Department of Geosciences

Development of a "Smart" Tracer

Microbial Respiration in Stream Sediment and the Resazurin-Resorufin System

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SUMMARY

Tracers are used for gathering information in hydrologic study but conventional tracers are limited to quantifying the average movement of water. It is our ambition to develop a "smart" tracer to measure ecologically relevant processes within moving stream water, such as the time spent in metabolically active versus inactive zones. We are working with resazurin, "Raz", a weakly fluorescent blue-colored, redox-sensitive dye which undergoes an irreversible reduction to the fluorescent red-colored resorufin, "Rru" when exposed to mildly reducing conditions. Previous research has shown that the decay of Raz and concurrent production and decay of Rru can be used as a "smart" tracer to detect metabolic activity in stream sediment for measuring the transient storage of nutrients.

We hypothesize that the reaction rate of Raz to Rru is proportional to respiration. We test this hypothesis by measuring the Raz reaction and respiration rates in sediments from the same stream under different temperature conditions.

Fig. 1 ISCO autosampler and DO/EC sonde at the last sampling point of the stream reach during a Raz injection in HJA at WS 1. Notice the water is tinted red due to the presence of Rru.

This experiment will utilize Raz as a "smart" tracer in attempt to model seasonal variations in the rate of Raz to Rru reduction in stream sediments. By employing a temperature controlled water column we can vary the respiration rate of microorganisms living in stream sediments without changing the system. The information gathered from these experiments will be critical in the development of Raz as a "smart" tracer and associated methodology for ecohydrological studies.

Fig. 2 Filtering samples during the Raz field experiment at WS1 in HJA.

REFERENCES

Haggerty, R., A. Argerich, and E. Martí (2008), Development of a "smart" tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: The resazurin-resorufin system, *Water Resour:Res., 44, W00D01*, doi:10.1029/2007WR00607.

Haggerty, R., E. Martí, A. Argerich, D. von Schiller, and N. B. Grimm (in review), Resazurin as a "smart" tracer for quantifying metabolically active transient storage in stream ecosystems, *Journal of Geophysical Research – Biogeosciences.*

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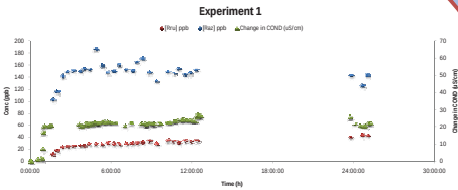
PRELIMINARY RESULTS

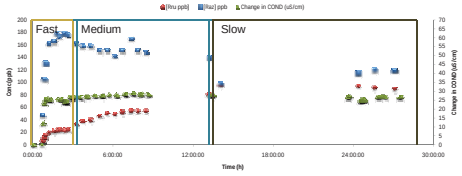
Experiment 1

Flow Rate	18.92 mL/h
Solution Temperature	21.8 ± 0.62°C
***Note : Recirculating heater-cooler not used and flow rate did not change	



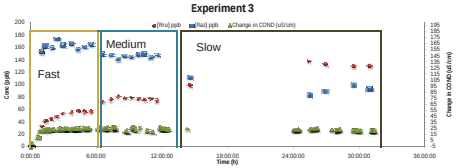
Experiment 2

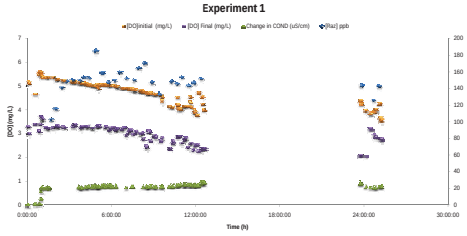
Flow Rate	*Fast*	33.2 mL/h
	Medium	20.4 mL/h
	Slow	5.05 mL/h
Solution Temperature	20.8 ± 0.23°C	

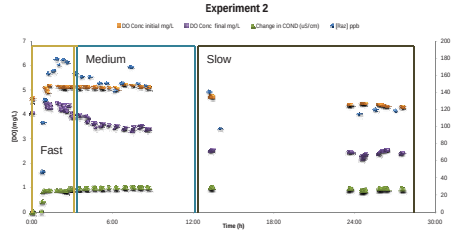


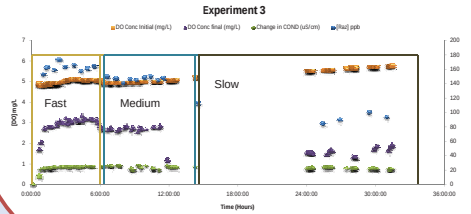
Experiment 3

Flow Rate	*Fast*	30.1 mL/h
	Medium	18.8 mL/h
	Slow	4.26 mL/h
Solution Temperature	10.83 ± 0.21 °C	

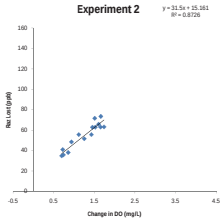


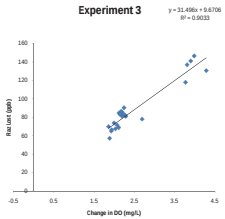






Experiment 3 yielded the largest production of Rru despite being run at 10 °C likely because that is a more optimal temperature for hyporheic activity than 20°C.





The above data shows a strong correlation between the production of Rru and increase in oxygen consumption. Additionally oxygen consumption and Rru production increase as the flow rate decreases.

METHODS

We are using flow-through column experiments to quantify the reaction and transport of Raz and Rru. Sediment is collected in the field and packed underwater in low-pressure chromatography columns then returned to the laboratory. We are using sediments and water from Oak Creek (44.567 N, 123.301 W) for all trials.

The experiment will be run in a temperature-controlled environment at approximately 25°C, 20°C, 15°C, 10°C, and 4°C to represent realistic environmental conditions.

Fig. 3 From left: data logger interface, recirculating heater-cooler, temperature controlled chamber, valve control panel and peristaltic pump

To equilibrate the column at specific temperatures the column, tracer, and water are placed in a temperature-controlled chamber for a minimum of 12 hours prior to each experiment. The column, tracer, and water remain in the chamber throughout the experiment. The chamber controls temperature using a recirculating heater-cooler to pump heated or chilled water through cooper tubing located on the inside of the chamber. Both the air temperature and tracer temperature are monitored during the experiments using thermistors.

Fig. 4 Uncovered column inside the temperature controlled chamber

A peristaltic pump is used to create flow through the column. Three flow rates are used for each experiment at approximately 5 mL/h, 15 mL/h, and 30 mL/h to optimize the amount of data collected. The flow rate is changed from higher to lower after 5 to 7 samples are collected at plateau. Plateau is determined from dissolved oxygen (DO) measurements taken at the outflow of the column

For each experiment a solution of Raz and NaCl in Oak Creek water is pumped through the column. The same approximate flow rates will be used for each experiment; however a balance is used to monitor exact flow rates. DO concentration and electrical conductivity are monitored at the input and output of the column. As Raz is photosensitive, the tracer reservoir and column are shielded from light with aluminum foil. Water samples are taken manually at regular intervals dependent on the flow rate. Samples are immediately filtered, refrigerated, and stored in the dark until fluorescence measurements of Raz and Rru can be taken. This occurs within 24 hours of the end of the experiment . We will complete three trials of live sediment and one trial of killed sediment for each temperature condition.

Fig. 5 Covered column and solution container following an experiment

Future trials will run repetitions of live sediment at the same time using 3 columns reducing variation between trials.