Possible Sources of Culture Failures Easily Eliminated

Inoculum Contamination

PBRs are designed to take a clean inoculum and automatically scale it up while maintaining a biosecure environment for the algae.

There are two primary means that the culture can be contaminated by the inoculum.

1. The inoculum used to inoculate the PBR itself is contaminated.
    a. Following proper sterile procedure for transferring cultures is the main way to avoid contaminated inoculum. There are a number of tools that make sterile transfer easier and more reliable:
        i. Autoclave - to sterilize glassware and growth media. Having a large enough autoclave to sterilize a carboy helps ensure sterility.
        ii. Bunsen burners (or flames) - to sterilize the mouths of glassware while transferring cultures and media.
        iii. Laminar flow transfer hood - helps establish a clean environment in which to perform culture transfers without introducing contamination.
        iv. Latex / nitrile gloves - help prevent introduction of contaminants from improper hand cleaning. It provides a much smoother surface to clean with alcohol when compared to bare hands.
        v. Hepa filtered positive air pressure around stock cultures - this helps keep contaminants away from flasks area in general.
        vi. Air conditioning - slows bacterial growth down, especially in warmer climates.
2. Contamination is introduced during the inoculation process.
    a. Equipping inoculum carboys with fQDCs to connect with the PBR's harvest pump is a simple way to ensure biosecurity is maintained during the transfer.
    b. Inoculum carboys should also be equipped with inlet air filter and vent filter (0.2 µm) to ensure that when the air replaces the culture leaving the vessel it doesn't contaminate the remaining culture in the vessel.
    c. Avoid drawing in sediment from the inoculum as it can provide a substrate for bacterial growth.

Ineffective PBR Cleaning

Ineffective PBR cleaning is another means of culture contamination. Chlorine cannot penetrate biofilms, so unless all biofilms are removed prior to moving to chlorine sterilization you can't be sure that the tank is actually clean.

1. Having both an acidic and basic biofilm removal agent will increase the efficiency in cleaning the PBR. We recommend having both muriatic acid and CIP 100 (from Steris) at your disposal.
2. Measuring the proper amount of cleaner for volume in the PBR increases the efficacy of cleaning by having the correct concentrations.
    a. Using the 75 L fill line with the proper volume of cleaning agent helps ensure you have the correct concentration in the PBR.
3. Make sure to close chlorine bleach containers after using and use new bottles to ensure the active ingredients will not dissipate. Chlorine bleach loses its active ingredients over time.
4. Ensure you use the correct amount of chlorine bleach for its concentration of sodium hypochlorite. The recommendations in the user manual are for 12%, so using the same amount of 4% bleach will not clean the PBR properly.

Nutrient Limitation

Algae cultures are very sensitive to nutrient limitation and this is the leading cause of crashes in the PBRs behind user introduced contamination.

1. Check that all nutrients (that are supposed to be going into the PBR) actually are when dosing nutrients.
    a. Seized nutrient tubing can stop flow if the nutrient pumps have been left with tubing in for long periods of time without use.
    b. The nutrient filters may clog over time with particulate in nutrients. The first sign of this is the larger diameter silicone tubing will swell when dosing nutrients.
        i. Replacing the filter will restore flow of nutrients.
        ii. Another option to avoid clogging is completely removing the filters. For this, nutrient concentrates must be pre-sterilized (autoclave) and kept sterile with air filters and sealed containers. Note that vitamins can easily denature in the autoclave, so it is best to add vitamins after autoclaving nutrients.
    c. Not adding enough major nutrients (nitrogen, phosphorus and silicate for diatoms) can cause clumping and crashes. This can be avoided by following recommended concentrations and generally running algae cultures nutrient replete. It is better to have healthy algae with too many nutrients than it is to have none at all and in almost all cases once algae from the PBR is diluted into another system the nutrients will be negligible. Dilution is the solution to pollution!

Fouling

When algae is grown in unfavourable conditions it will often cause fouling and may cause it to clump together. This may lead to a culture crash if it is not caught quickly.

1. pH fluctuations can cause clumping and flocculation. The closed loop pH control on the PBR should maintain the pH within a very tight tolerance (0.1 pH units). Fouling can cause the pH probe to read poorly and take longer to respond further exaggerating the effects of the pH fluctuations and crashing the culture. In the same regard if the CO2 supply runs out and the pH rises significantly it will cause a crash.
2. Over-aeration of sensitive cultures (generally flagellates) will cause excess shear stress on the cells and cause them to lose their flagella and can lead to a scum line developing on the top waterline as well as excess settling of dead cells. Though algae are very diverse staying between 20-40 SCFH for sensitive cultures is generally safe. Some cultures are so sensitive that they should not be aerated at all, in which case you'd need a stirring mechanism.
3. High temperatures can cause the cells to grow slower and even die. The other side effect of higher temperature is faster bacterial growth. Algae growing very quickly autotrophically can double up to 3 times per day, but bacteria can double up to 3 times per hour. Keeping a stable temperature in a range that is ideal for your strain of algae will keep culture from crashing due to heat. For most species of aquacultured algae, 18 ºC - 24 ºC is a safe range with slower growth starting above 25 and cell death starting around 27 ºC - 30 ºC generally.
4. Cultures will naturally adhere to the surface of the PBR over time. One way to maintain proper function of the Optical Density (OD) Sensor is to schedule regular harvests of 1-2 L every two hours (12 x / day). Because the harvest pump draws water from the lowest point directly after the OD Sensor this pulls out settled cells and cleans the sensor faces.

Software

We're constantly working to make our software more reliable and easy to use. This eliminates bugs and adds functionality. Having the most up-to-date code is a very easy way to ensure it is as easy as possible for you to effectively and easily culture algae. Write us with your software version found on the Advanced Settings screen in the lower right and arrange a time to update if your running an older code.