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To the Graduate Council:

I am submitting herewith a thesis written by Jack Joseph Parker entitled "The Effects of Dissolved Oxygen Concentration and Biological Solids Retention Time on Activated Sludge Treatment Performance." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental Engineering.

Dr. Kevin G. Robinson, Major Professor

We have read this thesis and recommend its acceptance:

Dr. Gregory Reed, Dr. Gary Sayler

Accepted for the Council: Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Dr. Gary Sayler

Accepted for the Council:

Dr. Anne Mayhew

Interim Vice Provost and Dean of The Graduate School

(Original signatures are on file in the Graduate Student Services offices)

The Effects of Dissolved Oxygen Concentration and Biological

Solids Retention Time on Activated Sludge Treatment

Performance

A Thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Jack Joseph Parker

December 2001

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Abstract

A bench scale treatment system with dissolved oxygen (DO) control was used to determine the effects of DO concentration and biological solids retention time (BSRT) on treatment performance using the activated sludge process. The four reactors, operating at BSRTs of 20, 10, 5, and 2 days, were fed settled municipal wastewater collected from the Kuwahee wastewater treatment plant in Knoxville, TN. The DO was maintained at different set points in each reactor ranging from 4.0 to 0.2 mg/L.

Experimental results indicate that carbon treatment performance improved, on average, with increasing BSRT but DO had little effect on carbon oxidation. Sludge volume index (SVI) and effluent suspended solids (ESS) values also indicated that BSRT not DO concentration, affected sludge settling. Complete nitrification occurred in the 20, 10, and 5 day BSRT reactors under excess DO conditions (\geq 2.0 mg/L). Nitrification was unaffected at a DO as low as 0.5 mg/L for the two longest BSRTs; however, nitrite buildup occurred in the 5 day BSRT during operation at 0.5 mg/L DO suggesting that nitrite oxidation can limit nitrification when insufficient DO is present. A 2 day BSRT was found to be insufficient for complete nitrification at all DO levels.

Kinetic coefficients for the nitrifiers were determined for Knoxville's municipal wastewater. The yield, decay coefficient, maximum substrate utilization rate, maximum growth rate, substrate half saturation coefficient, and oxygen half saturation coefficient were found to be 0.33 mg VSS/mg N, 0.17 day⁻¹, 2.2 mg N/mg VSS-day, 0.75 day⁻¹, 0.25 mg/L NH₄⁺, and 0.92 mg/L O₂ respectively. These values are within a published range identified in the literature.

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Nomenclature and Abbreviations

Nomenclature

C _{cr}	Critical Dissolved Oxygen Concentration
f_A	Active Biomass Fraction of MLVSS
f_b	Biodegradable Fraction of Active Biomass
f _{b,a}	Active Autotrophic Biomass Fraction
k	Maximum Specific Substrate Utilization Rate
K _d	Decay Rate Coefficient
Ko	Half-Saturation Constant for Oxygen
Ks	Half-Saturation Constant
PY _{obs}	Observed Growth Yield Index
Q	Flow Rate
q	Specific Substrate Utilization Rate
q _{nit}	Nitrifier Specific Substrate Utilization Rate
So	Influent Substrate Concentration
Se	Effluent Substrate Concentration
V	Volume of Sample
$W_{ m ff}$	Final Filter Weight from MLSS Analysis
W_{fv}	Final Filter Weight After MLVSS Analysis
WI	Initial Filter Weight
X _{B,A}	Active Autotrophic Biomass Concentration
X _T	Total Active Biomass Concentration
Y	Yield Coefficient
Y _A	Yield Coefficient for Autotrophs
γ	γ_1/γ_2
γ_1	Percentage of Maximum Growth Rate for Ammonia Oxidizers Under
	Different Dissolved Oxygen Conditions
γ_2	Percentage of Maximum Growth Rate for Nitrite Oxidizers Under
	Different Dissolved Oxygen Conditions
θ_{c}	Biological Solids Retention Time
μ	Specific Growth Rate
μ_{max}	Maximum Specific Growth Rate

Abbreviations

- BOD Biological Oxygen Demand Five Day Biological Oxygen Demand BOD₅ Biological Solids Retention Time BSRT Chemical Oxygen Demand COD
- CODt Total Chemical Oxygen Demand

COD _s	Soluble Chemical Oxygen Demand
DDE	Dynamic data Exchange
DO	Dissolved Oxygen
ESS	Effluent Suspended Solids
FA	Free Ammonia
HDPE	High Density Polyethylene
HART	Highway Addressable Remote Transducer
HRT	Hydraulic Retention Time
KHP	Potassium Hydrogen Phthalate
KUB	Knoxville Utilities Board
MET	Mass Estimation Technique
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NOD	Nitrogenous Oxygen Demand
SBR	Sequencing Batch Reactor
SSV	Settled Sludge Volume
SVI	Sludge Volume Index
TKN	Total Kjeldahl Nitrogen
WWTP	Wastewater Treatment Plant

Chapter 1.0

Introduction

Municipal wastewater treatment plants (WWTP) often use biological treatment processes to convert dissolved organic matter into settleable biological solids and carbon dioxide. Several biological treatment methods exist that are either suspended or attached growth processes. Activated sludge, the most widely used biological process for treating wastewater (Tchobanoglous and Burton, 1991), is an aerobic suspended growth process in which microorganisms biooxidize organic or carbonaceous compounds in the influent waste stream. The microorganisms, mainly composed of bacteria, form a flocculent slurry that settles under quiescent conditions. Due to the flocculation of biomass, organic solids can be reduced to low levels and a clear effluent can be produced (Grady et al., 1999). Organic compounds can impose a large oxygen demand on a receiving body of water if untreated. The goal of the activated sludge process is to reduce oxygen demand when bacteria utilize the organic compounds to yield energy for growth.

Activated sludge can also be used to oxidize inorganic compounds such as ammonia. The presence of reduced forms of nitrogen, specifically ammonia and organic nitrogen, is typical in municipal wastewater. Untreated, these compounds impose a large nitrogenous oxygen demand (NOD) on receiving waters, which can drastically lower the dissolved oxygen (DO) concentration. Additionally, ammonia is toxic to fish and other aquatic organisms at relatively low concentrations (Tchobanoglous and Burton, 1991). Biological oxidation of the ammonium ion (nitrification) is typically used to eliminate the NOD by conversion of ammonium to nitrate via nitrite. Nitrifying bacteria are very sensitive to operational factors such as BSRT and DO concentration (Benefield and Randall, 1985). Since nitrifying bacteria have a maximum growth rate nearly an order of magnitude lower than bacteria responsible for COD removal, they can be hydraulically washed-out of a bioreactor under conditions suitable for COD reduction (Grady et al., 1999). Consequently, the BSRT must be chosen carefully in systems incorporating nitrification because it cannot be assumed that conditions suitable for soluble organics removal are suitable for removal of ammonium. This problem is magnified by the fact that nitrifiers are more sensitive to DO concentration than other bacteria. Aeration is especially important in combined carbon removal/nitrification systems since approximately 4.33 mg of O_2 are consumed per mg of NH_4^+ oxidized to nitrate (Benefield and Randall, 1985).

Oxygen concentration is also important in determining the efficiency of activated sludge settling. In a non-DO limited system, bacteria agglomerate into flocs which rapidly settle in the clarifier. Capturing biomass in the clarifier and recycling the bacteria back to the aeration tank are key steps in the activated sludge treatment. Since poor floc formation is the most common cause of failure in activated sludge treatment systems, the DO must be kept at an acceptable level to assure good settling biomass (Rittmann and McCarty, 2001). Low DO concentrations enhance the growth of filamentous bacteria. These organisms are typically present even at excess DO conditions and form the backbone of floc particles. However, low DO conditions allow these bacteria to proliferate and thereby decrease the amount of biomass compaction. The excess of

filaments causes the formation of bulking sludge, which does not settle well and typically increases the amount of solids lost in the effluent.

The overall goal of this study was to examine how biological solids retention time and dissolved oxygen concentration influenced the processes that occur in a combined carbon removal/nitrification activated sludge system. Past studies have tended to focus on one particular area of treatment i.e. COD removal, settling, etc. rather than the whole picture. This study was intended to help determine the operational conditions (i.e. BSRT and DO concentration) necessary for effective carbon removal and nitrification to occur in an activated sludge system.

Biological solids retention time is important in activated sludge systems because it is functionally related to the specific growth rate of the biomass and because it is an operational parameter that can be physically controlled to maintain treatment performance. DO concentration is important because nitrifying bacteria are sensitive to low DO ($\leq 2.0 \text{ mg/L}$) and because aeration is a major associated with aerobic wastewater treatment (Grady et al., 1999). One primary objective of this study was to assess the minimum DO concentration and BSRT necessary to provide effective treatment performance. This was accomplished by operating reactors at BSRTs of 20, 10, 5, and 2 days and varying the DO concentration in a range from 40.2 mg/L while evaluating COD treatment, nitrification, and sludge settling.

The second objective was to determine the nitrification kinetic coefficients to make it possible to design an activated sludge system treating a similar waste stream. The yield coefficient, substrate half-saturation coefficient, decay coefficient, maximum specific substrate utilization rate, and maximum specific growth rate for the nitrifier population at steady state were determined. The oxygen half saturation coefficient was also calculated to evaluate the sensitivity of nitrifying bacteria to DO concentration. The coefficients for the nitrifiers were determined because they are typically the limiting factor in a combined carbon removal/nitrification system.

Chapter 2.0

Literature Review

2.1 Introduction to the Activated Sludge Process

The activated sludge process is utilized to convert most organic wastes to more stable inorganic forms or to cellular mass. In this process much of the organic matter remaining after primary sedimentation of wastewater is converted to carbon dioxide and water by a diverse group of microorganisms (Benefield and Randall, 1985) while the remainder of the organics are used for conversion to a cellular mass that can be separated from the waste flow by gravity settling.

Activated sludge is a heterogeneous microbial culture composed mainly of bacteria, protozoa, rotifers, and fungi. However, it is the bacteria that are mainly responsible for degradation of organic and nitrogenous compounds in the activated sludge treatment process (Benefield and Randall, 1985). Bacteria derive their energy and reducing power from oxidation reactions, which involves the removal of electrons. Heterotrophic bacteria use organic compounds as their electron donor and carbon source to synthesize new biomass in the presence of oxygen (Grady et al., 1999). Since the removal of organic compounds is the most important use of activated sludge, it follows that heterotrophic bacteria predominate in the system. Microorganisms that use inorganic compounds as their electron donor and carbon source are typically autotrophic bacteria. Nitrifiers are the most important autotrophic bacteria in

biochemical operations because they use ammonia and nitrite as an electron donor (Grady et al., 1999).

There are four factors common to all activated sludge processes: 1) a slurry of microorganisms (mixed liquor suspended solids [MLSS]) is used to treat soluble and particulate matter present in an influent waste stream, 2) quiescent settling is used to remove the MLSS and produce an effluent low in suspended solids, 3) settled solids are recycled from the clarifier back to the aeration basin, and 4) excess solids are wasted to maintain a particular biological solids retention time (BSRT) (Grady et al., 1999). Figure 1 shows the layout of a conventional activated sludge system. The reactor containing the MLSS (aeration basin) is aerobic throughout to provide the necessary oxygen for the microorganisms. Sufficient mixing energy must be provided in the bioreactor to keep the solids in suspension. The stream of solids being recycled from the settling tank, (return activated sludge (RAS)), is used to increase the biomass concentration in the reactor. Figure 1 shows the conventional method of solids removal for maintaining BSRT, from the clarifier, but solids can also be removed directly from the aeration basin.

Aeration basins are typically open tanks containing equipment to provide aeration and to provide sufficient mixing energy to keep the MLSS in suspension. The depth is mainly determined by oxygen transfer/mixing characteristics and usually ranges from 3 to 7.5 m (Grady et al., 1999). A single piece of equipment such as a diffused air, mechanical surface aerator, or jet aerator is used in many cases to provide aeration and keep the solids in suspension. Auxiliary mechanical mixers are used when the aeration does not provide sufficient mixing energy.



Figure 1 A typical design layout of an activated sludge system with recycle (reproduced from Benefield and Randall, 1985).

The secondary clarifier performs two functions in the activated sludge process. The first function, clarification, is the separation of MLSS from the treated wastewater to produce a clarified effluent that meets the effluent suspended solids goal. The other is the thickening of sludge for return to the bioreactor. Since both functions are affected by clarifier depth, the design depth must be selected to provide an adequate volume for both functions (Tchobanoglous & Burton, 1991). For instance, the volume must be sufficient to store the solids during periods of high flow.

2.2 BSRT Effects On Organic Carbon Treatment Performance

2.2.1 Carbon Treatment

Biological solids retention time has a principal effect on the performance and capabilities of an activated sludge system. Lawrence and McCarty's (1970) landmark paper linked BSRT and treatment efficiency thereby providing a means of maintaining treatment performance by manipulating physical attributes such as wastage rate. BSRT, shown in Equation 1, is defined as the average time a unit of biomass remains in the bioreactor.

$$\boldsymbol{q}_{c} = \frac{\boldsymbol{X}_{T}}{\left(\frac{\Delta \boldsymbol{X}_{T}}{\Delta t}\right)} \tag{1}$$

where:

 $\theta_c = BSRT$ (time) $X_T = reactor biomass concentration (mass/volume)$ $(\Delta X_T / \Delta t) = biomass removed from the treatment system (mass/(volume*time))$

Although a significant amount of biomass enters a municipal wastewater treatment system, the population of aerobic bacteria present is likely insignificant due to anaerobic conditions in the collection system. Therefore, it is typically assumed that no influent biomass is present for design purposes so that a materials balance for the net biomass rate of change in the system can be written as follows (Lawrence and McCarty, 1970):

Accumulation = Inflow – Outflow + Net Growth

$$V\left(\frac{dX}{dt}\right)_{N} = 0 - QX + \left(\frac{YkS}{K_{s} + S} - K_{d}\right)$$
⁽²⁾

where:

 $(dX/dt)_N =$ net bacterial growth rate (mass/(volume*time)) Q = flow rate of wastewater into the aeration basin (volume/time) V = reactor volume Y = biomass yield coefficient (mass/mass) S = wastewater substrate concentration, e.g. BOD₅ or COD (mass/volume) k = maximum specific substrate utilization rate (time⁻¹) $K_s =$ half-saturation constant (mass/time) $K_d =$ microbial decay coefficient (time⁻¹)

If the system is at steady state, the rate of biomass accumulation is equal to zero by definition (Benefield and Randall, 1985). The mass balance can then be rearranged to provide an equation in terms of BSRT when S is set to the effluent substrate concentration (S_e). Inspection of Equation 3 reveals the relationship between treatment performance and BSRT.

$$\frac{1}{\boldsymbol{q}_c} = \frac{YkS_e}{K_s + S_e} - K_d \tag{3}$$

Minimum BSRT is the value below which a group of microorganisms is unable to grow in an activated sludge reactor. The minimum value is a function of the influent substrate concentration and the kinetic parameters describing bacterial growth on that substrate. For a given set of kinetic parameters, the minimum BSRT for a particular waste stream can be calculated by replacing the effluent substrate concentration with the influent substrate concentration in Equation 3. The resulting value would be the BSRT at which no degradation occurred or the minimum BSRT.

A relationship between the microbial yield, maximum specific substrate utilization rate and maximum growth rate can be seen in Equation 4. Substitution of Equation 4 into Equation 3 reveals an important correlation between specific growth rate and BSRT. BSRT is inversely proportional to specific growth rate as seen in Equation 5.

$$\boldsymbol{m}_{\max} = Yk \tag{4}$$

$$\frac{1}{\boldsymbol{q}_{c}} = \frac{\boldsymbol{m}_{\max} \boldsymbol{S}_{e}}{\boldsymbol{K}_{s} + \boldsymbol{S}_{e}} - \boldsymbol{K}_{d}$$
(5)

The kinetic parameter that has the most prominent effect on BSRT is the maximum specific growth rate (μ_{max}) . Heterotrophic bacteria have a low minimum BSRT because of a high maximum specific growth rate. The typical range of BSRTs necessary for removal of soluble organic matter is between 0.5-1.5 days for municipal wastewater (Grady et al., 1999). However, a safety factor is typically employed to protect against process failure. Tchobanoglous & Burton (1991) propose that a design

BSRT of 4-10 days should be used for a conventional activated sludge system treating domestic wastewater.

Several studies have been conducted to assess the effects of BSRT on carbon treatment performance. Palm et al. (1980) found that complete mix activated sludge reactors operating at a BSRT of 1.9 days removed 85% of the influent COD. While the longer BSRTs removed a slightly higher percentage (90%) of COD, it was clear that effective carbon treatment could be accomplished at short BSRTs. Chuang et al. (1997) found that carbon treatment performance was similar for BSRTs of 5, 10, and 15 days treating an influent COD of 300 mg/L. The average effluent COD values for these three BSRTs were 13, 11, and 11 mg/L respectively. A full-scale municipal WWTP in Phoenix, AZ was forced to set the BSRT between 0.8 and 1.3 days because of foaming problems and limited clarification capacity (Albertson and Hendricks, 1992). However, this plant consistently reduced the BOD from 200 to 11.2 mg/L under low BSRT conditions.

2.2.2 Total Microbial Population

BSRT has been defined as the average length of time a particulate constituent (i.e. biomass) remains in a bioreactor. Therefore, it must be maintained at a sufficient level to provide the concentration of microorganisms necessary to effectively treat a waste stream. A minimum MLSS concentration is also necessary to allow the development of a flocculent biomass. Lawrence and McCarty (1970) found that the steady state mixed liquor microbial mass concentration could be obtained by a substrate mass balance on the reactor.

