ANAEROBIC TREATABILITY OF AN INDUSTRIAL INK REMOVAL WASTE STREAM

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NOMENCLATURE

ATA	anaerobic toxicity assay
BOD	biodegradable oxygen demand
CGP	cumulative gas production
COD	carbonaceous oxygen demand
CTAB	cetyltrimethylammonium bromide
DA	defoaming agent
HPLC	high performance liquid chromatograph
MCR	master culture reactor
mg/L	milligrams per liter
mL	milliliters
mM	millimolar
MRR	maximum rate ratio
NMB	nutrient mineral buffer
POTW	publicly owned treatment works
QAS	quaternary ammonium surfactant
SB	soaking bath
TOC	total organic carbon
WB	washing bath

CHAPTER I

INTRODUCTION

DEVELOPMENT OF RESEARCH

Industries that use plastic packaging are seeking to develop ways to efficiently and economically maximize the use of printed plastic materials by extending their life through possible reuse alternatives. Studies at the University of Oklahoma have been performed to determine the most efficient chemicals that are capable of deinking the printed plastics. Pilot scale research has shown that relatively small concentrations of a cationic surfactant, cetyltrimethylammonium bromide (CTAB), in conjunction with a defoaming agent can adequately deink plastic samples at pH levels of 11 to 12. The process involves soaking, washing, and dual rinse cycles that generate a liquid waste stream. The impact of this waste stream on typical biological wastewater treatment processes is very important to determine the potential impacts on both an industrial pretreatment plant and a Publicly Owned Treatment Works (POTW). The major component of concern is the CTAB, because it is the component which has the least amount of information known, and is potentially the most toxic.

CTAB is a cationic surfactant that has been typically used as a disinfectant or an emulsifying agent. CTAB is a highly purified, homogenous material that has been used for academic purposes in research over the past 30 years. CTAB falls into the category of

quaternary ammonium surfactants, which are primarily used as fabric softeners and disinfecting agents (Boethling, 1984).

Several studies have been performed on the aerobic treatability of CTAB in both lab scale and full scale systems (Pitter, 1961; OECD, 1976; Boethling, 1984; Dean-Raymond and Alexander, 1977; Larson and Vashon, 1983; Wierich and Gerike, 1978; van Ginkel and Kolvenbach, 1991; Ueno and Yokoya, 1996). There has been relatively little study of the effects of CTAB in anaerobic environments. For this reason, this paper will focus on the toxicity of CTAB, as well as the fate of CTAB under anaerobic conditions.

The anaerobic toxicity of CTAB needs to be determined in order to ascertain the potential negative impacts on anaerobic treatment processes. Anaerobic toxicity is defined as the adverse effect of a substance on methanogens, the predominant microbial species under anaerobic conditions (Owen et al., 1978). This toxicity can be exemplified by a simple procedure outlined by Owen et al. (1978), known as an anaerobic toxicity assay (ATA), where the rate of gas production of a reactor that contains a test chemical is compared to a control in the absence of the test chemical. These assays can be performed in batch or continuous reactors. Batch reactors have been chosen for this analysis, because they produce a relatively fast, reliable, and cost effective method to determine toxicity (Shelton and Tiedje, 1984). A decreased rate of gas production relative to an active control indicates an inhibitory test substance. The results are very important in determining the concentration of a substance that could exhibit toxic effects on an anaerobic treatment process.

OBJECTIVES

The concentration of this research was on the following:

1. Determine a toxicity threshold concentration of CTAB under methanogenic conditions.
2. Determine the biological treatability of simulated waste streams containing removed ink residue, a defoaming agent, and CTAB under methanogenic conditions.
3. Determine the fate of the aforementioned components in the simulated ink removal waste stream in anaerobic treatment processes.
4. Determine the fate of CTAB in a simulated wastewater treatment system.

CHAPTER II

LITERATURE REVIEW

The following literature review will briefly describe the uses of CTAB and biodegradation studies that have been performed on CTAB. Also covered will be a discussion of the adsorptive properties, complexing with anionic surfactants, and the influence of toxicity on biodegradation, and antimicrobial resistance. Finally, there is a discussion of biodegradation studies on CTAB under both aerobic and anaerobic conditions and the fate of CTAB in engineered systems.

CETYLTRIMETHYLAMMONIUM BROMIDE

Cetyltrimethylammonium bromide, CTAB, is a cationic surfactant typically used as a disinfectant or an emulsifying agent. CTAB is also known as either hexadecyltrimethylammonium bromide (HDTAB or HTAB), or cetrimide. It will only be referred to as CTAB here.

As with all cationic surfactants, the surface-active properties are contained in the cation, or positively charged group. Cationic surfactants first became important when the commercial potential of their bacteriostatic properties was recognized (Jungermann, 1970). CTAB is a highly purified, homogenous material that has primarily been used for academic purposes in research over the past 30 years.

CTAB falls into the category of quaternary ammonium surfactants. Quaternary ammonium surfactants (QAS) are cationic surfactant salts of quaternary ammonium hydroxide, where the hydrogen and ammonium ions have been replaced with different combinations of alkyl groups (Sawyer et al., 1994). CTAB has a molecular weight of 354.45 g/mol. QAS, as well as other cationic surfactants, are compounds containing at least one hydrophobic long chain group usually derived from either a fatty acid or a petrochemical source and a charged nitrogen. Quaternary ammonium compounds can be classified into one of four major categories: compounds with a single fatty alkyl chain, and compounds with two, three, and four long alkyl chains (Jungermann, 1970).

CTAB is one of the oldest known examples of surface-active quaternary ammonium compounds prepared by the alkylation of a low molecular tertiary amine, trimethylamine, with a fatty alkyl halide, hexadecyl bromide (Jungermann, 1970).

$$C_{16}H_{33}Br + N(CH_3)_3 \rightarrow C_{16}H_{33}N^{+}(CH_3)_3 \bullet Br^{-}$$

Since alkyl halides required for this process are derived from the corresponding fatty alcohol which in turn is obtained via hydrogenolysis of a fat, this process is rather awkward and somewhat expensive industrially (Jungermann, 1970).

Surfactant quaternary ammonium compounds have been used as fabric softeners, drilling muds, biocides, sanitizers, disinfectants, hair conditioners in shampoos and cream rinses, emulsifying agents and components of room deodorizers (Boethling, 1984). In the past, almost half of the QAS compounds have been added to laundry preparations as water softeners (Dean-Raymond and Alexander, 1977). The majority of cationic surfactants in laundry preparations and water softeners is expected to reach a Publicly Owned Treatment Works (POTW). Biodegradation studies that have been performed on

QAS compounds have primarily focused on concentrations that would be expected at wastewater treatment plants, where the concentration is typically in the range of 1-2 mg/L (Boethling, 1984). Studies for pretreatment plants with higher surfactant concentrations have not been as prevalent.

CTAB Biodegradation

There have been published studies on the biodegradation of CTAB, as well as many on various other types of QAS. Unfortunately not all of these studies were performed in a similar manner, making the results somewhat inconsistent. This has caused difficulties in interpreting results for several reasons. First, many studies have not distinguished removal by biodegradation and removal by sorption. Secondly, QAS's form complexes with anionic material which can lead to the confusion of complexation with primary degradation (Boethling, 1984). Therefore, before looking at the biodegradation of CTAB and quaternary ammonium compounds, it is important to understand the impact of adsorption and anionic complexation on CTAB removal.

Adsorption of CTAB

Many of the early biodegradation studies on cationic surfactants ignored the high sorption affinity of the compounds. Quaternary ammonium compounds are noted for characteristically strong adsorption to negatively charged surfaces (Karsa and Porter, 1994). QAS's strongly sorb to glass surfaces on test containers, to natural solids like clays, to bacterial cell walls, and to humic materials (Games et al., 1982; Salton, 1951).

The highly sorptive nature of QAS compounds complicates the fate and toxicity studies that have been performed in both the laboratory setting and in environmental systems.

Studies by Fujita and Koga (1961) investigated the binding of CTAB by yeast cells, in relation to the cation's germicidal action. Fujita and Koga found that adsorption of CTAB is very rapid, and was nearly completed in 2 minutes. The adsorption rate can be reduced by reducing the pH, which suggests that protons are competing for the same site to which the cell surface that the cation is attracted.

Other studies have shown that quaternary alkyl ammonium salts can be removed to an extent greater than 95% in less than 2 hours by adsorption onto sludge particles (Karsa and Porter, 1994). A major contributor to the rate of sorption is the solids content in the wastewater. It would seem apparent that the higher the solids concentration, the faster the sorption of CTAB, or any cationic surfactant, to solids. However, research by Games et al. (1982) indicates otherwise; they contend that adsorption is regulated by the types of solids. For example, an activated sludge would have a higher adsorption affinity than a raw wastewater with similar solids content because adsorption onto biological solids is apparently stronger than other types of solids.

Complexing with Anionic Surfactants

Many of the studies have been performed with the presence of anionic surfactants at concentrations similar to those found in raw wastewater. Typically in raw wastewater the ratio of cationic surfactants to anionic surfactants is on the order of 1:2. The presence of anionic surfactants is thought to reduce the toxicity by complexing the quaternary ammonium compounds, thus reducing their concentration in the water phase. In the

laboratory, these anionic surfactants can be precluded from the analysis, enabling the researcher to determine QAS removal efficiency in the absence of anionic surfactants. However, this can lead to difficult interpretation of cationic surfactant removal results in field-testing or in predicting an effluent concentration of a waste stream where the amount of anionic surfactants is unknown.

Influence of Toxicity on Biodegradation

In general, quaternary ammonium compounds have a broad spectrum of antimicrobial activity, with reported activity against both gram-positive and gram negative bacteria, yeast, mold, viruses, and protozoans (Swisher, 1987). The extent of these antimicrobial properties depends on the test conditions such as microbial density, presence of organic matter, temperature, exposure times, concentration, etc.

Another factor to consider in CTAB biodegradation studies is that at some concentration the QAS is inhibitory to biodegradation and/or toxic to microorganisms. One common element in CTAB degradation studies that cover a broad range of chemical concentrations, whether identified by the study or not, is that at some concentration the CTAB exhibits toxic characteristics that pose a significant risk to microorganisms in wastewater treatment systems.

The typical inoculum for aerobic and anaerobic laboratory biodegradation studies, activated sludge and digester sludge respectively, contains a limited diversity of microbial populations depending upon the characteristics of the influent waste stream. The microbial populations in POTW's are generally not the same for all treatment plants. However, most populations can be expected to be acclimated to low concentrations of

QAS's in sewage (van Ginkel et al., 1991). Nevertheless, sudden increases in QAS concentrations without proper acclimation can be fatal, even to microorganisms that are capable of degrading the test compound. Without knowing all of the chemicals and their respective concentrations in the waste stream at the POTW, it is difficult to assume that the microorganisms are acclimated to any given QAS, such as CTAB. For this reason it is important to understand the acclimation period required by microorganisms, as well as the concentration at which degradation is inhibited (Boethling, 1984).

Antimicrobial Resistance

The development of resistance to antibacterial agents has been widely reported since the early 1950's (Swisher, 1987). Accounts of QAS resistance have been reported in medical settings, where the QAS was used in sterilization, and also in meat and poultry processing plants. Resistance has been primarily associated with gram negative bacteria. This resistance to chemicals designed to be lethal to microorganisms can be explained by either intrinsic resistance or acquired resistance. Intrinsic resistance is related to the structural and chemical composition of the outer layers of the cells that may provide an effective barrier to the entry of antibacterial agents. Acquired resistance results from genetic changes in the bacterial cell by either a mutation or acquisition of genetic material from another cell (Swisher, 1987).

Biodegradation Studies

Test methods to determine the biodegradability of cationic surfactants generally include non-specific methods such as biological oxygen demand, carbon dioxide

production, and decrease in dissolved organic carbon. More specific analytical methods can be used to determine the concentration of quaternary ammonium salts, but caution should be taken when interpreting results. It should be noted that removal of a cationic surfactant from a test study does not indicate biodegradation without the accompaniment of the biochemical indicators previously mentioned.

Analytical methods for determining concentrations of quaternary ammonium compounds can be important in predicting expected effluent concentrations from laboratory analysis. One possible method is based on complexation with the colored anion, disulfine blue (Boethling, 1984). This complex can be extracted, and the color can be measured spectrophotometrically to determine concentrations. Another method of tracking surfactant removal is radiolabelling carbon on the compound and tracking its fate. A more recent analytical method uses a high-performance liquid chromatograph (HPLC) to determine concentration at a particular absorbance spectrum (Karsa and Porter, 1994). The HPLC method seems to be superior in sensitivity, specificity, and ease of performance when compared to the other methods (Boethling, 1984).

Aerobic Biodegradation

Studies performed on the aerobic degradation of CTAB vary greatly in their findings. Studies have shown results ranging from total inhibition to complete degradation. Some of the major inconsistencies between differing studies have been discussed in the previous section, such as confusing CTAB removal due to adsorption onto cellular solids as biodegradation.

A study by Pitter (1961) showed that CTAB could be effectively removed in a bench-scale activated sludge system only up to a concentration of 6 mg/l. When the CTAB concentration was raised to 20 mg/L, the sludge "lost activity" and flowed from the system. In Pitter's study, nitrification was strongly inhibited at 3 mg/L and completely blocked at 6 mg/L even though the microorganisms were still capable of reducing the BOD of the system. Pitter examined the wastewater effluent for content of CTAB, and found a very high removal percentage, but he did not attempt to account for adsorption in his experiments (Boethling, 1984).

An OECD Confirmatory Test (OECD) by Gerike, Fisher, and Jasiak (1978) to simulate activated sludge treatment demonstrates that CTAB was readily biodegradable at concentrations ranging from 5 to 15 mg/L. At 20 mg/L, the inhibitory limit had been reached and high levels of CTAB appeared in the effluent. The 15 mg/L sample had a retention time of 3 hours and achieved a dissolved organic carbon removal of 107% ± 19%, and with a retention time of 6 hours a DOC removal of 104% ± 6% with respect to the test substrate (Gerike et al., 1978). The results of this study neglected the possibility of adsorption of the compounds on the sludge, because only minor amounts of CTAB could be recovered from the sludge with a low pH methanol extracting solution.

Using standard BOD analytical techniques with a sewage inoculum, Dean-Raymond and Alexander (1977) found that microorganisms extensively metabolized CTAB at levels of 10 and 25 mg/L in time periods of 15 and 43 days, respectively. In a period of 60 days, it was found that a 100 mg/L initial concentration was not degraded at all. The lack of activity on the CTAB at a concentration of 100 mg/L was attributed to the toxicity at this higher concentration (Dean-Raymond and Alexander, 1977).

Larson and Vashon (1983) studied the kinetics of biodegradation of CTAB and other QAC's in natural water/sediment systems. They also attempted to quantify the adsorption of CTAB onto river water sediment using a modified form of the Freundlich isotherm to derive values for K_d, the solid/solution partition coefficient. Under the sediment conditions in river water, it was found that about 65% of the CTAB was bound to particulate matter (Larson and Vashon, 1983). Nevertheless, bound CTAB was readily accessible to degradative microorganisms, and sorption did not render it unavailable for biodegradation. These results differ from previous studies that stated that sorption of organics rendered the compounds unavailable for biodegradation (Larson and Vashon, 1983). The degradation rates of test chemical may, however, be dependent upon the sorption/partitioning properties. Based on the results of testing by Larson and Vashon (1983), extensive biodegradation occurs in river water samples with realistic environmental concentrations of less than 1 mg/L.

