

Algae Culturing

Rami Naddy <naddyrb.tre@gmail.com>

Mon, Jan
20, 11:13
AM

to me

conversation between Amanda (TRE) and Shannon (ND) about D. viridis growth rate.

----- Forwarded message -----

From: **Amanda Bidlack** <bidlackac.tre@gmail.com>

Date: Mon, Jan 20, 2020 at 10:56 AM

Subject: Fwd: D. viridis culture

To: Rami Naddy <naddyrb.tre@gmail.com>

----- Forwarded message -----

From: **Shannon Jones** <sjones32@nd.edu>

Date: Tue, Dec 3, 2019 at 1:17 PM

Subject: Re: D. viridis culture

To: Amanda Bidlack <bidlackac.tre@gmail.com>

Hi Amanda,

We normally culture at 120 ppt. I'm looking into why that jug was at a lower salinity - I'd been relying heavily on undergrads while I worked on a massive experiment for the last month, and one of them may have adjusted the salinity in error.

D. viridis grows very slowly. I set up a 1 gallon jug generally two to three months before I want to use it (generally I set it up at 1-2ug/l chl a, and after 2 months it's around 200-250 ug/l chl a). It doesn't grow fast.

Sorry that that may not be the answer you're looking for, but feel free to ask me whatever questions you have.

Shannon

On Sun, Dec 1, 2019 at 9:51 PM Amanda Bidlack <bidlackac.tre@gmail.com> wrote:

Hi,

I had a couple follow up questions. The jugs we received from you measured at 40ppt, is that where you normally culture? Also, how much do you expect the algae to grow in a week?

Thanks

On Tue, Nov 19, 2019 at 1:30 PM Amanda Bidlack <bidlackac.tre@gmail.com> wrote:
Thanks for the help!

- What temperature are you keeping your cultures at?

We are culturing at about 10C. They are in a walk in cooler and the temperature is pretty stable between about 9 and 12 degrees.

- How much are you aerating them (ex, 1 bubble every second, constant stream, etc.)

They are aerating at a constant stream.

- When you feed, what are you feeding and how much?

They are getting feed 2ml of nutrient stock per liter once a week. The nutrient stock is 10% blue solution, 10% P/N solution and 80% RO water.

- What are you using for your media?

The media is 120 ppt brine made with moton's water softener salt and instant ocean.

- What size containers are you using to enclose your cultures? Are they relatively closed systems?

Most of the cultures are in glass 1 gallon jugs. The smaller volumes may be in flasks, we only have one of those going at the moment. The jugs have a lid which the airline passes through, and there is a small hole in the lid to allow air to escape, but it is pretty closed. I have attached a picture of the current set up.

- When you receive a shipment of *D. viridis*, how are you treating it? Are you decanting and getting it bubbling immediately?

Yes, when we receive them, we get them bubbling right away. Depending on volume, it would either go into a flask or a jug. Typically under a liter goes into a flask and over a liter goes into a jug.

- Do you ever start new cultures, transfer cultures, or split old cultures? Or are you just trying to progressively grow what you have?

Whenever the volume on a culture in a jug starts to get near the capacity (probably when it is about 3L), I split it off into two containers. If a container seems to be developing a build up on the sides, I transfer the culture into a new container. In general, we are just growing progressively.

- How are you determining how much to dilute the culture you use before feeding it to the animals? If you have 12 L but it's above 40ug/L chlorophyll, you should probably be diluting it.

Prior to when I took over the culturing, a series of chlorophyll extractions vs absorbance levels was measured to establish the relationship. Now, when we are feeding the animals, I measure the absorbance and plug it into that formula and it tells me how much to add to get the desired test concentration. For the 150ml containers, this has typically worked out to be around 7ml of algae per testing vessel per day.

Thank you for sending the 4L, that will be extremely helpful. I'll get it aerating when we get it.