Accumulation = Inflow – Outflow + Net Growth

$$V\left(\frac{dS}{dt}\right)_{N} = S_{o}Q + RQS_{e} - \left(\frac{dS}{dt}\right)_{U}V - (1+R)QS_{e}$$

where:

 $(dS/dt)_N =$ net substrate utilization rate (mass/(volume*time)) (dS/dt)_U = overall substrate utilization rate (mass/(volume*time)) R = ratio of recycle flow rate to influent flow rate

The mass balance can be simplified for steady state conditions $((dS/dt)_N = 0)$ and divided through by X to develop an equation for specific substrate utilization rate (q):

$$q = \frac{\left(\frac{dS}{dt}\right)_{U}}{X} = \frac{Q(S_o - S_e)}{VX}$$
(6)

The specific substrate utilization rate can also be defined by the following equation (Benefield and Randall, 1985):

$$\frac{\left(\frac{dS}{dt}\right)_{U}}{X} = \frac{kS_{e}}{K_{s} + S_{e}}$$
(7)

Substitution of Equation 7 into Equation 3 yields Equation 8:

$$\frac{\left(\frac{dS}{dt}\right)_{U}}{X} = \frac{1+K_{d}}{\boldsymbol{q}_{c}Y}$$
(8)

Substituting Equation 8 in to Equation 6 results in an expression which links the MLSS concentration (X) to BSRT (θ_c) for a completely mixed activated sludge system with recycle.

$$X = \frac{Y(S_o - S)}{(1 + k_d \boldsymbol{q}_c)} \frac{\boldsymbol{q}_c}{\boldsymbol{q}}$$
(9)

It is evident from Equation 9 that MLSS is a function of the system BSRT. Once the BSRT for a process has been chosen, the steady state biomass level can be determined. Typical MLSS concentrations for the conventional activated sludge system range between 500-5000 mg/L (Grady et al., 1999).

Several studies have experimentally demonstrated that MLSS concentration increases with BSRT for a given waste stream. Grady and Williams (1974) conducted a set of experiments on chemostats treating synthetic wastewater at BSRTs of 0.17, 0.22, 0.3, and 0.46 days. It was discovered that the MLSS concentrations for an influent COD of 1000 mg/L were 317, 343, 353, and 369 respectively. Although the difference is moderate because no sludge recycle was involved, an upward trend in MLSS can be seen in the data. Chuang et al. (1997) produced similar results when operating a set of reactors at BSRTs of 5, 10, and 15 days. The MLSS concentrations were 920, 1690, and 2700 respectively for these reactors each treating an influent COD of 300 mg/L.

2.2.3 Settling

Successful operation of an activated sludge system requires the formation of a flocculent biomass that settles rapidly and compacts well. The presence of exocellular polymers (ECP), formed during microbial metabolism, is key in establishing flocculation (Surucu & Cetin, 1989). ECPs cause the aggregation of particles by acting as a bridge between cells. Several types of ECPs are involved in flocculation but polysaccharides and proteins are generally considered the most important (Grady et al., 1999).

Empirical observations suggest that a minimum BSRT must be maintained to successfully achieve flocculation. This observation is consistent with the role of ECP production by bacteria. Although ECP is produced continuously, its formation has been found to increase with increasing BSRT (Grady et al., 1999). Therefore, flocculation could be incomplete at short BSRTs because the generation of bacteria exceeds the rate of ECP production. By reducing the growth rate of bacteria to coincide with ECP production, effective flocculation can be achieved.

Bisogni and Lawrence (1971) conducted a study on the impacts of BSRT on settling performance for BSRTs ranging from approximately 0.5-12 days. The major finding was that a high percentage (10-30%) of the activated sludge solids did not settle when the BSRT was less than 1 day. Microscopic analysis of biomass showed well formed flocs for BSRTs greater than 2 days. Echeverria et al. (1993) obtained similar results when conducting a pilot plant study on municipal wastewater. Effective sludge settling (i.e. SVI values lower than 100) was found for a BSRT as low as 3 days for a conventional activated sludge reactor. Grady et al. (1999) recommend a minimum BSRT of 3 days for good flocculation but also state that several activated sludge plants have been successfully designed and operated at BSRTs as low as 1 day.

2.3 Fundamentals of Nitrification

2.3.1 Stoichiometric equations of nitrification

Nitrification is a two-step treatment process, performed by chemoautotrophs, that converts ammonia to nitrate in the presence of oxygen (Benefield & Randall, 1985). The initial step of nitrification $(NH_4^+ \rightarrow NO_2^-)$ has long been thought to be carried out by the

bacterial genera *Nitrosomonas*. However, *Nitrosococcus*, *Nitrosospira*, *Nitrosovibrio*, and *Nitrosolobus* can also sustain themselves by converting ammonia to nitrate (Rittmann and McCarty, 2001). The ammonia-oxidizers are all genetically diverse yet are related to each other, which suggests that the *Nitrosomonas* species is not necessarily dominant in a given system (Rittmann and McCarty, 2001). The second step, conversion of nitrite to nitrate, can be performed by *Nitrospira*, *Nitrospina*, *Nitrococcus*, *Nitrocystis*, and *Nitrobacter*. Although the latter organism is the most commonly referenced genus for this process, recent findings using molecular probes indicate that *Nitrospira* is the dominant nitrite-oxidizer in wastewater treatment processes (Rittmann and McCarty, 2001). Nitrification is desirable in wastewater treatment plants because ammonia consumes oxygen in receiving streams, is toxic to fish, and reacts with chlorine to form chloramines making drinking water treatment difficult. Since nitrifiers use inorganic carbon for cell synthesis, they are not in direct competition with heterotrophs for a carbon source.

Although nitrification is a two-step process, conversion of ammonia to nitrite is usually the rate-limiting step since nitrite does not typically accumulate in biological treatment systems under steady-state conditions (EPA, 1993). The lack of nitrite build-up can be attributed to the maximum growth rate for nitrite oxidizing population being considerably higher than the maximum growth rate for the ammonia oxidizing population (EPA, 1993).

2.3.2 Alkalinity

Alkalinity in wastewater results from the presence of hydroxides, carbonates, and bicarbonates of elements such as calcium and magnesium. Acting as a buffer, alkalinity

helps to resist changes in pH caused by the addition of acids. While pH should be maintained in the range of 7.0 to 8.5 for efficient nitrification, Grunditz and Dalhammar (2001) found that pH values of 8.1 and 7.9 provided optimum activities for *Nitrosomonas* and *Nitrobacter* respectively. Since 7.14 mg of alkalinity as CaCO₃ is consumed per mg of ammonia oxidized to nitrate (based on stoichiometry), the pH will rapidly drop if the concentration of alkalinity is insufficient (Grady et al., 1999). However, this is usually not a problem because domestic wastewater contains approximately 100-200 mg/L of alkalinity (Benefield & Randall, 1985).

2.4 Impacts of BSRT on Nitrification

When nitrification is to be incorporated into a wastewater treatment system, determination of the necessary BSRT becomes crucial. A BSRT of approximately 2-3 days has been established as the minimum for nitrification to occur (Benefield and Randall, 1985). A minimum BSRT for a particular waste stream can also be approximated by substituting typical nitrifier kinetic coefficients and the influent ammonia concentration into Equation 3. Since this value would not be sufficient for design purposes due to the dynamic nature of ammonia loadings in activated sludge WWTPs, Tchobanoglous and Burton (1991) recommend a BSRT between 8-20 days for combined carbon removal/nitrification systems.

BSRT becomes important because comparison of typical maximum growth rate values for heterotrophs and autotrophs reveals that the value for autotrophs is nearly an order of magnitude lower than for heterotrophs (Grady et al., 1999). This finding suggests that the minimum BSRT required for nitrification is nearly an order of magnitude larger than the minimum BSRT for heterotrophs. The situation is exacerbated by the fact that the nitrifier maximum specific growth rate can vary by a factor of two for a given temperature (Daigger and Parker, 2000). Since the variance can be attributed to several factors, it has been suggested that the maximum specific growth rate should be determined for each wastewater.

Because of the importance of nitrification in wastewater treatment, several studies have been conducted to determine the necessary BSRT for effective nitrification. Randall et al. (1992) conducted a study to compare nitrification kinetics in a conventional activated sludge system and a system accomplishing biological nutrient removal. The activated sludge system was operated at BSRTs of 1.5, 2.7, 5, and 15 days at a temperature of 20°C. It was found that complete nitrification could be achieved for BSRTs of 2.7 days or greater when treating an influent ammonia concentration of approximately 25 mg/L. It should be noted that 79% of the incoming ammonia was converted to nitrate in the 1.5 day BSRT reactor. Dincer and Kargi (2000) obtained similar results when treating a synthetic influent stream composed of 100 mg/L ammonia. The reactors for this experiment were operated at 20, 17, 15, 10, 8, 5, and 3 day BSRTs. Nitrification efficiency was found to increase up to a BSRT of 12 days. Any further increases in BSRT did not result in improved treatment performance. It was also discovered that almost 60% nitrification could be accomplished at a 3 day BSRT. Hanaki et al. (1990) also found that nitrification could be achieved at low BSRTs when conducting a study on the effects of DO on nitrification in a completely mixed activated sludge system. For the excess DO portion of their experiment, reactors were operated at BSRTs of 6.5, 5, 3.8, and 2 days for a synthetic influent feed containing 80 mg/L of ammonia. Complete nitrification was measured for all BSRTs greater than 3.8 days while approximately 50% of the influent ammonia was converted to nitrate in the 2 day BSRT reactor. This finding indicated that it was possible to nitrify nearly 40 mg/L of influent ammonia at a BSRT of only 2 days.

2.5 Dissolved Oxygen Effects on Treatment Performance

2.5.1 Carbon Treatment

In the presence of an easily degradable substrate, heterotrophic bacteria are able to grow at low DO concentrations. Although it is undesirable for oxygen to be rate limiting in the removal of organic compounds, only limited research has been done to establish the oxygen half saturation coefficient for mixed cultures of heterotrophs ($K_{O,H}$). A probable reason for the lack of work in this area is that population shifts in the microbial community, due to changes in DO concentration, make estimation of the value difficult (Grady et al., 1999).

The oxygen half saturation coefficient (K_0) has been defined as the oxygen concentration where nitrification takes place at one half of the maximum rate. Figure 2 shows the relationship between K_0 and the maximum substrate utilization rate (k). The half saturation coefficient is an indicator of a microorganism's affinity for oxygen. Therefore, a low value of K_0 indicates a high affinity for oxygen and the ability of a microorganism to effectively utilize substrate even at low DO conditions. Because of the disparity in value for heterotrophs and autotrophs, K_0 becomes especially important in combined carbon removal/nitrification activated sludge systems.



Figure 2 Saturation plot representing the correlation between oxygen concentration and bacterial substrate utilization rate.

Limited pure culture experimental data suggests that the heterotrophic oxygen half saturation coefficient is very low. Sinclair & Ryder (1975) conducted a study on the effects of DO on the behavior of *Candida utilis* grown in a chemostat. Using a glycerol medium, the $K_{O,H}$ for *C. utilis* was found to be approximately 0.08 mg/L. Similar results were obtained by Lau et al. (1984) when describing the growth kinetics of a floc former (*Citrobacter* sp.) and a filament (*Sphaerotilis natans*) obtained from activated sludge. The $K_{O,H}$ values for the floc former and filament were calculated to be 0.15 and 0.01 mg/L respectively. These low values indicate that a very low DO concentration would be required to affect carbon treatment performance, although it might influence competition between filamentous and floc forming bacteria. Henze et al. (1987) have adopted a standardized $K_{O,H}$ value of 0.2 mg/L for use in Activated Sludge Model No. 1.

Due the low K_0 value for heterotrophs, it is not surprising that several researchers have found that DO has little effect on carbon treatment performance. Chuang et al. (1997) conducted a study to determine the effects of DO on nutrient removal for concentrations ranging from 20-0.1 mg/L. A synthetic feed was introduced into the reactors at a COD concentration of 300 mg/L. The effluent CODs were 10, 11, and 11 mg/L for a 10 day BSRT reactor operating at 2.0, 0.5, and 0.1 mg/L DO, respectively. Similar treatment efficiencies for the other BSRTs showed that DO had no impact on carbon treatment for any of the BSRTs studied (5, 10 and 15 days). Munch et al. (2000) conducted a pilot plant study to establish the feasibility of upgrading a municipal WWTP to incorporate nitrification into a carbon treatment system. It was determined that effective carbon treatment could be accomplished when the reactor was set at a DO concentration of 0.5 mg/L. Lau et al. (1984) also found that effective carbon treatment could be accomplished at low DO in a chemostat treating synthetic wastewater. The reactor was operated at a 0.3 day BSRT for DO concentrations of 6.1, 0.35, and 0.09 mg/L. Operation at these DO concentrations produced effluent COD concentrations of 21, 40, and 30 mg/L. These findings indicated that DO concentration had no effect on carbon treatment efficiency.

2.5.2 Nitrification

Ammonia Oxidation

The dissolved oxygen concentration necessary for non-DO limited nitrification has been established at 2 mg/L (Benefield and Randall, 1985; Tchobanoglous and Burton, 1991), however, this value can vary with BSRT and organic loading. Recent findings have shown that oxygen requirements for nitrification are not the same for different BSRTs. The DO concentration required for high BSRTs is as little as 1 mg/L (Fillos et al., 1996; Stenstrom and Song, 1991). Dangcong et al. (2000) conducted a study on a sequencing batch reactor treating a high concentration of influent ammonia. When the DO was not controlled, it was discovered that a significant portion of the incoming ammonia was converted to nitrite even though DO levels in the rector were close to zero. The results indicate that the ammonia oxidizers adapted to the low DO.

The conditions change when organic shock loading is introduced into the system. Hanaki et al. (1990) found that ammonia oxidation could efficiently occur in a pure nitrification reactor even at a DO level of 0.5 mg/L and a BSRT of 3.8 days. However, it was found that ammonia oxidation was not as successful once a significant organic loading was introduced in the influent. Results showed that ammonia oxidation was inhibited at low DO levels for all BSRTs when 500 and 1000 mg/L COD was added. However, efficient ammonia removal was measured at a BSRT greater than 4 days when the organic loading was 160 mg/L. Hanaki et al. (1990) attributed the decrease in ammonia removal at high organic loadings to an increase in K_s . This meant a higher ammonia concentration was required at high organic loadings to maintain the growth rate at low DO (Hanaki et al., 1990).

Hanaki et al. (1990) studied the observed growth yield index (PY_{obs}) of ammonia and nitrite oxidizing bacteria to determine the effects of DO on growth. The proportional biomass determination factor (P) represented the specific substrate utilization rate of ammonia oxidizers from a batch test, in which harvested cells from mixed liquor consumed the substrate. The observed growth yield (Y_{obs}) was calculated by the following equation:

$$Y_{obs} = \frac{Y}{1 + K_d \boldsymbol{q}_c} \tag{10}$$

The first part of this study was conducted in a pure nitrification environment, which limited the amount of heterotrophs by restricting organic carbon. The results showed that PY_{obs} for ammonia oxidizers significantly increased at low DO levels while PY_{obs} for nitrite oxidizers did not change. Since the growth yield index increased, either P or Y_{obs} must have caused the increase. P can be influenced by heterotrophs, however, only a negligible amount were present so P was considered to be constant. This suggests that an elevated Y_{obs} caused the increase in the observed growth yield index. However, the substrate utilization rate decreased because of a decrease in the maximum substrate utilization rate when low DO conditions occur (Hanaki et al., 1990). Consequently, the high growth yield increased the amount of ammonia oxidizing biomass and compensated for the reduced ammonia oxidation rate per unit biomass.

Nitrite Oxidation

As previously mentioned, ammonia oxidation has historically been considered the rate-limiting step in nitrification. However, more recent findings seem to indicate that in the presence of low dissolved oxygen concentrations, the nitrite oxidizing bacteria are inhibited while the ammonia oxidizers are relatively unaffected (Hanaki et al., 1990; Fillos et al., 1996, Dangcong et al., 2000). Nitrite oxidation is inhibited in a low DO environment due to a specific affinity for oxygen that is lower than that of the ammonia oxidizers (Laanbroek et al., 1994). For this reason, nitrite oxidizers have difficulty competing for the available oxygen and adapting to the environment.

Bernet et al. (2001) have proposed using DO to limit the amount of nitrate produced in nitrogen removal processes because nitrite is cheaper to convert to N₂ gas via denitrification. To determine the effects of DO on nitrifiers the researchers introduced two parameters, γ_1 and γ_2 , to describe the percentage of maximum growth rate for ammonia and nitrite oxidizers. These two parameters can be defined by the following equations:

$$\boldsymbol{g}_{1} = \boldsymbol{m}_{1}^{'} / \boldsymbol{m}_{1} = \frac{DO}{K_{NH_{4}^{+}, O_{2}} + DO}$$
(11)

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$$\boldsymbol{g}_{2} = \boldsymbol{m}_{2}^{'} / \boldsymbol{m}_{2} = \frac{DO}{K_{NO\bar{2},O_{2}} + DO}$$
(12)

where:

 μ_1 = maximum ammonia oxidizer growth rate at excess DO (time⁻¹) μ'_1 = maximum ammonia oxidizer growth rate at low DO (time⁻¹) μ_2 = maximum nitrite oxidizer growth rate at excess DO (time⁻¹) μ_2 = maximum nitrite oxidizer growth rate at low DO (time⁻¹)

Equation 11 can be divided by Equation 12 to yield the variation of growth ratios between ammonia and nitrite oxidizers under different DO concentrations (Bernet et al., 2001):

$$\boldsymbol{g} = \boldsymbol{g}_1 / \boldsymbol{g}_2 = \frac{K_{NO_2^-,O_2} + DO}{K_{NH_4^+,O_2} + DO}$$
(13)

The researchers operated a nitrifying biofilm at 0.5 mg/L DO to determine whether or not nitrite accumulation could be sustained. The experiment was conducted over a 110-day period and nitrite accumulation occurred throughout. The results were that γ for the ammonia oxidizers was close to 1 (0.976) while the nitrite oxidation γ had decreased to 0.120. The findings indicated that nitrite oxidizers were only growing at 12% of their normal rate and consequently could not remove all of the nitrite. It was noted that once the DO was restored to 50% saturation, complete conversion of nitrite to nitrate once again occurred. This result clearly shows that nitrite oxidizers were always present in the biofilm (Bernet et al., 2001).

