BIODECOLORIZATION OF PAPER MILLS WASTEWATER USING

ANAEROBIC COMPOSTING

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BIODECOLORIZATION OF PAPER MILLS WASTEWATER USING ANAEROBIC COMPOSTING

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BIODECOLORIZATION OF PAPER MILLS WASTEWATER USING

ANAEROBIC COMPOSTING

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VITA

Noemí C. Méndez-Sánchez, daughter of Andrés Méndez-Medina and Noemí Sánchez-Desardén, was born on March 4, 1977 in the city of San Juan, Puerto Rico. She graduated high school from Escuela Superior Dr. Pedro Albizu Campus with Academic Excellence in the top of her class. In 2000, she graduated from the University of Puerto Rico – Mayagüez Campus with a Bachelor of Science in Chemical Engineering and a Minor in Biotechnology. She obtained her Master of Science in Civil Engineering in 2002 from The University of Akron. In fall 2002, she joined the Ph.D. program at Auburn University under the guidance of Dr. Clifford R. Lange. During 2005, she worked as a Research Scientist at Rayonier Inc. where she started her current research. Upon returning to Auburn in 2006, she worked as a GTA in the Civil Engineering Department, where she had the joy of teaching many of the future civil engineers from Auburn University. During her stay at Auburn University, she disseminated her research in national and international meetings and has submitted four papers summarizing her research.

DISSERTATION ABSTRACT

BIODECOLORIZATION OF PAPER MILLS WASTEWATER USING

ANAEROBIC COMPOSTING

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This research studied the feasibility of using anaerobic composting for decolorizing effluents in the pulp and paper industry. While color is regarded as an aesthetic problem, there are several adverse effects associated to it. The effects include reduced sunlight penetration, increases in water temperature, and decreases in dissolved oxygen. In addition, color is often associated with carcinogens. These problems along with stricter regulations have lead to increase research in this area. In this research, we studied a new method for reducing color using anaerobic composting.

Biologically active and gamma-sterilized compost was used to study the biological and abiotic adsorption and decolorization kinetics to demonstrate the feasibility of removing color from paper mill effluents using anaerobic composting. The results showed that higher decolorization was achieved using active compost in comparison with gamma sterilized compost.

The second set of experiments studied color removal sustainability and the degree and rate of decolorization of effluents of dissimilar color, pH, and oxidation reduction potential levels. More than 75% color reduction was achieved for effluents having color as high as 90,000 Platinum Cobalt Units. When the samples were aerated, the color reversion was minimal. The third part of this research presents experiments done to investigate the best conditions for biodecolorization. The parameters studied include compost-to-feed ratio, length of sequencing batch reactor cycles, mixing and shaking, temperature, flocculent effects, long-term operation, and nutritional requirements. The optimized conditions were applied to pilot scale reactors and decolorization exceeding 90% was achieved. The interaction between different anaerobic microbial communities was studied in the fourth part of this research. The results show that inhibition of sulfur reducing bacteria resulted in higher decolorization. An unknown culture was isolated and its 16S rRNA gene was sequenced for identification. This analysis resulted in 82.1% identity with *Aeromonas punctata*. Color reduction of 55% was achieved using *A. punctata* without additional sources of carbon. The last part of the research studied chromophores fate in the biodecolorization process using size exclusion chromatography. Treatment of the effluents showed that polymerization and biodegradation took place.

In general, the results from this study presented a novel, feasible, and readily implementable method of removing color using compost biosolids. The use of this process not only serves to decrease effluents color but will also reduce the treatment of solid waste by reusing waste fibers from the paper manufacturing industry. As new regulations for the color levels in effluents emerge, mills may focus on using this process to treat low-volume, high color wastes to make processing more economical.

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CHAPTER 1. GENERAL INTRODUCTION

Even with the most modern and efficient operational techniques, about 60 m^3 of wastewater is generated during the production of a ton of paper (Thompson et al. 2001). The effluents generated during the processing of pulp and paper are highly colored. One of the many adverse effects associated with the discharge of dark color wastewater into rivers and lakes is the reduction of photosynthetic activity as less sunlight can penetrate into the water. In addition, the temperature of the water increases leading to lower concentrations of dissolved oxygen.

Several physical, chemical, and biological methods are available to reduce the color of the wastewaters. Usually a combination of physical chemical processes is necessary in order to obtain satisfactory results. Biological methods under aerobic conditions focus mostly on the use of lignin-degrading fungi (Bajpai et al. 1993; Breen and Singleton 1999; Hatakka 2001; Livernoche et al. 1983; Murugesan 2003; Scott and Akhtar 2001). Some of the limitations with this approach are the nutrient and oxygen requirement of the fungi for effective decolorization. Additionally, the efficiency of these processes tends to be dependent on the characteristics of the mill wastewaters. Therefore, the conditions used for the removal of color in one paper mill will not necessarily work in another mill.

A feasible and sustainable decolorization process that can be applied in mills with different wastewater characteristic is highly desirable. Implementation of this process would guarantee the well-being of water ecosystems receiving the wastewaters.

1.1. Problem Definition

Recent studies have recognized the use of anaerobic biodegradation as a feasible approach for the reduction of chromophores in the textile, olive, and paper and pulp industries (Chuphal et al. 2005; Minke and Rott 1999; Panswad and Luangdilok 2000; Paraskeva and Diamadopoulos 2006; Pokhrel and Viraraghavan 2004). Still, reduction of color in paper and pulp mill wastewater has not been studied as frequently as in textile and olive mills. Research have been performed using combined aerobic and anaerobic processes for the treatment of pulp and mill effluents, however, the color removal is often done under aerobic conditions (Kortekaas et al. 1998; Singh 2007; Tezel et al. 2001). Recent experiments performed in our laboratory have shown that highly colored wastewater could be decolorized under anaerobic composting conditions (Lange and Mendez-Sanchez 2008; Méndez-Sánchez et al. 2007). The current literature has not reported color removal under anaerobic conditions using recycle materials with such effectiveness as those observed in preliminary studies. These results present a feasible and cost-effective method for removal of color. However, additional research is needed before applying this process at larger scale in other mills.

Questions addressed during this research focused in the aim of designing an anaerobic treatment for color reduction included: What variables affect the color removal process? What microorganisms are driving the color reduction process? What is the fate

of the compounds causing color in the wastewaters? Answer to these unknowns will help us improve the decolorization process and understand the mechanism of color removal observed in our experiments. The information obtained will also help in identifying how this decolorization process can be applied at larger scale and which operational variables must be controlled in order to guarantee its continuous operation. Therefore, *the overall objectives of this research focused on identifying and optimizing the process variables and environmental conditions that may affect the anaerobic decolorization process.*

1.2. Significance

Color removal from paper mill wastewater has become an important issue that many pulp mills have to accomplish before final disposal of their effluents. Decolorization of paper and pulp mill wastewater is critical for the well being of the receiving aquatic ecosystem (Del Giorgio and Peters 1994; Kirk 1994; Pellinen et al. 1988). At the moment there are no specific limits in the United States regarding the amount of color that can be discharged (Jensen 1999; USEPA 2002). Other countries in the world have set limitation to the color of the wastewaters they can discharge (Bajpai et al. 1999). However, this is only achieved by a combination of processes which generally are not economically feasible (Bhattacharyya and Sarma 1997; Tarlan et al. 2002).

The removal of color using the anaerobic compost biosystem we are proposing proved to be sustainable in studies performed in the laboratory. This process could be applied to wastewaters from other mills with similar success. This is a very significant finding as the processing from mill-to-mill is usually different. In turn, different processing in each mill makes its wastewater have dissimilar characteristics. Therefore,

processes that work efficiently in one mill may not work as well in another mill. Application of this decolorization process at other paper mills will prevent contamination of the receiving environment, as the wastewaters will be decolorized before being released into the aquatic ecosystem. Therefore, this process will help improve the current color situation of all water bodies receiving pulp and paper mills wastewater.

The results of this study will provide an efficient and cost-effective scheme for the removal of color. The elucidation of the different operational parameters (i.e., waste-to-compost ratio, mixing, shaking, etc.) for the optimization of the process is needed for an efficient, safe, and economical design. Identification of the decolorizing microbial community will help us understand the mechanism by which color is being removed. Identification of the fate of color forming compounds during biodegradation is necessary in order to ensure that color is removed permanently.

1.3. Objectives

The overall goal of this research was to investigate the feasibility of using anaerobic composting for the decolorization of paper mill wastewaters. The specific objectives included:

- Characterize the wastewaters and compost of samples in order to identify environmental conditions that may affect the anaerobic decolorization process.
- Determine the sorption capacity of active and virgin compost (new waste fibers that has not been in contact with the compost) under different environmental conditions and the effects they have on the decolorization process.

- Study the applicability of this decolorization process to diverse waste streams within a paper mill with different color, pH, and ORP levels.
- Identify and optimize key process variable that may affect the decolorization efficiency and reliability of this process.
- Study the interaction between methanogens, sulfur reducing bacteria, and other anaerobic microorganisms to in order to determine their impact to the biodecolorization process.
- Isolate and identify bacteria growing in paper mill anaerobic compost that may be involved in the decolorization process. In addition, test the extent and rate of decolorization of the isolated bacteria to corroborate its decolorization ability.
- Determine the fate of these chromophores in the anaerobic compost environment.

1.4. Organization

The results of this research are presented in Chapters 3 through Chapter 7. Chapters 3 is similar to the paper being reviewed for publication in the Journal of Environmental Engineering (Lange and Mendez-Sanchez 2008). Chapter 4 is similar to a research article presented in WEFTEC 2007: 80th Water Environment Federation's Annual Technical Exhibition and Conference and included within the proceedings of the conference (Méndez-Sánchez et al. 2007). In addition, this paper is under review for publication in the Journal of Environmental Engineering. Chapters 5 through Chapter 7 are manuscripts currently being prepared.

The potential for biological and abiotic color reduction of paper mill effluent using anaerobic composting was studied in Chapter 3. Chapter 4 presents the results for

the anaerobic decolorization of paper mill effluents with dissimilar characteristics. Important process variables in the anaerobic decolorization process are studied and optimized in Chapter 5. In addition, Chapter 5 presents a pilot-scale testing using the optimized parameters for decolorization that were assessed through the study. Chapter 6 presents the identification and isolation of microorganisms driving the anaerobic decolorization process. The last part of this research is presented in Chapter 7 where the changes in the size distribution of chromophores in effluents from paper mills during anaerobic decolorization are studied. Chapter 8 presents a summary of the results, implications, and recommendations for future research.

CHAPTER 2. LITERATURE REVIEW

2.1. Problems associated with pulp and paper mills wastewaters

There is great concern regarding the pollution generated at paper and pulp mills. While this industry discharges gaseous, liquid, and solid wastes, pollution of the aquatic ecosystem is the major problem as large volumes of wastewater is generated while producing paper (Bhattacharyya and Sarma 1997; Selvam et al. 2002). The characteristics of wastewater generated from various processes of the pulp and paper industry depend upon the type of process, wood material, process technology, recirculation of the wastewater for recovery of chemicals, and the amount of water to be used in each particular process. Parameters to be controlled before wastewater discharges into the environment include chemical and biological oxygen demand (COD and BOD), absorbable organic halides (AOX), and color.

2.1.1. COD, BOD, and AOX

Pulp and paper mill wastewater discharges into freshwater, estuarine, and marine ecosystems. This in turn can alter aquatic habitats and adversely impact human health (Ghoreishi and Haghighi 1997; Jha et al. 2002). These wastewaters are loaded with organic matter that without adequate treatment can have high chemical and biological oxygen demand (COD and BOD) in the receiving waters (Ali and Sreekrishnan 2001; Bhattacharyya and Sarma 1997; Pellinen et al. 1988; Vidal Saez 1999). Some

components in the wastewater contributing to COD and BOD loads include lignin and its degradation product, fatty acids, resin acids, tannins, and sulfur containing compounds (Jantsch et al. 2002; Lacorte et al. 2003; Sierra-Alvarez et al. 1994). Also, the effluents are characterized by having high concentration of chlorinated compounds in the form of AOX (Archibald et al. 1997; Tezel et al. 2001). AOX are usually formed when bleaching chemicals react with lignin and its degradation products.

Usually, most of the chemical compounds found in these effluents are naturally present in the environment. They tend to be slightly or non-toxic to the surrounding ecosystem. Toxicity in pulp and paper mills wastewater is usually attributed to the high concentrations of wood extracts and chlorinated organics (in the form of AOX) formed during the bleaching stages (Vidal Saez 1999). Some of the xenobiotic pollutants such as dioxins and furans are recalcitrant to degradation, while others such as sterols are known endocrine disruptors (Christov and van Driessel 2003; Dinel et al. 2004; Kostamo et al. 2004; Lehtinen et al. 1999). Current environmental laws in the US have forced pulp and paper mills to modify their bleaching process in order to reduce the formation and release of AOX into the environment (Breed et al. 1997; Hiltgen and Hinsey 1996; Jensen 1999). Unfortunately, these modifications are not completely efficient and still some toxicity is conveyed. In other parts of the world, similar laws have not been promulgated or standards for AOX discharge have not been established (Bajpai et al. 1999; Nagarathnamma and Bajpai 1999).

2.1.2. Color

While several alternatives exist for controlling COD, BOD, and AOX, decolorization of wastewaters is usually overlooked. Color in water inhibits the process of photosynthesis as particles in solution will scatter and absorb light, reducing the photosynthetically available radiation (Kirk 1994). In addition, brown color wastewaters increase water temperature leading to decreased levels of dissolved oxygen (Kringstad and Lindstroem 1984; Pellinen et al. 1988; Selvam et al. 2002). As a consequence, the colored substances in aquatic systems have been associated with changes in primary productivity (Del Giorgio and Peters 1994; Henebry and Cairns 1984; Ilmavirta and Huttunen 1989), phytoplankton species composition (Beauchamp and Kerekes 1989), and protozoan colonization rates. In addition, secondary production (Hessen 1985), macro-invertebrate behavior (Juarez et al. 1986) and macro-invertebrate community structure (Kullberg 1992) can be affected. Color can also alter the availability and hence toxicity of heavy metals to fish (Haines et al. 1995; Nilsson and Häkanson 1992; Orrego et al. 2005). These effects are enhanced in cases where the receiving stream has low or varying flow.

Currently, there are no specific restrictions regarding the color limits in pulp and paper mill wastewaters. However, due to the potential adverse effect this problem has in the ecosystem, it is expected in the near future that laws will be promulgated limiting the levels of color in wastewaters.

2.2. Formation of color and chromophores

Until recently, color was not considered a major problem, being classified as a non-conventional pollutant. Nowadays, decolorization of wastewaters must be performed in order to guarantee the health and safety of the receiving environment. One of the first steps to solve this problem is to determine how color is formed as well as to identify possible chromophores in the wastewaters.

2.2.1. Formation of color

Color in paper and pulp mills is often associated with the thermal, mechanical, and chemical conversion of wood into pulp. During this process, the cellulose and hemicelluose is separated from the lignin and other extractives in the wood. Some of the byproducts obtained are compounds such as residual lignin and lignin derivates along with polymerized tannins that impart color to the water (Crooks and Sikes 1990; Sarkanen et al. 1971). In addition, chromophores are created from the degradation of lignin (Jha et al. 2002; Kemeny and Banerjee 1997; Kringstad and Lindstroem 1984; Mounteer et al. 2005; Selvam et al. 2002; Tarlan et al. 2002).

During the processing of the pulp, delignification and brightening is performed in different bleaching stages. Bleaching of the pulp also contributes to the formation of colored compounds. Figure 2.1 shows a schematic diagram of color formation during the bleaching stage. As can be observed in the figure, bleaching chemicals react with lignin and other components of the pulp (Eriksson et al. 1985; Kringstad et al. 1985; Yin 1989). Such reactions result in the formation of chlorinated organics that further contribute to wastewater color (Bajpai et al. 1999).

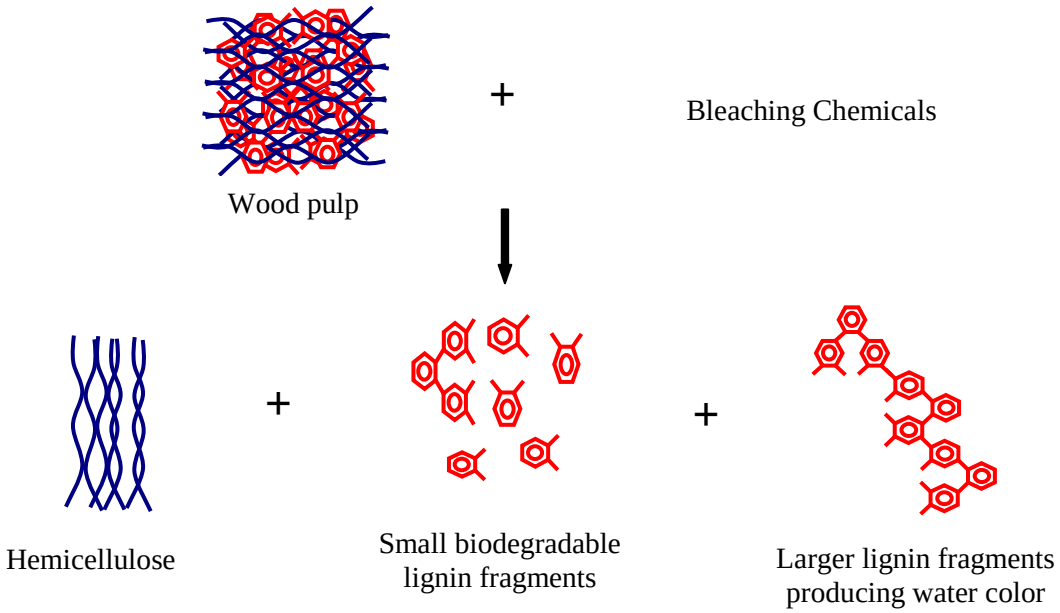


Figure 2.1 Schematic diagram of color formation during pulp bleaching.

2.2.2. Chromophores

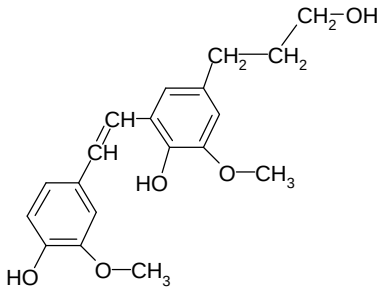
By definition, a chromophore is the part (atom or group of atoms) of a molecular entity capable of selective light absorption resulting in the coloration of certain organic compounds. The term originated in the textile industry referring to the molecule responsible for the dye's color. In dyes, the color is usually due to the azo-bond, which are two nitrogen atoms (N=N) in the center of the molecule. Cleavage of the azo-bond results in the decolorization of the dye (O'Neill et al. 2000; Panswad and Luangdilok 2000; Razo-Flores et al. 1997).

Contrary to the textile, where there is a usually a key chromophoric structure, there exist several chromophores in pulp and paper industry. In order to have an effective control of chromophores, it is necessary to elucidate what part of the lignin and its degradation products may cause color in the wastewaters of these paper mills.

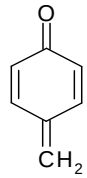
Adler (1977; 1966) summarized the main structure of lignin and studied its chemical reactions. Falkehag et al. (1966) identified the following as lignin components and degradation products as potential contributors to color:

a. CH=CH double bonds conjugated with aromatic ring
b. Quinomethides and quinones
c. Chalcone structures
d. Metal complexes with catechol structures

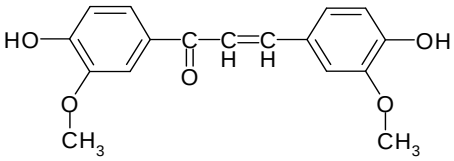
The representative structure of some of the chromophores identified is presented in Figure 2.2. Falkehag concluded in his studies that the last two structures contribution to color is of minor extent.



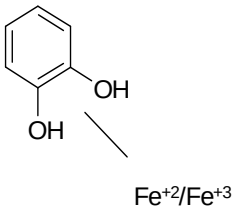
(a) *p,o'*-dihydroxistilbene



(b) *p*-quinonemethide



(c) Chalcone



(d) Catechol complexes with metals

Figure 2.2 Typical chromophores in paper and pulp mill wastewater effluents.