Boethling (1984) studied an activated sludge process with three QAC's, including CTAB, and determined that according to the mathematical model of Wierich and Gerike (1978), the removal by sorption could account for only 8-29% of the total elimination of the three tested QAC's, where removal in excess of 90% was observed. This study did not try to determine sorption in the laboratory analysis but based the sorption removal from the findings of Wierich and Gerike (1978).

In a relevant study by van Ginkel and Kolvenbach (1991), an inoculum of microorganisms was grown on CTAB in a carbon-limited continuous culture. The culture was acclimated to a feeding rate of 80 mg/L CTAB as the substrate and used to determine the biodegradation characteristics of other quaternary ammonium salts.

Neither the acclimation period nor the microbial populations in the study that were adapted to the high concentrations of CTAB were discussed in the paper.

In a recent study by Ueno and Yokoya (1996), the effects of CTAB on the metabolism of various microorganisms were investigated. The species resistance was correlated to the cell membrane permeability, with the microbial population of *Pseudomonas* described as the most resistant. The study showed that the maximum concentration of CTAB associated with enzyme activity was about 3 mg/L and inhibition of most species in aerobic conditions began at a concentrations between 5 and 10 mg/L.

Anaerobic Biodegradation

Considerably fewer studies have been reported that attempt to quantify the anaerobic degradation potential of quaternary ammonium surfactants. Karsa and Porter (1994) reported an analytical study which discovered that the concentration of QAS decreases only slightly in an anaerobic digester, implying minimal degradation. The study did not consider that the rate of biodegradation of QAS could be slower than typical digester sludge retention time allows; a high rate digester has a solids retention time of 10 to 20 days, while a single stage digester can have a solids retention time as long as 30 to 90 days (Tchobanoglous and Burton, 1991).

The generally accepted method of determining anaerobic degradation potential under methanogenic conditions is performed by comparing the cumulative net methane production of a compound to the theoretical methane potential. Battersby and Wilson (1983) employed a similar test method when comparing the degradation potential of many organic chemicals, including CTAB. They found that CTAB was initially

inhibitory to methanogenic degradation at a concentration of 50 mg of carbon per liter, which correlates to a total concentration of about 80 mg/L. They did not attempt to analytically track the fate of CTAB, but rather assumed that the biochemical indicator of gas production was a reliable indicator of anaerobic degradation. They found that CTAB required a detoxification or adaptation period of the sludge before any gas production took place. This was represented by the cumulative gas production curve, which showed an adaptation period where inhibition of gas production by CTAB caused a net negative gas pressure initially before any biogas was produced. The inhibitory effects of this study were hypothesized to be due to the lack of biodegradation of the surfactant at that concentration.

BEHAVIOR OF CTAB IN WASTEWATER TREATMENT

The presence of cationic surfactants has prompted extensive studies on the behavior of quaternary ammonium salts in activated sludge plants (Karsa and Porter, 1994). Acclimated biological wastewater treatment systems should generally remove QAS at nontoxic levels to an extent of 90% or better (Boethling, 1984). Removal normally occurs via a combination of biodegradation and sorption, with the majority of biodegradation occurring in sludge solids since sorption is typically faster than biodegradation (Karsa and Porter, 1994).

Primary Settling

The fact that CTAB is readily sorbed to solids causes a portion of the compound to be settled out in a primary settling unit. A study by Huber (1982) showed that 20-40%

removal of cationic surfactants was achieved in the primary clarifiers in full-scale activated sludge plants. The percent of CTAB sorbed to solids in the primary setting stage was not explicitly tested for, but the removal correlates to the fact that approximately 50-70% is a typical suspended solids removal efficiency in the primary settling phase of a treatment plant (Tchobanoglous and Burton, 1991).

Removal in Activated Sludge Reactors

The activated sludge treatment process has been investigated for the removal rates of quaternary ammonium surfactants in continuous activated sludge tests at concentrations ranging from 10-20 mg/L (Karsa and Porter, 1994). This range of surfactant concentration is much greater than the actual concentration in raw domestic wastewater, which is typically on the order of 1-2 mg/L. Nevertheless, removal has been greater than 95% in numerous studies. Since removal efficiencies were achieved within 3-24 hours, adsorption onto sludge particles seems to be the responsible mechanism. Because of low levels of cationic surfactants in waste activated sludge, it has been assumed that the majority of cationic surfactants are removed eventually through biodegradation, although often this biodegradation is not explicitly tested. Removal of CTAB from 91%-100% in continuous activated sludge (CAS) systems was reported by Karsa and Porter (1994).

Anaerobic Biodegradation

If anaerobic degradation of cationic surfactants is to play a role in wastewater treatment digesters, the digester must be capable of handling concentrations higher than

influent flow concentrations due to the fast adsorption rates and the slower degradation rates. The anaerobic degradation of cationic surfactants is an area where little research has been performed. It could be assumed, for the activated sludge treatment plants with anaerobic digesters that were tested with high levels of cationic surfactant, that if a major problem existed, then the anaerobic digesters would have been affected. No problems with the anaerobic digesters have been mentioned in systems that do contain low concentrations of cationic surfactants that have been found to be treatable. Further studies in this area should provide valuable information on anaerobic degradation, especially for industrial applications where high cationic surfactant concentrations could be expected.

CONCLUSION

Many studies have proven that by means of sorption and biodegradation, low levels of quaternary ammonium surfactants can be readily and efficiently attained in most wastewater treatment plants (Karsa and Porter, 1994; Games et al., 1982; Fujita and Koga, 1961). There are still many voids in the knowledge of biological interactions of CTAB in wastewater treatment. Studies have yet to find critical concentration ranges that are inhibitory or toxic to anaerobic degradation. The void in anaerobic studies could be very important if high adsorption rates are present at a plant; with an anaerobic digester receiving the greatest concentrations for the longest retention times.

CHAPTER III

MATERIALS AND METHODS

GENERAL METHODS

Plan of Research

The research for this paper was primarily focused on the anaerobic treatability of pure samples of cetyltrimethylammonium bromide (CTAB) and wastewater samples from the pilot scale deinking process at the University of Oklahoma. The anaerobic treatability was determined via an anaerobic toxicity assay (ATA) generally following the methods of Owen et al. (1978). In this procedure, gas production was measured from closed reactor bottles and methanogenic activity was determined by comparing the cumulative gas production of reactors with varying concentrations of CTAB, or dilutions of wastewater samples against controls. Also analyzed in this research was the effect adsorption had on a simulated activated sludge process. This aerobic adsorption study was performed by measuring total organic carbon (TOC) reduction. The presence of CTAB in the ATA reactors was quantified using a high performance liquid chromatograph (HPLC).

Materials

Deionized water was used for the nutrient/mineral/buffer stock solution, feedstock preparations, and subsequent reactor dilutions. The deionized water was Milli-Q water,

produced by a Milli-Q purification system through a deionization and reverse osmosis process.

The hexadecyltrimethylammonium bromide (CTAB) used in the all laboratory analysis was from Sigma Chemical Company. This CTAB was used to isolate the effects of CTAB from the effects of other wastewater sample constituents. The other chemicals needed in the preparation of the nutrient/mineral/buffer solution, as described under the Anaerobic Toxicity Assay section, were purchased from Fisher Chemicals and used as delivered.

All wastewater samples were collected from the University of Oklahoma in their deinking pilot plant. The four possible wastewater effluents were sampled from the pilot plant deinking process including a soaking bath, washing bath, and two rinsing baths. The soaking bath and washing bath contained CTAB to remove the ink from the plastic packaging, along with a defoaming agent to reduce the foaming of CTAB during agitation. The defoaming agent used was Trans-286 as received directly from the University of Oklahoma Laboratory. The ink removal process was performed at an elevated pH to approximately 12 using sodium hydroxide to help improve the deinking efficiency. The soaking and washing bath both consisted of a solution with 0.1% $^{w}/_{v}$ CTAB and 0.2% $^{w}/_{v}$ defoaming agent. The 0.1% $^{w}/_{v}$ CTAB correlates to a concentration of 1000 mg/L or 2.82 mM CTAB. The compositions of the wastewaters are shown in Table 3.1.

Table 3.1: Wastewater Characteristics

	CTAB ($^{w}/_{v}$ %)	Defoaming Agent ($^{w}/_{v}$ %)	COD	pH
Soaking Bath	0.1%	0.2%	2550 mg/L	11.8
Washing Bath	0.1%	0.2%	3155	11.3
Rinsing Bath 1	N/A	N/A	290	9.9
Rinsing Bath 2	N/A	N/A	25	7.6

Washing and Sterilization

All glassware was cleansed thoroughly with detergent, then rinsed with deionized water. Bioassay bottles were sterilized in an oven for not less than 60 minutes at a temperature of not less than 170 °C, as recommended by Standard Methods (1992).

pH Analysis

Fisher Accumet model 900 pH meters were used with electrodes calibrated by HACH Company buffer solutions with pH of 4.0, 7.0, and 10.0 before each use. The calibrated electrodes were typically placed directly into the master culture reactor for the pH readings.

Solids Analysis

Total solids and total volatile solids were analyzed according to the methods described in Standard Methods (1992). Porcelain dishes were thoroughly cleaned and ignited in a 550 °C ashing oven prior to the initial weighing. Samples were dried at 103-105 °C in a Thelco model 17 oven for at least two hours or until complete evaporation,

and then removed and allowed to fully cool in a dessicator. Volatile solids were analyzed by ashing at 550 °C for 30 minutes in a Lindberg Type 51894 oven.

COD Analysis

The COD analysis was performed according to the Reactor Digestion Method as described in Hach Water Analysis Handbook (Hach Chemical Co., 1992). The samples were incubated at 150 °C for two hours to insure complete digestion. The samples were then analyzed colorometrically at 620 nm in a HACH spectrophotometer.

HPLC Analysis

CTAB was analyzed by high performance liquid chromatography (HPLC) with a Beckman liquid chromatograph equipped with two model 127 solvent pumps, a model 166 absorbance detector set at 211 nm and a System Gold controller. The mobile phase was methanol-water (70:30 v/v). The flow rate was 1.0 mL/min. Centrifuged and filtered samples of 20 μL were injected into the Beckman C-18 Reverse phase, Ultrasphere ODS, 5 μm particle diameter, 4.6 mm x 25 cm column. The output was collected and integrated on a Hewlett Packard 3396a Series II integrator. The retention time for CTAB in the column was 2.8 minutes (Helboe, 1983).

Gas Chromatography

The biogas composition was analyzed from the reactors to ensure that the gas produced was due to biological and not abiotic activity, as determined by the presence and quantity of methane in the gas. Samples were taken from the reactors using a gas-

tight 5 mL syringe and injected directly into the Gow Mac model 350 thermal conductivity detector gas chromatograph. The chromatograph was fitted with a 6-foot stainless steel column packed with Porapak Q, 60/80 mesh. The temperature of the column was raised to 55 °C, the temperatures of the detector and injection port were maintained at 170 °C and 105 °C, respectively. The bridge current was maintained at 70 mA for the thermal conductivity detector. Helium was used as the carrier gas with a flow rate of 60 mL per minute. The sample size of 2 mL was used for all standards and reactor samples. Each time of use the instrument was calibrated with pure samples of methane to be used as the standard. A Hewlett Packard model 3380A integrator was used to interpret the output from the gas chromatograph.

Total Organic Carbon

The total organic carbon (TOC) was measured by a Shimadzu TOC-5000A Total Organic Carbon Analyzer with an ASI-5000A Autosampler. The TOC Analyzer was calibrated before each run with an organic carbon stock solution consisting of potassium bipthalate and an inorganic carbon stock solution that consisted of both sodium bicarbonate and sodium carbonate. Twenty-six microliter (26 µl) samples were injected into the analyzer, which was rinsed twice between each measurement.

ANAEROBIC TOXICITY ASSAYS

Bioassay techniques for measuring the presence of inhibitory substances and measuring the biodegradation potential are valuable in resolving anaerobic treatment problems because they are relatively simple and inexpensive. Batch assays were

performed on various test samples to evaluate their respective toxicity and biodegradability following the general procedure as outlined by Owen et al. (1978).

Master Culture Reactor

Primary digesting sludge was taken from the Stillwater municipal wastewater treatment plant and used as a seed for a master anaerobic culture maintained in a 20-liter continuously stirred tank reactor. The master culture reactor (MCR) was maintained at a temperature of 15-25 °C (unfortunately the room temperature was not consistent) with hydraulic and solids retention times of approximately 60 days. Once a positive pressure was attained in the reactor, gas was vented to a vacuum hood. Ample nutrients, minerals, and buffer capacity were incorporated in the MCR as described by Young and Khandaker (1992). A detailed list of the ingredients in the nutrient/mineral/buffer solutions that were added to the MCR is shown in Table 3.2.

Table 3.2: Nutrient/Mineral/Buffer Stock Solutions

Mineral Base I (Dilute to 1.0 L)

Mineral	Amount
$CoCl_2 \bullet 6H_2O$	0.25 g
$FeCl_2 \bullet 4H_2O$	2.0 g
$MnCl_2 \bullet 4H_2O$	0.05 g
H_3BO_3	0.025 g
$ZnCl_2$	0.025 g
$NaMoO_4 \bullet 2H_2O$	0.005 g
$NiCl_2 \bullet 6H_20$	0.025 g
Na_2SeO_4	0.025 g
$CuCl_2 \bullet 2H_20$	0.005

Mineral Base II (Dilute to 1.0 L)

Mineral	Amount
$CaCl_2$	38.0 g
$MgCl_2 \bullet 6H_2O$	50.0 g

Buffer Base (Dilute to 1.0 L)

Buffer	Amount
$NaHCO_3$	60.0 g

Nutrient Base (Dilute to 1.0 L)

Nutrient	Amount
KH_2PO_4	135 g
K_2HPO_4	175 g
NH_4Cl	53 g
Na_2SO_4	15 g

The MCR was prepared by adding 10 mL each of Mineral Base I, Mineral Base II and Nutrient Base, plus 100 mL of Buffer Base to each liter of diluted wastewater to be used as the seeding culture. The final wastewater dilution for inoculating the MCR was approximately 1 to 10.

A COD loading rate of 2 g/L-wk in the form of glucose was added to maintain microbial activity using a draw and fill procedure. The pH of the system was maintained between 6.7 – 7.4 with the addition of sodium bicarbonate when the pH dropped below 6.7.

Preparation of Test Reactors

Anaerobic toxicity was determined using 125 mL capacity serum bottles as described by Owen et al. (1978). The test compound was added initially as 10 mL in the

aqueous form, which included 50 mg/L of a readily degradable substance, glucose, which was used to ensure gas production and determine if inhibition took place. This was followed by the addition of 70 mL nutrient/mineral/buffer media, and 20 mL from the MCR. The MCR, which was being maintained by feeding with glucose, actually contained a much higher organic load than the aforementioned 50 mg/L addition of glucose that was added at the commencement of the assays. This initial residual organic load will be quantified in Chapter IV.

The headspace in the reactors was immediately purged for 5 minutes with argon, and finally the bottles were sealed using Supelco Teflon®/rubber septa and Supelco open center aluminum crimp seals. All test samples were tested in triplicate including the controls.

