Autotrophinator: A device for kinetic analysis of Autotrophic Organisms

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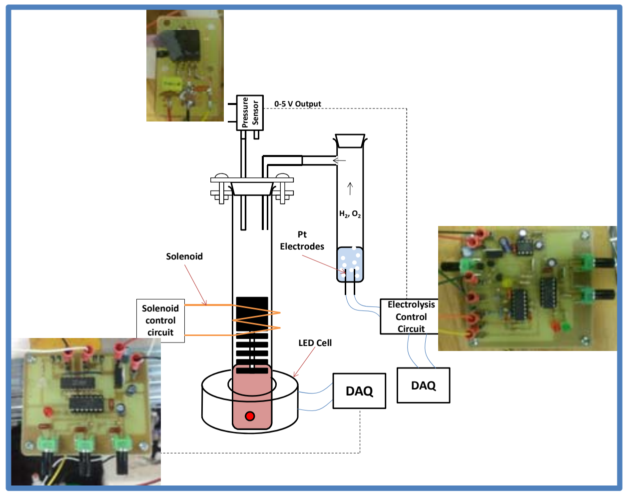
Background: Chemolithoautotrophic growth (growth on H_2, O_2 and CO_2) is difficult because of two major issues. One is *safety* – the mixture of H_2 and O_2 is explosive over the useful range of concentration. The second is *gas-liquid mass transfer*. Since >90% of the nutrients needed by the cells come from the gas phase, they need to be transferred to the liquid phase at a very high rate to match the rate at which they are consumed by growing cells. For these reasons, literature data on the kinetic parameters of autotrophic organisms are scarce. We have tried to design a device that could be used without too much difficulty to measure the important parameters, such as growth rate and yields, on different gases for an autotrophic organism.

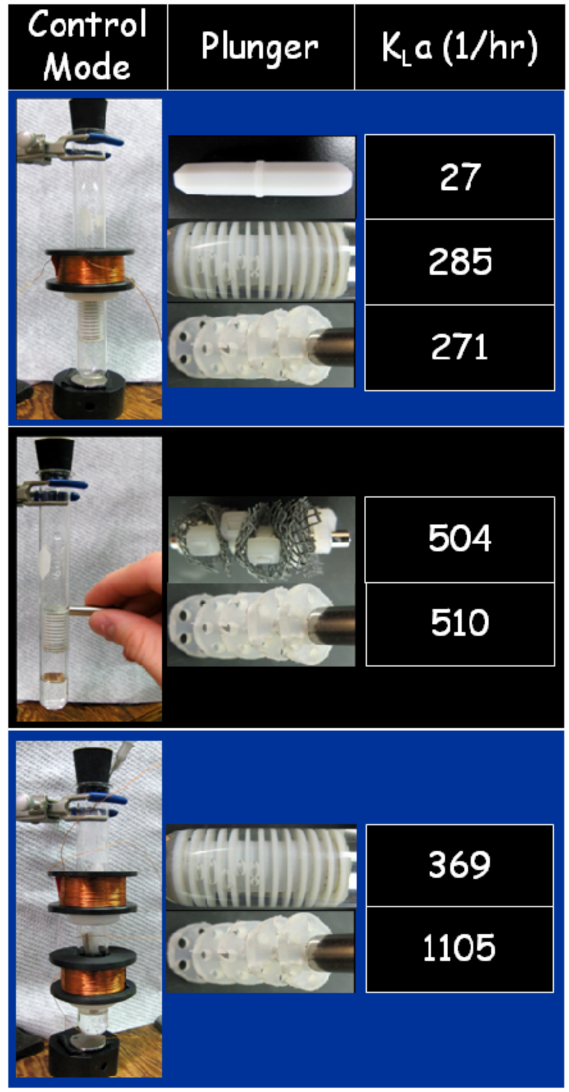
Description: There are three parts to the autotrophic growth device (aka "autotrophinator") that carry out three distinct functions – measuring growth kinetics (LED cell), delivering autotrophic gas mixture safely (electrolysis) and achieving very high mass transfer rates (solenoid-bobber system). Data acquisition systems can collect Optical Density (OD), pressure and electrolysis current data for calculation of kinetic parameters.



LED cell (growth kinetics): The LED cell measures the OD by shining an LED of the desired wavelength into the culture and measuring the intensity on the other side via a photodiode. As the cells grow up, the culture becomes more opaque to the light. By recording this change in light intensity (and therefore the OD) the growth rate of the cells can be calculated.

Solenoid-bobber arrangement (gas-liquid mass transfer): A solenoid around the culture tube causes the magnetic bobber inside to oscillate up and down and cause vigorous mixing and mass-transfer from gas to liquid phase. After a preset interval the bobber is held up by the solenoid while the OD reading is recorded by the LED cell. The frequency of oscillation of the bobber, the interval and length of the stop for OD reading are all adjustable via an electronic circuit that controls the current to the solenoid. There is also an output from this circuit to instruct a data acquisition device to start recording the data (with a 15 second delay) once the solenoid has stopped.

Pressure sensor-electrolysis arrangement (yield calculation): A pressure sensor connected to the headspace of the culture sends signal to a control circuit which controls the electrolysis current. If the pressure signal is below a setpoint, the electrolysis is triggered and stays on until the upper pressure setpoint is crossed. The upper and lower pressure setpoints as well as the electrolysis current (when it is triggered) can be adjusted via potentiometers on this circuit. It has outputs for pressure sensor voltage and electrolysis current for a data acquisition device. A gas phase material balance can easily be carried out by knowing the total electrolysis current, the initial and final headspace compositions and volume. This information can be used to calculate the yield on different gases.



Mass Transfer Improvements: To simulate the high rates of mass transfer anticipated in scale up, the mass transfer coefficient in the autotrophinator had to be drastically improved from the initial prototype. Two strategies were employed in order to enhance the mass transfer coefficient: changing the oscillation pattern and improving the design of the bobber.

Oscillation pattern improvements: The initial circuit and solenoid system functioned by pulling the bobber up and allowing it to drop back down into the liquid by means of gravity. Because this system did not push the bobber into the liquid with a strong force or provide a high frequency of oscillation, a system was considered in which forces were applied in both the upward and the downward directions. The utility of this feature was demonstrated by a manual (literally by hand) mode of operation in which the mass transfer coefficient was shown to improve. The two solenoid set up that followed allowed for an automated, high-rate-of-mass-transfer system that applied upward and downward forces. The final set up employed an improved circuit design using only one solenoid and allowed for the same upward and downward forces of the two-solenoid set up.

Bobber improvements: Various bobbers were constructed with the intention of improving mass transfer in several ways: creating thin films on the test tube walls, introducing small bubbles into the liquid phase, and pulling droplets of liquid into the gaseous phase. It is worth noting that a given bobber will not necessarily be the best performer under all operating conditions; ie. certain bobbers are better suited for high frequencies. As an example, the two bobbers tested with the two-solenoid set up demonstrated significantly different mass transfer coefficients even though they demonstrated very similar coefficients with the single-solenoid set up. The reason for this is that the finned bobber “bounced” off the surface of the liquid and did not produce the same degree of mixing as the other.