

**High-Intensity Shear as a Wet Sludge Disintegration Technology and a Mechanism  
for Floc Structure Analysis**

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# **High-Intensity Shear as a Wet Sludge Disintegration Technology and a Mechanism for Floc Structure Analysis**

by

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Environmental Engineering

## **(ABSTRACT)**

By shearing activated sludge using a high shear rotor stator device, bioavailable proteinaceous material can be produced. Operation at elevated temperatures serves to increase the amount of material that is rendered soluble ( $<0.45 \mu\text{m}$ ) and biodegradable. The storage of sludge under anaerobic conditions prior to shearing does not appear to enhance solubilization of solids, though deflocculation and deterioration of dewaterability was observed. Anaerobic digestibility appears to be enhanced by the addition of a high shear to material from an anaerobic digester as shown by increases in gas production and volatile solids destruction. The dewatering properties of activated sludge, measured by capillary suction time, deteriorated with the addition of sheared solids, but reaeration resulted in near complete recovery.

The role of iron and iron chemistry are thought to play an important role in the formation and properties of activated sludge flocs. Iron apparently selectively removes protein, in particular, material ranging in the  $1.5 \mu\text{m}$  to  $30\text{K}$  size range. It is hypothesized that the tri-valent metals, iron and aluminum, are locally concentrated within the floc structure affording the microorganisms protection from mechanical stress. Preliminary results suggest that the ferric/ferrous redox chemistry may serve to enhance floc structure. Increases in settling velocity and shear resistance were observed in sludges milled with a KADY mill and dosed with ferrous sulfate, while the addition of ferric iron decreased settling velocity and increased shear sensitivity. The data suggest that ferrous iron combines with solution colloids by a sequence of reactions to produce a more dense and tightly bound floc.

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## **Chapter 1: Literature Review**

### **A Review of Wet Sludge Disintegration Technology and Biosolids Reduction Research**

The EPA estimated that by the year 2000, the United States would produce, 12 million dry metric ton/year of wastewater sludge.(EPA 1987) The disposal of this quantity of biosolids can be expensive depending on which of the EPA accepted methods is used, land application, distribution and marketing, land filling, incineration or ocean disposal. Wet sludge disintegration is implemented to serve several goals, including waste solids reduction and generation of a carbon source for biological nutrient removal systems(Weemaaes et al. 1998). Mineralization is the mechanism for the reduction of solids that many systems try to take advantage of whether the process is done aerobically or anaerobically. Another benefit of disintegration of sludges is reduced digester foaming by the disruption of filamentous bacteria.(Müller 2000)

Many companies and researchers have invested time and money, developing and improving a multitude of different technologies. A recent review by Weemaaes et al. (1998), identified 5 general classes of wet sludge disintegration technologies, which includes mechanical, thermal hydrolysis, chemical and thermochemical hydrolysis, biological hydrolysis, and oxidative destruction. Within each of these classes is a myriad of technologies and approaches to render once stabilized material bioavailable. The following sections are a brief review of some of the technologies that are present in the market place today or are under development, classified per Weemaaes.

#### *Mechanical/Sonication*

Mechanical processes include devices such as sonicators, high pressure homogenizers, ultrasonic homogenizers, mechanical jet smash technique, high performance pulse technique, Lysat-Centrifugal-Technique (Müller 2000) and high shear devices such as the

KADY Mill. Means of disintegration for these systems are, impingement, shear and or cavitation. Mechanical disintegration devices have been reported to be enhanced at temperatures between 60°C and 120°C.(Müller 2000)

Springer et al (1996), investigated the use of the KADY Mill in a lyse-recycle system for solids reduction. It was demonstrated that the KADY Mill was capable of solublizing 61%+/-22% material processed. Reported COD reductions were 80% for the lyse-recycle while 87% for the control, little difference was noted in effluent TSS however elevated effluent soluble chemical oxygen demand (SCOD) was observed at 61 mg/L and 33 mg/L repectively. What the data appears to suggest is that there is a release of non-degradable material that travels out in the effluent stream, leading the author to suggest lyse-recycle for facilities well within EPA regulation.

The use of sonication has garnered more research than shear devices like the KADY Mill. Most applications of sonication are based on the pretreatment of WAS for enhance anaerobic digestion. Generally, volatile solids destruction, gas production, pathogen removal and volatile fatty acids are measures of enhanced digestion.

The effectiveness of sonication as a disintegration process prior to anaerobic digestion has had mixed levels of success. Quarmby et al. (1999) found that the addition of sonication on average only had moderate effects on VS destruction, average SCOD and methane production. It was noted however that storage of sludge prior to digestion in pilot scale systems might have had an impact on the perceived performance.

Tiehm et al. (2001), reported enhanced biogas production and 33.7% VS reduction at 41 kHz for 150 minutes as compared to the control at 21.5 %. Neis et al. (2000), found similar results to Tiehm et al.(2001), and reported successful operation of anaerobic fermentors a SRT = 4 days, suggesting that sonication can accelerate the stabilization process.



Disintegration of wastewater sludges is possible, and the process performance has been enhanced both aerobically and anaerobically. Beyond the consideration of the solubilization mechanism are the economic costs of running such systems, electrical power and servicing of the units can consume any savings realized from reduced solids handling.

### *Oxidation-Ozonation*

Ozone is an extremely strong oxidant that has a history in drinking water disinfection and the disinfection of recirculated water in aquaculture systems. The oxidizing power of ozone is used to render refractory compounds bioavailable.

Sakia et al. (1997) demonstrated that an activate sludge system could be operated with zero solids wastage utilizing ozonation, fed at 0.034 g-O<sub>3</sub>/g-feed-sludge to 510 g-SS/m<sup>3</sup>-sewage, as a side stream process in the return sludge line. Ozonation has also been applied to enhance anaerobic digestion (Weemmaes et al. 2000).

Scheminski et al. (2000), reported a 42% increase in biodegradation with an ozone dose of 0.2 g-O<sub>3</sub>/g-dry organic matter. Concurrent, with the enhanced degradation by partial oxidation, significant increases in polymer demand were noted for ozonated sludges but some recovery was noted with digestion.

### *Thermal Processes*

The addition of heat energy to enhance biodegradation has been used in many traditional wastewater processes. However, for wet sludge distintegration processes temperatures usually far exceed the upper limit of thermophilic processes.

Li and Noike (1992) investigated thermal pretreatment anaerobic digestion (TP/AD) as a viable technology for the enhancement of anaerobic digestion. TP/AD was found to an

optimal operating temperature of 170 °C, demonstrating 59% decrease in COD while approximately doubling methane production.

Cambis thermal hydrolysis is a full scale thermal disintegration process operating at HAIS WWTP in Hamar, Norway. Kepp et al (2000), demonstrated over the three year study that compared to conventional anaerobic digestion the thermal hydrolysis coupled digester, requires 50% less volume, increased solids reduction by 23%, and that the high temperature pretreatment produced a disinfected sludge that is stable even during storage allowing for agricultural application. Thermal processes as with many other disintegration technologies are invariably coupled to some biological process.

#### *Biological Processes and Manipulations*

Biological wet sludge disintegration technologies are as varied as all the other technologies in the application of different mechanisms for reduction. Rensink and Rulkens (1997) investigated the use of metazoa for the reduction of solids in trickling filter, essentially utilizing higher trophic organism to degrade materials that are refractory to microorganisms.

Abbassi et al. (2000) found that manipulation of oxygen concentrations in an activated sludge basin is a viable mechanism for the reduction of solids. By increasing the diffusion of oxygen into the floc matrix greater solids reduction was realized.

Sakia et al. (2000), demonstrated that the combination of thermophilic aerobic digestion and conventional activated sludge, essentially using the digester as a side stream for protein degradation and varying recycle rates, a zero sludge production process could be achieved.

### *Summary*

As demonstrated the field of solids reduction through sludge disintegration whether mechanical, thermal or biological is diverse. The common theme that appears throughout the literature is technologies that can be retrofit are preferred and being developed verses those that are entirely new treatment facilities, excluding Sakia et al (2000). Underlying the development of wet sludge disintegration technology is a basic understanding of the sludge floc. Though not initially presented an understanding of the components of the sludge floc and its structure is critical to understanding the performance or lack there of, of these technologies, therefore the following section is devoted to that purpose.

### **Activated Sludge Floc Structure and Implications for Shear Devices**

Many technologies specifically focus on the disintegration of waste activated sludge (Li et al. 1992; Springer et al. 1996; Sakia et al. 1997; Nah 2000; Tiehm et al. 2001). Therefore understanding of the composition of activated sludge flocs, structure, and potential sources of bioavailable is critical to understanding why certain results are observed in these processes.

Activated sludge flocs have been shown to have discrete structural components, the smallest being the microorganisms themselves, which form microcolonies the agglomeration of which form the activated sludge floc.(Snidaro et al. 1997, Jorand et al. 1995) Holding the floc structure together is complex matrix, of filamentous microorganisms, pores of water of various size, extracellular polymeric substances (EPS),(Li and Ganczarzyk. 1990) and particulate matter.

### *Microorganisms and Cellular Lysis*

Microorganisms and their subsequent lysis during sludge disintegration represent a rich source of readily biodegradable materials, including cytoplasm and nucleic acids. Lysis products as sources of biodegradable materials are considered the main reservoir of

biodegradable substrate driving wet sludge disintegration technologies. Several indicators of cellular lysis have been utilized including DNA and glucose-6-phosphate dehydrogenase activity (Frølund et al. 1995). However, care must be taken when using DNA because it has been shown to accumulate extracellularly in the floc matrix (Palmgren and Nielsen 1996).

#### *Extracellular Polymeric Substances (EPS), Biopolymer, Soluble Microbial Products (SMPs)*

The EPS present in activated sludge flocs has three basic sources, cellular lysis, biosorption from the waste stream and excretion (Boero et al. 1996; Jorand et al. 1998). Biopolymer of microbial origin that is of MW > 10,000 has been reported as refractory, having a BOD rate constant of  $0.04 \text{ d}^{-1}$ , in secondary effluents. (Hejzlar and Chudoba 1986) Boero et al. (1996) stated that some soluble microbial products are biodegradable in the time frame of many experiments done by researchers.

Biopolymer has been found to contain exoenzymes (Frølund et al. 1995), proteins, polysaccharides, humics (Frølund et al. 1996), DNA (Palmgren and Nielsen 1996) and uronic acids (Bura et al. 1998) of different ratios (Frølund et al. 1994). Irregardless of the initial bioavailability of this material, an effective disintegration device will disperse this material and render at least a fraction of it readily or slowly biodegradable.

#### *Particulate Matter*

Particulate material generally represents a dynamic variable in the activated sludge floc. The material that constitutes it depends heavily on the influent characteristics of the wastewater treatment facility and may be biodegradable or non-biodegradable. Considering this fact and the difficulty in determining whether a material is of particulate origin, a metabolic by product or waste product, particulate material is considered jointly with EPS.

## *Cations and Metals*

Divalent cations are critical participants in the bridging of negatively charged biopolymer in activated sludge (Higgins and Novak, 1997a). Higgins and Novak (1997b) also demonstrated that a monovalent to divalent cation ratio as well as magnesium to calcium ratio is important for determining settling and dewatering properties of activated sludge. The removal of calcium by EDTA addition has been found to cause deflocculation accompanied by decreased filterability and increased turbidity and solution carbohydrate concentration (Sanin and Vesilind 2000). Murthy et al. (1998) found that for industrial sludges, of with high pH, settling could be improved by the addition of magnesium as well as calcium. However there was an increase in lag time in between addition and observed improvement when calcium was used alone.

Metals such as aluminum (Muller and Novak 2001) and iron (Bura et al. 1998) are also known to present in activated sludge flocs. Iron is of particular interest in activated sludge flocs. Ferric and ferrous species have been reported to exist in sludge flocs under different oxidation conditions (Rasmussen and Nielsen 1996). Iron is capable of undergoing chemical reduction (Nielsen and Keiding 1998), biological oxidation in the presence of nitrate (Wilén et al. 2000), and biological reduction (Caccavo et al. 1996).

Iron reduction has been shown to cause deflocculation in activated sludge flocs (Caccavo et al. 1996; Nielsen and Keiding 1998). Nielsen and Keiding (1998) also demonstrated that shear sensitivity increased with decreased ferric iron concentration by formation of ferrous sulfide. Increased shear sensitivity under reduced conditions represents a potential mechanism for the enhancement of a mechanical disintegration process. The determination of points in the treatment processes where sludge flocs are most sensitive to mechanical disruption is critical to maximizing the effectiveness of devices like the KADY Biolysis<sup>tm</sup> System.

## **Solid/Liquid Separation and Solids Handling Considerations**

### *Solid/Liquid Separation*

Several factors control solid/liquid separation. Hilligardt and Hoffman (1997) stated that settling is influenced by density, particle shape, particle size distribution and porosity. Clauss et al. (1998), found the addition of talc a dense material, to activated sludge basins, improves settling velocity and sludge volume index. Similar results were observed in a pulp and paper mill process when Aquatal (a commercially marketed talc) was added and SVI decreased from 4000 ml/g to 1000 ml/g within hours (Clauss et al. 1999). In both cases, the improved solid/liquid separation was attributed to enhanced flocculation and the addition of high-density material.

The addition of shear may decrease overall floc size creating poorer settling floc or increase effluent COD as observed by Springer et al. (1996). Erdinçler and Vesilind (2000), found that the disruption of cells enhances the compactibility of sludges, by removing bound water, a benefit for a disintegration process.

Solid liquid/separation issues are more than likely going to depend on the amount of material that is processed in a given activated sludge basin. If only a small portion is processed periodically, one would expect that the deterioration in dewatering would be minimal.

### *Dewatering*

When considering wet sludge disintegration technologies, care must be taken not to offset cost savings realized by solids reduction with increased cost associated with dewatering cost, unless 100% reduction is realized. Nellenschulte and Kayser (1997) found that as mechanical stress is increased on a floc particle there is an increase in fines. Mechanical disintegration technologies especially high intensity shear devices like the KADY Mill are capable of producing such stresses.

Lawler et al. (1986) found that the removal of small particles by anaerobic digestion increased sludge dewaterability, when compared with undigested influent, therefore the generation of the fines by disintegration can be expected to adversely affect dewatering. Hall (1981) who observed that sonication of activated sludges for periods 4 seconds, served to increase capillary suction time from 14.3 seconds to 73.1 seconds.

The disruption of the activated sludge flocs by a mechanical device will in all likelihood cause some deterioration of dewatering. The degree of disintegration can be controlled, by volume processed and process time. It should be noted that shear sensitivity and resultant degradation of dewatering properties has been shown to be a function of the velocity gradient and not time (Novak and Bandak 1989).

#### *Conditioner Demand*

The addition of a conditioner, organic polymer or inorganic metal salts, usually occurs prior to dewatering. Dependent on the sludge the addition of a conditioner can be of considerable cost, thus any further deterioration of sludge properties can make a disintegration process unfeasible. Murthy et al. (2000) found that polymer demand is directly influence by solution protein concentration. The subsequent disruption of the floc matrix will release protein into solution and may increase polymer demand.

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**Chapter 2: An Investigation into the Applicability of Intensity High Shear as a Wet Sludge Disintegration Technology**

A manuscript prepared for anticipated publication.

# **An Investigation into the Applicability of Intensity High Shear as a Wet Sludge Disintegration Technology**

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## **Abstract**

By shearing activated sludge using a high shear rotor stator device, bioavailable proteinaceous material can be produced. Operation at elevated temperatures serves to increase the amount of material that is rendered soluble ( $<0.45 \mu\text{m}$ ) and biodegradable. The storage of sludge under anaerobic conditions prior to shearing does not appear to enhance solubilization of solids, though deflocculation and deterioration of dewaterability was observed. Anaerobic digestibility appears to be enhanced by the addition of a high shear to material from an anaerobic digester as shown by increases in gas production and volatile solids destruction. The dewatering properties of activated sludge, measured by capillary suction time, deteriorated with the addition of sheared solids, but reaeration resulted in near complete recovery.

## **Introduction**

Several technologies to reduce waste solids production in activated sludge systems have been investigated including the use of metazoa (Rensink 1997) excess oxygen concentrations (Abbassi 2000), ozonation (Sakia 1997), high shear devices (Springer 1996), sonication (Tiehm 2001, Chu 2001) and thermal pretreatment (Li 1992).