Incubation

After equilibration for one hour at incubation temperatures, excess pressure in the reactors was removed with a syringe and the bottles were ready for incubation and commencement of the test. Serum bottles were incubated in the dark in a Precision Scientific Co. Model 4 incubator at 35 ± 2° under quiescent conditions for a period of time until gas production had ceased for a two week period. Incubation at 23 °C was also tested in a Precision Scientific Model 805 incubator to determine if CTAB was more toxic at higher temperatures as previously reported (Jungermann, 1970).

Measurement of Gas Production

Gas produced by the microorganisms was measured once a week by manual means using either a 5-cc or 60-cc lubricated syringe depending on the amount of biogas present, so that the amount of biogas measured did not exceed the capacity of the syringe. The reactors were allowed to proceed for a duration long enough to ensure virtually complete decomposition of biodegradable organics as demonstrated by the cessation of gas production. Serum bottles were shaken vigorously before pressure measurement, and excess gas production was vented after measurement to avoid accumulation of gas pressures. During measurement of gas production, the syringe was held in a horizontal position while ensuring the needle stays within the gas space of the serum bottle. The syringe plunger was allowed to move freely to equalize the vessel and atmospheric pressures (ASTM, 1992). Negative pressure was not measured using the syringe and was recorded as zero gas production.

CHAPTER IV

RESULTS AND DISCUSSION

Anaerobic toxicity is defined as the adverse effect of a substance on the predominant methanogens (Owen et al., 1978). This can be exemplified by a simple procedure outlined by Owen et al. (1978), known as an anaerobic toxicity assay (ATA). In this procedure cumulative gas production (CGP) is measured from closed reactor bottles. A decreased rate of gas production in the test reactor bottles, relative to an active control, in the presence of an inhibitory test substance. The results are very important in determining the concentration of a substance that could exhibit toxic effects on an anaerobic reactor. The potential waste stream generated by the ink removal process consists of sodium hydroxide, CTAB, a defoaming agent, and the removed ink residue. The waste stream components analyzed individually to determine not only the potential anaerobic toxicity, but also the fate of the components after treatment in an engineered treatment system.

ANAEROBIC TOXICITY ASSAYS

The general procedure for determining the anaerobic degradability of a compound to CH_4 and CO_2 can be performed in a batch assay test similar to the anaerobic toxicity assay defined in the previous chapter. The gas production reported for the subsequent analyses will focus on the cumulative gas production (CGP) from a test reactor compared

to the CGP of a control. The method requires 50 mg/L carbon of an easily degradable source to promote biological activity in both the control and test reactors along with varying concentrations of the test chemical in the test reactors. Following the same procedure as Owen et al. (1978), the cumulative gas production is reported as total gas production, which is expected to include methane, carbon dioxide, as well as other minor constituents. The gas production from the reactors was periodically measured for methane using a gas chromatograph to ensure that the gas was due to methanogenic activity.

The cumulative gas production from sludge incubation with a test substance can be summarized by one of the following typical patterns shown in Figure 4-1. This figure is a modified version of a similar figure from Owen et al. (1978).

This figure can be generally summarized by the following:

1. If the cumulative gas production is similar to the control, then it can be said that the test substance is not inhibitory.
2. If the cumulative gas production is defined with an initial acclimation period with little gas production, followed by gas production that eventually equals that of the control, then it can be said that an acclimation period is required before methanogenic activity can take place in the presence of the test substance.
3. If the cumulative gas production of the reactor with the test chemical is not equal to that of the controls, then it can be said that the substance is partially toxic.
4. If a period of acclimation is followed by a cumulative gas production that does not reach that of the controls, then it can be said that the test substance requires an acclimation period and is partially toxic.
5. If there is little or no gas production throughout the test period, then the substance is inhibitory to biodegradation.
6. If the gas production of the reactor is greater than that of the control, then the test chemical is non-toxic and degradable.

Defoaming Agent Toxicity Study

In the deinking process a defoaming agent is added during the soaking and washing cycles in order to reduce the foaming of the surfactant in the waste stream during the agitation process. A toxicity study was performed on differing concentrations

of defoaming agent to determine if the substance would inhibit methanogenic degradation or be possibly degradable. The total gas production for each serum bottle is compared against the average of the controls containing glucose but no test chemical (defoaming agent) to generate a ratio designated as the maximum rate ratio (MRR), which is a percent of gas production for a sample versus the control (Owen et al., 1978). According to Owen et al. (1978), a MRR, or % of control, of less than 0.95 (or 95% of the control) suggests possible inhibition, and one of less than 0.90 (or 90% of the control) suggests inhibition.

The defoaming agent anaerobic toxicity assay lasted 146 days until no biogas was measured for a two week period. The cumulative gas production results (CGP) and percent methane composition of the biogas are shown in Table 4-1 and also plotted in Figure 4-2.

Table 4-1: Defoaming Agent Toxicity Assay

Sample	CGP (40 days) mL	% Methane	CGP (146 days) mL	% Methane	% of Control
Control	71.4	63.4	136.9	69.3	--
0.1% DA	80.6	61.8	138.0	56.7	100.8
0.5% DA	61.0	65.4	249.8	68.4	182.5
1.0% DA	154.8	71.9	328.4	68.1	239.9
2.0% DA	103.8	70.7	694.2	71.3	507.1

It can be seen from the data in Table 4-1, as well as Figure 4-2, on the following page, that as the amount of defoaming agent present in the batch assay was increased, the cumulative gas production of the reactors also significantly increased. It should be noted that the gas composition of the reactors, as analyzed on a gas chromatograph, consisted of

high methane content, which also indicates methanogenic activity. The control behaved in a reasonable manner which is discussed in a later section of this chapter as well as shown in Table 4-8. This leads to the conclusion that not only does the defoaming agent not inhibit anaerobic activity, but it is most likely readily converted to intermediates which serve as an energy source for methanogens.

CTAB Toxicity Study

Preliminary Studies

To test for CTAB toxicity, a wide spectrum of concentrations was selected to provide a range from non-inhibitory to severely toxic (Owen et al., 1978). Controls with no CTAB were also tested to produce the standard from which the inhibitory or non-inhibitory effects exerted by CTAB could be compared. Methane content in the gas was monitored periodically to ensure that the gas production was not abiotic.

After 146 days when gas production had ceased for two consecutive weeks in all of the reactors, the assay was considered finished. The results of the assay can be seen in Figure 4-2. The cumulative gas production data, as seen in Table 4-2, shows a vast difference in the gas production between the control and 0.01 mM CTAB reactors. However, it is very evident that the reactors with 0.05 mM CTAB, 0.10 mM CTAB, and 0.25 mM CTAB were inhibitory to gas production.

Table 4-2: Preliminary CTAB Toxicity Assay

Sample	CGP (40 days)	% Methane	CGP (146 days)	% Methane	% of Control
Control	168.3	72.1	332.8	69.2	--
0.01 mM CTAB	73.8	70.3	508.1	63.8	152.7
0.05 mM CTAB	5.9	25.8	6.0	11.5	1.8
0.10 mM CTAB	5.8	14.2	5.8	16.1	1.7
0.25 mM CTAB	3.7	26.4	3.7	12.4	1.1

Definitive results of this assay cannot be ascertained because of the inexplicable increase in gas production for both the control reactors and the 0.01 mM CTAB reactors. The gas production for the control was greater than anticipated, and this was most likely due to adding more than 50 mg/L glucose to the reactors. However, it does appear that the low concentration of 0.01mM CTAB does not seem to inhibit methanogenic activity.

CTAB Reactor Activity Assay

Because of the high gas production and the great discrepancy from the controls and the 0.01 mM CTAB reactors, another spike of 50 mg/L of carbon as glucose was added to the reactors. The gas produced from anaerobic biodegradation will be primarily divided between CO_2 and CH_4 along with by products of H_2O and growth of biomass ($C_5H_7O_2N$). Small amounts of hydrogen, some nitrogen, and traces of hydrogen sulfide are also typically present in gas produced during anaerobic digestion, but they are considered to be negligible during the theoretical analysis (Sawyer et al., 1994). The generalized equation for methane fermentation of glucose is given in the following Equation 4-1.

$$0.25CH_2O + 0.014HCO_3^- + 0.014NH_4^+ = \quad \text{(Eq 4-1)}$$

$$0.104CO_2 + 0.056H_2O + 0.09CH_4 + 0.014C_5H_7O_2N$$

In determining the gas production it was assumed that the portion of the electron donor used for synthesis and energy are 0.28 and 0.72, respectively (Sawyer et al., 1994). The carbohydrate in the reaction is glucose and the reaction assumes that ammonia is available for cell synthesis.

At 50 mg/L carbon, in 100 mL there are 12.5 mg glucose (5 mg carbon).

12.5 mg glucose = 0.0694 mmol

0.0125 g x 22.414 x 0.09 = 3.36 mL CH4

0.0125 g x 22.414 x 0.104 = 3.88 mL CO2

The correction for temperature must be made since the reactions are occurring at 35 °C. This adjustment corrects the volume of gas produced to 3.79 mL of methane and 4.38 mL of carbon dioxide, corresponding to a total gas production of 8.17 mL. Actual measured gas production is typically less than Eq. 3-1 shows, due to incomplete conversion of all of the organic carbon into CH_4 and CO_2, and the extent to which CH_4 and CO_2 remain solubilized in the aqueous phase (ASTM, 1992). Shelton and Tiedje (1984) proposed solubility corrections for CH_4 of 0.95 and 0.35 for CO_2 at 35 °C, which would lead to an expected gas production of 5.13 mL.

The reactors were monitored, and the gas production and percent methane in the headspace of the reactors were measured until the gas production had ceased for two consecutive weeks. The results are presented in Table 4-3 and plotted in Figure 4-3.

Table 4-3: CTAB ATA Reactor Activity Assay

Sample	CGP (20 days)	% Methane	% Theoretical
Control	4.9	72.1	95.5
0.01 mM CTAB	6.6	70.3	128.6
0.05 mM CTAB	0.0	15.8	0.0
0.10 mM CTAB	0.0	14.2	0.0
0.25 mM CTAB	0.0	16.4	0.0

The reactors with concentrations of 0.05 mM, 0.10 mM, and 0.25 mM CTAB continued to produce no biogas, which indicates that the CTAB was still inhibitory in the reactors. The control and 0.01 mM CTAB reactors responded to the organic carbon spike and had subsequent gas productions of 5.6 and 6.6 mL of biogas, respectively. This correlates reasonably well to the stoichiometrically expected production of approximately 5.13 mL of biogas. The increase in gas production for the 0.01mM CTAB reactors needed to be further analyzed to see if the CTAB could, at low concentrations, possibly stimulate methanogenic activity. It can at least be concluded, from these results, that the concentration of 0.01 mM CTAB was not inhibitory.

Toxicity Threshold

Using the data from the previous toxicity assay, a follow up toxicity assay was performed to more precisely define the toxicity threshold of CTAB. A range of concentrations from 0.001 mM to 0.05 mM was selected to better define the inhibitory threshold of CTAB. The results from this assay are shown in the following Table 4-4.

Table 4-4: CTAB Toxicity Assay

Sample	CGP (35 days) mL	% Methane	CGP (118 days) mL	% Methane	% of Control
Control	51.7	29.5	121.9	77.2	--
0.001 mM CTAB	48.1	32.8	116.9	81.1	95.9
0.005 mM CTAB	30.1	31.2	112.1	76.9	92.0
0.010 mM CTAB	31.8	34.9	113.2	74.4	92.9
0.020 mM CTAB	18.7	13.8	99.3	70.1	81.5
0.030 mM CTAB	8.3	11.0	73.9	75.8	60.6
0.050 mM CTAB	1.1	0.0	5.1	12.0	4.2

Upon looking at the data, it appears that CTAB concentrations of 0.02 mM and lower did not seem to inhibit methanogenic activity. The 0.020 mM CTAB reactors did produce less than 90% of the control, which Owen et al. (1978) determined to be inhibitory. However, the reactors did have a high methane content in the biogas, as well as have a cumulative gas production that was within 10%-15% for a good portion of the test period. For these reasons the concentration was determined to be not inhibitory with only 81.5% gas production with respect to the control. At 0.03 mM CTAB, the CTAB was not completely inhibitory to activity, but it did significantly reduce the amount of cumulative gas production. From the plotted data in Figure 4-5, the CTAB concentrations of 0.005 mM, 0.010 mM, 0.020 mM, and 0.030 mM all required an acclimation period where gas production was noticeably less than that of the control.

Effect of Temperature on Toxicity

A book by Jungermann (1970) stated that the bactericidal properties of cationic surfactants were greater at higher temperatures. This was important when CTAB was used as a sterilizing agent in the medical field. To test this theory, anaerobic toxicity assay reactors were similarly prepared in duplicate, and then separated with one set of reactors placed in a 35 °C incubator and the other set at 25 °C in another incubator. At both temperatures, both controls and 10 mg/L CTAB were tested. The concentration of 10 mg/L CTAB was selected because it was noticed to be more than slightly inhibitory during other assays at a temperature of 35 °C. The gas production at 25 °C was corrected for the volume difference due to temperature using Henry's Law. The results from the assay can be seen in Table 4-5 as well as plotted in Figure 4-6.

Table 4-5: CTAB Temperature Toxicity Assay

Sample	CGP (43 days) mL	% Methane	CGP (103 days) mL	% Methane	% of Control
Standard 35°	73.7	63.4	182.7	80.2	--
10 mg/L CTAB 35°	30.1	72.1	38.2	27.7	20.9
Standard 25°	20.2	70.3	113.1	80.6	--
10 mg/L CTAB 25°	3.6	25.8	28.2	42.7	24.9

There was not much difference in the reactors with 10 mg/L CTAB, the gas production of both the reactors with CTAB was significantly inhibited. The cumulative gas production of the control at 25 °C was significantly less than the control at 35 °C. The final gas production should have been similar for the toxicity assays at both temperatures, but with the 35 °C having a faster rate of degradation. The 10 mg/L CTAB

reactors at 25 °C did produce 24.9% of that of the control reactors at that temperature versus 20.9% for the 35 °C CTAB reactors versus their respective controls. This difference is very small considering the accuracy of the assay results. From the results of this assay, it does not appear that the effect of temperature should play a vital role on the toxicity of CTAB in anaerobic biological treatment.

Soaking Bath and Washing Bath Toxicity Assays

Preliminary Study

A preliminary study was performed on batch tests that included different percent compositions of the soaking bath and washing bath samples received from pilot testing from the University of Oklahoma. Because of the low solids concentration (low ink residue and minimal CTAB concentrations) the rinsing baths were not included in the toxicity studies. The rinsing baths should serve to dilute the loading of the ink removal process waste stream.

The soaking bath and washing bath samples were composed of sodium hydroxide, CTAB, defoaming agent, and the removed ink residue. The initial anaerobic toxicity assays were tested with concentrations of 1%, 5%, and 10% of the soaking and washing baths diluted with distilled water. Only the sample volume of 10 mL was diluted to arrive with the assay dilutions in the 100 mL total liquid volume of the reactors. Because of the 0.1 % w/v (0.282 mM) concentration of CTAB from the soaking bath and rinsing bath wastewater, the CTAB concentrations in the reactors were 0.0282 mM, 0.141 mM, and 0.282 mM, respectively. After 144 days, gas production had ceased in all of the

reactors for two consecutive weeks. The results of the toxicity assay are shown in following Table 4-6 and Figure 4-7.

