Cruise Report INT

R/V Thomas G. Thompson 4/20/21 – 05/17/20

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INT (Microbial Respiration)

**Motivation**

Heterotrophic Respiration is the fundamental process by which organisms obtain energy from organic matter and at the ecosystem level represents the largest sink for organic matter in the ocean. (Del Giorgio & Williams, 2005). Understanding the magnitude and variability of microbial respiration is critical for the understanding the metabolic balance of the ocean and the efficiency at which carbon is stored in the ocean. Measurements for respiration are rarely performed due to constraints in methodology and feasibility and thus respiration remains one of the least constrained parameters in contemporary oceanography (Robinson, 2019). However, recent advances in methodology via the Iodo-nitro-tetrazolium (INT) reduction assay have improved the ability to measure microbial respiration in aquatic environments (García-Martín et al., 2019). During this cruise, community and size-fractionated respiration rates were determined using the INT reduction method described below.

**Sampling**

**In Vivo Iodo-nitro-tetrazolium (INT) Reduction Assay**

This method is based on the reduction of INT, a water soluble, membrane permeable salt which passively penetrates into the cell, by dehydrogenase enzymes in the electron transport system forming insoluble formazan crystals (INT-*f*) (Martínez-García et al., 2009). The *in-vivo* method is based on a variation of the *in-vitro* method described by Packard et al., 1996. Whole seawater was collected from 5m using the ship’s underway seawater system at 30 stations across the transect. Five 250 ml polycarbonate bottles were rinsed with sample water and filled, two of which were immediately killed with 0.2 filtered formalin (2% v/v final concentration) and used as a control. The remaining 3 bottles were inoculated with 8mM INT solution to a final concentration of 0.2 mM. Samples were incubated within 1 degree of *in situ* temperature for 2-2.5 hours and subsequently fixed with formalin. Because the INT-*f* is formed internally, cells can be size-fractionated post-incubation and the INT-reduction rate determined for different size classes, specifically bacterial (0.2-0.8µm) and non-bacterial (>0.8µm). During A22 samples were filtered sequentially through a 0.8 and 0.2 μm polycarbonate filter which were then stored in 2ml sterile cryovials at -20°C until further analysis. Samples from A22 were stored on board until being shipped back to an in-shore laboratory at the University of California, Santa Barbara. The method uses the spectrophotometric absorption of the INT-*f* at 485 nm to determine the rate at which the insoluble crystals are formed inside the cell membranes. Absorbance of each sample will be determined by incubating the filters in 1 mL of 1-propanol, followed by sonication and centrifugation. The concentration of the INT-*f* in solution is calculated from its absorbance by applying a standard curve previously determined using twelve different concentrations of stock INT-*f* dissolved in pure 1-propanol.

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| --- | --- | --- | --- | --- | --- |
| Stations Sampled | | | | | |
| 2 | 10 | 14 | 18 | 20 | 22 |
| 32 | 34 | 38 | 41 | 42 | 44 |
| 48 | 50 | 52 | 54 | 58 | 62 |
| 64 | 68 | 70 | 72 | 73 | 74 |
| 76 | 78 | 80 | 84 | 86 | 88 |

**References**

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