Bioprocessing Fermentors Assembly

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Introduction

Biochemistry and biosystems are still relatively new fields with great demand in engineering. There are many opportunities for advancement and prosperity within this area for new engineers just coming out of school. Once students leave college so much can be done and learned, but it is still somewhat early in their development to get them started during their undergraduate years. With so many new things to learn and tackle, that is important to get these motivated students started as early as possible on undergraduate research. Little steps need to be taken to reach the goal of involving undergraduate students in research. This starts with a few motivated students and faculty working to lay down the groundwork to be followed by other interested individuals. Within biochemistry applications there is lots of research that stills need to be done and can involve undergraduate students. Starting up laboratories with specified and new equipment will help this along. One example would be using fermentors to grow microorganisms and even some macroorganisms. The organisms can be studied and manipulated to do certain functions and be used in other experiments.

Undergraduate Student Research Project: Fermentor System

During the 2011-12 academic year, and continuing in the current year, effort was spent on setting up and getting a new set of fermentors up and running for a bioprocessing lab. They are now all up and running, but there are still a few kinks to iron out. This new lab will help in teaching those Chemical Engineering students who hope to minor in biology. This lab will be a great way to get hands on experience in the area that these students will be going into and working at once they leave college and head into the real workforce. The fermentors could also be used to take in projects or conduct research from outside companies that don't have the capability themselves, and can be used for senior design projects within the foreseeable future. All these activities are a great way to introduce this technology to students and to help them start learning about this technology earlier than before.

The new fermentors were just pulled out of the box. Everything had to be hooked up on the electrical systems so they would work, and they had to be hooked up through a computer so that they could be controlled all together on a collaborative system. The three fermentors were each a 2.5 L BioFlo 110 Modular Benchtop Fermentor. The fermentors are run through a console to allow for individual control of each fermentor, and a computer is used to record the data for each batch or use of the fermentors. The collected data can be viewed at a later time and even printed out. The fermentors were setup and during the last couple weeks of the semester they were ready for testing.

Setting Up the Fermentor System

Before the fermentor test it was necessary to make sure everything was up and running. This meant hooking up all of the inlets and outlets of the fermentor systems. Fresh air from a builing compressed air line was run through a filter and regulator; and then run through the control console and into the fermentors. An inlet to the fermentors for water was also installed. To do this, deionized water was routed from the builing utility service through a regulator and filter and then through the console. The deionized water is used in the temperature control of the system to cool down the fermentor by running fresh water through cooling coils, which is done automatically by the system itself. The cooling water was piped out of the system from the fermentors and into a fume hood to be as safe and cautious as possible. A nitrogen tank was also used and hooked up through the air system to be used when a researcher wants to run an anaerobic fermentation or for when the calibration of the dissolved oxygen (dO_2) probe was needed.

In order to ensure that everything was safe and running before the final test, multiple intermediate tests were run to make sure every step along the way worked. This involved running a test of regular tap water in the fermentor vessel and pumping in pure nitrogen and filtered air at different times to not only calibrate the DI probe, but to ensure that it wasn't damaged in packaging and that it would continue working for extended periods of time. Hooking up the cooling water also tested the heating and cooling system and the heating jacket and heating the fermentors to a certain temperature and then cooling them down to another predetermined temperature. Multiple thermometers not connected to the control console were monitored to make sure that everything in the control and data recording system worked and was calibrated properly. This was done both with and without the agitation motor running to ensure that the agitation motor was also operating properly. These tests were done following strict safety guidelines and most worked without any issues. After this initial equipment testing phase, a more realistic final test was done by running a full scale fermentation and charting the collected data that was recorded with the computer hooked up to the system.

Experimental Testing of the Fermentor System

To test out the fermentor system a simple solution of Red Star Quick Rise Yeast was grown. The yeast was first inoculated overnight for 20 hours at 30ºC at about 300 rpm in an incushaker. There was 300 mL of growth medium and yeast grown in the incubator. Then the inoculate was mixed in the fermentor with another 1750 mL of growth medium. For the growth medium a mixture of powders were mixed in the water. The growth medium was purchased with funds from the NSF S-STEM grant program. The growth medium contained 10 g/L of D-glucose, 1.5 g/L of yeast extract, 4.8 g/L of ammonium sulfate, 0.75 g/L of potassium phosphate, 0.24 g/L of magnesium sulfate, and 0.036 g/L of calcium chloride. When the inoculate and growth medium were mixed in the fermentors they were set at 30ºC, 300 rpm, and air was pumped in at 5 L/min and a pH of between 3.5 and 4 was maintained for the length of the experiment naturally by the yeast. The fermentation was run for 5 hours and data collected to show that there was substantial growth of the yeast.