One such process for the reduction of waste solids using high intensity shear is the KADY mill. The principle on which this system is based is to render previously stabilized or non-bioavailable material bioavailable, resulting in increased mineralization. The KADY Model-L mill, the device used in this study, generates high shear forces and induces cavitation with a rotor-stator configuration in order to disintegrate waste activated sludge flocs. The application of this device as a wet sludge disintegration technology and its effect on dewatering were investigated.

To evaluate a wet sludge disintegration technology a working knowledge of activated sludge floc structure is required. Analysis of the structure of activated sludge flocs has shown that it consists of various sized pore spaces filled with water, extracellular polymeric substances (EPS), microbial communities (Li 1990) and particulate matter. The organic compounds commonly present in a floc matrix include proteins, polysaccharides, lipids, humic substances (Dignac 2000; Dignac 1998; Jorand 1995) and nucleic acids (Palmgren 1996).

Microorganisms, EPS and particulate matter represent potential sources of material for the generation of bioavailable substrate. The lysis of microorganisms by high shear processes would release the intracellular constituents, many of which are readily biodegradable. Indicators of cellular lysis include glucose-6-phosphate dehydrogenase activity (Frølund 1995) and the presence of nucleic acids in solution. There are several ways that shear may increase activated sludge bioavailability. These are by solubilizing floc material or intracellular components, increasing the activity of exoenzymes in floc, or by dispersing particulate solids trapped in floc. Each is described below.

Lysis, excretion and biosorption from wastewater are potential sources for the generation of EPS (Jorand 1998). It has been stated that soluble microbial products (SMP), a fraction of EPS, consists of both readily biodegradable and slowly biodegradable material (Boero 1996). Regardless of the source(s), it is expected that the application of high intensity shear should disperse the EPS allowing for the biodegradation of encapsulated bioavailable compounds and possibly rendering previously non-degradable materials biodegradable.

The presence of hydrolytic exoenzymes in EPS has been documented (Frølund 1995). Many of these are proteolytic in function and their subsequent release through the dispersion of the activated sludge floc matrix may enhance the hydrolysis rate via increased contact substrate further enhancing biodegradation.

In general, particulate matter present in waste activated sludge that passes through the activated sludge zone can be considered refractory. This material may be trapped so that it is unavailable for degradation or the conformation of the organic material comprising particulate matter may be such that biodegradation is extremely slow or nonexistent. The disruption of the floc matrix by high intensity shear may result in the release the encapsulated bioavailable material or changes the conformation of non-bioavailable substrate resulting in increased bioavailability.

All of the aforementioned components of the activated sludge floc matrix could potentially be solubilized to produce readily biodegradable material, dependent on the degree of disintegration and changes in structure of previously refractory compounds. The determination of which components of the activated sludge floc are contributing bioavailable material post shearing is crucial in gauging the effectiveness of high shear as a wet sludge disintegration technology. Consequently, particular attention was paid to determining which portions of the floc matrix were likely to be contributing bioavailable material.

Reducing the overall cost of solids disposal through mineralization of soluble substrate generated by the disintegration of WAS is the primary goal of utilizing a KADY mill for wet sludge disintegration. However, some consideration must be given to the effect that shearing of solids will have on solid/liquid separation. The deterioration of dewatering properties of sludges exposed to high shear could offset the cost savings realized by reduced solids disposal.

Novak and Bandank (1989), demonstrated that for unconditioned sludge, shear sensitivity was not dependent on time but rather, the mean velocity gradient (G). This was shown by increased capillary suction time with increasing G. The velocity gradient for the KADY Model-L Mill was estimated to be  $\sim 11,000 \text{ s}^{-1}$ . The solubilization of floc material will undoubtedly release colloidal and soluble protein into solution as well as other colloidal material. An increase in solution colloids, especially protein, has been found to increase cationic polymer demand (Murthy 2000).

## **Objective**

The objective of this research was to determine the applicability of high intensity shear device, the KADY mill, as a wet sludge disintegration technology. The identification of solubilization mechanism(s) of the KADY mill, cellular lysis or the dispersion of floc material, or both was of great importance in determining the potential for the generation of biodegradable substrate. Concurrent with the determination of the solubilization mechanism was the identification of the primary substrate that would be degraded and the extent of its bioavailability. These goals were accomplished by the measurement of soluble COD, solution protein, polysaccharide, and nucleic acids concentrations along with bulk solution intracellular enzyme activity and specific oxygen uptake rate.

Investigations were conducted to determine if changes operating conditions and parameters could be made to enhance process performance. These investigations included the variation of operating temperature, short-term anoxic/anaerobic storage and enhanced anaerobic digestion.

The effect of the addition of a high intensity shear device on dewatering in activated sludge a system was determined by measuring capillary suction time. These studies were conducted to determine if changes in dewatering properties would be impacted to the extent that the benefit of solids reduction is offset by increased dewatering costs.

## **Methods**

### *Shear Device*

The KADY Model L mill was utilized as the shear device in all the experiments for this study. The Model L consists of a 3 horsepower electric motor joined to a rotor stator assembly. It was calculated that the device has a  $G = \sim 11,000 \text{ s}^{-1}$ , assuming all of the

power generated by the motor is transferred to the water. The mechanism for disintegration is shear and impingement and the induction of cavitation. The mill was run for various lengths of time during the course of the study dependent on the degree of disintegration desired. A cooling jacket was provided with the mill and was utilized at all times to control the temperature. The amount of energy that is imparted to the sample during processing is capable of boiling water without the addition of a cooling jacket.

### *Samples*

Activated sludge samples were obtained from the Blacksburg and Virginia Polytechnic Institute Wastewater Treatment Plant (BVPI WWTP) located in Montgomery County, Virginia, USA. Samples were utilized on the same day for each experiment and if storage was required refrigeration at 4 °C was utilized.

Anaerobic sludges were obtained from the Peppers Ferry Regional Wastewater Treatment Facility (PFRWWTF) in Radford Virginia USA. Samples were taken from the mesophilic anaerobic digester located at that facility and processed the same day.

### *Chemical Analysis*

Chemical oxygen demand, soluble and total, was performed as stated in Standard Methods, Method 5220 C.(APHA 1995) Protein analysis was performed using the Hartree modification of the Lowry method (Hartree 1972, Lowry 1951), utilizing as the standard, Sigma protein standard bovine serum albumin (1mg/ml in 0.15 M NaCl with 0.05 % sodium azide). Polysaccharide measurements were conducted according to Dubois (1956), utilizing dextrose as the standard.

### *Solids Analysis*

Total Suspended Solids, Mixed Liquor Suspended Solids by Method 2450D (APHA 1995), Total Volatile and Volatile Suspended Solids by Method 2540E (APHA 1995) and Total Solids was performed per Method 2540B in Standard Methods(APHA 1995).

### *Specific Oxygen Uptake Rate (SOUR) Determination:*

SOUR was measured utilizing a YSI Model 57 Dissolved Oxygen Meter with a stirring probe attached. A sample was placed in 300 mL BOD bottle and the change in dissolved oxygen (DO) content was measured for 10 minutes or until the 2 mg/L-DO was reached. The oxygen uptake rate was determined by the slope of DO concentration over time (linear section only), and then normalized by MLSS (bioavailability studies) and MLVSS (process yield studies) to determine the specific oxygen uptake rate.



### *Dewatering Properties*

Capillary Suction Time was performed on a Triton-W.R.C. Type 165 CST-Apparatus and a Triton Electronics Ltd. Type P304M with Whatman 17CHR as the chromatography paper.

### *Nucleic Acid Analysis:*

Sludge obtained from BVPI WWTP was processed through the KADY Mill, followed by centrifugation at 12000 x g for 1 minute at full speed and then filtered through 0.45 µm Fisher membrane filter. RAS was disintegrated chemically to determine the amount of extracellular DNA present in the floc matrix. This was accomplished by utilizing a strong cation exchange resin (Fluka Chemica Dowex 50x8) to remove cations from the floc matrix. The procedure was based on studies conducted by Frølund (1996). Dowex 50x8, 109.5 g, was added to 300 ml of sludge to approximate 85 g-resin/g-VSS and mixed for 2 hours in an ice bath. UV-adsorption at 260 and 280 nm was measured on a Beckman DU-460 Spectrophotometer in 1-cm light path quartz cuvettes and calculations were performed per Gerhardt and Kreig (1994).

### *Glucose-6-Phosphate Dehydrogenase (G-6-PDH) Activity*

A modified analysis for G-6-PDH activity was based on the method prescribed by Maurer (1982) and conducted using a Beckman DU-460 Spectrophotometer. The method was modified by running the samples run at room temperature, rather than at 35 °C.

### *Metals Analysis*

Sludge samples were concentrated using centrifugation and dried at 105 °C. Solids digestion for metals analysis was based on EPA SW-846 Method 3050B, Acid Digestion for Metals Analysis of Soils, Sediments and Sludges. All samples were analyzed by flame atomic adsorption on a Perkin Elmer 5100PC Atomic Adsorption Spectrophotometer with resultant concentrations being expressed as mg-metal per gram total solids (TS) basis.

### *Aerobic Batch Reactor Design*

In order to investigate the biodegradability of activated sludges sheared by a KADY mill, bench scale aerobic digesters were constructed. The reactors contained mixed liquor (MLSS) and return activated sludge (RAS). The typical ratio of RAS to MLSS was 1:4, and depending on the status of the reactor, i.e. control or experimental, the RAS fraction was sheared in the KADY Mill for a period of time. The 1:4 ratio was utilized in this study to attempt to simulate field conditions.

### *Bioavailability Analysis Experiments*

To produce soluble material for SOUR and SCOD analysis, RAS was sheared using the KADY Model L for 15 minutes followed by centrifugation at 7240 x g for 15 minutes. The centrate was separated from the solids pellet and filtered through a Whatman 934-AH glass fiber filter and then filtered again through a Fisher 0.45 µm membrane filter. This filtrate was used for the determination of SOUR on the soluble fraction and for measurement of SCOD. As a control, a second RAS sample was processed in the same manner without first being sheared in the KADY Model L.

### *Analysis of Soluble Substrate Uptake Experiments*

RAS was sheared in the KADY mill for 7.5 minutes and then added to mixed liquor in a 1:4 ratio and aerated for a period of 4 hours. During aeration, soluble samples were taken and analyzed for SCOD, protein and polysaccharide on an hourly basis.

### *Soluble Product Production*

Multiple shear events were employed to determine if extended or multiple shear events could substantially increase the yield of biodegradable substrate. RAS was sheared for 15 minutes followed by centrifugation. The COD of the centrate was measured (SuCOD). The centrate was added in a 1:4 ratio to mixed liquor under aeration and the SOUR was measured to determine if readily biodegradable substrate was present. A second RAS sample, already sheared once for 15 minutes and flocculated by centrifugation, was resuspended in tap water and sheared for a second 15-minute period. Following centrifugation and measurement of SuCOD and SOUR, the pellet from the second shear event was resuspended in tap water and sheared for an additional 15 minutes. Following the measurement of SOUR and SuCOD, the pellet generated by centrifugation after the third shear event was resuspended in tap water and the SOUR was measured to determine if viable biomass was present.

### *Effect of Temperature on the Release of SCOD*

The effect of temperature on the release of SCOD with the addition of shear was analyzed in a two-step process. To determine the effect of temperature on the release of SCOD from RAS, return sludge from BVPIWWTP was heated using stirring hotplates with samples taken periodically for a 2-hour period. The effect of elevated temperature in conjunction with the addition high intensity shear was investigated on a batch basis in the KADY mill. The shear temperatures are reported as the maximum temperature obtained during the study period. Heating was accomplished by allowing the heat generated from the KADY mill to warm the sample. Temperature regulation was performed by manipulation of the water flow through the cooling jacket surrounding the shearing vessel. The effectiveness of each process, heating without shear and heating with shear, was based on the increase in SCOD above levels observed in the samples prior to any manipulation.

### *Effect of Anaerobic Storage on Solubilization Experiment*

To determine if anaerobic storage prior to shearing had an effect on the amount of SCOD produced from the KADY mill, the following protocol was employed. RAS from BVPIWWTP was split into 5 separate containers and sealed and stored at room temperature. Each day over the course of the study period, a container was opened and the contents sheared in the KADY mill for a period of 4-minutes. SCOD was measure prior to milling and after milling and total COD (TCOD) was measured prior to milling. The following equations were used to determine the solubilization by the KADY mill device under reducing conditions (Equation 1) and solubilization due to storage (Equation 2).

$$\% \text{ Solubilization Mill} = [(\text{SCOD}_m - \text{SCOD}_t)/(\text{TCOD}_t - \text{SCOD}_t)] * 100 \quad (1)$$

$$\% \text{ Solubilization Storage} = [(\text{SCOD}_t - \text{SCOD}_{t_0})/(\text{TCOD}_t - \text{SCOD}_{t_0})] * 100 \quad (2)$$

$\text{SCOD}_m$  = SCOD after milling

$\text{SCOD}_t$  = SCOD prior to milling at time = t days

$\text{SCOD}_{t_0}$  = SCOD in initial sample prior to storage

$\text{TCOD}_t$  = Total COD prior to milling at time = t days

### *Preliminary Invegstigations into High Intensity Shear Enhanced Anaerobic Digestion*

To determine if high intensity shear can enhance mesophilic anaerobic digestion batch digesters were constructed utilizing sludge obtained from the PFRWTF. Gas production was measured by water displacement and VS reduction was calculated as described later.

#### *Gas Production Analysis*

Four mesophilic 500 ml batch digesters were warmed in a water bath to maintain a temperature ~35 °C. Half the contents of two of the digesters were sheared for 10 minutes with the temperature reaching at 43 °C. After the sheared digester sludge was added to the batch digesters, a 4.8-hour period was allowed to pass prior to the measurement of gas production in order to allow for any entrained gas to be released. Gas production was then measured periodically for 3 days.

#### *Volatile Solids (VS) Reduction*

Batch mesophilic anaerobic digesters were used to determine if there is enhanced VS destruction as a result of high intensity shear. The treatment groups consisted of three anaerobic digesters, a non-sheared control, a single shear event reactor and a multiple shear event reactor. Shear events consisted of the shearing of ~1/3 of the total volume of the digester. Shearing was for 4 minutes with temperatures ranging from 27 °C to 47 °C as summarized in Table 1. Volatile solids content was measured for the original sample, with subsequent measurement occurring for all digesters prior to each shear event for the multiple shear digester. Volatile solids reduction was calculated using Equation 3.

$$\% \text{ Reduction in Volatile Solids} = [(VS_{t_0} - VS_t)/VS_{t_0}] * 100 \quad (3)$$

$VS_{t_0}$  = Initial Volatile Solids Content (%)

$VS_t$  = Volatile Solids Content at Time =t days

**Table 1 Shear Event and Summary of Maximum Temperature Achieved in the KADY Mill**

Shear Event #	Time (days)	Maximum Temperature (°C)
1**	0	42
2	4	27
3	6	38
4	9	42
5	11	38
6	18	36.5

\*\* Single Shear digester and multiple shear digester processed in the same initial batch

### *Statistics/Error Bars*

The error bars presented in this manuscript are +/- one standard deviation for all figures.

## **Results and Discussion**

### Determination of the Bioavailability of Sheared Activated Sludge

Activated sludge was sheared using the KADY Model L to determine if biodegradable substrate could be generated in order to assess the potential use of this device for solids reduction. Multiple experiments were conducted to determine the factors that were important in solubilization of activated sludge to produce readily biodegradable substrate. The parameters measured to determine the bioavailability of soluble material were specific oxygen uptake rate and soluble COD uptake.

### *Soluble Chemical Oxygen Demand Uptake Analysis*

Multiple experiments using bench scale batch aerobic reactors were utilized to determine SCOD uptake. The experimental reactors received RAS sheared in the KADY mill for a period of 15 minutes with the final temperatures reaching 54 °C in trial 1 and 42.5 °C in trial 2.

The exposure of the RAS to high intensity shear increased the soluble COD in both the 54 °C and 42.5 °C, shear reactors. Soluble COD increases of 149.8 and 85.5 mg/L as COD were found at 54 °C and 42.5 °C, respectively (Figure 1). During the 2-hour aeration period following the addition of sheared RAS, decreases in SCOD were observed, suggesting that the material is either biodegraded or is being reflocculated.