Sarkanen and Lundwing (1971) also identified compounds with double bonds conjugated within their aromatic ring, quinone methides, and quinone groups as possible sources of color in paper mill wastewaters solution. Sjostrom (1981) partially corroborated these findings and identified other possible color sources in pulp and paper mills. In addition to the structures identified by Falkehag, Sjostrom stated that leucochromophores (colorless chromophores) could be converted to chromophores by air oxidation. Kringstrad and Lindstroem (1984) identified high molecular weight chlorinated organics as carriers of chromophoric structures. Recently, Agarwal and Atalla (2000) used Raman spectroscopy as a tool to identify chromophores species difficult to isolate. In other studies, Zawadzki et al. (2000a; 2000b) used 31P-Nuclear Magnetic Resonance (NMR) to identify chromophores in lignin. Similarly to results from previous studies, they found that the main contributors to color were the C=C bonds conjugated with aromatic rings and C=O bonds such as in quinones molecules.

2.3. Methods for removal of color

Researchers are focusing lately on finding efficient ways for color removal. The alternatives used are very similar to those employed for color removal in textile wastewaters. These approaches lean toward methods where lignin and other chromophore(s) are further degraded. The methods are classified as physical-chemical or biological.

2.3.1. Physical-Chemical

Typical physical-chemical approaches for color removal include coagulation and flocculation (Dilek and Bese 2001), adsorption (Aziz et al. 2005; Tarlan et al. 2002;

Vidal et al. 2001), oxidation (Assalin et al. 2004; Hassan and Hawkyard 2002; Helmy et al. 2003; Moebius and Helble 2004; Perez et al. 1997b; Wingate et al. 2001), and membrane filtration (Joensson et al. 1996; Katkar and Sasidharan 2000; Laitinen et al. 2001). Reduction of color, high molecular weight chlorolignins, toxicity, and COD has been obtained with these processes. However, there are significant disadvantages associated with these approaches making them unfeasible for application. Some of them include little reduction of BOD and low molecular weight chlorolignins, generation of sludge, formation of toxic by-products, and high capital investment (Kahmark and Unwin 1998; Ochola and Moo-Young 2005; Pokhrel and Viraraghavan 2004; Zhou and Banks 1993).

2.3.1.1.Coagulation

Coagulation and flocculation is one of the most widely used methods for removal of color from wastewaters. Some of the coagulants used include aluminum sulfate and polyelectrolytes such as polyacrylamide, polyethyleneimine, and polyethylene oxide (Pokhrel and Viraraghavan 2004). One of the problems arising from their use includes the production of a large amount of sludge that is difficult to dewater (Bajpai et al. 1999; Zhou and Banks 1993). In addition, optimization of the process requires the use of extreme wastewater pH that must be adjusted before final discharge (Kahmark and Unwin 1998).

2.3.1.2.Adsorption

Plenty of adsorption materials have been tested for the reduction of color, COD, and AOX. They include activated carbon, fuller's earth, chitosan, agricultural residues,

algae, and fungi biomass (Bhattacharyya and Sarma 1997; Dixon et al. 1992; Tarlan et al. 2002; Vidal et al. 2001; Zhou and Banks 1993). Problems associated with the use of adsorption materials include long contact time as well as competitive interaction between different adsorbates and adsorbent. In addition, these adsorbents must be regenerated (reused) for the process to be economically feasible.

2.3.1.3. Oxidation

Advanced chemical oxidation processes make use of (chemical) oxidants to reduce COD/BOD levels and color. Some of these chemical agents include chlorine, ozone, and hydrogen peroxide (Assalin et al. 2004; Dugal et al. 1976; Wingate et al. 2001). Combining these processes such as O_2/ZnO/UV, O_2/TiO_2/UV, and O_3/UV has resulted in excellent color reduction (Catalkaya and Kargi 2007; Hassan and Hawkyard 2002; Helmy et al. 2003; Machado et al. 1999; Perez et al. 1997a). Also, the use of photo-fenton and Fenton reactions has been used as a pretreatment for biological decolorization (Gernjak et al. 2002; Perez et al. 2002). However, there are several limitations for the application of oxidation processes. For example, the use of oxidants such as chlorine can lead to the formation of chlorinated products that can be toxic to the aquatic ecosystem. Furthermore, advanced oxidation processes often have higher capital and operating costs compared to biological treatment.

2.3.1.4. Membrane Filtration

Ultrafiltration and reverse osmosis are some examples of membrane filtration technology for removal of color. Usually, they have been used to remove high molecular weight dissolved organic components from mills effluent streams. Filtration techniques

are now being used for the removal of color and to further reuse process wastewaters of this industry (Joensson et al. 1996; Katkar and Sasidharan 2000; Laitinen et al. 2001). Membrane techniques usually represent a large capital investment. This process requires pretreatment in order to prevent problems with fouling.

2.3.2. Biological

Certainly, the greatest disadvantage of physical-chemical treatments is that they take care of the problem temporarily. Those processes are not the ultimate solution to waste treatment problems, but rather a method of transferring the problem to another place, while creating toxic byproducts in some cases. In contrast, biological methods use different microorganisms to degrade lignin and chromophores without additional waste to be treated. In biological processes, color reduction can be performed under aerobic or anaerobic conditions.

2.3.2.1. Aerobic Degradation

Textile industries have tested the use of aerobic treatments for the color removal from their wastewaters. However, significant decolorization was only observed when the medium was supplemented with a secondary source of carbon. Experiments that categorically demonstrate that this can occur without any carbon sources have not been published (Stolz 2001).

Research of decolorization focused on the use of bacteria have identified *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Pseudomonas ovalis* as been able to remove color by degrading lignin and its byproducts (Blair and Davis 1980; Bourbonnais and Paice 1987; Kawakami et al. 1975). The maximum color reduction observed for *P.*

aeruginosa was 54%. However, most of the decolorization for *P. aeruginosa* and the other bacteria relied on biosorption with little lignin biodegradation. Low solubility and complex simultaneous biochemical reactions makes lignin biodegradation difficult (Iyer and Mahadevan 2002).

Algae have also been used for biodecolorization in textile and paper mills effluents. Lee et al. (1978) used pure and mixed cultures of algae obtaining approximately 70% color reduction within two months of incubation. Color removal was more effective during the initial 15-20 days of incubation and thereafter the removal efficiency was reduced. Dilek et al. (1999) and Tarlan et al. (2002)studied the removal of AOX and color using mixed algae culture as well. Results from those experiments show that up to 70% of AOX and 80% color removal could be obtained. Lee et al. claimed that color reduction was due to the transformation of colored compounds to non-colored compounds rather than degradation. In contrast, Dilek et al. and Tarlan et al. stated that decolorization was due to degradation of the chromophores. While good decolorization was observed using algae, limited research and lack of congruency regarding the degradation mechanism have prevented the application of this process (Huang et al. 2000; Liu and Liu 1991).

Certainly, the use of white-rot fungi presents most studied method for removal of AOX and wastewater decolorization (Bilgic et al. 1997; Lankinen et al. 1991; Pallerla and Chambers 1997; Wingate et al. 2005), since these fungi are the only organisms able to mineralize lignin to carbon dioxide and water. Color reductions in the wastewater result as lignin and its byproducts are degraded (Murugesan 2003; Van Driessel and Christopher 2004). The overall mechanism and enzymes involved in these degradation

reactions are well documented (Ben Hamman et al. 1997; Ben Hamman et al. 1999a; Kirk and Farrell 1987; Kirk et al. 1978). Fungi produce lignin modifying extracellular enzymes to degrade lignin in wood cell walls.

While application of this process seems promising, several problems still exist preventing the use of fungi at larger scales. First, optimal conditions for fungi growth require pH around 4.0-5.0, producing effluents that must be neutralized before disposal. In addition, the enzymatic activity is reduced by nutritional deficiencies or excesses (Ben Hamman et al. 1997; Ben Hamman et al. 1999a; Garg and Modi 1999; Kirk and Farrell 1987; Perez et al. 1998). For example, a secondary carbon source along with higher copper and manganese enhances enzymatic activity (Yin et al. 1989). High concentrations of nitrogen, zinc, iron, and molybdenum reduces enzymatic activity. Other parameters that must be monitored to obtain satisfactory results include dissolved oxygen concentration and agitation. Several researchers have suggested the use of surfactants such as Tween 80 to increase the decolorization by increasing the dispersion of the enzymes (Joyce et al. 1989; Pellinen et al. 1988).

2.3.2.2. Anaerobic Degradation

Significant decolorization of textile effluents under anaerobic conditions has been reported by several authors (An et al. 1996; Beydilli et al. 1998; Brown and Laboureur 1983; Fontenot et al. 2002; Lee et al. 2005; Quan et al. 1991; Razo-Flores et al. 1997; Yoo et al. 2001). However, due to the complexity of chromophores in the pulp and paper mills, this industry does not follow the same trend. Some researchers state that bleached effluents are not suitable for anaerobic decolorization due to the low biodegradability and

the presence of toxic compounds that can affect methanogens (Pokhrel and Viraraghavan 2004). Most literature regarding biological decolorization states that lignin degradation does not take place under anaerobic conditions (Feijoo et al. 1995; Kirk and Farrell 1987). Therefore, anaerobic decolorization of effluents containing lignin chromophores is not expected.

Despite these arguments, literature presents cases of lignin degradation using anaerobic treatments. Benner and Hodson (1985) did studies using a mixed population isolated from anaerobic compost and found that it could mineralized 2-4% of lignin and 14-22% of Kraft lignin under anaerobic conditions at 55°C. Similarly, Colberg and Young (1985), used a mixed population isolated from activated sludge that was able to degrade low molecular weight lignin in anaerobic conditions. Colberg (1988) reviewed the degradation of lignin and its degradation product under different anaerobic conditions. Key findings of this review include the slow but significant rate of polymeric lignin mineralization in anoxic sediments. Ziomek and Williams (1989) studied the modification of lignin under sulfate reducing conditions. They found that under those conditions, lignin could be partially depolymerized as its polyphenolic groups and parts of its functional groups were degraded. Pareek et al., (2001) found similar results in experiments using lignin and lignin model compounds under sulfate reducing conditions. In these studies, cellulose and newspapers were used as an alternate source of carbon. In both cases, complete degradation of the lignin model compounds was reported. Tartakovksy et al., (2003) studied the biodegradation of lignin under mesophilic and thermophilic conditions. As high as 59% of the lignin was degraded under thermophilic conditions due to higher solubility and availability of lignin.

The limited research done using anaerobic conditions for color removal usually focused on the combination of this treatment with aerobic processes. Feijoo et al. (1995) used a combination of fungi and anaerobic bacterial for the decolorization of high molecular weight compounds in Kraft pulp effluents. In this study, decolorization was obtained only after fungal pretreatment of the effluent. This pretreatment in turn enhanced the removal of high molecular weight compounds by as much as 79%. Tezel et al. (2001) studied sequential aerobic/anaerobic treatment of pulp and paper effluents. This combination enhanced the total removal of COD by 91%, AOX by 58%, and color by 90%. Chuphal et al. (2005) studied the use of anaerobic decolorization followed by aerobic treatment of the effluents. In this case, the author found up to 88% color reduction when using a three step anaerobic-aerobic sequential reactor. However, it must be observed that only 59% color reduction was observed in the anaerobic phase and this was supplemented with dextrose as a source of carbon.

As was remarked in the cases above, decolorization under anaerobic conditions was achieved. However, the degree of decolorization during the anaerobic phases was not enough for the system to stand on its own in mills with strict color regulations. While integrated methods is the current trend, a system that could achieve higher degree of anaerobic decolorization is desired as it will result in a process that requires less energy, produces less sludge, and represents a potential source of energy production.

CHAPTER 3. BIOLOGICAL AND ABIOTIC COLOR REDUCTION OF PAPER MILL EFFLUENTS DURING ANAEROBIC COMPOSTING

This chapter presents the biological and abiotic adsorption and kinetic experiments to demonstrate the feasibility of removing color from paper mill effluents using anaerobic composting. Experiments were performed using active and gamma sterilized compost and two different effluents: Pulp Mill Upset Tank (PMU) and E Stage Filtrate (ESF). Effluent and compost characterization was performed as part of this study. In addition, the effect of pH and aging in adsorption was assessed in this experiment. Finally, reactors were prepared and run as batch to determine the kinetic parameter for the biodecolorization of PMU and ESF.

3.1. Introduction

Pulp and paper mill discharges to receiving waters are characterized as being highly colored (Bajpai and Bajpai 1994; Bajpai et al. 1999). The generation of color is often associated with thermal, mechanical, and chemical conversion of wood into pulp (Garg et al. 2005; Lo et al. 1991). During this process, the cellulose and hemicelluose is separated from the lignin and other extractives in the wood. Some of the byproducts obtained are compounds such as residual lignin and lignin derivates along with polymerized tannins that impart color to the water (Adedayo et al. 2004). In addition,

chromophores are created from the partial degradation of lignin (Kringstad and Lindstroem 1984). Contrary to the textile industry, where there is a usually a key chromophoric structure, several chromophores exist in the pulp and paper industry. Within lignin structure, some chromophores include: CH=CH double bonds conjugated with aromatic ring, quinomethides and quinones, and chalcones. Additional a contribution to the color problem arise during the process of pulp bleaching, chemicals react with lignin and other components of the pulp (Eriksson et al. 1985; Kringstad et al. 1985). Such reactions result in the formation of chlorinated organics that further contribute to wastewater color.

In 1998, the Environmental Protection Agency (EPA) proposed amendments to the pulp paper production source category regulations, known as the "Cluster Rules" (USEPA 1998). These new standards linked air and water standards for the pulp and paper industry into a single set of coordinated regulations. It was stated in these rules that color should not be controlled under federal regulations because it is an aesthetic concern more appropriately addressed in individual permits based on local applicable water quality standards.

Despite the Cluster Rules allegation that color is just an aesthetic problem, literature review shows that there are other significant problems related to color. For example, color particles in water inhibits the process of photosynthesis as particles in solution will scatter and absorb light, altering the intensity and spectrum of the light thereby reducing the photosynthetically available radiation (Kirk 1994). In addition, brown color effluents absorb infrared radiation and increase water temperature leading to decreased levels of dissolved oxygen (Pellinen et al. 1988; Selvam et al. 2002). Colored

substances in aquatic systems have been associated with changes in primary productivity (Del Giorgio and Peters 1994; Henebry and Cairns 1984), phytoplankton species composition (Beauchamp and Kerekes 1989), and protozoan colonization rates. Color can also alter the availability and hence toxicity of heavy metals to fish (Haines et al. 1995; Orrego et al. 2005). Furthermore, color is often associated with other organic toxins such as wood extracts and chlorinated organics formed during the bleaching stages (Vidal Saez 1999). Some of those xenobiotic pollutants such as dioxins and furans are recalcitrant to degradation, while others such as sterols are known endocrine disruptors (Kostamo et al. 2004; Lehtinen et al. 1999). These effects are enhanced in cases where the receiving stream has low or varying flow.

Recognition of the problems associated with color as well as stricter effluent limits imposed by local government has led to the development of new technology for the efficient removal of color. These decolorization methods are classified as chemical-physical methods or biological methods. Typical physical-chemical approaches for color removal include coagulation and flocculation, adsorption, oxidation, and membrane filtration. Reduction of color, high molecular weight chlorolignins, toxicity, and COD has been obtained with these processes. However, there are significant disadvantages associated with these approaches making them unfeasible for application. Some of them include little reduction of BOD and low molecular weight chlorolignins, generation of sludge, formation of toxic by-products, and high capital investment (Ochola and Moo-Young 2005; Pokhrel and Viraraghavan 2004).

Regarding biological approaches, use of white-rot fungi presents the most studied method for removal of AOX and wastewater decolorization (Bilgic et al. 1997; Wingate

et al. 2005). Color reductions in the effluent result as lignin and its byproducts are degraded (Murugesan 2003; Van Driessel and Christopher 2004). The mechanism and enzymes involved in these degradation reactions are well documented (Ben Hamman et al. 1999a; Kirk and Farrell 1987; Kirk et al. 1978). However, several problems still exist preventing its use at larger scales. First, optimal conditions for fungi growth require pH around 4.0-5.0, producing effluents that must be neutralized before disposal. In addition, the enzymatic activity is reduced by nutritional deficiencies or excesses (Ben Hamman et al. 1997; Ben Hamman et al. 1999a; Garg and Modi 1999; Kirk and Farrell 1987; Perez et al. 1998). For example, a secondary carbon source along with higher copper and manganese enhances enzymatic activity (Yin et al. 1989). High concentrations of nitrogen, zinc, iron, and molybdenum reduces enzymatic activity. Other parameters that must be monitored to obtain satisfactory results include dissolved oxygen concentration and agitation.

Our current study is part of a series of interrelated research focused on determining what chemical-physical or biological method is driving the color reduction observed in anaerobic compost pits at a paper mill in Georgia. The process was developed after significant decolorization was observed when highly colored wastewater was diverted into these compost pits. The compost pits contain aged primary solids, high in fiber, in an anaerobic environment. Due to the complexity and heterogeneity of this system, two mechanisms were proposed as performing the decolorization: adsorption of color by the fibers and biological transformation and degradation of the color molecules.

In the first proposed color reduction mechanism, we hypothesized that the color forming bodies are absorbed to the surface of the fibers. Aging of fibers by bacteria may

increase adsorptive surface area or create functional groups that aid in adsorption of the color molecules. Literature presents several studies were decolorization of dyes and paper mill effluents was performed by adsorption of color bodies to waste biomass, activated carbon, fuller's earth, chitosan, agricultural residues, algae, and fungi biomass (Anjaneyulu and Bindu 2000; Bhattacharyya and Sarma 1997; Vidal et al. 2001; Zhou and Banks 1993). Based on these observations, adsoption could be an important mechanism in the removal of color.

The second mechanism proposed is the reduction of color by bacterial transformation and degradation of the color bodies. The color transformation had some characteristics that may indicate the compost environment selects a bacterial consortium that can biodegrade the lignin groups. This phenomenon will be a unique characteristic of the system due to its predominant anaerobic conditions. While complete decolorization of dyes under anaerobic conditions have been reported by several authors (An et al. 1996; Beydilli et al. 1998; Razo-Flores et al. 1997; Yoo et al. 2001), reports from the pulp and paper industry do not demonstrate the same trend. Most literature regarding biological decolorization states that lignin degradation does not take place under anaerobic conditions (Feijoo et al. 1995; Kirk and Farrell 1987). Therefore, anaerobic decolorization of effluents containing lignin chromophores is not expected.

The focus of this paper is to quantify the rate and degree of anaerobic color removal occurring in the anaerobic compost and to elucidate the mechanism of color removal. Although the literature suggests that biological transformation of lignin and color bodies under anaerobic conditions is not likely, the possibility that both sorption and biotransformation play significant roles in color removal were investigated. The

results obtained in this study were integrated into the biodecolorization process that is currently being used at this paper mill for the reduction of color in a semi-continuously flow operation (Méndez-Sánchez et al. 2007).

3.2. Materials and Methods

As part of determining the mechanism by which color is being removed, characterization of the aqueous and fiber/compost environment was performed. Adsorption studies were done to determine the role of compost adsorption on decolorization. The isotherm tests were done using compost with different microbial activity (i.e. abiotic and biologically active compost) and at various pH. Additional isotherm experiments were conducted using virgin compost to determine if weathering and biological transformation of the compost alter the degree of color removal. Finally, kinetic experiments were performed to determine the time require for decolorization as well as the extent of decolorization.

3.2.1. Wastewater Characterization

Two different highly colored waste streams within the paper mill were selected for this study. These streams represent around 10% of the wastewater produced in the paper mill and contribute to 25-30% of the color. Samples from the Pulp Mill Upset Tank (PMU) as well as E Stage Filtrate (ESF) were analyzed for nutrients (N, P, and S) color, pH, ORP, TOC, BOD, COD, lignin, humic acid, fulvic acid, and humin).

3.2.2. Compost Characterization

The compost used in this study came from solids disposal pits located on the mill site. These pits received primary clarifier underflow solids and were between 20 and 25 feet in depth and 10 acres each in area. Samples were taken from the surface of three anaerobic compost pits at the wastewater inflow, mid-line, and discharge area of the pit. Also, samples were collected the area across the depth of the pit, until a dense impenetrable layer was reached. The samples collected were characterized for solid content, organic content, surface area, hydraulic conductivity, nutrients (S, N, and P), color, pH, and lime content.