Table 4-6: Preliminary Soaking and Washing Bath Toxicity Assay

Sample	CGP (42 days) mL	% Methane	CGP (144 days) mL	% Methane	% Control
Control	56.8	67.5	138.1	57.8	--
1% Soaking Bath	36.6	83.4	138.9	81.4	100.6
5% Soaking Bath	2.9	0.0	2.9	0.0	2.1
10% Soaking Bath	3.1	0.0	3.1	0.0	2.2
1% Washing Bath	25.1	62.6	129.4	72.1	93.7
5% Washing Bath	3.5	0.0	3.5	0.0	2.5
10% Washing	0.2	0.0	0.2	0.0	0.1

It is obvious that both the soaking and washing bath anaerobic reactors that were diluted to 5% and 10% were completely inhibitory to methanogenic activity, as seen by the extremely low cumulative gas production and the 0% methane in the biogas. However, the 1% soaking and washing bath reactors both had gas production that was consistent with the standard that was run in parallel with the percent of control being 100.6% and 93.7%, respectively. This assay indicates that when the soaking and washing baths are diluted to 1%, which correlates to 0.0282 mM CTAB, they are not inhibitory to gas production. From this assay the diluted concentrations that are not inhibitory to anaerobic activity can be further examined by evaluating dilutions less than 5% (0.141 mM CTAB) of soaking and washing baths.

Soaking and Washing Bath Toxicity

To further define the toxicity threshold of the deinking waste stream, dilutions of 0.5% (0.0141 mM CTAB), 1.0% (0.0282 mM CTAB), 2.0% (0.0564 mM CTAB), and 4.0% (0.1128 mM CTAB) from both the soaking bath and the washing bath were tested with the anaerobic toxicity assay. After 110 days, when gas production had ceased for two weeks, the assay was considered complete. The results from the assay are shown in Table 4-7 below as well as in Figure 4-8 on the following page.

Table 4-7: Soaking and Washing Bath Toxicity Assay

Sample	CGP (44 days) mL	% Methane	CGP (110 days) mL	% Methane	% Control
Control	54.9	76.6	134.3	78.4	--
0.5% Soaking Bath	47.8	80.8	136.3	79.7	101.5
1% Soaking Bath	19.6	42.9	80.5	71.6	59.9
2% Soaking Bath	1.2	43.5	7.3	53.2	5.4
4% Soaking Bath	0.0	0.0	0.8	0.0	0.6
0.5% Washing Bath	43.7	75.9	134.9	80.1	100.4
1% Washing Bath	15.7	41.6	40.6	58.9	30.2
2% Washing Bath	1.3	2.7	1.3	0.0	1.0
4% Washing Bath	1.0	0.0	1.0	0.0	0.7

The 0.5% dilutions of the soaking bath and washing bath were not inhibitory to methanogenic activity in the reactors. However, unlike the previous preliminary soaking bath and washing bath anaerobic toxicity assays, the 1% soaking bath and 1% washing bath did inhibit gas production in the reactors. The 1% dilutions decreased the gas production in the soaking bath to 60% of that of the control and 30% of that for the washing bath. The raw data from the washing bath control in Appendix F shows a 100% difference between the two reactors at 1% washing bath. This inconsistency in data is

indicative of the fact that the assay was being performed very near the toxicity threshold. It was very evident that soaking and washing bath dilutions of 2% and 4% were completely inhibitory to methanogenic activity.

Adaptation of Reactors to CTAB

Anaerobic studies by Battersby and Wilson (1983) showed that at a concentration of 80 mg/L CTAB was initially inhibitory to methanogenic degradation, but that after an initial inhibitory period of adaptation the methanogens could somewhat adapt and become active. This was not seen during the anaerobic toxicity assays performed for this study, except for the fact that there was a lag period in the reactors with concentrations that were not toxic, in which gas production was considerably less than that of the controls. The concentrations that were completely toxic, greater than 0.0282 mM or 10 mg/L CTAB, did not show signs of activity after the test period that showed inhibition. To ensure that this adaptation did not occur, the reactors were maintained for several months after the assay had completed and periodically tested just to make sure that this "adaptation period" had not occurred.

Expected Gas Production During Batch Assays

An analysis of the expected gas production on the controls was necessary to determine if the anaerobic tests were behaving in a typical and predictable manner. This analysis of the control reactors was performed by checking if the measured cumulative gas production correlates well with theoretical gas productions. The theoretical gas productions were determined by calculating the amount of gas production that would be

expected from the anaerobic degradation of glucose into CO_2 and CH_4 at 35 °C while accounting for cell synthesis and the solubility of gases, as described before in Eq. 4-1.

The background organic concentration in the master culture reactor needs to be determined in order to be able to predict a theoretical gas production for the control reactors. The MCR was initially seeded with inoculum from the Stillwater Wastewater Treatment Plant anaerobic digester along with a spike of 20,000 mg/L ethanol to stimulate activity. This initial spike along with a 2 g/l-wk of glucose feeding rate to the master reactor meant that there would be residual organic material in the reactors.

The production from the controls of all aforementioned anaerobic toxicity assay has been evaluated with respect to the theoretical amount of gas expected, by utilizing the initial measurements of COD in the reactors compared to final measurements of COD. In the absence of additional information, all of the chemical oxygen demand was assumed to be in the form of glucose, and the subsequent analysis of expected gas production is based on that assumption. The amount of organic material that was converted includes the material that was converted to cell mass and the by-product of water. An example of the calculations for theoretical gas production is shown in Appendix H. The analysis of the control gas production, including the average of measured production in the controls, is presented in Table 4-8.

Table 4-8: Analysis of Control Gas Production

Test Control	Theoretical Gas Production (mL)	Actual Measured Gas Production (mL)	Percent Gas Produced (%)
Defoaming Agent ATA	150.0	136.9	91.3%
Preliminary CTAB ATA	151.9	332.8	219.1%
CTAB Toxicity Threshold	143.4	121.9	85.0%
SB and WB Preliminary	140.4	138.1	98.4%
SB and WB Toxicity Threshold	145.7	134.3	92.1%

The residual organics, in the form of glucose, played a very large part of the gas production for the samples. Assuming that the initial COD was due solely to glucose, an initial glucose concentration in the reactors of between 1500 and 3000 mg/L was determined to be present. The reason for the high initial COD would be that feeding schedule for the master culture reactor was taken from Young and Khandaker (1993). The schedule was based on the active culture being incubated at 35 °C. Because of inconsistent room temperature, the master culture reactor was maintained at a room temperature from 15 to 25 °C, at which the anaerobic digestion process is not as efficient because of slower reaction rates. The feeding rate was slowly reduced from 1 g COD/day to 2 g COD/wk. Because the reaction for degradation of glucose to acetic acid is faster than the degradation of acetic acid into methane and carbon dioxide, the pH was consistently dropping. Altering the COD loading corrected this.

The gas production from the controls seems very reliable with respect to the initial and final COD measurements, taking into account the assumptions for expected

gas production as described previously, except for the Preliminary CTAB control reactor. The Preliminary CTAB ATA was the test that had much greater gas production for the 0.01 mM CTAB reactors than what was expected (219.1% of theoretical gas production). As seen by the control from that assay, the results show that an error must have been made during the initial glucose spike providing a much greater initial organic loading than what was expected. For this assay only, the initial COD of the reactors was measured prior to the addition of glucose making the amount of COD increase due to the glucose addition unknown. However, overall the gas production from the controls can be said to have behaved in a reasonable and predictable manner. Therefore, it can be assumed that the gas production data obtained during analysis is representative given the conditions.

FATE OF CTAB

Adsorption in Aerobic Completely Mixed Reactor

Cationic surfactants are strongly adsorbed onto surfaces that are mostly negatively charged. The cellular biomass in wastewater treatment plants is slightly negatively charged, making sorption to microbial solids a major factor in wastewater treatment. Studies by Sullivan (1983) and Games et al. (1982) showed that quaternary ammonium surfactants (like CTAB) can be removed from solution to an extent greater than 95% within 2 hours by adsorption. Given that quaternary ammonium surfactants are strongly sorbed by a wide variety of materials, it is difficult to distinguish removal among sorption, complexation, and biodegradation. Since for most quaternary ammonium surfactants, like CTAB, adsorption is much faster than biodegradation it is important to

know the removal efficiency by sorption. In order to demonstrate this strong sorption to aerobic biomass, an aerobic culture that was originally seeded with municipal wastewater was developed and a sorption study was undertaken. This study was meant to demonstrate the fate of CTAB in a completely mixed aerobic reactor, to simulate an activated sludge tank.

Aerobic Culture

Raw wastewater influent and wastewater effluent were taken from the Stillwater municipal wastewater treatment plant and used as a seed for a master aerobic culture maintained in a continuously aerated tank reactor. The aerobic master culture reactor (AMCR) was maintained at a temperature of 20°C with a hydraulic and solids retention times of 5 days. Ample nutrients, minerals, and buffer capacity were incorporated in the AMCR. A BOD loading rate of 200 mg/L-d in the form of glucose was added to maintain microbial activity. The solids concentration of the raw master culture was maintained between 1500-2000 mg/L, which is a typical mixed liquor suspended solids (MLSS) for a conventional activated sludge process.

Determination of Sorption Removal

The rate and extent of adsorption of CTAB at 0.1 mM CTAB to wastewater solids was measured using a total organic carbon analyzer, Shimadzu model TOC-5000A. Samples from the master culture reactor were placed in 1-liter glass vessels and agitated on a magnetic stirring plate to maintain uniform suspension of solids. After CTAB was added to the mixture, samples were drawn at various intervals for a 30-minute period.

The samples were immediately filtered with 0.45 μm filters to remove cell biomass, which is generally of a size greater than 1.0 μm, and adsorbed CTAB. The amount of CTAB adsorbed to the solids was calculated by taking the initial soluble TOC of the wastewater sample and adding the known TOC value for 0.1 mM CTAB, then subtracting the final soluble TOC. In the 30-minute period, removal by biodegradation was neglected and it was assumed that removal would only occur by adsorption.

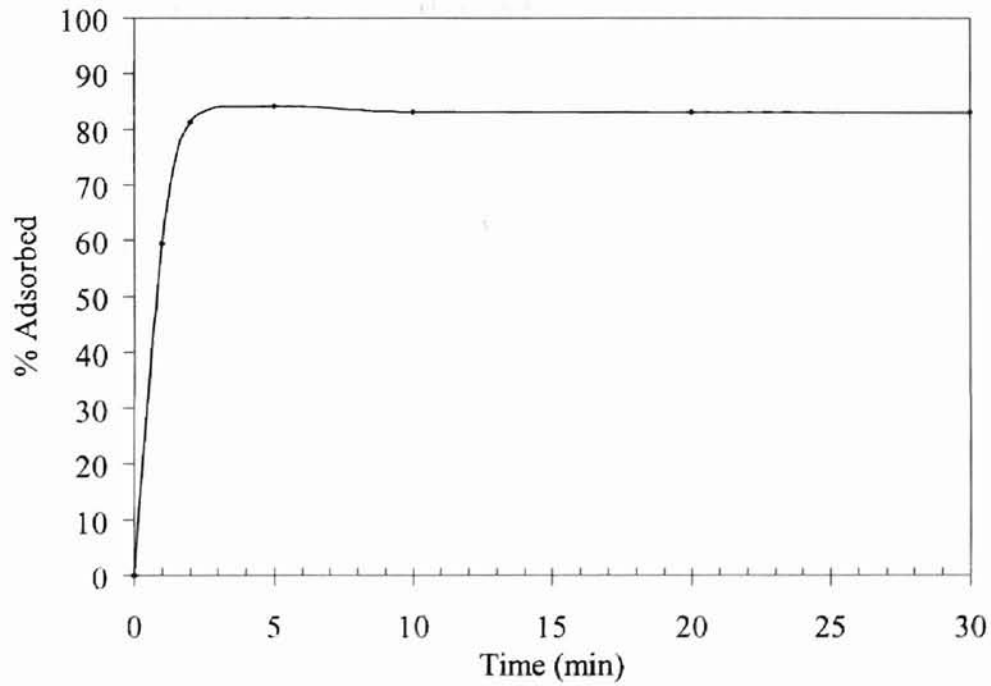


Figure 4-9: CTAB Adsorption Removal. Removal of CTAB by adsorption in a completely mixed aerobic reactor.

The averaged results of two adsorption tests with the raw wastewater seeded culture are shown in Figure 4-9. Both tests were very similar with the 30-minute adsorbed CTAB percentages of 82.1 and 84.2%, as measured by TOC reduction. As

expected, the data showed that the removal by adsorption was very fast, with over 80% removal in 2 minutes.

The rapid attainment of equilibrium was consistent with other studies that have been performed on quaternary ammonium surfactants (Karsa and Porter, 1994; Games et al., 1982; Fujita and Koga, 1961). The rapid adsorption rates during wastewater treatment can cause increased concentration of CTAB on solids. In an activated sludge tank that recycles a mixed liquor, the buildup of CTAB on solids would need to be kept below toxic concentrations. The CTAB adsorbed to wasted solids would be further treated by acrobic or anaerobic digestion, or other methods of sludge stabilization.

CTAB in ATA Effluents

CTAB ATA Reactor Effluent Samples

The anaerobic toxicity assays that were performed give an indication of whether a compound is toxic, but they do not indicate whether the compound is degraded. Better indicators for determining the anaerobic biodegradation potential are available (Shelton and Tiedje, 1984, ASTM, 1992). The general procedure for determining the anaerobic degradability of a compound to CH_4 and CO_2 can be performed in a batch assay test similar to the anaerobic toxicity assay previously defined. In a procedure outlined by ASTM (ASTM, 1992), a test compound with concentration of 50 mg/L as organic carbon is analyzed along with other compounds at the same concentration that are known to be readily degradable under anaerobic conditions, and the gas measurements are compared. In the case of CTAB, the concentration of 50 mg/L as organic carbon correlates to a concentration of 0.26 mM. This concentration is well above 0.03 mM, which was shown

to be inhibitory to methanogenic activity. Even though a standard biochemical methane potential test cannot be performed on the CTAB because of its low toxicity threshold, the question of whether CTAB is found in the effluent of an anaerobically treated waste stream is still very pertinent.

The effluents from the batch reactors were periodically sampled and analyzed on a high performance liquid chromatograph (HPLC). All samples were centrifuged on an International Equipment Clinical Centrifuge Model CL and filtered with Acrodisc CR PTFE 0.45 μm syringe-tip filters (Gelman Sciences). A standard curve was generated with a range of CTAB concentrations from 0.01 mM to 0.5 mM. This curve was created to determine the CTAB concentration in the anaerobic toxicity assay reactors immediately after the commencement of the assay, during the assay, and after the assay had gone to completion.

The results from the HPLC analysis were difficult to interpret because of the interference of other components at the CTAB retention time in the low wavelength of 210 nm (Helboe, 1983). The difference in peak areas of samples from blank reactors in comparison to the reactors that had low concentrations of CTAB was not discernable. Since no peak for CTAB was found in the test reactors, with respect to the control, CTAB was added directly to the reactor bottle samples to confirm that the retention time was around 2.8. The peak area at a retention time of 2.8 greatly increased when CTAB was added directly to the filtered reactor samples confirming that that is where the CTAB peaks in the HPLC analyses. The results from the HPLC analysis can be viewed in Table 4-10. The table shows a relatively wide variance (±12%) of averaged peak areas from the

reactors around the control. However, the range of areas does not seem to be correlated with the concentration of CTAB present in the sample.

