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Automated Bench-Scale Bioreactor

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Automated Bench-Scale Bioreactor Design

A Major Qualifying Project

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the Requirements for the

Degree of Bachelor of Science

Date: April 24, 2008

Submitted by:

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Abstract

The objective of this project was to design and build an automated bench-scale sequencing batch reactor to demonstrate the treatment of wastewater using an activated sludge process. LabVIEW 8.0 development software was used to build a user interface to control timing sequence, pumping, aeration, and temperature of the physical reactor setup.

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This project required a great deal of time and effort, not only on our behalf, but also from several others. Without their help, this project could not have been successfully completed.

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2.1 Treatment of Wastewater	Nicholas Wilbur	Nicholas Erickson
2.2 The Activated Sludge Process	Nicholas Erickson	Nicholas Erickson
2.3 Bioreactor Design Parameters	Jason Mello	Jason Mello
2.4 Bench Scale Systems	Nicholas Wilbur	Nicholas Erickson
3.0 Materials and Methods	Nicholas Erickson	Nicholas Erickson
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1.0 Introduction

The goal of this project was to build an automated bench-scale biological batch reactor that will continuously run in the Environmental Laboratory of Kaven Hall. The reactor is intended to mimic a wastewater treatment process where biological organisms are used to degrade organic waste. This process breaks down the organic waste into organic-free liquid and suspended solids. The reactor is completely controlled by computer software called LabVIEW 8.0. This program allows the operator to monitor the reactor by simply using a computer, and to easily control all of the properties of the reactor. For sanitary reasons the reactor will not degrade real organic waste; instead a simple sugar and glucose mix will be used to keep the bacteria alive. The bench-scale reactor will allow research on the microorganism's ability to degrade various wastes under different conditions.

Wastewater treatment plants are designed to treat the sewage released by local residents, businesses and industry that are local to the treatment plant. Sewage can contain pathogens or disease-causing organisms, minerals, metals, and nutrients such as ammonia and phosphorous. Most wastewater is 99.94% water and 0.06% dissolved and suspended solid material. Strength is often measured by the biochemical oxygen demand, or BOD_5. BOD_5 represents the oxygen demand for microorganisms to break down sewage in 5 day testing period. Typical sewage BOD_5 measurements range from 100 mg/L to 300 mg/L. The goal of a treatment plant is to remove most of the organic matter, solids, nutrients, disease-causing organisms, and other pollutants before it is discharged into a receiving body of water, into the ground, or to another use. Discharge permits, also called National Pollutant Discharge Elimination System (NPDES) permits, list the allowable levels of BOD_5, suspended solids, bacteria, and other pollutants.

After entering the treatment facility, wastewater undergoes preliminary treatment, primary treatment, secondary treatment, and tertiary treatment. Preliminary treatment eliminates debris and large particles by screening and settling. Primary treatment separates and removes the suspended solids and greases from the wastewater. This is done by allowing the waste to sit long enough for greases to float to the top and solids to sink to the bottom. Secondary treatment uses one of various methods to remove the dissolved organic matter from the wastewater. Final treatment in a wastewater treatment plant removes harmful bacteria and disease-causing organisms. The wastewater is disinfected by adding chlorine or using ultraviolet light. The discharge from a treatment plant must meet regulated standards set by their NPDES permit.

There are two secondary treatment methods: fixed film systems and suspended growth systems. In a fixed film system, the microorganisms are grown on rocks, sand, or plastic film. Organic matter and nutrients passing over the film are absorbed by the microorganism, causing the film to grow and thicken. Suspended growth systems mix and aerate microorganisms that are suspended in the wastewater. The microorganisms grow in size and numbers as they absorb organic matter and nutrients from the wastewater. It can take several hours, depending on the contents of the wastewater and temperature, for the microorganism to absorb the organic matter and nutrients. The microorganisms are then allowed to settle to the bottom as sludge. The reactor decants organic-free liquid from the surface and excess sludge from the bottom of the bioreactor. A portion of the sludge is saved to act as a seed for the next batch of incoming waste.

The bench-scale bioreactor designed in this project is a small model of a sequencing batch reactor. The bench-scale biological batch reactor goes through multiple stages in order to break

down the organic waste and nutrients to produce an organic-free liquid. In stage one, replicated wastewater is pumped into the system until the reactor is full. During stage two, air is pumped through the mix while the microorganisms absorb the organic matter and nutrients. Stage three separates the sludge from the treated liquid through a settling process. Finally, stage four removes the treated liquid from the surface and the excess sludge from the bottom, leaving enough sludge to act as a seed for the next batch reaction. It is necessary that these four stages continuously cycle for the microorganisms to survive and continue to effectively degrade organic waste.

The bioreactor initially starts the batch cycle with about 200 mL of leftover sludge solids from the previous batch. The leftover sludge from the previous batch contains the microorganisms needed to degrade the organic waste and nutrients in the wastewater. During the first stage, about 600 mL of a sugar-glucose food mix is pumped in, filling the reactor to the top. A float valve is used to stop the pump when the reactor is full. The sugar-glucose food mix represents the wastewater that has already passed through preliminary and primary wastewater treatment. The microorganisms in the sludge will grow and reproduce as they degrade the organic matter and nutrients found in the replicated wastewater.

Stage two is the aeration process that provides the microorganisms with oxygen. Oxygen is vital to degradation of waste and the survival and reproduction of the microorganisms because it is a respiration requisite. During respiration, sugar or starch and oxygen are converted into carbon dioxide, water, and the energy an organism uses to survive and reproduce. An aeration stone is used in this stage to disperse air in the form of small bubbles to help with how well mixed the

contents of the reactor are, and how much dissolved oxygen is present inside the reactor. Smaller bubbles are ideal for aeration because they have a larger surface area per volume. After the organisms have had enough time to digest the waste, the reactor enters the settling stage.

Stage three, the settling stage, separates the suspended solids from the organic-free liquid. The compressed air is turned off and the contents of the reactor are allowed to settle. The denser sludge settles to the bottom of the reactor wile the organic-free liquid stays on the top. The liquid that is settled on top of the sludge is known as the supernatant. After enough time for proper settling has elapsed, the reactor enters the decanting stage.

Stage four, the decanting stage, removes the supernatant water from the reactor and excess sludge from the bioreactor. The supernatant at the top of the bioreactor gets pumped out while another pump removes excess suspended solids from the bottom of the reactor. In an actual wastewater treatment plant, the organic-free supernatant liquid would receive further treatment and sterilization before being discharged from the plant. The suspended solids would go through a squeezing process to separate any liquid from the sludge. The removed liquid is recycled back through the bioreactor while the solids are discarded as solid waste sent away from the plant, typically to a landfill. Since neither of these processes are part of this bench-scale bioreactor, both the liquid and sludge will be sent down the drain to be retreated at the Upper Blackstone Wastewater Treatment Facility.

The bench-scale bioreactor created in this project will allow for in-depth research to be executed on the activated sludge treatment process. The design of the bench scale bioreactor allows the

operator to adjust volumes, timing, and temperature in the reactor. The operator is also able to run a variety of tests to check the effects of these variables on the efficiency of waste removal. The full adjustability of the reactor allows the microorganisms to be studied under a broad scale of conditions and settings.

The bioreactor can be adjusted either manually or with the LabVIEW 8.0 programming software. The LabVIEW 8.0 computer software allows the operator to control the volume of organic-free water being removed, the volume of wasted sludge, and the volume of remaining sludge remaining at to bottom used seed the next batch. The float valve is also threaded through the top to allow to the operator can control maximum fluid level in the reactor. The elevation of the aeration stone is also similarly adjustable. The LabVIEW program also allows the operator to adjust the aeration time and temperature of the solution within the reactor. Temperature in the bioreactor is controlled by a surrounding water jacket and monitored by a temperature sensor in the center of the reactor. When temperature drops below the minimum desired temperature, a pump circulates water through the jacket from a heated reservoir until the desired temperature is reached. Temperature affects the rate that the microorganisms degrade the organic matter.

The operator of the bench-scale reactor is also able to replicate different types of waste and monitor its effect on the microorganism's ability to degrade the organic matter. The contents of the bioreactor can be tested for biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), dissolved oxygen (DO), acidity (pH), and mixed liquor suspended solids (MLSS). The BOD_5 represents the amount of organic carbons the microorganisms can oxidize over five days. This test is becoming less common in practice because the five-day period required for the test is

a much longer test period than required for COD testing, which also provides information about the amount of organic material removed. The COD is the total measurement of all chemicals in the solution that can be oxidized. This test takes only 2 hours and is used to see the remaining organic matter in the water. Microorganisms and bacteria living in the solution produce dissolved oxygen through respiration. The level of dissolved oxygen found in a DO test indicates the growth production of biomass in solution. The acidity or alkalinity of a solution is also important to monitor because the microorganisms will not survive in a waste that is too acidic or basic. Testing for pH will let the operator know if acid or base solutions need to be added to return the pH to around 7.0. The MLSS test gives the concentration of suspended solids in the activated sludge mixed liquor. It is used to determine the amount of sludge needed to consume the desired volume of waste. These tests are crucial to correctly operate and monitor the microorganism's activity within the reactor.

2.0 Background

A bench-scale bioreactor is a laboratory tool intended to mimic certain biological treatment processes used in modern wastewater treatment. The proper design of a bench-scale bioreactor, therefore, necessitates a complete understanding of these biological treatment processes. The following chapter is intended to illustrate the role of biological treatment in the modern wastewater treatment process and to describe in detail the biological treatment processes around which a bench-scale bioreactor must be designed.

2.1 Treatment of Wastewater

Treatment of wastewater is a relatively modern practice. Although techniques for waste removal and disposal have been used for several millennia, methods for disinfection have been practiced for only the past two centuries. Towards the start of the 1800s, as cities across the globe began to grow exponentially, the amount of pollutants in source waters due to contaminated wastes became a growing cause for concern. Many of the most populated cities began experiencing outbreaks of life-threatening diseases caused by high concentration of pathogens in their water, and so began a worldwide effort to develop an efficient process to effectively treat and clean large amounts of water at a time (Pfafflin, 2000).

Early attempts at wastewater treatment used chemical processes to remove large suspended particles, but this failed to remove disease-causing microorganisms. In the late 1800s, methods for primary settling and biological treatment were introduced and these were found to be far more effective at treating waste. As the treatment process continued to develop and improve, cities and

countries began building treatment plants to handle large volumes of waste. By 1970, wastewater treatment techniques had become so effective that standards were implemented by governments all over the world to control and minimize pollution caused by wastewater discharges (Cooper, 2002).

2.1.1 Modern Wastewater Treatment

Modern treatment plants are typically built to handle several million gallons of wastewater per day and discharge an estimated 50 to 100 gallons of water per person per day. They generally utilize some combination of physical, chemical, and biological processes to remove pollutants from influent wastewater (Bio World Products, 2007). Figure 2.1 illustrates a typical activated-sludge wastewater treatment process.

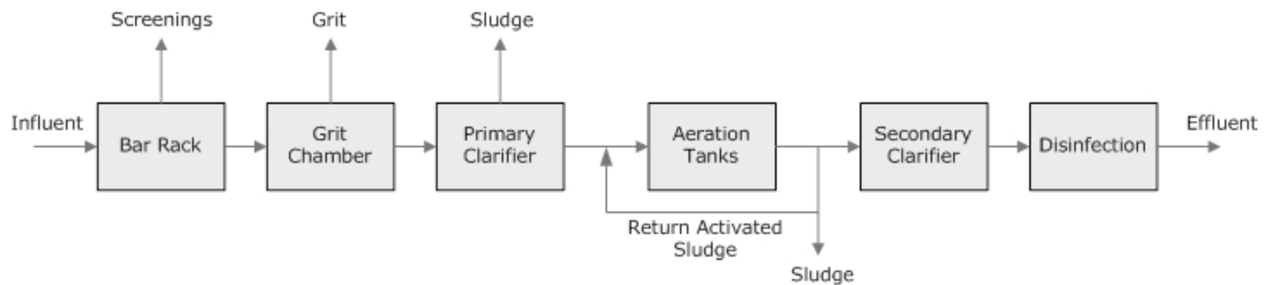


Figure 2.1: Flow diagram for a typical activated-sludge treatment process (Lenntech, 2008)

The first stage of the modern treatment process, known as primary treatment, is the physical treatment of the wastewater. The influent is passed though a series of bar racks, grit chambers, and clarifying basins to remove large particles by means of filtration and settling. Primary treatment is effective at removing about 50% of the pollutants from influent wastewater.

The next stage of the modern treatment process, known as secondary treatment, is the biological treatment of the wastewater. In this process, wastewater is pumped into a reactor and microorganisms are added to the wastewater to remove colloidal and dissolved organics and certain nutrients. Secondary treatment is effective at removing about 90 percent of the pollutants from influent wastewater (Droste, 1997).

There are two different techniques commonly used in secondary treatment: suspended growth processes or fixed growth processes. In the suspended growth process, wastewater is pumped into a large basin, called a reactor, which contains microorganisms in the form of sludge. Treatment occurs as the microorganisms are mixed with the wastewater. Suspended growth processes may be used in both aerobic and anaerobic environments. In an aerobic suspended growth process, usually referred to as an activated sludge process, aeration is used to provide mixing. In an anaerobic suspended growth process, however, an auxiliary mixer may be required. The activated sludge process is the most commonly used form of secondary treatment; anaerobic suspended growth processes are typically reserved for industrial or agricultural wastewaters (Droste, 1997). The treatment process shown previously in 2.1 uses an activated sludge process.

In the fixed growth process, microorganisms grow and accumulate on some solid support media. Wastewater is brought into contact with the media, and treatment occurs as it passes slowly over the thin film of microorganisms. There are two different treatment methods that are commonly used for fixed growth processes: trickling filters, and rotating biological contactors (RBCs). In a

trickling filter, wastewater is sprayed over a bed of rocks or synthetic media upon which microorganisms have grown and accumulated. As the wastewater drains off of the media, it is treated by the microorganisms. Trickling filters may be used in both aerobic and anaerobic environments. In an RBC, microorganisms are grown and accumulated on large disks that are partially submerged in wastewater. As the disks are rotated, microorganisms are aerated and allowed to react with the water. RBCs may only be used in an aerobic environment. Fixed growth processes are not as common as suspended growth processes in secondary treatment (Droste, 1997).