3.2.3. Adsorption Studies

3.2.3.1. Isotherm Testing

EPA amber glass vials (40 mL) were used to perform isotherm testing. The vials were filled with compost masses ranging from 0.01 to 20.0 grams. The vials were then filled with 20 mL of wastewater (PMU or ESF). The vials were agitated in a TCLP tumbler for 48 hours and the aqueous phase color concentrations were measured following standard methods. Isotherm testing was done using both biologically active compost and gamma irradiated compost due to the possible role of biological activity on decolorization. For those experiments, the gamma sterilization of the compost was performed using a cobalt – 60 gamma cell and a total dose of 0.8 Mrad. After the gamma irradiation, the compost total viable bacteria count was less than 10^2 cfu/mL, which is significantly lower compared to the biologically active material with bacteria count above 10^7 cfu/mL.

3.2.3.1.1. Effect of pH on Sorption

Similar isotherm experiments were conducted to test the effect of pH on decolorization. Sample vials were prepared using pH values of 4, 7, and 10. pH adjustment was performed using H_2SO_4 and NaOH.

3.2.3.1.2. Effects of Aging on Sorption

Isotherm testing was conducted using virgin compost to determine if weathering and biological transformations alter the degree of color removal by compost. By virgin compost, we refer to composting material that has not been in contact with the compost pits. Isotherm testing was performed using both abiotic (gamma sterilized) and biologically active virgin compost.

3.2.4. Color Removal Studies

3.2.4.1. Batch Color Removal Testing

EPA amber glass vials (40 mL) were used to perform isotherm testing. The vials were filled with compost masses ranging from 0.01 to 20.0 grams. The vials were then filled with 20 mL of wastewater (PMU or ESF). The vials were agitated in a TCLP tumbler for 48 hours and the aqueous phase color concentrations were measured following standard methods (NCASI 1999). Color removal testing was conducted using both biologically active compost and gamma irradiated compost due to the possible role of biological activity on decolorization.

3.2.4.2. Kinetic Rate of Color Removal

Kinetic experiments were performed using two liter batch reactors. The reactors were seeded with 2 kg of compost and 1L of wastewater (PMU or ESF). At different time intervals, 5 mL aliquot was removed for color analysis. This experiment was performed using both active and gamma sterilized compost. Integration method using the graphical approach will be used to calculate the reaction rate (Levenspiel 1972). The goal of kinetic testing was to determine if color removal by anaerobic compost was kinetically limited or an equilibrium process. This information would aid in differentiating between sorption and biotransformation as the means of color reduction.

3.2.5. Analytical Methods

The color of the samples was measured using a Hewlett Packard Diode Array spectrophotometer at a wavelength of 465 nm and following standard procedures (NCASI 1999). The term "color" represents the true color of an aqueous sample from which turbidity has been removed. A platinum cobalt standard was used to quantify the concentration of color in PCU. The TOC (SM 5310), BOD (SM 5210), COD (SM 5220) of the wastewater were measured during this experiment following standard methods (American Public Health Association et al. 1998). An ATI Orion expandable ion analyzer (EA 940) was used to measure both the ORP and pH. Standard Method (SM) 4110 was followed to measure the concentration of sulfate, nitrite, and nitrate were measured using a Dionex DX-120 Ion Chromatograph (Dionex Corporation, Sunnyvale, CA). Total Kjeldahl Nitrogen (TKN) and total Phosphorus were measured following SM 4500. Lignin concentration was measured using an Agilent 6890 Gas Chromatographer

following standard method 5550-B. The concentration of humic acid, fulvic acid, and humin was measured following standard methods (SM 5510). The surface area was analyzed using a NOVA 1000 B.E.T. analyzer in accordance with the manufacturer's standard operating procedures. The hydraulic conductivity of the soil was determined using a column percolation test. Lime content of the compost was determined by titration (AASHTO T 232-90).

3.3. Results and Discussion

3.3.1. Wastewater Characterization

Table 3.1 presents the results for characterization of the PMU and ESF effluents. The concentration of sulfate as the wastewater exited the pond (PMU) was 9.7 g/L, while the sulfide concentration was approximately 368 mg/L. The presence of high sulfide concentrations indicates the presence of a strongly reducing environment in the PMU. Based on the sulfide/sulfate couple, the redox of the influent averages approximately -255 mV which means the waste is devoid of oxygen and will sustain highly anaerobic reactions such as sulfate reduction. The PMU has a low amount of nitrogen as indicated by the low TKN and small concentrations of nitrate and nitrite. The available phosphorous is also too low to sustain biodegradation of large amounts of organic carbon. The PMU contained a large amount of organic carbon, with COD averaging 1250 mg/L. The BOD of the PMU was 960, which is much higher than the available nutrients. Since the COD is much higher than the BOD, it can be concluded that a large portion of the COD is refractory. The measured color for PMU averaged 5,300 PCU. Large amounts of lignin (1,300 mg/L) and humic acid like compounds (1,000 mg/L) were measured in the

Table 3.1 Characterization of wastewater (all results mg/L, unless specified).

	Pulp Mill Upset Tank	E Stage Filtrate
Sulfide	9.7 (±0.1) †	0.02 (±0.004)
Sulfate	368 (±8.0)	89.5 (±2.2)
Ammonia	0.600 (±0.04)	3.37 (±0.06)
Nitrate	0.150 (±0.014)	5.05 (±0.03)
Nitrite	0.078 (±0.01)	1.22 (±0.05)
TKN	2.100 (±0.071)	7.95 (±0.07)
Phosphate	0.310 (±0.007)	8.00 (±0.115)
Chloride	89.00 (±1.58)	744.50 (±3.7)
Calcium	8.900 (±0.091)	9.40 (±0.04)
Iron	4.775 (±0.131)	2.70 (±0.04)
TOC	1562.5 (±22.9)	680.00 (±4.1)
BOD	960 (±45)	450 (±18)
COD	1250 (±76)	980 (±55)
Color (PCU)	5342.5 (±44)	10340.0 (±60)
pH	7.200 (±0.01)	6.800 (±0.06)
Humic acid	1040.00 (±24.9)	1721.25 (±24.2)
Lignin	1310.00 (±62.7)	1983.75 (±5.2)
Humin	130.00 (±4.1)	160.000 (±7.4)
Fulvic acid	71.250 (±5.5)	95.000 (±1.1)
Total Chlorinated Organics as Cl	2.50 (±0.06)	41.9 (±0.87)

†Values in parenthesis indicate standard error (n=5).

waste using the technique of Stumm (1987) It is likely that both tests measure many of the same compounds in the waste and that the lignin has partially degraded to resemble humic acid. Smaller amounts of fulvic and humin like compounds were detected.

The E Stage Filtrate was also characterized, and the results are presented in Table 3.1. The presence of low sulfide concentrations in the ESF indicates that a slightly oxidizing environment was present in this waste. Based on the sulfide/sulfate couple, the calculated redox of the ESF was nearly zero mV which means that it will not sustain highly anaerobic reactions such as sulfate reduction unless the redox is reduced. The ESF influent has much higher concentrations of nitrogen than the PMU wastewaters as indicated by the moderate TKN (7.98 mg/L) as well nitrate (5.1 mg/L) and nitrite (1.2 mg/L). The available phosphorous (8.0 mg/L) is also higher than those measured for PMU and could sustain biodegradation of larger amounts of organic carbon than the strong pond wastewaters. The ESF contained large amount of organic carbon, with COD averaging 980 mg/L. However, this COD is less than two thirds the measured for PMU. BOD for the ESF was less than 550 mg/L which is higher than the available nitrogen compounds. The measured color for ESF averaged 10,340 PCU. Large amounts of lignin (1,980mg/L) and humic acid like compounds (1,720 mg/L) were measured. It is likely that both tests measure the same compounds in the waste and that the lignin has partially degraded to resemble humic acid. Smaller amounts of fulvic and humin like compounds were detected.

Table 3.2 Characterization compost samples from surface and average across depth.

Parameter Measured (units)	Surface	Across Depth
Solid Content (%)	18.0 (±1.9) †	17.6 (±1.6)
Surface Area (m^2/gram)	10.04 (±0.1)	14.00 (±0.45)
Hydraulic Conductivity (m/hr)	2×10^{-3}	8×10^{-4} ($\pm 4.5 \times 10^{-5}$)
Sulfide (mg/kg)	37.0 (±1.9)	66.0 (±1.8)
Sulfate (mg/kg)	107.0 (±1.9)	24.2 (±0.7)
Total P (%)	0.352 (±0.008)	0.650 (±0.03)
TKN (%)	0.954 (±0.03)	1.282 (±0.022)
Lime (%)	2.88 (±0.08)	1.80 (±0.04
Organic Content (%)	82.0 (±1.3)	76.1 (±1)
Pore Water Color (PCU)	520.0 (±3.5)	510.0 (±2.3)

†Values in parenthesis indicate standard error (n=5).

3.3.2. Compost Characterization

Results for the compost characterization are presented in Table 3.2. The solid content for the surface and depth averaged compost samples had a similar range. Solid content varied from 12% (thick liquid) to 23% (solid/firm). The average surface area of the samples taken from the surface of the compost pits was 10 m^2/g, while samples from the across depth had a slightly higher surface area with 14 m^2/g. In both cases, the average surface area was similar to that of top soils (Carter et al. 1994). The measured surface area was very low in comparison to the surface area of activated carbon. Typical surface area for activated carbon is in the range of 600-1200 m^2/g (Gregg and Sing 1982). While it is not likely that the compost has a high capacity for physical adsorption, absorption and ion exchange type removal cannot be rule out on this basis. The hydraulic conductivity of the samples averaged across depth were significantly lower ($8x10^{-4}$ m/hr) than those obtained for the surface samples ($2x10^{-3}$ m/hr). However, the hydraulic conductivity in both cases was characteristic to clay sands (Masch and Denny 1966) making the makes use as a filter type media very difficult.

The concentration of sulfide in the pore water was two times higher for the depth averaged samples (66 mg/kg) compared to those taken from the surface of the pits (37 mg/kg). The high concentrations observed on the across depth samples represent conditions characteristic of anoxic sulfur reduction. Total phosphate and nitrogen were lower for the surface samples (P = 0.35% and TKN = 0.95%) compared to the concentrations measured for the across depth samples (P = 0.65%. and TKN = 1.28%). The ratio of nutrients measured on the samples is within the range of nutrient

concentration suitable for composting of mixed paper mill sludge (Haug 1980) and would help provide N & P for degradation of organic carbon in the studied wastewaters. Lime concentration was 60% higher on samples from the surface of the compost pits (2.9%) compared to lime levels for the depth averaged samples with lime contents of approximately 1.8%. The lime level for both samples is high enough as to provide a good buffering capacity to the compost.

Samples were very rich in organic matter as evidenced by the high values obtained in both surface and across depth. The organic content for the surface samples was 82%, while the average of samples taken across the depth of the compost pit was slightly lower with 76%. Pore water color was 522 PCU for surface samples, while color for average across depth was 510 PCU. The color of pore water is at least ten times lower than the color of the wastewaters being applied to the compost pits, indicating that color removal was actively occurring in the area where the compost samples were obtained.

3.3.3. Adsorption Studies

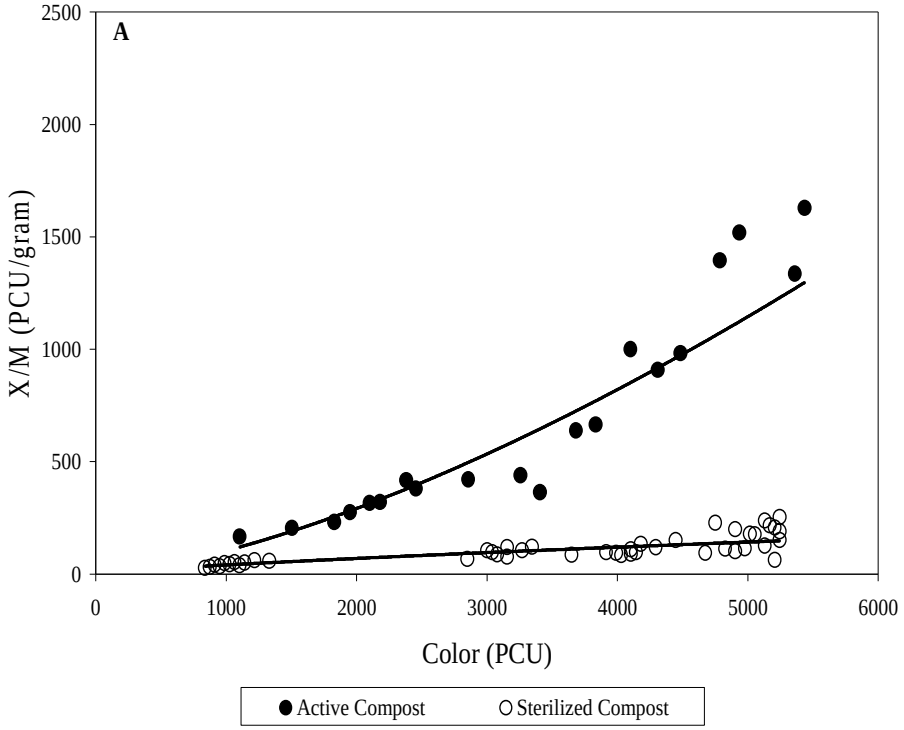
Based on the assumption that physical sorption phenomena were the dominant mechanism for color removal, the Freundlich isotherm model (Biswas et al. 1992) was used analyze the data obtained from the color removal tests. The Freundlich isotherm that models adsorption at equilibrium is in the form of:

$$x/m = K_f C_e^n \qquad \text{(Eq. 3.1)}$$

Where x is the mass of color adsorbed on the compost (PCU), m is the mass of compost (g), K_f is the equilibrium constant indicative of adsorptive capacity, C_e is the

color concentration at equilibrium (PCU/L), n is a constant indicative of adsorption intensity. The Freundlich constant K_f represents the adsorption capacity of the carbon for specific adsorbate (color), at a given equilibrium concentration C_e. In general, a high value for K_f is desirable for higher color reduction.

Results for the adsorption experiments testing the decolorization of different wastewaters are presented in Figure 3.1. The kinetic parameters for the sorption isotherms are presented in Table 3.3. In general, a linear profile was obtained for the abiotic material that had been gamma-sterilized. The biologically active compost had "sorption" isotherms that had significantly higher X/Ms than those for the abiotic material at any given equilibrium concentration. The amount of color removed by the biologically active material was almost one order of magnitude higher than those attained by the gamma irradiated abiotic samples. This indicates that biological activity plays a major role in removal of color from PMU by anaerobic compost with almost ten times more removal achieved via biological activity. The relative difference between the abiotic and biological isotherms is greater at higher equilibrium concentrations. This may be a result of first order biological color removal, which would have higher rates at higher color concentrations. The implications of the biological activity are two fold. First, biological activity increases the sorptive capacity of the compost by almost ten times. This means that the rate of exhaustion of compost would be ten times lower and the same mass of compost could treat ten times more waste than by sorption alone. Second, the biological component of color removal may not have a finite capacity. Biological color removal may be a continuous and sustainable process. This would allow even greater



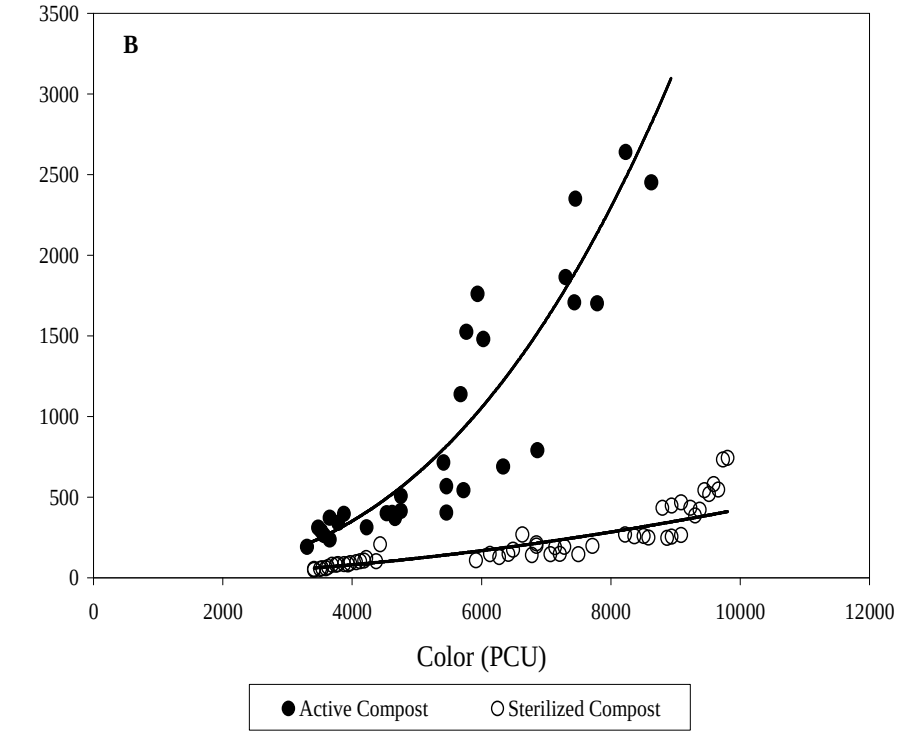


Figure 3.1 Sorption isotherm of wastewater decolorization at pH = 7.0 for (A) PMU, (B) ESF.

Table 3.3 Freundlich isotherm model constants for color adsorption to compost using different wastewaters.

Effluent	Active Compost			Gamma – Sterilized Compost		
	K_f	n	R^2	K_f	n	R^2
PMU	$3.5x10^{-3}$	1.50	0.909	0.182	0.782	0.763
ESF	$3.0x10^{-8}$	2.81	0.852	$2.0x10^{-5}$	1.816	0.847

amounts of color removal per unit mass of compost and possibly preclude removal or replacement of compost when exhaustion occurs. However, if a finite capacity does not exist, isotherm modeling (i.e., equilibrium modeling) of color removal is not valid and kinetic modeling needs to be used.

3.3.3.1. pH

Figures 3.2 – 3.4 present the results for the adsorption isotherms of PMU at pH 5, 7 and 10. As depicted in Figure 3.2, under low pH conditions the abiotic color removal is increased by more than five times for all equilibrium concentrations. Therefore, abiotic removal of color is favored at lower pHs and higher degrees of abiotic color removal can be attained by lowering pH. The biologically active removal at pH 5 also increased at the same degree as the abiotic removal. Isotherms for color removal of PMU at pH 10 in Figure 3.4 shows that at elevated pH the removal by abiotic compost solids is almost identical to that achieved at pH 7 (see Figure 3.3). Similarly, the degree of color removal by the biological compost under anaerobic conditions is almost identical to the attained at pH 7. It appears that elevating pH to 10 has almost no effect on abiotic or biological color removal by the anaerobic compost.

