PH CONTROL OF *MONORAPHIDIUM MINUTUM* GROWN IN A BATCH CULTURE ENVIRONMENT[1]

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Abstract

Research at the National Renewable Energy Laboratory (NREL) is being conducted to develop low-cost technology for coupling the exhaust from fossil fuel-fired power plants to the growth of microalgae for the dual purpose of recycling carbon dioxide emissions and producing a renewable fuel source. Microalgae are unicellular photosynthesizers with tremendous potential for rapid division (growth) and high lipid production. The carbon assimilated into lipids provides a renewable source for biodiesel fuel. Experiments are being conducted to explore the growth rates of microalgae exposed to simulated flue gas. Interest is focused on the effectiveness of pH control for regulating gas dosage to optimize cell growth. Preliminary results using the species, *Monoraphidium minutum*, indicate no advantage to this method.

Introduction

The planet earth with its atmosphere of greenhouse gases (carbon dioxide, water, ozone, methane, and nitrous oxide) is unique among the other planets in this solar system. Indeed it is the existence of this atmosphere that supports the life forms of the planet. Recent industrialization, though, has dramatically increased levels of fossil fuel burning and deforestation. The combination of these two activities has significantly increased the atmospheric levels of greenhouse gases. This increase of atmospheric gases is disrupting the delicate energy balance maintained by the sun and the earth's atmosphere. Heat from the sun reaches the earth's surface and part of this energy is reflected back into space, while the earth's atmosphere traps the rest of this energy. As atmospheric levels of gas increase, so too does the atmosphere's ability to trap the infrared radiation, causing the energy equilibrium to be reached at increasingly higher temperatures on the surface of the planet. This process is known as the greenhouse effect.

The National Renewable Energy Laboratory (NREL) in Golden, Colorado, is conducting research to minimize carbon dioxide emissions. The goal of this research is to develop low-cost technology for coupling the exhaust of fossil fuel-fired power plants to the growth and cultivation of microalgae. The carbon dioxide generated by these power plants would serve as the primary carbon source for these photosynthetic microorganisms. Once this carbon is assimilated, the algae may then be co-combusted in the power plant, or converted to biodiesel - a substitute for conventional diesel fuel. Either method allows for efficient recycling of the carbon.

The selection of microalgae stems from its tremendous potential for rapid division (growth) and high lipid production. In addition microalgae are the best photosynthetic carbon dioxide assimilators with yields of biomass per acre threefold to fivefold greater than that from typical crop plant acreage (Neenan, 1986). The ultimate goal of this project is to develop a zero-cost method of reducing the amount of carbon dioxide

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contributed to the atmosphere by fossil fuel-fired power plants. A recent economic analysis performed by the laboratory (Zeiler, 1995a) indicates that increasing growth rate is the most important parameter for the attainment of this goal. This economic model allows comparisons between microalgal technology and other currently used carbon dioxide mitigation technologies. Model predictions state that "for an assumed cost of \$66/t for CO_2 collection, the overall CO_2 mitigation cost for microalgal technology is \$45.6/t for a targeted lipid content of 50% and a cell productivity of 45 $g/m^2/d$ (Zeiler, 1995a). When compared with other CO_2 remediation methods such as absorption with MEA (monoethanolamine) (Herzog, 1994) this cost is competitive.

Bench scale exploration of parameters including selection of algal species, determination of environmental tolerances, and genetic enhancement of lipid production and growth rates is now underway. Additional study is needed to simulate, on a small scale, the natural environments for algal growth that will be utilized in the implementation of this emerging biotechnology. The information outlined by this document represents work in progress on one small aspect of this exciting project - pH control of batch microalgal cultures.