A significant buildup of nitrite in the effluent is problematic because nitrite is toxic to aquatic life at concentrations as low as 0.5 mg/L (Balmelle et al., 1992). High nitrite concentrations can also inhibit growth of floc forming organisms, which could cause settling problems (Kappeler et al., 1994). Another side effect of excess nitrite is the production of N₂O gas. Zheng et al. (1994) used a 10-day BSRT and DO concentrations of 0.1, 0.2, 0.5, 1.7, and 6.8 to examine the effect of dissolved oxygen on nitrous oxide formation. At a DO of 0.1 mg/L, approximately 5.4% of the nitrified nitrogen was converted to N₂O gas. The conversion rate increased to 7.0% at the 0.2 mg/L DO concentration and then declined back to 5.4% at 0.5 mg/L DO. The actual amount of N₂O produced at 0.5 mg/L DO was higher than at 0.2 mg/L but more ammonification occurred so the percentage dropped. It appears that N₂O will be produced to some extent no matter what the DO level. However, conversion rates are highest at low DO levels. This condition correlates to the DO concentration where nitrite oxidation is inhibited suggesting a close relationship between N_2O production and nitrite buildup in wastewater (Zheng et al., 1994).

2.5.3 Simultaneous Nitrification/Denitrification

In a reactor attempting to nitrify at low DO concentrations, it is possible for nitrification and denitrification to occur simultaneously. Denitrification is performed by facultative heterotrophic bacteria, which are able to use nitrate as a terminal electron acceptor for the oxidation of carbon substrates (Benefield & Randall, 1985). The conditions in the reactor must be anoxic for denitrification to occur. The term anoxic refers to the use of nitrate as an electron acceptor instead of oxygen and is used rather than anaerobic because it is a modification of aerobic pathways (Tchobanoglous & Burton, 1991). Denitrification is a two-step process in which nitrate is converted to nitrite then to nitrogen gas. Because nitrate is used as an electron acceptor, denitrifiers must have an easily degradable carbon and energy source available. Methanol typically has served as a carbon and energy source after BOD removal and nitrification have occurred (Benefield & Randall, 1985; Tchobanoglous & Burton, 1991). Stoichiometry of the denitrification process with methanol as the substrate is given by the following:

$$Step1 \; 3NO_3^- + CH_3OH \rightarrow 3NO_2^- + CO_2 + 2H_2O \tag{14}$$

$$Step 2 \ 2NO_2^{-} + CH_3OH \rightarrow N_2 + CO_2 + 2OH^{-}$$

$$\tag{15}$$

$$Overall \ 6NO_3^- + 5CH_3OH \to 3N_2 + 5CO_2 + H_2O + 6OH^-$$
(16)

The typical DO limit for denitrification has been reported to be between 0.1 and 0.2 mg/L (Fillos et al., 1996; Lie & Welander, 1994). However, denitrification has been found to occur at DO concentrations up to 3 mg/L, although the rate was less than 25% of the maximum rate (Oh & Silverstein, 1999).

A series of SBR experiments on denitrification were conducted by Oh & Silverstein (1999) at DO concentrations of 0.09, 0.4, 0.8, 1.2 and 2.0 mg/L. At a DO concentration of 0.4 mg/L, the average rate of denitrification was 0.0108 mg NO_x-N/mg-MLVSS/h. This was a 50% reduction in rate from the anoxic value of 0.0214 mg NO_x-26

N/mg-MLVSS/h. Even at values of 0.09 mg/L DO, a significant (35%) inhibition of denitrification rates occurred during the experiment. Small anoxic microzones form in the floc allowing denitrification to proceed at low DO concentrations (Fillos et al., 1996).

One method of determining whether simultaneous nitrification/denitrification occurs is to monitor the ratio of alkalinity consumed per ammonia converted to nitrate. Nitrate concentration, not ammonia, should be used in this ratio because it is not subject to any further reactions other than denitrification (Marsili-Libelli & Giovanni, 1997). The theoretical rate of alkalinity production is 3.57 mg alkalinity per mg NO₃⁻ reduced (Fillos et al., 1996). Thus, the effect of both reactions occurring at the same time would be a linear decrease in the theoretical alkalinity consumption ratio (7.14) until it reached 3.57 at 100% concurrent denitrification.

2.5.4 Total Microbial Population

When treating a waste stream, it is important to maintain a sufficient microbial population for degradation of organic and inorganic compounds. Heterotrophic bacteria comprise the majority of the mixed liquor in activated sludge systems (Grady et al., 1999). As discussed previously, it has been found heterotrophic bacteria are capable of effective carbon treatment even at very low DO levels. Therefore, major changes in the mixed liquor concentration would not be expected to occur at low DO unless the influent COD concentration varied.

A number of studies have determined that mixed liquor was more a function of influent organic carbon than DO. The study of DO and carbon removal by Lau et al. (1984) revealed that no significant changes in MLSS occurred for a chemostat treating a

consistent synthetic waste stream at 6.1, 0.35, and 0.9 mg/L DO. The MLSS values were 502, 525, and 490 respectively for this experiment. These findings indicated that DO had no effect on MLSS. Chuang et al. (1997) obtained similar results when treating a synthetic influent feed comprised of 300 mg/L COD at 2, 0.5, and 0.1 mg/L DO. Operating the system at a 5 day BSRT, MLSS concentrations of 920, 1010, and 1030 were obtained for the three DO levels respectively. The findings also reinforce the notion that organic carbon levels rather than DO exert the most influence on MLSS concentration. Ng et al. (1989) conducted a study on biological treatment of a pharmaceutical wastewater using activated sludge. The reactor was operated at BSRTs of 20, 6.67, 4, and 2.86 days with a DO concentration of approximately 0.3 mg/L. It was discovered that an increase in COD loading produced a corresponding rise in mixed liquor concentration. Therefore, the increase in biomass was attributed to the change in influent COD.

2.5.5 Settling Characteristics

Sludge Settling

Sludge bulking, foaming, floc formation, and turbidity are all parameters that affect settleability. These parameters are negatively impacted by low dissolved oxygen concentrations and therefore settling characteristics are affected as well (Akca et al., 1993; Surucu & Cetin, 1990; Foot, 1992; Wilen & Balmer, 1998; Wilen & Balmer, 1999; Surucu & Cetin, 1989). However, it has been reported that DO concentration alone may not be the cause of poor settling. Palm et al. (1980) noted that the DO concentration that would hinder settling was a function of the organic loading rate. Surucu and Cetin (1990) conducted a study in which the DO concentration varied from set points 0.5, 1.0, 1.5, 2.0, and 5.0 mg/L. Their findings were that DO levels less than 2.0 mg/L significantly and adversely affect settling characteristics of the activated sludge. Suspended solids concentrations increased from 67 mg/L to 410 mg/L at DO concentrations of 5 and 0.5 mg/L respectively. Surucu and Cetin (1990) proposed that the eucaryote population was inhibited below 2 mg/L DO, which in turn hindered settling. However, the high organic loading rate used in this experiment could have been responsible for the settling problems. Palm et al. (1980) reported that the COD/biomass ratio played a larger role than DO in causing poor sludge settling.

Floc Structure

A useful property of activated sludge is its adsorption ability (Wilen & Balmer, 1998). Guellil et al. (2001) have found that biosorption is a fast process in which a large portion (45% on average) of the non-settleable fraction of wastewater can be transferred to activated sludge flocs within a few minutes. However, when DO concentration is low (<1 mg/L), the sludge flocs tend to lose much of their adsorption capacity. This results in a much more turbid effluent. The study by Wilen and Balmer (1998) involved decreasing the DO to zero and monitoring the turbidity over a 1-4 hour time period. It is believed that the increase in turbidity was not caused by inhibition of eucaryotes for these experiments because the anaerobic periods were too short. Rather, it was speculated that the anaerobic period either affected adsorption of contaminants in the wastewater or caused floc dispersion.

Surucu and Cetin (1989) discovered that low DO concentrations affected compressibility and filterability of activated sludge. Reactors were run at five different DO levels: 0.5, 1.0, 1.5, 2.0, and 5.0 mg/L. The poor filterability at low DO can be attributed to a reduction in particle size and the formation of dispersed solids. It has been found that a well-formed floc contains water, which is easily removed by filtration (Surucu and Cetin, 1989). Poor settling or pin flocs do not filter well because of their smaller floc size. The particles are not only small but the water is also in the form of capillary water, which is much more difficult to remove and causes a high resistance to compressibility (Surucu and Cetin, 1989). A low DO concentration also produces smaller floc populations (Wilen and Balmer, 1999). Low DO levels can also cause the floc to become irregular in shape and porous. Wilen and Balmer (1999) found that these poorly shaped flocs caused an increase in SVI values for reactors operating at a 5-day BSRT.

Filamentous Bulking

Bulking sludge can also be a problem in activated sludge treatment plants. Bulking occurs when aggregates do not compact and form a loose, low-density floc (Clauss et al., 1998). Higher recycle rates are required because the sludge concentration becomes low. Bulking sludge can cause huge losses of biomass and thereby reduce effluent BOD quality. Filamentous organisms have been identified as a major cause of sludge bulking. The filaments form bridges between flocs and prevent them from compacting in the clarifier (Foot, 1992). Bridging prevents the flocs from compacting and traps water between the flocs. Recent studies have found that *Microthrix parvicella* is the filament most responsible for bulking and foaming (Madoni et al., 2000; Wanner et al., 2000). The presence of these microorganisms has been attributed to low DO, organic loading rate, and sludge age.

A DO concentration of 2.0 mg/L has long been established as the level necessary to prevent excessive growth of filamentous bacteria in activated sludge (Grady et al., 1999). Several studies have found that settling efficiency can be severely inhibited at low DO concentrations. However, data by Echeverria et al. (1992) has shown that SVI values of <100 could be achieved at DO levels lower than 1 mg/L. The landmark study by Palm et al. (1980) demonstrated that the major factor controlling low DO bulking is the organic loading rate. The experiment involved controlling the DO at different set points and increasing the substrate removal rate until the SVI increased. The substrate removal rate needed to cause settling problems at DO concentrations between 0.1 to 0.5 mg/L was 0.30 mg COD/mg VSS-day. It appears that even at DO concentrations below 0.5 mg/L, filamentous organisms do not dominate the system provided the organic removal rate is low enough.

BSRT can also be a factor in determining the DO concentration required to avoid sludge bulking. Akca et al. (1993) have created the following relation between BSRT and dissolved oxygen concentration based on converted data:

$$C_{cr} = 6.705 e^{(-0.2034q_x)} \tag{17}$$

where:

 C_{cr} = critical dissolved oxygen concentration in aeration tank (mass/volume) θ_x = BSRT (time)

This equation shows that a particular BSRT has a critical DO concentration. If that concentration is not maintained, sludge bulking can occur. This equation also suggests that the DO must be higher at short BSRTs and lower at long BSRTs in order to avoid bulking sludge (Akca et al., 1993). However, it should also be noted that filaments have a minimum BSRT of approximately 1.5-3.0 days so bulking might not occur at the lower biological solids retention times (Wanner, 1998).

2.6 Determination of Kinetic Coefficients

2.6.1 Nitrification Kinetics

The growth rate of nitrifiers has been found to follow Monod type kinetics. The rate of this process can be expressed by the following double-substrate limiting equation (Bae and Rittman, 1996):

$$\boldsymbol{m} = \boldsymbol{m}_{\max} \left(\frac{S_1}{K_1 + S_1} \left(\frac{S_2}{K_2 + S_2} \right) \right)$$
(18)

where:

 μ = specific growth rate (time⁻¹) μ_{max} = maximum specific growth rate (time⁻¹) S_1 and S_2 = limiting concentrations of each parameter (mass/volume) K_1 and K_2 = half-saturation constants for parameters S_1 and S_2 (mass/volume)

This is an interactive model because it assumes that both parameters, oxygen and ammonia substrate, can influence the rate of nitrification at the same time (Grady et al., 1999). This equation could also be modeled non-interactively. In that case, it is assumed that the substrate utilization rate can only be affected by one parameter at a time. For example, the nitrification process is not limited by oxygen at concentrations greater than 2 mg/L, so the second term would drop out of the equation. The interactive model is more conservative for the type of situation likely to be encountered in wastewater treatment. It is also more appropriate when one parameter is the electron donor (substrate) and the other is the electron acceptor (oxygen) (Bae and Rittmann, 1996).

Although the interactive model can be used for describing the effects of duallimitation on cell growth, research conducted in the past has used the non-interactive model for determining kinetic coefficients (Ryder and Sinclair, 1975;Hanaki et al., 1990;Beccari et al., 1992). Ryder and Sinclair (1975) obtained their kinetic coefficients by providing one substrate in excess so one term in the Monod model could be neglected. Therefore, the kinetic analysis for determination of μ_{max} , k, K_d, Y_A, and K_S for the nitrifiers was conducted at excess DO conditions. The K_O value was evaluated last using data from excess and low DO concentrations.

2.6.2 Determination of Y_A and K_d

Since the only source of active biomass is from growth due to substrate utilization, its concentration can be calculated by the following steady state mass balance on substrate (Grady et al., 1999):

Accumulation = Inflow – Outflow + Net Growth

$$Q^*S_o - Q_w^*S_e - (Q - Q_w)^*S_e - \frac{\mathbf{m}_A}{Y_A}^*X_{B,A}^*V = 0$$
⁽¹⁹⁾

where:

Q = influent flow rate (mass/time) $Q_w = wastage flow rate (mass/time)$ $S_o = influent substrate concentration (mass/volume)$

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$$\begin{split} S_e &= effluent \; substrate \; concentration \; (mass/volume) \\ X_{B,A} &= active \; autotrophic \; biomass \; concentration \; (mass/volume) \\ V &= volume \; of \; reactor \end{split}$$

Rearrangement of the mass balance yields the following equation for the steady state concentration of active autotrophic biomass:

$$X_{B,A} = \frac{\boldsymbol{q}_c Y_A (\boldsymbol{S}_o - \boldsymbol{S}_e)}{1 + k_d \boldsymbol{q}_c}$$
(20)

The linear form (Equation 21) of Equation 20 relates substrate utilization to the inverse of BSRT. Inspection of Equation 21 reveals that the $(S_o-S_e)/X\theta$ term is the specific substrate utilization rate (q) as seen in Equation 6. Therefore, a plot of q versus inverse BSRT can be plotted to calculate the autotroph yield (Y_A) and decay coefficients (K_d). A plot of $1/\theta_c$ vs. q provides a straight line with a slope of Y_A and a y-intercept of – K_d.

$$\frac{1}{\boldsymbol{q}_{c}} = Y_{A} \frac{S_{o} - S_{e}}{X_{B,A} \boldsymbol{q}} - K_{d}$$
(21)

$$\frac{1}{\boldsymbol{q}_c} = \boldsymbol{Y}_A \boldsymbol{q} - \boldsymbol{K}_d$$

The active biomass concentration is not easily measured. Several methods have been proposed to quantify this parameter. The use of MLVSS as a gross measure of biomass concentration has long been employed. One method proposed by EPA (1993) involves relating active biomass to the mixed liquor volatile suspended solids (MLVSS). The relationship between these two quantities can be seen in Equation 22:

$$f_{A} = \frac{1}{1 + (1 - f_{b})\boldsymbol{q}_{c}}$$
(22)

where:

 f_A = active biomass fraction of MLVSS

 f_b = biodegradable fraction of active biomass (typically = 0.8)

b = decay coefficient (typically = 0.25 d^{-1} for heterotrophic bacteria treating municipal wastewater)

The active fraction shown in Equation 22 is for all microorganisms and should be multiplied by the MLVSS to obtain the total active biomass concentration (X_T). Identification of the fraction of nitrifiers to the total population is also difficult. Rittman et al. (1999) determined the ratio of ammonia oxidizers to heterotrophs using 16S rRNA probes and found that ammonia oxidizers accounted for approximately 6% of the total active population. The ratio was very stable for the five municipal WWTPs used in the study although they were operated at different BSRTs. This finding suggests that if X_T could be calculated then the active ammonia oxidizer population could be estimated using the aforementioned ratio.

The Rittman et al. (1999) data was insufficient to determine the total nitrifier active biomass because no information on the nitrite oxidizer population was given. However, Copp and Murphy (1995) employed a mass estimation technique (MET) to determine the mass of ammonia and nitrite oxidizers in activated sludge treating municipal wastewater. Results showed that the *Nitrosomonas* and *Nitrobacter* populations had average concentrations of 16.6 and 6.0 mg VSS/L respectively. This indicates that ammonia oxidizing bacteria account for approximately 74% of the nitrifier population. Therefore, the amount of total active nitrifiers can be calculated by dividing the ratio of ammonia oxidizers to the total population (0.06) by the ratio of ammonia oxidizers to total nitrifiers (0.74). The result is an active autotrophic biomass fraction ($f_{B,A}$) of 0.08. This value can be multiplied by X_T to obtain $X_{B,A}$ for use in the determination of Y_A and K_d .