After secondary treatment, the water is either discharged directly into a lake, stream, or reservoir, or it is treated with a third process. The third stage of the modern treatment process, known as tertiary treatment, uses UV light, ozone, or chemicals such as chlorine to kill pathogens remaining in the water after secondary treatment. Tertiary treatment is effective at deactivating about 99 percent of remaining microorganisms in the wastewater. Water is discharged to a lake, stream, or reservoir after tertiary treatment (Bio World Products, 2007).

Secondary treatment is the most effective of the three stages in the modern wastewater treatment process because it combines the removal of colloidal and dissolved organic matter. Primary treatment removes a significant amount of colloidal matter, but it does not remove dissolved organic matter. Tertiary treatment deactivates 99% of pathogens, but it does not specifically remove remaining colloidal or dissolved organic matter. Secondary treatment combines a 90% pathogen removal rate with the ability to remove both colloidal and dissolved particles. For this

reason, secondary treatment is considered the most important step in the wastewater treatment process.

Because secondary treatment is so vital, it is important to optimize the process to obtain the optimum removal rates of organic matter. Bench-scale tests are used in the treatment industry to test biological treatment processes for removal efficiency. In most cases, scale reduction techniques are used to shrink the process for use in a small laboratory bench-top reactor. Typical bench-scale reactors range in volume from several hundred milliliters to twenty liters or more, and can be completely automated.

2.2 The Activated Sludge Process

A bench-scale bioreactor is essentially a scale model of a reactor needed for a suspended growth biological treatment process. Since the activated sludge process is the most commonly used technique for biological treatment, activated sludge reactors were used as the basis for this project's bench-scale bioreactor design.

2.2.1 Typical Activated Sludge Reactors

In order to decide which activated sludge reactor was most suitable for use in this project, it was necessary to first become familiar with the major types that are commonly used. The first is called a plug flow reactor. In a plug flow reactor, influent is pumped into a pipe or a channel as a constant stream. Fluid particles are assumed to pass through the tank in sequence: mixing may occur in the radial direction, but not in the axial direction. The particles leave the reactor in the order that they entered. This type of system allows for a fine-tuning of the aeration process, as

oxygen demand is usually higher towards the end of the reactor, but is often costly and can easily be affected by temperature (Droste, 1997).

The second type of activated sludge reactor most commonly used is called a complete mix reactor. In a complete mix reactor, influent is pumped in as a constant stream, and the influent flowrate equals the effluent flowrate. When the influent enters the reactor, it is assumed to be completely and instantaneously mixed with the rest of its contents. The concentration of the effluent is assumed to be the same as that within the tank. Although complete mix reactors are generally very efficient, they have a tendency to cause the growth of filamentous microbes that cause sludge bulking (Droste, 1997).

The last type of activated sludge reactor most commonly used is called a sequencing batch reactor. Unlike plug flow reactors and complete mix reactors, a batch system does not have a constant influent rate. Instead, wastewater is pumped into a basin containing microorganisms in the form of sludge and allowed to react for a certain period of time. Mixing occurs until reaction is complete, at which point microorganisms are settled and the treated water is drawn off. This method is both effective and inexpensive; for these reasons, it is one of the most widely used reactors in activated sludge treatment.

Although these three are the major types of reactors used in activated sludge processes, there are variations thereof that are also used. Sequencing batch reactors, for example, are very common.

2.2.2 Sequencing Batch Reactors

The sequencing batch reactor system is a hybrid system with some characteristics of plug flow and complete mix systems, but it also has characteristics that are unique. The activated sludge process utilized by a sequencing batch reactor involves four distinct operations: fill, aerate, settle, and decant (Alleman, 2002). These operations are defined as follows:

1. *Fill:* *The influent wastewater is pumped into the aeration basin, which contains a layer of sludge.*
2. *Aerate*: *Aeration times vary according to the plant size and the composition of the incoming wastewater, but are typically between sixty and ninety minutes. Microorganisms are mixed with the wastewater and oxygen is supplied to the process by aeration. Within the aeration step, the organic matter in the wastewater is metabolized to produce end products and new microorganisms (Droste, 1997). Mixing must be adequate within the basin to prevent the sedimentation of microorganisms.*
3. *Settle*: *Aeration is turned off and settling begins. The settling stage is usually the same length in time as aeration. During this stage, the sludge formed by the bacteria is allowed to settle to the bottom of the tank. As the bacteria multiply and then die, the sludge within the tank increases over time and a waste activated sludge pump removes some of the sludge during the settle stage to a digester for further treatment. The "age" of sludge within the tank is closely monitored, as this can have a marked effect on the treatment process. The sludge is allowed to settle until clear water is on the top 20%-30% of the tank contents.*

4. *Decant: Aeration remains off and the volume of clear water on top of the settled sludge, known as supernatant water, is withdrawn from the system and placed into storage.*

A typical sequencing batch reactor process cycle requires four to eight hours to complete (Mikkelson, 2003). In most sequencing batch reactor systems, two batch reactors are usually operated in parallel. As one tank fills and then aerates, the second settles and decants, and then the process is reversed: the second fills and aerates while the first settles and decants (Droste, 1997).

The preliminary designs for this bench-scale bioreactor included two sequencing batch reactors operated in parallel. After doing some background research, however, the conclusion was drawn that a project of such scope would cost too much money. Instead, the final design settled upon a single sequencing batch reactor with a fill, aerate, settle, and decant sequence.

2. 3 Bioreactor Design Parameters

In designing a bioreactor, multiple operating parameters must be considered. These parameters are focused around maintaining a healthy and stable microorganism population while maximizing substrate utilization. In practice, some of these parameters, such as influent substrate concentration and, are random and depend on varying influent characteristics. Others, such as operating temperature and dissolved oxygen (DO) concentrations, can be controlled by treatment system operators.

2.3.1 Influent Characteristics

The main objective of the biological treatment of wastewater is to remove biodegradable constituents. It is important to understand influent wastewater properties. Domestic wastewater has many different sources such as residences, commercial buildings, and industrial operations, as well as stormwater. From these sources both inorganic and organic wastewater components are generated. Common wastewater components include solids, organic carbon, nitrogen, phosphorus, volatile organic compounds (VOCS), sulfate, and petroleum-based products (Droste, 1997). Pathogens such as *Giardia lamblia, Cryptosporidium*, and coliforms are also present in wastewater.

The primary source of organics in wastewater is human excreta which is composed of fecal matter, urine, and cleansing materials (Droste, 1997). On average, an adult contributes 198 grams of COD to wastewater each day (Burton et al., 2003). Human urine contains ammonia, which is a large source of nitrogen (Droste, 1997). Additionally, the presence of pathogens in wastewater is largely due to human waste. A table in the appendix details the concentration of various wastewater constituents generated by residences.

With respect to biological treatment, the concentration of nutrients such as nitrogen and phosphorous and the biodegradable organics, or substrate, are especially important. As nutrients can be contributed by multiple sources, they are often measured in terms of total availability. The concentration of degradable organics can be measured in two ways, biological oxygen demand (BOD_5), or chemical oxygen demand (COD). Biological oxygen demand is a measurement of the oxygen required by microorganisms to decompose organic matter, while chemical oxygen

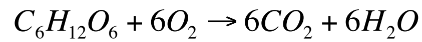
demand is a measurement of the oxygen required to stabilize organic matter through oxidation (Droste, 1997). Typically, raw wastewater has a BOD_5/COD ratio of 0.3 – 0.8 (Burton et al., 2003). The table below details the composition of various strength common raw wastewaters. A more detailed table can be found in the appendix.

Table 2.1: Composition of Common Raw Wastewater (Burton et al., 2003)

Constituent	Units	Concentration		
		Low Strength	Medium Strength	High Strength
Total Solids (TS)	mg/L	390	720	1230
Total Dissolved Solids (TDS)	mg/L	270	500	860
Total Suspended Solids (TSS)	mg/L	120	210	400
BOD_5	mg/L	110	190	350
Total Organic Carbon	mg/L	80	140	260
COD	mg/L	250	430	800
Total Nitrogen As N	mg/L	20	40	70
Total Phosphorus as P	mg/L	4	7	12
VOCs	µg/L	<100	100 - 400	>400
Total Coliforms	#/100mL	$10^6 - 10^8$	$10^7 - 10^9$	$10^7 - 10^{10}$

COD and BOD_5 are used to measure the strength of wastewater, as they are representative of the primary focus of biological treatment, biodegradable organic matter. As COD is based on chemical oxidation, the COD of wastewater can be calculated based on the substrate present in

the wastewater. The stoichiometric reaction on the next page is representative of oxidation of glucose, with $C_6H_{12}O_6$ representing glucose (Burton et al., 2003).



Oxidation of Glucose

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

This reaction can then be used to form a relationship between the oxygen used to oxidize glucose and the amount of glucose that is oxidized, or the COD of glucose. Equation 1 is used to calculate COD based on the substrate being used. For example, for glucose:

$$COD = \frac{\Delta(Electron\ \ Acceptor)[g/mole]}{\Delta Substrate[g/mole]} \qquad Equation\ 1$$

$$COD = \frac{\Delta(O_2)}{\Delta(C_6H_{12}O_6)}$$

$$COD = \frac{6(32g/mole)}{(180g/mole)}$$

$$COD = 1.07g\ \ O_2/g\ \ glucose$$

$$COD = 1070mg\ \ O_2/1000mg\ \ glucose$$

COD is often expressed in terms of concentration, or mg/L. In the example above, if glucose were present in at a concentration of 1000 mg/L, the COD concentration would be 1070 mg/L.

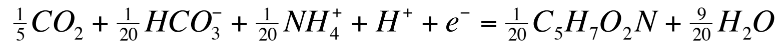
2.3.2 Reactor Biomass and Growth

The degradation of biodegradable constituents in a bioreactor is due to their utilization by microorganisms. Various kinds of microorganisms are present in a bioreactor, although not all are useful in terms of the oxidation of organic matter. Bacteria are single-cell prokaryotic organisms that reproduce mainly through fission. Bacteria are the main sources of

biodegradation in bioreactors (Droste, 1997). Additionally, bacteria produce biofilms, which can be settled out of the water and result in a further decrease of organic matter (Burton et al., 2003). Due to their diversity and minimal growth requirements, bacteria proliferate in wastewater (Droste, 1997). Protozoa also play an important role in biological treatment. Protozoa are eukaryotic, mostly single-cell organisms. Through the consumption of bacteria and organic particulates, protozoa clarify wastewater. Viruses, yeast, and fungi are also present in bioreactors, but do not aid in biological treatment. When describing populations, the microorganisms in the reactor are measured in terms of mg/L of mixed liquor suspended solids (MLSS).

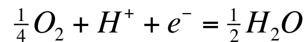
The removal of biodegradable organics from wastewater occurs through their digestion by microorganisms. Microorganisms oxidize substrate to produce energy and new cells. Organisms that rely on chemical reactions to create energy, such has the bacteria and protozoa present in a bioreactor, are referred to as chemotrophs (Burton et al., 2003). Oxidation-reduction reactions are the chemical reactions that chemotrophs use to produce energy. These reactions depend on an electron acceptor and donor. In aerobic biological treatment, the majority of microorganisms have a respiratory metabolism, meaning that they use oxygen as the electron acceptor in energy producing oxidation-reduction reactions. However, some bacteria involved in aerobic biological treatment may use nitrate or nitrite in the absence of oxygen. These bacteria are referred to as facultative aerobic bacteria (Burton et al., 2003). Microorganisms rely on these same reactions to reproduce. The reactions below detail the oxidation-reduction reactions that occur in an aerobic bioreactor (Burton et al., 2003). In these reactions, $C_{10}H_{19}O_3N$ represents raw domestic wastewater and $C_5H_7O_2N$ represents new biomass.

Reaction of Bacterial Cell Synthesis with Ammonia as Nitrogen Source



$$\tfrac{1}{5}CO_2 + \tfrac{1}{20}HCO_3^- + \tfrac{1}{20}NH_4^+ + H^+ + e^- = \tfrac{1}{20}C_5H_7O_2N + \tfrac{9}{20}H_2O$$

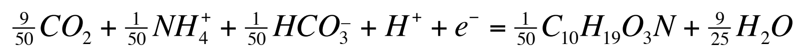
Oxygen Electronic Acceptor Reaction



$$\tfrac{1}{4}O_2 + H^+ + e^- = \tfrac{1}{2}H_2O$$

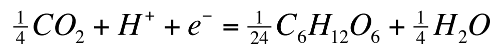
Electron Donor Reactions

Raw Domestic Wastewater



$$\tfrac{9}{50}CO_2 + \tfrac{1}{50}NH_4^+ + \tfrac{1}{50}HCO_3^- + H^+ + e^- = \tfrac{1}{50}C_{10}H_{19}O_3N + \tfrac{9}{25}H_2O$$

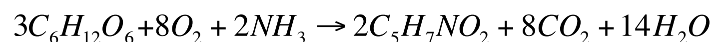
Glucose



$$\tfrac{1}{4}CO_2 + H^+ + e^- = \tfrac{1}{24}C_6H_{12}O_6 + \tfrac{1}{4}H_2O$$

The synthesis of new biomass is directly related to the substrate concentration in the wastewater being treated. The reaction below details the definite stoichiometric relationship between cell synthesis and the digestion of glucose as substrate.

Bacterial Cell Synthesis through Glucose Digestion (Burton et al., 2003)



$$3C_6H_{12}O_6 + 8O_2 + 2NH_3 \rightarrow 2C_5H_7NO_2 + 8CO_2 + 14H_2O$$

An expression detailing the mass of cells produced through the utilization of substrate (in terms of COD) can be developed. Equation 2 is used to calculate the theoretical yield of biomass in terms of COD. For example, for glucose:

Theoretical Biomass Yield with Glucose Substrate (Burton et al., 2003)

$$Y = \frac{\Delta(Biomass)[g/mole]}{\Delta(Substrate\ \ as\ \ COD)[g/mole]} \qquad Equation\ \ 2$$

$$Y = \frac{\Delta(C_5H_7NO_2)}{\Delta(C_6H_{12}O_6\ \ as\ \ COD)}$$

$$Y = \frac{2(112g/mole)}{3(180g/mole)(1.05g\ \ COD/g\ \ glu\cos e)}$$

$$Y = 0.39g\ \ biomass/g\ \ COD\ \ used$$

The growth of biomass in a reactor is based on the yield factor of the substrate utilized and the rate at which the substrate is utilized. Equation 3 is used to calculate the production rate of biomass in a reactor.

Biomass Production Rate (Droste, 1997)

$$r_{xp} = -(Y)[g\ \ biomass/g\ \ COD\ \ used](r_s)[mg/L\cdot day] \qquad Equation\ \ 3$$

With;

r_{xp} = biomass production rate

Y= substrate yield factor

r_s = rate of substrate utilization

As bioreactors are operated under limited substrate concentrations, decay in biomass due to starvation, death, predation, and auto oxidation occurs (Droste, 1997). Equation 4 is used to calculate the decay rate of biomass in a reactor.