On Tue, Nov 19, 2019 at 12:37 PM Shannon Jones <sjones32@nd.edu> wrote:
Hi again,

How are you determining how much to dilute the culture you use before feeding it to the animals? If you have 12 L but it's above 40ug/L chlorophyll, you should probably be diluting it.

Shannon

On Tue, Nov 19, 2019 at 9:45 AM Shannon Jones <sjones32@nd.edu> wrote:
Hi Amanda,

First of all, I'm going to overnight you ~4L of *D. viridis* today. It should arrive tomorrow, I'll give you the tracking number once it's ready to go out.

Gary and I spoke yesterday, and I have some questions for you.

- What temperature are you keeping your cultures at?
- How much are you aerating them (ex, 1 bubble every second, constant stream, etc.)
- When you feed, what are you feeding and how much?
- What are you using for your media?
- What size containers are you using to enclose your cultures? Are they relatively closed systems?
- When you receive a shipment of *D. viridis*, how are you treating it? Are you decanting and getting it bubbling immediately?
- Do you ever start new cultures, transfer cultures, or split old cultures? Or are you just trying to progressively grow what you have?

If you'd rather, we can discuss this on the phone.
Shannon

On Fri, Nov 15, 2019 at 7:24 PM Amanda Bidlack <bidlackac.tre@gmail.com> wrote:
Thanks.

We are trying to generate as much as possible right now. Currently we have about 12L and we will burn through that pretty quickly with the brine shrimp tests, so we are trying to stay ahead.

Right now, I feed it once a week and add about 10% more media each week.

On Fri, Nov 15, 2019 at 7:11 AM Shannon Jones <sjones32@nd.edu> wrote:

Hi,

Slow growth is fairly standard here as well. What volume are you trying to get to, and what rate are you using the culture at?

On Thu, Nov 14, 2019 at 5:22 PM Amanda Bidlack <bidlackac.tre@gmail.com> wrote:

Hello,

I am working on the *D. viridis* cultures for TRE. Our cultures have been maintaining well, but we are seeing very slow grow, so Rami suggested I reach out to you and see if you had any techniques to speed up the process. We were considering aeration with CO₂ as a possibility.

Thanks,
Amanda

--

Amanda Bidlack
Quality Assurance Manager/ Project Specialist
TRE Environmental Strategies, LLC
T [970.416.0916](tel:970.416.0916) F 970.490.296
bidlackac.tre@gmail.com

Confidentiality Notice: This electronic message and any attachment may contain confidential and privileged information belonging to the sender or intended recipient. This information is intended only for the use of the persons or entities named therein. If you are not the intended recipient or the agent or employee responsible to deliver this message to the intended recipient, you are hereby notified that any disclosure, copying, use, distribution, or taking of any action in reliance on the contents of this information is strictly prohibited. If you have received this transmission in error, please immediately advise the sender by reply email and delete this message from your system. Thank you for your cooperation.

Yeast (Fleishmans)

Shannon Jones <sjones32@nd.edu>

Thu, Feb
20, 3:23
PM

to Rami, me

Hi Rami and Chris,

Here's the basic protocol we were using for our latest yeast experiments, which were done in individual 400 ml jars. Let me know if you have any questions!

Shannon

Brine shrimp should be fed a dissolved yeast solution once every two days.

1. Fill a 1L beaker with 500 ml RO water
2. Add yeast - Stock solution = 1 g yeast in 500 ml RO
3. Add a stir bar to the beaker, and place it on a stir plate for at least 10 minutes, or until the yeast is thoroughly dissolved.
4. Add variable volumes of this mixture to 400 ml brine solution in each bottle:

Volume added to each bottle & mass equivalent

7.5 ml = 10 mg

3.75 ml = 7.5 mg

2.5 ml = 5 mg

1.25 ml = 2.5 mg

0.5 ml = 1 mg

0.01 ml = 20 ug

--

Shannon Jones

Belovsky Lab Research Technician

Lab phone: 574-631-0949

096/098 Galvin Life Science Center

University of Notre Dame

Notre Dame, IN 46556