The results of testing conducted at high and low pH demonstrate that abiotic color removal is favored at slightly acidic pH. Other studies presented in literature shows that color adsorption was maximized at pH levels below seven, while pH higher than ten resulted in release of the sorbed color back into solution (Anjaneyulu and Bindu 2000; Bhattacharyya and Sarma 1997; Vidal et al. 2001). However, biologically active color removal appears to be robust with respect to pH and is largely unaffected by pH over a

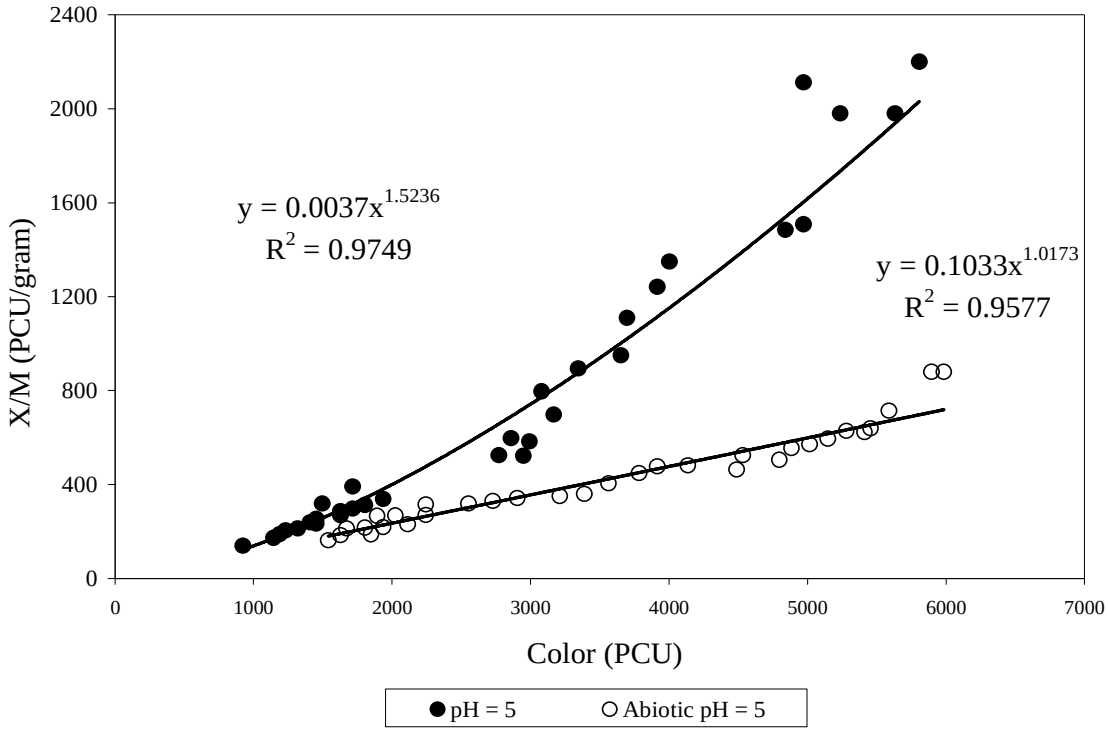


Figure 3.2 Sorption isotherm for decolorization of PMU at pH = 5.0.

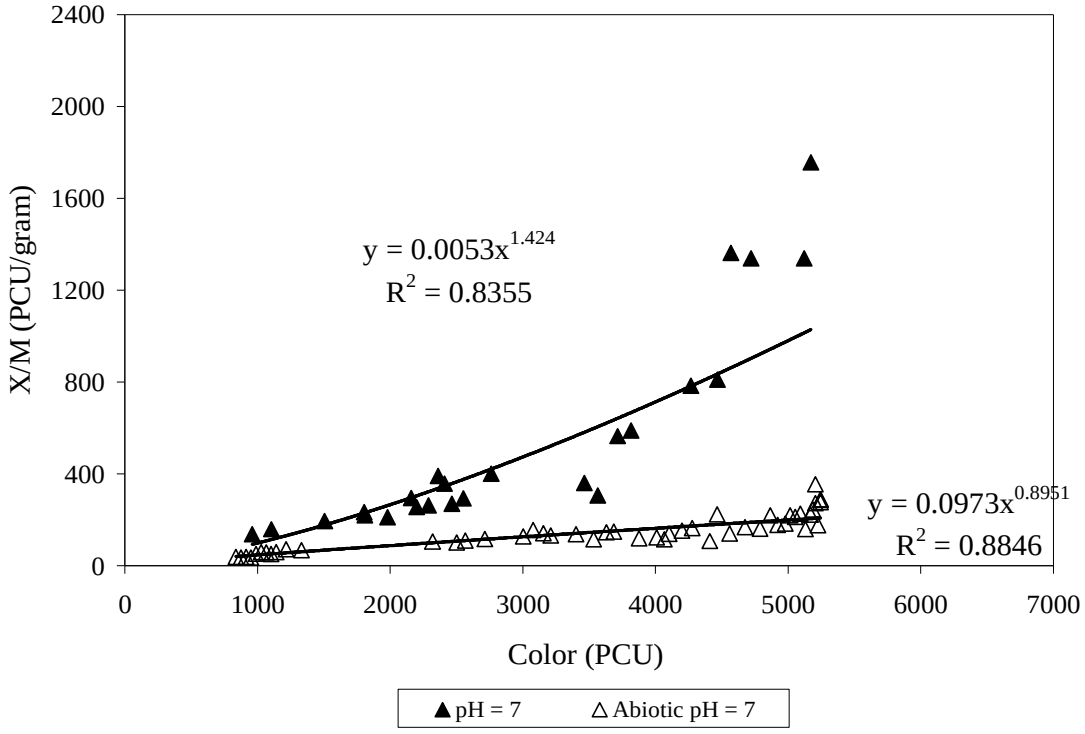


Figure 3.3 Sorption isotherm for decolorization of PMU at pH = 7.0.

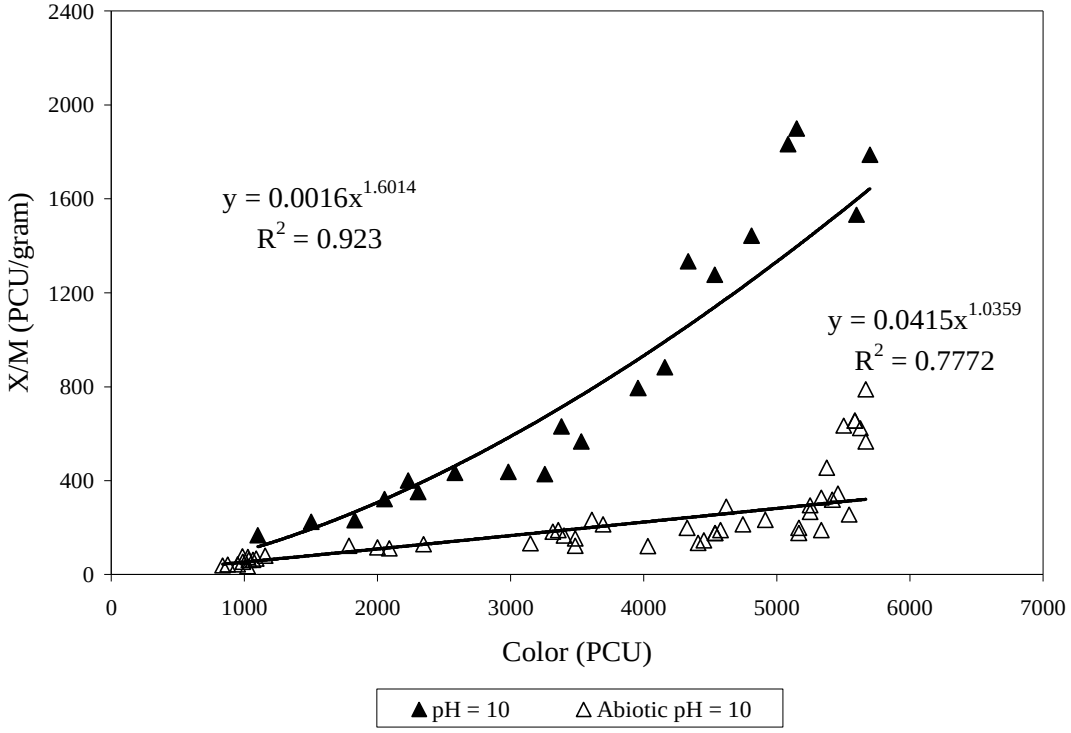


Figure 3.4 Sorption isotherm for decolorization of PMU at pH = 10.0.

range from pH 5 to pH 10. This is important operational information, since pH control may be difficult and a process that is relatively insensitive to pH is desirable. However, it should be noted that the long-term effect of pH on the biological activity of the process cannot be discerned from these tests.

3.3.3.2. Effects of Aging on Sorption

In order to test the effects of aging in decolorization, a sequencing batch reactor (SBR) was seeded with virgin compost operated for 25 cycles using PMU. Isotherm testing was performed using the solids from the SBR. Results in Figure 3.5 shows that sterilized virgin compost had a similar sorption isotherm to the abiotic compost. Using the isotherm Equation 3.1 and the isotherm constants obtained in Table 3.4, the color adsorption capacity for the virgin sterilized compost would be 200 PCU/g. The virgin compost that had undergone repeated exposures to PMU had an isotherm that was similar to the biologically active compost. In this case the color adsorption capacity for the virgin compost will be 1890 PCU/g, while the aged compost had an adsorption capacity of 1913 PCU/g. These results show that it is possible to establish a microbial population in the virgin compost material capable of color reduction in less than 50 days.

3.3.4. Color Removal Studies

To assess the rate of color removal by biologically active compost, batch biodegradation tests were performed. The rate of decolorization for PMU and ESF wastewater was determine using Monod kinetics following a classical graphical technique. Figure 3.6 – 3.7 present the results for the decolorization test. Results for PMU

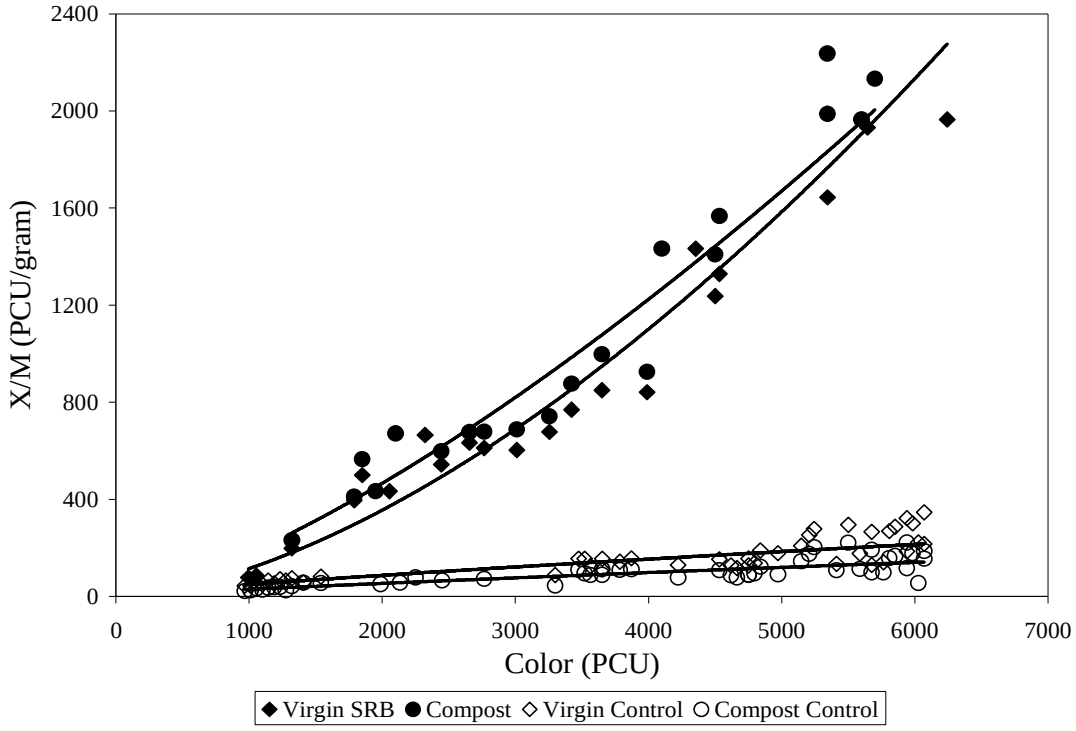


Figure 3.5 Sorption isotherms for decolorization experiments using Virgin Compost.

Table 3.4 Freundlich isotherm model constants for color adsorption using different compost ageing.

Compost	Active Compost			Gamma – Sterilized Compost		
	K_f	n	R^2	K_f	n	R^2
Virgin	0.0015	1.6310	0.94	0.1672	0.8226	0.82
Aged	0.0118	1.3929	0.94	0.0681	0.8769	0.78

presented in Figure 3.6 shows that the maximum rate constant (k) was 25 /hr and the half velocity constant (K_s) was 4160 PCU. Figure 3.7 shows that the maximum rate constant (k) for ESF was 72 /hr and the half velocity constant (K_s) was 8,100 PCU. The magnitude of k value indicates that color is reduced at a maximum rate of 25 PCU/hour for PMU, while the rate for ESF is higher at 72 PCU/hr. The K_s value means that the maximum rate will occur only when the color is much higher than 4,160 PCU for PMU and at 8,100 PCU for ESF. When color is at the Ks values, the rate of color removal is only ½ of the maximum rate. Since the color in PMU is typically in the 4,000 – 6,000 PCU range, the rate of color removal will average about half of the maximum. In contrast, since the color in ESF is typically in the 8,000 – 10,000 PCU range, the rate of color removal would be at or below half of the maximum.

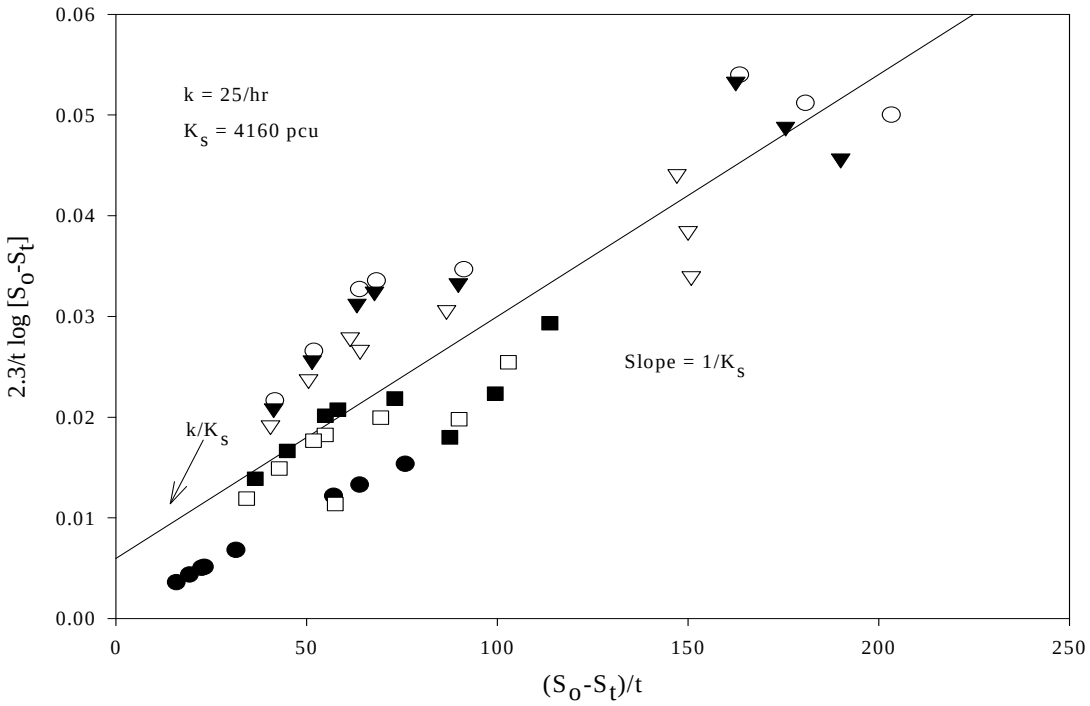


Figure 3.6 Determination of reaction rate constants for the decolorization of Pulp Mill Upset Tank by Anaerobic Compost. Results presented above are the combination of six bioreactors.

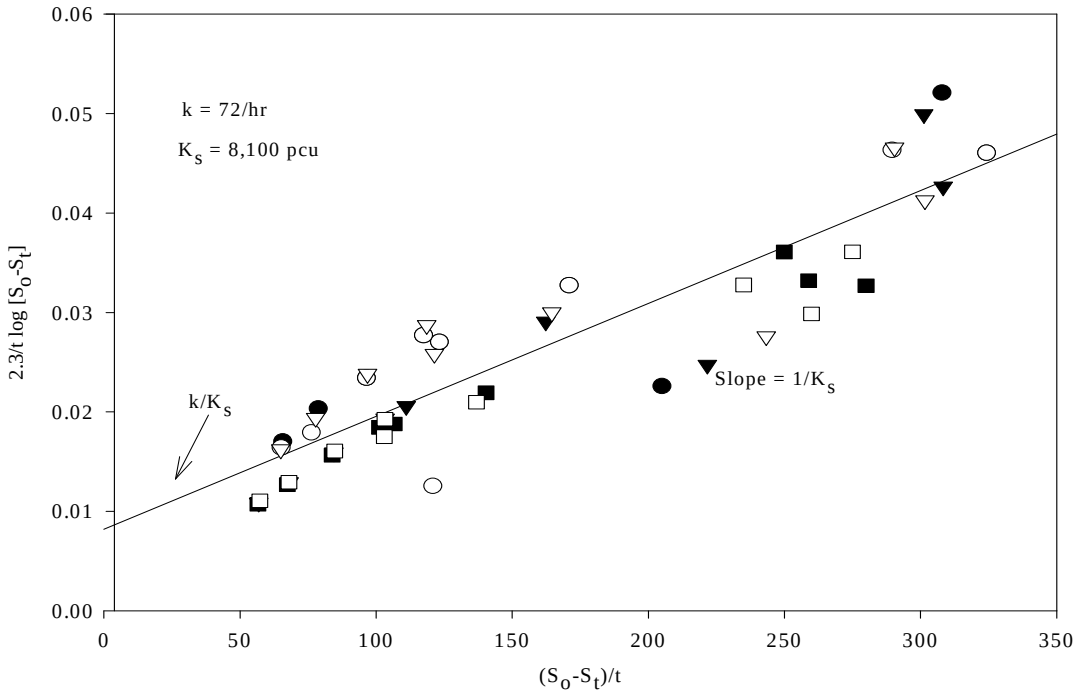


Figure 3.7 Determination of reaction rate constants for the decolorization of E Stage Bleach Filtrate by Anaerobic Compost. Results presented above are the combination of six bioreactors.

3.4. Summary and Conclusions

Experiments showed that anaerobic decolorization of paper mill effluents with different characteristics was feasible using anaerobic composting. The tests performed to assess the contribution of adsorption on decolorization shows that higher adsorption capacity for color was obtained when using biological active compost in comparison with gamma sterilized compost. This means that biological activity increases the sorptive capacity of the compost. Therefore the rate of exhaustion of compost would be lower which means that the same mass of compost could treat waste than by sorption alone. Kinetics experiments showed that the final percentage of decolorization was higher in reactors with active compost than in reactors containing gamma sterilized compost. This showed that anaerobic biodecolorization is a more effective color reduction mechanism when compared to adsorption alone.

The results from this study presents a novel, feasible, and readily implementable method of removing color using compost biosolids. The use of this process not only serves to decrease effluents color but will also reduce the treatment of solid waste by reusing waste fibers from the paper manufacturing industry. Fundamental information that is needed for successful and continuous operation of the process will be obtained in this study. The project will lead to a new, cost-effective, and environmentally friendly solution for the bioremediation of colored effluents. The supplementary research will help to identify other areas which may be benefited from this anaerobic composting process such as the bioremediation of chlorinated compounds and the reduction of selenium in wastewaters.

CHAPTER 4. DECOLORIZATION OF PAPER MILL EFFLUENTS WITH DISIMILAR CHARACTERISTICS USING ANAEROBIC COMPOSTING

This chapter presents experiments to study the sustainability of color removal and the degree and rate of decolorization of four different effluents: Pulp Mill Upset Tank (PMU), E Stage Filtrate (ESF), and Pre-Hydrolized Liquid Grade A (PHL A) and Pre-Hydrolized Liquid Grade B (PHL B). These effluents are characterized by having dissimilar color, pH, and ORP levels. The experiments were performed using anaerobic sequential batch reactors. In addition, experiments were conducted to study the color reversion and to confirm that color reduction was due to anaerobic biodegradation.

4.1. Introduction

There is great concern regarding the discharge of pulp and paper mill effluents into freshwater, estuarine, and marine ecosystems. These effluents contain large amounts of organic matter that without adequate treatment can exert high chemical and biological oxygen demand (COD and BOD) in the receiving waters (Ali and Sreekrishnan 2001; Bhattacharyya and Sarma 1997; Pellinen et al. 1988; Vidal Saez 1999). Additionally, the exposure of these organics to free chlorine and combined chlorine can result in the formation of adsobable organic halide (AOX) compounds. While several alternatives

exist for controlling COD, BOD, and AOX, decolorization of effluents is usually a secondary objective of treatment. Color has been perceived by many as a purely aesthetic contaminant. However, color in water inhibits the process of photosynthesis as particles in solution will scatter and absorb light, reducing the photosynthetically available radiation (Kirk 1994). In addition, brown color effluents increase water temperature leading to decreased levels of dissolved oxygen (Kringstad and Lindstroem 1984; Selvam et al. 2002). As a consequence, the colored substances in aquatic systems have been associated with changes in primary productivity (Del Giorgio and Peters 1994; Ilmavirta and Huttunen 1989), phytoplankton species composition (Beauchamp and Kerekes 1989), and protozoan colonization rates. In addition, secondary production (Hessen 1985), macro-invertebrate behavior (Juarez et al. 1986) and macro-invertebrate community structure (Kullberg 1992) can be affected. Color can also alter the availability and hence toxicity of heavy metals to fish (Haines et al. 1995; Orrego et al. 2005). These effects are enhanced in cases where the receiving stream has low or varying flow. Currently, there are no federal restrictions regarding the color limits in pulp and paper mill wastewaters effluents. However, due to the potential adverse effect this problem has in the ecosystem, it is expected in the near future that laws will be promulgated limiting the levels of color in effluents.