Project Overview

Microalgae are photosynthesizers, meaning they require carbon dioxide (CO_2), light and water for survival. As CO_2 is supplied to an algal batch culture, carbonic acid is produced and the pH of the aqueous media is lowered (becomes more acidic). As the algae utilize the CO_2, carbonic acid is removed and the pH rises (becomes more basic). The need to feed the algae and the solution chemistry of this process led to the inception of a pH control-based feeding process. Figure 1 illustrates the general setup of the microalgal pH control system. The microalgae are grown in a vessel containing aqueous growth media which simulates one of various ground waters from different geographical areas. Within the growth vessel is a pH probe and a port for introducing gas into the medium - a process called sparging. The pH probe is connected to a pH meter. The output voltage from this meter is sent to a data acquisition and control program via an I/O
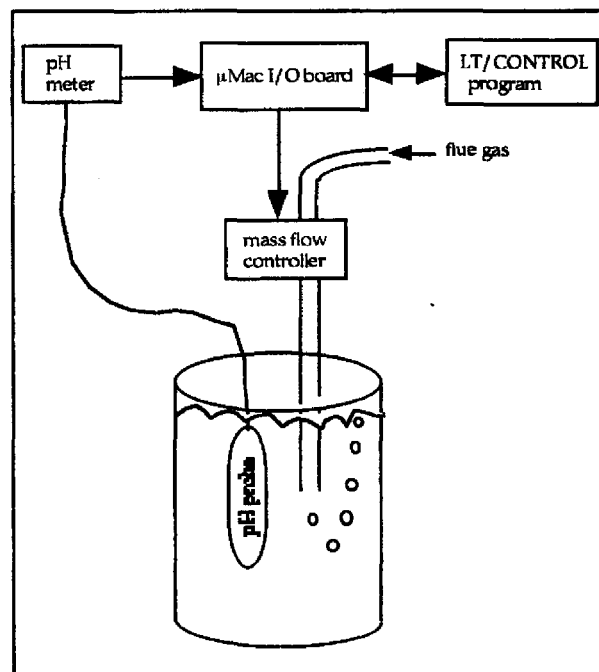

Figure 1. Simplified pH control system showing growth vessel with pH probe and gas sparging apparatus, both of which are monitored by a data acquisition and control system.

board. The software was programmed to process the input pH data and send output to a mass flow controller (MFC) which meters gas into the vessel.

As the block diagram in Figure 2 relates, the system uses closed-loop control since feedback comparison is present. The system works as follows: (1) A desired pH is preset as a constant input. The actual pH of the system (2) is then subtracted from this value. The result is called the error (3) which is sent to the control system (4). From the control system, an output voltage (5) is sent to the MFC. This voltage regulates the flow of gas into the vessel (6) which changes the pH of the system. This change is then detected by the pH probe and the loop begins anew.