Biomass Decay Rate (Droste, 1997)

$$r_{Xe} = -(k_e)[1/day](X_V)[mg/L] \qquad Equation\ 4$$

With;

r_{Xe}= biomass decay rate

k_e= decay rate constant

X_V = MLSS concentration

Equations 3 and 4 can be combined to detail the net growth rate of microorganisms within a reactor. Equation 5 is used to calculate the net growth rate of reactor biomass.

Net Biomass Growth Rate (Droste, 1997)

$$r_x = -(Y)[g\ \ biomass/g\ \ COD\ \ used](r_s)[mg/L \cdot day] - (k_e)[1/day](X_V)[mg/L] \qquad Equation\ 5$$

In order to maximize the number of digesting microorganisms in a reactor, biomass is removed from the reactor. The rate at which biomass is removed from the reactor is based on the age, or residence time, of the microorganisms. Equation 6 is used to calculate the age of biomass in a reactor

Biomass Residence Time

$$\Theta_x[day] = \frac{(V_{reactor})[L](MLSS)[mg/L]}{(Biomass\ \ \mathrm{Re}moval\ \ Rate)[mg/day]} \qquad Equation\ 6$$

With respect to reactor biomass growth, another important parameter exists. The food to microorganism ratio (F/M) is used to describe the degree of starvation of microorganisms within

the reactor (Droste, 1997). This parameter is often used in designing reactors and characterizing their performance. Equation 7 details the calculation for the food to microorganism ratio in a reactor. This equation is very useful in designing bioreactors, as it can be rearranged to solve for the MLSS concentration in a reactor given a certain substrate concentration or the substrate concentration necessary to maintain a certain MLSS concentration.

Food to Microorganism Ratio (Burton et al., 2003)

$$(F/M)[1/day] = \frac{(S)[mg/L)(Q)[L/day]}{(X_v)[mg/L](V_{reactor})[L]} \qquad \textit{Equation 7}$$

With;

F/M = food to microorganism ratio

S = influent substrate concentration

Q = influent flow rate

X_V = MLSS concentration

$V_{reactor}$ = reactor volume

Nutrients are also essential to the oxidation of substrate by microorganisms. Principle inorganic nutrients required by microorganisms for the oxidation of substrate include nitrogen, phosphorus, sodium, potassium, magnesium, calcium, sulfur, iron, and chlorine (Burton et al., 2003). The mass ratio of COD, nitrogen, and phosphorus in a reactor should be around COD:N:P =100:5:1 (Droste, 1997). Sufficient quantities of these nutrients occur naturally in domestic wastewater.

2.3.3 Substrate Utilization Rate

The utilization of substrate in biological treatment occurs through microbiological oxidation. As this is the primary objective of biological treatment, understanding the rate at which substrate is utilized in a reactor is extremely important. In the early 1949, Jacques Monod developed an equation that relates substrate utilization rate to substrate concentration and reactor biomass (Droste, 1997). The processes in which substrate is degraded during biological treatment are referred to as Monod kinetics. Equation 8 is the expression formulated by Monod.

<u>Substrate Utilization Rate, Monod (Droste, 1997)</u>

$$(r_s)[mg/L \cdot day] = -\frac{(k)[1/day](X_v)[mg/L](S)[mg/L]}{(K)+(S)[mg/L]} \qquad \textit{Equation } 8$$

With;

r_s = rate of substrate utilization

S = influent substrate concentration

X_V = MLSS concentration

k, K = maximum and half-velocity constants

Under low substrate concentrations, the Monod equation reduces to a first-order form. As the influent entering a bioreactor is usually low in biodegradable substrate concentration, first-order Monod kinetics can be used to determine the theoretical substrate utilization rate. Equation 9 details the rate of substrate utilization in bioreactor according to first-order Monod kinetics.

<u>Substrate Utilization Rate, First-Order Monod (Droste, 1997)</u>

$$(r_s)[mg/L \cdot day] = -(k)[1/day](S)[mg/L] \qquad \textit{Equation } 9$$

As biological treatment processes occur for a definite period of time, it is important to understand substrate concentration at the end of treatment. Integration of Equation 9 with respect to time yields Equation 10. Equation 10 is used to calculate substrate concentration at the end of biological treatment.

<u>Substrate Concentration After Biological Treatment</u>

$$(S_e)[mg/L] = (S)[mg/L](e^{-kt_r}) \qquad \textit{Equation } 10$$

$k \text{ in terms of } 1/day$

$t \text{ in terms of } days$

With:

S_e = substrate concentration following biological treatment

S = influent substrate concentration

k = maximum substrate utilization rate

t_r = reaction time

Through the use of Equation 10, the theoretical effluent substrate concentration can be determined. This parameter details the efficiency of biological removal and is monitored extensively in wastewater treatment plants.

2.3.4 Process pH, Dissolved Oxygen, and Temperature

While biomass growth and substrate utilization are the primary parameters used when designing a bioreactor; pH, dissolved oxygen, and temperature all contribute to the overall function of biological treatment. The pH present in a reactor directly affects the biomass within the reactor. The optimum growth for various microorganisms occurs within a small pH range, generally

between 6.5 and 7.5, while carbon-oxidizing reactions required a pH range of 6.0 – 9.0 (Burton et al., 2003). Additionally, the majority of bacteria cannot tolerate pH levels outside of a range of 4.0 – 9.5 (Burton et al, 2003). As microorganisms are especially sensitive to shifts in pH, it is important to control and monitor the pH within a reactor as much as possible.

In aerobic treatment, dissolved oxygen is the primary electron acceptor used by microorganism in digesting substrate through oxidation. Dissolved oxygen is also required by aerobic microorganisms for respiration. Microorganisms require a surprisingly small quantity of dissolved oxygen, around 2.0 mg/L (Burton et al., 2003). The dissolved oxygen concentration in a reactor can be controlled via aeration or agitation. The addition of DO to a reactor is common, as DO plays such an important role in biological degradation.

Reactor temperature can affect a number of other parameters. Temperature directly affects both biomass growth rates and substrate utilization (Burton et al., 2003). With respect to microbial growth rates, it has been observed that growth rates double every 10 °C until the optimum temperature is reached. Temperature also plays a part in substrate utilization. At higher temperatures, microorganisms utilize substrate much quicker. This in turn improves the overall function of a bioreactor. In addition to biomass growth and substrate utilization, temperature also has an effect on the settling characteristics of biosolids and gas transfer rates (Burton et al., 2003).

2.4 Bench-Scale Systems

Bench-scale testing has long been a valuable way to perform research on large-scale processes that cannot be feasibly tested without a scale reduction. The first bench scale bioreactor was constructed by Sir Gilbert John Fowler in the year 1914, and improved upon by his students Ardern and Lockett shortly after in the same year. It was initially designed to test the activated sludge process, which has just been introduced as a concept, before building a full-scale treatment plant based on this method of biological treatment. The results of this laboratory test proved to be extremely helpful to Ardern and Lockett when trying to maximize the efficiency of their activated sludge process (Dhir, 2001). Modern bench-scale testing has become an essential tool in process optimization and design of wastewater treatment processes.

2.4.1 Automated Control Systems

The advancement of technology over the past century has lent itself to vast improvements in bench-scale bioreactor design. It is now possible to completely automate a bench-scale process using a computer and the appropriate combination of hardware and software. This has resulted in improved bench-scale testing accuracy and improved consistency among test variables. An automated bench-scale bioreactor can also be designed for remote operation, potentially reducing labor costs by eliminating the need to have a person in the laboratory monitoring the reactor.

The automation of this bench-scale bioreactor was restricted to the use of LabVIEW 8.0, as this was the only development software available from Worcester Polytechnic Institute. LabVIEW 8.0, nonetheless, is a very powerful program that utilizes a unique graphical programming language. Programmers can create a general user interface called a "front panel" to control or

monitor processes as defined in a data flow chart called a “block diagram.” LabVIEW 8.0 includes built-in functionality for simulation, data acquisition, instrument control, measurement analysis, and data presentation.

The LabVIEW 8.0 software would, however, be useless without the appropriate hardware. A data acquisition device is needed to interface the software with the hardware that it is controlling. Data acquisition devices typically connect to the computer and can be used by the LabVIEW 8.0 software to generate, read, or simulate electrical signals. Most sensors, relays, and controllers are plugged directly into the data acquisition device and can be controlled or monitored using these electrical signals.

There were two different data acquisition devices available for use in the automation of this bench-scale bioreactor. The first was a National Instruments cDAQ-9172 USB chassis with an NI-9481 relay module and an NI-9205 input module, while the second was a National Instruments PCI-6036E PCI card connected via serial cable to a terminal block for wiring. Figures 2.2 and 2.3 show a picture of each of these devices. Both data acquisition devices are fully supported by the LabVIEW 8.0 software and seamlessly integrate with process control.

Figure 2.2: National Instruments cDAQ-9172 and Various Modules

Figure 2.3: National Instruments PCI-6036E

A thermocouple was also available to be used in temperature monitoring of the reactor. This was a Type J thermocouple, and was wired directly into the National Instruments PCI-6036E PCI. LabVIEW 8.0’s thermocouple wizard makes it extremely easy to add one to a project.

2.4.2 Other Bench-Scale Bioreactors

Although bench-scale reactors started out as relatively simple home-made devices, they have evolved into very complex automated systems. Technology has allowed not only for the creation of automated control processes, but also for the manufacturing and sale of complete bench-scale bioreactor systems. Companies such as New Brunswick Scientific and Infors currently offer bench top systems complete with a built-in automated control system. Figure 2.4 shows just a few of the pre-manufactured bench-scale bioreactors currently available for purchase.

Figure 2.4: Bench-scale bioreactor systems available directly from a manufacturer

There have also been many university-level studies performed using bench-scale bioreactor testing to accurately model biological treatment processes. One recently completed bench-scale system was designed and built by a group of students at Cornell University. Their computer-controlled bench-scale bioreactor was successfully used to execute tests in order to optimize the performance of an actual wastewater treatment plant. Several different types of hardware were utilized, including peristaltic pumps, centrifugal pumps, solenoid valves, pressure sensors and flow sensors. The peristaltic pumps were used to control inflow and outflow of the reactor, while the centrifugal pumps were used to control the velocity of the influent and the solenoid valve was used to control aeration. LabVIEW software was used for the creation of a control interface and a data acquisition device was used to connect and control all of these pieces of hardware electronically. A relay control box was built to provide power to all of the hardware used by the bioreactor based on a voltage input from the computer (Cornell, 2007).

Many tests were completed using this computer-controlled reactor and the results were used to optimize the performance of an actual wastewater treatment plant. Testing results indicated

optimum aeration level, optimum timing sequence, and optimum sludge levels for a specific flow of wastewater. All of the results from the tests performed were then scaled up to represent true values to be used in an actual-sized wastewater treatment plant (Cornell, 2007).

3.0 Materials and Methods

The purpose of this project was to design and build a functional and versatile bench-scale bioreactor. The design had to incorporate certain characteristics in order to be suitable for a wide range of laboratory testing. These characteristics included the following:

- Small scale reactions to reduce sludge volumes
- Minimal operational cost
- Corrosion resistance
- Minimal displacement by internal parts
- Consistent fill and decant cycles
- Adjustable drawdown level
- Adjustable aeration
- Adjustable sludge waste
- Controllable temperature
- Easily cleanable

Preliminary setups for this bench-scale bioreactor did not necessarily address all of these design needs. It was only through a process of trial and error that these needs were identified and the final components were chosen.

Once constructed, the bench-scale bioreactor needed to be tested for treatment effectiveness. Several different laboratory tests were used for this purpose, including COD and Mixed Liquor Suspended Solids. Both pH and dissolved oxygen were also monitored to ensure proper sludge growth. Much like with the design of the reactor, optimizing the biological treatment process of the reactor was a trial and error process. It was not until a significant amount of testing had been done and many adjustments had been made that the reactor consistently treated influent wastewater. This chapter describes the methods and materials used in the design, construction, and testing of this bench-scale bioreactor.

3.1 Component Selection

The construction of this bench-scale bioreactor was rather straightforward and mostly involved assembling pre-manufactured parts. Therefore, one of the most important aspects of this project was the selection of components to be used in assembly. Each part of the bench-scale bioreactor had to include any applicable design characteristics (as discussed above) and had to be of some reasonable cost, as there was a limited budget for the completion of this project. The following sections describe each of the components chosen for use in the bench-scale bioreactor.

3.1.1 Reactor

The design of this bioreactor and its treatment sequence was ultimately based upon the reactor that was chosen. Although this was the first component purchased, its features really dictated what other components would be necessary and how the reactor would function. The reactor selected was a jacketed 1 L glass beaker. A jacketed beaker can contain two fluids separately inside it at once, one being contained internally and one externally. One small tubing inlet and one small tubing outlet control the flow of fluid through the external section of the beaker, while the top of the internal section remains open.

Since research shows that microorganisms perform more efficiently at higher temperatures, the external section of the jacketed beaker was used for temperature control. Heated water was cycled through the external section to raise the temperature in the reactor. The water was kept in a water bath heated to 40ºC. Figure 3.1 shows the bench-scale bioreactor with its lid, pumps, and hot water bath.

Figure 3.1: The bench-scale bioreactor setup

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The activated sludge and sample wastewater were allowed to react in the internal section of the jacketed beaker. The design batch size for our bench-scale reactor was to be 800 milliliters, based on the capacity of the internal beaker. A beaker of this size was chosen for this project because of the practicality for laboratory testing. A 1 L beaker with an 800 mL batch size is rather small and will not require a large amount of sludge for operation.

3.1.2 Reactor Lid

The lid for the reactor was the one component manufactured specifically for this project. It was machined down from a square piece of gray high-density Plexiglas that originally measured 12”x 12” and 2.5” thick. The piece was then cut down into four pieces with a table saw; each piece could be made into a spare or replacement top. The plastic was then cut down on a lathe so that it was circular, fit snug on the reactor, and had round edges. Holes were then drilled and tapped to 7/16”–20 to fit the pipes and 3/8”–24 to fit the float sensor. The lip around where the bottom of the lid rests on the reactor was coated with a Liquid Electrical Tape. The Liquid Tape hardens to

form a soft rubber layer that provides protection for the glass reactor. Figure 3.2 shows a picture of the completed reactor lid.