2.6.3 Estimation of u_{max}, K_s, and k

Equation 23 relates the maximum specific growth rate (μ_{max}) and half saturation coefficient (K_s) to bioreactor effluent substrate concentration (Grady et al., 1999). Once the value of K_d has been determined, Equation 23 can be linearized to obtain values for μ_{max} and K_s.

$$S_{e} = \frac{K_{s} \left(\frac{1}{\boldsymbol{q}_{c} + K_{d}} \right)}{\boldsymbol{m}_{\max} - \left(\frac{1}{\boldsymbol{q}_{c} + K_{d}} \right)}$$
(23)

Three techniques are available for linearization of Equation 23 including the Hanes, Hofstee, and Lineweaver-Burke methods. The Lineweaver-Burke technique was used to develop Equation 24. This method involves plotting $1/S_e$ vs. $1/(\theta_c+K_d)$ to obtain a slope of μ_{max}/K_s and y-intercept of $-1/K_s$.

$$\frac{1}{S_e} = \frac{\boldsymbol{m}_{\text{max}}}{K_s} \left(\frac{1}{1/\boldsymbol{q}_c + K_d} \right) - \frac{1}{K_d}$$
(24)

Once these parameters have been calculated, Equation 4 can be rearranged to determine the maximum substrate utilization rate (k).

$$k = \frac{\mathbf{m}_{\max}}{Y_A} \tag{25}$$

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2.6.4 Estimation of Ko

A value for K_0 can be calculated using data from respirometric batch tests or by conducting a traditional kinetic analysis. When using the respirometry method, a respirogram is taken by adding a sample of wastewater to a vessel containing a volume of endogenous respiring activated sludge (Brouwer et al., 1998). The respiration rate is then monitored over time and used in the determination of K_0 . Since respirometry equipment was unavailable for this study, K_0 was estimated using kinetic data.

Kinetic analysis has been used by researchers to estimate K_0 in activated sludge systems. Sinclair and Ryder (1975) studied the effects of DO limited conditions on bacteria treating organic carbon. Kinetic data was used to calculate a K_0 for heterotrophic bacteria growing in a CSTR. Similarly, Hanaki et al. (1990) used this technique while evaluating K_0 for ammonia oxidizers. The method employed by Hanaki et al. (1990) involved monitoring treatment performance at excess and low DO levels. A kinetic analysis was then conducted on data from the excess and low DO data separately, as outlined in Sections 2.6.1 and 2.6.2, to determine the kinetic coefficients μ_{max} and Y. The purpose of the analysis was to obtain a value of k using Equation 25.

Decrease of substrate utilization rate by low DO has often been formulated by a decrease in maximum substrate utilization rate as follows (Hanaki et al., 1990):

$$\frac{k_{low DO}}{k_{excess DO}} = \frac{DO}{K_0 + DO}$$
(26)

Hanaki et al. (1990) used Equation 26 to calculate a K_0 of 0.32 mg/L value by substituting the k values for low and excess DO conditions and inserting a value of 0.5 mg/L for the DO concentration.

2.7 Nitrification Kinetic Coefficients

Investigations of nitrification in lab experiments and wastewater treatment systems have produced a variety of coefficient values due to different methods of estimation. Table 1 is a list of the nitrifier kinetic parameters gathered from a review of the literature that was used as a basis for comparison with the coefficients obtained in this treatability study. The effect of method on coefficient value can be seen by the difference in k values provided by Massone et al. (1998) and Dincer and Kargi (2000). The work of Massone et al. (1998) involved the use of a titrimetric technique for coefficient determination while Dincer and Kargi (2000) employed the traditional parameter estimation technique, outlined in the previous section, to calculate a value for k. Conversely, Gee et al. (1990) used the same method of coefficient determination as Dincer and Kargi (2000) resulting in very comparable yields. Another difference relating to these findings was that most values were for activated sludge while values provided by Tchobanoglous & Burton (1991) were for pure cultures. Since temperature affects the rate of nitrification, the values were also different due to a temperature range of 20-25°C for the different experiments.

Process		Source						
	μ _{max}	Y	k	K _{SN}	K _{SO}	K _d	Source	
Overall	1.06 day ⁻¹	-	0.076 mg N/mg VSS- day	0.85 mg/L [NH4 ⁺ -N]	-	-	Massone et al. (1998)	
Overall	-	0.34 mg VSS/mg N	1.15 day ⁻¹	5.14 mg/L [NH ₄ ⁺ -N]		0.021 day ⁻¹	Dincer and Kargi (2000)	
Overall	-	0.36 mg VSS/mg N	-	-	-	0.12 day ⁻¹	Gee et al. (1990)	
Ammonia Oxidation	0.78 day^{-1}	-	-	1.1 mg/L [NH4 ⁺ -N]	0.32 mg/L O ₂	0.039 day ⁻¹	Hanaki et al. (1990)	
Overall	0.77 day^{-1}	0.17 ^a mg VSS/mg N	0.22 ^b mg N/mg VSS- day	1.0 mg/L [NH4 ⁺ -N]	0.75 mg/L O ₂	0.096 day ⁻¹	Grady et al. (1999) @20°C	
Overall	0.48 day^{-1}	-	-	1.0 mg/L [NH4 ⁺ -N]	0.5 mg/L O ₂	0.12 day ⁻¹	Stenstrom and Poduska (1980)	
Nitrite Oxidation	0.33 day^{-1}	0.015 mg VSS/mg N	0.66 day^{-1}	2.8 mg/L [NO ₂ ⁻ -N]	-	0.14 day ⁻¹	Copp and Murphy (1995)	
Overall	0.55 day ⁻¹	0.13 mg VSS/mg N	0.16 day ⁻¹	3.0 mg/L [NH ₄ ⁺ -N]	-	0.17 day ⁻¹	@20°C	
Ammonia Oxidation	1.45 day^{-1}	-	-	3.59 mg/L [NH4 ⁺ -N]	0.63 mg/L O ₂	-	Jayamohan et al. (1988)	
Nitrite Oxidation	1.32 day^{-1}	_	-	1.55 mg/L [NO ₂ ⁻ -N]	1.32 mg/L O ₂	-		

Table 1 Kinetic parameters for nitrifying bacteria in municipal wastewater treatment systems

Table 1 (continued)

Process	Nitrification Kinetic Constants						
	μ_{max}	Y	k	K _{SN}	K _{SO}	K _d	Source
Overall	0.32-0.77day ⁻¹	0.05-0.22 mg biomass/mg N	0.63-4.3 mg N/mg-VSS- day	-	0.43-2.0 mg/L O ₂	-	Randall et al. (1992) @20°C
Nitrite Oxidation	_	-	0.19 mg [NO ₂ ⁻ N]/mg VSS- day	1.2 mg/L [NO ₂ ⁻ -N]	-	-	Ficara et al. (2000)
Ammonia Oxidation	0.76 day^{-1}	0.34 mg VSS/mg [NH4 ⁺ -N]	-	1.0 mg/L [NH4 ⁺ -N]	0.5 mg/L O ₂	0.11 day^{-1}	Rittman and McCarty (2001) @20°C
Nitrite Oxidation	0.81 day^{-1}	0.08 VSS/mg [NO ₂ ⁻ -N]	-	1.3 mg/L [NO ₂ ⁻ -N]	0.68 mg/L O ₂	0.11 day^{-1}	
Overall	-	-	0.17-0.22 mg [NH4 ⁺ -N]/mg VSS-day	0.28-0.61 mg/L NH4 ⁺ -N	-	-	Drtil et al (1993)
Overall	$0.3-3.0 \text{ day}^{-1}$	0.1-0.3 mg VSS/mg N	0.2 mg N/mg VSS-day	0.2-5.0 mg/L [NH4 ⁺ -N]	1.3 mg/L O ₂	0.03-0.06 day ⁻¹	Tchobanoglo
Ammonia Oxidation	$0.3-2.0 \text{ day}^{-1}$	-	-	0.2-2.0 mg/L [NH4 ⁺ -N]	-	-	(1991), pure culture @20°C
Nitrite Oxidation	$0.4-3.0 \text{ day}^{-1}$	-	-	0.2-5.0 mg/L [NO ₂ ⁻ -N]	-	-	
Overall Range	0.3-3.0 day ⁻¹	0.05-0.36 mg VSS/mg N	0.076-4.3 mg N/mg VSS- day	0.2-5.14 mg/L [NH4 ⁺ -N]	0.32-2.0 mg/L O ₂	0.03-0.17 day ⁻¹	

^a 1.42 mg biomass COD/mg COD conversion factor used; ^b Computed from the reported μ_{max} and Y_T as μ_{max}/Y_T

Chapter 3.0

Materials and Methods

3.1 Collection and Storage of Influent Wastewater

The influent for this treatment study was obtained in batches from the Knoxville Kuwahee WWTP. The waste was collected from the overflow of the plant's primary clarifiers. This procedure was carried out using two steel 208.2 L (55-gallon) drums. The combined volume of the drums was sufficient to feed the reactors for four days. Therefore, waste was collected every four days throughout the treatment study. The drums were filled by a sump pump submerged in the WWTP overflow. A rubber hose connected to the pump was placed inside the drum once the bung had been removed. Upon collection of the wastewater, the drums were transported to the lab and placed in a 5°C cold room for storage. Typically, the waste was allowed to cool for one day and then pumped into a 378.5 L (100 gallon) HDPE feed tank (5°C). The influent line to the reactors was placed at the bottom of this tank. A small submersible pump was also placed at the bottom of the tank to minimize solids settling and maintain a homogeneous waste feed.

3.2 Experimental Treatment System

Figure 3 shows one of the treatment units, consisting of a complete mix reactor and external secondary clarifier, used in this study to treat municipal wastewater. The treatment unit was designed to mimic the hydraulic and physiochemical characteristics



Figure 3 A diagram of one of the treatment units, which consisted of a complete mix reactor and secondary clarifier.

of a conventional activated sludge wastewater treatment. The entire system (Figure 4) was composed of four identical treatment units along with other equipment such as influent and recycle pumps and a DO control system.

The reactor illustrated in Figure 3 was constructed entirely of Plexiglas. Each reactor consisted of a 30.48 cm (12" square) top plate, a 30.48 cm (12" square) bottom plate, and a 30.48 cm (12") section of 25.4 cm (10" diameter) Plexiglas tube. The bottom and top plates each had a 25.4 cm (10") circular etching to provide a better fit for the tube section. The tube was bonded to the bottom plate to prevent leakage but the top plate was removable for cleaning purposes. A large magnetic stir plate, approximately 30.48 cm (12") x 30.48 cm (12"), was used to mix the reactor contents. The plate turned a 5.08 cm (2") stir bar inside the reactor. To achieve complete mixing, the stirrer was set at or above $\frac{1}{2}$ full speed. It was determined that this speed would easily provide the volumetric power input of 14 kW/1000 m³ necessary to completely mix an activated sludge reactor (Grady et al., 1999). The calculation for complete mixing can be seen in Appendix A. The reactor's contents drained via gravity overflow to a secondary clarifier through a 1.27 cm ($\frac{1}{2}$) diameter port. The drain port was positioned to provide a liquid volume of 10-L in each reactor. Sludge recycle and influent flows were introduced continuously into the reactors through a 0.635 cm ($\frac{1}{4}$ ") port located 5.08 cm (2") above the surface level of the reactor contents. To avoid short-circuiting, the influent and sludge recycle flows were introduced above the mixed liquor by a piece of tubing that extended approximately $3.81 \text{ cm} (1.5^{\circ})$ from the sidewall into the reactor.



Figure 4 A picture of the treatment system consisting of the reactors and clarifiers.

The clarifier was composed of two components, a main body and a scraper arm assembly. The main body was constructed from a 15.24 cm (6") diameter glass tube by a commercial glass blowing shop. One end was heated and drawn down from the original 15.24 cm (6") diameter to a 3.18 cm (1 ¼") diameter, forming a conical bottom section. A 7.62 cm (3") section of 3.18 cm (1 ¼") glass tube was then welded onto the main body to serve as a reservoir for sludge scrapings. The conical section sloped approximately 70° from horizontal. The overflow line from the reactor was submerged inside the clarifier to minimize mixing and disruption of settling. The effluent drain consisted of a 0.953 cm (3/8") diameter 2.54 cm (1") long glass tube welded over a precut hole in the side of the clarifier. The drain was located to provide a liquid volume of 2.90 \pm 0.05 liters within the clarifier's main body.

The second clarifier component was the scraper arm assembly, which was mounted on top of the clarifier's main body via a grooved circular trough cut into a 17.78 cm (7") square, 1.27 cm ($\frac{1}{2}$ ") thick Plexiglas base plate. A 10.16 cm (4") long section of 15.24 cm (6") diameter Plexiglas tube was bonded to this base plate and enclosed the scraper arm motor. This 1-rpm motor was bolted to the base plate with the motor shaft penetrating into the clarifier's main body through a hole drilled in the base plate. The sludge scraper arm consisted of a 0.318 cm (1/8") diameter stainless steel wire, which was shaped to conform to the side of the clarifier wall and mounted to the motor shaft with setscrews. An automobile wiper blade was placed on the scraper arm to provide a snug fit against the clarifier wall. The scraper motor was controlled with a timer and

every five minutes the motor would engage long enough to rotate the scraper arm approximately ¹/₂ turn.

To discourage photosynthetic growth in the reactors and clarifiers, each was fitted with a black nylon shroud. In addition, exposed influent and effluent tubes were jacketed with an opaque cover. Influent and sludge recycle tubing within the pump heads could not be covered. However, this tubing was replaced periodically due to wear. Air was provided to each treatment unit through two 15.24 cm (6") long non-clogging porous aquarium aerator tubes mounted to the bottom of the eactors by a suction cup. A laboratory compressed air valve provided the air supply for the reactors. Air from this valve passed into a pressure regulator, a filter assembly, and into a 20-L carboy fitted with an airtight cap. Airflow was directed to the bottom of the carboy where it bubbled up through a water column. This process served to both humidify the air and equalize the air temperature, preventing excessive evaporation from the reactors during aeration. From the carboy, air flowed through a condensate trap to a four-way airflow control metering assembly. This manifold allowed the airflow rate in each reactor to be adjusted independently. The dissolved oxygen content was maintained using a control system, which will be discussed in Section 3.3.

The influent and recycle flows were conveyed to the reactor by two identical peristaltic pumps. One pump was dedicated to each of the flows. The pump rotors were fitted with four pump heads so that each reactor would receive influent and recycle flows at exactly the same rate. The influent flow rate was set to 19 mL/min, which provided an HRT of 8.8 hours in the reactor and 2.5 hours in the clarifier (excluding the recycle flow).

The pumps were continuous duty Standard Drive® (Cole Parmer, Inc.) designed for precise control of liquid flow rates, which are electronically adjusted based on tubing size.

An important feature of the flow system was the influent and effluent sampling ports, which allowed collection of grab samples of the influent flow to all reactors and the effluent flow from each clarifier. Grab samples were used because a composite sampler was not available. The sample ports were placed to yield the most representative grab samples possible. For example, the influent sample port was located immediately prior to the influent waste entering the 5-day BSRT reactor. This placement effectively eliminated concerns about degradation in the storage tank and in the influent lines. The effluent grab sample ports were located immediately following clarifier overflow. This placement avoided collection of the effluent after it had passed several feet through effluent tubing prone to photosynthetic growth.

3.3 Description of DO Control System

To better maintain DO levels in each reactor, a control system (Great Lakes Instruments), consisting of four (Model 5500-series) DO probes, two (Model D53) DO analyzers, four solenoid valves and four air blast units was installed. The analyzers, air blast boxes, and solenoid valves were all mounted on a 1.91 cm (³/₄") piece of birch plywood. The airflow was derived from the in-house compressed air system in the laboratory and was regulated to approximately 104.4 kPa (15 psi) using a constant pressure valve.

Designed specifically for use in WWTPs, the probes had a hydrophobic membrane that resisted fouling. The membrane, electrolyte, and electrodes were all contained within a removable cartridge. This was a useful feature because several of the original membranes were faulty and had to be replaced. The sensor consisted of a gold anode, silver cathode and silver reference. Using the principle that current was a function of the partial pressure of the DO in solution, the sensor measured current across two electrodes to determine the DO concentration. The DO moved through the membrane and into the electrolyte solution. A constant voltage was applied to reduce DO at the cathode and produce a current. The current was directly proportional to the DO concentration in solution. Since the reference electrode was not used to conduct current flow, it was used to provide a potential that remained constant over time. Calibration of the probes was essential in maintaining accuracy of measurement. During calibration, the probe tips were rinsed and wiped with a damp cloth to remove bacterial growth on the membrane. Calibration was accomplished using an air calibration method. Each probe was placed in a plastic calibration bag, which provided a stable environment around the sensor membrane. The analyzer then computed the mg/L value based on atmospheric pressure and temperature of the air.

Each DO probe was equipped with an air blast apparatus to minimize attached growth by periodically blasting air across the membrane. The analyzers turned on the air blast boxes once every hour for a duration of 10 seconds. The air blast boxes provided air via a hose attached to the probe. Once the DO logging system was completed, it was discovered that the air blast system was causing huge increases in each reactor DO. The air blast system was then turned off in order to avoid affecting treatment performance. As an alternative, the probes were rinsed daily during solids analysis to minimize attached growth. Calibration was also performed once every 1-2 weeks rather than the recommended 1 month so that bacterial growth could be removed from the tips. Elimination of the air blast also decreased the amount of foam in the reactors.