Table 4-9: HPLC Analysis of CTAB Assay Reactors

Reactor Sample	Averaged Retention Time (sec.)	Averaged Peak Area
Control	2.837	8,127,048
0.01 mM CTAB	2.822	8,467,236
0.05 mM CTAB	2.814	9,112,812
0.10 mM CTAB	2.863	7,893,094
0.25 mM CTAB	2.805	8,873,901

Since it is difficult to distinguish between the averaged peak area of the control reactor samples and the averaged peak areas of the reactors that contained CTAB, it can be concluded from the analysis that CTAB was not present in measurable quantities in the centrifuged and filtered samples due to sorption.

Soaking Bath and Washing Bath ATA Reactor Effluent Samples

The results from the HPLC analysis of the soaking bath and washing bath anaerobic toxicity assay reactors (Table 4-10) were not easily discernable, similar to the CTAB reactor HPLC analysis. The averaged peak areas of the controls did not seem to portray a lesser area at the retention time of 2.8 seconds than the soaking and washing bath reactor samples. In order to ensure that CTAB was measured at the retention time of 2.8 seconds, measured quantities of pure CTAB were added to the centrifuged and filtered samples. Also soaking bath and washing bath samples were added directly to the

centrifuged and filtered reactor samples. In both cases the peak area at 2.8 seconds increased in response to the respective addition.

Table 4-10: HPLC Analysis of Soaking Bath and Washing Bath Assay Reactors

Reactor Sample	Averaged Retention Time (sec.)	Averaged Peak Area
Control	2.831	10,443,286
1% Soaking Bath	2.840	11,517,344
5% Soaking Bath	2.835	9,247,600
10% Soaking Bath	2.800	10,343,736
1% Washing Bath	2.811	10,312,024
5% Washing Bath	2.829	9,742,170
10% Washing Bath	2.824	9,631,187

Although no quantitative data could be taken from running the HPLC analysis, it does seem that the data indicate low, if any, concentrations of CTAB in the filtered effluent samples. This would seem to validate the aerobic analysis that showed removal by adsorption to biological solids, as well as other studies with similar findings (Karsa and Porter, 1994; Games et al., 1982; Fujita and Koga, 1961).

FATE OF INK RESIDUE

The ink residue removed from the plastic packaging in the soaking and washing bath processes consists of extremely fine particles that are not soluble and therefore most likely not readily available for biological degradation. The soaking bath was a blue-greenish color and the washing bath was a deep bluish color as received from the University of Oklahoma. The fate of these colored ink particles was tracked by both a solids analysis, as well as by analyzing the color of the wastewater.

Ink Residue Solids Tracking

The solids in the washing and soaking bath wastewater samples consist of the CTAB, defoaming agent, and the removed ink residue particles. According to the University of Oklahoma, which prepared the wastewater samples, the soaking and washing baths contained 1000 mg/L CTAB and 2000 mg/L defoaming agent (DA). Table 4-11 summarizes the solids in the soaking bath, washing bath, the first rinsing bath, and the second rinsing bath. Appendix A has a more detailed analysis of the wastewater samples.

Table 4-11: Solids Content of Wastewater Samples

	Expected CTAB	Expected DA	Actual Solids Measurement	Ink Residue
Soaking Bath	1000 mg/L	2000 mg/L	2800 mg/L	N/A
Washing Bath	1000	2000	2400	N/A
Rinsing Bath 1	N/A	N/A	96	N/A
Rinsing Bath 2	N/A	N/A	60	N/A

The actual measured solids in the wastewaters was less than that expected from the information provided about the wastewater samples. Without having an excess amount of solids that could be the ink residue particles, the quantity of ink residue is not available (N/A). Without being able to quantify the initial solids concentration of ink residue particles, trying to track fate of the ink residue in the form of solids analyses was made virtually impossible for the soaking bath and washing bath toxicity assays. At the beginning of the washing and soaking bath toxicity test, the solids concentration of the soaking bath reactors was measured. The removed ink residue particles were very fine and could not all be captured as suspended solids by syringe filtration with 0.45 μm and

0.20 μm syringe tip filters. This initial solids concentration was compared against the final solids concentration (which accounts for growth of biomass and conversion of organic substrate). The calculations for the soaking bath solids analysis results shown in Table 4-12 are given in Appendix I.

Table 4-12: Soaking Bath ATA Solids Analysis

Soaking Bath Reactor	Initial Volatile Solids	Final Volatile Solids	Expected Final Volatile Solids	% Expected
Control	4980	5040	5179	97.3
1% Soaking Bath	5040	4840	5236	92.4
5% Soaking Bath	5120	5020	5123	98.0
10% Soaking Bath	5160	4940	5172	95.5

The variation in volatile solids was very slight in the anaerobic toxicity assay reactors from the beginning to the completion. Because of the high concentrations of solids in the reactors and the low percentage of those solids that could be removed ink particles, the results of the solids analysis are not very meaningful. The expected error in the solids analysis is 5-7% according to Standard Methods (1992), which means that tracking the fate of ink residue which constitute less than 1% of the total solids would be very difficult through a solids analysis.

Ink Particle Color Tracking

Another approach to tracking the fate of the ink residue particles was performed by colorometric analysis of the wastewater reactors using a spectrophotometer. As mentioned before, the soaking bath, as received from the University of Oklahoma, was a deep bluish green, and the washing bath was a deep bluish color from the removed ink

residue particles. When the anaerobic toxicity reactors were made up, it was observed that the 5% and 10% soaking bath dilutions had initially discolored the inoculated reactors as compared with the 1% dilution of soaking bath and the control. At the end of the assay the bluish discoloration was no longer present in the 5% and 10% soaking and washing bath reactors. This visual change in color was also measured using a spectrophotometer by measuring the absorbance in both the blue (600 nm) and green (650 nm) absorbance spectrum for 0.45 μm syringe tip filtered samples. The results from this test are shown in the following Table 4-13.

Table 4-13: Spectrophotometric Analysis of Washing and Soaking Bath Samples

Reactor	Blue Absorbance (600 nm)	Green Absorbance (650 nm)
10% Soaking Bath Influent	0.019	0.022
10% Soaking Bath Effluent	0.002	0.004
10% Washing Bath Influent	0.031	0.027
10% Washing Bath Effluent	0.010	0.008
ATA Control Reactor	0.003	0.002

It is seen from the results of the colorometric analysis that the anaerobically treated reactors had a reduced color when compared to the 10% soaking and washing bath standards that were diluted with distilled water to the same percentage as the experimental reactors. Whether the ink particles were biologically degraded, settled, or adsorbed and settled along with biological solids was not determined in this analysis. But the analysis did show that the color that the ink particles produce was significantly reduced during anaerobic treatment.

CHAPTER V

CONCLUSIONS

SUMMARY OF ANAEROBIC TOXICITY ASSAY RESULTS

The final wastewater from the removal of ink on printed plastic materials consists of 4.5% volume soaking bath, 4.5% volume washing bath, 45.5% volume rinsing bath 1, and 45.5% volume rinsing bath 2. This produces an effluent with a final concentration of 182 mg/L defoaming agent, 0.26 mM CTAB (91 mg/L), as well as the removed ink particles, and sodium hydroxide. The following section will compile and summarize the results from the anaerobic toxicity assay analyses of these samples.

Defoaming Agent

The results from the defoaming agent, Trans-286, anaerobic toxicity assay (ATA) clearly showed that not only did the defoaming agent not inhibit gas production, but it appeared to stimulate the production of gas. This could be expected because of the fact that the defoaming agent used is an oily, organic liquid that has a food grade status.

Removed Ink Residue

The removed ink residue resulting from the washing and soaking processes for ink removal was determined to be removed during anaerobic treatment by using a colorometric analysis of the treated wastewater. The color reduction was noticeable both

by inspection and by indication from the decreased absorbance at the blue and green wavelengths. The mechanism of color removal was not determined.

CTAB

The results from the preliminary anaerobic toxicity assay for CTAB were inconclusive due to the much higher than expected gas production for both the control and the 0.01 mM CTAB reactors. However, it was evident from the assay results that the concentration of 0.01 mM CTAB was not inhibitory, and that the concentrations of 0.05 mM and greater were completely inhibitory to methanogenic activity. A second ATA was conducted to further pinpoint the inhibitory concentration of CTAB, using concentrations from 0.001 mM to 0.05 mM. The results from this assay showed that concentrations of 0.02 mM CTAB and under were not inhibitory. The concentration of 0.03 mM was not completely inhibitory, but it did significantly reduce the cumulative gas production. The concentration of 0.05 mM again was completely inhibitory.

Soaking and Washing Bath Effluents

The soaking bath and washing bath wastewaters were analyzed similarly with the anaerobic toxicity assay procedure. The preliminary study examined dilutions of 1%, 5%, and 10% of both the soaking and washing bath reactors. The results from the assay showed that for 5% and 10% solutions, complete inhibition was observed in both soaking and washing bath reactors. At 1%, which correlates to 0.0282 mM CTAB, the soaking bath and washing bath reactors were not inhibited from methanogenic activity, as seen by the cumulative gas production of these reactors being similar to that of the controls. This

concentration of 0.0282 mM CTAB is very near the concentration of 0.030 mM CTAB that showed signs of partial inhibition in the CTAB toxicity assays.

After the preliminary soaking and washing bath assay was finalized, another assay with dilutions of 0.5%, 1%, 2%, and 4% of both soaking and washing baths was performed. This assay again showed that concentrations of greater than 0.0282 mM CTAB were completely inhibitory to methanogenic activity. However, at the 1% dilution (0.0282 mM), the reactors were somewhat inhibited by the CTAB from the wastewaters. The 0.5% dilutions were not inhibited by the presence of 0.0141 mM CTAB, as was expected from the results of the CTAB toxicity assays.

The two separate sets of toxicity assays showed that concentrations of greater than 0.0282 mM, which is 10 mg/L were inhibitory to anaerobic activity, and concentrations of less than 0.020 mM, or 7.1 mg/L, were not inhibitory. The consistency of the results from these independent assays supports the findings.

The finding that concentrations of 10 mg/L CTAB and greater are toxic to methanogenic bacteria is also consistent with aerobic findings of CTAB toxicity. Pitter (1961) showed that a bench-scale activated sludge system could only remove CTAB at concentrations of up to 6 mg/L. At 20 mg/L the sludge "lost activity" and flowed from the system, which indicates complete toxicity. A study by Gerike et al. (1978) found that CTAB could be removed at concentrations from 5 to 15 mg/L but at 20 mg/L was toxic. A recent study by Ueno and Yokoya (1996) showed that the maximum concentration associated with enzyme activity in aerobic conditions was about 3 mg/L and inhibition of most species began at concentrations between 5 and 10 mg/L.

RECOMMENDATIONS FOR FURTHER STUDY

Further research leading to practical application of the technology of removing ink for the purpose of recycling printed plastic products is needed to further define a practical, cost-effective solution for removing ink, and subsequently treating the ink removal waste stream. The recycling of printed plastic materials is an environmentally sound idea that is currently limited by the economics involved in recycling and reusing the plastic. Any such process will likely result in the production of an aqueous wastestream, one which must be dealt with in a manner that is economically and environmentally sound if the recycling process is to be practical. If the current process using CTAB is applied on a large scale, additional research will be needed to optimize the waste treatment process.

The research from this paper has led to the following recommendations for further study.

1. An attempt should be made to try to quantify the concentration of CTAB that is sorbed to biological solids in both aerobic and anaerobic treatment processes at various intervals throughout treatment.
2. The long-term fate of CTAB sorbed onto biomass should also be investigated.
3. Continuous tank reactors should be used to model both activated sludge treatment, as well as anaerobic digestion, in order to better define the effects of accumulation of CTAB onto biological solids. Mean cell residence times should be varied to determine if cell residence time plays a factor in treatment.

4. A study should be performed to see if an anaerobic continuous tank reactor can be acclimated to increasing concentrations of CTAB as indicated by the aerobic studies from van Ginkel and Kolvenbach (1991).
5. Further studies using HPLC or other instrumental analysis should be performed to better characterize the biological fate of CTAB.
6. The use of an adsorbent (like activated carbon) should be tested for its ability to prevent toxicity in biological processes by selectively sorbing CTAB and avoiding buildup to toxic levels in the biomass.

CHAPTER VI

ENGINEERING SIGNIFICANCE

The wastewater from the plastic packaging ink removal process includes soaking and washing baths, combined with the water from the two rinsing baths. The following chapter will attempt to describe what would be likely to happen to the CTAB in the wastewater in a typical activated sludge treatment plant with an anaerobic digester by utilizing the findings from this paper.

Primary Settling

As seen in both the aerobic and anaerobic adsorption studies, CTAB readily sorbs to biological solids. Primary settling processes should remove 50-70% of suspended solids in a typical municipal wastewater treatment plant (Tchobanoglous and Burton, 1991). The fact that CTAB readily sorbs to organic solids would lead one to believe that a portion of the CTAB would be removed in this initial physical process. Huber (1982) found that 20-40% removal of cationic surfactants was achieved in the primary clarifiers in full-scale activated sludge plants.

No attempt was made in the laboratory to quantify the removal of CTAB in a settling reactor. However, the CTAB sorption studies for both aerobic and anaerobic reactors showed high percent removal efficiency, and in the case of the aerobic study in a period of less than two minutes. This leads to the conclusion that in a primary settling

basin, or primary clarifier, a high percent of CTAB sorbed to the organic solids in the wastewater would most likely be removed. Because of the suspended solids removal efficiency of primary clarifiers, typically 50-70%, it should be expected that Huber's (1982) estimate of 20-40% removal of CTAB would be conservative based on the sorption studies performed in this study.

Removal in Activated Sludge Reactors

With activated sludge treatment having a typical hydraulic retention times of 4 to 8 hours in the aeration tank (Tchobanoglous and Burton, 1991), degradation of CTAB in soluble form is expected to be minimal. The fact that the aerobic adsorption tests showed that CTAB was readily adsorbed to biological solids in a completely stirred tank reactor shows that the mean cell residence time is probably the more important variable in determining toxicity in an activated sludge reactor. Typical mean cell residence time for activated sludge tanks may vary from 6 to 15 days (Tchobanoglous and Burton, 1991). The mean cell residence time would possibly be the limiting factor of toxicity, because the adsorption of CTAB onto the mixed liquor suspended solids that are recycled to maintain microbial activity could not reach toxic levels.

Although several studies suggest that CTAB along with other quaternary ammonium surfactants can be biologically degraded in an activated sludge process (Pitter, 1961; OECD, 1976; Boethling, 1984; Dean-Raymond and Alexander, 1977; Larson and Vashon, 1983; Wierich and Gerike, 1978; van Ginkel and Kolvenbach, 1991; Ueno and Yokoya, 1996), these studies typically considered removal solely by biological degradation, and did not consider removal by adsorption to biological solids.