### *Specific Oxygen Uptake Rate Analysis*

Several studies utilizing SOUR and SCOD uptake were conducted to determine if the soluble product generated in the KADY mill was being metabolized or if the observed SCOD reduction was due to reincorporation of soluble organics back into the floc matrix. The addition of soluble products from the KADY Mill to batch aerated reactors containing mixed liquor increased the SCOD from 33.8 mg/L-COD to 134 mg/L-COD and the SOUR from 6.7 mg-O<sub>2</sub>/g-MLSS to 22.7 mg-O<sub>2</sub>/g-MLSS-hr as shown in Figure 2. As a positive control, the addition of unsheared RAS filtrate was added to mixed liquor. At the conclusion of three-hours of aeration, the SCOD and SOUR in the reactor receiving milled filtrate had decreased to 58.8 mg/L-COD and 10.6 mg-O<sub>2</sub>/g-MLSS-hr, while the control with RAS filtrate was relatively unchanged. The elevation of SOUR and its subsequent decrease back to near the control level suggests that the material produced by the KADY Mill is at least some readily biodegradable material.

A positive linear relationship between SCOD concentration and SOUR,  $R^2 = 0.65$ , was observed when all of the data for reactors receiving the KADY mill product was plotted (Figure 3). These data, in conjunction with the observed trend found in Figure 1, demonstrates that the KADY Mill is capable of producing a readily biodegradable product.

### Determination of the Origin of Bioavailable Materials

It is important to determine the origin of the degradable material solubilized by the KADY mill. EPS, particulate matter, and cellular lysis are the obvious sources for potentially biodegradable material that results from the shearing activated sludge. These studies were undertaken to determine the source of biodegradable material; especially to determine if cellular lysis could contribute degradable organic matter.

### *Induction of Cellular Lysis*

The role of cellular lysis and the subsequent degradation and mineralization of intracellular material is considered a potentially important component in solids reduction. The quantification of cellular lysis was investigated by measuring glucose-6-phosphate dehydrogenase activity and solution nucleic acids concentrations. Glucose-6-phosphate dehydrogenase and nucleic acids are both intracellular components and would be present at elevated levels in the bulk solution only if lysis has occurred.

As indicated in Figure 4, UV adsorption at 260 nm for nucleic acids showed no significant increase in solution concentration of nucleic acids between sheared sludge, (6.32 µg/L) and that exposed to a strong cationic exchange resin (7.42 µg/L). Along with the adsorption at 260nm, adsorption at 280 nm was measured for each sample to determine the interference from solution protein. Interference from solution protein can be detected as a deviation in the 260-nm/280-nm ratio. A ratio of 1.8-1.9 was accepted as indicative of no protein interference (Ausubel 1999). The observed 260-nm/280-nm ratio for the sheared RAS was 1.30 and 1.52 for the unsheared. This indicates that there is

some adsorption interference from solution proteins in the analysis and another method or sample purification maybe required for a more accurate measurement

Extracellular glucose-6-phosphate dehydrogenase activity did not increase appreciably after shearing (Figure 5). These data in combination suggest that lysis products do not contribute a significant amount of material to the observed release of SCOD.

#### *EPS and Particulate Matter Solubilization Analysis*

The elimination of lysis products as a significant source of biodegradable material indicates that EPS and particulate matter are the most likely sources of biodegradable material released or solubilized by milling. Due to difficulties encountered when trying to separate sheared EPS from sheared particulate material, these two fractions are considered as one material in this analysis. EPS from activated sludge has been reported to be composed of proteins, polysaccharides, humic material and nucleic acids for activated sludge municipal wastewater treatment ( Frølund 1996). The composition of particulate matter is heavily influenced by influent wastewater characteristics.

The introduction of SCOD, either from solubilized EPS or solubilized particulate matter, has been shown to elevate SOUR initially followed by a decrease during batch aeration. The nature of the material being degraded has not been thoroughly investigated, whether it is protein or polysaccharide and in what proportions are they being degraded. An aerobic batch reactor receiving 20% by volume of RAS sheared in a KADY mill and was utilized answer these questions.

As SCOD decreased from 69.8 mg/L-COD to 20.9 mg/L-COD over the 4-hour experimental period, protein concentrations declined as well, from 42.9 mg/L to 11.2 mg/L (Figure 6). Polysaccharide concentrations remained relatively constant, decreasing from 16.8 mg/L to 11.4 mg/L during the same period. A conversion of protein and polysaccharides to COD based on COD stoichiometry shows a reduction of 48 mg/L-as COD and 6 mg/L for protein and polysaccharide COD, respectively. The total reduction in measured SCOD was 49 mg/L- as COD, therefore the reduction is primarily associated with protein.

The relatively modest increases in soluble substrate generated during shearing suggest that activated sludge flocs are more resistant to mechanical disintegration than previously thought. The elimination of the lysis products as the source of biodegradable material suggests that bacteria present in the sludge floc are afforded some protection by the floc matrix, therefore making lysis difficult. The most likely mechanisms for the generation of soluble substrate are disruption and dispersion of particulate matter and EPS, generating a soluble and biodegradable proteineaceous substrate.

#### Operational Considerations and Factors Controlling Floc Disintegration

A fraction of the substrate produced by the KADY mill is biodegradable and proteinaceous in nature. However when RAS from BVPIWWTP was exposed to a G =

$\sim 11,000 \text{ s}^{-1}$  for 4 minutes with temperatures reaching  $47 \text{ }^\circ\text{C}$ , a 6.7 % net release of SCOD resulted, based on calculation using Equation 1. At a  $G = \sim 11,000 \text{ sec}^{-1}$ , a greater level of solubilization was expected even at relatively short times. The fact that less than 10% solubilization was realized suggests that the structure of an activated sludge floc affords some protection from mechanical disintegration or that some minimal required energy level was not attained to disintegrate the floc (Chu 2001).

The low solubilization levels generated by the KADY mill would suggest the mill, as currently operated, would not be a good candidate for the disintegration of WAS. In the following sections, consideration was given to operational parameters that may be manipulated in order to alter the performance of a high intensity shear device. These included, multiple shear passes, temperature increases, holding of sludge under reduced oxygen supply and anaerobic conditions.

### *Multiple Shear Passes*

It was of interest to determine if there is greater potential for the solubilization of the floc material if multiple passes through the KADY mill were used. To study this, RAS was sheared for 15 minutes, producing a supernatant COD (SuCOD) of 429 mg/L. A second and third shear event produced a SuCOD of 84.5 and 49.6 mg/L for each subsequent shearing event. After each successive shear and centrifugation event, the supernatant was added to mixed liquor and an observed SOUR was recorded 26.4, 16.4 and 10 mg- $\text{O}_2/\text{g-VSS-hr}$  for each mill event. The SOUR and COD data are summarized in Figure 7.

The SOUR increased 219 % above the background rate of mixed liquor when the supernatant from the first shear event was added to mixed liquor. The percent increase in SOUR above background level decreased after each successive pass through the KADY mill, as can be seen in Figure 7. After the third and final pass the percent increase in SOUR was 4.2 % over that of mixed liquor.

After each successive mill period, SOUR was measured on the resuspended pellet to determine if viable biomass was present. The SOUR after the third shear event was 9.84 mg- $\text{O}_2/\text{g-VSS-hr}$  which is very similar to the mixed liquor control SOUR (9.61 mg- $\text{O}_2/\text{g-VSS-hr}$ ), demonstrating that biomass viability is unchanged by shearing.

The data suggests that there is potential for further release of bioavailable material beyond that generated by an initial pass. However, the yields decrease with each pass and by the third milling event little additional release occurs.

### *Temperature*

Elevated temperatures are known to cause sludge disintegration and has been the focus of several wet sludge disintegration technologies (Li 1992; Kepp 2000). The KADY Mill generates heat as a by-product of the large quantity of energy that is imparted to the water. Increases in temperature were investigated as to the effect of temperature on process performance.

The susceptibility of BVPI WWTP RAS to heat induced solubilization was initially investigated in order to separate the effect of mechanical disintegration from thermal disintegration. Return activated sludge was heated to 24 °C, 35 °C and 51 °C while SCOD were taken over a 2-hour period. It was found that only at 51 °C, was considerable release of SCOD measured after 2.0 hours (Table 2).

**Table 2 Net Release of SCOD over Time due to Increased Temperature**

<b>Mixing Time</b>	<b>24 °C mg/L-as SCOD</b>	<b>34 °C mg/L-as SCOD</b>	<b>51 °C mg/L-as SCOD</b>
0	0.0	0.0	0.0
10	3.2	-1.3	0.0
20	0.8	-6.9	44.3
30	-8.5	-5.0	115.8
60	-10.6	-4.0	248.6
90	-8.2	4.6	359.7
120	-10.8	15.3	407.3

\*net release =  $SCOD_{initial} - SCOD_t$

The KADY Mill was allowed to heat to 29 °C, 35 °C and 45 °C by manipulation of the cooling water flow through the water-cooling jacket. As Figure 8 illustrates, increased temperature during milling releases more SCOD. The observed 40 % in released SCOD between the 45 °C operating temperature and the 29 °C demonstrates that increased operational temperature can contribute additional soluble COD release.

The data suggests that not only is the mechanical energy imparted to the system critical to floc disintegration but operating temperature as well. This was not unexpected due to previous studies where elevated temperatures in conjunction with sonication have been shown to enhance floc disintegration as well (Chu 2001).

Elevated operating temperatures could be obtained in the field by the addition of a heat exchanger upstream of the mill or increasing the residence time of sludge in the mill. However before either change is made to the operation of the mill, the biodegradability the excess material released must be determined and the cost effectiveness of the additional energy inputs must be analyzed.

#### *Anoxic/Anaerobic Storage of Return Activated Sludge*

Deflocculation of activated sludge has been shown to occur under reduced conditions (Wilén 2000). Iron has been shown to be involved in deflocculation by undergoing chemical reduction (Nielsen 1998; Wilén 2000) and biological reduction (Caccavo 1996). BVPIWWTP return activated sludge was found to contain 5.12 mg-Fe/g-TS. If iron is reduced, deflocculation and consequently increased shear sensitivity should be observed.



In order to determine when deflocculation had occurred during storage, the percent SCOD prior to milling was monitored. An increase in SCOD prior to milling was observed to have occurred between day 2 and day 4 (Figure 9). At that time an increase in shear sensitivity was expected. Weakening of the floc structure by the solubilization of iron should allow for greater solubilization of the floc by the KADY mill. Though deflocculation did occur, shear sensitivity did not appear to increase. The net release of SCOD increased from 6.73% to 7.09 % over the course of the study (Figure 9), suggesting that short-term anaerobic storage will not appreciably enhance KADY Mill performance.

It is possible that some fraction of the floc is highly resistant to shear as well due to interaction with metals other than iron. Iron is known to undergo oxidation and reduction as previously mentioned and floc strength is related to its oxidation state. Aluminum is the same valence state as ferric iron and it has been found in quantities greater than iron, 8.38 mg-Al/g-TS for BVPIWWTP sludge versus 5.12 mg-Fe/g-TS for iron. Aluminum could bind in a similar fashion as iron with protein and since it does not undergo oxidation and reduction localized concentration of aluminum could be generated creating dense granules that are resistant to shear and thus provide protection to microorganism from mechanical forces such as shear. Additional research into floc structure is needed to help elucidate the role of aluminum in activated sludge flocs.

#### *Preliminary Investigations into Enhanced Anaerobic Digestion*

Many of the wet sludge disintegration technologies have been applied to anaerobic digestion to enhance volatile solids destruction and gas production. Generally the application of the disintegration technology has been to the WAS being fed to a digester. In the studies involving the KADY Mill, the mill was utilized as a side stream process, shearing solids directly from the digester and returning them for further degradation. The shearing of the digester solids as opposed to influent WAS, was done due to the assumed weakened nature of the anaerobic floc structure under reduced conditions which would maximize mill effectiveness.

The shearing of digested sludge from PFRWTF enhanced total gas production in bench scale reactors. Net gas production from both the experimental and control reactors is plotted in Figure 10. The observed 62.7% average increase in total gas production from the sheared digesters was attributed to the addition of high shear.

A second series of batch experiments were undertaken to determine if volatile solids destruction was enhanced by the addition of shear. The digesters under investigation included a control, non-sheared, a single shear event digester and a multiple shear event digester. At the conclusion of the experiment a 20.1%, 19.8% and 14.3% reduction in volatile solids was observed in single shear, multiple shear and control digesters respectively. The average 39.5 % increase in VS destruction was attributed to the use of the KADY Model L.

Analysis of the data suggests that the application of shear devices is an effective technology for enhancing mesophilic anaerobic digestion. These findings could prove beneficial to facilities that are having difficulty meeting regulated level of VS reduction. However this data is preliminary and requires considerably more research before the exact mechanisms behind these observations are understood.

### *Dewatering*

An increase of small particulate matter in solution decreases the dewaterability of sludge. (Bruus 1992) The generation of colloidal material during the shear process is expected to have a negative impact on the dewatering characteristics of a system. What is not known is how reaeration, coupled with degradation of solubilized materials will impact sludge dewatering.

Bench scale activated sludge systems were set up to investigate the effect that a high shear device would have on dewatering properties. Initial investigations focused on the dewatering rate as measured by capillary suction time.

Several general trends were found when investigating the effect the KADY Model L had on CST. When RAS is sheared the CST initially increased. However, when aerated there was a steady decrease in CST over time. This trend can be observed in Figure 12. RAS, was sheared three times for 4-minutes each time and between each shearing event the RAS was aerated for 2-hours. After each shear event the CST increased followed by a recovery period where the CST approached the initial or non-sheared values.

In full-scale operation, an aeration basin would not contain 100% sheared flocs. Therefore an experiment was designed to determine the effect that different sheared volume ratios would have on CST. The treatments consisted of varying the volume of RAS that was sheared and added to a specific volume of MLSS. One reactor consisted of only sheared RAS, while a second consisted of 1/2 sheared RAS and 1/2 unsheared mixed liquor. Two controls were also designed with the same mixtures but the RAS was unsheared. Milling of the RAS only reactor increased the CST from 17.2 second to 1733.1 seconds, a 9976 % increase. When milled RAS was combined with mixed liquor in a 1:1 ratio the initial CST of the reactor increased from 15.9 second to 553.1 seconds, or by 262 %.

Over the 4-hour test period complete recovery of the CST back to the control values was never observed for the reactor containing only sheared RAS, the final value after 4-hours of aeration was 579 seconds. The mixed liquor/sheared RAS reactor recovered to 17.9 seconds, which is very close to its initial value of 15.9 seconds. What these data suggest is that the addition of a high shear device will negatively impact the dewatering properties of activated sludge. However the degree of deterioration is will depend on the fraction of the basin that is processed and therefore may be minimal in full-scale operation.

The recovery of the CST observed in both Figure 12 and Figure 13 after aeration suggests that reflocculation may play a role in the observed reductions in SCOD. Reflocculation or recovery in CST has been observed in sonicated sludges as well (Hall 1981).

Accepting that a fraction of the observed decreases in SCOD are due to reflocculation and not to biodegradation alone a mechanism for enhancing the dewatering properties with minimal effect the fraction of biodegradable material should be investigated. The additions of flocculation aids such as talc (Claus 1998) or iron may be beneficial depending on the initial dewatering properties of the sludge.

## Conclusions

The exposure of RAS to a device that is capable of generating a  $G = \sim 11,000 \text{ s}^{-1}$ , was found at 46 °C to solubilize 6.7% of total COD with no observed cellular lysis. The soluble substrate that was produced was biodegradable and consisted mostly of protein. The data from this study suggests that the KADY mill operates by dispersion of floc materials and not cellular lysis, and it is the solubilization of EPS and particulate matter that is the likely source of biodegradable material leading to sludge reduction. The observed decrease in SCOD and SOUR from 429.4 mg/L COD solubilized and 26.4 mg-O<sub>2</sub>/g-VSS-hr after a single pass through the mill to 49.6 mg/L-COD solubilized and 10.0 mg-O<sub>2</sub>/g-VSS-hr after a third pass, suggests that there is only a finite amount of material that can be solubilized from the activated sludge at the G of the mill.

High intensity shear as a wet sludge disintegration technology is limited in applicability for activated sludge systems. The apparent lack of lysis products in solution suggests that reduction in total waste solids will be a function of quantity of EPS and particulate matter solubilized from the floc matrix. The levels of EPS and particulate matter in the floc matrix that can be solubilized and biodegraded are related to influent characteristics and the metabolic state of the microorganisms; therefore performance would be expected to vary from facility to facility.