The two DO analyzers were each capable of receiving input from two DO probes. The analyzer read the current produced by a probe and converted it to DO concentration. The analyzer then compared that value with a previously specified set point concentration. If the DO read higher than the set point, the analyzer flipped a relay controlling a solenoid valve and closed it. This stopped the airflow to the reactor until the analyzer read a value below the set point. Once the DO concentration fell below the set point, the analyzer opened the solenoid valve and allowed airflow into the reactor. In this manner, the analyzer maintained tight DO control in each reactor. Upon installation of this system, the study of DO effects on activated sludge treating municipal wastewater was begun.

3.4 Verification of DO Control Capability

In order to verify the accuracy of the DO control system in maintaining a set DO concentration, a computer logging system was developed to monitor DO concentration over time. The system used a Highway Addressable Remote Transducer (HART) protocol to transmit a signal from the DO analyzers to a personal computer. Each analyzer was equipped with positive and neutral analog (4-20mA) HART outputs. These outputs sent a signal via a Bell 202 loop to the PC. HART used the Bell 202 loop to

superimpose digital signals at a low level on top of the 4-20 mA. The Bell 202 loop typically consists of one or more analyzers, a current sensing resistor connected in series to a power supply, and an RS232-Bell 202 (mini-modem) converter connected to a PC. The mini modem was connected across the resistor. These analyzers were connected in series to complete the loop.

A software package (H-View), produced by Arcom Control Systems, was installed on the PC. This program was designed to allow a user to monitor changes in DO over time in each of the reactors using the input from the RS232 mini modem. The first step was to have the software search for analyzers in the system. The software was then able to locate and individually tag each of the analyzers. Since the software would not store data, the next step was to use Dynamic Data Exchange (DDE) to log DO over time. DDE allowed the changing data to be linked to a spreadsheet in Microsoft Excel. Using a macro written in visual basic, the data was then copied and pasted into a database every 30 seconds for each reactor. This method was a vast improvement over the initial method, which consisted of taking discrete DO measurements daily. However, there was a problem with the logging system in that it shut off every night at midnight. The program was restarted every morning before the treatment performance samples were taken. This problem is currently being investigated but there is a sufficient amount of DO data to confirm the performance of the control system.

3.5 Operating Procedures

3.5.1 Choice of reactor BSRTs

Reactor BSRT was a primary experimental variable for this study. This parameter dictated the biomass growth rate in each of the reactors. The relationship between growth rate and BSRT is given as the following (Benefield and Randall, 1985):

$$\boldsymbol{m} = \frac{1}{\boldsymbol{q}_c} \tag{27}$$

where:

 μ = biomass growth rate, day⁻¹ θ_c = reactor BSRT, day

In order to assess the wastewater treatment kinetic coefficients, BSRTs representative of full-scale WWTPs were chosen. The BSRTs chosen for the study (20, 10, 5, and 2 days) were controlled in reactors operating simultaneously. The 10- and 20day BSRTs are representative of full-scale single-stage nitrifying systems. The 2-day BSRT placed one reactor near the maximum specific growth rate for nitrifiers.

3.5.2 Solids sampling and sludge wastage procedure

Solids analysis was performed daily after the collection of all treatment performance samples to avoid disruptions in system operation caused by the procedure. This also allowed treatment performance samples to be collected after nearly 24 hours of undisturbed operation. Only the sequence and procedures for solids sampling and wastage will be discussed in this section. The techniques for solids sample analysis will be discussed in Section 3.6.1. Following collection of treatment performance samples, the influent and solids recycle pumps were shut off. The effluent port of each reactor was then stoppered with a thick wire brush and the recycle pump was turned on at full capacity to return all sludge in the clarifier to the reactor. After all solids were returned to the respective reactors, the recycle pump was again shut off along with the aerator and stirrer. The aeration and magnetic stirrer were only turned off for a few minutes to allow the sludge to settle below the effluent port. The reactors were then unstopped and clear reactor effluent was then allowed to flow into the clarifier. This process allowed for the retention of all biomass in the reactor.

Samples were taken from the 40-L carboys for 24-hr averaged effluent suspended solids (ESS) determination. The contents were stirred to obtain a representative sample for ESS analysis. The samples were withdrawn with a pump into volumetric flasks at sample volumes ranging from 100 to 400 mL. Note that photosynthetic growth was discouraged by occasionally cleaning the carboys with a dilute bleach solution. The carboys were carefully rinsed afterward to remove the bleach solution. This measure was taken to minimize the amount of wall attached growth that could slough off and affect ESS measurements.

Once the reactors were unstopped, the aeration and mixing units were turned back on to resuspend the biomass. After a couple minutes of mixing, 10 or 20 mL samples of mixed liquor were taken from each reactor using wide-mouth serological pipettes. During sampling, the pipettes were placed near the center of each reactor. The samples were withdrawn with a battery-powered auto-pipette and sample volumes were noted on a daily solids analysis/sludge wastage procedure log. Following MLSS sampling, mixed liquor was collected and stored in an -80°C freezer for molecular analysis at a later time. At this point, sludge volume index (SVI) analysis was performed on each of the reactors. A 1 L sample of mixed liquor was pumped into a graduated cylinder and allowed to settle for 30 minutes. Upon completion of the SVI tests, the samples were mixed and placed back in each corresponding reactor.

Influent and recycle pumps were then restarted after which daily cleaning and maintenance of the reactors was performed. After analysis of MLSS and ESS samples was complete, the wastage volume was determined using the following equation:

$$WastageVolume = \left(\frac{MLSS * V_R}{\boldsymbol{q}_c} - Q * ESS\right) * MLSS$$
(28)

where:

$$\begin{split} MLSS &= mixed \ liquor \ suspended \ solids \ (mg/L) \\ V_R &= volume \ of \ reactor \ (L) \\ \theta_c &= desired \ BSRT \ (day) \\ ESS &= effluent \ suspended \ solids \ concentration \ (mg/L) \\ Q &= influent \ flow \ rate \ (L/day) \end{split}$$

The calculated volumes were then removed from each reactor and immediately replaced with the same volume of effluent from the respective carboys. Wastage volumes were replaced by reactor effluent to eliminate any effects that adding tap or deionized water might have on the activated sludge. The procedure was modified for the 2-day BSRT reactor due to its relatively low MLSS concentration and high wastage volume. The wastage volume was split into two equal parts that were removed approximately eight to twelve hours apart. One wastage event was thought to present too much of a disturbance for proper steady–state operation of the reactor.

3.5.3 Treatment Performance Sampling Procedure

The types of samples collected to assess reactor treatment performance are listed in Table 2. Influent samples were collected as grab samples by unclamping a tube that was connected, by a tee, to the 5-day BSRT reactor influent line. The tube was placed in a container and the influent pump provided the necessary volume of sample. Effluent samples (except ESS) were collected by removing the tubing that ran from the clarifier to the carboy. The samples were then collected by gravity flow from the clarifier to a sample container. This procedure was followed on a daily basis, with the exception of a few days, from the start of the treatment study in June until its conclusion in January. It should be noted that only 50 mLs of sample were required to conduct the alkalinity analysis. Therefore, volumes obtained for anion and COD analysis were taken from the sample volume collected for alkalinity analysis.

Table 2Sample volumes collected for each type of analysis

Analysis	Volume Collected		
Ammonium	100 mL		
Alkalinity	75-100 mL		
Chemical Oxygen Demand (COD)	5-7 mL		
Anions (NO ₃ ⁻ and NO ₂ ⁻)	3-5 mL		

3.6 Sampling and Analysis Procedures

3.6.1 Solids Sampling and Analysis Procedure

MLSS and ESS

Daily samples of the reactor MLSS and ESS were taken as described in Section 3.5.2. The mixed liquor was carefully removed from each reactor using a 10 mL pipette and then discharged and rinsed onto a 1.5 μ m x 47 mm glass fiber filter (Proweigh[®] by Environmental Express). The samples were analyzed to determine the MLSS and ESS according to standard Method 2450 D, Total Suspended Solids Dried at 103-105°C (APHA, 1998). MLSS and ESS concentrations were then calculated using the following equation:

mg suspended solids /
$$L = \frac{W_{FF} - W_I}{V} * 1000$$
 (29)

where:

mg suspended solids/L = MLSS or ESS (mg/L) W_{FF} = final weight of filter (mg) W_{I} = initial weight of filter (mg) V = volume of sample (L)

MLVSS

MLVSS values were determined to obtain a closer approximation of the biological component in each reactor. This analysis was conducted daily according to Standard Method 2540 e, Fixed and Volatile Solids Ignited at 550°C (APHA, 1998). The filter containing dried solids used for MLSS analysis was placed in a muffle furnace and

combusted for a minimum of 15 minutes at 550°C. MLVSS was calculated using the following equation:

mg volatile suspended solids /
$$L = \frac{W_{FF} - W_{FV}}{V} * 1000$$
 (30)

where:

mg volatile suspended solids/L = MLVSS (mg/L) W_{FF} = final filter weight from the MLSS analysis (mg) W_{FV} = final filter weight after MLVSS analysis (mg) V = volume of sample (L)

SVI

The settling characteristics of the each reactor were monitored daily using a modification of Standard Method 2710 D, Sludge Volume Index, (APHA, 1998). The term SVI relates the volume in milliliters occupied by 1 gram of a biological suspension after 30 minutes of settling. Modification of Standard Method 2710 D was necessary because a settling column was not available with the stirring mechanism specified in the Standard Method. The modified procedure involved pumping completely mixed activated sludge from each reactor into 1-L volumetric graduated cylinders. The settled volume was recorded after 30 minutes of quiescent settling. The following equation was used to determine SVI:

$$SVI = \frac{SSV}{MLSS} * 1000 \tag{31}$$

where:

SVI = sludge volume index (mL/g) SSV = settled sludge volume (mL/L) MLSS = mixed liquor suspended solids (mg/L)

3.6.2 Chemical analyses to document wastewater treatment performance

Ammonium sampling and analysis procedures

Samples were collected on a daily basis throughout the treatability study to document the ammonia oxidizer performance for the various DO concentrations. An effluent grab sample from each of the four reactors and one influent grab sample were collected in 150 mL Erlenmeyer flasks from the sample locations discussed in Section 3.5.3. Immediately following sample collection, 100 mLs were withdrawn with a volumetric pipette and placed in another flask. The samples were allowed to reach room temperature prior to analysis. The purpose of this procedure was to assure that the sample quantities and temperatures were the same as the ammonium standards. On days when ammonium analysis was not performed, samples were preserved by addition of 200 μ L of concentrated H₂SO₄, stoppered, and refrigerated at 4°C. Four standards, 0.1, 0.5, 5 and 50 mg/L, and a quality control were always analyzed with the samples collected from the reactors. Analysis of ammonium concentration was conducted in accordance with Standard Method 4500 D, Ammonia-Selective Electrode Method (APHA, 1998). An Orion Model 95-12 probe was used for the analysis as suggested by the method.

Alkalinity sampling and analysis procedures

Since nitrification consumes alkalinity at a high rate, alkalinity analyses of influent and reactor effluent samples were used to verify that the wastewater was sufficiently buffered to prevent depression of pH. This analysis was also used to verify the theoretical alkalinity consumption ratio of 7.14 mg alkalinity as CaCO₃/ mg of NH₄⁺ oxidized to nitrate. Alkalinity samples were collected as grab samples in 150 mL Erlenmeyer flasks. Using a volumetric pipette, 50 mLs were then taken from the flask and used for analysis according to Standard Method 2320 B, Titration Method (APHA, 1998).

Anions sampling and analysis procedure

Anions samples were collected to confirm the removal of ammonia and assess the performance of nitrite oxidizers at the various BSRTs and DO concentrations. The influent and effluent grab samples used for this analysis were taken from the same 150 mL Erlenmeyer flasks used for alkalinity analysis. Three to five mLs of sample were taken from the flask with a syringe and filtered (0.45 µm Gelman filter) into auto-sample vials. The samples were analyzed on a Dionex DX 100 and a Dionex DX 500 Ion Chromatograph (IC) fitted with an Ionpac[®] AS9HC 4mm anion column according to Standard Method 4110 B, Ion Chromatography with Chemical Suppression of Eluent Conductivity (APHA, 1998). For each batch of samples, the IC was first calibrated with four standards. Each standard contained a different concentration of all anions of interest. One QC sample was also analyzed during each analytical run. Samples not analyzed immediately were stored at 4°C for a maximum of 48 hours.

COD sampling and analysis procedures

Carbon treatment efficiency was documented with analysis of influent Total Chemical Oxygen Demand (COD_t) and effluent Soluble Chemical Oxygen Demand (COD_s) . The reasoning behind using COD_s for the effluent rather than COD_t was that filtration of effluent samples would remove the variability in the test caused by daily fluctuations in ESS. Due to cost and labor considerations, this analysis could not be performed on a daily basis. The samples were preserved as they were collected with concentrated H₂SO₄. The acid was added at a rate of 2 mL per liter of sample collected. These samples also came from the flasks used to obtain alkalinity samples. The effluent samples were filtered at the same time as the samples used for anion analysis. Samples were ultimately placed in sterile 15 mL centrifuge tubes. The preservative was then added (30 μ L) and the tube was vigorously hand shaken. Samples were stored for a maximum of 28 days at 4°C. This sample preservation method follows the recommendations given by Standard Methods (APHA, 1998).

Analysis of COD_t and COD_s was conducted using Standard Method 5220 D, Closed reflux Colorimetric Method (APHA, 1998). Specifically, micro-COD test vials from Hach, Inc. were used to minimize disposal waste and testing time. For each set of COD samples analyzed, five COD standards were simultaneously analyzed to establish a standard absorption curve. COD standards were prepared using dried potassium hydrogen phthalate (KHP) in accordance with the Standard Method.
Chapter 4.0

Results and Discussion

4.1 COD Treatment Performance

The study of DO and BSRT effects on activated sludge treatment performance of municipal wastewater was begun on June 6, 2000 with the DO set at 4.0 mg/L to minimize the impact of DO on treatment performance. The 20, 10, 5, and 2 day BSRT reactors were operated, during the course of this study, at each of the following DO concentrations: 4.0, 3.0, 2.0, 1.5, 1.0, and 0.5 mg/L. In addition, the 20, 10, and 5 day BSRT reactors were also operated at 0.2 mg/L DO. The low DO portion of the study was concluded on January 10, 2001 when it was determined that the 5 and 2 day BSRT reactors had failed with respect to nitrification. The DO was raised back to 3.0 mg/L at the end of the study (from January 11th-24th) to reestablish steady state nitrification in all four reactors. The date ranges for operation of each BSRT reactor at various DO concentrations are shown in Table 3. Actual average DO levels in each reactor over time are presented in Appendix B.

Reactor	4.0	3.0	2.0	1.5	1.0 mg/L	0.5 mg/L	0.2 mg/L
BSRT	mg/L	mg/L	mg/L	mg/L			
20	6/6-6/30	7/1-7/27	7/28-9/6	9/7-10/5	10/6-11/2	11/3-11/29	11/30-1/10
10	6/6-6/30	7/1-7/27	7/28-9/6	9/7-10/5	10/6-11/2	11/3-11/29	11/30-1/10
5	6/6-6/30	7/1-7/27	7/28-9/6	9/7-10/5	10/6-11/2	11/3-12/15	12/16-1/10
2	6/6-6/30	7/1-7/27	7/28-	10/28-	11/30-	12/28-1/10	
			10/27	11/29	12/27		

Table 3DO operation date ranges for the course of the treatability study.

To ensure that the control system actually maintained the DO levels shown in Table 3, a logging system was installed to monitor DO control performance. Logging of DO data began on December 12, 2000. It was discovered that DO control of the 20-day BSRT reactor was inefficient at the 0.2 mg/L set point. This problem has been attributed to the high solids content interfering with the probe. The probe in this reactor could regularly be found reading 0.05 mg/L. This condition would have kept air running to the reactor at all times. Upon shaking the probe, the DO would quickly increase to well over 2 mg/L. For this reason, 0.2 mg/L data for the 20-day BSRT reactor was excluded from analysis.

Conversely, the 10- and 5-day BSRT DO control systems provided tight control throughout the period of logging. The average DO concentration and standard deviation for the 10-day BSRT reactor were 0.19 mg/L and 0.04 mg/L respectively. An average DO level of 0.23 mg/L was found in the 5-day BSRT reactor with a standard deviation of 0.08 mg/L. The 2-day BSRT reactor was switched from 1.0 to 0.5 mg/L DO concentration about two weeks after logging began. The average DO concentrations were 0.99 and 0.57 mg/L respectively with standard deviations of 0.10 and 0.15. It should be noted that the 2-day BSRT reactor DO probe became very dirty and malfunctioned during the period of December 24th-January 1st. All data on these days has been excluded from consideration. Upon cleaning and calibration of the probe, DO control was restored in the 2-day BSRT reactor.

Once the low DO study was concluded and the probes were returned to 3.0 mg/L, the 20-day BSRT control system began to function correctly again. The 20-day BSRT reactor had an average DO of 2.97 with a standard deviation of 0.09 during this time. This finding reinforces the assumption that high solids might have disrupted DO control at low concentrations (0.2 mg/L).

Steady state is defined as the condition at which the net rate of change of biomass concentration over time is zero (Benefield & Randall, 1985). Since settled wastewater from KUB's Kuwahee WWTP was used to more closely mimic full-scale WWTP conditions, the influent characteristics of the waste (i.e. COD, nitrogen content, etc.) varied each time it was collected from the WWTP. Figure 5 shows a plot of influent and effluent COD over the course of the treatment study. While total COD (COD_t) was used to monitor the influent, the effluent samples were filtered so that only soluble COD (COD_s) was considered. The main reason for filtration was to minimize the influence of ESS suspended solids on effluent COD values.