In anticipation of future color limits for discharge effluents, researchers are focusing on finding efficient ways for color removal. Typical physical-chemical approaches for color removal include coagulation and flocculation, adsorption, oxidation, and membrane filtration. Reduction of color, high molecular weight chlorolignins, toxicity, and COD has been obtained with these processes. However, there are

disadvantages associated with these approaches making them unfeasible for application. They include: little reduction of BOD and low molecular weight chlorolignins; generation of sludge; formation of toxic by-products; and high operational costs (Ochola and Moo-Young 2005; Pokhrel and Viraraghavan 2004).

The use of white-rot and brown-rot fungi represents the most studied method for removal of AOX and decolorization (Lankinen et al. 1991; Wingate et al. 2005). These fungi are the only organisms reported as being capable of mineralizing lignin to carbon dioxide and water. Color reductions in the effluent result as lignin and lignin byproducts are degraded (Murugesan 2003; Van Driessel and Christopher 2004). Problems still exist preventing the use of white-rot and brown-rot fungi at larger scales. First, optimal conditions for fungi growth require pH around 4.0-5.0, resulting in effluents that must be neutralized before disposal. In addition, the enzymatic activity is reduced by nutritional deficiencies or excesses (Ben Hamman et al. 1999a; Perez et al. 1998). Consistent growth of the fungi is problematic and operational stability of the fungal process is poor (Moreira et al. 2004).

Most literature regarding biological decolorization states that lignin degradation does not take place under anaerobic conditions (Feijoo et al. 1995; Kirk and Farrell 1987). Therefore, anaerobic decolorization of effluents containing lignin chromophores is not expected. Recent experiments conducted in our laboratory tested decolorization using anaerobic composting after field observations of this phenomenon in a paper mill at Georgia (Lange and Mendez-Sanchez 2007). Decolorization of highly colored pulp mill waste streams was demonstrated to be a result of anaerobic biological activity.

The objective of the current study focused on testing the applicability of this decolorization process to different waste streams within a paper mill. Usually, wastewaters with diverse physical, chemical, and color characteristics are obtained along the different stages of paper and pulp processing. Some of the parameters that differ between them include residual chlorine concentrations, nutrients (i.e., sulfate, nitrogen, phosphate, etc.), pH, oxidation reduction potential (ORP), and color. Due to these differences the anaerobic decolorization of the effluent may be hindered or activated. In addition, samples were aerated to test that anaerobic biodecolorization took place and that color reduction was not due to the reduction of chromophores in the wastewater.

4.2. Materials and Methods

4.2.1. Wastewater Characterization

Wastewaters from four different sources in the pulp mill were studied in this experiment including: 1) Pulp Mill Upset Tank (PMU), 2) E Stage Filtrate (ESF), 3) Pre-hydrolysis Liquid Grade A (PHLA), and 4) Pre-hydrolysis Liquid Grade B (PHLB). The total flow of PMU and ESF streams represent less than 10% of the wastewater produced in this paper mill but contribute over 25-30% of the total color. The PHL wastewaters are two different waste streams produced during pre-pulping hydrolysis of the wood chips. Table 4.1 presents some of the characteristics of the wastewaters studied.

The average color for PMU sample was 5,680(±170) PCU. The pH for PMU was slightly basic at 10.1(±0.1). This wastewater had a highly reductive potential with an average ORP of -210(±10) mV. ESF had the lowest levels of color for the studied streams at 2,970(±18) PCU. In addition, the pH for this stream was very basic at 11.2(±0.1). ORP

Table 4.1 Characterization of different paper mill effluents.

Effluent	pH	ORP (mV)	Color (PCU)	BOD (mg/L)
PMU	10.1 (±0.02)*	-211 (±5.7)	5,680 (±120)	960 (±45)
ESF	11.2 (±0.05)	-15 (±4.4)	2,970 (±166)	450 (±18)
PHLA	4.3 (±0.01)	+73 (±3.6)	43,250 (±188)	62,250 (±2,045)
PHLB	3.0 (±0.02)	+90 (±3.2)	89,500 (±450)	42,000 (±2,615)

* Values in parenthesis indicates standard error (n=3).

values for this stream was -115 mV which is slightly reductive in comparison with results for PMU. The PHL had the highest color of the waste streams tested. The first sample had a color of 43,000 PCU, while a second sample had a color of 89,500 PCU. The pH of the waste was acidic and ranged from 3.9 – 4.3. The ORP was between 73 and 90 mV indicating moderately oxidizing conditions.

4.2.2. Compost Characterization

The solids content of the compost ranged from 12% (thick liquid) to 23% (solid/firm). All of the compost material had high organic contents which comprised over 80% of the solids as determined by ignition at 550ºC. The compost surface area was between 10 – 14 m^2/g (Lange and Mendez-Sanchez 2007), which is relatively low compared to sorbents like activated carbon. This indicates that it is not likely to be a good adsorbent. The compost has sufficient lime (2.9%) to yield a good buffering capacity**.**

4.2.3. Reactors setup and operation for decolorization

Figure 4.1 presents a diagram for the reactor preparation. Large debris and roots were removed from the compost. The compost was mixed with reject fibers in a 2:1 ratio (w/w) in order to improve the porosity and hydraulic characteristics of the compost. A 1:2 ratio of wastewater to compost was added to 2 liter settlometer jar (Nalge Nunc International). Each test condition was tested in triplicate reactors for reproducibility and reliability purposes.

The wastewater was amended with a salt solution to satisfy nutritional requirements. The nutrient solution contained in (g/L): KH_2PO_4 (0.053), K_2HPO_4

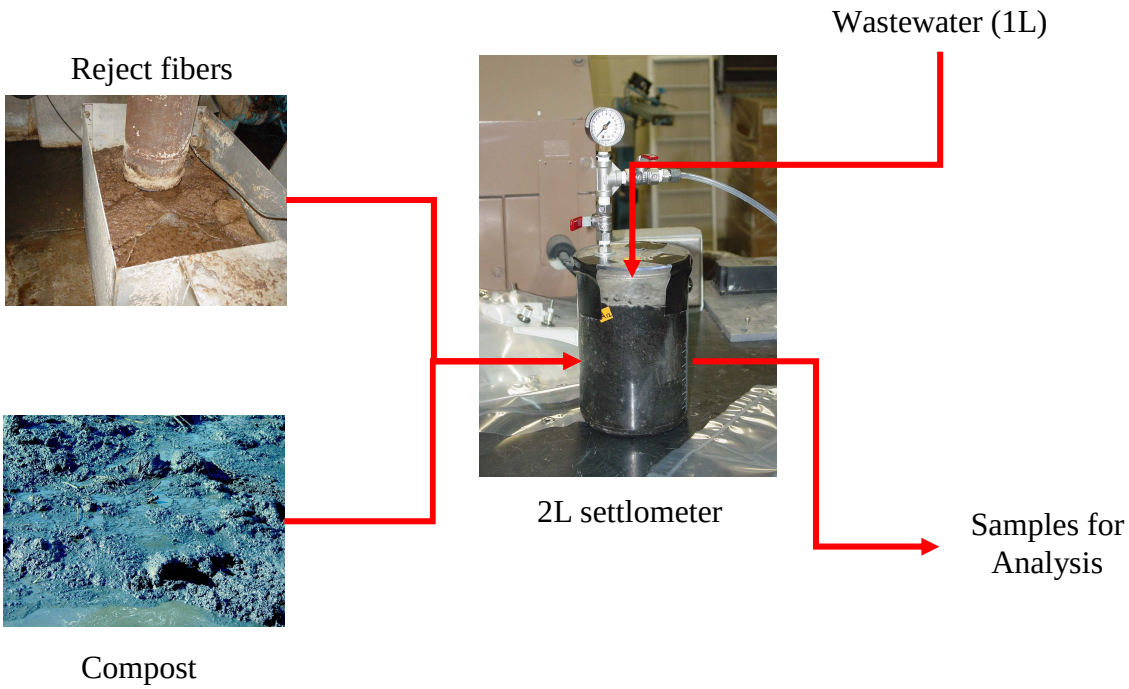


Figure 4.1 Schematic diagram of reactors preparation.

(0.1068), NH_4Cl (1.00), Na_2SO_4 (2.00), KNO_3 (2.00), $CaCl_2$ (0.735), $MgSO_4 \cdot 7H_2O$ (0.20). As a control for the effect of the compost solids, reactors without compost were prepared to study the biodecolorization of the effluents by the normal microbial community of the effluent.

Decolorization reactors were operated as anaerobic sequencing batch reactors (AnSBRs). During the react phase, the contents were incubated for two days on a shaker at 60 RPM. The SBR contents were allowed to settle for two hours. After settling, approximately 50% of the supernatant was decanted and the reactors were refilled with fresh wastewater. Reactors were operated as AnSBR during 5 cycles of 48 hrs and then as batch for 5 days. Wastewater samples were taken at the beginning and end of each cycle and daily when reactors were operated as a batch. Kinetic parameterization was performed with the data obtained during batch phase of operation.

4.2.4. Aeration

At the end of decolorization experiments, aeration testing was performed using 250mL Erlenmeyer flasks to determine if color reversion (color growth) occurred under aerobic conditions. For each wastewater studied, 100mL samples of decolorized effluent was collected and diluted with 100mL of distilled water. The flasks were covered with paraffin to minimize evaporation of the samples. Aeration was achieved using aquarium aeration stones, an associated connection apparatus. Air supply was adjusted to maintain minimal aeration that resulted in a dissolved oxygen concentration that remained above 2mg/L at all times. The samples were aerated for two days. Samples for color testing were taken every 12 hr.

4.2.5. Analytical Methods

Wastewater samples were taken at the beginning and end of each cycle and daily when reactors were operated as a batch. The samples were analyzed for pH and ORP using a Thermo Orion Model 720Aplus meter (Thermo Electron Corporation). The color of the sample was measured using a Hach DR/2500 Spectrophotometer at a wavelength of 465 nm and following standard procedures (NCASI 1999). The spectrophotometer was operated and calibrated following manufacturer's guidelines. The term "color" represents the true color of an aqueous sample from which turbidity has been removed. A platinum cobalt standard was used to quantify the concentration of color in PCU.

4.3. Results and Discussion

4.3.1. pH

The variations for pH during the decolorization process for PMU and ESF are presented in Figure 4.2 – 4.3. As can be observed in these figures, pH is reduced for the two effluents during the decolorization of chromophores and degradation of solids (i.e., wood sugars from fibers) within the compost. We hypothesize that this leads to the production of organic acids and the lower pH levels observed just before the reactors are refreshed. The lowest pH reached for PMU and ESF was 6.5. Previous experiments (Lange 2004) have shown that neutral to mild acidic levels are optimum for anaerobic decolorization of the wastewaters using anaerobic composting. Reduction of pH in control reactors was significantly lower than the observed for reactors with compost.

Figure 4.4 shows the variation of pH for PHLA and PHLB. As can be observed the initial pH levels for PHLA and PHLB were significantly lower than those for PMU

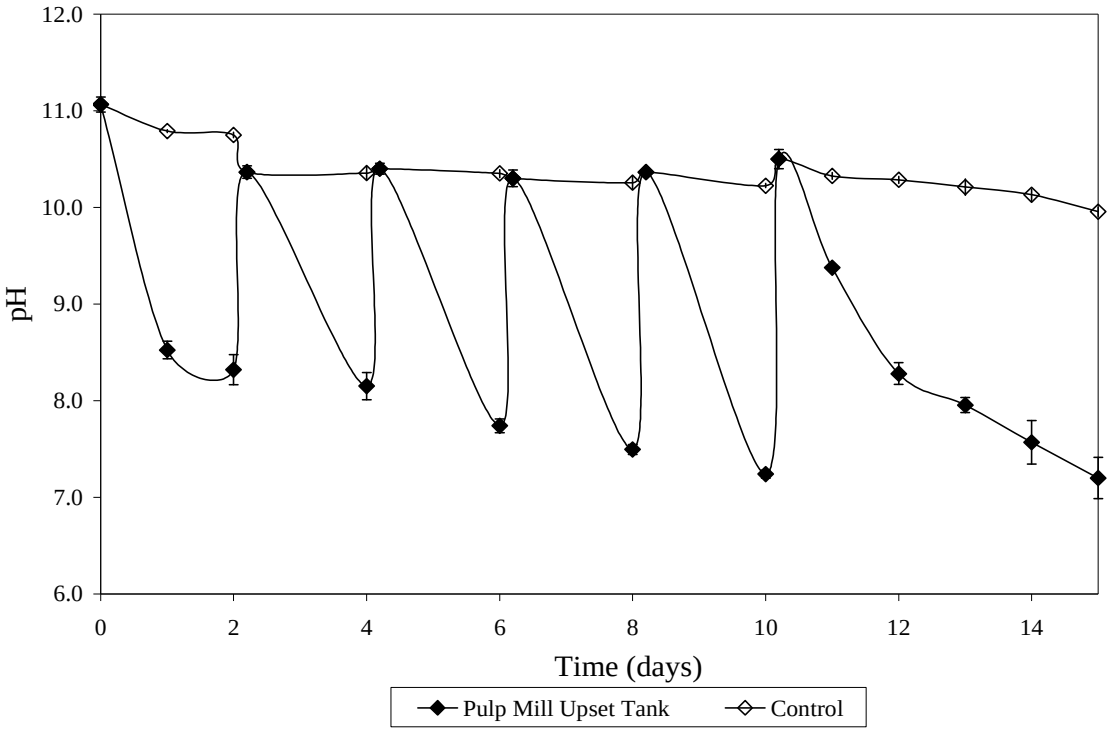


Figure 4.2 pH variations with time for Pulp Mill Upset Tank.

Error bars represent ± standard error (n=3).

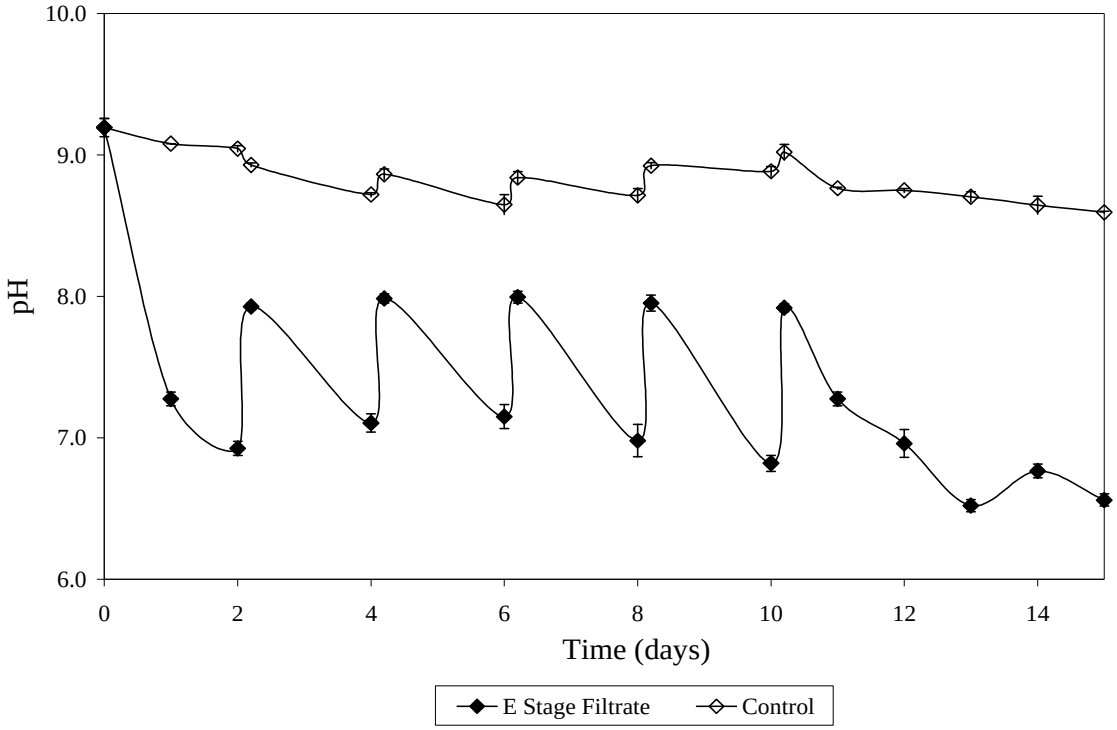


Figure 4.3 pH variations with time for E Stage Filtrate.

Error bars represent ± SE (n=3).

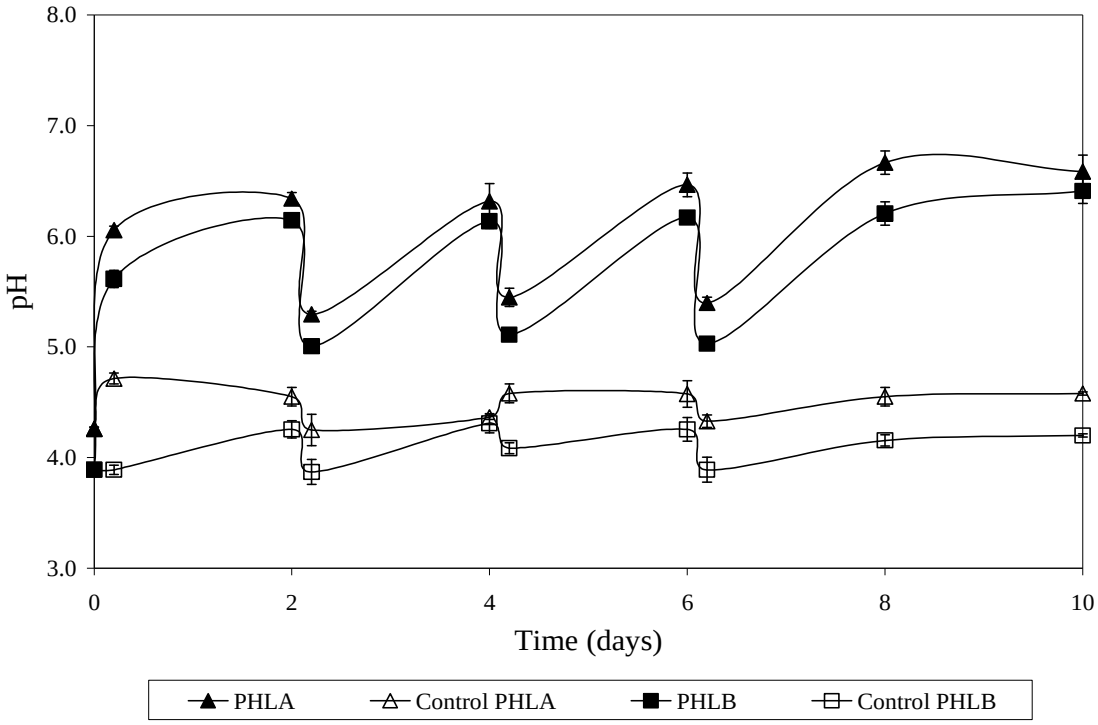


Figure 4.4 pH variations with time for PHLA and PHLB.

Error bars represent ± SE (n=3).

and ESF. Contrary to the pH variation for PMU and ESF, the trend observed for both PHL's shows that pH actually increased. The higher pH levels (~ 6.5) reached at the end of the decolorization cycle are within the optimal levels for decolorization to take place.

4.3.2. ORP

ORP variations for the four effluents are presented in Figure 4.5 – 4.7. The initial ORP for PMU reactors was -200 mV. Generally, while wastewater was being decolorized, the ORP for biologically active PMU reactors with remained under -300 mV. The only exception to this was observed when reactors were refreshed. During this process, the reactors were open and mixed with wastewater used for their refreshment resulting in higher ORP. However, the strong anaerobic conditions were recovered fast as ORP was reduced by more than 100 mV within a day. It can also be observed in Figures 4.5 – 4.7 that the reduction of ORP in control reactors was lower. Thus, the presence of the compost matrix was essential for creating and maintaining strong anaerobic conditions in the reactors. This is likely due to the degradation of large amounts of readily biodegradable organic compounds in the composted fibers.