Studies of the enhancement of the shear process found that operating at elevated temperatures enhanced the performance of the KADY Mill. Milling at a temperature of 45 °C increased the release of SCOD by 40% over milling at 29 °C. The combination of temperature and high shear appears to be a more effective process than shear alone. The storage of activated sludge does not appear to promote the release of soluble material, only increasing solubilization by 5 % after 5 days of storage. Milling of anaerobically digested sludge is promising, with an observed 67% increase in total gas production for batch milling and an average 39.5% increase in VS reduction with the addition of shear.

The addition of high shear increases the CST dramatically 9967% when 100% of the sludge is sheared. However mixing the sludge with un-sheared mixed liquor dampens the effect of shear on dewatering. The apparent controlling mechanism for observed dewatering properties is the fraction of shear product added to mixed liquor as well as the duration of aeration.

## **Acknowledgements**

KADY BIOLYSIS DIVISION of KADY INTERNATIONAL funded the research conducted at Virginia Tech. Special appreciation is extended to D. Holbrook, S. Phipps, J. Fraga, C. Bott, J. Petruska, and J. Smiley for their assistance completing this research.

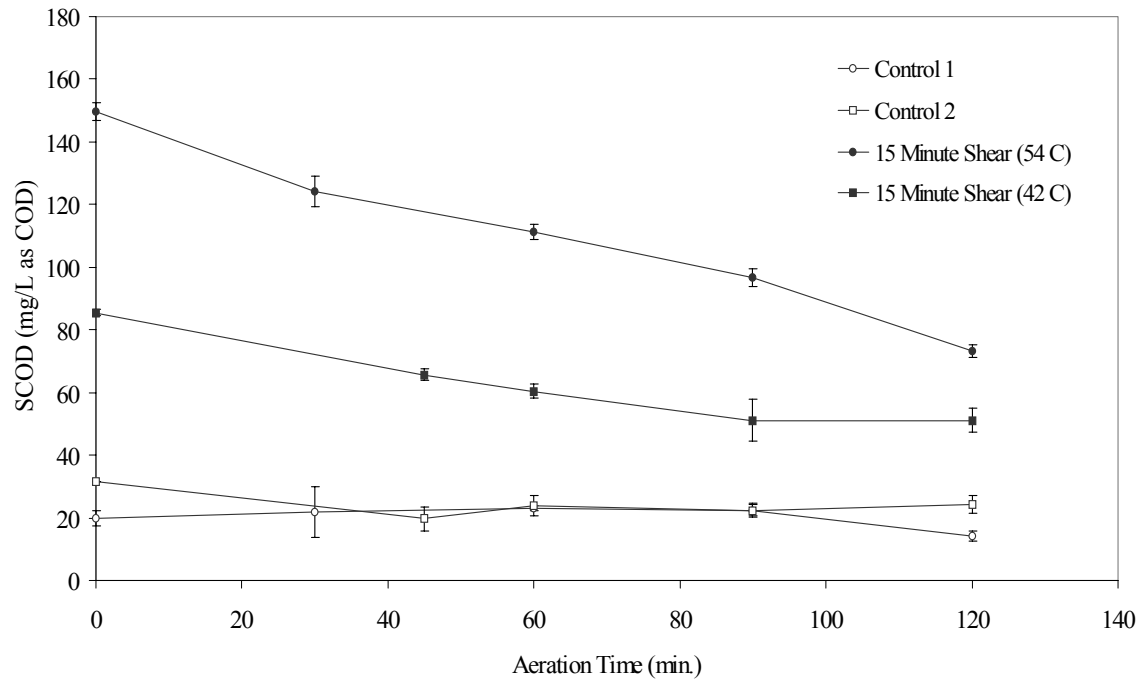
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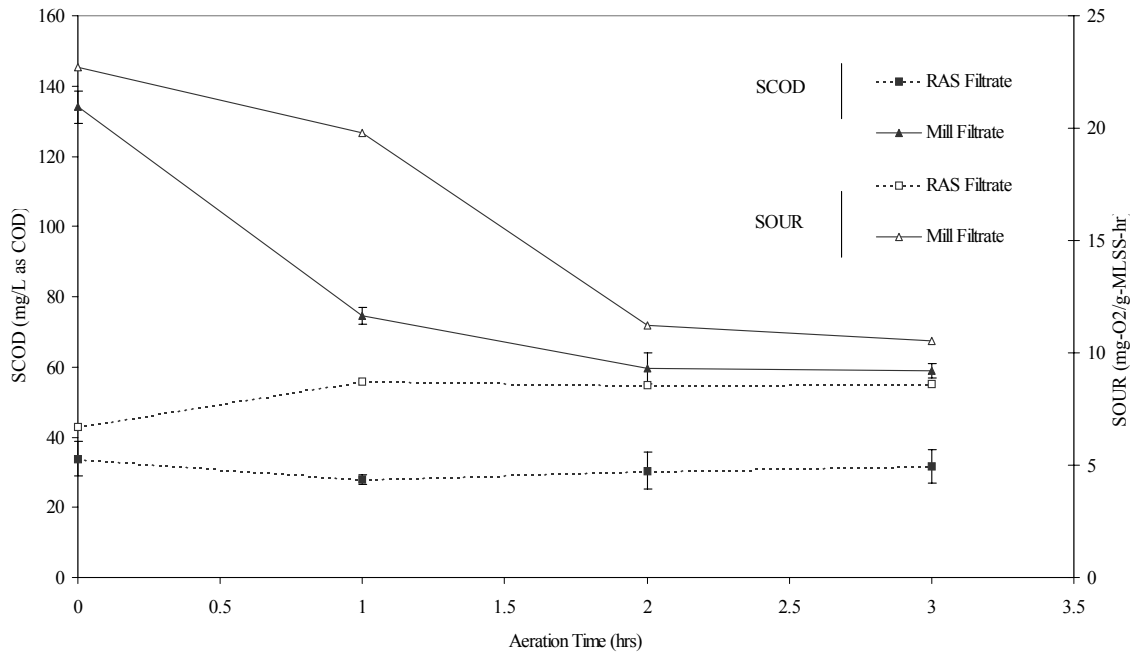
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**Figure 1 The Effect of High Shear on Soluble COD Concentrations in Batch Activated Sludge Reactors**

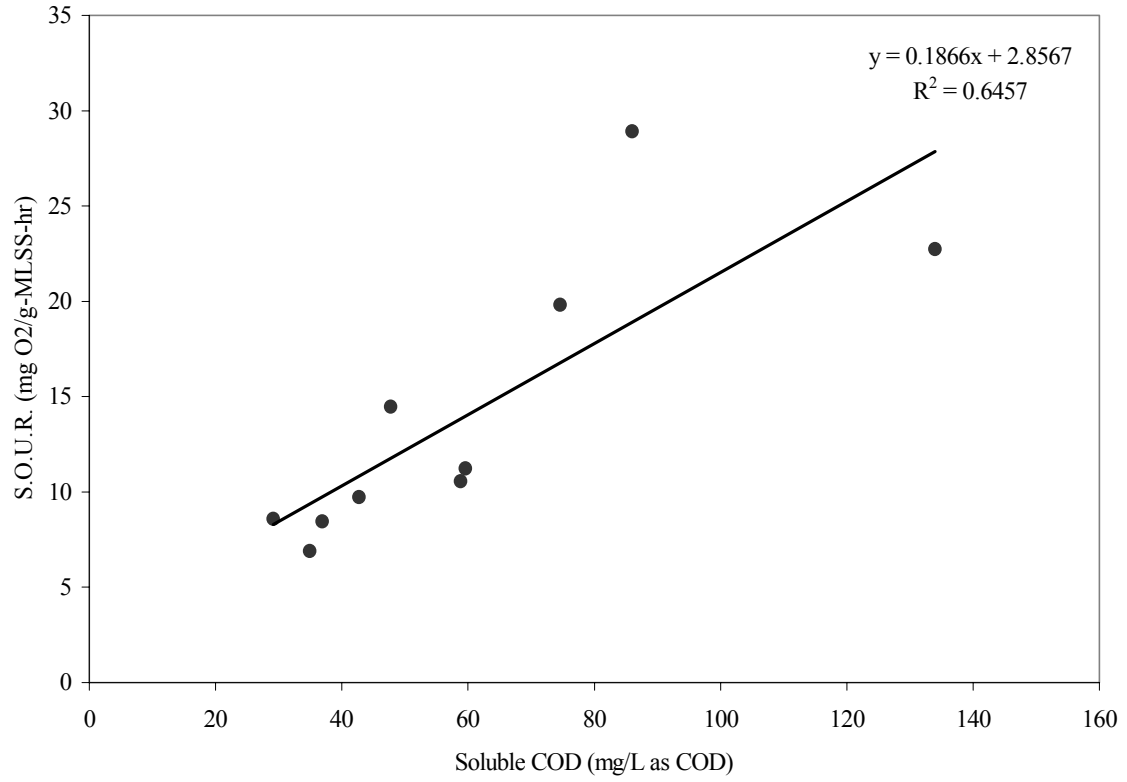




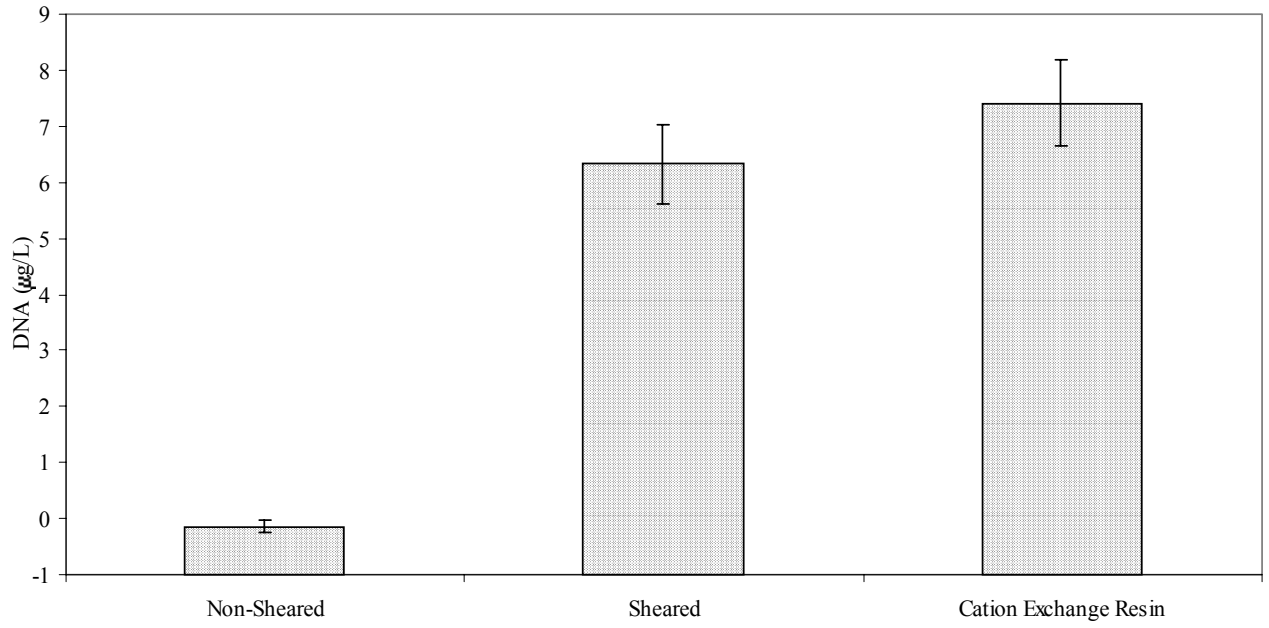
**Figure 2 The Addition of Soluble KADY Mill product and the Subsequent Changes in SCOD and SOUR during Aeration**



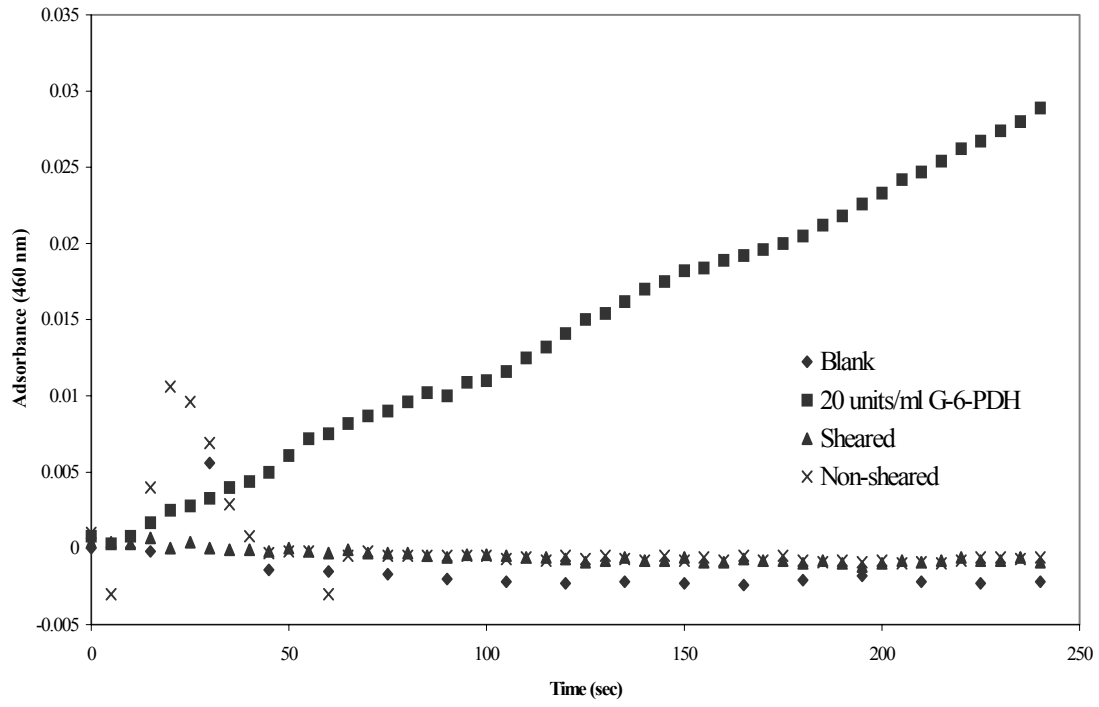
**Figure 3 The Relationship Between Specific Oxygen Uptake Rate and SCOD Produced by a KADY Mill during Aeration**



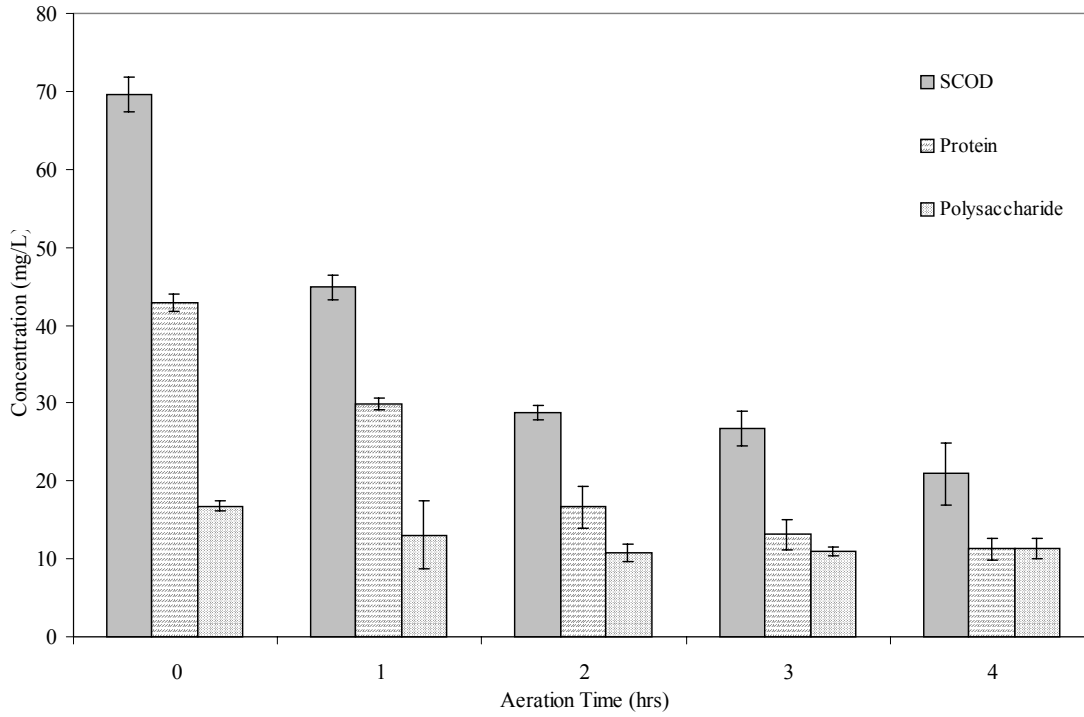
**Figure 4 Soluble Deoxyribonucleic Acids Concentrations by different Extraction Techniques as Determined by Ultraviolet Absorbance at 260 nm**