COD removal was excellent in all four reactors during the entire study except for the week of July 28th to August 4th. The presence of what is believed to have been a surfactant decreased the degradability of the influent and caused high effluent COD values for all BSRTs. Once the surfactant finished passing through the system, effective COD treatment resumed. It should be noted that the KUB WWTP experienced similar problems with this unknown compound and actually violated their maximum daily discharge BOD concentration four times during the week of July 17th to July 24th. This corresponds to approximately the time waste would have been collected from the KUB plant. Figure 6 is a plot of KUB's effluent BOD data showing the disturbance.



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Figure 5 Effect of DO concentration (ranging from 4.0-0.2 mg/L) on COD treatment performance.

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Figure 6 Effect of an unknown surfactant on KUB's BOD treatment performance.

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Although carbon treatment would likely not be affected until a very low DO was reached, a distinction between excess and low DO was made since nitrification can be limiting at DO concentrations less than 2.0 mg/L (Benefield and Randall, 1985). Carbon treatment averages during steady state operation for excess (\geq 2.0 mg/L) and low (\leq 2.0 mg/L) DO are presented in Table 4. The low standard deviations can be attributed to the use of COD_s since it has been found that ESS can play a major role in effluent COD determination (Grady et al., 1999). Since the effluent was filtered to minimize the impact of effluent suspended solids, treatment performance in this study related to the influent waste stream.

A paired t-test of the data was conducted to determine differences in carbon treatment performance. This type of statistical analysis was used to analyze pairs of data to determine the likelihood that the mean of one data set was equal to the mean of another for a given confidence interval. Since it was assumed that the data was random and normally distributed, the distribution of data was first analyzed according to the method

Reactor	Excess DO Condition			Low DO Condition		
	COD, (mg/L)	σ, (mg/L)	Eff. (%)	COD, mg/L	σ, mg/L	Eff. (%)
Influent	229	77		234	57	
20 day θ_{c}	23	15	88	25	7	90
10 day $\theta_{\rm c}$	26	16	87	27	7	89
5 day θ_{c}	31	17	84	35	10	86
$2 \text{ day } \theta_{c}$	38	15	83	39	10	83

Table 4Average COD treatment performance during steady state operation.

described by D'Agostino et al. (1990). The data for all BSRTs was found to be normally distributed at a 99% confidence interval when excess and low DO treatment efficiencies were compared. The results of the paired test showed that there was no statistical difference in COD treatment efficiency at a 99% confidence interval between high and low DO concentrations at each BSRT. These findings indicate that effective carbon treatment can be accomplished low DO levels (0.2 mg/L).

Chuang et al. (1997) also found that effective COD treatment could be achieved in a system operating at DO levels as low as 0.1 mg/L. Reactors operated at BSRTs of 5, 10, and 15 days each treated an influent COD concentration of approximately 300 mg/L, which is comparable to the average influent COD values in Table 4. Similar results were obtained by Munch et al. (2000) when conducting a pilot plant study at the Oxley Creek WWTP. Effective carbon treatment was achieved at a BSRT of 45 days when the bioreactor was aerated at 0.5 mg/L DO.

The 20-day BSRT reactor consistently provided the highest average treatment efficiency under both excess and low DO conditions, whereas the 2-day BSRT reactor provided the poorest treatment efficiency. Using the paired t-test, average treatment efficiencies of the 20 and 10 day BSRT reactors were found to be statistically the same for a 99% confidence interval. However, a paired t-test analysis between the 20 and 2 day BSRT data and 20 and 5 day BSRT data under excess and low DO conditions revealed that the average values were statistically different at a 99% confidence interval. All compared data in this analysis were found to be normally distributed for a 99% confidence interval. These results indicate that carbon treatment is a function of BSRT

and therefore a BSRT of 10 days or greater should be maintained to achieve the highest treatment efficiency.

Bernal-Martinez et al. (2000) also found that COD treatment efficiency increased with BSRT for a reactor treating domestic wastewater. COD treatment efficiencies of 81, 91 and 99% were obtained when the reactor was operated at BSRTs of 3, 6, and 23 days, respectively. Similar findings were reported by Palm et al. (1980) for bioreactors operating at BSRTs of 1.9 and 11 days. The treatment efficiencies were found to be 85% and 90% respectively for the reactors treating settled municipal wastewater.

4.2 Solids Analysis Data

Daily solids analysis was conducted starting March 20th to ensure that the reactors were at steady state with respect to solids concentration before beginning a complete analysis of performance. Reactor suspended solids data are shown in Figure 7. As demonstrated in Equation 9, biomass concentration is a direct function of BSRT and influent substrate concentration. Since the bacterial population is mainly comprised of heterotrophic bacteria, changes in influent COD can cause large variations in biomass concentration for any BSRT. Using typical values for Y and $k_{\rm d}$ of 0.42 and 0.24 in Equation 9 for a BSRT of 2 days, it can be seen that an increase in influent COD of only 100 mg/L could change the biomass concentration by almost 130 mg/L (Grady et al., 1999). Therefore, it was not possible to maintain a strict steady state condition due to variable influent wastewater characteristics.



Figure 7 Fluctuations in MLSS concentration due to changing DO and influent COD concentrations throughout the treatability study.

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As seen in Figure 7, changes in MLSS concentration were noted over time. The first major change in solids concentration began to occur on August 27th. The reason for this abrupt drop in solids concentration was the changing of tubing in the influent pump. In order to prolong the life of the tubing used in the peristaltic pumps, a thick walled type of tubing was used to replace the worn tubing. However, the tubing was too thick and did not allow the full flow to pass into the reactors. The 20-day BSRT reactor was the most affected dropping from a concentration of 2310 mg/L to 1310 mg/L in a matter of three days. The 10-day BSRT reactor also saw a significant solids decrease from 1460 mg/L to 1040 mg/L. The 2- and 5-day BSRT reactors were not as affected since their smaller microorganism populations required a lower substrate concentration for survival. Installation of the proper tubing increased the substrate feed rate, which resulted in an explosion of growth in all four reactors.

During the period from October 5th to November 2nd solids concentrations, particularly in the 20- and 10-day BSRT reactors, underwent major changes. These changes can be attributed to a consistent foaming problem that occurred over this time. The foaming did not cause settling problems but often caused the reactor effluent port to clog. Solids would remain in the reactor until the foam dissipated at which point a significant amount of mixed liquor would flood into the clarifier. This overloading of the clarifier caused high amounts of solids to be lost in the effluent on several occasions and affected the MLSS concentrations. It was later discovered, after turning off the air blast units, that this equipment might have caused some of the foaming. Considering the two previously mentioned time periods, steady state conditions for each reactor were determined using Figure 7. The 20-day BSRT reactor was estimated to have two steady state time periods during this study. The first ranged from June 22nd to August 24th, which corresponded to the period of excess DO operation; the second ranged from November 11th to January 10th, which is in the low DO operational period. A similar situation was found for the 10-day BSRT reactor with steady state date ranges for excess and low DO operation of July 1st to August 27th and November 10th to January 10th, respectively. For the 5-day BSRT reactor, the MLSS concentration was at steady state from June 22nd to September 1st and October 8th to January 10th. Because MLSS concentration did not significantly vary at any time during the study in the 2 day BSRT reactor, steady state Occurred from June 6th to January 10th. Averages and standard deviations for the steady state MLSS concentrations are shown in Table 5.

The data in Table 5 indicate that a decrease in DO resulted in an increase in MLSS concentration. A paired t-test was conducted on the two data sets for the 20 day BSRT reactor to determine whether or not the mean values were different. It was discovered that the means for the two steady state operational ranges for a 99%

Reactor	Excess DO Co	ondition	Low DO Condition		
	MLSS,	Std. Deviation, mg/L	MLSS,	Std. Deviation, mg/L	
	mg/L		mg/L		
20 day θ_c	2762	349 (13%)	3265	368 (12%)	
10 day θ_{c}	1440	149 (10%)	2061	230 (11%)	
5 day $\theta_{\rm c}$	768	105 (14%)	1047	184 (18%)	
$2 \text{ day } \theta_{c}$	420	96 (23%)	420	96 (23%)	

Table 5Average MLSS concentrations during steady state operation.

confidence interval were not the same. A similar result was obtained when the respective steady state averages of the 10 and 5 day BSRT reactors were analyzed.

A synthetic influent waste feed is often used in treatability studies to help maintain a stable MLSS concentration. Lau et al. (1984) found that MLSS concentration remained fairly constant for a BSRT of 8 days when the DO was varied from 6.1 to 0.8 mg/L when a synthetic feed was used. The same finding was observed when a 3.25 day BSRT reactor was operated in a range of 6.1 to 0.09 mg/L DO. The MLSS variation in this study for the 20, 10, and 5 day BSRT reactors was initially thought to be a result of changes in influent COD. However, a paired ttest conducted on the influent COD averages for excess and DO steady state operation revealed that the values were not statistically different for a 99% confidence interval.

An increase in bacterial yield could contribute to an increase in MLSS concentration. Hanaki et al. (1990) found that the nitrifier observed yield coefficient increased when the conditions were varied from excess to low DO conditions. The observed yield was found to increase from approximately 0.3 to 0.6 when DO in the reactor was dropped from 6.0 to 0.5 mg/L. Although an increase in nitrifier population would not significantly impact MLSS, a larger population of heterotrophs would increase MLSS. Lishman et al. (2000) conducted a study of wastewater treatment under aerobic and anoxic conditions. The researchers found that heterotrophic yield increased from 0.25 to 0.35 for the two conditions respectively. The increased yield resulted in an increase in MLVSS concentration.

A kinetic analysis was conducted on COD treatment data to determine the effects of DO on heterotrophic yield using a method similar to the one outlined in Section 2.6.1. The major difference was that the residual oxygen demand had to be considered when conducting an analysis of carbon treatment kinetics (Grady et al., 1999). Benefield and Randall (1985) suggest that the residual COD can be estimated by plotting effluent substrate concentration versus specific substrate utilization rate. A residual of approximately 20 mg/L was found. The yield coefficients were then calculated for the previously defined excess and low DO conditions using a plot of specific growth rate versus specific substrate utilization rate. Values of 0.42 and 0.43 mg VSS/ mg COD were calculated for the excess and low DO operational periods respectively. Although a slight increase occurred, it was not found to be significant. Therefore, it is unclear exactly why MLSS apparently increased with decreasing DO.

The MLVSS concentrations, shown in Figure 8, were monitored throughout the treatment study. MLVSS is typically used as a more accurate measure of biomass because it only accounts for the organic portion of the MLSS. The MLVSS/MLSS ratio varied slightly in the 20-, 10-, and 5-day BSRT reactors from the excess DO steady state period to the low DO period. The volatile portion of the MLSS in the 20-day BSRT reactor ranged from an average of 83% (Std. Dev. = +/-2%) to an average of 79% (Std. Dev.=+/-2%). For the 10-day BSRT reactor, MLVSS accounted for averages of 86% (Std. Dev.=+/-5%) and 81% (Std. Dev.=+/-3%) of the MLSS during the excess DO and low DO time periods. The volatile portion of MLSS for the 5-day BSRT reactor ranged from an average of 89% (Std. Dev.=+/-3%) to an average of 84% (Std. Dev.=+/-2%).



Figure 8 Fluctuations in MLVSS concentration due to changing influent COD concentrations during the treatability study.

5%). For the 2-day BSRT reactor, the volatile portion of the MLSS averaged 88%(Std. Dev.=+/-5%) over the course of the treatment study.

4.3 Sludge Settling and Effluent Suspended Solids

The SVI of each reactor over the time course of the treatment study is displayed in Figure 9 and average steady state SVIs for each reactor are shown in Table 6. Sludge settling was excellent throughout most of the treatment study. Good settling was expected for the operational periods where DO was at least 2 mg/L (Surucu & Cetin, 1989). Treatment performance in this study related to the occurrence of SVI values less than 100 mL/g and effluent suspended solids concentrations below 45 mg/L. However, settling problems did occur in the 5-day BSRT reactor from August 15th to August 22nd and in the 2-day BSRT reactor from August 18th to September 3rd. Inspection of Figure 9 shows that the week of poor settling in the 5-day BSRT reactor caused the high SVI average and standard deviation in the excess DO steady state period. The periods of poor settling in the 5 and 2 day BSRT reactors indicate that settling was not stable at times for the shorter BSRTs. However, the averages in Table 6 are well below the SVI value of 80 mL/g necessary for good settling sludge (Grady et al., 1999).

Reactor	Excess DO Condition		Low DO Condition	
	SVI (mL/g)	Std. Dev. (mL/g)	SVI (mL/g)	Std. Dev. (mL/g)
20 day θ_c	38	10	37	8
10 day θ_{c}	45	10	38	9
5 day $\theta_{\rm c}$	71	70	54	16
$2 \text{ day } \theta_{c}$	53	65	44	21

Table 6Steady state SVI values during the two DO time periods.



Figure 9 Effect of DO concentration (ranging from 4.0-0.2 mg/L) on settling performance.

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Since settling efficiency has been found to vary with BSRT, a paired t-test analysis was conducted to assess the impact of BSRT on sludge settling. Average SVI values in the 20 and 10 day BSRT reactors for the two steady state time periods were found to be statistically the same for a confidence interval of 99%. However, a significant difference was found when comparing steady state data for the 20 and 5 day and the 20 and 2 day BSRT reactors. These findings indicate that solids settled more efficiently at longer BSRTs. Similar results were obtained by Echeverria et al. (1993) when conducting a pilot plant study on municipal wastewater. It was found that SVI values were the lowest (approximately 50 mL/g) when operating at BSRTs greater than 8 days. Bisogni and Lawrence (1971) also found that settling velocity increased with BSRT and therefore settling became more efficient for longer BSRTs.

A paired test analysis was also conducted on data from each reactor at excess and low DO conditions to determine whether or not DO affected sludge settling. It was found that the average steady state SVI values were statistically equivalent at a 99% confidence interval for the 20 day BSRT data. The same results were found when the analysis was performed on the 10 and 2 day BSRT data. Conversely, a t-test analysis on the 5 day BSRT reactor revealed that the values were statistically different for a 99% confidence interval.

Although DO might be expected to have a negative impact on sludge settling, it has been found that the main factors which affect sludge settling are BSRT and organic loading (Surucu and Cetin, 1990). Therefore, the DO itself might not cause poor SVI values unless a sufficient organic loading occurred. It was discovered in the classic study by Palm et al. (1980) that an organic removal rate of 0.2-0.3 mg COD/mg MLVSS/d at DO concentrations of 0.1-0.5 mg/L would not negatively affect SVI. However, organic loadings above this range would cause significant settling problems. For DO concentrations between 0.5-1.0 mg/L, a substrate removal rate greater than 0.45 mg COD/mg MLVSS/d was required to affect SVI values. Figure 10 shows the DO concentration and corresponding organic removal rate necessary for sludge bulking. The average substrate removal rate of the 20-day BSRT reactor at 0.5 mg/L DO was calculated to be 0.10 mg COD/mg MLVSS/d. This value is well below the organic loading of 0.45 mg COD/mg MLVSS/d reported for sludge bulking. Therefore, the organic loading was insufficient to cause SVI problems in the 20-day BSRT reactor during the 0.5 mg/L operational period.

For the 10-day BSRT reactor, the average substrate removal rates for the 0.5 and 0.2 mg/L operational periods were 0.12 and 0.12 mg COD/mg MLVSS/d, respectively. Therefore, the 10-day BSRT reactor was well below the organic loadings of 0.45 mg COD/mg MLVSS/d and 0.3 mg COD/mg MLVSS/d reported to cause settling problems, which might explain why sludge settling remained effective even at such low DO concentrations. The substrate removal rates in the 5-day BSRT reactor were slightly higher due to its bwer solids concentration. The average substrate removal rates for the 0.5 and 0.2 mg/L periods of operation were 0.23 and 0.26 mg COD/mg MLVSS/d. The average loading of 0.23 mg COD/mg MLVSS/d during the 0.5 mg/L phase was well below the boundary for sludge bulking. Since the SVI values in the 5-day reactor



Figure 10 Effect of organic removal rate and DO concentration on sludge settling (adapted from Palm et al., 1980).

remained low during the 0.2 mg/L operational period, the organic loading was not sufficient to cause sludge bulking in the 5-day BSRT reactor.

The average substrate removal rate in the 2-day BSRT reactor was 0.57 mg COD/mg MLVSS/d. This value exceeded the upper substrate removal rate boundary of 0.45 mg COD/mg MLVSS/d for effective settling at a DO concentration of 0.5 mg/L, however, settling was not a problem in this reactor. Echeverria et al. (1992) have obtained similar findings for activated sludge reactors treating domestic wastewater at a loading rate of 0.36-0.56 mg BOD₅/mg MLVSS/d. For reactors operating at BSRTs ranging from 4-12 days, it was observed that SVI values less than 100 could be achieved while maintaining reactor DO at approximately 0.5 mg/L.

Figure 11 depicts a plot of effluent suspended solids (ESS) over the time course of the study. The ESS for each of the four reactors was below 45 mg/L, the maximum daily discharge limit for KUB's Kuwahee WWTP, on most days. However, the maximum discharge limit was exceeded on several occasions indicating that another process e.g. chlorination would be needed for full-scale operation. Poor solids separation on some days was likely influenced by the difficulty in scaling down secondary clarifiers for bench scale studies. Hence, solids separation was not as effective as that of a full-scale treatment facility.

Because the SVI values remained low, it was not surprising that ESS values were also low during the study. However, there were a couple of periods in which ESS values were high. The first major incident occurred when the influent pump tubing was replaced with thick-walled tubing. The high ESS is consistent with the drop in solids in the four



Figure 11 Effect of DO concentration (ranging from 4.0-0.2 mg/L) on effluent suspended solids.