Gerike et al. (1978) found that CTAB was removed using an activated sludge plant model with an efficiency from 91.4% to 97.5% for concentrations ranging from 5 to 20 mg/L. The 20 mg/L was toxic when an attempt was made to treat the solution with fresh unacclimated sludge, however after the sludge was allowed to acclimate to concentrations of 5 mg/L, the CTAB was removed without difficulty (Gerike et al., 1978). This analysis did try to account for the removal of CTAB by adsorption, and determined that since a very low percentage could be found in the sludge, biological degradation must have been the major mechanism of removal. On the other hand, Karsa and Porter (1994) state that since high removal efficiencies are achieved within 3-24 hours, adsorption onto sludge particles seems to be the responsible mechanism. Pilot tests should be performed for a plant simulating the activated sludge process to determine the toxic level of CTAB using a seed culture from the plant that will be receiving the potential wastestream. According to studies, it should be possible for an activated sludge tank to receive a low concentration of CTAB (perhaps 5 to 10 mg/L) without becoming upset.

Anaerobic Digestion

Anaerobic digestion involves the decomposition of organic matter in the absence of oxygen. Because of the adsorptive properties of CTAB, accumulation onto biomass from aerobic treatment and primary settling sludges coupled with no, or slow, degradation, the digester must be capable of handling concentrations higher than influent flow concentrations.

The findings presented from the anaerobic toxicity assays performed as a part of this paper showed that for the Stillwater, Oklahoma wastewater treatment plant anaerobic

digester sludge used as the inoculum, concentrations of CTAB from 7 mg/L to 10 mg/L were partially inhibitory to biogas production in anaerobic reactors, and concentrations greater than 10 mg/L were completely inhibitory. This inhibitory concentration is reasonably consistent with the toxic concentration found in earlier aerobic studies. Battersby and Wilson (1989) found that an adaptation or detoxification by the sludge could be observed after 2 weeks for samples with CTAB concentration of 50 mg/L carbon (approximately 0.23 mM), but that the concentration significantly inhibited gas production. Battersby and Wilson (1989) studied a wide variety of chemicals and did not attempt to find a toxicity threshold for CTAB in their study.

Pretreatment Alternatives

Because of the high toxicity of CTAB in both aerobic and anaerobic systems at relatively low concentrations, industries that will discharge wastewater with concentrations greater than 5-10 mg/L should pretreat the wastewater to prevent deleterious effects on POTWs. Because of the high adsorptive properties of CTAB an adsorption column containing activated carbon, zeolite, or another adsorbent would seem to be an effective and economical way of removing CTAB down to concentrations that would not be biologically inhibitory. An adsorptive process should precede a biological pretreatment plant, when concentrations of CTAB are expected to be greater than inhibitory concentrations. The use of an activated carbon adsorption column would have to be economically practical depending on the efficiency of carbon regeneration after the adsorptive capacity has been reached (Tchobanoglous and Burton, 1991).

Emergency Release Plan

One engineering application for the research results of this study is the development of information needed for response to an emergency release of a CTAB-containing wastestream. In the case of a plant upset resulting in release of a toxic concentration of CTAB to the environment, an emergency release plan should be in place. The results presented will allow wastewater treatment plant personnel to determine if a "shock load" of CTAB waste will be toxic, and if so, what an appropriate response will be. Because of the highly adsorptive nature of CTAB, an adsorptive process, such as activated carbon treatment, should be available to help remove toxic loads of CTAB from treated or untreated wastewater (Bele et al., 1998). This provision should be made available in the primary as well as final clarification stages. Activated carbon, powder or granular, could be added in the primary clarifier to reduce the toxicity of incoming wastewater that has a high concentration of CTAB. This could reduce toxicity by allowing the CTAB to adsorb to the activated carbon and not directly onto the active biomass used in biological treatment processes. In the case of treatment plant effluent, where the CTAB concentration may be greater than the discharge permit or the allowable environmental levels, a contacting basin prior to final clarification could be added to the treatment process to enable the addition of activated carbon prior to final clarification.

REFERENCES

ASTM, E 1196-92. Standard Test Method for Determining the Anaerobic Biodegradation Potential of Organic Chemicals. (1992). *Annual Book of ASTM Standards*, Vol 11.01, 166-170.

Battersby, N.S., and Wilson, V. (1989). Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. *Applied Environmental Microbiology*. Vol. 55, 433-439.

Bele, Kodre, Arcon, Grdadolnik, Pejovnik, and Besenhard (1998). Adsorption of cetyltrimethylammonium bromide on carbon black from aqueous solution. *Carbon*. Vol. 36, No. 6-7, 1207-1212.

Boethling, R.S. (1984). Environmental fate and toxicity in wastewater treatment of quaternary ammonium surfactants. *Water Research*. Vol. 18, 1061-1076.

Dean-Raymond, D. and Alexander, M. (1977). Bacterial metabolism of quartenary ammonium compounds. *Applied Environmental Microbiology*. Vol. 33, 1037-1041.

Fujita, T. and Koga S. (1961). The binding of a cationic detergent by yeast cells, in relation to its germicidal action. *J. Gen. Appl. Microbiol.* Vol. 12, 229-237.

Games, L.M., King, J.E. and Larson, R.J. (1982). Fate and distribution of a quaternary ammonium surfactant, octadecyltrimethylammonium chloride (OTAC) in wastewater treatment. *Environmental Science Technology*. Vol. 16, 483-488.

Gerike, P., Fischer, W.K. and Jasiak, W. (1978). Surfactant quaternary ammonium salts in aerobic sewage digestion. *Water Research*. Vol. 12, 1117-1122.

Hach Chemical Co. (1992). Hach Water Analysis Handbook.

Helboe, P. (1983). Separation and quantitative determination of long-chain alkyltrimethylammonium ions by reversed-phased ion-pair liquid

chromatography using ultraviolet-absorbing counter ions. *Journal of Chromatography.* Vol. 261, 117-122.

Huber, L. H. (1982). Cationic detergents and their significance for the environment. *Tenside Detergent.* Vol. 19, 178-180.

Jungermann, E. (1970). *Cationic Surfactants*. New York: Marcel Dekker.

Karsa, D.R., and Porter, M.R. (1994). *Biodegradability of Surfactants*. New York: Blackie Academic and Professional.

Larson, R.J. and Vashon, R.D. (1983). Adsorption and biodegradation of cationic surfactants in laboratory and environmental systems. *Dev. Ind. Microbiol.* Vol. 24, 425-434.

Owen, Stuckey, Healy, Young, and McCarty. (1978). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research.* Vol. 13, 485-492.

Pitter, P. (1961). Synthetic surface-active agents in wastewaters. III. Biological degradation of cationic surfactants in laboratory activated sludge units. *Sb. vys. Sk. Chem. Technol. Praze, Technol. Vody.* F14, 19-25.

Salton, M.R.J. (1951). The adsorption of cetyltrimethylammonium bromide by bacteria, its action in releasing cellular constituents and its bactericidal effects. *Journal of Genetic Microbiology*, Vol. 5, 391-404.

Sawyer, McCarty, and Parkin. (1994). *Chemistry for Environmental Engineering*, 4th Ed. New York: McGraw-Hill.

Shelton, D. and Tiedje, J. (1984). General method for determining anaerobic biodegradation potential. *Applied and Environmental Microbiology.* Vol. 47, No. 4, 850-857.

Standard Methods for the Examination of Water and Wastewater. (1992) American Public Health Association. New York.

Sullivan, D.E. (1983). Biodegradation of a cationic surfactant in activated sludge. *Water Research.* Vol. 17, No. 9, 1145-1151.

Swisher, R.D. (1987). *Surfactant Biodegradation, 2nd Ed.* New York: Marcel Dekker.

Tchobanoglous, G., and Burton, F. (1991). *Wastewater Engineering: Treatment, Disposal, and Reuse*, Metcalf and Eddy, Inc. 3rd Ed. Boston, MA: McGraw-Hill.

Ueno, M. and Yokoya, F. (1996). Inhibition of microbial metabolism by CTAB and its effects on cytoplasm enzymes. *Revista de Microbiologia.* Vol. 27, 171-176.

van Ginkel, Dijk, and Kroon (1992). Metabolism of hexadecyltrimethylammonium chloride in *Pseudomonas* strain B1. *Applied and Environmental Microbiology.* Vol. 58, No. 9, 3083-3087.

van Ginkel, C.G., and Kolvenbach, M. (1991). Relations between the structure of quaternary alkyl ammonium salts and their biodegradability. *Chemosphere.* Vol. 23, No. 3, 281-289.

Wierich, P. and Gerike, P. (1978) The fate of soluble, recalcitrant, and adsorbing compounds in activated sludge plants. *Ecotoxic. Envir. Saf.* Vol. 5, 161-170.

Young, J.C., and Khandaker, N.R. (1992). Anaerobic protocol for assessing industrial waste treatability. University Park, PA: The Pennsylvania State University.

APPENDICES

APPENDIX A

Analysis of Wastewater

Appendix A
Analysis of Wastewater

	pH	COD (mg/l)	Total Solids (mg/l)	Volatile Solids (mg/l)	Suspended Solids (mg/l)	Volatile Suspended Solids (mg/l)
Soaking Bath	11.8	2550	2800	2288	120	120
Washing Bath	11.3	3155	2400	2068	200	200
Rinsing Bath 1	9.9	290	96	60	4	4
Rinsing Bath 2	7.6	25	60	24	0	0

APPENDIX B

Gas Production for Defoaming Agent ATA

Appendix B
Gas Production for Defoaming Agent ATA

Day	1	5	6	8	13	15	22	27	35	43	50	57	66	73	78	85	91	99	108	118	126	133	139	146
Blank 1	4.2	19.6	12.8	22.2	24.4	4.4	0.8	0.2	0.6	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Blank 2	5.2	16.8	10.4	17.6	5.8	2.2	2.0	2.2	4.6	2.0	4.0	0.0	3.2	3.0	6.4	8.6	15.6	31.6	23.6	4.2	0.0	0.0	0.0	0.0
Blank 3	3.6	7.2	3.6	4.0	15.0	12.4	7.0	0.2	3.0	0.2	3.6	0.4	0.0	0.6	2.6	0.0	1.8	0.2	10.2	19.4	32.2	22.4	2.6	0.2
Blank Average	4.3	14.5	8.9	14.6	15.1	6.3	3.3	0.9	2.7	0.7	2.6	0.1	1.1	1.2	3.0	2.9	5.8	10.6	11.3	7.9	10.7	7.5	0.9	0.1
Cumulative	4.3	18.9	27.8	42.4	57.5	63.8	67.1	67.9	70.7	71.4	74.0	74.1	75.2	76.4	79.4	82.3	88.1	98.7	109.9	117.8	128.5	136.0	136.9	136.9
0.1% DA 1	4.8	10.8	5.4	6.8	9.0	6.0	16.4	22.4	3.2	1.4	0.8	18.8	10.4	8.8	8.6	4.0	2.0	0.4	0.6	0.0	0.0	0.0	0.0	0.0
0.1% DA 2	6.0	13.2	2.6	6.4	8.2	4.8	13.6	16.0	2.8	1.4	2.4	6.8	18.0	16.0	12.2	4.8	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
0.1% DA Average	5.4	12.0	4.0	6.6	8.6	5.4	15.0	19.2	3.0	1.4	1.6	12.8	14.2	12.4	10.4	4.4	1.0	0.2	0.4	0.0	0.0	0.0	0.0	0.0
Cumulative	5.4	17.4	21.4	28.0	36.6	42.0	57.0	76.2	79.2	80.6	82.2	95.0	109.2	121.6	132.0	136.4	137.4	137.6	138.0	138.0	138.0	138.0	138.0	138.0
0.5% DA 1	4.6	6.4	2.8	8.4	11.8	5.6	4.0	0.2	8.2	0.4	6.4	6.2	16.4	24.4	34.8	8.6	2.2	8.0	18.8	26.4	48.6	12.4	3.2	0.0
0.5% DA 2	5.4	10.0	2.4	6.8	28.2	6.8	4.8	0.6	4.6	0.0	4.0	3.8	12.0	10.0	21.2	3.8	0.6	22.4	24.8	22.0	15.4	15.6	4.8	0.0
0.5% DA Average	5.0	8.2	2.6	7.6	20.0	6.2	4.4	0.4	6.4	0.2	5.2	5.0	14.2	17.2	28.0	6.2	1.4	15.2	21.8	24.2	32.0	14.0	4.0	0.4
Cumulative	5.0	13.2	15.8	23.4	43.4	49.6	54.0	54.4	60.8	61.0	66.2	71.2	85.4	102.6	130.6	136.8	138.2	153.4	175.2	199.4	231.4	245.4	249.4	249.8
1.0% DA 1	8.2	26.8	10.0	6.6	1.8	0.6	1.8	4.8	54.2	24.8	28.4	36.2	32.8	20.2	24.6	16.8	15.4	14.0	12.8	4.6	0.0	0.0	0.0	0.0
1.0% DA 2	2.6	17.6	9.2	8.2	4.2	3.0	7.4	5.6	28.2	16.4	30.0	47.8	39.6	23.4	20.2	12.0	10.6	10.4	4.8	4.2	0.0	0.0	0.0	0.0
1.0% DA Average	5.4	22.2	9.6	7.4	3.0	1.8	4.6	5.2	41.2	20.6	29.2	42.0	36.2	21.8	22.4	14.4	13.0	12.2	8.8	4.4	0.0	0.0	0.0	0.0
Cumulative	5.4	27.6	37.2	44.6	47.6	49.4	54.0	59.2	100.4	121.0	150.2	192.2	228.4	250.2	272.6	287.0	300.0	312.2	321.0	325.4	325.4	325.4	325.4	325.4
2.0% DA 1	4.2	8.8	13.0	8.4	6.0	4.2	9.8	3.4	28.4	64.2	72.8	64.2	42.4	26.4	22.8	31.0	52.0	62.4	72.0	38.6	26.4	28.2	14.4	0.0
2.0% DA 2	5.4	11.6	15.4	24.8	7.2	3.4	6.2	0.6	5.6	44.6	59.6	54.2	18.0	34.4	39.6	59.4	68.0	83.6	75.2	28.2	17.6	15.8	2.4	0.0
2.0% DA Average	4.8	10.2	14.2	16.6	6.6	3.8	8.0	2.0	17.0	54.4	66.2	59.2	30.2	30.4	31.2	45.2	60.0	73.0	73.6	33.4	22.0	22.0	8.4	0.0
Cumulative	4.8	15.0	29.2	45.8	52.4	56.2	64.2	66.2	83.2	137.6	203.8	263.0	293.2	323.6	354.8	400.0	460.0	533.0	606.6	640.0	662.0	684.0	692.4	692.4