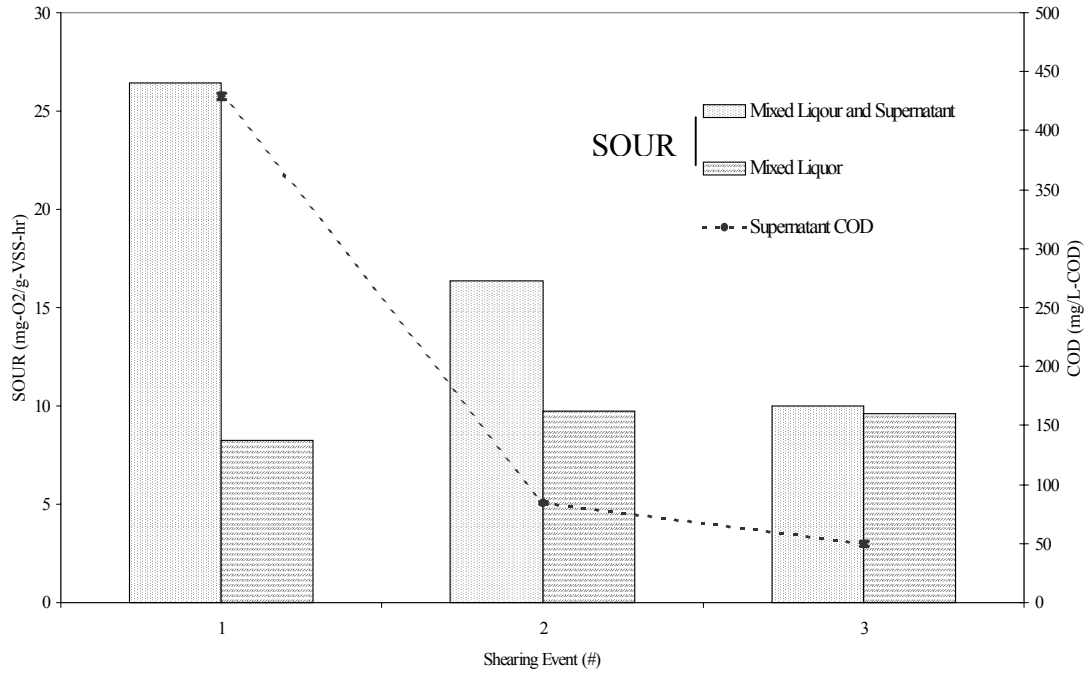
**Figure 5 Solution (<0.45 μm) Glucose-6-phosphate Dehydrogenase Activity of Sheared RAS**



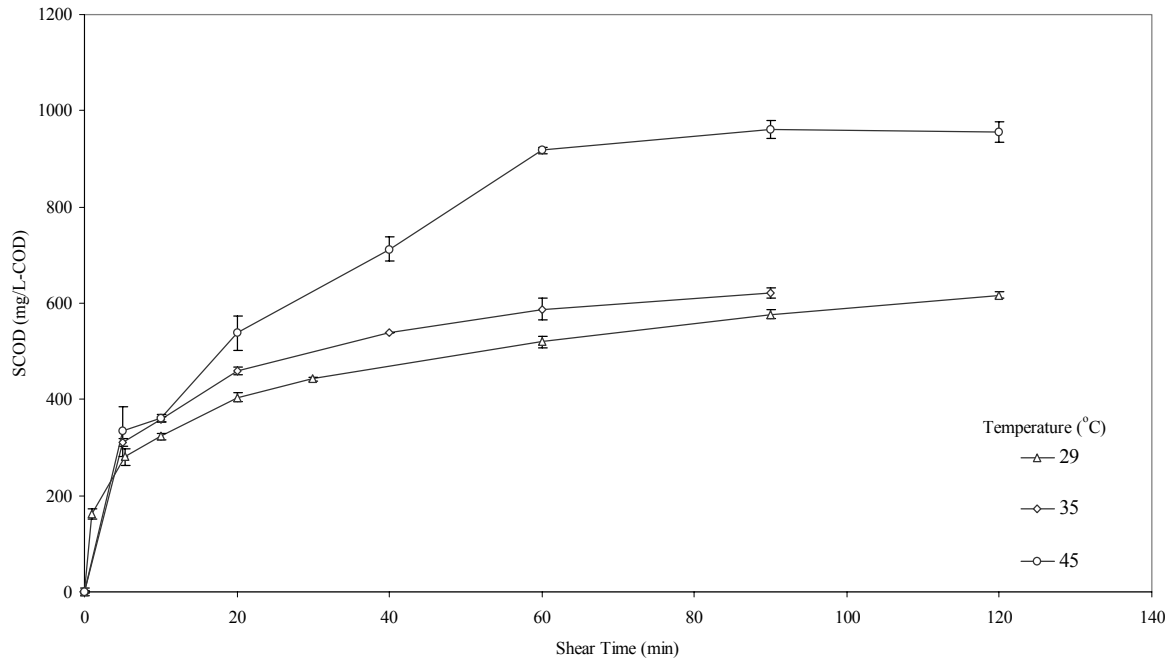
**Figure 6 Uptake of SCOD, Protein and Polysaccharide in an Aerobic Batch Reactor Receiving 20% by Volume KADY Mill Product**



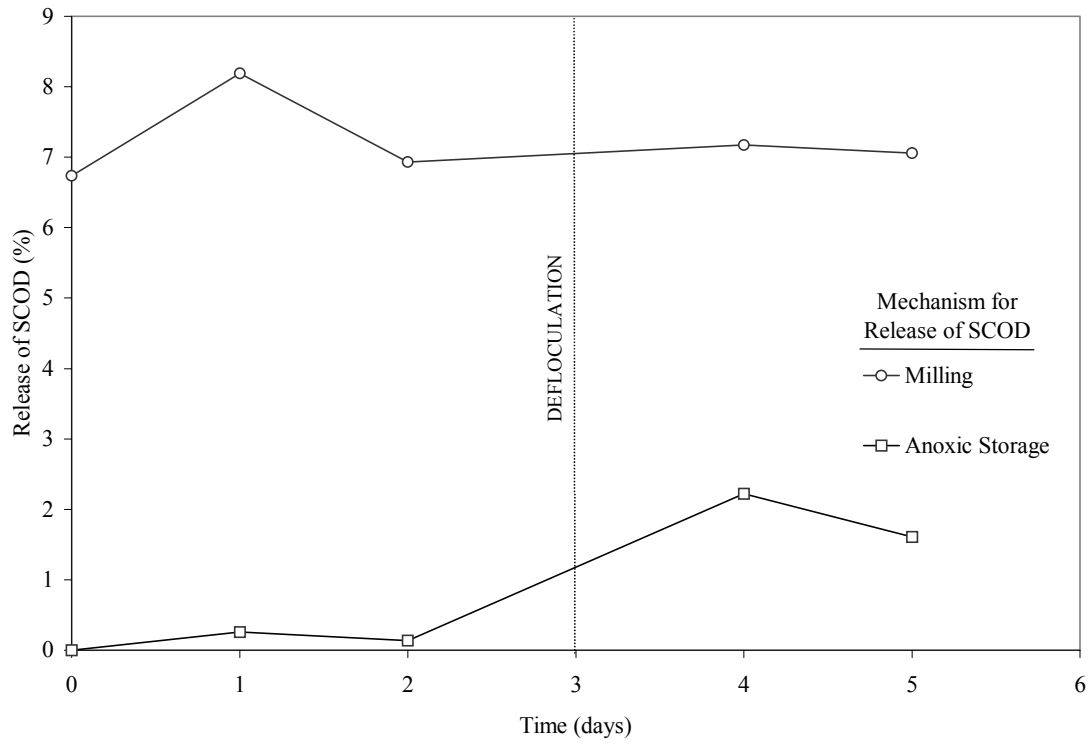
**Figure 7 The Release of COD from RAS Subjected to Multiple Shearings in a KADY Mill and its Bioavailability as Increase in SOUR above Endogenous Levels of Activated Sludge**



**Figure 8 The Effect of Operating Temperature on Soluble COD Release from Return Activated Sludge**

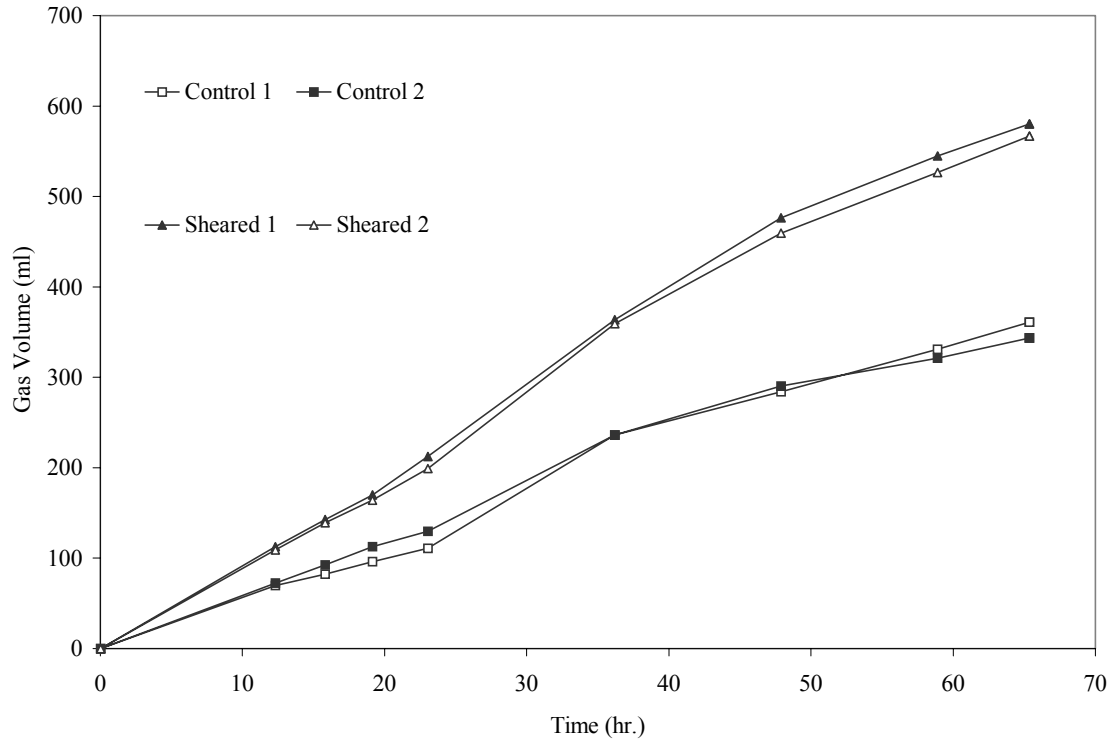


**Figure 9 The Effect of Anoxic Detention Time on SCOD Release and Solubilization Fraction by a KADY Model L Mill**

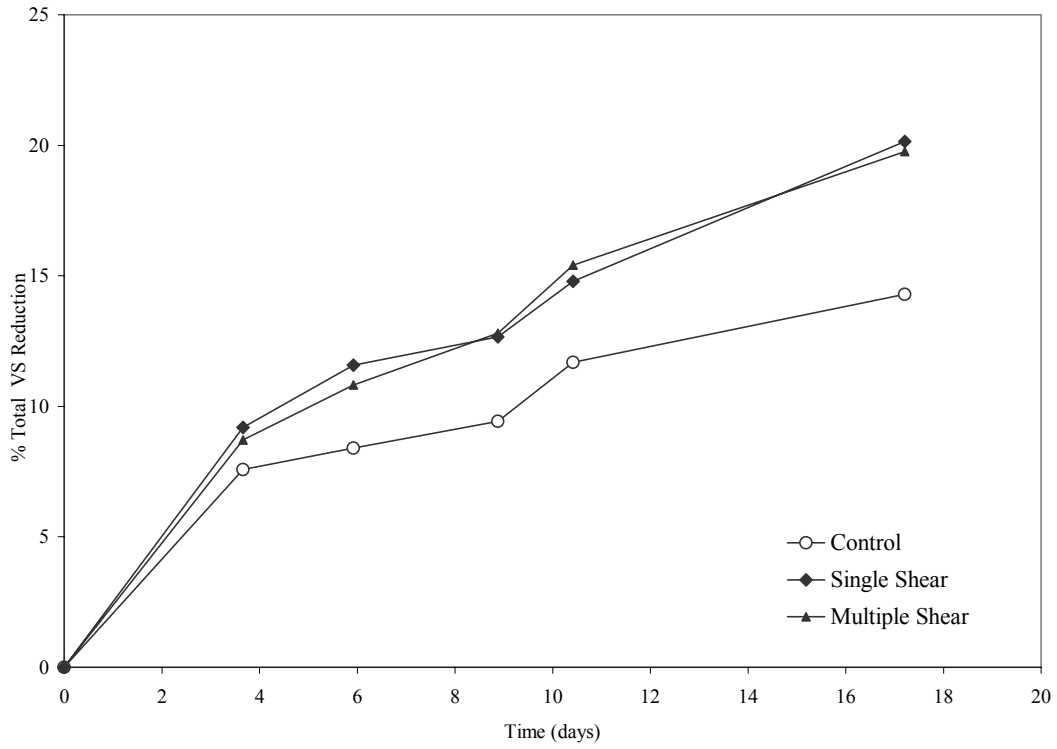




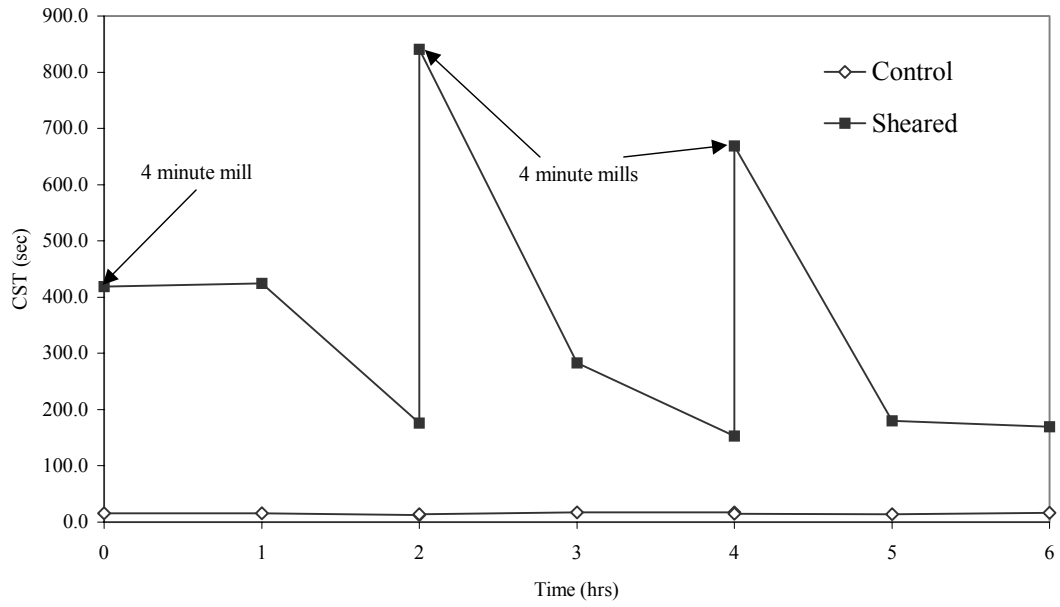
**Figure 10 Effect of High Intensity Shear on Gas Production from Batch Mesophilic Anaerobic Digesters, when Shearing 50% of the Total Volume**