Data Analysis and Discussion

To measure the growth of the yeast, and thereby prove that the fermentors do indeed work, the dynamic method was used. This is a simple way to measure the growth, since the fermentors have a probe to measure the amount of dissolved oxygen (dO_2) in the solution. The incoming air was stopped for a short period of time, and a measurement was taken of how long it took the dO_2 to drop from about 21% by mole, where it is when it is well supplied with air, to about 10%. This decrease in dissolved oxygen is due to the consumption of oxygen by the yeast as it grows. It is important not to go any lower than about 10% dO_2 because this could possibly damage the yeast growing in the tank. There is growth of the yeast if the amount of time that it takes to lower the dissolved oxygen content is less over time, which means there is a larger amount of yeast present in the fermentor and consuming oxygen. After a dO_2 measurement, air is returned to the system (21% O_2 content) for a period of time, until air is again shut off and the time recorded that it takes the dO_2 to fall to10%. The test did prove to grow the yeast sufficiently and the data is shown in Figure 1. Figure 1 shows a steady decline in the amount of time needed for the dO_2 measurement to fall from 21% to 10%, as the time of day increases, which means additional yeast is present in the latter parts of the experimental run.

Figure 2 shows the fermentors as they were used in this experiment. They had a 2.5 L capacity glass tank. The fermentor has many different ports that can support many attachments. The dO_2 probe monitored the amount of dissolved oxygen. The pH probe kept an accurate measurement of the pH level in the tank, and this actually dropped slightly over time during this experiment, from about 4 to about 3 by the end of the experiment. The temperature was monitored with an automatic thermometer, and a motor ran the agitator in the tank that was controlled by the console. These were all monitored and controlled by the panel shown in Figure 3. Figure 4 shows the fermentor and its control console together as they were operated regularly next to each other.

Future Use of the Fermentor Systems

Now that these fermentors have proven to work, they can be used in many different ways to grow many different things. This will greatly help to give an edge to students at this university who are looking to go into biochemistry after graduation. These fermentors can be used in bioprocessing classes or in senior design projects with real world applications for companies outside of the campus. This outsourcing of the work is cheaper and can be helpful at times, especially when the projects are not a top priority for the company and lots of time is available. This experience of setting up these fermentors has been great and creates a good familiarity with the equipment and what it takes to install and setup new equipment. It definitely gives a leg up in this field and also instills a confidence to go out and work in the field later on without hesitation. This can also help to improve the university by giving more options and giving a greater depth in the education of this specific topic.

Conclusions

Biochemistry is definitely on the rise in industry, especially with all of the production of food, pharmaceuticals, and biofuels. This calls for a greater demand for chemical engineers with experience in this area. The earlier this training can be started the better and setting up bioprocessing research labs is a great way to do this. Fermentors are a key component of any bioprocessing lab. The assembly and use of these in a lab can be a great teaching opportunity. It is important to test and ensure that this equipment is working properly though before too much use and all of these steps can be a great learning opportunity.