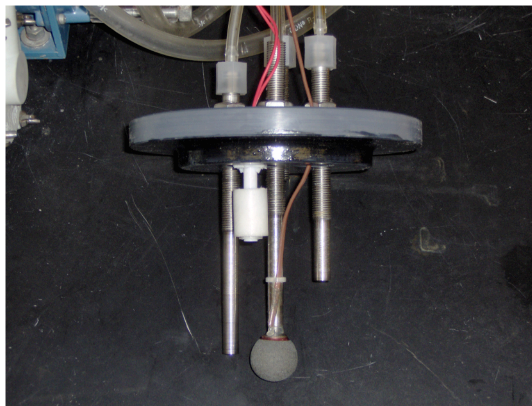

Figure 3.2: Bench-scale bioreactor lid

The pipes used in the reactor are Standard-Wall (Schedule 40) Stainless Steel Threaded-On-One-End Pipe. Six pipes with a pipe size of 1/8" were ordered for the project. They each measure 6" long, 3/8" outside diameter, 1/4" inside diameter, and have a standard tapered 1/8" pipe thread in the end. The pipes were then threaded on the outside with a 7/16–20 dye. The pipes were then put on a lathe where any extra thread that wasn't used was trimmed off. This was done to reduce their displacement and make them smoother and easier to clean. The pipes were then cleaned of oil lubricant and metal fragments from fabrication. The end with the standard 1/8" tapered pipe thread was fitted with a nylon barbed hose end adapter which fit the 1/4" outside diameter Tygon tubing used in the pumps and air hose. The pipes are completely adjustable and thread into the reactor lid. A nylon washer and a stainless steel nut on top of the lid are used to lock the pipe in position.

The aeration stone is attached to a fabricated stainless steel pipe without a nylon barbed hose end adapter. The aeration stone connects to 1/4" outside diameter Tygon tubing with a barbed hose connection. The Tygon tubing was sent through the pipe and the stone was pressed onto the end.

A liquid level sensor was integrated into the lid to prevent overflow. The sensor had to be countersunk into the lid to gain full capacity of the reactor. A 1” diameter hole was drilled to a depth of 3/8" on the bottom of the lid. This allowed the float sensor to be positioned high enough that the reactor can completely fill before triggering the sensor. The sensor height can be adjusted by inserting nylon washers between the lid and the sensor.

Because the lid took a significant amount of time to construct, testing on the reactor began before the lid had been finished. It was necessary to get the reactor functioning early on to run tests, try different artificial wastes, and get a feel for what the final design would need to encompass. This initial setup used glass stirring rods suspended in the reactor, supported by stands and clamps. Influent, effluent, and waste tubes and the aeration line were zip-tied to the glass rods. There was no top and over flow was controlled by wrapping Para film around the opening. This method proved to be cumbersome, messy, and inconsistent. The addition of a proper lid vastly improved the cleanliness, ease of operation, and consistency of the reactor.

3.1.3 Pumps and Controllers

Pumps and controllers were used to manage all of the inflows and outflows of the reactor. The pumps used were peristaltic pumps manufactured by the laboratory supply company Cole Parmer. A peristaltic pump is a pump used for positive displacement of fluids. The fluid is

contained within a flexible tube fitted inside a circular pump casing. A rotor is used to compress the flexible tube, and as the rotor turns, the fluid is forced to move through the tubing. 1/8” Tygon tubing was used for each pump.

The peristaltic pumps used for this project plug into a standard wall outlet. Two of the pumps used can operate between 6 and 600 revolutions per minute, and the third pump used can operate between 1 and 100 revolutions per minute. The speeds of the peristaltic pumps are adjusted by using pump controllers, which were also manufactured by the company Cole Parmer. Figure 3.3 shows a picture of the pumps and controllers used for this project.

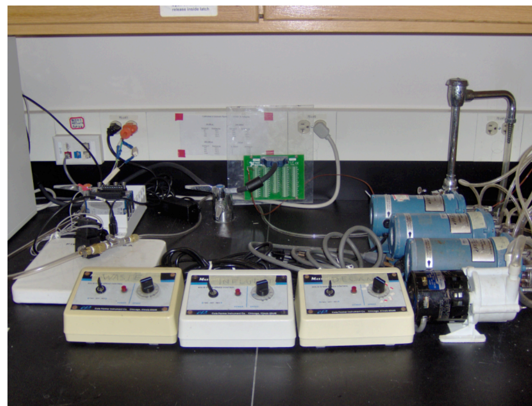

Figure 3.3: Pumps and controllers used with this bench-scale bioreactor

Each of the pumps was automated using an external relay control box which was connected to a computer (as discussed below). Because of the limited availability of extra outlets on the relay control box, the number of pumps used in the system had to be reduced to a total of three. One pump was dedicated to the influent, one pump was dedicated to both the effluent and the sludge waste, and one pump was devoted to temperature control. The successful combination of effluent and sludge waste lines into one pump was achieved by careful placement of the effluent/sludge

waste tube extending down into the reactor. It was positioned such that, after completing a batch reaction and settling, it drew off any excess sludge waste then began to draw off the treated water. The only drawback in the combination of the two lines is with respect to sampling. Samples of treated wastewater must not be taken from the effluent/sludge waste tube but instead may be taken from the sampling port in the reactor lid.

3.1.4 Air Supply and Aeration Valve

Aeration is the most essential part of the activated sludge process. As the dissolved oxygen content in the reactor increases, so does the efficiency of microorganisms in degrading organic waste. For this bench-scale bioreactor, aeration was provided by a gas outlet in the laboratory. Flow was controlled using a two-way normally closed solenoid valve and a 12 Volt DC electrical coil. An external DC power supply was wired to valve and current was turned on and off using the cDAQ-9172 data acquisition device with the NI-9481 relay module. Figure 3.4 shows the solenoid valve used.

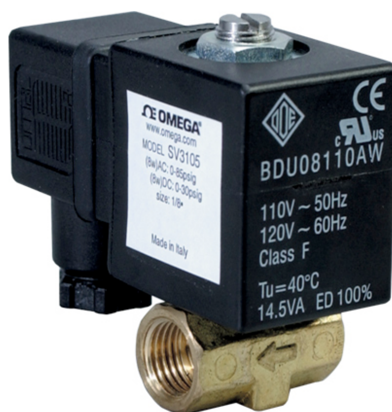

Figure 3.4: Solenoid valve used to control aeration

Initially, there were problems with the valve overheating after being used to aerate for an extended period of time. This problem was resolved by wiring two 10 Ohm resistors in line with the positive lead of the power supply.

3.1.5 Relay Control Box

Because the three pumps used in this project are powered using a standard wall outlet, there was no feasible way to directly control their function with the computer. Instead, a relay control box had to be built to allow for automated pump control. This relay control box is compatible with both data acquisition devices used in this project. In most cases, however, the National Instruments PCI-6036E was used for automation and control of the relay control box.

Dean Daigneault, one of the laboratory managers in the Civil Department at Worcester Polytechnic Institute, constructed the relay control box using an old, empty PC tower, a power supply, three electrical sockets, three Crydom single-state electromechanical relays, and some wiring. Each of the three electromechanical relays was wired to the computer, to the power supply, and to one of the three electrical sockets. With the relay control box's power source plugged into an existing wall outlet, current flows from the power source to each of the three electromechanical relays. Because the relays are normally open, however, this current dead-ends and nothing happens.

When a voltage of 3 V or greater is applied to one of these electromechanical relays, its internal circuitry closes and allows the current coming from the existing wall outlet to reach the

connected electrical socket. Thus any device that plugs into a standard wall outlet may be plugged into the relay control box and turned on or off using the computer.

If this relay control box was to be expanded, two major things could happen. First, influent, effluent, sludge waste, and hot water could each be controlled using separate pumps. And second, the solenoid valve used for aeration could be plugged directly into and the relay control box to avoid the use of the cDAQ-9172 data acquisition device for its relay function.

3.2 Automated Control Process

LabVIEW 8.0 is a very easy to use and effective software development tool. Many different features of the program were utilized to create the automated control process and general user interface for this project. Figure 3.5 shows a screenshot of the front panel developed to control this bench-scale bioreactor. Figure 3.6 shows a screenshot of the block diagram built to control the reactor.

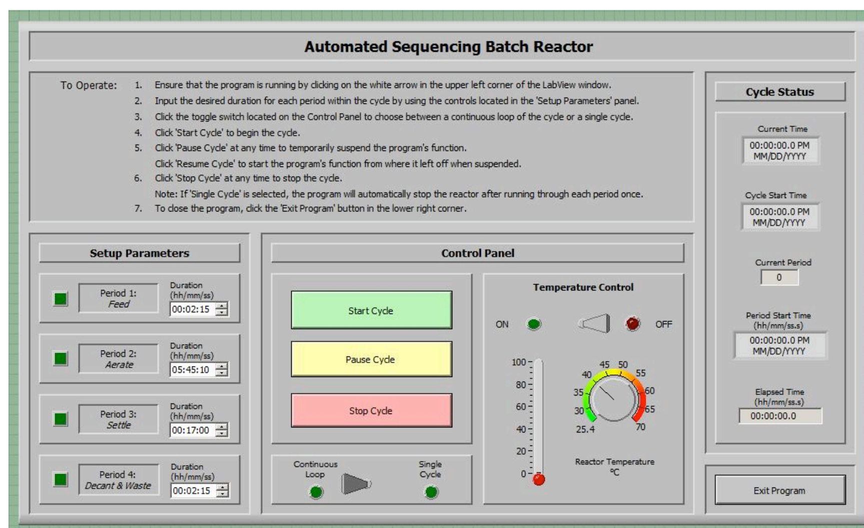

Figure 3.5: General user interface for bench-scale bioreactor automation and control

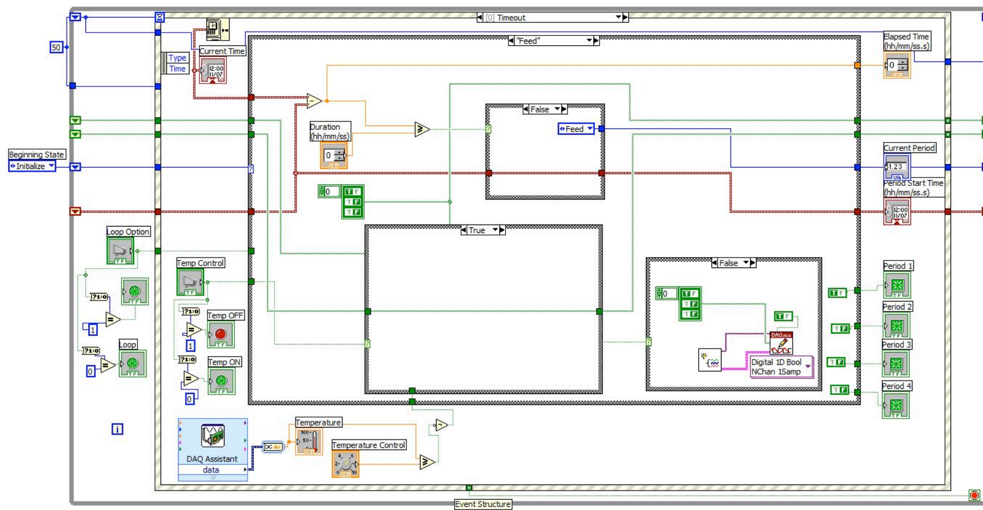

Figure 3.6: Block diagram for bench-scale bioreactor automation and control

The control program built with LabVIEW 8.0 provides the user with flexible options to accommodate a variety of testing. The user can set a custom timing sequence and can choose to run the reactor either continuously or for only one cycle. The temperature inside the reactor is measured using a thermocouple and is continuously displayed on the thermometer located in the “Temperature Control” field. Temperature control may be turned on or off, and the adjusting dial allows for a wide range of operating temperatures. The “Cycle Status” field continuously displays the current time, the cycle start time, the current period within the cycle, the current period’s start time, and the current period’s elapsed time. The reactor may be paused or stopped at any time.

3.3 Experimental Laboratory Procedures

Extensive laboratory work and calculations are necessary in designing and operating a bench scale biological reactor. Before laboratory procedures can be started, several calculations and determinations must be made. These include desired effluent substrate concentration, reactor MLSS concentration, and reaction time. Experimental procedures involved with bench scale biological treatment include reactor seeding, artificial substrate production, and parameter monitoring. When working with a bench scale bioreactor and activated sludge, safety is very important. Activated sludge is comprised of a wide variety of microorganisms, some of which are potentially harmful to humans. As such, personal protection equipment (PPE) such as gloves, goggles, and laboratory coats were worn at all times when working directly with the reactor or its contents.

3.3.1 Operating Parameters

When designing a bench scale bioreactor, several operating parameters must first be decided on. The batch volume, or volume of water to be treated, reactor biomass volume must be selected, and biomass concentration (MLSS) must be chosen. The selection of these parameters is based on the reactor size and Monod kinetics. A food to mass ratio, reaction time, and operating temperature must also be selected. The team decided on a batch volume of 500 mL, biomass volume of 300 mL, MLSS concentration of 3,000 mg/L food to mass ratio of 0.07, reaction time of 5.45 hrs, and operating temperature of 35 °C. The influent flow rate of the reactor was determined using Equation 12:

Influent Flow Rate

$$(Q)[L/day] = \frac{(V_{batch})[L]}{(T_{cycle})[hrs]}(24hrs/day) \qquad Equation\ 12$$

$$Q = \frac{.5L}{6hrs}(24hrs/day)$$

$$Q = 2.0L/day$$

Additionally, reaction coefficients and constants had to be selected. The table below details the values selected by the team for these constants and coefficients.

Table 2.2: Reaction Constants and Coefficients (Droste, 1997) (Burton et al., 2003)

Constant/Coefficient	Unit	Value
k	1/day	13.2, 3.76
k_e	1/day	0.02
Y	-	0.39

3.3.2 Reactor Seeding

Before all experimental procedures could begin, the reactor had to be seeded with an activated sludge sample. These samples were obtained from a number of treatment plants including the Upper Blackstone Pollution Abatement District wastewater treatment facility, the Marlboro-Westerly wastewater treatment facility, and the Somerset wastewater treatment facility. Samples were gathered from the various facilities using a 2 L Erlenmeyer flask. Parafilm was used to cover the flasks during transport to the laboratory. Once in the laboratory, the flasks were placed on a counter and left alone for approximately 30 minutes. During this time, the activated sludge settled to the bottom of the flask. Once the sludge was sufficiently settled, the supernatant was drawn off using a peristaltic pump and pumped down the drain. Next, the desired biomass

volume of 300 mL was transferred from the flask to a graduated cylinder. Any remaining sludge was pumped down the drain. Lastly, the biomass was transferred to the reactor.