APPENDIX C

Gas Production for Preliminary CTAB ATA

Appendix C

Gas Production for Preliminary CTAB ATA

Day	1	5	6	8	13	15	22	27	35	43	50	57	66	73	78	85	91	99	108	118	126	133	139	146
Control 1	9.4	9.6	3.4	2.4	5.4	2.2	11.4	25.2	42.0	40.2	56.2	35.0	12.8	3.2	4.8	2.0	0.0	0.0	6.8	1.8	1.4	1.8	0.2	0.0
Control 2	4.4	5.2	1.6	2.2	2.0	3.2	29.2	33.0	54.4	69.0	47.7	10.4	7.6	4.2	6.2	5.4	33.0	41.0	20.2	9.0	3.4	1.4	1.2	0.2
Control 3	5.4	5.0	2.0	3.6	5.0	2.6	9.4	24.0	47.4	45.2	45.0	13.0	8.4	4.4	5.0	2.8	4.2	27.8	38.2	17.6	5.8	2.4	1.8	0.2
Control Avg.	6.4	6.6	2.3	2.7	4.1	2.7	16.7	27.4	47.9	51.5	49.6	19.5	9.6	3.9	5.3	3.4	12.4	22.9	21.7	9.5	3.5	1.9	1.1	0.1
Cummulative	6.4	13.0	15.3	18.1	22.2	24.9	41.5	68.9	116.9	168.3	218.0	237.4	247.0	251.0	256.3	259.7	272.1	295.0	316.8	326.2	329.8	331.6	332.7	332.8
0.01 CTAB 1	7.8	12.0	12.2	13.6	5.0	1.4	1.4	3.6	43.6	11.8	15.0	15.2	22.8	14.0	16.8	38.2	110.0	97.0	16.8	1.2	2.0	1.8	0.0	0.0
0.01 CTAB 2	4.8	4.6	1.4	2.2	0.6	1.2	5.4	1.8	6.0	20.2	16.8	20.2	25.0	9.8	8.0	9.8	26.4	135.0	158.6	21.2	4.0	1.2	1.2	0.0
0.01 CTAB 3	5.2	7.6	10.0	15.2	10.0	4.0	0.4	0.0	4.8	3.6	5.4	5.0	22.0	25.2	35.2	32.8	52.4	84.0	66.2	96.0	63.4	16.6	5.2	5.6
0.01 CTAB Avg	5.9	8.1	7.9	10.3	5.2	2.2	2.4	1.8	18.1	11.9	12.4	13.5	23.3	16.3	20.0	26.9	62.9	105.3	80.5	39.5	23.1	6.5	2.1	1.9
Cummulative	5.9	14.0	21.9	32.2	37.4	39.6	42.0	43.8	61.9	73.8	86.2	99.7	122.9	139.3	159.3	186.2	249.1	354.5	435.0	474.5	497.6	504.1	506.3	508.1
0.05 CTAB 1	2.4	2.6	0.4	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.05 CTAB 2	2.6	1.4	0.4	0.2	0.0	0.2	0.0	0.0	2.0	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.05 CTAB 3	2.2	0.4	0.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.05 CTAB Avg	2.4	1.5	0.3	0.9	0.0	0.1	0.0	0.0	0.7	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cummulative	2.4	3.9	4.2	5.1	5.1	5.1	5.1	5.1	5.8	5.9	5.9	5.9	5.9	5.9	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
0.1 CTAB 1	3.0	1.6	0.2	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 CTAB 2	3.0	2.0	0.2	0.4	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 CTAB 3	4.0	0.4	0.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1 CTAB Avg.	3.3	1.3	0.2	0.8	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cummulative	3.3	4.7	4.9	5.7	5.7	5.7	5.7	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
0.25 CTAB 1	3.0	0.6	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25 CTAB 2	2.6	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25 CTAB 3	3.2	0.4	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.25 CTAB Avg	2.9	0.4	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cummulative	2.9	3.3	3.5	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7	3.7

Appendix C (continued)
Activity Analysis of ATA Reactors

Days	3	8	14	24	31
Control 1	0.2	3.2	1.8	0.0	0.0
Control 2	0.2	2.6	0.0	0.0	0.0
Control 3	0.6	5.2	1.0	0.0	0.0
Control Average	0.3	3.7	0.9	0.0	0.0
Cummulative	0.3	4.0	4.9	4.9	4.9
0.01 CTAB 1	0.4	5.6	2.0	0.0	0.0
0.01 CTAB 2	2.8	2.6	0.2	0.0	0.0
0.01 CTAB 3	2.2	4.0	0.0	0.0	0.0
0.01 CTAB Averag	1.8	4.1	0.7	0.0	0.0
Cummulative	1.8	5.9	6.6	6.6	6.6
0.05 CTAB 1	0.0	0.0	0.0	0.0	0.0
0.05 CTAB 2	0.0	0.0	0.0	0.0	0.0
0.05 CTAB 3	0.0	0.0	0.0	0.0	0.0
0.05 CTAB Averag	0.0	0.0	0.0	0.0	0.0
Cummulative	0.0	0.0	0.0	0.0	0.0
0.1 CTAB 1	0.0	0.0	0.0	0.0	0.0
0.1 CTAB 2	0.0	0.0	0.0	0.0	0.0
0.1 CTAB 3	0.0	0.0	0.0	0.0	0.0
0.1 CTAB Average	0.0	0.0	0.0	0.0	0.0
Cummulative	0.0	0.0	0.0	0.0	0.0
0.25 CTAB 1	0.0	0.0	0.0	0.0	0.0
0.25 CTAB 2	0.0	0.0	0.0	0.0	0.0
0.25 CTAB 3	0.0	0.0	0.0	0.0	0.0
0.25 CTAB Averag	0.0	0.0	0.0	0.0	0.0
Cummulative	0.0	0.0	0.0	0.0	0.0

APPENDIX D

Gas Production for CTAB Toxicity ATA

Appendix D

Gas Production (ml) for CTAB Toxicity ATA

Days	2	5	10	17	23	35	49	56	63	70	75	90	96	103	110	118
Standard 1	2.6	0.4	1.0	1.8	14.8	17.8	24.8	17.0	5.2	0.2	0.0	25.2	8.8	4.4	0.4	0.0
Standard 2	1.0	0.4	0.4	0.4	13.2	49.4	30.2	0.4	0.2	0.6	0.2	6.4	6.2	6.8	0.2	0.0
Standard 3	1.2	0.4	1.4	2.8	14.4	31.8	34.8	15.2	0.4	1.8	2.2	9.8	6.2	2.8	0.0	0.0
Standard Average	1.6	0.4	0.9	1.7	14.1	33.0	29.9	10.9	1.9	0.9	0.8	13.8	7.1	4.7	0.2	0.0
Cummulative	1.6	2.0	2.9	4.6	18.7	51.7	81.7	92.5	94.5	95.3	96.1	109.9	117.0	121.7	121.9	121.9
0.001 CTAB 1	1.2	0.2	0.4	0.4	5.2	19.4	8.2	16.2	0.8	1.8	0.2	15.0	16.4	8.8	6.6	0.4
0.001 CTAB 2	0.8	0.2	0.6	1.0	7.6	43.2	23.6	0.6	0.6	0.0	0.0	20.2	9.4	10.6	2.8	0.0
0.001 CTAB 3	0.4	0.2	0.6	0.4	5.4	35.4	37.4	8.4	1.6	0.4	2.8	3.0	1.8	6.2	6.2	0.0
0.001 CTAB Average	0.8	0.2	0.6	0.7	6.5	39.3	30.5	4.5	1.1	0.2	1.4	11.6	5.6	8.5	5.2	0.1
Cummulative	0.8	1.0	1.6	2.3	8.8	48.1	78.6	83.1	84.2	84.4	85.8	97.4	103.0	111.5	116.7	116.9
0.005 CTAB 1	0.8	0.2	0.4	0.4	4.6	15.0	24.2	30.4	9.4	0.0	0.0	9.6	9.0	6.8	2.2	0.2
0.005 CTAB 2	0.8	0.2	0.6	0.2	4.6	27.6	31.4	19.8	8.6	0.0	0.0	0.2	5.2	1.8	0.4	0.0
0.005 CTAB 3	0.4	0.6	0.4	0.4	5.0	28.2	36.8	25.0	5.8	1.8	0.0	5.8	6.2	4.4	0.8	0.0
0.005 CTAB Average	0.7	0.3	0.5	0.3	4.7	23.6	30.8	25.1	7.9	0.6	0.0	5.2	6.8	4.3	1.1	0.1
Cummulative	0.7	1.0	1.5	1.8	6.5	30.1	60.9	86.0	93.9	94.5	94.5	99.7	106.5	110.9	112.0	112.1
0.01 CTAB 1	0.4	0.2	0.2	0.2	5.8	25.4	28.6	18.4	4.2	0.6	2.4	8.2	9.6	4.4	0.4	0.0
0.01 CTAB 2	0.6	0.4	0.4	0.4	6.4	30.2	19.6	16.0	4.6	2.0	2.0	7.6	10.2	3.6	0.0	0.0
0.01 CTAB 3	0.6	0.2	0.6	0.4	4.4	18.6	17.0	32.8	9.8	2.6	0.6	12.4	17.4	8.4	0.8	0.0
0.01 CTAB Average	0.5	0.3	0.4	0.3	5.5	24.7	21.7	22.4	6.2	1.7	1.7	9.4	12.4	5.5	0.4	0.0
Cummulative	0.5	0.8	1.2	1.5	7.1	31.8	53.5	75.9	82.1	83.9	85.5	94.9	107.3	112.8	113.2	113.2
0.020 CTAB 1	0.4	0.2	0.4	0.2	0.2	16.4	27.2	34.4	16.8	5.2	0.2	1.2	1.2	0.8	0.0	0.0
0.020 CTAB 2	0.6	0.2	0.2	0.2	2.6	16.0	23.2	32.0	12.2	0.2	1.8	0.8	0.2	0.0	0.0	0.0
0.020 CTAB 3	0.6	0.4	0.2	0.2	3.0	14.2	21.4	30.2	15.2	0.4	2.6	5.4	7.0	2.2	0.0	0.0
0.020 CTAB Average	0.5	0.3	0.3	0.2	1.9	15.5	23.9	32.2	14.7	1.9	1.5	2.5	2.8	1.0	0.0	0.0
Cummulative	0.5	0.8	1.1	1.3	3.2	18.7	42.7	74.9	89.6	91.5	93.1	95.5	98.3	99.3	99.3	99.3
0.030 CTAB 1	0.6	0.2	0.2	0.4	0.2	0.0	13.0	9.4	17.8	7.2	4.6	8.2	0.2	0.0	0.0	0.0
0.030 CTAB 2	0.4	0.2	0.2	0.2	0.2	18.2	16.2	20.4	10.2	0.0	2.4	11.6	0.0	0.0	0.0	0.0
0.030 CTAB 3	0.8	0.2	0.4	0.2	0.2	2.2	13.2	6.2	1.8	0.0	0.6	22.2	22.2	8.4	0.8	0.0
0.030 CTAB Average	0.6	0.2	0.3	0.3	0.2	6.8	14.1	12.0	9.9	2.4	2.5	14.0	7.5	2.8	0.3	0.0
Cummulative	0.6	0.8	1.1	1.3	1.5	8.3	22.5	34.5	44.4	46.8	49.3	63.3	70.8	73.6	73.9	73.9
0.050 CTAB 1	0.6	0.2	0.2	0.0	0.0	0.0	0.0	0.0	5.8	5.4	0.4	0.0	0.0	0.0	0.0	0.0
0.050 CTAB 2	0.6	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
0.050 CTAB 3	0.8	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.050 CTAB Average	0.7	0.2	0.2	0.1	0.0	0.0	0.0	0.0	1.9	1.9	0.1	0.0	0.0	0.0	0.0	0.0
Cummulative	0.7	0.9	1.1	1.1	1.1	1.1	1.1	1.1	3.1	4.9	5.1	5.1	5.1	5.1	5.1	5.1

APPENDIX E

Effect of Temperature on Gas Production for CTAB ATA

Appendix E
Effect of Temperature on Gas Production for CTAB ATA

Days	2	7	16	26	34	43	49	57	64	70	82	96	103	110
Temperature 38 C														
Blank 1	4.2	2.2	0.4	19.8	12.6	40.6	33.6	13.0	13.4	26.2	8.2	3.2	0.6	0.0
Blank 2	4.2	1.2	1.2	21.2	5.6	5.2	42.0	55.2	13.2	21.0	13.8	3.6	0.0	0.0
35 Blank Average	4.2	1.7	0.8	20.5	9.1	22.9	37.8	34.1	13.3	23.6	11.0	3.4	0.3	0.0
Cummulative	4.2	5.9	6.7	27.2	36.3	59.2	97.0	131.1	144.4	168.0	179.0	182.4	182.7	182.7
CTAB 1	3.8	0.4	0.4	7.2	16.2	4.2	1.2	0.0	0.0	0.0	0.0	0.8	3.0	0.0
CTAB 2	3.2	1.2	0.2	4.8	14.8	3.8	0.4	0.0	0.0	0.2	0.0	5.8	4.8	0.0
38 CTAB Average	3.5	0.8	0.3	6.0	15.5	4.0	0.8	0.0	0.0	0.1	0.0	3.3	3.9	0.0
Cummulative	3.5	4.3	4.6	10.6	26.1	30.1	30.9	30.9	30.9	31.0	31.0	34.3	38.2	38.2
Temperature 28 C														
Blank 3	1.2	1.2	0.2	0.4	5.6	15.4	6.8	22.6	30.4	15.0	7.4	16.8	0.2	0.0
Blank 4	1.4	1.4	0.0	0.2	5.4	18.0	6.3	17.8	16.4	17.8	16.8	1.6	0.0	0.0
28 Blank Average	1.3	1.3	0.1	0.3	5.5	16.7	6.5	20.2	23.4	16.4	12.1	9.2	0.1	0.0
Cummulative	1.3	2.6	2.7	3.0	8.5	25.2	31.7	51.9	75.3	91.7	103.8	113.0	113.1	113.1
CTAB 3	0.6	0.6	0.2	0.2	0.2	2.6	2.8	5.6	6.2	3.8	0.0	0.0	0.0	0.0
CTAB 4	0.4	0.4	0.2	0.2	0.2	1.4	3.4	5.0	5.2	10.2	7.0	0.0	0.0	0.0
28 CTAB Average	0.5	0.5	0.2	0.2	0.2	2.0	3.1	5.3	5.7	7.0	3.5	0.0	0.0	0.0
Cummulative	0.5	1.0	1.2	1.4	1.6	3.6	6.7	12.0	17.7	24.7	28.2	28.2	28.2	28.2