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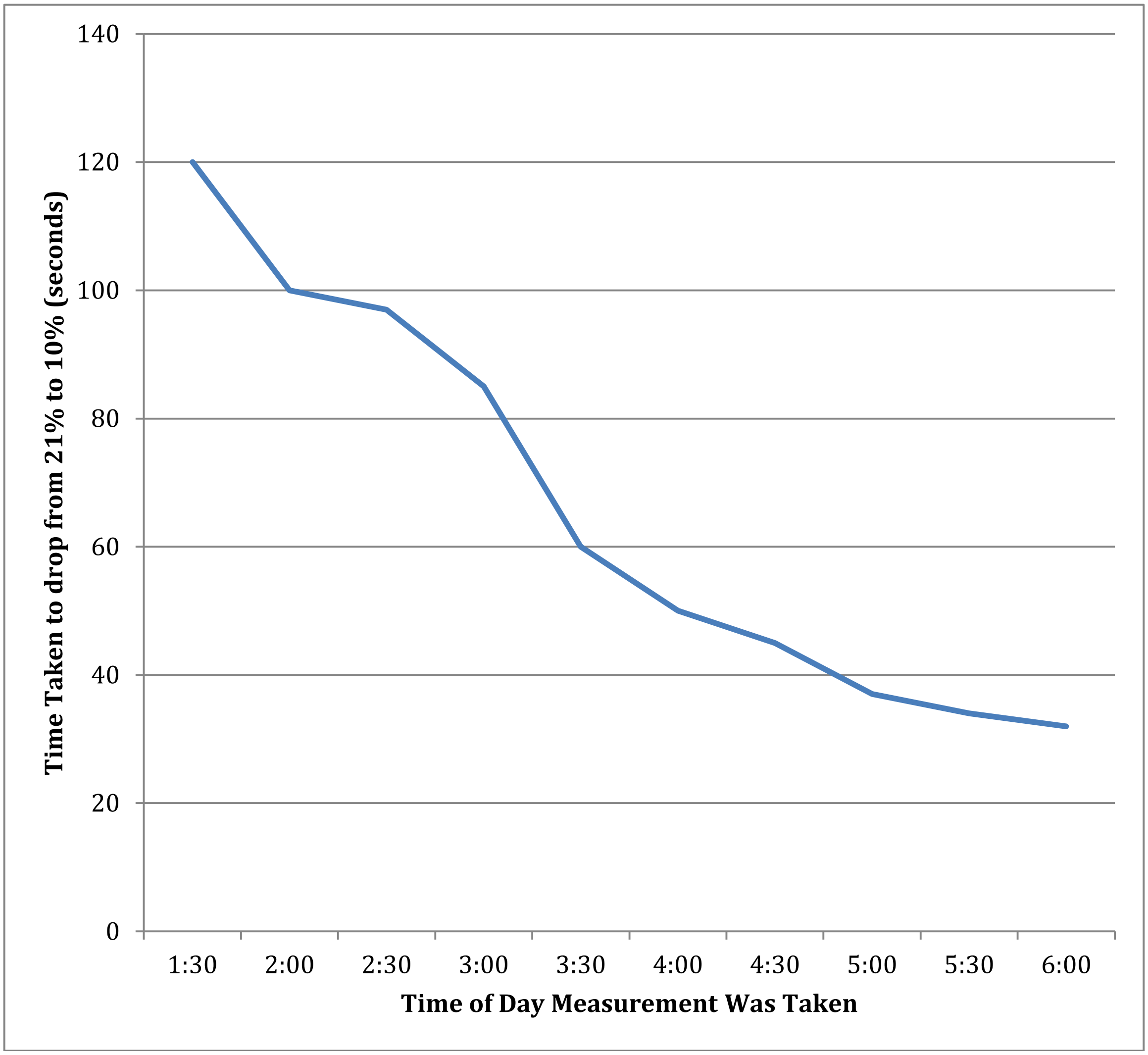

