

Par measurements

11%

13.35

11.65

12.55

2am in= 12 hour dark exposure for thalli-par(13 hours and 48 minutes)

3:48pm wednesday in to the par

Friday at 3:48 am we switch to moderate

par

3:48am August 8 36 hours in,

Par light set to 35%

Before par = 13 hours 45 mins dark

Turning on first set of par 3:48 pm

Air humidity-81.6

Air temp 2.2 degrees

3:48am August 8 36 hours in,

Par light set to 35%

5:27pm

Air humidity-79.7

Air temp- 5.2 degrees celcius

Water 1ml each

2:48pm August 9

Air temp - 4.9 degrees

Air humidity - 80.5%

Par off 4:16pm August 10

1ml of water given to each

Treatment 6 removed from column 10 and placed under uv table

UV A on at 4:18pm-24 hour cycle begins

UV B on at 4:18pm. 64minute cycle begins

Clock indicates 96 hours of pre cycle in total on ipad.

Air temp- 4.9 degrees

Air humidity - 82.2%

August 11

UVB on 4:18pm

Give 1 ml AFTER UVB TIME

Air temp- 5.3 degrees

Air humidity- 82.1%

UVB off at 5:24pm

August 12

Photo pre taken of uv exposed and control

UVB on at 4:18

Air temp- 4.6

Air humidity - 80.4

UVB turned off at 5:22

1ml given to uv exposed
None given to control(sheen)

August 13

Photo taken pre uv

Uvb on 4:18

Uvb off 5:22

1ml to uv exposed

0.5ml to control

Air temp- 2.7

Air humidity - 82%

Photo taken post

August 14

Uvb on 4:18

Uvb off 5:22

Air temp- 4.6

Air humidity 81.4

Pre photos taken

1ml given to control

1ml given to uv dosed

August 15

Uvb on 4:20

Uvb off 5:24

Air temp 2.2

Air humidity 82.4

Pre and post photos taken

1 ml given to control

1 ml given to uv exposed

Dessication ij control apparent, uv exposed far away seem si*ificantly more moist.than close to uv samples

August 16

UVB on 4:18

UVB off 5:22

Air temp 3.1

Humidity 81.8%

Pre and post photos taken

0.5 ml given only to first row uv exposed

Only the outlying square of control given 0.5

All rest were with sheen and moist water pool

August 17

Air temp 2.6

Air humidity 74.0

UVB on 4:18

UVB off 5:22

No water to control 0.5ml selectively given to uv exposed based on moisture - if dry 0.5, if sheen and moist 0ml. Last row received no water.

This is the last day of watering and exposure

○

August 18

UVa off at 9:14 am

Final temp 4.0 degrees

Final humidity 79.8

Final photos taken

Bacteria into -80 at 11:29am

Freeze drier warm up at 12:03pm

Trays in freezer at 12:20pm

Start main drying test at 12:43pm

-80 into dry ice at 2:01pm

Samples out of dry ice at 2:28pm for weighing includes weight of vial

Empty vial - 13.026 no tape or label yes lid

12.9854

13.131

12.866

1. Scytonemin — Stored Set

Treatment 1

D 13.524

F 13.401

G 13.086

I 13.220

J 13.810

Treatment 2

B 13.398

E 13.469

G 13.195

I 13.407

J 13.227

Treatment 3

A 13.638

C 13.472

D 13.739

E 13.878

H 13.141

Treatment 4

A 13.666

B 13.569

F 13.245
G 13.468
H 13.959
Treatment 5
B 13.888
D 13.759
E 13.868
F 14.125
I 13.524
Treatment 6 (Control)
C 13.476
D 13.303
F 13.382
H 13.427
J 12.9934

| 2. Porphyra-334 — Stored Set |

Treatment 1
D 13.317
F 13.142
G 13.170
I 13.696
J 13.226
Treatment 2
B 13.295
E 13.397
G 13.0818
I 13.0988
J 13.3481
Treatment 3
A 14.263
C 13.8515
D 13.9112
E 13.6141
H 13.4074
Treatment 4
A 13.432
B 13.723
F 13.846
G 13.4050
H 13.6542
Treatment 5
B 13.4999

D 13.5043

E 13.3769

F 13.8908

I 13.8738

Treatment 6 (Control)

C 13.1402

D 13.4887

F 13.475

H 13.446

J 13.289

Trays removed from freezer at 3:02 pm

Bacteria put in at 3:06pm Cyanobacteria program

4:12 remove spectroscopy bacteria from dark room and into transport- still in the 0 degree room

At terraluma 5:16 - note. We have 2 calibration panels not 4, i found a 5% as well. We should use the data from china to determine the exact reflectance of the 2 panels in our region of interest so we can standardize.