Figure 3 shows the laboratory configuration. Experiments utilize two types of mixed gases, a simulated flue gas and a control gas. Some cultures receive control gas which contains 13.6% CO_2, 5% O_2, balance N_2. The remainder of the cultures receive simulated flue gas containing 1990 Clean Air Act Amendment (1990 CAAA) levels of sulfur and nitrogen oxides. This simulated flue gas specifically contains
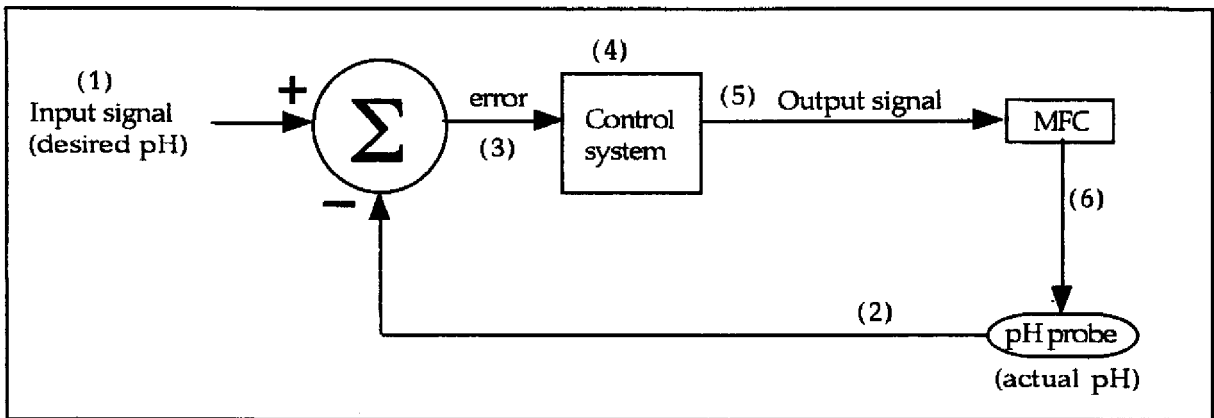
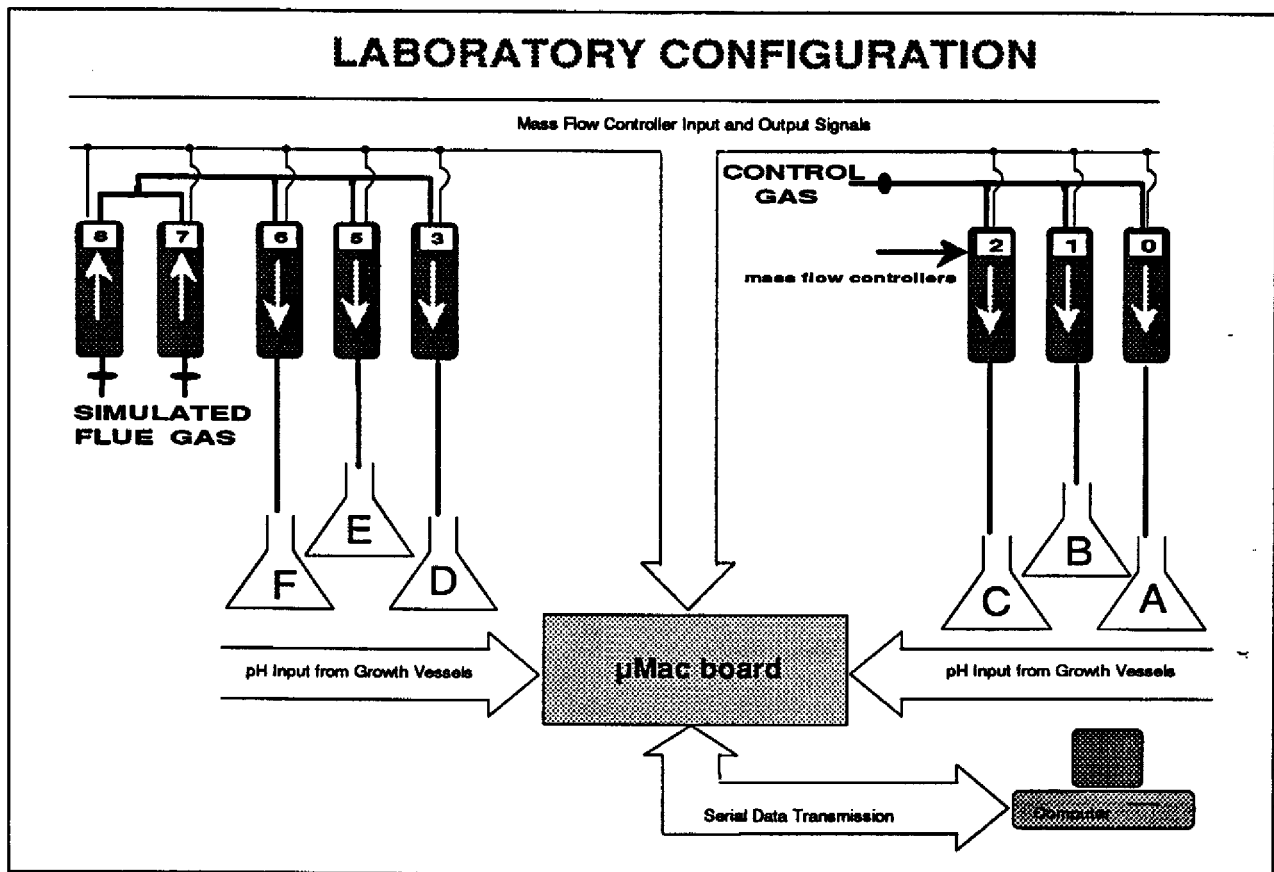Figure 2. Block diagram of a closed-loop pH control system.