Figure 1: Growth of Yeast in New Fermentors

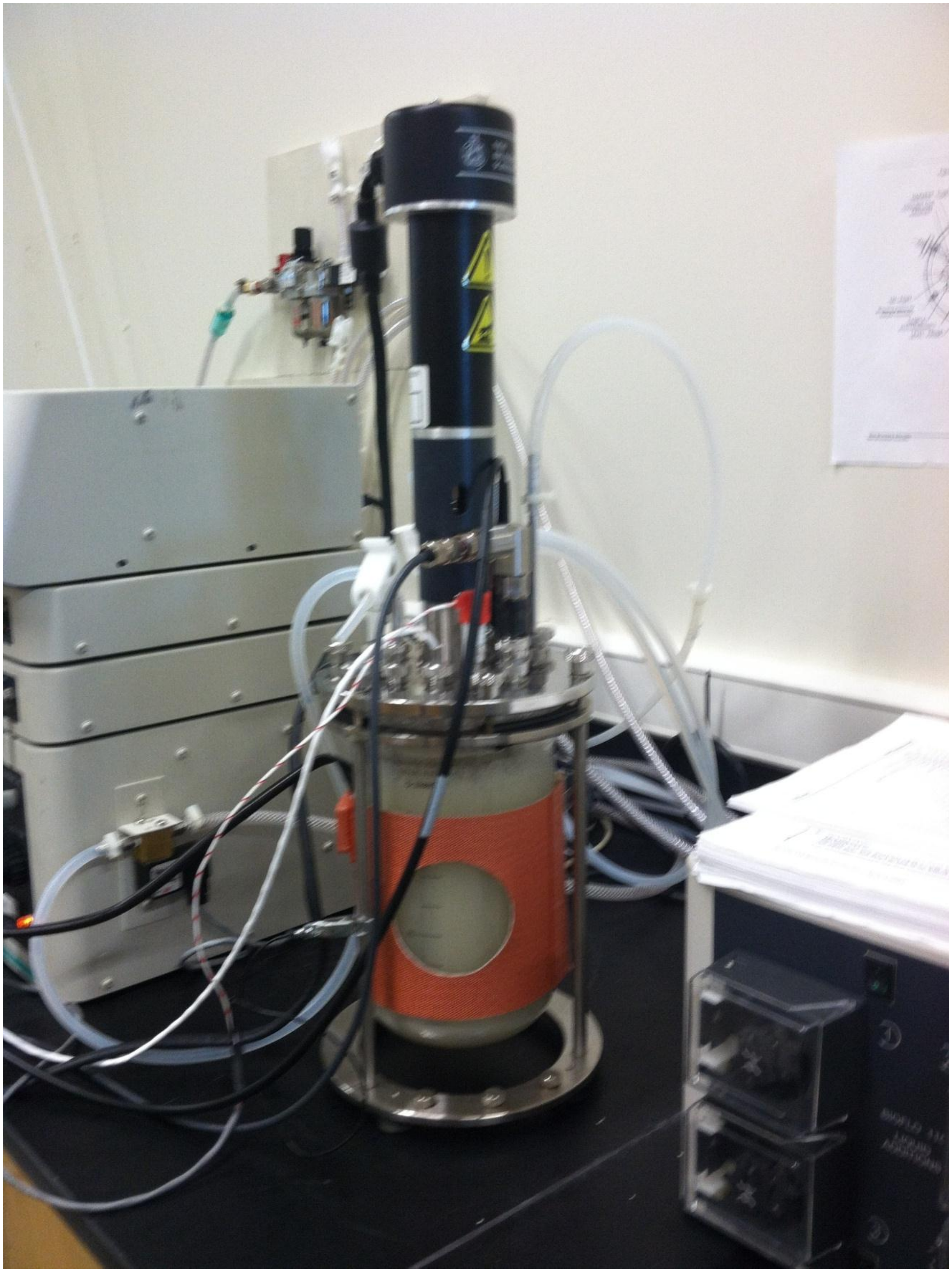

Figure 2: Fermentor Tank with Yeast Growing

Figure 3: Fermentor Control Console

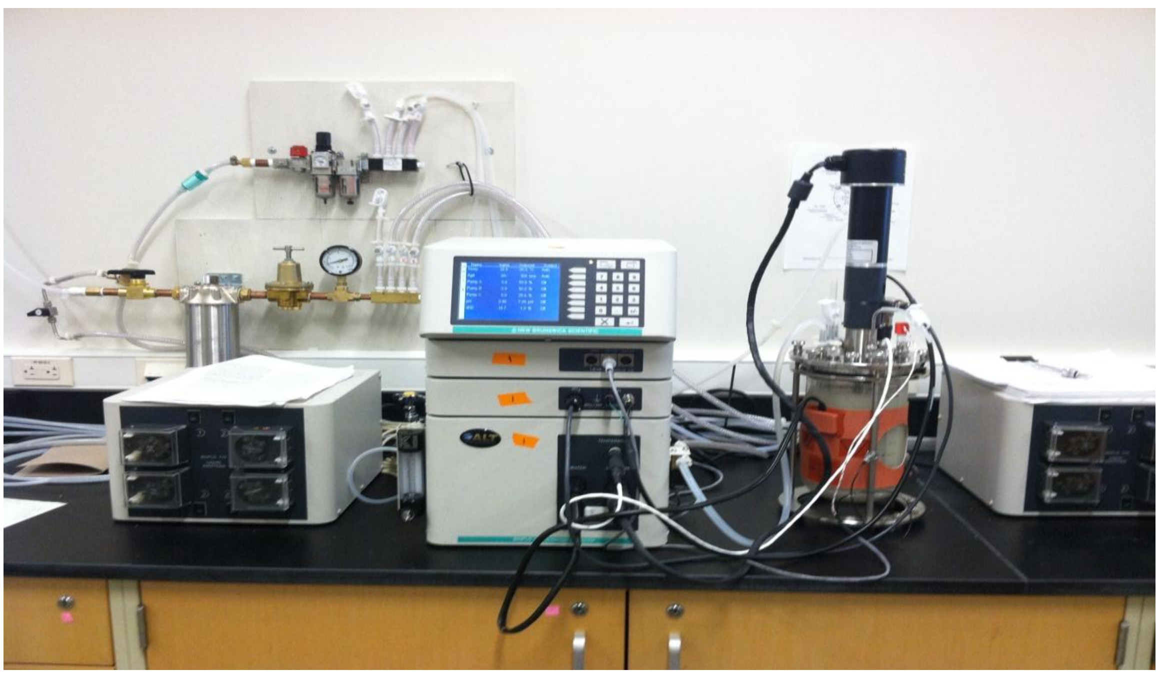

Figure 4: Console and Fermentor Side-by-Side