

Nitrobacter experiment

Have been growing *Nitrobacter winogradskyi* (ATCC strain #) in ATCC480 medium with shaking for several weeks. For the past week, I have monitored the nitrite concentration (chemically) every day and added nitrite (up to 10 mM) every time it exhausted the supply.

3/6/2007

This morning (11AM), I harvested 400 mL culture by spinning in (sterile) GSA centrifuge bottles for 15 minutes at 7000 rpm (room temp). I removed all but ~100 mL, resuspended cells in that and transferred to (sterilized) chamber. Added 700 mL fresh medium. Started bubbling with air (150 mL/min) at noon. Covered with Al foil to protect from light.

[Left the other 400 mL of culture shaking in flasks after adding NaNO_2 to 10 mM final concentration at noon.]

At 4:30PM today, we added ~12.5 mL (sterile) 1 M NaNO_2 to culture. (Now should have about 15-20 mM nitrite.)

At ~5 PM today, we took a ~10-mL sample of culture and filtered with 0.2- μm filter to remove cells. We measured NO_3^- with the nitrate ISE after having calibrated it with 7.14 mM NO_3^- (100 mg/L) and 0.0714 mM (1 mg/L). [NO_3^-] \approx 6.8 mM. The file is called "20070306exp1". (Note: this represents the residual nitrate present in original culture after the 8-fold dilution. Thus, that culture had accumulate ~50 mM nitrate from oxidizing nitrite. Remember that the medium contains 3 mM nitrite, but I had been spiking in extra NaNO_2 every day. This is about what I expected.)

Used the same sample to measure nitrite.

First we calibrated the electrode (after having let it sit ~ 2 hours in NESS = Nitrite electrode storage solution):

- 1) Prepared serial dilutions of NaNO_2 into water: 30, 10, 3, 1 mM.
- 2) Diluted each standard 1:1 with NISS (usually 7 mL + 7 mL)
Also diluted 7 mL of filtered culture with 7 mL NISS (resulting solution was somewhat cloudy, unlike standards).
- 3) Measured voltage from Nitrite-ISE (using Vernier electrode amplifier) of each standard, from lowest to highest.
- 4) Measured voltage of sample – voltage was between that of 30 and 10 mM standards, as expected.

Saved file as "20070306exp2". Also made calibration file (20070306exp2_calibrn). Estimated nitrite concentration in culture to be 15.2 mM.

Left Nitrite electrode in NESS. Left Nitrate electrode in humid bottle.

3/7/2007

Took time points at 11:45AM and 8:45PM and processed similarly:

- 1) Removed ~15 mL culture.
- 2) Filtered through 0.2- μ m filter.
- 3) Measured nitrate in filtrate after calibrating Nitrate-ISE.
- 4) Mixed filtrate 1:1 with NISS and measured nitrite after calibrating Nitrite-ISE with standards.

Basic result: culture is not doing much...

3/8/2007

Took time point at noon and processed as before – nitrite has not convincingly dropped.

Conclusion: culture is dead for some reason. (Centrifugation killed it??)

We decided to check the flasks that we had left in the old medium (+ 10 mM nitrite) shaking in flasks 48 hours ago. The nitrite was gone from the culture – they are still alive!

Estimated volume of culture = ~400-450 mL. Added sterile 1M NaNO₂ to 3 mM final concentration. We added about 200-250 mL of medium 480 (containing 3 mM NO₂⁻) and split the culture into 3 flasks (~200 mL each) and shook in dark at around 12:30PM. We took a sample of culture at 1PM and measured nitrite (no point in measuring nitrate, since we know it will be high). The concentration in the culture was just under 3 mM (see the late time points in file “20070308exp1”).

The last 2 things were done in class. We took a sample of culture at 2:20PM and measured nitrite. We also did a calibration, which is in the Excel file.

At around 4:45 PM we put all the culture into the chamber, and then took a sample to measure nitrite as before. The chamber had air running through at 500 mL/min at first. I started running the exhaust from the chamber through the O₂ and CO₂ sensors at t = ~500 s. Then I hit the bypass at t = ~2100 s. I was not convinced of the measurement, so I reduced the flow rate of air to 50 mL/min to make it more sensitive for detecting CO₂ uptake. First I had it bypassing the chamber (50 mL/min), then I switched to the chamber. The CO₂ slowly dropped. At the time I did not know if it was just the long equilibration with the slower rate (which also takes longer, *because* the rate is so slow), or if it was the sensor drifting (*again*), so I bypassed the chamber again – as you can see, it immediately rose back up, so the drop in CO₂ was real! (You can even see a slight drop in O₂, but I am not sure how much to believe this..., so do the calculation for yourself!) By the way, all the ice had melted by around t = 6400, and then everything began to change, so do not believe any data beyond that point.

Thus, you should only consider the data from “Day 3” for your calculations – try to calculate consumption rates for nitrite, CO₂, and O₂ (if possible).