3.3.3 Artificial Substrate Production

Biological reactions are dependant upon the digestion of substrate. In practice, substrate is derived from the constituents of wastewater. As it is impractical and posses a health risk, actual domestic wastewater was not used in laboratory experiments. In its place, a synthetic wastewater was used. The recipe for the synthetic wastewater used was taken from *Standard Methods for the Examination of Water and Wastewater, 19th Edition*. This recipe includes both a biodegradable organic carbon substrate, as glucose, and nutrients. The table in the below details this recipe details this recipe.

Table 3.1: Synthetic Wastewater Composition (Eaton et al., 1995)

Constituent	Components	Component Concentrations (g/L)	Constituent Volume (mL/L of synthetic WW)	Function
Phosphate Buffer	KH_4PO_4	8.5	1.0	nutrient
	K_2HPO_4	21.75	1.0	
	$Na_2HPO_4 \cdot 7H_2O$	33.4	1.0	
	NH_4Cl	1.7	1.0	
Magnesium Sulfate	$MgSO_4 \cdot 7H_2O$	22.5	1.0	nutrient
Calcium Chloride	$CaCl_2$	27.5	1.0	nutrient
Ferric Chloride	$FeCl_3 \cdot 6H_2O$	0.25	1.0	nutrient
Glucose-Glutamic Acid	$C_6H_{12}O_6$	0.15	1000	substrate
	$C_5H_9NO_4$	0.15	1000	nutrient

Before use, the team had to confirm that the concentration of substrate present in the synthetic wastewater was high enough to maintain the desired MLSS concentration. First, the substrate COD required to maintain the desired MLSS concentration was determined using Equation 7:

Required Influent Substrate Concentration

$$(F/M)[1/day] = \frac{(S)[mg/L)(Q)[L/day]}{(X_v)[mg/L](V_{reactor})[L]}$$

$$S = \frac{(F/M)(X_v)(V_{reactor})}{Q}$$

$$S = \frac{(.07\ 1/day)(3000mg/L)(.8L)}{(2.0L/day)}$$

$$S = 84mg/L$$

Using the ratio of COD to glucose established by Equation 1, COD == $1070mg/L\ O_2/1000mg/L\ glucose$, the team cross multiplied to find the concentration of glucose necessary to maintain an influent COD concentration of 84 mg/L:

Glucose Concentration, COD = 48 mg/L

$$\frac{1070mg/L\ O_2}{1000mg/L\ glucose} = \frac{84mg/L\ O_2}{x\ mg/L\ glucose}$$

$$(x\ mg/L\ glucose)(1070mg/L\ O_2) = (1000mg/L\ glucose)(84mg/L\ O_2)$$

$$1070x = 84{,}000$$

$$x = 78.5mg/L\ glucose$$

It was found that the concentration of glucose in the synthetic wastewater was high enough to maintain the desired MLSS concentration, and thus the synthetic wastewater recipe was acceptable for use in our experiments.

As the reactor has a flow rate of 2.0 L/day and laboratory experiments would take coarse over several days, a large volume of synthetic wastewater needed to be produced at one time. The team decided an 18 L batch of synthetic wastewater would fulfill all experimental needs. To determine the required volume of each constituent, the constituent volume ratio was multiplied by the batch size. The table below details the required volume of each synthetic wastewater constituent to produce a 18 L batch.

Table 3.2: Required Constituent Volumes

Constituent	Batch Volume (L)	Constituent Volume Ratio (mL/L of synthetic WW)	Volume Required for Batch Size (mL)
Phosphate Buffer	18	1.0	18
Magnesium Sulfate		1.0	18
Calcium Chloride		1.0	18
Ferric Chloride		1.0	18
Glucose-Glutamic Acid		1,000	18,000

The initial step in the production of synthetic wastewater was the production of its nutrient components. First, one 1 L sealable container per component was gathered, filled with 500 mL of distilled water (DI), and labeled. The compounds necessary to make the components (Detailed in Table 3.2) were then retrieved from the chemical storage room in the lab. The necessary mass of each compound was then weighed using a ceramic crucible, metal scoop, and electronic balance. The crucibles containing the various component constituents were placed adjacent to the appropriate 1 L container immediately after weighing to prevent confusion. Using the metal scoop, the compounds were then added to their respective 1 L containers. An additional 500mL

of DI water was then added to each container, and the containers were inverted to allow for mixing. The nutrient constituent containers were then placed in the refrigerator for storage.

The glucose-glutamic acid solution was then prepared. As a large volume of this constituent was required, the entire solution was prepared in one batch, rather than 18 1 L batches. Reagent grade glucose and glutamic acid were dried in the kiln at 103 °C for one hour. A 20 L carboy was then filled with 18 L of DI water. After removal from the kiln, the necessary mass of each compound was then weighed using a ceramic crucible, metal scoop, and electronic balance. The compounds were then transferred to the carboy. The mechanical mixer in the carboy was then turned on at a rapid pace to dissolve the glucose and glutamic acid and promote mixing. Once the glucose and glutamic acid were dissolved, the nutrient constituents were added to the carboy in the volumes prescribed in Table 3.2 This was done using a graduated cylinder. The mixer was then turned down to a slower rate.

3.3.4 Mixed Liquor Suspended Solids Concentration

In order to monitor and analyze microbial population and growth within the reactor, the MLSS concentration of the reactor had to be known. The first step in a MLSS test is the gathering of a sample. An automatic pipette was used to transfer mixed liquor from the reactor to a small glass beaker. Samples were gathered at roughly one-half the height of fluid in the reactor and the pipette tips used for gathering the sample had a small length of material removed from their end to allow for larger size particles to be collected. During MLSS sampling, aeration was paused to allow for the collection of a more representative sample. In total, 20 mL of mixed liquor was removed from the reactor to perform each MLSS test. Next, the mass of the weighing boat and

filter paper to be used was measured using an electronic balance and recorded as $M_{Filter,\ Boat}$. The filter paper was then formed into a cone, placed in a funnel, and moistened with DI water. Following this, funnel was placed atop a small Erlenmeyer flask and the sample was filtered through the filter paper. The filter paper was then placed on the weighing boat and dried in the kiln at 103 °C for 45 minutes. The mass of the dried sample, filter paper, and weighing boat was then measured using an electronic balance and recorded as $M_{Dried\ Sample,\ Filter,\ Boat}$. Following this measurement, the dried sample, filter paper, and weighing boat were discarded. The appendix contains more detailed directions for this procedure. Equation 13 was then used to determine the MLSS concentration in the reactor.

MLSS Concentration

$$(Conc._{Reactor\ MLSS})[mg/L] = \frac{(M_{Dried\ Sample,\ Filter,\ Boat})[g] - (M_{Filter,\ Boat})[g]}{(20mL)} \times (1000mg/g) \times (1000mL/L) \quad Equation\ 13$$

3.3.5 Chemical Oxygen Demand Measurement

In order to monitor and analyze the efficiency and effectiveness of the bioreactor, the COD of both the influent and effluent were measured. First, a calibration curve was created. This curve relates the absorbance of a sample to its COD concentration. An existing calibration curve was obtained and altered to match our experimental parameters. This was done through the use of the existing curve's trend line. The original calibration curve can be found in the appendix. Figure 3.7 below is the calibration curve that was created for use in our experiments

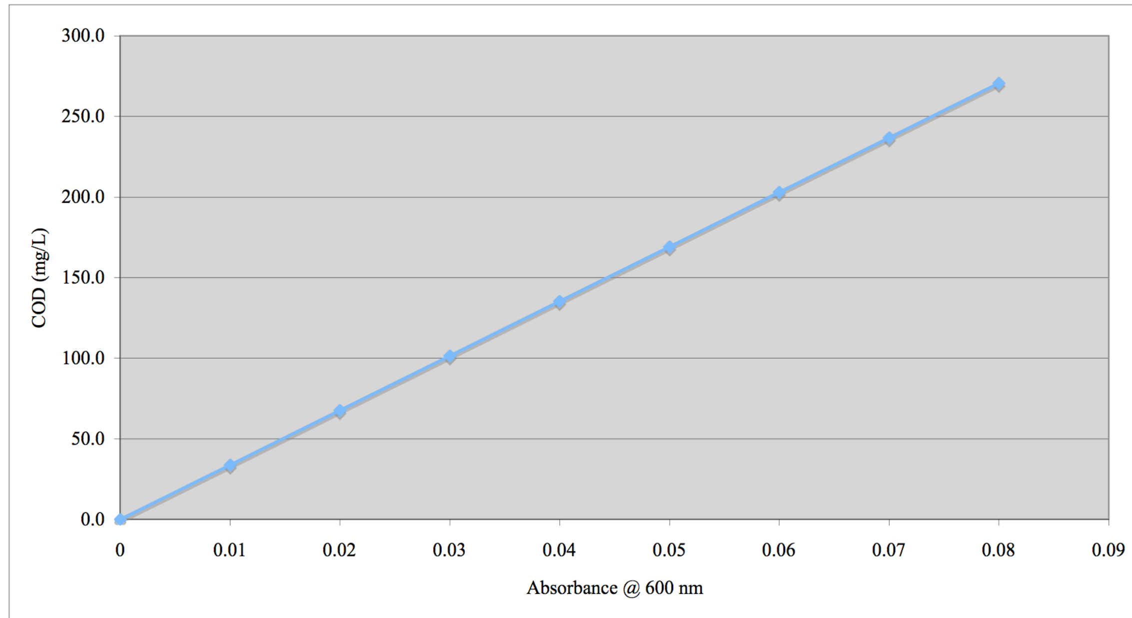

Figure 3.7: COD – Absorbance Calibration Curve

To determine influent and effluent COD, samples of both were taken. When taking samples for COD analysis, a sample volume of 10 mL is sufficient. Influent samples were collected by diverting the influent line to a small glass beaker and effluent samples were collected directly from reactor supernatant at the end of the settling period using an automatic pipette. A 10 mL sample of DI water was also collected. This was used to create a test blank. The test blank was used for calibration purposes later in the procedure. One COD vial per sample was then obtained and labeled. Next, 0.5 mL of each sample was pipetted into the appropriate COD vial. The COD vials were then placed in a COD digester, which was preheated to 150°C. The COD vials were digested for 2 hours at 150°C. Following this time, the vials were removed from the digester and allowed to cool to room temperature. Next, 3.5 mL of each sample was transferred to individual spectrophotometer cavets. The *Simple Reads* program was then started on the spectrophotometer-linked computer and set to read absorbance at 600 nm. The spectrophotometer was calibrated for use by placing the blank cavet in the spectrophotometer and pressing “ZERO” in *Simple Reads*.

The cavet was then removed from the spectrophotometer and its contents were transferred back to its respective COD vial. For continuity, the sample blank was used when analyzing all influent and effluent samples, and as such was stored in the refrigerator. The absorbance of influent and effluent samples was then determined and recorded. This was done by placing each cavet in the spectrophotometer and pressing "Read" in *Simple Reads*. The COD concentration of the samples could then be determined using Equation 14, which was developed from the COD – Absorbance Curve trend line. A more detailed procedure for completing a COD test can be found in the appendix.

COD Concentration based on Absorbance

$$(COD)[mg/L] = (absorbance)[1/cm](3385) \qquad \textit{Equation } 14$$

3.3.6 pH Measurement

Measurements of reactor pH were done using an electronic pH meter. Prior to use, the pH meter was calibrated. The pH probe was rinsed with DI water before being placed in the reactor to insure that residual calibration fluids on the probe did not taint measurements. The reactor pH was then measured and recorded. Following measurement, the probe was rinsed with DI water.

3.3.7 Dissolved Oxygen Measurement

The dissolved oxygen concentration within the reactor was measured using an electronic DO meter. First, the meter was calibrated and set to read in mg/L. Next, aeration was paused and the probe was inserted into the reactor. The DO concentration was then measured and recorded. After measurement was completed, the probe was rinsed with DI water.

3.3.8 Temperature Measurement

The reactor temperature was measured using an electronic thermometer. Prior to use, the thermometer was set to read in °C. The thermometer was then inserted into the reactor and the temperature was measured and recorded. The thermometer was rinsed with DI water following its use.

4.0 Results and Discussion

During the course of this project, several experimental trials were conducted. These trials ranged in time from 3 to 5 days. During these experiments, MLSS concentration, influent and effluent COD, and reactor pH, DO concentration, and temperature were continually monitored.

During each trial, the MLSS concentration of the reactor was measured at the beginning of the first cycle after seeding and once a day each subsequent day. A table in the appendix contains the measured MLSS concentration during the various experimental periods. Figure 4.1 below details the MLSS concentration of the reactor during experimental periods.