LABORATORY CONFIGURATION

Mass Flow Controller Input and Output Signals

CONTROL GAS

mass flow controllers

SIMULATED FLUE GAS

µMac board

pH Input from Growth Vessels

pH Input from Growth Vessels

Serial Data Transmission

Figure 3. Laboratory set-up used for pH control. Mass flow controllers deliver gas to growth vessels based on pH.

0.015% NO, 0.02% SO_2, 13.6% CO_2, 5% O_2, balance N_2. Experiments must be performed in a hood since components of the simulated flue gas are toxic. Six growth vessels are used. Three of the vessels receive simulated flue gas, while the other three receive control gas. Each vessel is sparged by an MFC. Additionally two MFCs are required to mix the component parts of the simulated flue gas.

The MFCs are hard wired to an I/O board and

3

controlled by data acquisition and control software. pH meters are similarly wired to input channels of the I/O board.

Electrical Control

The electrical system is also shown in Figure 3. Analog I/O functions are accomplished using the μMAC-1050 from Analog Devices which supports 16 analog input channels and two analog output channels. Eight additional analog output channels are available on an STB-AOT expansion panel to control the MFCs. RS-232C communication mode is used which is a standard communications protocol for asynchronous serial data transmission using a baud rate of 9600.

The software used for the pH control process is LT/CONTROL by LABTECH. The software runs on a x486 PC. LT/CONTROL uses both icon and menu-based programming which is well suited for modularity of program design. For each of the six vessels, the routines are very similar since for each pH, data must be collected, compared to a preset value, and an output voltage to the MFCs calculated.

As noted above, gas is delivered to the algal growth vessels by MFCs. One MFC feeds one individual growth vessel. Thus six MFCs are required for the growth vessels and two additional MFCs are required to mix the simulated flue gas. The eight total MFCs are MKS type -1259C. The MFCs are calibrated for nitrogen flow from the factory. The two mixing MFCs have a range of 0-200 sccm N_2 while the six remaining MFCs have a range of 0-100 sccm N_2. Both ranges correspond to 0-5 VDC Set Point Input values. The MFC compliance to this set point input voltage is monitored by a flow signal output voltage.

pH measurements within each of the six growth vessels are obtained with autoclavable pre-pressurized gel-filled pH electrodes manufactured by Mettler - Toledo Process Analytical, Inc. These electrodes are connected to portable digital pH meters manufactured by Jenco Electronics, LTD. The pH meters provide 0.01 pH resolution. The analog output from these instruments is 100 mV/pH (0 to 1400 mV for 0 to 14.00 pH). These output voltages are connected to the μMAC-1050 analog input channels and provide the actual pH signals.

pH Control

The goal of this project is to explore the potential benefits of pH control on microalgal cell growth. Figure 2 shows that feedback control is used in this project. The actual pH of the system (batch culture vessel) is compared to a desired pH. The difference (error) between the actual and desired values dictates whether or not gas sparging should occur. The two types of closed-loop control that have been targeted are On-Off and Proportional control (P-control).

On-Off control is the simplest form of control. A desired pH (or set-point pH) is selected based on the physiological optimum of the pH species. High and low threshold pH values are also selected. When the pH of a culture vessel rises above the high threshold pH value, the MFC is activated, and gas flows into the system. Sparging continues until the pH within the vessel drops below the low threshold pH value. The difference between the turn-on and turn-off pH values is called the hysteresis or dead band. Hysteresis is used to prevent noise from switching the MFC rapidly and unnecessarily when the pH is near the set-point. For this project, different pH ranges were tested to see if growth was affected.