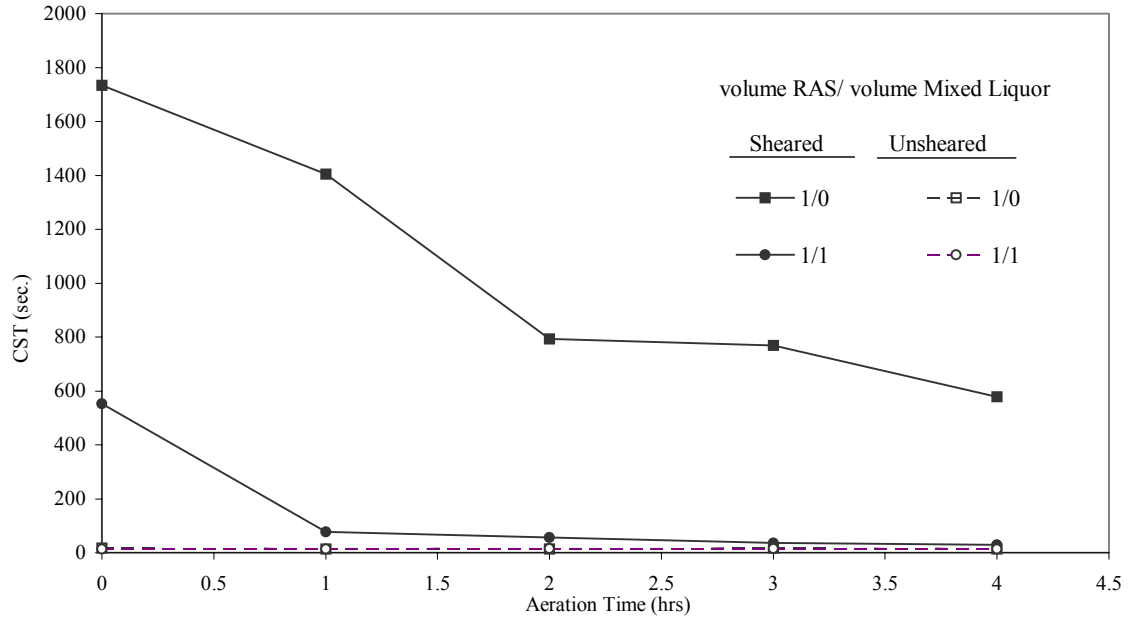
**Figure 11 The Effect of High Intensity Shear on Volatile Solids Reduction in Batch Mesophilic Anaerobic Digesters, when Shearing 33% of the Total Digester Volume**



**Figure 12 The Recovery of CST during Aeration of RAS Sheared Multiple Times in a KADY Mill**



**Figure 13 Effect of High Intensity Shear on Capillary Suction Time for Batch Activated Sludge Reactors of Varying RAS/MLSS Ratios**



### **Chapter 3: The Role of Iron in Wastewater Sludges with Regard to Floc Structure and Solid/Liquid Separation**

A manuscript prepared for anticipated publication.

# The Role of Iron in Wastewater Sludges with Regard to Floc Structure and Solid/Liquid Separation

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## Abstract

The role of iron and iron chemistry are thought to play an important role in the formation and properties of activated sludge flocs. Iron apparently selectively removes protein, in particular, material ranging in the 1.5  $\mu\text{m}$  to 30K size range. It is hypothesized that the tri-valent metals, iron and aluminum, are locally concentrated within the floc structure affording the microorganisms protection from mechanical stress. Preliminary results suggest that the ferric/ferrous redox chemistry may serve to enhance floc structure. Increases in settling velocity and shear resistance were observed in sludges milled with a KADY mill and dosed with ferrous sulfate, while the addition of ferric iron decreased settling velocity and increased shear sensitivity. The data suggest that ferrous iron combines with solution colloids by a sequence of reactions to produce a more dense and tightly bound floc.

## Introduction

Cations have been investigated extensively to determine their role in activated sludge floc structure. Divalent cations are critical participants in the bridging of negatively charged biopolymer in activated sludge (Higgins and Novak, 1997a). Higgins and Novak (1997b) also demonstrated that a monovalent to divalent cation ratio as well as magnesium to calcium ratio is important for determining settling and dewatering properties of activated sludge. The addition of divalent cations, calcium and magnesium, have been shown to enhance poorly setting industrial sludges as well (Murthy, 1998). The removal of calcium by the introduction of EDTA has been demonstrated to increase turbidity and decrease the rate of cake filtration (Sanin 2000).

Metals such as iron and aluminum that are present in activated sludge have been studied little until recently. Iron is known to be associated with the activated sludge floc matrix (Bura 1998). Iron in sludge flocs can be found in several forms, solid ferrous

sulfide, associated with biopolymer, or as vivianite (Frossard 1997). Ferric and ferrous species have been reported to present in sludge flocs under different oxidation conditions (Rasmussen 1996). Iron is capable of undergoing chemical reduction (Nielsen 1998), biological oxidation (Wilén 2000), and biological reduction (Caccavo 1996).

It is the objective of this research to determine if metals, especially iron, play a critical role not only in floc structure but observed properties. It has been hypothesized that iron and other metals are not uniformly distributed throughout the floc structure. Instead of a more homogeneous distribution there are localized pockets of metals creating material that is highly resistant to biological and mechanical disintegration.

The research was conducted to elucidate what role iron plays in floc structure, flocculation, dewatering and settling, utilizing high shear and reducing conditions.

## **Methods**

### *Samples:*

Samples were collected from the Blacksburg and Virginia Polytechnic Institute wastewater treatment plant (BVPIWWTP) located in Montgomery County, Virginia. Sludge samples were also collected from the Peppers Ferry Regional Wastewater Treatment Facility (PFRWWTF). If storage was required it occurred at 4 °C for no more than 1 day.

### *Measured Parameters*

Mixed liquor suspended solids (MLSS), chemical oxygen demand (COD), capillary suction time (CST) and the sludge volume index (SVI) were all measured according to Standard Methods (APHA 1995). Iron and aluminum were measured on a Perkin Elmer 5100 PC Atomic Adsorption Spectrophotometer, following acid digestion per USEPA Method 3050B (EPA 1996). Protein was measured utilizing the Hartree modification of the Lowry method (Lowry 1951; Hartree 1972), with absorbance being measured on a Beckman DU-460, and bovine serum albumin as the standard.

### *Fractionation Studies*

RAS was sheared in a KADY mill for 5 minutes reaching temperatures of 45 °C. The contents were split into 5 aliquots, then dosed with ferrous and ferric iron. Following iron addition the aliquots underwent centrifugation at 2830 x g for 4 minutes, followed by filtration through Whatman 934-AH, (1.5 µm), Fisher Brand Membrane Filters (0.45 µm), and ultrafiltration with nitrogen as the pressurizing gas through Amicon YM-1 and YM-30 filters.

### *Shear Tests*

Shear tests were conducted using a high-speed rotor-stator mill manufactured by Kady International. The mill, Model L, can process up to 3L of sludge on a batch basis at a  $G = \sim 11,000\text{s}^{-1}$ . The shear in the mill is such that cooling is required to prevent excess heating. Without cooling, the contents of the sample vessel will boil after 20 minutes.

The purpose of the sludge testing using the KADY mill was to determine if sufficient shear could be provided to deflocculate activated sludges and thereby make them more biodegradable (Muller and Novak, 2001, in preparation). Samples of activated sludge were milled at different times and then either mixed with fresh activated sludge and aerated to evaluate biodegradability or aerated with addition of additional unmilled activated sludge. The sludges were also characterized with regard to their CST and settling characteristics during these tests.

### *Soluble COD Uptake*

The uptake of soluble chemical oxygen demand (SCOD),  $< 0.45\ \mu\text{m}$ , was measured in aerobic batch reactors that were composed of mixed liquor (80% reactor volume) and RAS sheared in a KADY mill (20% reactor volume). Each reactor was dosed with a ferric or ferrous sulfate at the initiation of the 2-hour aeration period. Samples were taken immediately after iron addition and every 30 minutes for the duration of the study. The sheared RAS was generated using a KADY mill.

### *Error Bars*

All error bars presented in this manuscript represent +/- one standard deviation.

## **Results and Discussion**

### *Iron and Floc Component Interactions*

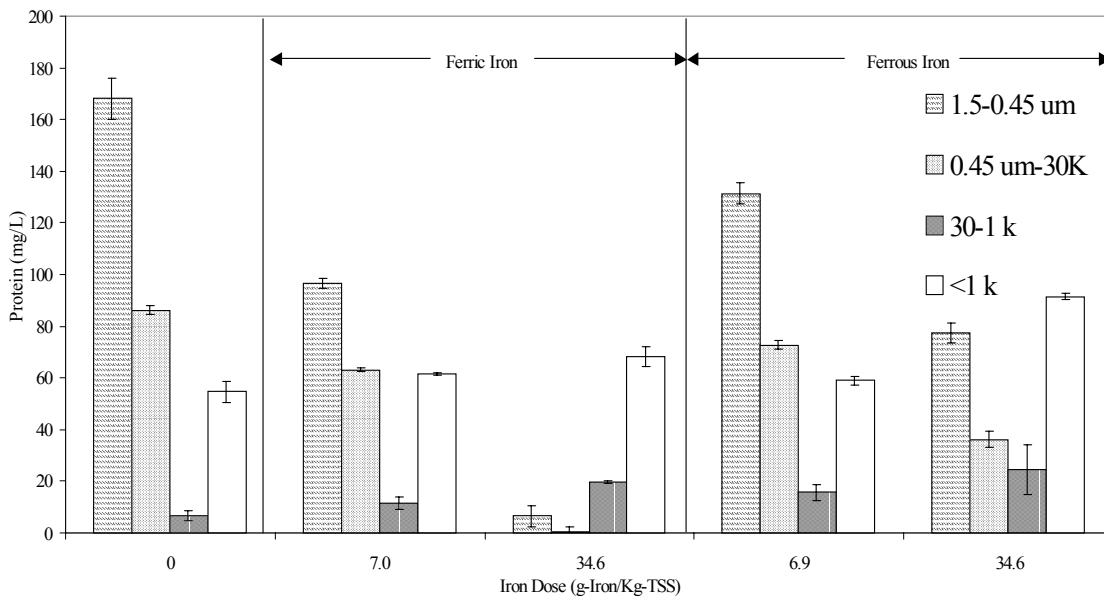
When iron undergoes reduction in activated sludge deflocculation occurs (Caccavo 1996; Nielsen 1998; Wilén 2000). Activated sludges from BVPIWWTP and PFRWWTF, when held in the absence of air, were found to release material that was  $\sim 2/3$  protein and  $\sim 1/3$  polysaccharide in composition (Rust 1998). Iron has been shown to selectively remove protein from solution when added to autothermal thermophilic aerobic digester (ATAD) sludges (Murthy et al, 2000). These observations when taken in total would suggest that iron and protein are intimately involved in floc structure.

The selective binding of proteins by iron and the  $\sim 2/3$  protein content found in released biopolymer would suggest that iron is bound exclusively to the proteinaceous fraction of the activated sludge floc structure. This is supported by the fact that deflocculation has been observed when iron is reduced by a variety of mechanisms, as previously noted. However the interactions between protein and the different iron species is not known.



To further investigate iron and protein interactions high shear was employed to disperse the activated sludge floc matrix of BVPI WWTP sludge. Following dispersion dosing with ferric sulfate and ferrous sulfate occurred at similar mg-Fe/Kg-TSS levels. The addition of the two iron species allows for comparison of the effectiveness of each in binding protein.

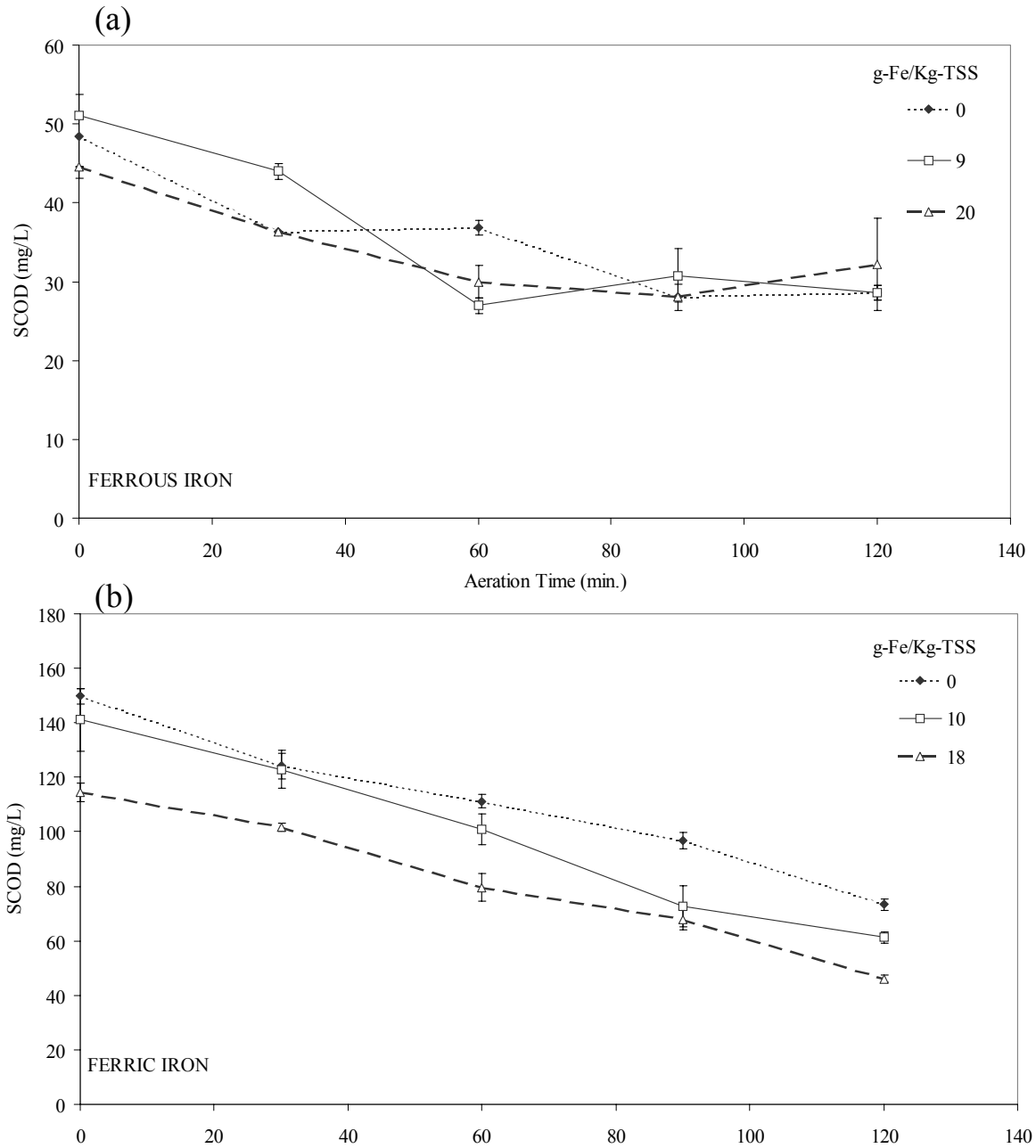
The data in Figure 1 shows a removal of protein in the 1.5µm-0.45µm and 0.45 µm-30K, for both iron species. The smallest size fractions, 30 K-1 K and <1K, were unaffected by iron addition. Interestingly at similar iron doses the effectiveness of ferric iron over ferrous is noticeable. Ferric iron removed 96.1% and 99.2% of the 1.5µm-0.45µm and 0.45 µm-30K size fractions while ferrous iron removed 54% and 58% of the 1.5µm-0.45µm and 0.45 µm-30K size fractions at the 34.6 mg-Fe/kg-TSS dose. What the data suggests is that iron selectively binds to material that is in the 1.5µm-30K size range.



**Figure 1 Selective Removal of Different Protein Size Fractions at Varying Ferric and Ferrous Iron Doses;  $\alpha=0.05$**

The selective binding of iron to material greater than 30K in size would suggest that the biodegradable material, <30K, is unaffected by the addition of iron. This was observed in the soluble COD uptake during aeration of reactors that received sheared RAS and were dosed with either ferric or ferrous iron. As Figure 2 shows there is an initial uptake of SCOD, with the addition of iron. This was expected since soluble was defined as < 0.45 µm in size. However after the initial uptake of SCOD, due to iron addition there is continued uptake over the 2-hour aeration period due to biodegradation and reincorporation of substrate to the floc matrix. The SCOD uptake curves observed in

Figure 2 are similar uptake curves were noted by Muller and Novak (2001) in reactors receiving sheared sludge with out iron addition.