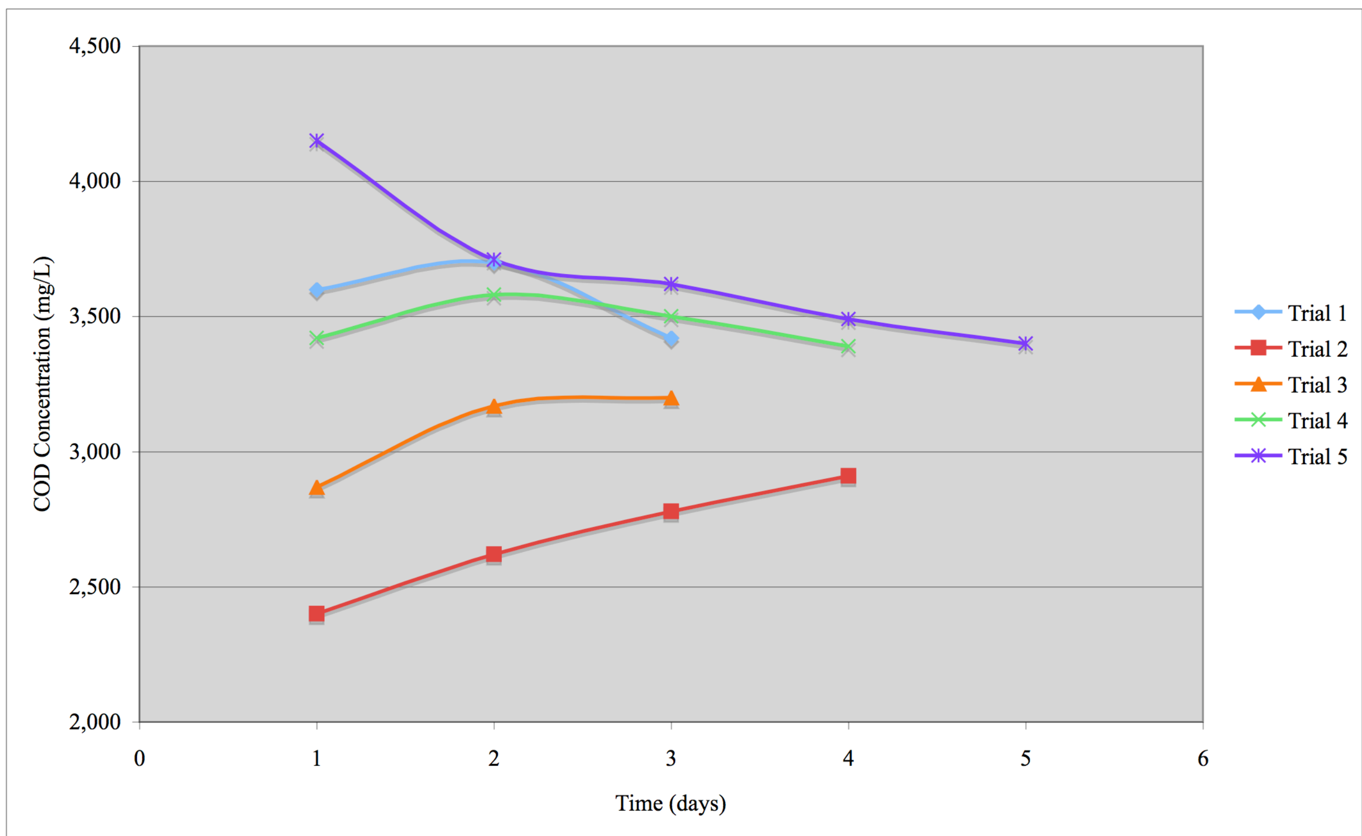

Figure 4.1: MLSS Concentration in reactor during Experimental Periods

The concentration of MLSS in the reactor behaved according to three trends. These trends are directly related to growth and decay within the reactor and are follow patterns of:

1. Continued increased
2. Continued decrease
3. Increase followed by decrease

Trial 2 follows trend 1, while Trial 4 follows trend 2 and Trials 1, 3, and 5 follow trend 3. Through analysis of this data and knowledge of biomass growth patterns, correlations can be made between the trend exhibited during a trial, microbial growth patterns, and the MLSS concentrations of the trial. For each trial, the theoretical net growth rate within the reactor over a period of one day can be calculated using Equation 5. The appendix contains a table that lists the theoretical and observed growth rates for each For example, for Trial 2:

Theoretical Biomass Growth Rate Based on Day 1 MLSS

$$r_x = -(Y)[g \ \ biomass/g \ \ COD \ \ used](r_s)[mg/L \cdot day] \ -(k_e)[1/day](X_V)[mg/L]$$
$$r_x = -(0.39)(-3.76\ {}^1\!/_{day})(160.5mg/L) - (0.02\ {}^1\!/_{day})(2400mg/L)$$
$$r_x = 187\ {}^{mg/L}\!/_{day}$$

A set of tables can be found in the appendix that contains both theoretical and observed growth rates of the various experimental trials for all day to pay periods. Relative error can be used to compare the theoretical and observed growth rates. Large values of relative error imply that the experimental data does not correlate well with theory, while small values imply the opposite. Table 4.1 below lists the relative error for each trail based on average growth rates. Equation 15 is used to calculate relative error. For example, for Trial 5, day 4 – 5:

Relative Error in Theoretical and Observed Growth Rates

$$(relative\ \ error)[\%] = \left(\frac{observed\ \ value - true\ \ value}{true\ \ value}\right) \times 100\% \qquad Equation\ 15$$

$$relative\ \ error = \left(\frac{-112.5 - 166}{166}\right) \times 100\%$$

$$relative\ \ error = -168\%$$

Table 4.1: Average Theoretical and Observed Growth Rates and Relative Error

Trial	Experiment Period	Experiment Length (days)	Avg Theoretical Growth Rate	Averaged Observed growth rate	Relative Error in growth rates
1	12/3/07 - 12/5/07	3	162.4	-112.5	-169
2	12/14/07 - 12/17/07	4	183.4	212.5	16
3	1/10/08 - 1/12/08	3	175.0	206.3	18
4	1/25/08 - 1/28/08	4	165.4	-12.5	-108
5	2/6/2008 - 2/10/08	5	160.5	-234.4	-246

Both the observed MLSS trends and the relative error in growth rates show large discrepancies between theoretical and experimental data. Theoretically, positive growth rates will yield an increasing trend while negative growth rates yield negative trends. All five trials should have followed trend one, as the calculated theoretical growth rates are all positive. The relative errors that exist between the theoretical and observed growth rates signify that the reactor was not functioning as designed.

Several factors exist that can explain the unusual growth trends observed in Figure 4.1 and the rather large values of relative error calculated. Microorganisms used in biological treatment grow

accustom to the typical wastewater characteristics in which they operate. Often, the microorganism population in a reactor transforms over time to become more efficient at degrading the wastewater it comes into contact with. As the synthetic wastewater used in these experiments is vastly different than true wastewater, it is possible that the microorganisms present in the seed sludge were not as efficient at degrading glucose as they are at domestic wastewater.

When calculating theoretical growth rates, F/M ratio, substrate utilization, and microorganism decay, several coefficients and constants had to be assumed. It is possible that the values chosen for these numbers were incorrect, which would lead to the reactor not functioning as designed and the theoretical analysis being incorrect.

During the course of these experiments, pH, DO, and reactor temperature were all monitored. Tables in the appendix list the pH, DO concentration, and temperature measured in the reactor during each trial, while Figures 4.2, 4.3, and 4.4 below display reactor pH, DO concentration, and temperature, respectively, with respect to time for each trial.

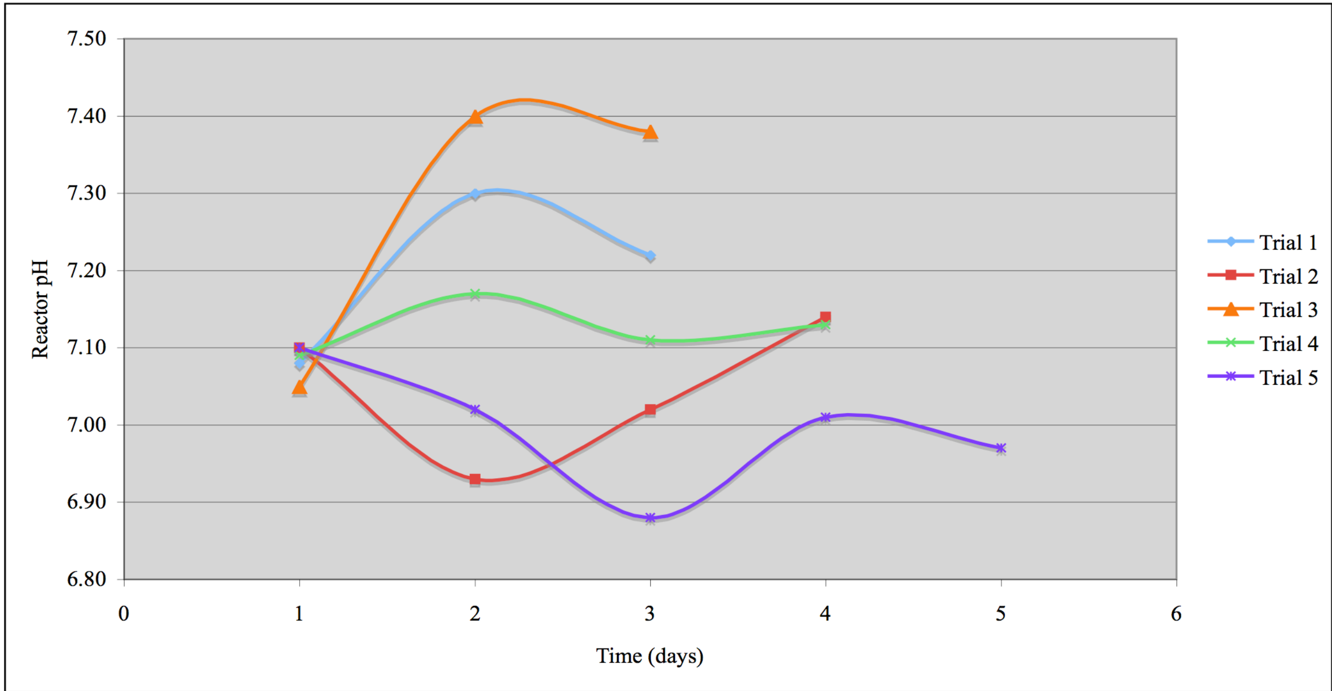

Figure 4.2: Reactor pH During Trial Periods

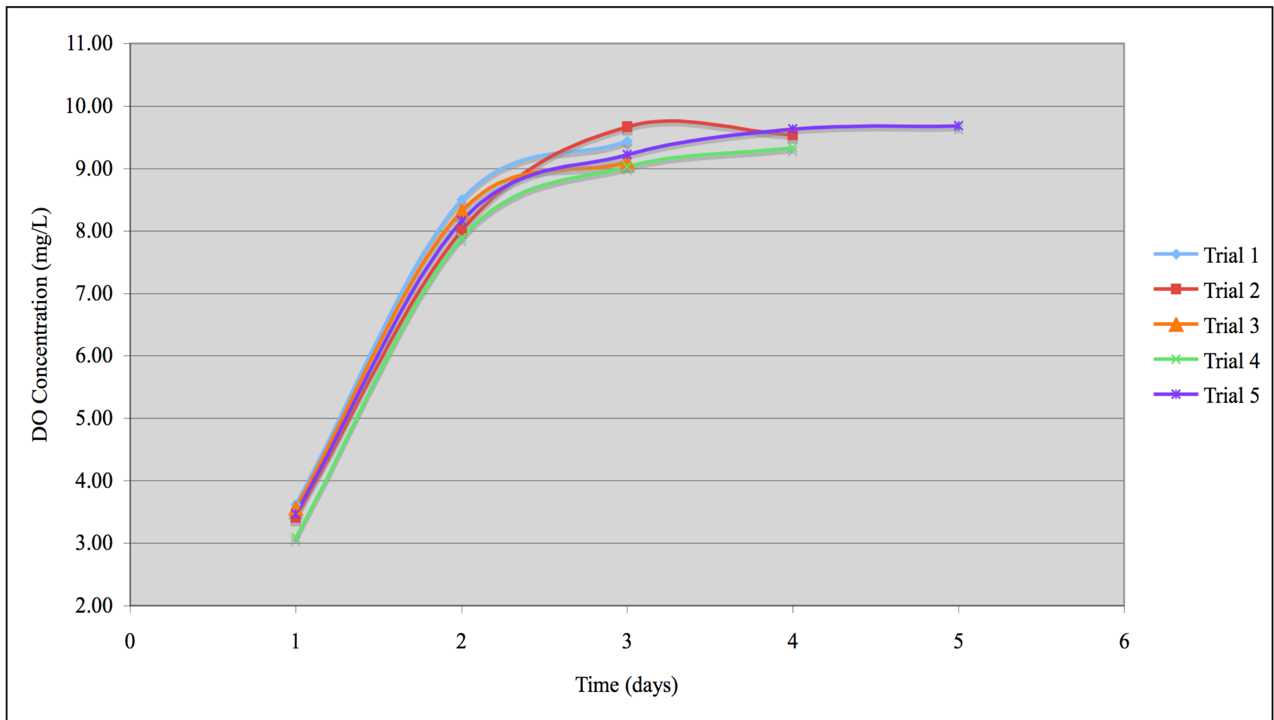

Figure 4.3: DO Concentration During Trial Periods

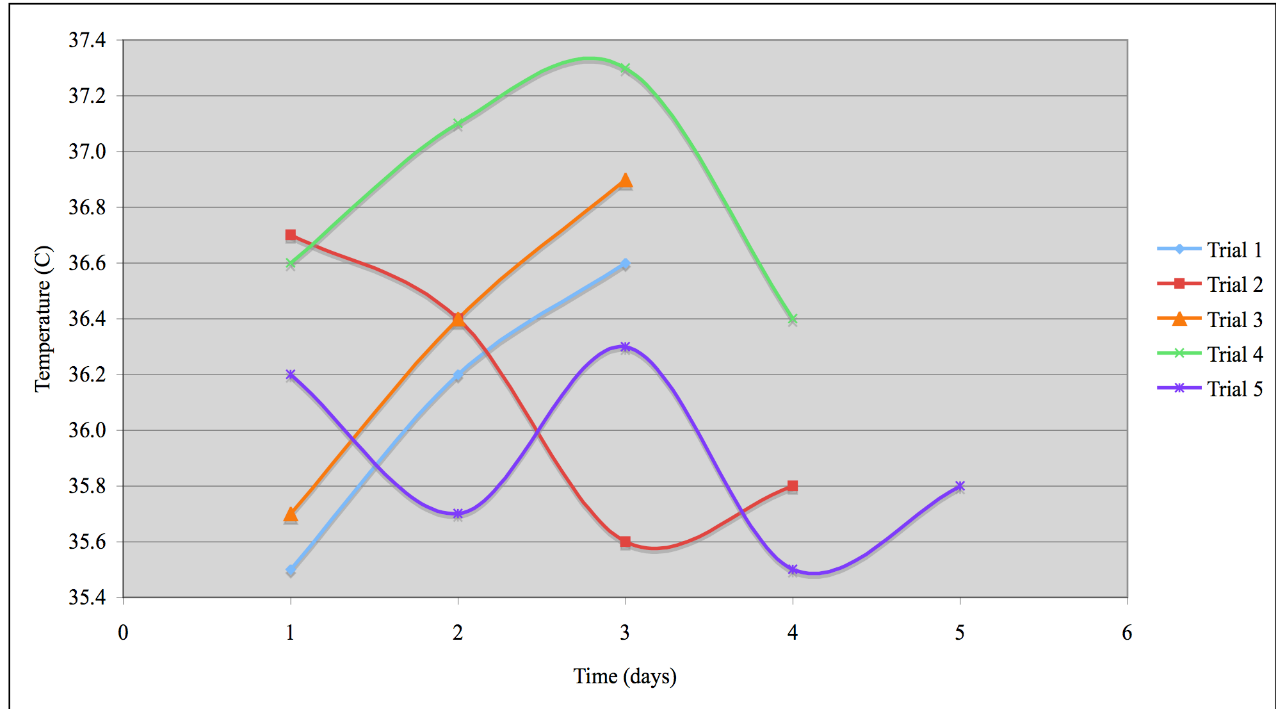

Figure 4.4: Reactor Temperature During Trial Periods

With respect to typical operating parameters of bioreactors, the pH, DO concentration, and reactor temperature measured during the trials can all be considered normal. For the removal of biodegradable carbon organics, a pH range of 6.0 – 9.0 is acceptable (Burton et al, 2003). During all trials the reactor was found to operate within a range of 6.88 to 7.38, which is well within the acceptable 6.0 – 9.0 range.

