lower the risk of contamination. Combining some of these tools can be a good idea to avoid contamination of a culture.

Inoculum Carboy
Recommendations:

- Sterilize the culture vessel and the media inside (Autoclave it all together if possible)
- Use hydrophobic 0.2µm filters on the inlet and vent
- Install full shut-off sterile quick disconnect fittings to attach it to the PBR

Bacteria
Some people prefer to grow axenic cultures of algae. This means growing a culture that is free from living organisms other than the species required (ie your species you want to grow). Using an axenic culture to inoculate the PBR is a fantastic way to keep bacteria out, but it may not be reasonable to achieve in most production environments. It is far more reasonable to grow a carboy that has some bacteria, and is free of pathogens than it is to get a truly axenic culture and for most intents and purposes this is good enough.

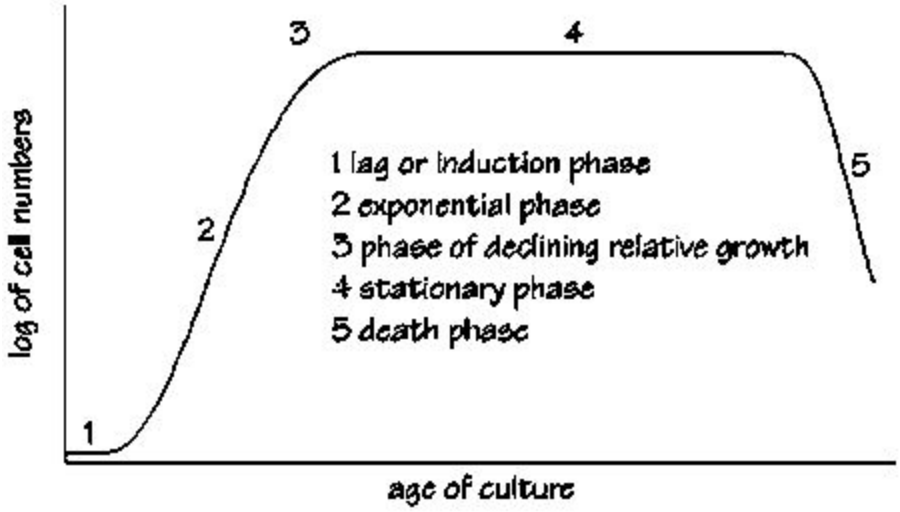
Bacteria can have beneficial, neutral, or harmful effects on algae growth and downstream processes and their effect largely depends on how the bacteria live. Incorporating probiotic bacterial cultures into your algae can decrease the abundance of pathogenic bacterial by limiting the niche space available for pathogens. Probiotics can be especially beneficial when circumstances prevent you from properly following algae protocols, which are made to reduce the risk of contamination.

Training your inoculum
Algae can be trained to perform better. By using serial dilutions over time and exposing it to conditions that you want it to grow well in, you can select for algae that grows faster under those parameters. To train algae for the PBR, it is ideal to grow your inoculum under the PBR's spectrum of LED lights, and at the temperature and pH you would like to run the PBR at. It can take generations to properly train algae, but the production out of the PBR makes it worth doing eventually.

Nutrients
Nutrients are the biggest source of variability in PBR productivity. We recommend using Proline's F/2 Part A and B. If you're growing diatoms you need a source of Silica as well. For our diatom cultures we make a stock solution made from Anhydrous Sodium metasilicate at 99g dissolved into 1L of distilled water. We then add each of Part A, B and the silica solution at a 1:1:1 ratio. This ensures that Silica is in excess in the media. If you intend on using any other nutrient media please be aware that the volumes referenced are not relevant and your volume of nutrient added **must be adjusted accordingly**. If you are using Sodium metasilicate pentahydrate or Sodium metasilicate nonohydrate you will need to use 172g or 231g, respectively instead of 99g of anhydrous sodium metasilicate.

Nutrients can be a source of contaminants so it is important to filter or otherwise sterilize the nutrients prior to being added to the culture media. If you choose to autoclave your nutrients, you should be aware that the vitamins will get denatured by the heat, so will need to be added after autoclaving. Also, if autoclaving, you must seal the containers that hold the nutrients and install hydrophobic air filters, so that air replacing the liquid leaving the containers does not bring contaminants with it.

Inoculation with Diatoms
You should have 20-30L of healthy inoculum in a carboy equipped with full shutoff sterile quick disconnect fittings. The algae used for inoculating the PBR should be quite dark in colour and free of lumps or foam when you visually inspect the carboy. Ideally it would have been trained with rapid dilutions (every ~3 days) and near the end of its exponential growth phase.



http://www.fao.org/docrep/003/W3732E/w3732e06.htm

The PBR should be cleaned properly and prepared with media (nutrients and saltwater) in a ratio of 10:1 media to algae volume in the inoculum carboy. Follow the instructions in the manual and ensure to follow sterile procedure when transferring the algae into the PBR. We recommend starting with at least 20L of inoculum into 200L of media (200L of saltwater, with 100mL of each of Part A, B and silica solution).

Scale-up with Diatoms
We add all three nutrients (Part A, Part B and Silica solution) at 0.5mL/L of saltwater. After inoculation we start the culture with a saltwater flow rate of 10LPH (~2.5 GPH) and match that with 5mL/hour dosed from the dosing control box (underneath the main control box).

Harvest
Once the reactor is full we recommend that you keep the flow at 10L/hour but adjust the nutrients to 1.0mL/L (10mL/hour) on the dosing control box until you get up to a the harvest-density desired. Once at harvest-density you can increase the saltwater flow rate. Ensure you're still dosing roughly at 1mL/L of new water coming in. It will require some trial and error to find the optimal output of the PBR but 400L/day flowthrough is a conservative amount to aim for.

Lighting
For the lighting we try to increase the light as rapidly as possible without causing damage to the cells. Once we start seeing a growth rate on the main page (usually midway through day 1, the 1st day after inoculation), any time that number is less than 0.020, we add 1% light. It should respond with an increase to the growth rate within 2 hours. We increase the light by 1% at a time until at ~20% light then we change to 3% increments until 50%, then to 5% up to 75% then 10% increases to 100%.

Other parameters
We recommend

- Air-flow of 55 SCFH on the air control dial
- A pH setpoint of 8.0 with closed loop control on
- Temperature set on the chiller at 24 Celsius