**Figure 2 Uptake of SCOD Produced by a KADYMill during Aeration After the Addition of Iron (a) Ferrous Iron (b) Ferric Iron**

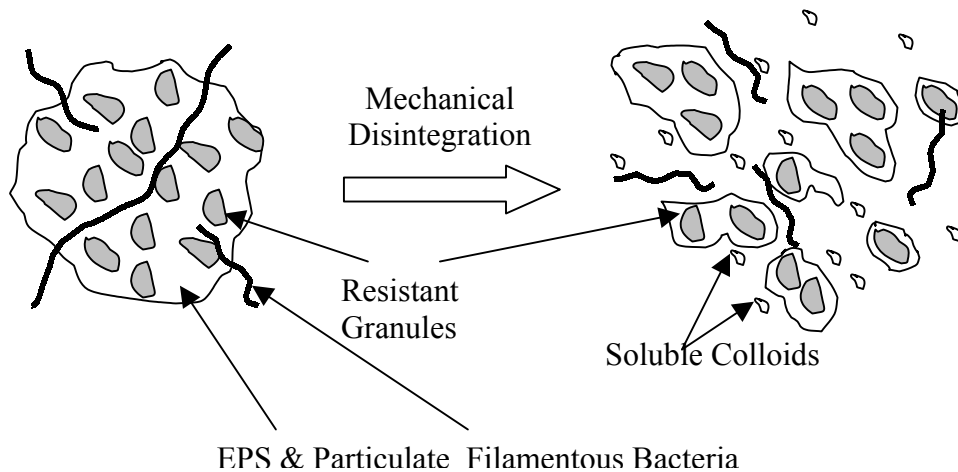
*Iron Distribution in Activated Sludge*

Investigations into high shear sludge disintegration using the KADY Mill,

$G = \sim 11,000 \text{ s}^{-1}$ , showed that significant lysis in activated sludge did not occur (Muller and Novak 2001). At that power input level, lysis would be expected. However the apparent binding of iron and protein in the colloidal range suggests that floc is somewhat different in structure than generally thought. It is hypothesized that iron and other high valent metals such as aluminum could be localized in high concentration in the flocs within bacterial aggregates. When activated sludge from BVPI WWTP was acid digested and analyzed by flame atomic adsorption, it was found to contain, 8.38 mg-Al/g-Solids and 5.15 mg-Fe/g-solids. The result of the presence of these metal-bacterial-protein aggregates would be granules that are more resistant to mechanical disruption within the floc.

Figure 3, is an illustration of the, hypothesized, granulized activated sludge floc, and its subsequent dispersion after the application of high intensity shear. The mechanical disintegration of activated sludge floc is such that the EPS, filamentous organisms, and particulate matter are dispersed. However the ferro-protein complexes afford the associated microorganisms some protection denoted by the gray darkened areas in the figure.

Tri-valent metals such as ferric iron and aluminum are present at concentrations of 8.38 mg-Al/g-Solids and 5.15 mg-Fe/g-solids however the distributions of these metal in the activated floc matrix are not well understood.



**Figure 3 Depiction of Ferro-Protein Granule Structure in an Idealized Activated Sludge Floc**

### *Iron effect on Settling, Dewatering and Floc Strength in Activated Sludge*

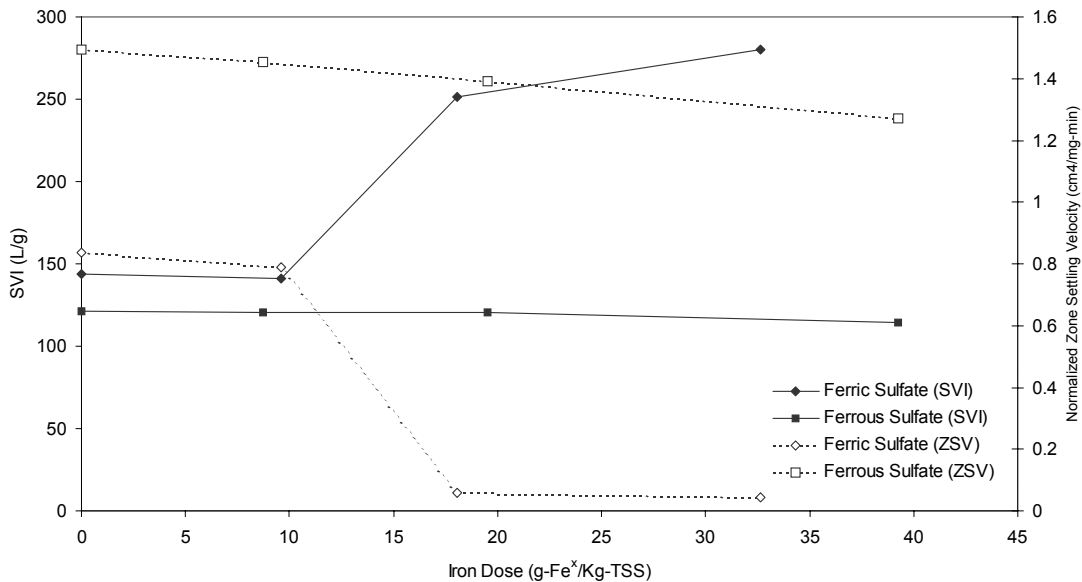
The selective removal of protein in the 1.5  $\mu\text{m}$ -30 K size range suggest that iron could be added to a wastewater stream to enhance flocculation and dewatering without detriment to the bioavailable fraction in solution. This may be particularly effective for systems with mechanically disintegrated or weak flocs.

Both ferric and ferrous irons were studied using ferric sulfate and ferrous sulfate. One-liter batch reactors were fed sludge milled in at KADY Mill for 15 minutes at 25% by volume. The SVI was measured after 2-hours of aeration.

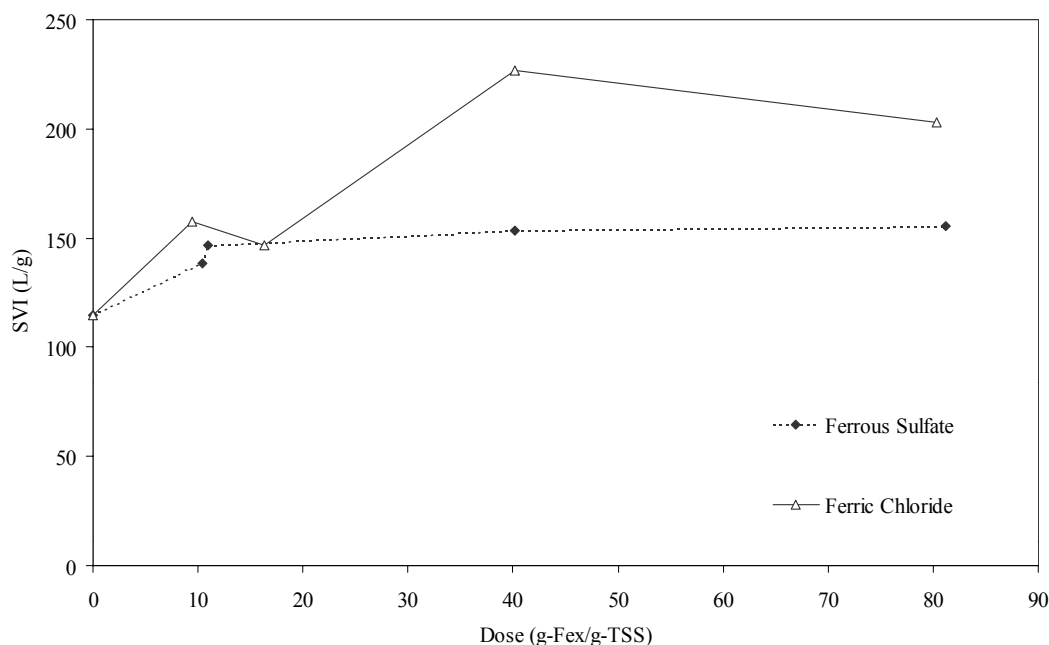
The SVI data are summarized in Figure 4. What can be readily observed is that ferric iron serves to increase the SVI at doses above 10 g-Fe/Kg-TSS, while ferrous iron does not appear effect SVI appreciably at any dose above 13.5 g-Fe/Kg-TSS. This trend of increased SVI was also observed in reactors that were dosed with ferrous sulfate and ferric chloride after 4-hours of aeration, in reactors receiving 25% by volume milled RAS (Figure 5).

The apparent deterioration in settling of the sludge would suggest that ferrous iron would be a better candidate for the strengthening of sludge flocs and recovery from mechanical disintegration. However, referring back to Figure 1, the enhanced removal of colloidal material at similar iron doses gives a possible explanation for this phenomenon. The increased binding capacity of the ferric iron removes more material and places it back with the floc. The material from the disintegrated sludge when added to the existing flocs would not be bound as tightly thus decreasing the overall density of the floc. Essentially the bound material serves to retard settling and thus compaction. Ferrous dose not bind material as well and thus this effect is dampened.

This can be observed in ZSV data in Figure 4. At the 10 g-Fe<sup>3+</sup>/Kg-TSS dose the normalized zone settling velocity decreases from 0.79 cm<sup>4</sup>/mg<sup>4</sup>-min to 0.06 cm<sup>4</sup>/mg<sup>4</sup>-min, while there is little observed change in the ZSV over the entire dose range for the ferrous reactors.



**Figure 4** Effect of Ferric and Ferrous Sulfate on Reactors with 25% by Volume Sheared RAS



**Figure 5 Sludge Volume Index for Activated Sludge with 25% by Volume Disintegrated RAS, Dosed with Ferric Chloride and Ferrous Sulfate**

If the bonding of disintegrated material produces lower density flocs, what should be observed is an increased sensitivity to shear forces. During a four-hour aeration period, reactors receiving 20% by volume of sludge sheared for 7.5 minutes, CST was monitored. An increased sensitivity to shear should be reflected by an increase in CST as material is sloughed off the floc structure.

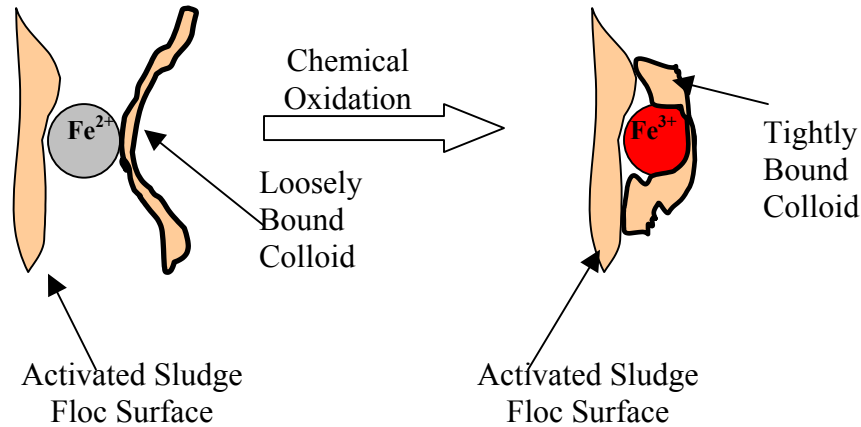
Over the four hour period there was an observed increased in the CST of all of the ferric reactors including the unsheared control (Table 1). In the ferrous dosed reactors only moderate increases in CST were observed. What the data suggest is that the material bound to the floc by the addition of ferric iron is not as well bound as that bound in biological flocculation.

**Table 1 Summary of Change in CST of Varying Iron Dosed Reactors Receiving Milled Product**

	Initial CST	Final CST	Increase %		Initial CST	Final CST	Increase %
Ferrous Sulfate g-Fe/Kg-TSS	Seconds	seconds	%	Ferric Sulfate g-Fe/Kg-TSS	seconds	seconds	%
Non Sheared	11.3	15.05	33	Non-sheared	12.4	16.6	34
14	16	18.3	14	10	32.6	39	20
27	16.05	15	-7	20	18.8	30.9	64
55	14.7	15.2	3	30	12.7	21.1	66

Figure 1 demonstrated ferrous iron removes material in the 1.5 $\mu$ m-0.45 $\mu$ m size range to a lesser extent than that of ferric iron, however there appears to a resistance to shear imparted (Table 1), even over that of sludge the control sludge, 33% increase in CST.

A possible explanation for the observed resistance to shear in ferrous iron flocculated sludges lies in the chemistry of iron. The addition of iron in its ferrous state, assuming complete mixed reactors, will bring the iron in contact with both disintegrated material and activated sludge flocs. The reaction kinetics between the organics and the ferrous iron could be faster than those observed for iron going from ferric to the ferrous state. Thus ferrous iron could bind colloidal material to the floc becoming entrapped and then transforming to the ferric state in this bound condition. Therefore increasing the bond strength between the added material and the floc as illustrated in Figure 6.



**Figure 6 Increased Floc Strength By Ferrous to Ferric Redox Chemistry in Floc Structure**

Assuming this ferrous to ferric bonding is occurring on the surface of the floc, it would explain the observed settling trends in Figures 4 & 5. The binding of colloidal material more tightly to a floc would serve to decrease the surface area to volume ratio making the floc more dense thus would be able to settle more quickly and therefore the extreme changes in SVI and ZSV would not be observed.

Though this data is preliminary and requires extensive follow up work it does suggest that metals such as iron can play a critical role in floc structure and the observed properties of sludges in wastewater. More importantly it is the chemistry that each is capable of that gives flocs their unique properties.

## Conclusions

The role of iron and other metals in sludge flocs requires extensive research, which may explain how and why certain floc attributes are observed. The selective removal of proteins by iron in solution suggests that that particular selectivity would hold for the floc structure itself. More importantly iron selectively titrates proteins in the 1.5  $\mu\text{m}$ –30 K range which is considered to be important in dewatering properties for colloidal material, particularly proteins, are thought to control filtration.

Iron, whether ferric or ferrous removes protein in the 1.5  $\mu\text{m}$  -30 K size range, however their apparent effects on dewatering and settling are markedly different. Ferric iron at doses above 10 g-Fe<sup>3+</sup>/Kg-TSS to 18 g-Fe<sup>3+</sup>/Kg-TSS was found to increase SVI, 78% and reduce ZSV by 93 %. Ferrous iron showed no such trend, at all dose ranges, however fractionation studies showed that similar doses of ferrous iron does not remove as much material as ferric. Though not as much material is removed there appears to be an increase in floc strength denoted by a decrease in shear sensitivity. The CST data for ferrous iron showed a decrease in sensitivity below that of the parent mixed liquor and well below that of ferric iron.

Though this data is preliminary in nature it raises some interesting questions about floc structure. Does ferric and ferrous iron chemistry affect floc structure to a significant degree? Are there portions of the activated sludge floc that are resistant to mechanical disintegration? Do metals play a role in this resistance? Can some inferences about settling and dewatering be made based on internal floc iron concentrations and can influent metals concentration be utilized to predict settling and dewatering properties? The answers to these questions will be useful for providing a full description of floc structure and in gaining insight into the role of metals in conditioning, dewatering and digestion of sludges.