The DO concentration within the reactor was observed to increase drastically within a short period of time. This sharp jump in DO directly related to the aeration method used for this reactor. Simply by aerating the reactor, a large quantity of oxygen became dissolved in it. The DO values increase far slower towards the end of the trials because the water inside the reaction is reaching the DO saturation point. A DO concentration of 2.0 mg/L is often used for reactors

(Burton et al., 2003). At no point did the DO concentration in the reactor fall below 3.00, and as such the reactor was functioning properly with respect to DO.

The temperature in the reactor was directly controlled via a heat sync, water bath, and the jacket surrounding the reactor. Most biological treatment operations occur at a temperature of approximately 20 °C. A change in temperature above or below 20 °C will affect the rate at which a reactor utilizes substrate, and in turn, the rates at which microorganisms reproduce. Raising the operating temperature above 20 °C will increase substrate utilization while lowering it below this level will have the inverse effect. The team decided to operate the reactor at a temperature of 35 °C. The operating temperature of the reactor stayed within a range of 35.4 – 37.4 °C, which is acceptable and within the reactor design.

When monitoring the efficiency and effectiveness of a bioreactor, the utilization of substrate is the primary parameter that should be analyzed. Substrate utilization was monitored through measuring influent and effluent COD concentrations. To measure COD, the absorbance of a sample was taken and related to the COD – Absorbance Calibration Curve via Equation 14. For example, for Trial 1, day 1 effluent COD:

COD Concentration Based on Absorbance

$$(COD)[mg/L] = (absorbance)[1/cm](3385)$$
$$COD = (0.0201\,{}^{1}/_{cm})(3385)$$
$$COD = 68.04\,mg/L$$

A table in the appendix details the absorbance, as well as COD concentration for each sample taking during the trials. Figure 4.5 below displays the effluent COD concentrations of the various trials with respect to time.

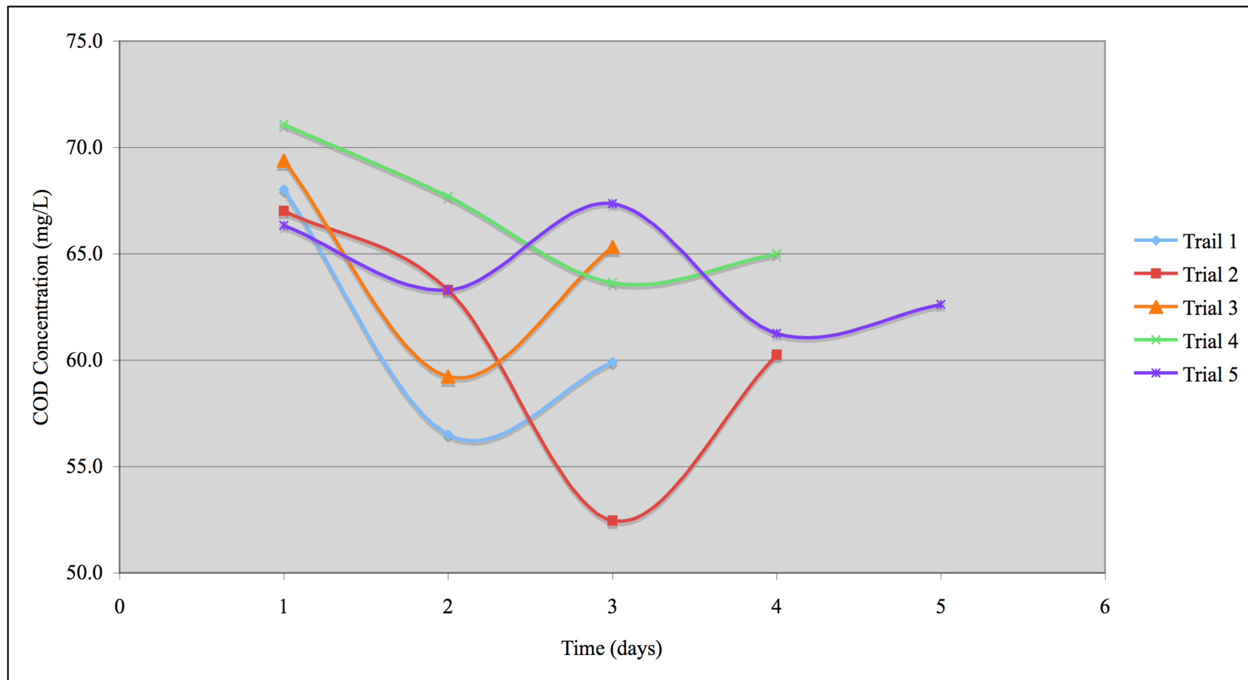

Figure 4.5: Effluent COD Concentration During Trials

By comparing the influent COD concentration to that of the effluent, the percent removal of organic matter by the bioreactor can be determined. Percent removal is an important parameter to monitor when considering the efficiency of biological treatment as it directly relates influent and effluent COD concentrations. Equation 16 is used to calculate the percent removal of COD through biological treatment. For example, for the Trial 1 average:

Percent Removal of COD

$$Percent\ Removal_{COD} = \frac{(COD_{Influent})[mg/L] - (COD_{Effluent})[mg/L]}{(COD_{Influent})[mg/L]} \times 100 \qquad Equation\ 16$$

$$Percent\ Removal_{COD} = \frac{(157.9 mg/L - 61.5 mg/L)}{(157.9 mg/L)} \times 100$$

$$Percent\ Removal_{COD} = 61.0\%$$

Table 4.2 details the average percent removal of COD for the various trials conducted. A table containing the percent removal on a day-by-day basis for all trials is available in the appendix.

Table 4.2: Average Percent Removal of COD

Trial	Trial Length (days)	COD Concentration (mg/L)		Percent Removal (%)
		Influent	Effluent	
1	3	157.9	61.5	61.0
2	4	161.0	60.8	62.2
3	3	153.1	64.6	57.8
4	4	155.3	66.9	56.9
5	5	165.3	64.2	61.2

When analyzing COD oxidation, it is important to have knowledge of the theoretical effluent COD concentration first. Using Equation 10, the theoretical effluent COD concentration can be determined:

Theoretical COD Concentration

$$S_e = (S)(e^{-kt_r})$$

$$S_e = (160.5 mg/L)(e^{-(13.2)(5.75 hrs/24 hrs)})$$

$$S_e = 6.79 mg/L$$

This value can then be used to evaluate the relative error that exists between the theoretical and experimental effluent COD concentrations. Relative error is calculated using Equation 15. The table below details the relative error that exists between the average theoretical and experimental COD concentrations.

Table 4.3: Average Theoretical and Experimental COD Concentrations and Relative Error

Trial	Trial Length (days)	Theoretical COD Concentration (mg/L)	Average Experiment COD Concentration (mg/L)	Relative Error (%)
1	3	6.79	61.5	805
2	4	6.79	60.8	795
3	3	6.79	64.6	852
4	4	6.79	66.9	885
5	5	6.79	64.2	845

The percent removal of COD and the relative error calculated for the various trials show that the reactor was not working according to design. According to the theoretical values for influent and effluent COD concentration, the bioreactor should be capable removing almost 96% of influent COD. While the reactor removed a relatively constant percentage of COD (50.7– 68.2%), it did not perform as it was designed. When taking relative error into account, the reactors performance could be judged even more poorly. The lowest percent error with respect to theoretical and experimental effluent concentrations was 795%. A relative error this large is unacceptable are displays how far the reactor was from functioning according to its design. This issue also affects various other parameters of the reactor, namely the biomass growth rate. The presence of large concentrations of COD in the effluent means that the microorganisms present in the reactor did

not digest the substrate in an effective fashion. This led to the decrease in biomass growth in the reactor displayed in the table above.

The possible sources of error with respect to COD utilization are the same as those that could have affected the biomass growth, sludge characteristics and reaction constants and coefficients. While glucose is a major source of energy in aerobic digestion, it is not present in abundant quantities in domestic wastewater. As such, the microorganisms in the seed sludge were most likely not used to digesting glucose as their only form of substrate. Over an extended period of time the microorganism population present in the reactor would adapt to the influent concentration. However, the relatively short trial periods did not permit this. With respect to reaction constants and coefficients, these values could be altered to more appropriately fit the reactor. Determining exact, or even close values for these parameters is extremely difficult in a laboratory setting, as laboratory reactors are usually small and do not operate for extended periods of time.

5.0 Conclusion

The bench-scale sequencing batch reactor that was built can be effectively used for scaled-back laboratory testing for an actual wastewater treatment plant. This was proven by the results of the chemical oxygen demand testing that was performed daily in the laboratory. These laboratory tests showed a significant decrease in the COD of the wastewater, on average about 60 percent. Optimally, an actual wastewater treatment plant needs to remove more than 60 percent of the COD from the influent wastewater. Therefore, there were a few potential improvements that were determined to increase the efficiency of our bench-scale reactor.

Potential improvements for our bench-scale reactor include automated pH monitoring, automated dissolved oxygen monitoring, a fully automated feed system, and more accurate temperature control. Automated pH and DO monitoring would allow for higher reaction efficiency, as the microorganisms consume more organic waste at optimum pH and DO levels. A fully automated feed system would improve our bench-scale reactor, as it would provide a constant flow of food to the microorganisms at all times keeping the amount of new sludge higher than the amount of old sludge inside the reactor. More accurate temperature control would improve our bench-scale reactor because microorganisms consume organic waste more effectively at high, constant temperatures. The heated water bath used in this project cannot be the most efficient way of keeping the contents of the reactor at a high, constant temperature as many errors can occur with the bath. Automated pH and DO monitoring can be easily fixed for our reactor, as these parts can be simply purchased from a laboratory company. However, further research and experimentation must be performed to determine the most effective way to have an automated feed system and more accurate temperature control.

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7.0 Appendix

7.1 Laboratory Procedures

7.1.1 Mix Liquor Suspended Solids (MLSS) Test

Objective: Determine the MLSS concentration inside the reactor

Equipment

1. 2-10 mL Automatic Pipette
2. Modified 2-10mL Automatic Pipette Tip
3. Scissors
4. Glass Erlenmeyer Flask
5. Glass Beaker
6. Plastic Funnel
7. Whatman #4 110mm Diameter Qualitative Filter Paper
8. Aluminum Weighing Boat
9. Mettler Toledo AB104-S Electronic Balance
10. Lindberg/Blue Kiln

Method

1) Turn on the kiln and set it to 103 °C.
2) Using the electronic balance, determine the mass of the aluminum weighing boat and filter paper in grams. Record this mass as $M_{Filter,\ Boat}$.
3) Use the scissors to modify the pipette tip as required.
4) Near the end of the react phase, pause aeration, remove 20 mL of mixed liquor in 5 mL increments, and transfer the samples to the glass beaker. Take samples roughly at one-half the depth of the liquid volume.
5) Shape the filter into a cone as shown, moisten it with DI water, and place it in the funnel. Place the funnel in the Erlenmeyer flask.
6) Filter the sample through the filter, collecting the solids in the filter and the excess liquid in the Erlenmeyer flask. Filter the sample in several increments to avoid overloading the filter.
7) Place the filter on the weighing boat. Place the filter and weighing boat in the kiln and allow them to dry for approximately 45 minutes to one hour.
8) Remove the dried filter paper and weighing boat from the kiln. Determine the mass of the dried MLSS, filter paper, and weighing boat using the electronic balance. Record this mass as $M_{Dried\ \ MLSS,\ Filter,\ Boat}$.
9) Determine the MLSS concentration in the reactor using Equation 11.

7.1.2 Chemical Oxygen Demand (COD) Test

Objective: Determine the COD of reactor influent or effluent.

Equipment

1. Bioscience Inc. accu-TEST Chemical Oxygen Demand Micro-COD Vials
2. 100-1000 μL Automatic Pipette
3. 100-1000 μL Automatic Pipette Tip(s)
4. 2-10 mL Automatic Pipette
5. 2-10 mL Automatic Pipette Tip(s)
6. Thermometer
7. Spectrophotometer Cavet(s)
8. Glass Beaker
9. Hach COD Reactor
10. Cary 50 Scan UV-Visible Spectrophotometer
11. Spectrophotometer-linked Computer, *Simple Reads* Program

Method

1) Place the thermometer in the COD reactor. Set the COD reactor to infinite time and 150 °C. Allow the reactor temperature to reach 150 °C.
2) Obtain a small volume (< 20 mL) of DI water in the glass beaker. Using the 100-1000 μL Automatic Pipette, transfer 0.5 mL (500 μL) to a COD vial. Cap the vial and shake to mix. This vial will serve as the test blank.
3) For effluent, near the end of the settle phase, transfer 0.5 mL of supernatant from the reactor to a COD vial using the 1000 μL Automatic Pipette. For influent, collect a 10 mL by diverting the influent line to a small glass graduated cylinder, then transfer 0.5 mL of the sample to a COD vial. Cap the vials and shake to mix. These vials are the test sample vials.
4) Place all vials in the COD reactor and allow two hours for reaction.
5) After two hours, remove both vials from the reactor. Allow them to cool to room temperature and allow any particulates to settle.
6) Turn on the Spectrophotometer linked computer and start *Simple Reads*. Set the program to read the sample absorbance at 600 nm.
7) Using the 2-10 mL Automatic Pipette, transfer 3.5 mL of the blank vial to a cavet. Place the cavet in the spectrophotometer. Press “Zero” on the program. The spectrophotometer is now zeroed. Removed the blank cavet and transfer the contents back into the zero COD vial. Save this vial for use during later COD tests.
8) Transfer 3.5 mL of the each sample COD vial to a cavet. Place the cavet in the spectrophotometer and read the absorbance of the sample by pressing, “Read” on Simple Reads. Record the absorbance value.
9) Empty the contents of the COD vial and cavet into a hazardous waste container and place the container in the hazardous waste locker.
10) Determine the sample COD by using Equation 13.