BSRT reactors. MLSS concentrations dropped the most in the 20- and 10-day BSRT reactors. Consequently, the ESS was also high once the proper tubing was installed due to the explosion of growth in the reactors. This growth likely overloaded the clarifiers and caused excess solids to be lost in the effluent. The second period of poor settling occurred from November 1st to November 21st primarily in the 20- and 5-day BSRT reactors. Once again these high ESS values corresponded to drastic changes in the MLSS concentration. As stated earlier, foaming during significant portions of October and into November likely influenced results. Average ESS values for the two steady state periods are reported in Table 7.

A paired ttest was conducted to determine if the differences in average ESS values were significant for the four BSRT reactors. A paired t-test between 20 and 2 day BSRT values revealed that the averages were statistically the same at a 99% confidence interval. Similar results were obtained when the 20 day BSRT data was analyzed with the 10 and 5 day BSRT average values respectively. Palm et al. (1980) also found that ESS values were similar despite varying BSRTs in a complete mix activated sludge system. The researchers operated reactors at BSRTs of 11, 9, 5.5, and 1.9 days and measured average ESS values of 20, 18, 23, and 13, respectively.

Table 7Average steady state ESS values during the two DO operational periods.

Reactor	Excess DO Condition		Low DO Condition		
	ESS, mg/L	Std. Deviation, mg/L	ESS, mg/L	Std. Deviation, mg/L	
$20 \text{ day } \theta_c$	19	13	15	16	
10 day θ_{c}	18	13	19	5	
5 day $\theta_{\rm c}$	19	11	20	19	
$2 \text{ day } \theta_c$	14	10	13	9	

The average excess and low DO effluent suspended solids values in Table 7 were also analyzed to determine whether DO had any impact on settling performance. A paired t-test for the 20 day BSRT data revealed that the averages were statistically the same for a 99% confidence interval. The same result was found for the 10, 5, and 2 day BSRT averages respectively when they were subjected to a paired t-test. Palm et al. (1980) produced similar findings when operating an 11 day BSRT reactor at DO concentrations ranging from 0.1-0.5 mg/L. Nowak et al. (1986) also found that low ESS values could occur in low DO environments. The study was conducted at the Gold Bar WWTP by maintaining a BSRT of 6 days in the aeration basin. It was found that ESS concentrations were maintained at less than 25 mg/L during the study even at a DO concentration of 0.8 mg/L.

4.4 Nitrification Performance Data

4.4.1 Reactor Ammonia Removal Performance

A narrower range of dates had to be chosen in order to evaluate nitrification treatment performance in the four reactors. Treatment performance related to the complete removal of ammonia from the waste stream. The excess DO date ranges for the 20-, 10-, 5-, and 2-day BSRT reactors remained the same. Since dissolved oxygen can become a limiting factor for nitrification at concentrations below 2.0 mg/L (Fillos et al., 1996), any data collected below that concentration could be considered low DO operation. For the 20-day BSRT reactor, the low DO date range was during 0.5 mg/L operation (November 2nd-November 29th). The low DO date range for the 10-day BSRT reactor was November 2nd to January 10th, which corresponded to 0.5-0.2 mg/L

operation. November 2nd to December 15th was used for the 5-day BSRT reactor because virtually no ammonia removal occurred once DO was dropped to 0.2 mg/L. The lack of ammonia removal at 0.5 mg/L in the 2-day BSRT reactor precluded the use of that data for analysis so the 1.0 mg/L (November 29th-December 27th) operational period was used. Upon completion of the low DO study on January 10th, the DO in all four reactors was increased to 3.0 mg/L to reestablish steady state treatment performance. Figure 12 shows the influent and reactor effluent ammonia concentrations during the course of this study.

It was readily apparent that complete ammonia removal occurred in the 20-day BSRT reactor at all DO concentrations ranging from 4.0-0.5 mg/L (June 6^{h} -November 29th). There are only a few days in which effluent ammonia was present. The first occurred on July 1st as a result of a spike in ammonia concentration from 17.4 to 25.1 mg/L, which temporarily affected ammonia removal in all reactors. The second incident (July 31st) was believed to be caused by the unknown surfactant passing through the system. Ammonia removal was once again affected in all reactors during this time. The 20-day BSRT reactor also discharged ammonia on August 29th, 30th, and 31st due to the improper tubing which resulted in a lowering of the solids concentration. Complete ammonia removal also occurred in the 10-day BSRT reactor throughout 4.0-0.5 mg/L DO operation. However, operation at 0.2 mg/L DO resulted in ammonia discharge from the 10 day BSRT reactor. Complete ammonia removal once again took place upon elevation of the DO back to 3.0 mg/L.



Figure 12 Ammonia removal as a function of DO concentration over the course of the treatability study.

Figure 12 shows that complete ammonia removal occurred in the 5-day BSRT reactor during the 4.0-1.0 mg/L operational periods (June 6th-November 2nd). However, ammonia was regularly discharged from the 5-day BSRT reactor during 0.5 mg/L operation. An average of 87% of influent ammonia was removed during this DO period. At 0.2 mg/L, ammonia removal was greatly impaired in the 5 day BSRT reactor. During the time period of January 6th to January 10th, an average of 4.3 mg/L of the influent ammonia was being removed in the 5-day BSRT reactor. Most of the ammonia removed was likely uptaken by bacteria for synthesis. In order to verify this assumption, the bacterial uptake (biomass-N) was computed assuming that the only cell loss was in the wastage volume and ESS. The following equation was used to calculate biomass-N:

$$Biomass - N = \frac{(MLVSS)*(NitrogenContent)*(WastageVol)}{FlowRate} + (ESS)*\left[\frac{MLVSS}{MLSS}\right]*(NitrogenContent)$$
(32)

In December, MLSS samples were taken to Eastman WWTP for analysis of nitrogen content. TKN and TSS analysis were then used to compute the %N present in each reactor. The samples were run in duplicate and the average nitrogen content for the 20-, 10-, 5-, and 2-day BSRT reactors were found to be 3.65%, 4.03%, 4.25%, and 4.63% respectively. Using Equation 32, an average bacterial nitrogen uptake of 2.8 mg/L was calculated in the 5 day BSRT reactor for the period of January 6th-January 10th. After January 10th, DO was restored to 3.0 mg/L and complete ammonia removal occurred in the 5 day BSRT reactor after three days.

Complete ammonia removal rarely occurred in the 2-day BSRT reactor during the entire course of the treatment study. A downward trend toward complete removal can be seen in Figure 12 near the end of the 4.0 mg/L DO period. Although it might appear that dropping the DO to 3.0 mg/L caused the disruption in ammonia removal, it has been concluded that the spike in influent ammonia concentration caused the high concentration of ammonia to be discharge in the effluent. Near complete ammonia removal occurred during the last two weeks of 3.0 mg/L operation with an average effluent ammonia concentration of 1 mg/L. From the time the DO was changed to 2.0 mg/L until the end of the study, near complete ammonia removal took place in the 2 day BSRT reactor. This inconsistency is very characteristic of such a low BSRT reactor (Grady et al., 1999). Although near complete ammonia removal occurred on August 26th-28th, this phenomenon was due to the thick walled tubing which allowed less influent to flow into the reactor. During the period from December 23rd to January 1st, almost complete ammonia removal took place in the 2-day BSRT reactor as well. This can be attributed to a malfunction in the DO control system. The probe became very dirty and read a low concentration almost constantly. Since the reading was below the DO set point, the control system fed air into the reactor almost constantly on those days allowing ammonia removal to be possible. Figure 12 clearly shows that once the aeration problem was solved on Jan 1st, ammonia removal ceased at 0.5 mg/L DO concentration. After failing to remove any appreciable amount of ammonia from January 2nd to January 10th, DO in the 2-day BSRT reactor was increased to 3.0 mg/L. Nearly two weeks were required for ammonia removal to occur at a level comparable to the previous 3.0 mg/L period.

Although BSRT is an important consideration when incorporating nitrification into an activated sludge system, BSRTs of 8-20 days have been found to be sufficient for effective removal of ammonia nitrogen (Tchobanoglous and Burton, 1991; Randall et al., 1992). Therefore, it was not surprising that complete ammonia removal occurred in the 10 and 20 day BSRT reactors at excess DO. On the other hand, ammonia oxidation might not have been expected to take place in the 5 and 2 day BSRT reactor because these low BSRTs can approach the minimum solids retention time for ammonia removal to occur (Benefield and Randall, 1985).

It has been found in several investigations that at least partial ammonia removal can occur at low BSRTs. Randall et al. (1992) investigated the impact of BSRT on nitrification in activated sludge by operating reactors at BSRTs of 1.5, 2.7, 5, and 15 days. Complete ammonia removal was observed at a BSRT as low as 2.7 days while 79% ammonia removal occurred at a 1.5 day BSRT. Hanaki et al. (1990) also found that nitrification could be achieved at low BSRTs when conducting a study on the effects of DO on nitrification in a completely mixed activated sludge system. For the excess DO portion of the experiment, reactors were operated at BSRTs of 6.5, 5, 3.8, and 2 days for a synthetic influent feed containing 80 mg/L of ammonia. Complete nitrification was observed for all BSRTs greater than 3.8 days while approximately 50% of the influent ammonia was converted to nitrate in the 2 day BSRT reactor. Dincer and Kargi (2000) also found that ammonia removal occurred at low BSRTs. The researchers operated reactors at 20, 17, 15, 10, 8, 5, and 3 day BSRTs. Although more effective ammonia

removal took place at longer BSRTs, it was discovered that almost 60% of the 100 mg/L influent ammonia could be removed at a 3 day BSRT.

Dissolved oxygen concentration can also have a major impact on ammonia removal in activated sludge. Since a DO concentration at or above 2 mg/L has been established as the minimum necessary to prevent inhibition (Benefield and Randall, 1985, Tchobanoglous and Burton, 1991), it was somewhat unanticipated that effective ammonia removal would occur at DO levels as low as 0.5 mg/L. However, previous studies indicate that low DO ammonia removal is possible even at relatively short BSRTs. Hanaki et al. (1990) conducted research to determine the effects of DO on nitrification and discovered that only a 3.8 day BSRT was required to efficiently remove approximately 80 mg/L of influent ammonia at a DO of 0.5 mg/L. Similar results were obtained by Chuang et al. (1997) when operating reactors at BSRTs of 5, 10, and 15 days. Those researchers discovered that ammonia removal occurred to some degree for all BSRTs at a DO of 0.5 mg/L. A 15 day BSRT was also found to be sufficient to remove ammonia at a DO of approximately 0.1 mg/L. Jayamohan et al. (1988) conducted a study on the effects of DO on ammonia removal by operating a 1.5 day BSRT reactor at DO concentrations of 8.8, 1.3 and 0.76 mg/L. The researchers discovered that near complete ammonia removal took place at even at 0.76 mg/L DO.

4.4.2 Nitrogen Mass Balances

Figure 13 shows a mass balance for the 20-day BSRT reactor using influent and effluent ammonia, nitrate, nitrite, and nitrogen lost as cell mass (biomass-N). Although the absence of TKN precludes this from being a true mass balance, the main



Figure 13 A nitrogen balance used to establish the occurrence of complete nitrification in the 20 day BSRT reactor.

purpose of this analysis was to verify the conversion of ammonia to nitrate and determine the extent to which ammonification was occurring. The %N values obtained from Eastman were assumed to be representative of each reactor and were used to compute the biomass-N component of each day's nitrogen balance for the course of the treatment study.

The presence of an effluent nitrate concentration comparable to the influent ammonia (on a nitrogen basis) and the lack of effluent nitrite for DO concentrations ranging from 4.0-0.5 mg/L indicates that complete nitrification consistently took place in the 20-day BSRT reactor. There were several days when the effluent nitrate was significantly higher than the influent ammonia, which suggests the occurrence of organic nitrogen conversion to ammonia.

Upon inspection of Figure 14, it can be seen that treatment performance in the 10day BSRT reactor was comparable to the 20-day BSRT reactor during the 4.0-0.5 mg/L operational periods. The presence of a sufficient effluent nitrate concentration indicates that complete nitrification occurred virtually every day. One notable exception was July 31st when ammonia and nitrite were found in the effluent due to the surfactant. The 10day BSRT reactor also discharged an average of 0.6 mg/L of nitrite during the kst few days of 0.5 mg/L operation. Upon dropping the DO to 0.2 mg/L, treatment performance was impacted in the 10 day BSRT reactor and complete nitrification did not occur. Figure 14 shows that high concentrations of ammonia were regularly discharged in the effluent during this time. The presence of nitrate indicates that nitrification was still taking place to some degree. Treatment performance rebounded quickly once the DO



Figure 14 A nitrogen balance for the 10 day BSRT reactor indicating the occurrence of complete nitrification.

was restored to 3.0 mg/L and complete nitrification once again occurred in the 10-day BSRT reactor after two days.

The nitrogen balance for the 5-day BSRT reactor is shown in Figure 15. Although effluent nitrite averaged 0.3 mg/L during the 4.0-1.0 mg/L periods of operation, near complete nitrification did occur because the effluent nitrate levels were comparable to the influent ammonia. A loss of treatment performance took place in the 5-day BSRT reactor once the DO was dropped to 0.5 mg/L. This DO concentration impacted ammonia and nitrite oxidation. Although ammonia oxidation is supposed to be the rate-limiting step for nitrification, Figure 13 indicates that a build up of nitrite in the effluent occurred during 0.5 mg/L operation. It was discovered by Laanbroek et al. (1994) that ammonia oxidizers had a higher affinity for oxygen (i.e. a lower half-saturation constant) than nitrite oxidizers. The nitrite oxidizers apparently had difficulty competing for the available oxygen. A downward trend in effluent nitrite suggests that the nitrite oxidizers began to acclimate to the low DO conditions.

Nitrification was severely inhibited once the DO in the 5 day BSRT reactor was lowered to 0.2 mg/L. No more than 50% of the influent ammonia was ever removed during this period and an average of only 4.3 mg/L was removed during the January 6th-10th portion of the study. The average effluent nitrate and nitrite concentrations on those five days were 0.8 and 0.4 mg/L respectively. Upon raising the DO back to 3.0 mg/L, complete nitrification occurred in the 5 day BSRT reactor after only a few days. At this



Figure 15 A balance of influent ammonia, effluent ammonia, effluent nitrite, effluent nitrate, and reactor biomass nitrogen used to confirm the occurrence of complete nitrification in the 5 day BSRT reactor.

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point, complete ammonia removal was taking place and effluent nitrite levels decreased to levels comparable to the original 3.0 mg/L period of operation.

The mass balance for the 2-day BSRT reactor, shown in Figure 16, is much different than the mass balances for the other reactors. Even at excess DO conditions, complete nitrification rarely occurred in the 2 day BSRT reactor. Significant ammonia and nitrite concentrations averaging 6 and 1.2 mg/L respectively were regularly found in the effluent. Because of the unstable nature of nitrification at such a short BSRT, quantification of DO impact was difficult. Near complete nitrification (95%) occurred in the 2-day BSRT reactor for a few days during each of the 4.0, 3.0, and 2.0 mg/L operational periods. After operating at 2.0 mg/L DO from July 28th-October 27th, it was concluded that complete nitrification was not possible for the 2-day BSRT reactor at this concentration. Complete nitrification never occurred again in the 2-day BSRT reactor for the remainder of the treatment study. During the 1.5 and 1.0 mg/L periods of operation, the 2-day BSRT reactor averaged 44% and 50% ammonia removal, respectively. Although the waste was changing every few days, the average influent ammonia during both periods was between 20-21 mg/L. Effluent nitrate and nitrite concentrations were also virtually the same for the two operational periods. The similarity of these numbers suggests that DO concentrations of 1.5 and 1.0 mg/L had the same impact on nitrification. Operation of the 2-day BSRT reactor at 0.5 mg/L completely inhibited nitrification once the aeration problem was fixed. Data from January 2nd-10th clearly shows that ammonia removal with the exception of bacterial uptake had ceased. For



Figure 16 A balance of the 2 day BSRT nitrogen data used to determine the occurrence of nitrification during the course of the treatment study.
these dates only traces amounts of nitrate and nitrite averaging 0.4 and 0.4 mg/L respectively could be found in the effluent. Once nitrification had almost ceased, the DO in the 2-day BSRT reactor was restored to 3.0 mg/L. The 2-day BSRT reactor required a longer period of adjustment than the other reactors but a treatment level comparable to previous excess DO conditions did occur after approximately two weeks.

The impact of BSRT on ammonia removal was discussed in the previous section. Figure 12 clearly showed that complete ammonia removal occurred in the 20, 10 and 5 day BSRT reactors for excess DO conditions. Since the first step of nitrification, conversion of ammonia to nitrite, is typically considered to be limiting (Benefield and Randall, 1985), it was not surprising that complete nitrification took place in the 20, 10 and 5 day BSRT reactors at excess DO. The difficulty in achieving complete nitrification at a BSRT as low as 2 days has been well documented (Grady et al., 1999; Benefield and Randall, 1985; Randall et al., 1992). Therefore, the remainder of the analysis will focus on the impact of DO on nitrification.