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## Appendix A: Data Associated with Chapter 2

### Soluble Chemical Oxygen Demand Uptake Data (FIGURE 1)

Time minutes	Control 1 mg/L as SCOD	Standard Deviation mg/L	15 Minute Shear (54 C) mg/L as SCOD	Standard Deviation mg/L
0	19.728	2.357	149.80	2.83
30	21.905	8.218	124.22	4.71
60	22.993	0.943	111.16	2.49
90	22.449	1.633	96.73	2.94
120	14.286	1.633	73.33	2.05

Time minutes	Control 2 mg/L as SCOD	Standard Deviation mg/L	15 Minute Shear (42 C) mg/L as SCOD	Standard Deviation mg/L
0	31.48	0.00	85.51	0.91
45	19.67	3.93	65.70	1.67
60	23.87	3.18	60.33	2.27
90	22.30	2.27	51.15	6.81
120	24.39	2.84	51.15	3.93

### Specific Oxygen Uptake Rate and SCOD Uptake Experiment (FIGURE 2)

Time (hrs)	RAS Filtrate			Mill Filtrate		
	SCOD (mg/L)	Standard Deviation mg/L	SOUR mg O <sub>2</sub> / g MLSS-hr	SCOD (mg/L)	Standard Deviation mg/L	SOUR mg O <sub>2</sub> / g MLSS-hr
0	33.8	4.9	6.7	134.0	4.5	22.7
1	27.9	1.5	8.7	74.6	2.3	19.8
2	30.4	5.4	8.5	59.6	4.5	11.2
3	31.7	4.7	8.6	58.8	2.0	10.6

**Relationship Between SCOD and SOUR for Mill Product Added to Batch Aerobic Reactors (FIGURE 3)**

<b>Soluble COD</b> <b>mg/L as COD</b>	<b>S.O.U.R.</b> <b>mg-O<sub>2</sub>/g-MLSS-hr</b>
134.0	22.7
74.6	19.8
59.6	11.2
58.8	10.6
86.0	28.9
47.8	14.5
42.7	9.7
29.1	8.6
36.9	8.4
35.0	6.9

**Release of DNA from Activated Sludge by Shearing and the Addition of Cation Exchange Resin (FIGURE 4)**

	<b>Mean</b> <b>Absorbance</b> <b>260 nm</b>	<b>DNA</b> <b>µg/L</b>	<b>Standard</b> <b>Deviation</b> <b>µg/L</b>
Non-Sheared	0.00	-0.14	0.10
Sheared	0.13	6.32	0.69
CER	0.15	7.42	0.78

CER = Cation Exchange Resin

**Glucose-6-Phosphate Dehydrogenase Activity Data of RAS Shear in a KADY Mill  
(FIGURE 5)**

Adsorbance @ 340 nm

<b>Time (sec)</b>	<b>Non- sheared</b>	<b>Sheared</b>	<b>20 units/ml G-6- PDH</b>
0	0.001	0.0004	0.0008
5	-0.003	0.0004	0.0003
10	0.0004	0.0003	0.0008
15	0.004	0.0007	0.0017
20	0.0106	0	0.0025
25	0.0096	0.0004	0.0028
30	0.0069	0	0.0033
35	0.0029	-0.0001	0.004
40	0.0008	-0.0001	0.0044
45	-0.0003	-0.0002	0.005
50	-0.0002	0	0.0061
55	-0.0002	-0.0002	0.0072
60	-0.003	-0.0003	0.0075
65	-0.0005	-0.0001	0.0082
70	-0.0002	-0.0003	0.0087
75	-0.0005	-0.0003	0.009
80	-0.0005	-0.0003	0.0096
85	-0.0005	-0.0005	0.0102
90	-0.0005	-0.0006	0.01
95	-0.0005	-0.0004	0.0109
100	-0.0005	-0.0004	0.011
105	-0.0007	-0.0005	0.0116
110	-0.0006	-0.0006	0.0125
115	-0.0008	-0.0006	0.0132
120	-0.0005	-0.0007	0.0141
125	-0.0007	-0.0009	0.015
130	-0.0005	-0.0008	0.0154
135	-0.0007	-0.0006	0.0162
140	-0.0008	-0.0008	0.017
145	-0.0005	-0.0008	0.0175
150	-0.0008	-0.0006	0.0182
155	-0.0006	-0.0009	0.0184
160	-0.0008	-0.0009	0.0189
165	-0.0005	-0.0007	0.0192
170	-0.0008	-0.0008	0.0196

175	-0.0005	-0.0008	0.02
180	-0.0008	-0.001	0.0205
185	-0.0009	-0.0008	0.0212
190	-0.0008	-0.001	0.0218
195	-0.0009	-0.0012	0.0226
200	-0.0008	-0.001	0.0233
205	-0.001	-0.0008	0.0242
210	-0.0009	-0.0009	0.0247
215	-0.001	-0.0008	0.0254
220	-0.0008	-0.0006	0.0262
225	-0.0006	-0.0008	0.0267
230	-0.0006	-0.0008	0.0274
235	-0.0007	-0.0006	0.028
240	-0.0006	-0.0009	0.0289

**Uptake of Protein, Polysaccharide and SCOD during Aeration after Shearing in a KADY Mill (FIGURE 6)**

<b>Time</b>	<b>Polysaccharide</b>	<b>Standard Deviation</b>
<b>hr</b>	<b>mg/L</b>	<b>mg/L</b>
0	13.64	0.52
1	10.53	0.22
2	9.31	0.63
3	8.93	0.27
4	9.03	1.55

<b>Time</b>	<b>SCOD</b>	<b>Standard Deviation</b>
<b>hr</b>	<b>mg/L COD</b>	<b>mg/L COD</b>
0	49.56	5.41
1	34.47	3.25
2	23.02	5.41
3	21.72	3.15
4	21.98	8.60

<b>Time hr</b>	<b>Protein mg/L</b>	<b>Standard Deviation mg/L</b>
0	39.96	2.77
1	24.21	1.35
2	8.80	1.33
3	7.23	3.25
4	14.03	5.20

**Effect of Multiple Shear Events in a KADY Mill on the Release of Supernatant COD and SOUR of RAS (FIGURE 7)**

<b>Mill #</b>	<b>Supernatant COD mg/L as COD</b>	<b>Standard Deviation mg/L as COD</b>
1	429	2.8
2	84.5	1.4
3	49.6	2.3

Supernatant Addition Reactor (Experimental)

<b>Mill</b>	<b>Slope mg/l-min</b>	<b>MLSS mg/L</b>	<b>MLVSS mg/L</b>	<b>S.O.U.R. mg-O<sub>2</sub>/g- biomass-hr</b>	<b>SOUR Elevation Above Endogenous %</b>
1	0.977	2640	2220	26.4	219.7
2	0.360	1550	1320	16.4	68.2
3	0.344	2415	2060	10.0	4.2

Mixed Liquor Only Reactor (Control)

<b>Mill</b>	<b>Slope mg/l-min</b>	<b>MLSS mg/L</b>	<b>MLVSS mg/L</b>	<b>S.O.U.R. mg-O<sub>2</sub>/g- biomass-hr</b>
1	0.349	3145	2535	8.26
2	0.411	3145	2535	9.74
3	0.406	3145	2535	9.61

**Release of SCOD as a Function of Shearing Time and Temperature in a KADY Mill  
(FIGURE 8)**

**35 °C Milling Data**

<b>Time min</b>	<b>SCOD mg/L</b>	<b>Standard Net Release Devaitio</b>	
		<b>SCOD mg/L</b>	<b>SCOD mg/L</b>
0	15.1	0.0	1.6
5	325.3	310.2	7.8
10	373.3	358.2	4.6
20	473.7	458.6	7.9
40	553.4	538.3	0.0
60	603.0	587.9	21.5
90	637.1	622.0	10.5

**45 °C Milling Data**

<b>Time min</b>	<b>SCOD mg/L</b>	<b>Standard Devaitio</b>	
		<b>SCOD mg/L</b>	<b>SCOD mg/L</b>
0	19.8	0.0	0.0
5	353.1	333.2	50.5
10	380.8	361.0	6.9
20	558.0	538.2	35.8
40	732.6	712.7	25.5
60	938.0	918.1	6.9
90	981.2	961.3	18.3
120	975.9	956.0	21.0

**29 °C Milling Data**

<b>Time min</b>	<b>SCOD mg/L</b>	<b>Standard Devaitio</b>	
		<b>SCOD mg/L</b>	<b>SCOD mg/L</b>
0	50.0	0.0	6.7
1	211.8	161.9	9.6
5.4	330.8	280.9	16.9
10	372.9	322.9	7.0
20	454.6	404.6	8.3
30	493.1	443.1	4.0
60	570.0	520.1	11.7
90	627.2	577.2	8.5
120	667.2	617.3	7.0

**Effect of Anoxic/Anaerobic Storage Storage on SCOD Release by a KADY Mill (FIGURE 9)**

<b>Time d</b>	<b>Total COD mg/L</b>	<b>Milled Soluble mg/L</b>	<b>Unmilled Soluble mg/L</b>	<b>Anoxic Percent Soluble %</b>	<b>Mill Percent Soluble %</b>
0	4122.03	306.44	30.92	0.00	6.73
1	4393.22	398.64	42.31	0.26	8.19
2	4040.68	314.03	36.34	0.14	6.93
4	3834.58	382.37	115.53	2.22	7.18
5	4111.19	379.93	96.54	1.61	7.06

**Effect of High Intensity Shear on Anaerobic Digester Gas Production (FIGURE 10)**

<b>Time hrs</b>	<b>Control 1 ml</b>	<b>Control 2 ml</b>	<b>Mean Production ml</b>	<b>Sheared 1 ml</b>	<b>Sheared 2 ml</b>	<b>Mean Production ml</b>
0.017	0	0	0	0	0	0
12.3	70	72.5	71.25	112.5	109	110.75
15.8	82.5	92.5	87.5	142.5	139	140.75
19.2	96	112.5	104.25	170	164	167
23.1	111	129.5	120.25	212.5	199	205.75
36.2	236	236	236	363.5	359	361.25
47.9	284	290.5	287.25	476.5	459.5	468
58.9	331	321	326	545	526.5	535.75
65.4	361	343.5	352.25	580	566.5	573.25

**The Effect of the Addition of High Intensity Shear on the Reduction of Volatile Solids during Anaerobic Digestion (FIGURE 11)**

<b>Time Days</b>	<b>Single Mill Reduction in Volatile Solids %</b>	<b>Control Reduction in Volatile Solids %</b>	<b>Multiple Mill Reduction in Volatile Solids %</b>
0	0	0	0
3.7	9.2	7.6	8.7
5.9	11.6	8.4	10.8
8.9	12.7	9.4	12.8
10.4	14.8	11.7	15.4
17.2	20.1	14.3	19.8

**The Recovery of Dewatering Properties with Aeration after Multiple Shearing Events as Measured by CST (FIGURE 12)**

<b>Time</b>	<b>Control CST</b>	<b>Sheared CST</b>
<b>hour</b>	<b>seconds</b>	<b>seconds</b>
*0	15.6	419.1
1	15.4	424.4
2	12.6	176.0
*2	14.1	841.0
3	17.1	282.8
4	17.2	153.3
*4	14.5	668.5
5	14.1	179.9
6	16.4	169.2

\*indicates shear event

**Effect of High Intensity Shear on Capillary Suction Time (FIGURE 13)**

*Ratio: RAS/MLSS*

<b>Time</b>	<b>1/0</b>		<b>1/1</b>	
	<b>Control CST</b>	<b>Experimental CST</b>	<b>Control CST</b>	<b>Experimental CST</b>
<b>hr</b>	<b>(sec)</b>	<b>CST (sec)</b>	<b>(sec)</b>	<b>CST (sec)</b>
0	17.2	1733.1	13.6	553.1
1	14.3	1403.9	13.2	76.9
2	13.9	792.3	16.2	55.9
3	17.2	768.9	16	35.5
4	14.2	578.9	14.4	29.8



## Appendix B: Data Associated with Chapter 3

### Fractionation of Proteins after Shear in a KADY Mill and the Addition of Iron: (FIGURE 1)

Sample (mg-Fe/Kg- TSS)	Standard		Ferric	Standard		Ferric	Standard
	0	mg/L-	Iron	mg/L-	mg/L-	Iron	mg/L-
Size	mg/L- Protein	mg/L- Protein	mg/L- Protein	mg/L- Protein	mg/L- Protein	mg/L- Protein	mg/L- Protein
1.5-0.45 $\mu\text{m}$	168.2	7.9	96.6	2.1	6.6	4.0	
0.45 $\mu\text{m}$ -30K	86.3	1.6	63.2	0.5	0.7	1.8	
30-1 k	6.8	2.0	11.6	2.4	19.8	0.5	
<1 k	54.7	4.1	61.7	0.5	68.4	3.9	

Sample (mg-Fe/Kg-TSS)	Ferrous		Ferrous	Standard	
	Iron	Standard	Iron	mg/L-	Standard
Size	6.9	mg/L-	34.6	mg/L-	mg/L-
	mg/L- Protein	mg/L- Protein	mg/L- Protein	mg/L- Protein	mg/L- Protein
1.5-0.45 $\mu\text{m}$	131.5	4.1	77.4	3.6	
0.45 $\mu\text{m}$ -30K	72.7	1.6	36.2	3.2	
30-1 k	15.7	3.1	24.5	9.5	
<1 k	58.9	1.6	91.4	1.2	

### Uptake of SCOD during Aeration in Aerobic Batch Reactors Receiving Sheared RAS from a KADY Mill and Dosed with Iron (FIGURE 2)

Ferric Iron Addition

g-Fe/Kg- TSS	0		10		18	
	SCOD	Standard	SCOD	Standard	SCOD	Standard
Time minutes	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
0	150	3	141	12	114	3
30	124	5	123	7	102	1
60	111	2	101	6	80	5
90	97	3	73	8	68	4
120	73	2	61	2	46	1

Ferrous Iron Addition

g-Fe/Kg-TSS	0		9		20	
	SCOD mg/L	Standard Deviation mg/L	SCOD mg/L	Standard Deviation mg/L	SCOD mg/L	Standard Deviation mg/L
Time minutes						
0	48	5	51	0	45	0
30	36	0	44	1	36	0
60	37	1	27	1	30	2
90	28	2	31	3	28	0
120	29	1	29	1	32	6

**Effect of Immediate Iron Dosage to Aerobic Batch Reactors on SVI and ZSV (FIGURE 4)**

**Ferric Sulfate**

Iron Dose g-Fe/Kg-TSS	30-minute		S.V.I. L/g	ZSV cm/min	Normalized ZSV on TSS cm <sup>4</sup> /mg-min
	Volume mL/L	TSS mg/L			
0	400	2417	166	0.805	0.33
0	320	2220	144	1.855	0.84
10	328	2327	141	1.8375	0.79
18	544	2163	251	0.1225	0.06
33	672	2397	280	0.105	0.04

**Ferrous Sulfate**

Iron Dose g-Fe/Kg-TSS	30-Minute		S.V.I. L/g	ZSV cm/min	Normalized on TSS cm <sup>4</sup> /mg-min
	Volume ml/L	MLSS mg/L			
0	224	1967	114	2.6425	1.34
0	224	1851	121	2.765	1.49
9	224	1853	121	2.695	1.45
20	224	1863	120	2.59	1.39
39	224	1957	114	2.485	1.27

**Effect of Iron Addition to Aerobic Batch Reactors on SVI After 4-hour Aeration Period (FIGURE 5)**

**Ferric Chloride**

<b>Iron Dose lb Fe/lb solids</b>	<b>g-Fe/Kg- TSS</b>	<b>30 minute Settled Volume</b>	<b>TSS</b>	<b>SVI</b>
		<b>ml/L</b>	<b>mg/L</b>	<b>L/g</b>
0	0	212	1851	115
0.009	9.4	292	1851	158
0.016	16.3	272	1851	147
0.040	40.1	420	1851	227
0.080	80.4	376	1851	203

**Ferrous Sulfate**

<b>Iron Dose g-Fe/Kg- TSS</b>	<b>30-Minute Settled Volume</b>	<b>TSS</b>	<b>SVI</b>
	<b>ml/L</b>	<b>mg/L</b>	<b>L/g</b>
0	224	1967	114
0	224	1851	121
9	224	1853	121
20	224	1863	120
39	224	1957	114

**Summary of Change in CST of Varying Iron Dosed Reactors Receiving Milled Product (TABLE 1)**

<b>Ferrous Sulfate g-Fe/Kg- TSS</b>	<b>Initial CST</b>	<b>Final CST</b>	<b>Increase</b>	<b>Ferric Sulfate g-Fe/Kg-TSS</b>	<b>Initial CST</b>	<b>Final CST</b>	<b>Increase</b>
	<b>Seconds</b>	<b>seconds</b>	<b>%</b>		<b>seconds</b>	<b>seconds</b>	<b>%</b>
Non							
Sheared	11.3	15.05	33	Non-sheared	12.4	16.6	34
14	16	18.3	14	10	32.6	39	20
27	16.05	15	-7	20	18.8	30.9	64
55	14.7	15.2	3	30	12.7	21.1	66

## **VITA**

### Christopher Dustin Muller

Christopher Dustin Muller was born on August 1, 1973 in New Hampshire. He graduated Manchester High School West in Manchester, NH in 1992. Following graduation, he attended the University of New Hampshire, in Durham, NH, receiving a Bachelors of Science in Marine and Freshwater Biology, in May of 1996. After graduating from UNH, he worked a short stint in the aquaculture industry and then enrolled at Virginia Polytechnic Institute and State University in August 1998 to begin masters work. Upon completion of his Masters, he plans to pursue a doctorate in the same field at VPI & SU.