A proportional controller attempts to maintain tighter control than an On-Off controller. A voltage, V, is applied to the MFC in proportion to the difference in pH between the culture vessel and the set-point.

$$V = P \times (pH_{set} - pH_{culture}) \qquad (1)$$

P represents the proportional gain of the controller.

Experimental Results

To date three experiments have been conducted to determine the effectiveness of pH control on microalgal growth. These experiments used a green alga, *Monoraphidium minutum* (NREL Strain Monor02). The lighting scheme for the three experiments was 12 hours of light followed by 12 hours of darkness. Lighting was provided by Cool White fluorescent bulbs at an intensity of approximately 200 mE $m^{-2}s^{-1}$. The cultures were continuously stirred with a magnetic stirrer at 150 rpm and the temperature was maintained at 25°C. The three vessels sparged

with control gas were labeled A, B, and C. Similarly vessels D, E, and F were sparged with simulated flue gas. The initial inoculation levels for the first two experiments was approximately 2.8×10^6 cells/mL. Increased levels of nitrate and phosphate were added to the initial growth medium as suggested by Zeiler et al. (1995a).

Experiment 1

The initial experiment ran for 144 hrs. (6 days), and examined only On-Off control. The main goal of this experiments was to determine an optimum dead band range for maximized growth. Vessels A and D were maintained at a pH range between 8.95 to 9.05. Vessels B and D were maintained at a pH range between 8.9 to 9.1. Cultures C and F were maintained at a pH range between 8.75 to 9.25. The sparging flow rate to all six growth vessels was approximately 10 mL of gas per minute. The flow rate was chosen to be as low as possible within the accuracy of the MFCs. A low flow rate is important because it allows for better absorption of the gas into the growth media. Approximate flow rates were calculated using formulas provided by the MFC manufacturer. Verification and fine-tuning of flow was performed empirically using a soap bubble flowmeter. Since it is important to know the amount of carbon delivered atmospheric pressure must be considered with volume measurements. The average atmospheric pressure at NREL's altitude is approximately 82.1 kPa.

Cell density growth curves are shown in Figure 4.

Table I contains a summary of biomass
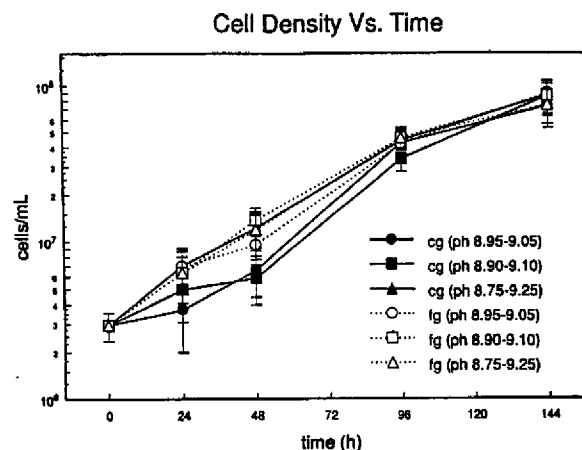

Cell Density Vs. Time

Figure 4. Cell density growth curves. Closed symbols represent vessels A, B, and C which were sparged with control gas. Open symbols represent vessels D, E, and F which were sparged with simulated flue gas.

data. Ash-free dry weights (AFDW) are measured in grams per liter and are obtained by subtracting a weight of ash from a dry weight value. Theoretically this biomass data represents the weight of the organic content of the algae with approximately 50% of this weight contributed by carbon. This ratio was verified by subsequent elemental analysis (unpublished results). From these ash-free dry weight values, the increase in biomass has been calculated.

Figure 5 shows a representative graph of data collected during the first 24 hours of Experiment 1 for growth vessel A. Superimposed

Table I. The Increase in Biomass (as Ash-Free Dry Weight) of Cultures Exposed to Control Gas or Flue Gas Under Different pH Control Regimes (Experiment I).