It has been argued that DO can be the limiting factor for nitrification at concentrations below 2.0 mg/L. However, previous work has indicated that nitrification can occur at DO levels at or below 1 mg/L (Fillos et al., 1996). Chuang et al. (1997) conducted a study on the impacts of DO on nutrient removal using BSRTs of 5, 10, and 15 days. They found that complete nitrification took place at 0.5 mg/L DO for a 15 day BSRT, which is comparable to the 20 and 10 day BSRTs used in this study. The study also revealed that partial nitrification took place at that DO concentration for a BSRT of 5 days. Hanaki et al. (1990) also found that nitrification was possible at a DO

concentration of 0.5 mg/L for BSRTs ranging from 3.8 to 6.5 days. However, the researchers also discovered that the conversion of nitrite to nitrate became the limiting step at low DO. This phenomenon caused a build up of nitrite in the effluent similar to the one seen in the 5 day BSRT reactor. Jayahoman et al. (1988) obtained the same result when operating a CSTR at a 1.5 day BSRT and 0.76 mg/L DO. Nitrate levels in the effluent were very low and an effluent nitrite concentration close to influent ammonia level was observed. Balmelle et al. (1992) have also found that insufficient DO levels can result in a build up of nitrite in the effluent. It was discovered that the rate of nitrite conversion decreased by approximately 50% when the DO was dropped from 4.0 to 0.5 mg/L. These findings indicate that a sufficient mass of nitrite oxidizers must be present for effective conversion. As the DO is decreased, an increased biomass is needed in order to compensate for the decrease in nitrite conversion rate. Therefore, longer BSRTs would be required for effective nitrification to occur. This would explain why nitrite buildup occurred in the 5 day BSRT reactor but did not take place in the longer BSRTs.

4.4.3 Alkalinity

Influent and effluent alkalinity values are presented in Figure 17. These measurements were taken to ensure that enough buffering capacity existed to avoid major pH depression. Reactor pH must remain in the range of 7.0-8.5 to keep from affecting nitrification (Tchobanoglous & Burton, 1991). Figure 17 shows that the 2-day BSRT reactor regularly discharged a higher concentration of alkalinity than the other reactors. This finding reinforces the fact that complete nitrification did not regularly take place in



Figure 17 Fluctuations in effluent alkalinity as a result of decreasing DO concentration during the study.

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the 2-day BSRT reactor during the course of the study. Table 8 shows the average alkalinities for the influent and reactor effluents. The alkalinity measurements, along with effluent nitrate and nitrite concentrations, were also used to verify the stoichiometric ratio of 7.1 mg alkalinity (as CaCO₃) consumed per mg of ammonia converted to nitrate. The difference in influent and effluent alkalinity divided by the sum of effluent nitrate and nitrite was used to compute the ratio. It can be seen in Figure 18 that the ratio was upheld for the duration of the treatment study in the 20- and 10-day BSRT reactors. This finding supports the assumption that no significant amount of simultaneous nitrification/denitrification was occurring since that would decrease the alkalinity consumption ratio (Fillos et al., 1996).

A t-test analysis was conducted on excess and low DO data for the influent alkalinity and the 20, 10, 5 and 2 day BSRT reactors to determine any statistical differences. It was found that the influent averages were statistically the same for a 99% confidence interval. Conversely, the averages for the 20, 10, 5, and 2 day BSRT were not the same statistically when their excess and low DO averages were compared. This result was not surprising since alkalinity consumption is a function of nitrification and

Reactor	Excess	DO Condition	Low DO Condition		
	Average	Std. Deviation	Average	Std. Deviation	
Influent	230	14	239	21	
20 day θ_{c}	105	14	118	25	
10 day θ_{c}	104	14	136	32	
5 day $\theta_{\rm c}$	106	14	146	46	
$2 \text{ day } \theta_{c}$	137	13	174	37	

Table 8Average alkalinity (mg/L as CaCO3) during excess and low DO operation.



Figure 18 Change in alkalinity consumption ratio due to the inconsistency of nitrification at low DO.

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nitrification was found to be affected by low DO concentrations. The 5 and 2 day BSRT reactors have the highest low DO effluent alkalinity averages because they were impacted the most by decreases in DO concentration.

The 5-day BSRT reactor maintained the ratio until it began to fail with respect to nitrification at 0.2 mg/L. At this point, only trace amounts of nitrate and nitrite were found in the effluent. The 2-day reactor showed similar results during the 0.5 mg/L operational period. Once the DO was raised to 3.0 mg/L, nitrification resumed within a few days in the 5-day BSRT reactor and the ratio returned to normal. The 2-day reactor took longer to respond but the ratio eventually returned to stoichiometric levels after approximately two weeks.

4.5 Kinetic Analysis of Nitrification Data

4.5.1 Estimating Y and K_d

The yield and decay coefficient for the overall nitrifier population was determined by plotting the substrate utilization rate versus the inverse of BSRTs growth rates. The plot can be seen in Figure 19. The specific substrate utilization rate for each BSRT was determined using the following equation:

$$q_{nit} = \frac{dS/dt}{(X_a)_{nit}}$$
(33)

where:

$$\begin{split} dS &= \text{change in ammonia concentration (mg/L as N)} \\ dt &= \text{hydraulic retention time (0.366 day)} \\ (X_a)_{nit} &= \text{active nitrifying biomass (8% of X_T)} \\ q_{nit} &= \text{specific substrate utilization rate (day}^1) \end{split}$$



Figure 19 Steady state plot of nitrification substrate utilization rate versus specific growth rate that was used to determine the autotrophic yield and decay rate for excess DO conditions.

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Confidence intervals (99%) are shown for the mean specific utilization rates. The yield coefficient was determined to be 0.33 mg VSS/mg N, with a 99% confidence interval ranging from 0.31-0.35 mg VSS/mg N. Although it is higher than some values in the literature (Table 1), the yield is still within the overall range. The yield is particularly similar to the value of 0.34 mg VSS/mg N calculated by Dincer and Kargi (2000) using kinetic data. Hawkins (2000) obtained a yield coefficient value of 0.26 mg VSS/mg N while using the same reactor setup and treating the same waste stream as this study. The decay coefficient was then determined by multiplying the value obtained for Y and the y-intercept of the best-fit line. The decay coefficient was estimated to be 0.17 day⁻¹ with a 99% confidence interval ranging from 0.15-0.18 day⁻¹. This value compares favorably with the ones obtained in the literature (Table 1) and is identical to the value of 0.17 day⁻¹ obtained by Hawkins (2000).

4.5.2 Estimating K_s, **m**_{hax}, and k for the overall nitrifiers

The half saturation coefficient and maximum growth rate were calculated using Equation 24 to plot the inverse of the effluent substrate concentration against values of $(1/(\theta_c+K_d))$. The decay coefficient determined in previous calculations (0.17 day^{-1}) was used in this analysis. The data provided a linear regression with an R² value 0.98 as seen in Figure 20. The linear regression analysis provided a half saturation coefficient value of 0.25 mg/L NH₄⁺ with a 99% confidence interval ranging from 0.24-0.27 mg/L NH₄⁺. Although this value is quite small, it is still within the range reported in the literature (Table 1). Hawkins (2000) obtained a value of 0.2 mg/L for K_s, which is very similar to the value calculated in this study. The nitrifier maximum specific growth rate was found



Figure 20 Steady state plot of nitrification data used to determine the maximum specific growth rate and half saturation constant for excess DO conditions.

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to be 0.75 day⁻¹ with a confidence interval of 0.68-0.82 day⁻¹. Dividing the maximum growth rate by the yield (0.33 mg VSS/mg N) provided a maximum substrate utilization rate of 2.2 mg N/mg VSS-day. The confidence interval range was 2.1 to 2.3 mg N/mg VSS-day. Both μ_{max} and k compare favorably with values reported in the literature (Table 1). Once again these values were very similar to the values of 0.6 day⁻¹ and 2.3 mg N/mg VSS-day estimated by Hawkins (2000) for μ_{max} and k.

4.5.3 Estimating K₀ for the nitrifiers

One focus of this study was to calculate the oxygen half saturation coefficient for nitrifiers. The method outlined by Hanaki et al. (1990) was used for determination of K_0 . A kinetic analysis similar to the one performed in Sections 4.5.1 and 4.5.2 was conducted on data obtained at 1.0 mg/L DO. Since a minimum of four BSRTs is typically used for determining kinetic coefficients (Grady et al. (1999), the analysis had to be conducted at 1.0 mg/L because it was the lowest DO level at which significant substrate utilization occurred in all BSRT reactors.

The yield coefficient was calculated by plotting specific substrate utilization versus inverse BSRT. The data provided a linear regression with an R² value 0.95 as seen in Figure 21. The linear regression analysis provided a yield coefficient value of 0.66 mg VSS/mg N with a 99% confidence interval ranging from 0.49-0.83 mg VSS/mg N. The calculated yield was much higher than the value determined at excess DO. Hanaki et al. (1990) also found that the yield coefficient increased in the presence of low DO conditions. Although the yield appeared to increase, comparison of the yield at excess and low DO conditions did not take into account population shifts which would



Figure 21 Steady state plot of nitrification substrate utilization rate versus specific growth rate that was used to determine the autotrophic yield for low DO conditions.

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affect the concentration of nitrifiers in the system. For this study, the assumption was made that the nitrifier population was a constant fraction of the MLVSS concentration. It should be noted that there was no means of evaluating this assumption so care should be taken when considering these results. New techniques, such as genetic probes, are being developed to better quantify the amount of nitrifiers present in activated sludge systems. Rittman et al. (1999) have used 16s RNA probes to determine the percentage of ammonia oxidizers relative to the overall microbial population. These tools will provide a more accurate means of determining a value for kinetic coefficients such as the yield.

The maximum specific growth rate was calculated by plotting the inverse of the effluent substrate concentration against values of $(1/(\theta_c+K_d))$. Figure 22 contains a plot of the average data fit with a simple linear regression. The poor linear fit produced by this technique has been problematic in past bench scale studies due to low effluent substrate concentrations (Grady et al., 1999). A similar fit was produced by Hawkins (2000) when estimating a value for μ_{max} . The nitrifier maximum specific growth rate was found to be 0.75 day⁻¹ with a 99% confidence interval of 0.64 to 0.86 day⁻¹. The maximum specific growth rate did not vary from the value obtained at excess DO. Hanaki et al. (1990) also found that μ_{max} for nitrifiers did not significantly vary when DO conditions were altered. Using the Y and μ_{max} values, a maximum specific substrate utilization rate of 1.14 mg N/mg VSS-day with a 99% confidence interval of 0.91 to 1.51 mg N/mg VSS-day was obtained.



Figure 22 Steady state plot of nitrification data used to determine the maximum specific growth rate for low DO conditions.

A value of K_0 was then calculated using Equation 26 by substituting 2.2 and 1.14 mg N/mg VSS-day for the excess and low DO maximum substrate utilization rates respectively. A value of 1.0 mg/L was used for the DO term to yield a K_0 of 0.92 mg/L with a 99% confidence interval of 0.46 to 1.3 mg/L. Although this value varies from the one estimated by Hanaki et al. (1990), it is well within the range reported in the literature (Table 1). The difference in values between this study and the study by Hanaki et al. (1990) has been attributed to the fact that this study used a combined carbon/nitrification system for estimation while a pure nitrification system was employed in the previous work.

Chapter 5.0

Conclusions

5.1 Evaluation of Carbon Treatment Performance

One part of the first objective of this treatability study was to assess the impact of BSRT and DO on carbon removal efficiency. Statistical analysis indicated that treatment performance was a function of BSRT since the average effluent COD in the 20 day BSRT reactor was significantly lower than the averages for the 5 and 2 day BSRT reactors. Because treatment performance in the 10 and 20 day BSRT reactors did not differ statistically, it appeared that COD removal was not enhanced by BSRTs longer than 10 days.

Conversely, DO had no impact on carbon treatment performance. Statistical analysis showed no significant variance in the average effluent COD values in the excess and low DO periods of any BSRT. This occurrence has been attributed to the low K_O value associated with heterotrophic bacteria utilizing an easily degradable substrate. Based on the data collected in this study, optimum COD removal took place at a DO of only 0.2 mg/L when the BSRT is maintained at 10 days or longer.

5.2 Evaluation of Sludge Settling Performance

Another part of the first objective was to carry out bench scale treatment experiments to examine the effect of low DO (0.2-0.5 mg/L) and BSRT on activated sludge settling. Statistical analysis of SVI values showed that BSRT had no effect on

settling performance. With the exception of a short period during the 2.0 mg/L operational period, low SVI values were found for all BSRTs during the course of the study. DO concentration had no impact on sludge settling even when the 10-day BSRT reactor was operated at 0.2 mg/L.

The consistency of ESS values during the treatability study is further evidence that sludge settling was not impacted by DO concentration. Each reactor regularly discharged an ESS concentration lower than the 45 mg/L discharge limit imposed on the Kuwahee WWTP. However, all four reactors violated the discharge limit on several occasions indicating that the bench-scale system was not as effective as a full-scale plant. The incidents of high ESS were caused by operational problems. The first was the installation of improper tubing. The second occurrence was the result of foaming in the reactors. The foam was likely caused by the injection of air provided by the air blast units and not low DO since foaming ceased once the units were turned off.

5.3 Evaluation of Nitrification Performance

The final part of the first objective was to determine the effects of DO and BSRT on nitrification. Based on the data collected in this study, complete nitrification can occur in systems with a BSRT of 10 days or longer at DO levels as low as 0.5 mg/L. However, as evidenced by the presence of the surfactant (July 31st), treatment performance is based on the composition of the influent. The build-up of nitrite in the 5-day BSRT reactor at 0.5 mg/L DO suggests that the nitrite oxidizers can become the rate-limiting factor for nitrification at low DO. However, the lack of nitrite build-up in the 20 and 10 day BSRTs at 0.5 mg/L DO also indicates that effective nitrite oxidation can

occur if a sufficient amount of biomass is present. The 2-day BSRT was insufficient to consistently achieve complete nitrification at any DO level. Therefore, a minimum BSRT of 5 days should be maintained to establish nitrification at excess DO but a 10 day or greater BSRT is necessary for effective nitrification at low DO (≤ 1.0 mg/L).

The second objective of this study was to calculate nitrifier kinetic coefficients to make it possible to design an activated sludge system treating a similar waste stream. The estimated overall kinetic coefficients are within a range published in the literature for municipal wastewaters (Table 9) and very similar to those obtained by Hawkins (2000) while treating the same waste stream. These facts indicate that the values obtained from the treatability study are reliable. Therefore, it is believed that these values could be used in the design of an activated sludge treatment facility with a similar influent waste stream. Since the stoichiometric ratio of alkalinity consumed per ammonia converted to nitrate was upheld during the course of the study, it appears that denitrification did not

Table 9Comparison of nitrification coefficients obtained during the treatability
study and coefficients taken from the literature.

Activated Sludge Kinetic	Current Study [99%	Range Reported in the	
Constant	Confidence Interval]	Literature	
K _d	$0.17 \ [0.15 - 0/18] \ day^{-1}$	$0.03-0.17 \text{ day}^{-1}$	
K_{SN}	0.25 [0.24-0.27] mg/L	0.2-5.0 mg/L [NH4 ⁺ -N]	
	$[NH_4^+-N]$	-	
Ko	0.92 [0.46-1.3] mg/L O ₂ *	0.32-2.0 mg/L O ₂	
Y	0.33 [0.31-0.35] mg	0.1-0.36 mg VSS/mg N	
	VSS/mg N		
k	2.2 [2.1-2.3] mg N/mg	0.076-4.3 mg N/mg VSS-	
	VSS-day	day	
μ_{max}	$0.75 \ [0.68-0.82] \ day^{-1}$	0.3-3.0 day ⁻¹	
k µ _{max}	2.2 [2.1-2.3] mg N/mg VSS-day 0.75 [0.68-0.82] day ⁻¹	0.076-4.3 mg N/mg VSS- day 0.3-3.0 day ⁻¹	

occur to any significant degree in any of the reactors. Analysis of the mass balances indicates that the nitrifiers used primarily the influent ammonia as a substrate. Conversion of organic-N to ammonia was negligible except for a few days when the effluent nitrogen was well above the influent ammonia-N.

Based on the data, it appears that a 10 day BSRT and a DO concentration of 0.5 mg/L are necessary for effective COD treatment, sludge settling, and nitrification However, WWTPs cannot afford to violate their permits so a factor of safety should be introduced to reduce the risk of operational failure. For this reason, WWTPs typically operate their aeration basins at excess DO (≥ 2 mg/L) conditions when nitrification is part of the treatment process. It is recommended that a treatability study be conducted for a particular waste if lower levels of DO are to be employed in operation.

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Appendices

Appendix A. Calculation Showing Complete Mixing

The stir plates were typically set to approximately one-half their maximum setting of 1000rpm, which corresponds to a power output of 15 watts.

$$P_{Vol} = \frac{P}{V} = \frac{0.015 \, kW}{0.01 \, m^3} = 1.5 \, kW / m^3 = 1500 \, kW / 1000 \, m^3$$

Appendix B. Average DO Concentrations

Reactor	4.0	3.0	2.0	1.5	1.0 mg/L	0.5 mg/L	0.2 mg/L
BSRT	mg/L	mg/L	mg/L	mg/L			
20	N/A	3.04	1.96	1.53	0.99	0.52	N/A
10	N/A	2.97	1.98	1.51	1.00	0.51	0.19*
5	N/A	2.96	1.95	1.52	0.99	0.53	0.23*
2	N/A	3.02	1.97	1.51	0.99*	0.57*	N/A

Table B.1Average DO concentrations during the treatability study.

*Values obtained by DO data logging.

Vita

Jack Parker was born in Smyrna, Tennessee on January 8, 1976. He attended the public system of schools in Rutherford County, where he graduated from Smyrna High School in May 1994. He entered The University of Tennessee-Knoxville in August 1994, receiving a Bachelor of Science in Civil Engineering in May 1999. Mr. Parker entered the Master's program in Environmental Engineering as a full time student in August of 1999. Working as a graduate research assistant, he officially received his Master of Science Degree in Environmental Engineering in December 2001.