4.3.3. Decolorization of Pulp Mill Upset Tank

During operation of the reactors as AnSBR, the color of pulp mill upset tank wastewater (PMU) was reduced by more 45% during the first cycle (Figure 4.8). The degree of decolorization of the effluent of the AnSBRs increased in subsequent cycles and 65% higher color reduction was achieved in the AnSBRs than was observed in the control reactors. Reactors were operated in batch E Stage Filtrate mode after five cycles.

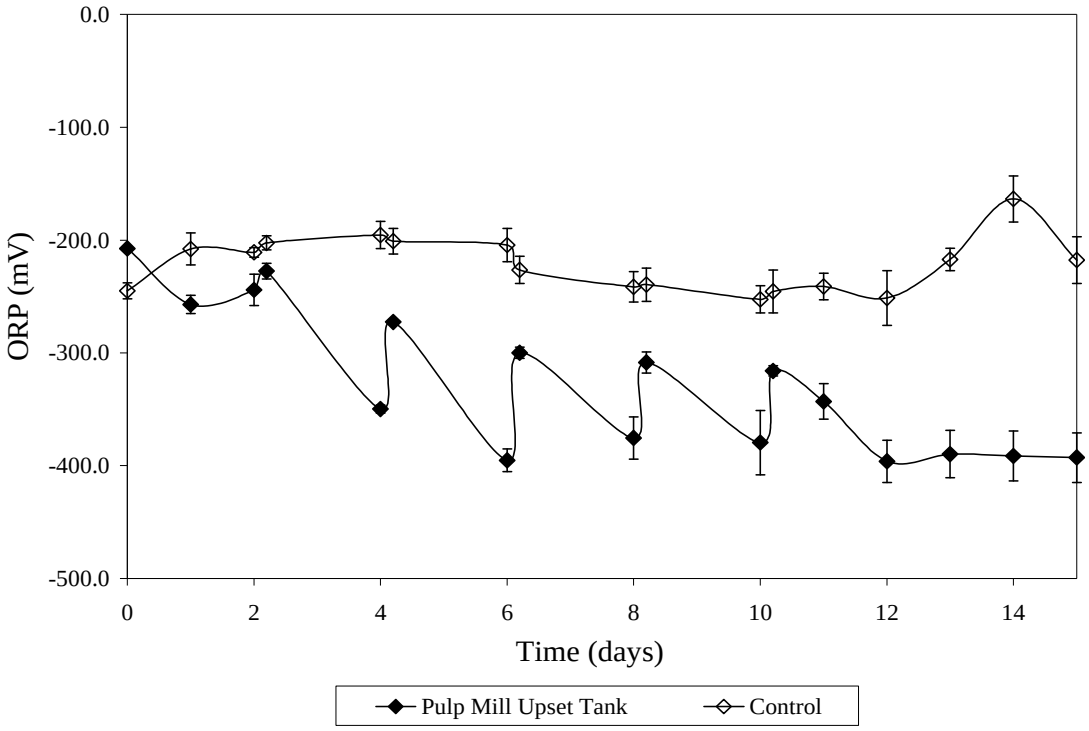


Figure 4.5 ORP variations with time for Pulp Mill Upset Tank.

Error bars represent ± SE (n=3).

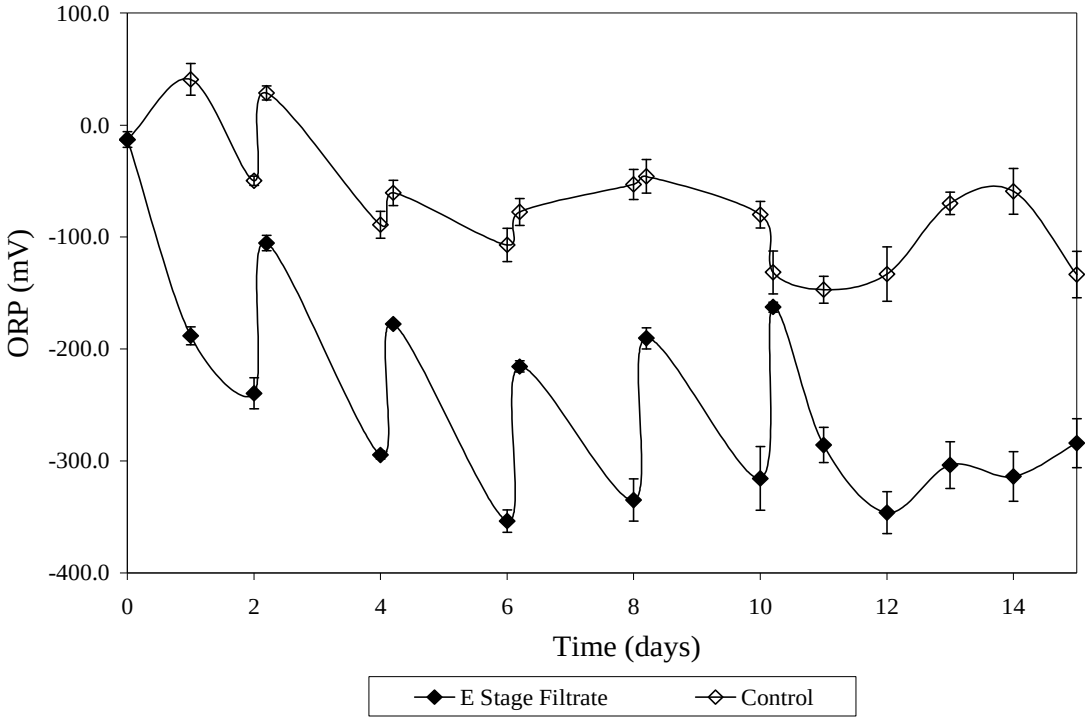


Figure 4.6 ORP variations with time for E Stage Filtrate.

Error bars represent ± SE (n=3).

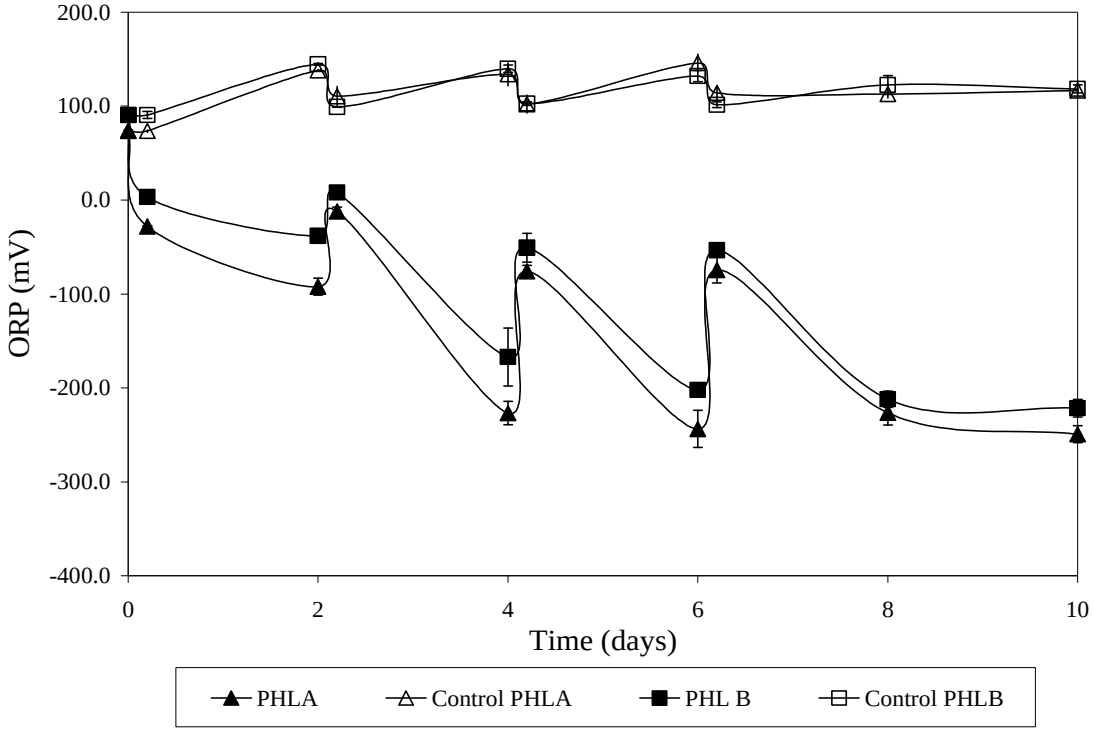


Figure 4.7 ORP variations with time for PHLA and PHLB.

Error bars represent ± SE (n=3).

At the end of the 5 day batch testing period color was reduced by 79%, while control reactors resulted in only 2% color reduction. The low decolorization of non-compost control reactors shows that the compost matrix provides conditions and/or microflora that remove color.

4.3.4. Decolorization of E Stage Filtrate

Initial decolorization experiments for ESF using new compost (non-acclimated compost) resulted in an increase in color often termed color reversion (Figure 4.9 – non-acclimated compost). The data in Figure 4.10 shows that the ORP for the non-acclimated compost reactors increased during the experiments. This increase in ORP in the non-acclimated compost SBRs, ultimately resulting in positive ORPs, was in stark contrast to the highly negative ORPs measured during successful color reduction. This indicates that the reduction conditions in the non-acclimated compost were not optimal for the biodecolorization of ESF.

The decolorization experiments for ESF were repeated using acclimated compost that was previously used with successful results for the decolorization of PMU. The results for the second set of ESF decolorization are also presented in Figure 4.9 (acclimated compost). Decolorization of ESF shows that color reduction was higher (61%) during the first cycle in contrast to the results observed for PMU. However, the degree of decolorization during the remaining of the cycles was only 55% when comparing with the color levels of the control reactors. Batch operation resulted in color reduction from 1900 PCU to 570 PCU. Compared to the control reactors, the color

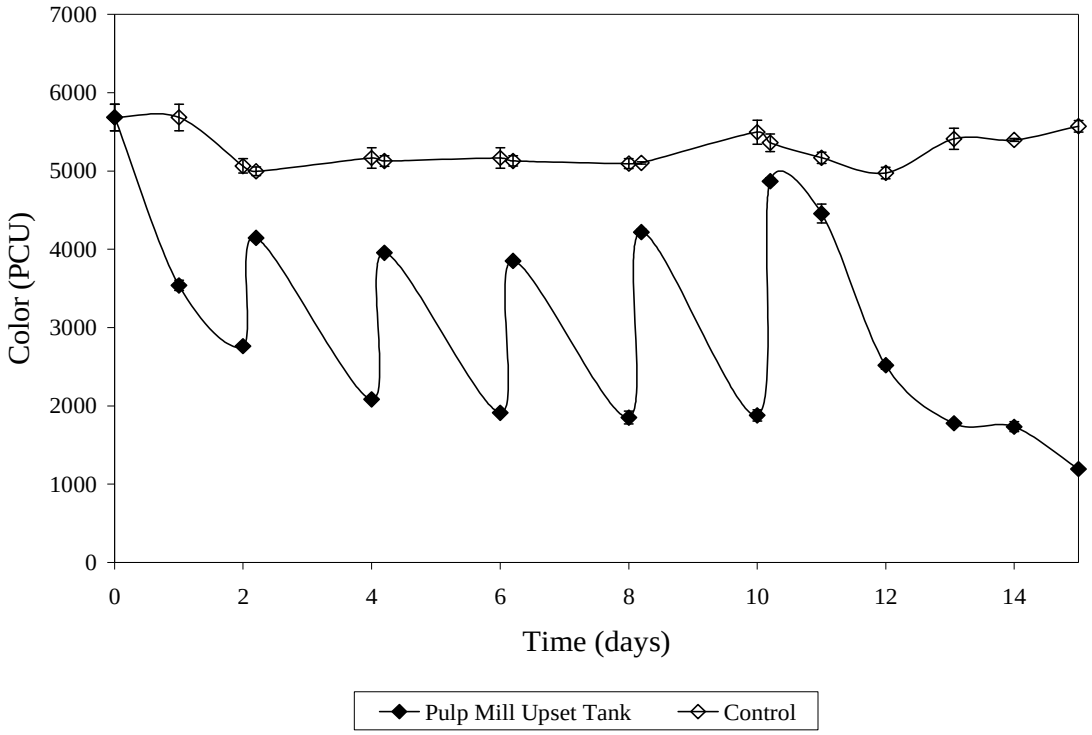


Figure 4.8 Decolorization of Pulp Mill Upset Tank.

Error bars represent ± SE (n=3).

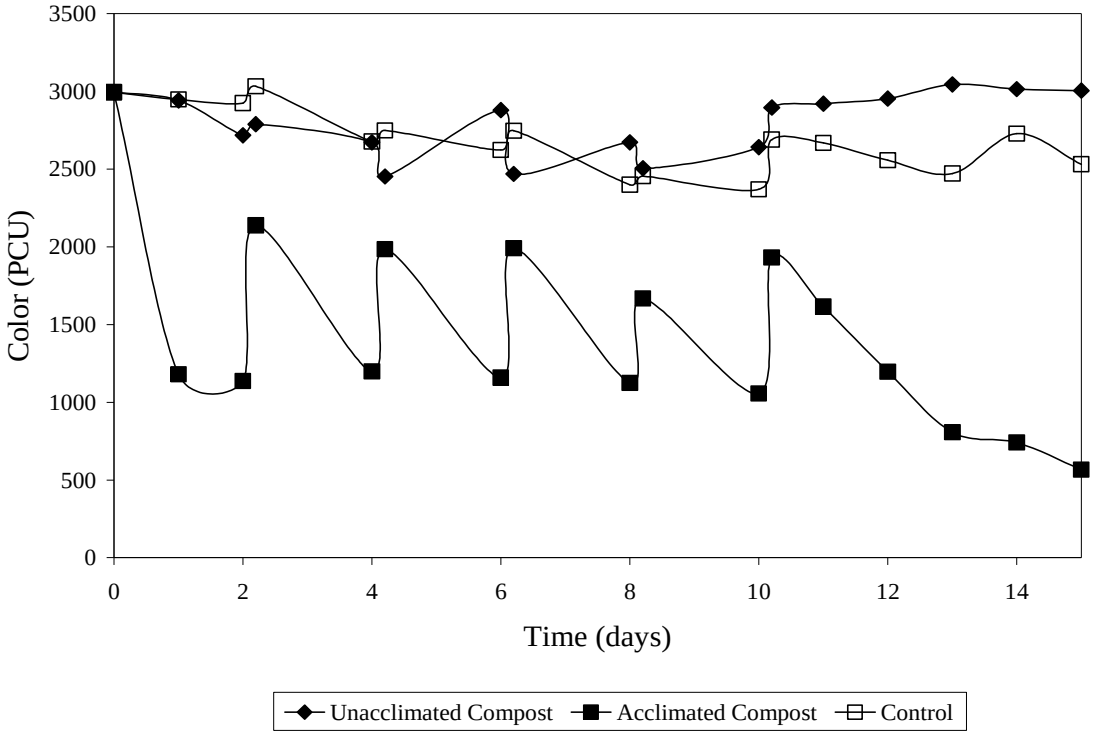


Figure 4.9 Decolorization of E Stage Filtrate using acclimated and unacclimated compost. Error bars represent ± SE (n=3).

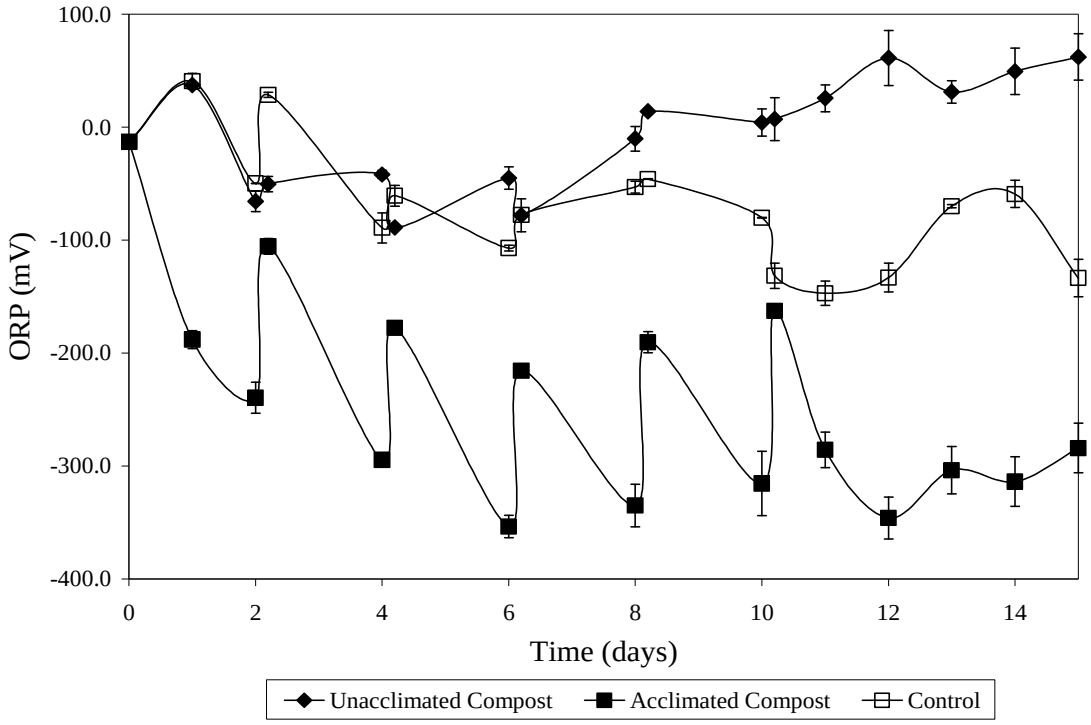


Figure 4.10 ORP variations with time for E Stage Filtrate using acclimated and unacclimated compost. Error bars represent ± SE (n=3).

reduction for ESF using acclimated compost was 78%, while color in compost free control reactors was only reduced by 6%.

Additional experiments were performed to test the effect of using acclimated compost for the decolorization of PMU. Those experiments (data not show) resulted in similar decolorization when using either acclimated or unacclimated compost as decolorization only improved by 5%. However, as observed for ESF, acclimation of the compost was needed for difficult or highly colored effluents. This acclimation period is essential for the reproduction of microflora responsible for the reduction of color. In addition, the acclimation of compost leads to the formation of the required conditions (i.e., ORP below -200 mV) for the anaerobic biodecolorization of slightly reductive effluents.

4.3.5. Decolorization of Pre-hydrolyzed Liquid

Experiments performed in our laboratory tested the effect in the number of AnSBR cycles with the final degree of decolorization (data presented in Chapter 5, Figure 5.3). It was found that after three AnSBR cycles little difference in the final decolorization levels was achieved during subsequent cycles. The similarity of color reduction between cycles three and subsequent cycles are also obvious in Figures 4.8 and 4.9. Therefore, experiments for decolorization of PHL were conducted using three AnSBR cycles instead of five cycles. Results for decolorization experiments of the PHL are presented in Figure 4.11. This graph shows that color was reduced from 43,200 to 11,700 PCU. This represents a decrease in color levels of 73% which is significantly

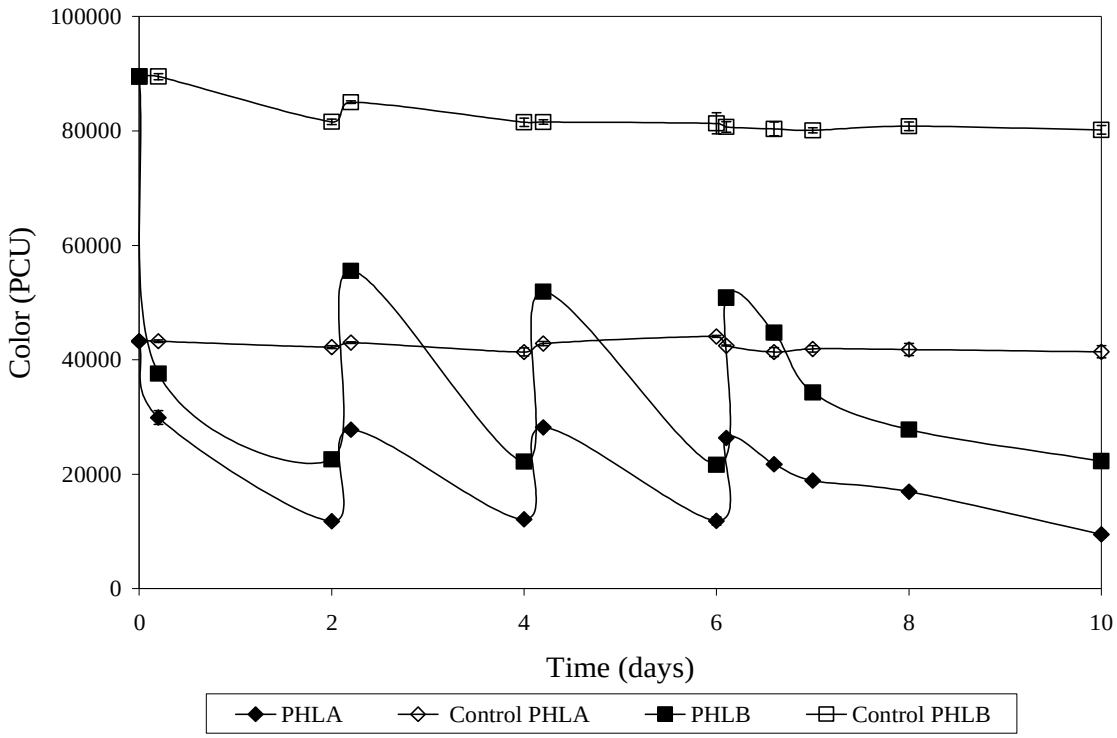


Figure 4.11 Decolorization of Pre-hydrolyzed Liquids.