Xenon warmed up for 25 minutes

Integration times for calibrated 6% didn't work... too bright-makes tiny tiny reflectance for bacteria

Troubleshooted and started again at 10:38pm

250 darks - integration time 665370 us

25 minute warm up

Make note of 5G which was damaged in transit

Observations: perhaps the farther away from source (less potent) signals could be seen more clearly if the sensor was closer to the sample

Lamp off for a couple of hours. I finished treatment 3 and then got coffee around 8 am after an hour of sleep.

Lamp is warming up for 25 minutes

Treatment 4 beginning

REMEMBER TO TAKE A PHOTO OF THE SETUP FOR A CONCEPTUAL DRAWING! Good lighting required!

12:53pm process for removing samples from freeze drier begins

Sample weighing 1:57 pm

1. Scytonemin — Stored Set

Treatment 1

D 13.173

F 13.175

G 13.0281

I 12.9412

J 13.0826

Treatment 2

B 13.0516

E 13.1291

G 13.1039

I 13.2820

J 13.028

Treatment 3

A 13.2522

C 13.0163

D 13.189

E 13.1978

H 12.9739

Treatment 4

A 13.136

B 13.011

F 12.829

G 13.060

H 13.251

Treatment 5

B 13.334

D 13.180

E 13.286

F 13.250

I 12.990

Treatment 6 (Control)

C 13.186

D 12.954

F 13.080

H 13.182

J 12.933

| 2. Porphyra-334 — Stored Set |

Treatment 1

D 12.991

F 13.049

G 13.049

I 13.419

J 13.014

Treatment 2

B 13.0167

E 13.1501

G 13.006

I 13.0066

J 13.1682

Treatment 3

A 13.161

C 13.1926

D 13.1527

E 13.0536

H 12.9352

Treatment 4

A 12.9418

B 13.3463

F 13.3076

G 13.1356

H 13.0261

Treatment 5

B 13.1227

D 13.103

E 12.890

F 13.054

I 13.321

Treatment 6 (Control)

C 12.929

D 13.155

F 13.271

H 13.110

J 13.178

Transported via dried ice and stored in -80 at 2:50pm

Spectroscopy commences: details elsewhere-

25 minute lamp warmup

Lamp off again 7:02pm

Warm up for 25 starting again at 8:28

Done 11:25

HPLC pre-process: 25/8/2025

Glassware in acid wash 10% Hcl at 5:00pm

Glassware flipped 6:22 August 26

Glassware drying in laminar at 11:13 am August 27

Glassware out August 29-

New batch placed in 9:15am

Same settings used :program 2: overnight bake 12 hours at 400

Glassware out at 3:00pm over done 30 August , 2025

First 10 samples removed from -80 at 4:45pm. Each vial placed in a ziplock bag to prevent humidity buildup. Left bench top to allow time for temperature of vials to reach the room 60 minutes left. Then dry weight - vial to weigh boat - Amber vial

2I: 0.087g

2J: 0.065g

1D:0.072g

1F: 0.094g

1J: 0.080g

3D: 0.069g

3A: 0.043g

3H: 0.052g

3E: 0.080g

2B: 0.080g

Sonicated samples are on shaking rack at 7:09 pm . August 30. Air temp- 4.2 degrees

Rpm - 50

Removed t 1:15pm august 31

1D is destroyed, shattered.

Supernatant extract successful for all others. 1D liquid still taken from bottom of centrifuge basket(likely contaminated, we will make a note and test still..)

Sonicate 2 starting at 4:08pm

In shaker 50rpm

Time 4:27pm

Air temp is 4 degrees celcius

Operation rescue the og batch...

We decided to not risk the use of the glass amber vials and their glass stoppers. After the two casualties and the death of replicate 1D... re went with a borosilicate pyrex glass vial and a ptfe lined cap... this does compromise our plastic free workflow but i decided to risk the plastic contamination as opposed to further sample losses..

We have jjust washed the vials and the ptfe lined caps(interior) with tripple wash of our acetic acid, methanol, ethyl acetate solvent mix. 1ml in each shake for 37 seconds, pour out, repeat 3 times. They are drying now starting at 4:27pm. The plan is that after they are dried we will carefully as possible transefer the thalli pellet from the shaker table and the solvent into this new vial and use that in the centrifuge. All future batches will then use these new style of vial and no longer the amber vial. We will be standardizing to the shaker table time however and as such, this will mean almost 24 hours for each, which is unfortunate for timing, but such is life. I will also proceed with a third extraction pass.but this third one will be shorter is the plan. I will then put the supernatant overnight until tomorrow mornign when i will 1, start the rotary evaporator, and 2, begin extraction of the next batch

- both can be done simultaneously,

One complication in this is the transfer to the new vials which may amount to some loss and be a confounding variable in our experimnt. Unfortunate indeed. We will also need to figure out how to get our pellet transferred effectively. Given its shape and structure and the reduced diameter of the new vials, this may prove difficult indeed.

Og batch removed at 5:53 pm sptember 1, to attempt transfer into new vials.