	Grams per Liter **	Grams per Liter	Fold Increase	Grams per Liter	Relative %	fold increase (from t=24)	fold increase (from t=0)
Culture* A	0.112	0.272	2.43	1.08	100	3.97	9.64
B	0.128	0.252	1.97	0.88	82	3.49	6.88
C	0.116	0.236	2.03	0.88	82	3.73	7.59
D	0.128	0.228	1.78	0.92	85	4.04	7.19
E	0.124	0.248	2.00	1	93	4.03	8.06
F	0.132	0.264	2.00	1.08	100	4.09	8.18
time, h	0	24		144			

* Cultures A, B & C were exposed to control gas. Cultures D, E & F were exposed to simulated flue gas.

Cultures A & D were controlled between pH 8.95-9.05, B & E between pH 8.9-9.1, and C & F between pH 8.75-9.25.

** Biomass values are the average of 4 replicates with all values having a standard error of <5%.
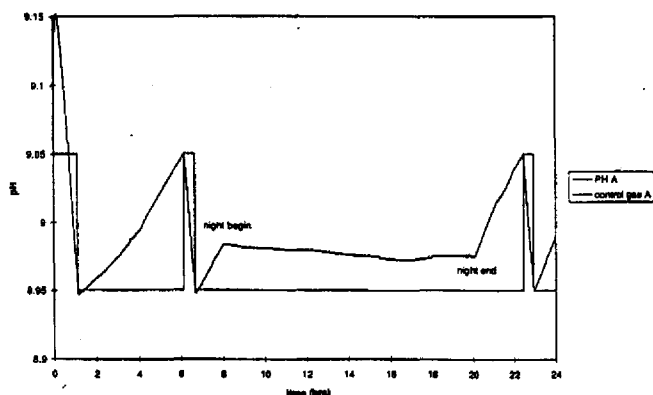
pH vs. Time for Vessel A

Figure 5. pH trends for first day of Experiment 1 for vessel A (control gas). Pulse wave represents output to the MFC.

on the pH data is a pulse wave that represents the voltage output to the MFC. The initial pH was above the high threshold (pH 9.05) thus the MFC was initially triggered on at a flow rate of approximately 10 mL control gas per min. The pH in vessel A then proceeded to decrease until the low threshold value (pH 8.95) was reached. At this point the MFC was triggered off. Recalling that the experiment alternated between light and darkness, the middle region of the graph indicates little change in pH. The data collected at "night" thus provides a visual confirmation that photosynthesis does, indeed, require light.

Experiment 2

Experiment 2 ran for 166 hrs. (7 days). As in Experiment 1, pH for vessels A and D were On-Off controlled between 8.95 to 9.05, and vessels C and F were On-Off controlled between a pH range of 8.75 to 9.25. The sparging for vessels B and D, however, was not pH controlled. Instead a timed gas delivery regime was used. This timed delivery was intended to match the carbon delivery used in previous experiments (Zeiler, 1995b). The delivery contained an amount of carbon sufficient to achieve an estimated yield of approximately 1g dry wt. biomass $L^{-1}day^{-1}$. Since the vessels used in this experiment were larger than those in the past, a revised timed delivery regime to provide the same approximate grams of carbon per volume was developed. At the 10 mL per minute flow rate this regime alternates between approximately five minutes of sparging followed by approximately nine minutes without sparging. Final cell densities are summarized in Table II. Ash-free dry weights and fold increase data are also included.

Experiment 3

Experiment 3 ran for 216 hrs. (9 days). This experiment examined On-Off control, timed gas delivery (no pH control), and P-control. Vessels A and D were maintained between pH 9.01 to 8.99 with On-Off control. Vessels B and E maintained the same sparging regime as in Experiment 2. Vessels C and F used P-control

Table II. Final cell concentrations and biomass (AFDW)accumulations for cultures exposed to either control- or simulated flue gas with delivery controlled by timed schedule or pH range(Experiment II).