Error bars represent ± standard error (n=3).

higher than the decolorization observed during the first cycle for the other wastewater studied in this set of experiments. During the other cycles color reduction was also around 73% (±4.1%). Similarly, PHLB had 75% (±3.2%) reduction of color during the three cycles. Final decolorization for PHLA was 78% while for PHLB color was reduced by 75%. Color Reductions in this highly colored waste stream represent a significant reduction in mill color, compare to other waste effluents.

4.3.6. Kinetic Studies

The data used to perform the kinetics studies for the batch experiments is presented in Figure 4.12. Integration method using the graphical approach was used to calculate the reaction rate constants (Levenspiel 1972). The results for these kinetics studies are summarized in Table 4.2. The kinetic constant for PMU decolorization was calculated as 0.0122/hr. For ESF, the kinetic constant was lower at 0.0105/hr. While decolorization of PHL gives the impression of having higher reaction rates, kinetics studies reveal that their reaction constant is similar (PHLA = 0.0107/hr) or if not smaller (PHLB = 0.0083/hr) than those obtained for PMU and ESF.

4.3.7. Aeration

Previous decolorization treatments for color reduction under anaerobic conditions have been unsuccessful and/or unreliable due to color reversion during aeration of the treated effluent. Studies performed by Milestone et al., (2004; 2007) showed that increase in color concentration was observed in the inlet to the main treatment pond and in polishing ponds that followed the main treatment pond. Both of these areas receive little

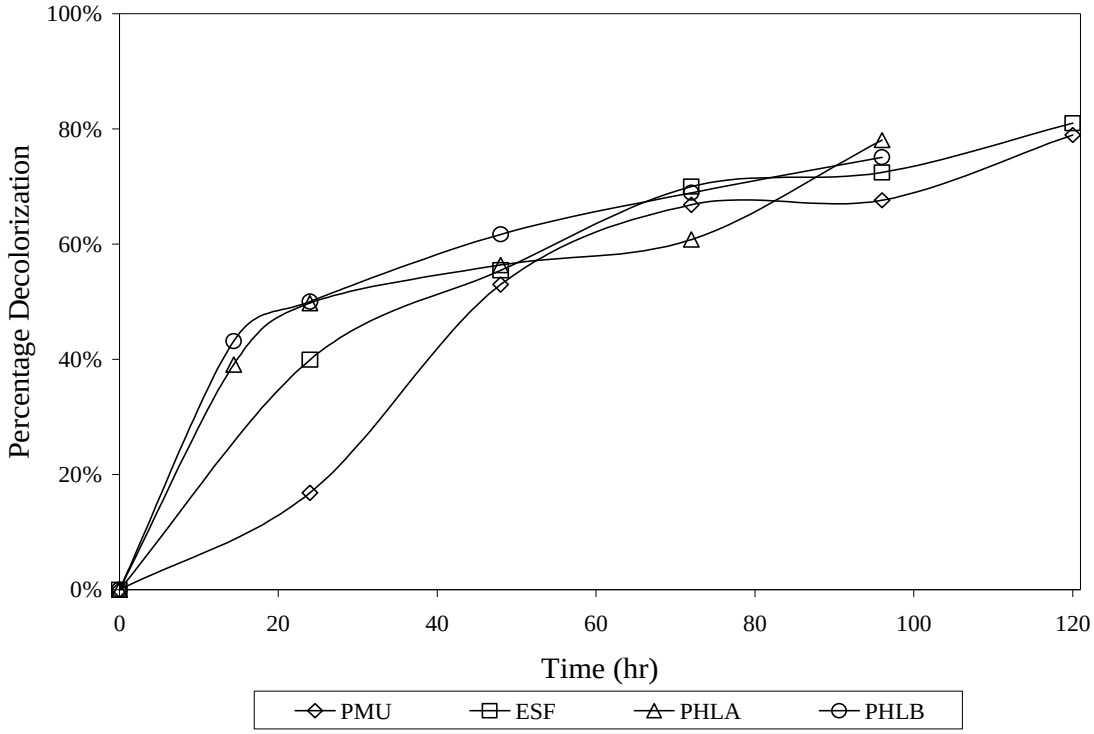


Figure 4.12 Percentage of decolorization during operations of reactors as batch.

Table 4.2. Final decolorization and reaction rates.

Wastewater	Color Reduction (%)	Reaction Rate Constant (1/hr)
PMU	79 (±0.004)*	0.0122 (0.921)†
ESF	81 (±0.006)	0.0105 (0.980)
PHLA	78 (±0.011)	0.0107 (0.973)
PHLB	75 (±0.017)	0.0083 (0.914)

* Numbers in parenthesis indicates standard error (n=3).

† R^2 for calculation of reaction rate constant.

or no aeration suggested that redox conditions play a major role in influencing color behavior.

In order to test the successfulness of our decolorization process and investigate the effect of redox conditions, samples of the treated wastewater were aerated for 48 hrs. Table 4.3 includes the color at the beginning of aeration and the percentage of color reversion at the end of the aeration period. The results for these experiments are summarized in Figure 4.13. Within 3 hrs of aeration, color for all the samples was reduced by approximately 13.7%. Afterwards color for PMU and ESF increased continuously. Comparing the color at the end of 48 hrs with the color at the start of aeration, color increased in PMU and ESF by1.8 and 5.1, respectively. This color increment is not significant considering the initial color levels of the effluents.

Aeration of the PHL shows that there was no color reversion. In the case of PHLA, color decreased by 4.7%. Statistical analysis of for this data shows that this number was not significantly different from the color at the beginning of the aeration period. Similarly, there was no color reversion for the PHLB wastewater. The observed phenomenon was that color decreased in this effluent by 48%. This observation indicates that aerobic decolorization of the effluent is facilitated by the previous anaerobic biodecolorization step.

4.4. Summary and Conclusions

The biodecolorization process studied presents a feasible and economical method of treating colored effluents. Results show that wastewater having diverse characteristics could be decolorized effectively under anaerobic conditions. Higher decolorization was

Table 4.3 Color before aeration and percentage of color reversion after aeration of the samples for 48 hrs.

Wastewater	Color (PCU)	Color Reversion (%)
PMU	1194.6(±22.4)	+1.8
ESF	567.8(±28.4)	+5.1
PHLA	9470(±230)	-4.7
PHLB	22280(±480)	-48.2

* Numbers in parenthesis indicates standard error (n=3).

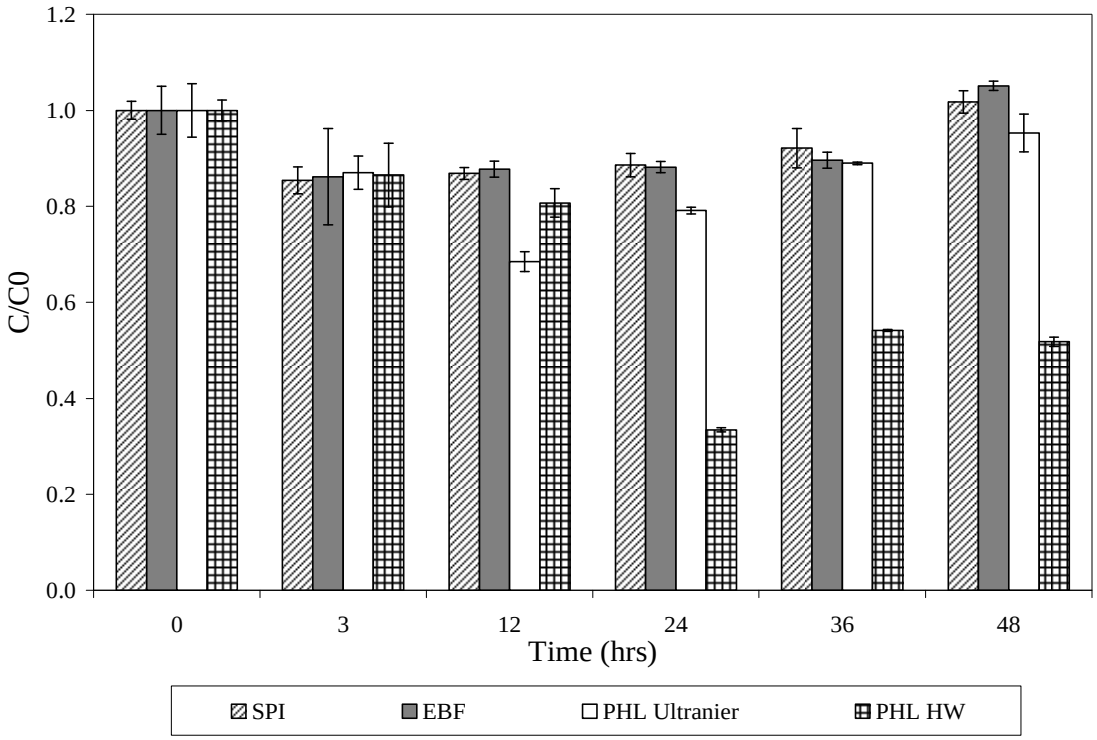


Figure 4.13 Aeration of decolorized wastewater. Error bars represent ± SE (n=3).

achieved when compost matrix as well as acclimation of the compost was used. In the case of PMU and ESF, color was reduced by 79% and 81%, respectively. Wastewater with higher levels of color could also be treated by this process. For example, color levels for PHLA were reduced from 43,250 to 9,470 PCU which represents 78% color reduction. Similarly, color for PHLB was reduced by 75% from levels that were initially at 89,500 PCU. Aeration of the samples indicates that color reversion was minimal (i.e. less than 6%).

In general, the studied process showed that similar percent in color reduction could be obtained regardless of the initial color of the effluent. As new regulations for the color levels in effluents emerge, mills may focus on using this process to treat low volume high color wastes to make process more economical.

CHAPTER 5. ANAEROBIC COMPOSTING OPTIMIZATION FOR THE BIODECOLORIZATION OF PAPER MILL EFFLUENTS

In this chapter, different operational parameters were investigated in order to select the best conditions for the biodecolorization process. The parameters studied include compost-to-feed ratio, length of sequencing batch reactor cycles, mixing and shaking, temperature, flocculent effects, long term operation and nutritional requirements. Finally, we validate these results by running decolorization experiments on pilot scale reactor.

5.1. Introduction

Composting is a widely used method for the treatment of biological waste. The use of anaerobic composting has proven efficient in the removal of color from paper effluents. In previous papers (Lange and Mendez-Sanchez 2008; Méndez-Sánchez et al. 2007), we described an anaerobic composting process based on the sequencing batch reactor (SBR), which significantly reduced the color of paper mill effluents. It was shown that this process could be used to decolorize effluents with high levels of color [<89,500 platinum cobalt standard (PCU)]. In addition, the composting process demonstrated the ability to function under conditions which are typically adverse to anaerobic processes

such as high concentrations of chlorinated compounds and transient high oxidation-reduction potential (ORP) events. As a robust and inexpensive process for decolorization of pulp and paper wastewater, this process could prove to be an important tool for meeting ever-stricter color regulations.

It is our aim in this paper to identify and quantify different parameters needed for the optimization, final design, and operation of this anaerobic decolorization process. Identification and quantification of the suitable environmental conditions is imperative for the success of this process. Decolorization efficiency can be increased by identifying and manipulating key operational variables affecting color removal process. The operational variables selected for testing included: feed wastewater-to-compost ratio (F:M), batch starting time, shaking rate, mixing, temperature, effect of pretreatment using flocculent, and nutrient requirements. The study of these variables provides needed information for the scale-up of the process as well as its application in other paper mills. The information obtained from these experiments gives us an indication of the capacity of the system (i.e., how much water it can treat and for how long), energy requirements, and time required for setup of the system.

Mixing in anaerobic process is usually used to provide direct contact between microorganisms and substrate. This helps reduce the mass transfer limitations in the reactor. In addition, mixing minimizes the buildup of inhibitory reaction intermediates and prevents the formation of scum at the water level (Dague 1994; Rodrigues et al. 2003; Sarti et al. 2007).

Anaerobic bacteria have a temperature range in which they are most productive in terms of process growth rates and substrate degradation performance (Chynoweth et al.

2006; El-Mashad et al. 2004; Kim and Speece 2002a; Kim and Speece 2002b). The several groups of bacteria involved in anaerobic digestion have two different temperature optimums. Bacteria growing between 20 – 40°C are known as mesophilic, while those growing between 45 – 80°C are known as thermophilic. This results in two main temperature ranges in which digestion usually can be performed optimally. The temperatures selected for this study were 30ºC, 40ºC, and 50ºC.

Coagulation and flocculation is one of the most commonly used methods for removal of color. Many companies use this approach as a first step in the process of color reduction. Some of the flocculents used include aluminum sulfate and polyelectrolytes (Rohella et al. 2001) such as polyacrylamide, polyethyleneimine, and polyethylene oxide (Pokhrel and Viraraghavan 2004). While color reduction using flocculent is often remarkable, some of the problems arising from coagulants use includes high operational cost as well as production of high volumes of sludge (Bajpai et al. 1999; Cruz et al. 1984; Zhou and Banks 1993). As an inexpensive means of disposing of this sludge, which is loaded with remnants of flocculent, introduction into the compost cells used for decolorization is desireable. The current literature presents inconsistency on the toxicity of organic polyeletrolytes on anaerobic microorganisms (Chu et al. 2003). Consequently, the study of flocculent effect in the anaerobic compost is needed.

Both nitrogen and phosphorus are essential nutrients in microorganism metabolism. Limitations of either of these nutrients in anaerobic process could lead to loss of performance of the biodecolorization process (Dinel et al. 2004; Parker and Laha 2005; Takashima and Speece 1989). An example of this is presented in the use of white-rot fungi, which is the most studied method for removal of absorbable organic halides

(AOX) and wastewater decolorization (Bilgic et al. 1997; Lankinen et al. 1991; Pallerla and Chambers 1997; Wingate et al. 2005). The enzymatic activity of white-rot fungi is reduced by both nutritional deficiencies and nutrient excesses (Ben Hamman et al. 1997; Ben Hamman et al. 1999a; Garg and Modi 1999; Kirk and Farrell 1987; Perez et al. 1998). Macronutrients may also affect the performance of the anaerobic decolorization process. For example, a secondary carbon source along with higher copper and manganese enhances the enzymatic activity of white – rot fungi (Yin et al. 1989). High concentrations of nitrogen and the trace nutrients zinc, iron, and molybdenum reduces enzymatic activity (Ben Hamman et al. 1997; Ben Hamman et al. 1999b; Garg et al. 1999; Garg and Modi 1999; Kirk and Farrell 1987; Perez et al. 1998).

The last objective of this paper focused in incorporating the optimum operational parameters into the operation of a pilot scale reactor. This test served to validate that our results were the optimum for the operation of the anaerobic decolorization process.

5.2. Materials and Methods

5.2.1. Wastewater and Compost Characterization

The wastewater studied in this experiment was E-Stage Filtrate (ESF). The total flow of ESF streams represent less than 10% of the wastewater produced in the Rayonier mill but contribute over 25-30% of the total color. The ESF average color for this study ranged between 3,000 to 6,000 PCU with an average of 4,200 PCU. Changes in the processing of pulp during these experiments resulted in samples with different concentrations of color. The average pH for ESF stream was 11.2 which is considered to

be very basic. The average ORP was -115 mV which indicates a slightly reductive environment.

The solids content of the compost ranged from 12% (thick liquid) to 23% (solid/firm). All of the compost material had high organic contents, which comprised over 80% of the solids as determined by ignition at 550ºC. The compost surface area was between 10 – 14 m^2/g (Lange and Mendez-Sanchez 2007), which is relatively low compared to sorbents like activated carbon. This indicates that the compost is not likely to be a good adsorbent. The compost has sufficient lime (2.9%) to yield a good buffering capacity.

5.2.2. General Reactors Setup

Large debris and roots were removed from the compost by hand. Acclimated compost was mixed with reject fibers in a 2:1 ratio (w/w) in order to improve the porosity and hydraulic characteristics of the compost. For the first experiment, different ratios of wastewater and compost were added to 2 liter settlometer jars **(**Nalge Nunc International). Each test condition was tested in triplicate reactors for reproducibility and reliability purposes.

The wastewater was amended with a salt solution to satisfy nutritional requirements. The nutrient solution contained in (g/L): KH_2PO_4 (0.053), K_2HPO_4 (0.1068), NH_4Cl (1.00), Na_2SO_4 (2.00), KNO_3 (2.00), $CaCl_2$ (0.735), $MgSO_4 \cdot 7H_2O$ (0.20). As a control for the effect of the compost solids, reactors without compost were prepared to study the biodecolorization of the wastewaters by the normal microbial community of the wastewater.

Decolorization reactors were operated as anaerobic sequencing batch reactors (AnSBRs). During the react phase, the contents were incubated for two days (48 hrs) on a shaker at 60 RPM. The SBR contents were allowed to settle for two hours. After settling, 50% of the supernatant was decanted and the reactors were refilled with fresh wastewater. Before starting the incubation periods reactors were mixed with a glass rod. Afterwards, the reactors were capped and vigorously shaken by hand. The same mixing procedure was used when refreshing the reactors after sampling.

Reactors were operated as AnSBR during 5 cycles (48 hrs) and then as batch for 5 days. Wastewater samples were taken at the beginning and end of the cycles and daily when operating as a batch. Kinetic parameterization was performed with the data obtained during the batch phase of operation. Modification of the general reactor setup and operation was done based on the parameters that were being studied. The effects of changes in: feed-to-compost ratio, number of SBR cycles, shaking and mixing, temperature, nutrient content, and polymer addition were studied. These modifications are described below.

5.2.3. Feed-to-Compost Ratio

To determine the wastewater feed (L/d) to compost (kg) ratio (F:M) that will produce the highest decolorization, SBRs were operated with different amounts of solids. The five F:M combinations tested in this study were (in L/d of waste/kg wet compost): (a) 1:3, (b)1:2, (c) 1:1, (d) 2:1, and (e) 3:1.

5.2.4. Number of SBR Cycles

The effect of SBR cycle length on decolorization was studied by starting the batch phase of the reaction at different days (cycles). Each SBR cycle took 48 hrs. After filling the reactors they were let react for 46 hrs followed by settling for 2 hrs. At then end of each cycle, 50% of the treated wastewater was decanted and the same volume of new wastewater was used for refreshed the reactors. All reactors were setup similarly and the SBR length was varied to 3, 4, and 5 cycles.

5.2.5. Shaking and Mixing

Effects of mixing on decolorization were first tested by shaking reactors at different rates. The shaking rates selected for this experiment were (RPM = 0), medium shaking (RPM = 60), and high shaking (RPM = 120).