Batch in sonicator at 8:13pm

8:31 pm batch on 50rpm shaker in cooler box. Air temp 4.7 degrees celcius

Off shaker at 12:30pm

Decanting done, superb stent collected, now stored in 4 degree fridge until tomorrow for the rotary evaporation. Time now is 1:11am

4:35

First rotary evaporation - water bath set to 45 degrees celcius. , rotations of the rotrary evaporator aare close to maximum, but a little under.

Sample 3E is first

Paused at 6:10 still not done

New glassware set in oven program 2 at 6:11pm

We will count how many transfers into the proper vial. Then transfer one of them into another for the acetone. Use that proportion. 2mL max is to be used

3E:

200 uL saved separate meaning 1.8mL in vial

Give or take a bit given we are going to near dryness...

We have decided to put it back into the same vial and do a nitrogen blowdown for later resuspending.

Now 3H

Now 2J in progresss

Removing second batch of thalli from fridge: 3:48pm September 3

We will wait 60 minutes for acclimatisation

We are setting up 1J in the rotary evaporator at 4:54pm

I am now weighing out the new samples

4B-0.071g

4H-0.070g

1I- 0.074g

5F- 0.068g

6F- 0.077g

4A- 0.070g

1G- 0.081g

2G- 0.081g

2E- 0.073g

3C- 0.074g

Samples weighed now adding solvent

1J not finished. Removing until tomorrow

Air temp 3.3 degrees

Batch 2 on shaker table 6:50pm

All round bottom vials transferred to -20 freezer for storage. Extract is also stored there.

9:51am September 4, putting 1J back on the evaporator.

1J is now done at 12:25pm September fourth. Now putting 2I onto the rotary evaporator.

Batch 2 is off the shaker table at 12:50pm September 4th. I am now taking them for centrifuging!

In sonicator for 10mins at 2:43 pm

We reran the sonicator for an extra 4 minutes out of paranoia that something happened.

Pass 2

In shaker table 3:03 pm. Air temp 3.6 degrees

September 5th

2B going into roto- evap at 9:42am

2B paused at 11:15am back in -20 degree

2B back in rotary evaporator at 12:15pm

Batch 2 pass 2 removed from shaker 4:37pm September 5. I am now weighing and preparing for centrifuge

Batch 2 is in dominator at 6pm for 10 mins

Batch 2 is on shaker table for pass 3 at 6:15pm

2B done 8:35pm

Taking batch 2 off shaker from pass 3 10:57pm

Batch 2 done and in -20 11:45 pm

The fucked up (spilled) 1D is on the rotary evaporator at 10:42am September 8th

3D is now in at 11:27am

1F is in September 9 10:26am

September , 12:45pm

4B is in

New technique with higher vacuum is to be used after discussion with David. The time per sample shouldn't be hours. It should be on a scale of minutes

4H on at 4:20pm

6F done 5:14

5F done 5:43

4A done 6:08

1I done 6:49

1G done

2G done 11:01am sep11

3C done 11:36am

2E

Just as a reminder for myself the rotary evaporator is a Buchi Rotavapor R-114 R-124. The round bottom flasks used are 100ml Duran

We now need to do our final batch of 10. I am putting a load of glass into the oven after DI water wash.

Then will rinse with methanol

Glass out Saturday the 13th. Removing final 10 + extra 1D from -80 at 2:04pm thaw for an hour

Final batch

1D =0.060g

4G =0.062g

6C = 0.074g

6H =0.046g

5E =0.079g

5D =0.072g

4F =0.080g

5B =0.068g

6D =0.071g

6J =0.071g

5I =0.058g

Now solvent put in 8ml each

Out of sonicator 4:36pm

Into 50rpm shaker 4:40pm

Air temp 3.5 degrees celcius

Remove at 10:46am = 18 hours and 6 minute pass

Removed at 10:59 from shaker. Now measuring for centrifuge

Basket with 6C, 4G, 1D is 230.898g

Basket with 6H, 5E, 5D is 230.915g

Basket with 4F, 5B, 6D is 230.725g

Basket with 6J, 5I, blank is 230.806g

In centrifuge at 11:10am

Into sonicator and now going back into shaking bath at 12:16pm

Air temp 3.9 degrees

18hour extraction. Coming out at 6:16am September 15.

I

Batch 3 removed from shaking table at 6:45am September 15.

Now weighing for centrifuge

In shaker table 8:02am for last extraction pass-4 hours

Air temp- 4 degrees celcius

Removed from shaker table at 4:42pm oops.. thst was a 8 hour almost 9 hour extraction instead of the proposed 4.

Doing the measurments for centrifuge basket

Basket with 6H, 6D, 5B is 231.876

Basket with 5E, 5D, 6C is 233.767

Basket with 6J, 5I, 4G is 231.532

Basket with 4F, 1D, and blank is 233.438

Centrifufed.. transfered.

Now extrzcting from 6:09p,:

5I done

6H done

6C done

6J done

5E done

4G done

5D done

6D done

1D done

4F done

5B done