	Grams per Liter	Grams per Liter	Fold Increase	Grams per Liter	Fold Increase (from t=0)	Grams per Liter	Fold Increase (from t=0)	Grams per Liter	Relative %	Fold Increase (from t=24)	Fold Increase (from t=0)	Cells/mL*** (t=168)
Culture*												
A	0.108	0.164	1.52	0.296	2.74	0.568	5.26	1.21	84	7.4	11.2	8.54E+07
B	0.108	0.168	1.56	0.372	3.44	0.688	6.37	1.44	100	8.6	13.3	1.02E+08
C	0.108	0.164	1.52	0.304	2.81	0.612	5.67	1.22	85	7.4	11.3	1.02E+08
D	0.108	0.172	1.59	0.312	2.89	0.628	5.81	1.28	89	7.4	11.9	9.21E+07
E	0.108	0.180	1.67	0.328	3.04	0.700	6.48	1.36	94	7.6	12.6	1.12E+08
F	0.108	0.152	1.41	0.260	2.41	0.560	5.19	1.06	74	7.0	9.8	8.24E+07
time, h	0	24		48		96		168				

* Cultures A, B & C were exposed to control gas. Cultures D, E & F were exposed to simulated flue gas.
  Cultures A & D were controlled between pH 8.95-9.05, B & E by timed gas delivery, and C & F between pH 8.75-9.25.

** Biomass values are the average of 4 replicates with all values having a standard error of <5%.

*** Cell counts are the average of duplicates counted in duplicate (20 counts per sample) having a std. error <10%.

6

with a set point value of pH 9.00 and a proportional value of 1. Flow rate values for vessels A, B, D, and E were again 10 mL gas/min. Flow rates for vessels C and F varied during the experiment. Difficulties concerning the accuracy of the MFCs at low flow rates and the software implementation of P-control were encountered, and still must be resolved. The inoculation level for all six growth vessels was 0.028 g/L (AFDW). Final AFDW values were 0.99 g/L for vessel A, 1.08 g/L in vessel B, 1.04 g/L for vessel C, 1.04 g/L for vessel D, 1.04 g/L for vessel E, and 0.99 g/L for vessel F.

Conclusion

Only basic observations and preliminary conclusions may be drawn from the three experiments. The data from Experiment 1 (Table I) indicates that the cultures grew best (as measured by AFDW) in control gas having delivery controlled in the narrowest pH range. The interpretation for the control gas seems to indicate that more delivered gas results in increased growth. In vessels D, E, and F which received simulated flue gas, however, cells grew best with sparse gas delivery which occurred in widest pH range (vessel F). This is probably because they were exposed to less SO_2, which is toxic to the algal cells.

The data from Experiment 2 (Table II) is more interesting in that it suggests that On-Off pH control regimes tested to date are not as efficient in stimulating growth (and thereby carbon assimilation) when compared to sparging procedures used in the past. This timed delivery system supplied a much greater amount of gas to vessels B and E than was delivered to any of the pH controlled vessels. As can be seen, this extra gas severely dropped the pH in these vessels. Thus the benefit of surplus carbon seems to outweigh the negative effects of a lower pH - at least for this organism. The data is quite significant in that the timed delivery resulted in from 15% to approximately 25% greater growth for both control and simulated flue gas treated cultures.

The data for Experiment 3 also indicate greater growth for the timed delivery system in vessels B and E. The distinction in this case, though, is less clear. Indeed the algae show no clear preference for any sparging regime or mixed gas.

If future work yields similar results, it will be necessary to reevaluate the need for pH control of gas dosing altogether. The implementation of P-control must still be perfected. Different ranges for on/off control should also be attempted. Ultimately data collected should be used for system identification to provide a model to guide further pH control directions. Most importantly other algal species should be used to verify that trends found for *Monoraphidium minutum* are universal. It is expected that this is not the case, and that the response of other species that are more sensitive to pH and simulated flue gas may differ significantly.

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