7.1.3 pH Measurement

Objective: Determine the pH within the reactor

Equipment
1. Orion Model 420A pH Meter
2. pH Meter Calibration Liquids
3. DI Rinse Water Squirt Bottle

Method
1) Turn on the pH Meter. Calibrate the meter using the calibration liquids, rinsing the probe with DI water after insertion in each liquid.
2) Insure that the pH meter is not set to "Auto-Off".
3) Insert the probe into the reactor and record the reactor pH.
4) Remove the probe from the reactor and rinse the probe with DI water.
5) Place the probe back in its storage liquid and turn the pH meter off.

7.1.4 DO Measurement

Objective: Determine the DO concentration within the reactor

Equipment
1. Thermo-Electron Corp Orion Star 3 DO Benchtop Meter
2. Stir Plate and Bar
3. DI Rinse Water Squirt Bottle

Method
1) Turn on the DO Meter. Calibrate the meter using the stirring plate and stir bar.
2) Insure that the DO meter is set to read in mg/L.
3) Insert the probe into the reactor and record the reactor DO.
4) Remove the probe from the reactor and rinse the probe with DI water.
5) Place the probe back in its storage container and turn the DO meter off.

7.1.5 Temperature Measurement

Objective: Determine the temperature within the reactor

Equipment
1. Electronic Thermometer
2. DI Rinse Water Squirt Bottle

Method

1) Turn on the thermometer.
2) Place the thermometer in the reactor and record the reactor temperature.
3) Turn the thermometer off and rinse it with DI water.

7.2 Tables

Table 7.1: Composition of Common Raw Wastewater (Burton et al., 2003)

Constituent	Units	Concentration		
		Low Strength	Medium Strength	High Strength
Total Solids (TS)	mg/L	390	720	1230
Total Dissolved Solids (TDS)	mg/L	270	500	860
- Fixed	mg/L	160	300	520
- Volatile	mg/L	110	200	340
Total Suspended Solids (TSS)	mg/L	120	210	400
- Fixed	mg/L	25	50	85
- Volatile	mg/L	95	160	315
Settable Solids	mg/L	5	10	20
BOD_5	mg/L	110	190	350
Total Organic Carbon	mg/L	80	140	260
COD	mg/L	250	430	800
Total Nitrogen As N	mg/L	20	40	70
- Organic	mg/L	8	15	25
- Free Ammonia	mg/L	12	25	45
- Nitrites	mg/L	0	0	0

- Nitrates	mg/L	0	0	0
Total Phosphorus as P	mg/L	4	7	12
- Organic	mg/L	1	2	4
- Inorganic	mg/L	3	5	8
Chlorides	mg/L	30	50	90
Sulfate	mg/L	20	30	50
Oil and Grease	mg/L	50	90	100
VOCs	μg/L	<100	100 - 400	>400
Total Coliforms	#/100mL	$10^6 - 10^8$	$10^7 - 10^9$	$10^7 - 10^{10}$
Fecal Coliforms	#/100mL	$10^3 - 10^5$	$10^4 - 10^6$	$10^5 - 10^8$
Cryptosporidium oocysts	#/100mL	$10^{-1} - 10^0$	$10^{-1} - 10^1$	$10^{-1} - 10^2$
Giardia lamblia, cysts	#/100mL	$10^{-1} - 10^1$	$10^{-1} - 10^2$	$10^{-1} - 10^3$

Table 7.2: Typical Residential Wastewater Constituent Concentrations (Burton et al., 2003)

Constituent	Mass (g/ capita·day)	Concentration (mg/L)	
		Volume (L/capita·day)	
		190	460
BOD_5	85	450	187
COD	198	1,050	436
TSS	95	503	209
NH_3 as N	7.8	41.2	17.2
Organic N as N	5.5	29.1	12.1
TKN as N	13.3	70.4	29.3
Organic P as P	1.23	6.5	2.7
Inorganic P as P	2.05	10.8	4.5
Total P as P	3.28	17.3	7.2
Oil and Grease	31	164	68

Table 7.3: MLSS Concentrations during Experimental Trials

Trial	Trial Period	Trial Length (days)	MLSS Concentration (mg/L				
			Day 1	Day 2	Day 3	Day 4	Day 5
1	12/3/07 - 12/5/07	3	3,600	3,700	3,420	-	-
2	12/14/07 - 12/17/07	4	2,400	2,620	2,780	2,910	-
3	1/10/08 - 1/12/08	3	2,870	3,170	3,200	-	-
4	1/25/08 - 1/28/08	4	3,420	3,580	3,500	3,390	-

Table 7.4: Theoretical and Observed Growth Rates during Experimental Trials

Trial	Trial Period	Day 1	Day 2	Day 1 to 2		
		MLSS (mg/L)	MLSS (mg/L)	Theoretical Growth Rate ($\frac{mg/L}{day}$)	Observed Growth Rate ($\frac{mg/L}{day}$)	Relative Error in growth rates (%)
1	12/3/07 - 12/5/07	3,600	3,700	163	125	-23
2	12/14/07 - 12/17/07	2,400	2,620	187	275	47
3	1/10/08 - 1/12/08	2,870	3,170	178	375	111
4	1/25/08 - 1/28/08	3,420	3,580	167	200	20
5	2/6/2008 - 2/10/08	4,150	3,710	152	-550	-461

Trial	Trial Period	Day 2	Day 3	Day 2 to 3		
		MLSS (mg/L)	MLSS (mg/L)	Theoretical Growth Rate	Observed Growth Rate	Relative Error in growth rates
1	12/3/07 - 12/5/07	3,700	3,420	161	-350	-317
2	12/14/07 - 12/17/07	2,620	2,780	183	200	9
3	1/10/08 - 1/12/08	3,170	3,200	172	37.5	-78
4	1/25/08 - 1/28/08	3,580	3,500	164	-100	-161
5	2/6/2008 - 2/10/08	3,710	3,620	161	-112.5	-170

Trial	Trial Period	Day 3	Day 4	Day 3 to 4		
		MLSS (mg/L)	MLSS (mg/L)	Theoretical Growth Rate	Observed Growth Rate	Relative Error in growth rates
2	12/14/07 - 12/17/07	2,780	2,910	180	162.5	-10
4	1/25/08 - 1/28/08	3,500	3,390	165	-137.5	-183
5	2/6/2008 - 2/10/08	3,620	3,490	163	-162.5	-200

Trial	Trial Period	Day 4	Day 5	Day 4 to 5		
		MLSS (mg/L)	MLSS (mg/L)	Theoretical Growth Rate	Observed Growth Rate	Relative Error in growth rates
5	2/6/2008 - 2/10/08	3,490	3,400	166	-112.5	-317

Table 7.5: Reactor pH During Trial Periods

Trail	Trial Period	Trial Length (days)	pH				
			Day 1	Day 2	Day 3	Day 4	Day 5
1	12/3/07 - 12/5/07	3	7.08	7.30	7.22	-	-
2	12/14/07 - 12/17/07	4	7.10	6.93	7.02	7.14	-
3	1/10/08 - 1/12/08	3	7.05	7.40	7.38	-	-
4	1/25/08 - 1/28/08	4	7.09	7.17	7.11	7.13	-
5	2/6/2008 - 2/10/08	5	7.10	7.02	6.88	7.01	6.97

Table 7.6: Reactor DO Concentration during Trial Periods

Trial	Trial Period	Trial Length (days)	DO Concentration (mg/L)				
			Day 1	Day 2	Day 3	Day 4	Day 5
1	12/3/07 - 12/5/07	3	3.62	8.50	9.44	-	-
2	12/14/07 - 12/17/07	4	3.41	8.01	9.67	9.54	-
3	1/10/08 - 1/12/08	3	3.56	8.32	9.11	-	-
4	1/25/08 - 1/28/08	4	3.09	7.89	9.03	9.33	-
5	2/6/2008 - 2/10/08	5	3.47	8.16	9.22	9.63	9.68

Table 7.7: Reactor Temperature during Trial Periods

Trial	Trial Period	Trial Length (days)	Reactor Temperature				
			Day 1	Day 2	Day 3	Day 4	Day 5
1	12/3/07 - 12/5/07	3	35.5	36.2	36.6	-	-
2	12/14/07 - 12/17/07	4	36.7	36.4	35.6	35.8	-
3	1/10/08 - 1/12/08	3	35.7	36.4	36.9	-	-
4	1/25/08 - 1/28/08	4	36.6	37.1	37.3	36.4	-
5	2/6/2008 - 2/10/08	5	36.2	35.7	36.3	35.5	35.8

Table 7.8: Influent Absorbance and COD Concentration

Trial	Trial Length (days)	Day 1		Day 2		Day 3		Day 4		Day 5	
		Absorbance (1/cm)	COD (mg/L)	Absorbance (1/cm)	COD (mg/L)	Absorbance (1/cm)	COD (mg/L)	Absorbance (1/cm)	COD (mg/L)	Absorbance (1/cm)	COD (mg/L)
1	3	0.0455	154.0	0.0467	158.1	0.0477	161.5	-	-	-	-
2	4	0.0481	162.8	0.0492	166.5	0.0488	165.2	0.0441	149.3	-	-
3	3	0.0416	140.8	0.0473	160.1	0.0468	158.4	-	-	-	-
4	4	0.0475	160.8	0.0464	157.1	0.0435	147.2	0.0461	156.0	-	-
5	5	0.0489	165.5	0.0492	166.5	0.0484	163.8	0.0495	167.6	0.0481	162.8

Table 7.9: Influent Absorbance and COD Concentration

Trial	Trial Length (days)	Day 1		Day 2		Day 3		Day 4		Day 5	
		Absorbance (1/cm)	COD (mg/L)	Absorbance (1/cm)	COD (mg/L)	Absorbance (1/cm)	COD (mg/L)	Absorbance (1/cm)	COD (mg/L)	Absorbance (1/cm)	COD (mg/L)
1	3	0.0201	68.0	0.0167	56.5	0.0177	59.9	-	-	-	-
2	4	0.0198	67.0	0.0187	63.3	0.0155	52.5	0.0178	60.3	-	-
3	3	0.0205	69.4	0.0175	59.2	0.0193	65.3	-	-	-	-
4	4	0.0210	71.1	0.0200	67.7	0.0188	63.6	0.0192	65.0	-	-
5	5	0.0196	66.3	0.0187	63.3	0.0199	67.4	0.0181	61.3	0.0185	62.6

Table 7.10: Percent Removal of COD during Trials

Trial	COD Concentration (mg/L)		Percent Removal (%)	COD Concentration (mg/L)		Percent Removal (%)	COD Concentration (mg/L)		Percent Removal (%)	COD Concentration (mg/L)		Percent Removal (%)	COD Concentration (mg/L)		Percent Removal (%)
	Influent	Effluent		Influent	Effluent		Influent	Effluent		Influent	Effluent		Influent	Effluent	
1	154	68	55.8	158.1	56.5	64.3	161.5	59.9	62.9	-	-	-	-	-	-
2	162.8	67	58.8	166.5	63.3	62.0	165.2	52.5	68.2	149.3	60.3	59.6	-	-	-
3	140.8	69.4	50.7	160.1	59.2	63.0	158.4	65.3	58.8	-	-	-	-	-	-
4	160.8	71.1	55.8	157.1	67.7	56.9	147.2	63.6	56.8	156	65	58.3	-	-	-
5	165.5	66.3	59.9	166.5	63.3	62.0	163.8	67.4	58.9	167.6	61.3	63.4	162.8	62.6	61.5

7.3 Figures

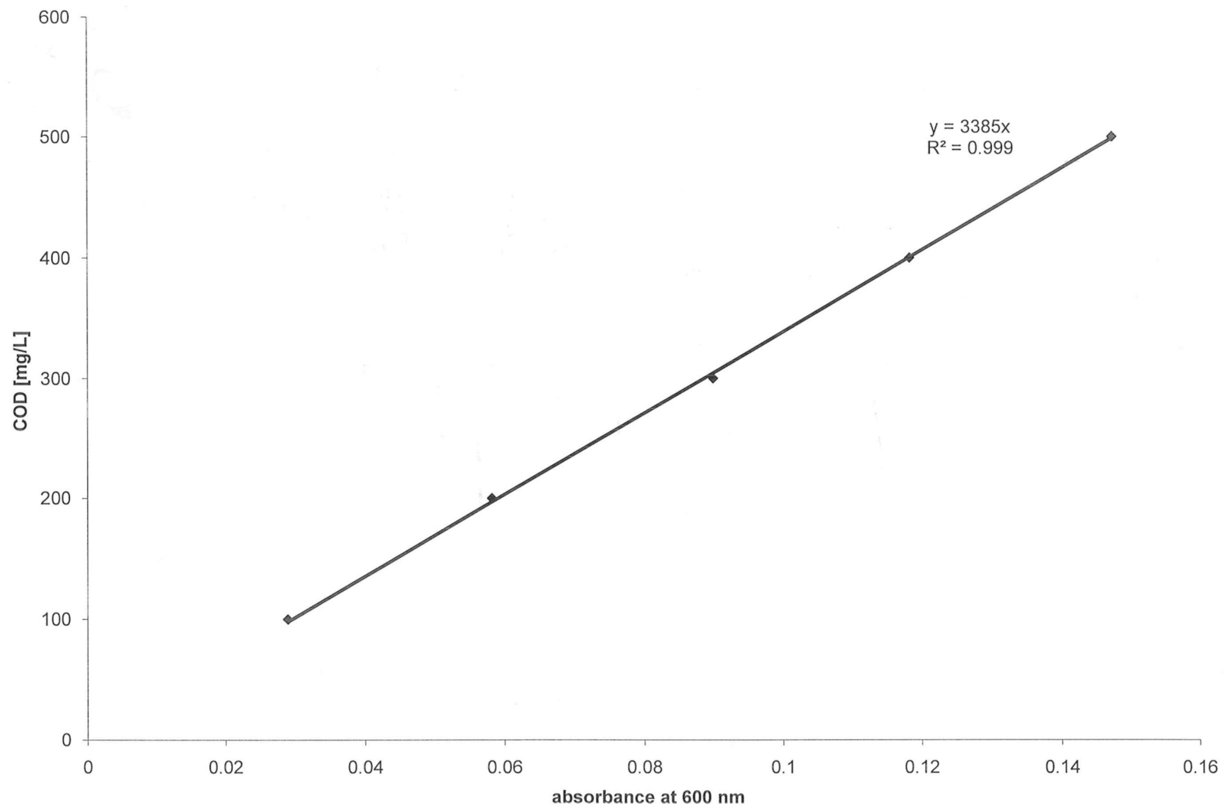

Figure 7.1: Existing COD – Absorbance Calibration