APPENDIX F

Gas Production for Preliminary Soaking and Washing Bath ATA

Appendix F

Gas Production for Preliminary Soaking and Washing Bath ATA

	2	6	13	21	27	35	42	48	56	66	76	84	91	97	105	112	118	130	144
Standard 1	2.4	0.8	0.0	13.8	8.8	8.0	23.2	25.4	9.8	10.8	31.6	6.4	1.8	0.0	0.0	0.0	0.0	0.0	0.0
Standard 2	2.2	0.8	0.2	15.2	9.2	14.6	14.2	23.6	6.0	8.2	32.0	7.2	3.0	0.8	0.2	0.6	0.2	0.2	0.0
Standard 3	2.0	0.6	0.0	10.2	5.0	20.0	19.2	14.2	7.8	8.0	12.8	25.0	6.2	1.4	0.2	0.2	0.4	0.0	0.0
AVG	2.2	0.7	0.1	13.1	7.7	14.2	18.9	21.1	7.9	9.0	25.5	12.9	3.7	0.7	0.1	0.3	0.2	0.1	0.0
CUM	2.2	2.9	3.0	16.1	23.7	37.9	56.8	77.9	85.7	94.7	120.2	133.1	136.7	137.5	137.6	137.9	138.1	138.1	138.1
WB 1% 1	2.8	0.6	0.0	2.2	14.6	6.2	15.8	12.8	17.4	9.4	6.4	11.2	11.8	2.8	7.2	5.4	4.8	3.4	0.2
WB 1% 2	2.2	0.4	0.0	6.4	12.8	3.6	0.0	17.0	8.6	13.4	28.8	28.6	3.4	5.4	0.8	3.0	0.2	0.0	0.2
WB 1% 3	2.6	0.4	0.0	5.6	13.6	5.6	14.4	14.2	10.8	16.0	7.2	7.0	6.6	8.2	9.2	6.8	4.0	5.2	9.2
AVG	2.5	0.5	0.0	4.7	13.7	5.1	10.1	14.7	12.3	12.9	14.1	15.6	7.3	5.5	5.7	5.1	3.0	2.9	3.2
CUM	2.5	3.0	3.0	7.7	21.4	26.5	36.6	51.3	63.5	76.5	90.6	106.2	113.5	118.9	124.7	129.7	132.7	135.6	138.8
WB 5% 1	1.6	0.6	0.2	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WB 5% 2	2.2	0.6	0.0	0.2	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WB 5% 3	2.0	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVG	1.9	0.5	0.1	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CUM	1.9	2.5	2.5	2.7	2.7	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9
WB 10% 1	1.8	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WB 10% 2	2.4	0.6	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WB 10% 3	2.4	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVG	2.2	0.9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CUM	2.2	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
SB 1% 1	2.6	0.6	0.0	2.8	13.0	4.8	4.6	8.4	12.2	16.8	14.4	8.2	6.8	6.8	7.8	8.8	5.4	3.8	0.0
SB 1% 2	3.2	1.2	0.0	4.4	14.8	1.2	1.6	14.6	21.6	20.8	7.4	7.2	7.0	7.8	8.6	8.0	4.2	3.6	0.0
SB 1% 3	2.8	1.0	0.0	2.8	15.2	1.6	0.4	0.4	1.4	8.2	18.0	40.6	14.6	10.2	5.2	0.2	0.6	0.0	0.0
AVG	2.9	0.9	0.0	3.3	14.3	2.5	2.2	7.8	11.7	15.3	13.3	18.7	9.5	8.3	7.2	5.7	3.4	2.5	0.0
CUM	2.9	3.8	3.8	7.1	21.5	24.0	26.2	34.0	45.7	61.0	74.3	92.9	102.4	110.7	117.9	123.5	126.9	129.4	129.4
SB 5% 1	2.6	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SB 5% 2	3.2	0.2	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SB 5% 3	2.4	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVG	2.7	0.7	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CUM	2.7	3.4	3.4	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
SB 10% 1	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SB 10% 2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SB 10% 3	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVG	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CUM	0.0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

APPENDIX G

Gas Production for Soaking and Washing Bath Toxicity Assay

Appendix G
Gas Production (ml) for Soaking Bath and Washing Bath ATA

Days	4	16	30	37	44	51	56	71	77	82	89	96	103	110
Blank 1	0.2	1.6	7.6	11.4	14.6	23.2	28.4	31.2	10.4	4.2	7.2	1.6	0.0	0.0
Blank 2	0.2	1.2	16.0	33.2	23.8	12.2	10.2	19.2	2.6	0.0	7.6	0.8	0.0	0.0
AVG	0.2	1.4	11.8	22.3	19.2	17.7	19.3	25.2	6.5	2.1	7.4	1.2	0.0	0.0
CUM	0.2	1.6	13.4	35.7	54.9	72.6	91.9	117.1	123.6	125.7	133.1	134.3	134.3	134.3
0.5% SB 1	0.2	0.8	13.8	10.4	19.6	27.6	32.8	36.4	4.4	0.4	0.0	0.0	0.0	0.0
0.5% SB 2	0.2	1.4	13.6	11.6	24.0	18.8	14.6	27.6	11.6	2.6	0.2	0.0	0.0	0.0
AVG	0.2	1.1	13.7	11.0	21.8	23.2	23.7	32.0	8.0	1.5	0.1	0.0	0.0	0.0
CUM	0.2	1.3	15.0	26.0	47.8	71.0	94.7	126.7	134.7	136.2	136.3	136.3	136.3	136.3
1% SB 1	0.4	0.8	0.0	6.6	4.2	0.4	0.2	1.6	1.4	6.4	6.8	4.4	0.4	0.0
1% SB 2	0.2	1.0	0.2	0.0	11.4	12.6	9.8	19.8	0.0	3.0	3.2	6.4	0.6	0.0
AVG	0.4	1.0	0.2	6.6	11.4	12.6	9.8	19.8	1.4	6.4	5.0	5.4	0.5	0.0
CUM	0.4	1.4	1.6	8.2	19.6	32.2	42.0	61.8	63.2	69.6	74.6	80.0	80.5	80.5
2% SB 1	0.2	0.8	0.0	0.0	0.0	0.6	3.8	7.6	0.0	0.0	0.0	0.0	0.0	0.0
2% SB 2	0.2	1.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVG	0.2	1.0	0.0	0.0	0.0	0.3	2.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0
CUM	0.2	1.2	1.2	1.2	1.2	1.5	3.5	7.3	7.3	7.3	7.3	7.3	7.3	7.3
4% SB 1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4% SB 2	0.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVG	0.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CUM	0.2	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
0.5% WB 1	0.2	1.2	11.6	18.6	19.2	40.6	14.0	17.6	4.2	0.0	0.0	0.0	0.0	0.0
0.5% WB 2	0.2	0.8	10.2	11.8	13.6	13.2	21.6	41.2	29.8	0.2	0.0	0.0	0.0	0.0
AVG	0.2	1.0	10.9	15.2	16.4	26.9	17.8	29.4	17.0	0.1	0.0	0.0	0.0	0.0
CUM	0.2	1.2	12.1	27.3	43.7	70.6	88.4	117.8	134.8	134.9	134.9	134.9	134.9	134.9
1% WB 1	0.4	0.6	0.2	0.0	9.2	9.4	2.8	6.0	6.4	8.8	8.2	6.8	0.8	0.0
1% WB 2	0.2	1.6	0.4	8.2	10.6	0.0	0.2	0.2	0.0	0.0	0.0	0.2	0.0	0.0
AVG	0.3	1.1	0.3	4.1	9.9	4.7	1.5	3.1	3.2	4.4	4.1	3.5	0.4	0.0
CUM	0.3	1.4	1.7	5.8	15.7	20.4	21.9	25.0	28.2	32.6	36.7	40.2	40.6	40.6
2% WB 1	0.2	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2% WB 2	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVG	0.2	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CUM	0.2	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
4% WB 1	0.2	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4% WB 2	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
AVG	0.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CUM	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

APPENDIX H

Expected Gas Production From Anaerobic Toxicity Assays

Appendix H
Expected Gas Production From Anaerobic Toxicity Assays

ATA	CODi (mg/l)	CODe (mg/l)	COD Used (mg/l)	Equivalent Glucose (mg/l)	Expected Gas Production (ml)	Actual Gas Production (ml)	% Gas Produced
Defoaming Agent	5980	2070	391	365.4	150.0	136.9	91.3%
Preliminary CTAB ATA	6040	2080	396	370.1	151.9	332.8	219.1%
CTAB Toxicity Threshold	5840	2100	374	349.5	143.4	125.2	87.3%
SB & WB Preliminary AT/	5800	2140	366	342.1	140.4	138.1	98.4%
SB & WB Toxicity Thresh	5620	1820	380	355.1	145.7	134.3	92.1%

APPENDIX I

Fate of CTAB in Simulated Activated Sludge Reactor

Appendix I

Fate of CTAB in Simulated Activated Sludge Reactor

Trial #1

Time (min)	TOC Reading (mg/l)	Dilution	Adjusted TOC (mg/l)
0	24.52	0.2	122.6
0.1	39.07	0.2	195.4
1	38.42	0.2	192.1
2	36.69	0.2	183.5
5	33.76	0.2	168.8
10	35.57	0.2	177.9
20	35.71	0.2	178.6
40	36.93	0.2	184.7
60	37.52	0.2	187.6
120	40.25	0.2	201.3
240	45.98	0.2	229.9
480	45.87	0.2	229.4
1100	53.17	0.2	265.9
1440	244.6	1.0	244.6

CTAB 500 mg/l = 312.8 TOC reading

Trial #2

Time (min)	TOC Reading (mg/l)	Dilution	Adjusted TOC (mg/l)
1	42.80	0.2	214.0
2	47.32	0.2	236.6
10	46.19	0.2	231.0
20	46.01	0.2	230.1
40	48.28	0.2	241.4
240	52.57	0.2	262.9
480	49.42	0.2	247.1
1100	54.97	0.2	274.9
1440	56.15	0.2	280.8
2880	43.35	0.2	216.8

CTAB 500 mg/l = 312.8 TOC reading

APPENDIX J

Fate of CTAB Through ATA Reactors (HPLC Analysis)

Appendix J
Fate of CTAB Through ATA Reactors (HPLC Analysis)

Standard Curve for CTAB on HPLC at 210 nm

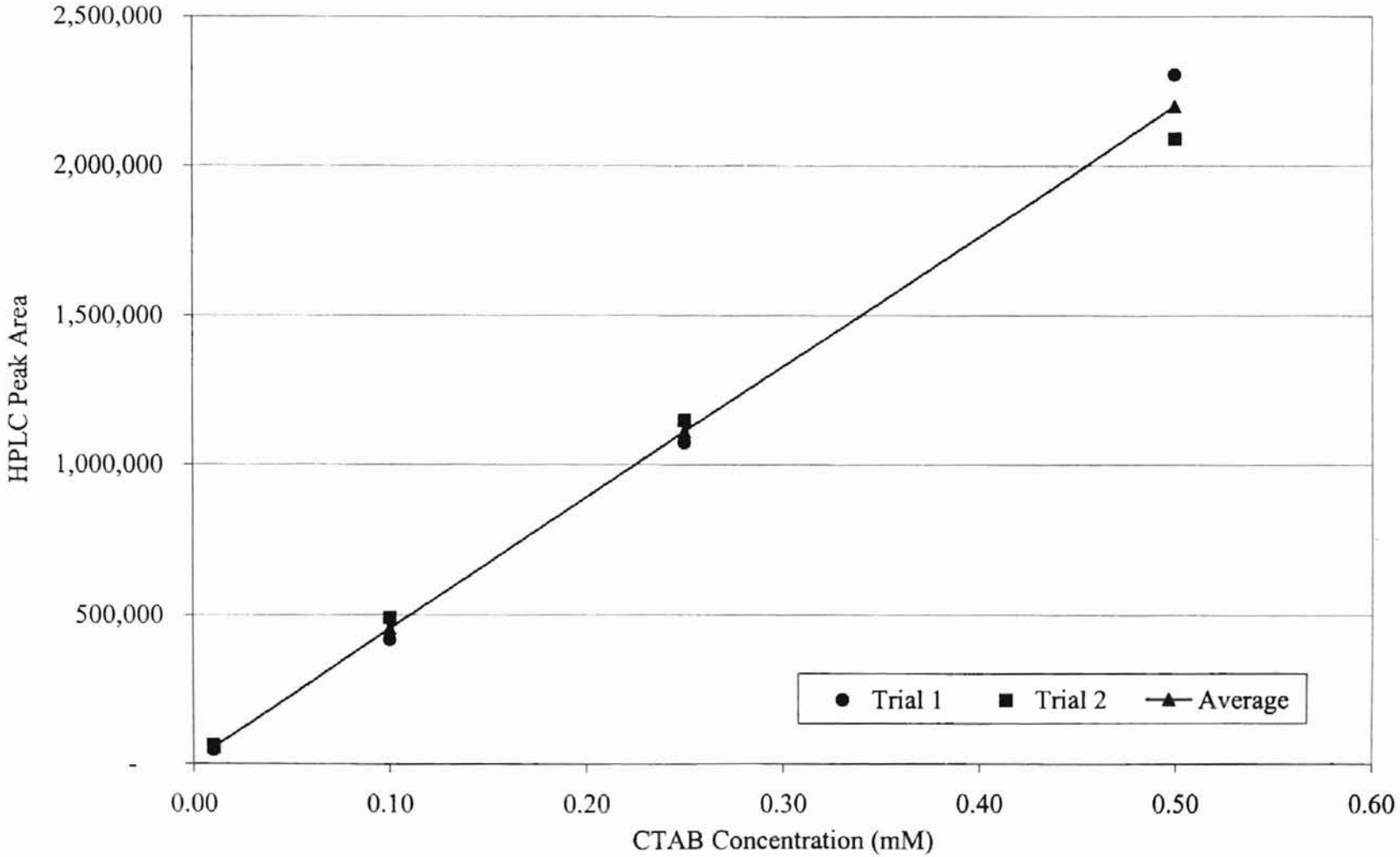
Sample	Trial 1 Peak Area	Trial 2 Peak Area	Average Peak Area
0.01 mM CTAB	46,002	64,017	55,010
0.10 mM CTAB	416,767	489,967	453,367
0.25 mM CTAB	1,072,324	1,145,651	1,108,988
0.50 mM CTAB	2,300,186	2,087,855	2,194,021

HPLC Analysis of CTAB Assay Reactors

Reactor Sample	Trial 1 Peak Area	Trial 2 Peak Area	Average Peak Area
Control	8,532,844	7,721,252	8,127,048
0.01 mM CTAB	8,734,880	8,199,592	8,467,236
0.05 mM CTAB	8,925,091	9,300,533	9,112,812
0.10 mM CTAB	7,689,054	8,097,134	7,893,094
0.25 mM CTAB	9,105,674	8,642,128	8,873,901

HPLC Analysis of Soaking and Washing Bath Assay Reactors

Reactor Sample	Trial 1 Peak Area	Trial 2 Peak Area	Average Peak Area
Control	9,879,532	11,007,040	10,443,286
1% Soaking Bath	10,783,420	12,251,268	11,517,344
5% Soaking Bath	9,349,845	9,145,355	9,247,600
10% Soaking Bath	11,348,051	9,339,421	10,343,736
1% Washing Bath	12,084,112	8,539,936	10,312,024
5% Washing Bath	9,568,732	9,915,608	9,742,170
10% Washing Batl	10,045,742	9,216,632	9,631,187



Standard Curve for CTAB HPLC Analysis by measuring the absorbance at 211 nm at varying concentrations of CTAB.

APPENDIX K

Fate of Ink Residue

Appendix K
Fate of Ink Residue

Reactor	Initial			Final		
	Total Solids (mg/l)	Volatile Solids (mg/l)	COD (mg/l)	Total Solids (mg/l)	Volatile Solids (mg/l)	COD (mg/l)
Control	13220	4980	5800	13400	5040	2140
1% SB	13880	5040	5640	13260	4840	2040
5% SB	14880	5120	5920	14480	5020	5860
10% SB	14520	5180	5980	14980	4940	6120

Reactor	Equivalent Glucose Uptake (mg)*	Theoretical Biomass Growth (mg)*	Expected Final Volatile Solids (mg/l)	Actual Final Volatile Solids (mg/l)	% Theoretical
Control	343.125	542.1375	5179.0	5040.0	97.32%
1% SB	337.5	533.25	5235.8	4840.0	92.44%
5% SB	5.625	8.8875	5123.3	5020.0	97.98%
10% SB	-13.125	-20.7375	5172.4	4940.0	95.51%

*Negative values for equivalent glucose uptake and biomass growth merely indicate that the final COD reading was in excess of the initial COD reading. These negative numbers do not reflect what would be expected to actually happen in an anaerobic toxicity assay reactor.

VITA

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Master of Science

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