A second set of experiments were conducted in order to determine if mixing was needed for the decolorization process. The general reactor setup was followed to prepare reactors for this experiment. In this case, three different treatments were used to determine the effect of mixing on decolorization. Treatments with mixing indicate that compost and feed were mixed initially with a glass rod. For those reactors that were shaken, the shaking rate was constant at 60 RPM.

5.2.6. Temperature

Three different reactor temperatures (30°C, 40°C, and 50°C) were used to determine the optimum for decolorization. Compost reactors were constructed following the general reactor setup using a ratio of 1:2 (kg/L) of compost to wastewater. Afterwards, reactors were placed in water baths at 40°C or 50°C. The results for these

decolorization experiments were compared to results for reactors operating at 30°C (average temperature at which all decolorization experiments were conducted).

5.2.7. Long-Term Operation and Nutrient Limitation

To determine the effects of nutrient concentration on AnSBR performance, nitrogen and phosphate salt concentrations were modified to achieve various target concentrations. The ratios tested ranged from a relatively large excess to a moderate deficiency of both nitrogen and phosphate. Ratios of carbon-to-nitrogen-to-phosphorus (C:N:P) of 100:10:1, 100:4:1, 100:15:2, and 100:10:0.5 were examined. Experiments for long term decolorization and nutrient limitations were run for 45 days.

5.2.8. Polymer Effects

To determine if polymers used to decolorize wastewater are inhibitory to the AnSBR decolorization process, polymer-pretreated wastewater was added to operating AnSBR. A polyectrolyte flocculent HCP-55 (Polymer Ventures, Inc.) with a concentration of 350 ppm was used in the first treatment since this concentration is similar to the used in the activated sludge ponds. In addition, concentrations five (1750 ppm) and ten (3500 ppm) times higher than currently being used were tested. For each treatment, wastewater was prepared my mixing the flocculent and letting it react for five minutes. Afterwards, the wastewater was mixed again and added to the compost reactors.

5.2.9. Pilot Scale Study

Validation of optimization experiments was performed by running semi-continuous pilot-scale reactors treating 200 L/days (compared to 1 L/day for bench). A

schematic diagram of the reactors setup is presented in Figure 5.1. Two 250-gallon plastic totes were used as reactors and operated in series. The reactors were filled with unacclimated compost that had been mixed with reject fibers in a 2:1 ratio (w/w). River rocks were used in the bottoms of the totes for sustaining the compost and allowing solid liquid separation. Influent and effluent samples were taken daily for 20 days. The color of the feed ranged between 2,050 to 10,700 PCU with an average color of 5,400 PCU.

5.2.10. Analytical Methods

The samples were analyzed for pH and ORP using a Thermo Orion Model 720Aplus meter (Thermo Electron Corporation). The color of the sample was measured using a Hach DR/2500 Spectrophotometer at a wavelength of 465 nm and following standard procedures (NCASI 1999). The spectrophotometer was operated and calibrated following manufacturer's guidelines. The term "color" represents the true color of an aqueous sample from which turbidity has been removed. A platinum cobalt standard was used to quantify the concentration of color in PCU. Nitrogen concentrations were measured following Hach Method 10023 (Reardon et al. 1966) and phosphate concentrations were measured following Hach Method 8048 (American Public Health Association et al. 1998).

5.3. Results and Discussion

5.3.1. Feed-to-Compost Ratio

The results for experiments to determine the optimal feed to compost ratio (F:M) are shown in Figure 5.2. The initial color concentration for this study was 5,000 (±112)

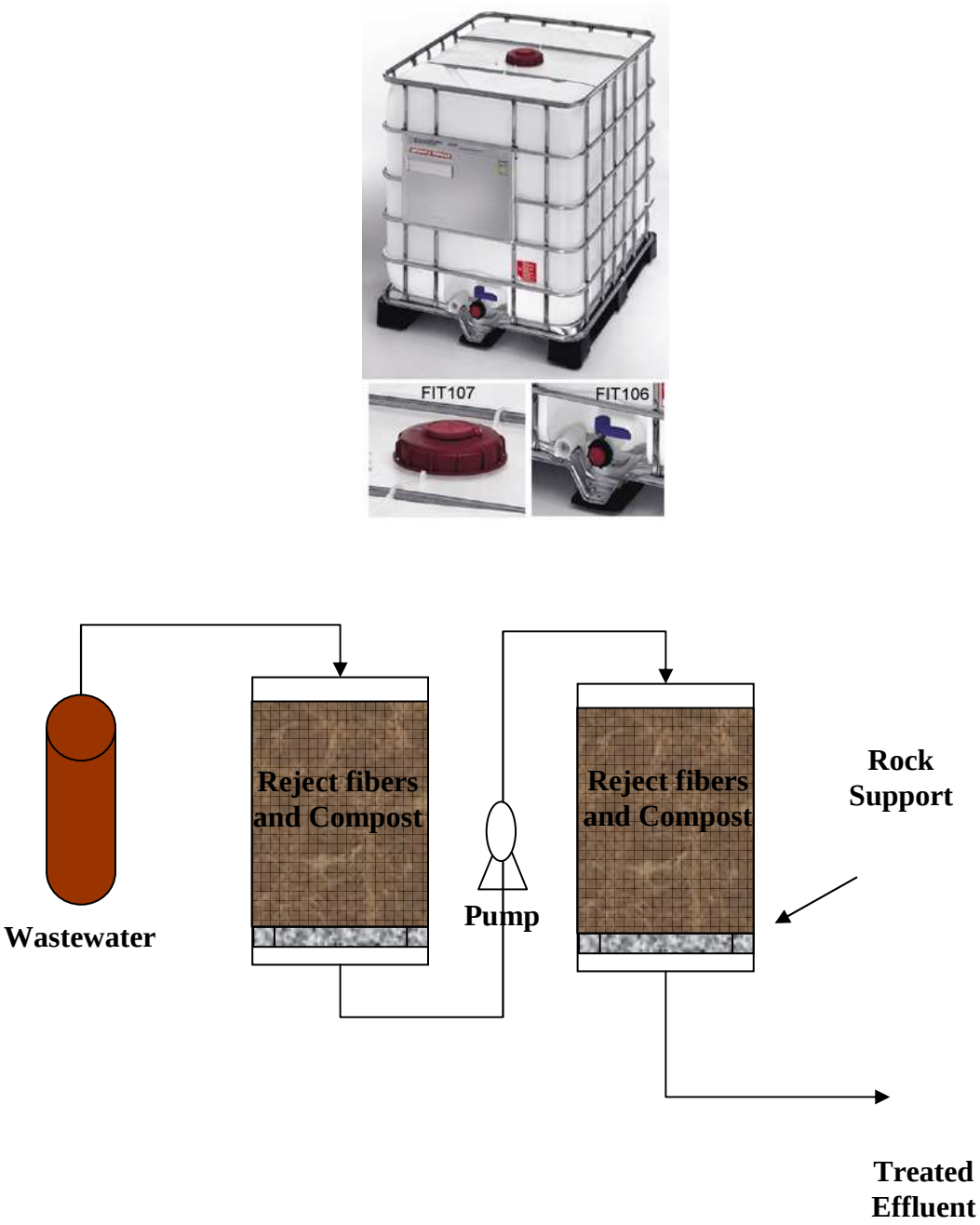


Figure 5.1 Schematic diagram of pilot scale reactors set-up.

PCU. Reactors with higher compost concentration showed higher decolorization. Within 48 hrs of experiments, 61% (±2.8%) of the color was reduced in reactors using the highest solids (1:3 feed to compost ratio). Reactors using smaller amounts of solids demonstrated similar trends in decolorization. However, as the amount of solids decreased (F:M increased) the degree of decolorization decreased. The reactor with 1:2 F:M achieved almost the same degree of color reduction as that with a 1:3 F:M, although two additional cycles were needed to reach 60% color reduction. Analysis of the data shows that for the final two cycles there was no statistical difference ($p<0.05$) for reactors with 1:3 and 1:2 feed to compost ratio. Reactors with lowest concentration of compost (3:1) only achieved 40% color reduction between cycles. In contrast, the control reactor decolorization was minimal at only 8%. Based on the results, it is apparent that a ratio-of-feed to compost of 1:2 or greater is needed to achieve the highest rate and degree of decolorization.

Presented in Figure 5.3 are the first order rate constants obtained from the data collected during the operation of the reactors in batch mode (t> 10 days in Figue 5.2). As observed during SBR operation, the ultimate percent of decolorization for 1:3 and 1:2 reactors was also very similar with 73% (±1.5%) and 69% (±1.8%), respectively. The decolorization rates presented in Table 5.1 also show that rate constants for these two treatments (1:3 = 0.0091hr^{-1} and 1:2 = 0.0088hr^{-1}) were very close with only a 3% difference between values. The rate and degree of decolorization achieved in reactors with lower concentration of compost were all significantly lower than those measured in reactors with reactors 1:3 and 1:2 F:M ratios. Based on these results, and considering that there was no statistical difference between F:M ratios of 1:3 and 1:2, it was determine

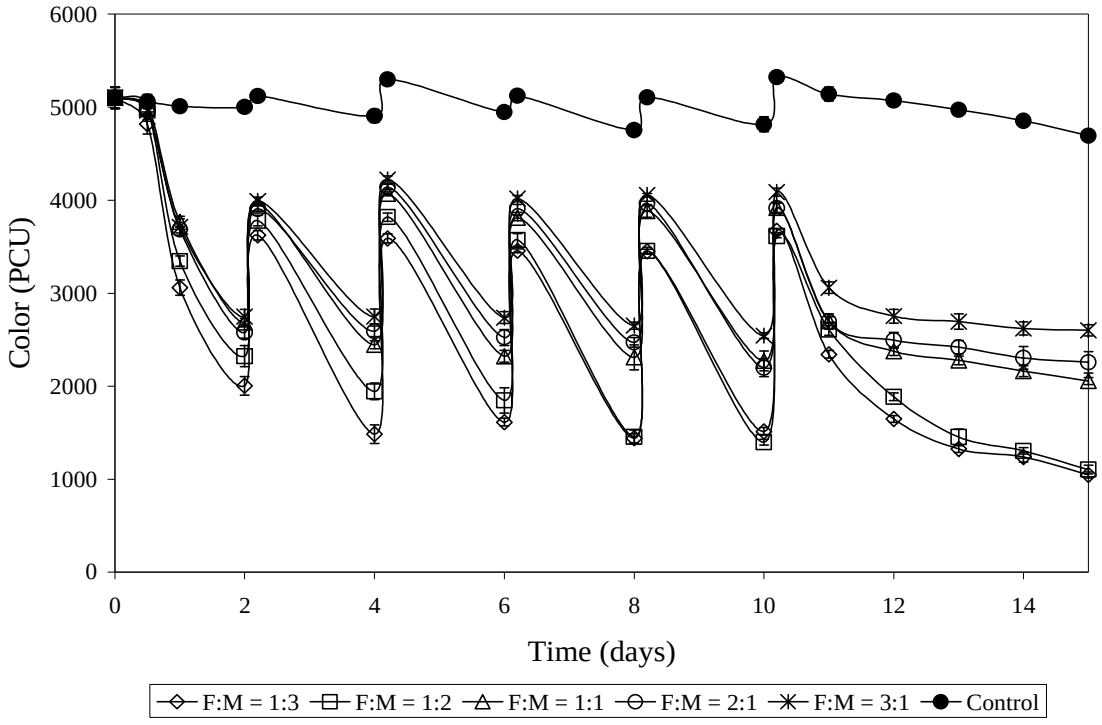


Figure 5.2 Decolorization of ESF at different F:M combinations.

Error bars represent ± standard error (n=3).

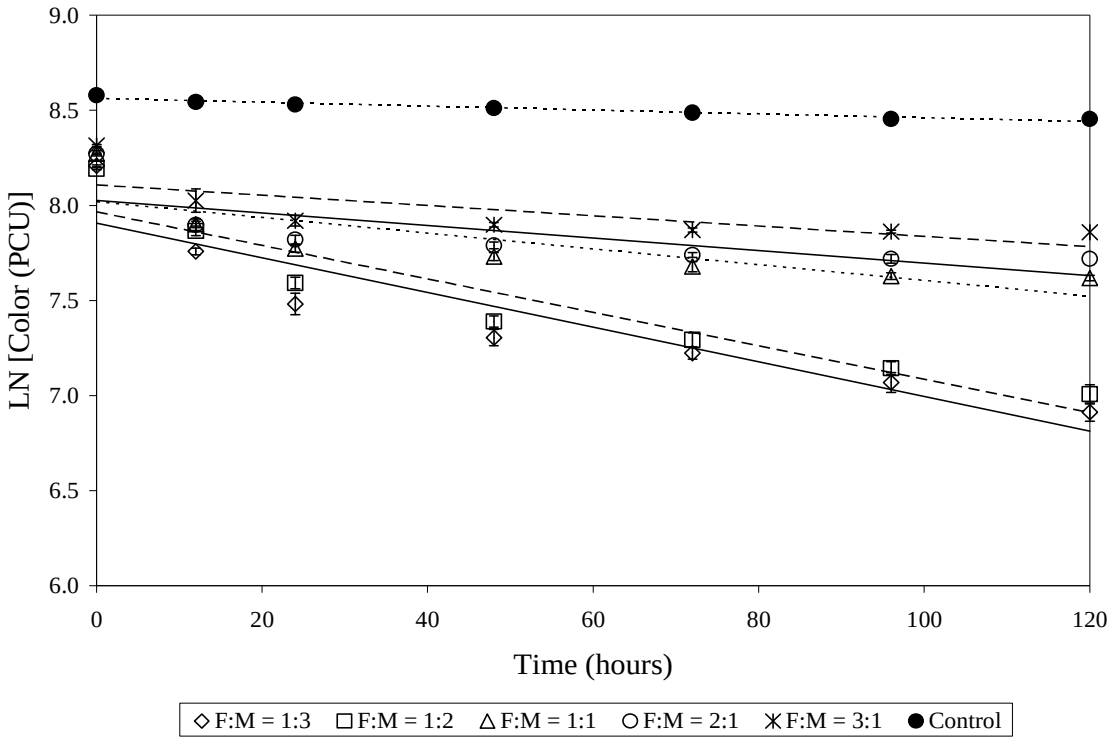


Figure 5.3 Determination of reaction rate constant for decolorization using different F:M. Error bars represent ± standard error (n=3).

Table 5.1 F:M Decolorization results and reaction rates in batch phase.

F:M	Initial Color (PCU)	% Final Decolorization	Decolorization Constant (hr^{-1})
1:3	3670(±20)†	73 (±1.5)	0.0091 (±0.00028)
1:2	3610(±15)	69 (±1.8)	0.0088 (±0.00043)
1:1	3920(±50)	48 (±1.6)	0.0041 (±0.00036)
2:1	3910(±60)	42 (±3.0)	0.0033 (±0.00042)
3:1	4090(±40)	37 (±1.2)	0.0027 (±0.00037)
Control	5320(±40)	12 (±0.3)	0.0010 (±0.00009)

†Values in parenthesis indicate standard error (n=3).

that the optimum F:M was 1:2. During subsequent studies, the AnSBR reactors were operated to using a 1:2 of feed to compost.

5.3.2. Number of SBR Cycles

The results for the variation of number of SBR cycle are presented in Figure 5.4. The main differences observed in this case are regarding the final color reduction achieved by each treatment. As seen in Figure 5.4, increasing the time for starting the batch phase resulted in higher decolorization. At the end of the experiments, the color reduction for reactors that started batch in the 5^{th} cycle was 78% (±1.2%) compared to 75% (±0.4%) and 74% (±0.7%) for reactors started at the 3^{rd} and 4^{th} cycle, respectively. Final color levels were 760 (±7.8) PCU for the reactors started in the 3^{rd} cycle, 800 (±25.2) PCU for the 4^{th} cycle, and 680 (±35.1) PCU for the 5th cycle. A difference of only 4% in decolorization for reactors run 3 cycles is insignificant compare to the additional time and expenses involved in running the process for 5 cycles.

5.3.3. Shaking and Mixing

The effects of shaking on the decolorization of EBF was studied by incubating reactors with no shaking (RPM = 0), normal shaking (RPM = 60), and high shaking (RPM = 120). Shown in Figure 5.5 are the results for the shaking experiments. It can be observed that there was no difference in the degree of decolorization for the three treatments using compost, while decolorization in control reactors was insignificant. The final decolorization for the different shaking speed was of 82% (±4.5%), 81% (±1.3%), and 79% (±2.5%) for 0, 12, and 120 RPM, respectively. These values are not considered to be statistically different ($p = 0.121$) since the relative error in the color determination

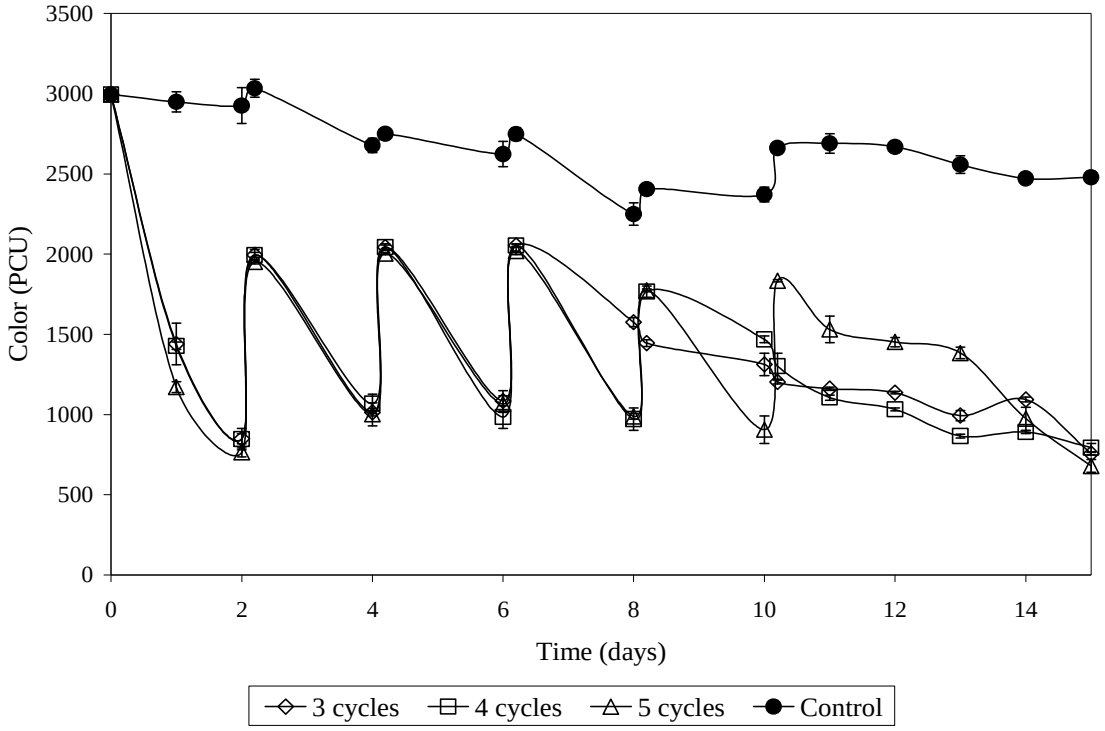


Figure 5.4 Effects of number of SBR cycle in decolorization.

Error bars represent ± standard error (n=3).

may be as much as 5%. Thus, shaking was concluded to make little observable difference in color removal.

After finding no difference in decolorization degree using the three different shaking rates, it came into question whether mixing using the glass rod and shaking by hand was needed at all in the decolorization process. Reactors were operated with no agitation, shaking only, and or mixing and shaking with the results presented in Figure 5.6. During the operation of the reactors as SBR, decolorization for reactors that were neither mixed nor shaken during incubation was as high as 90% (±5.6%). Batch operation shows that in reactors that were not mixed or shake the color levels was reduce by 78% (±2.3%). Biodecolorization for reactors that were not mixed but shaken during incubation was 81% (±1.3%). In contrast, reactors that were mixed and shaken had color reduction of only 70% (±1.9%).

While shaking had no impact on decolorization, it appears that mixing may have actually decreased biodecolorization slightly. It was hypothesized that mixing may have introduced significant quantities of air into the reactors, and this raised the redox to less favorable levels. It can be concluded mixing is not needed in the anaerobic process and maybe somewhat detrimental if excess air is entrained into the reactor contents. We hypothesized that diffusion-based processes and turbulence by the formation of gas bubbles and their transport into the top of the bioreactors provides adequate contact between the compost and feed wastewater.

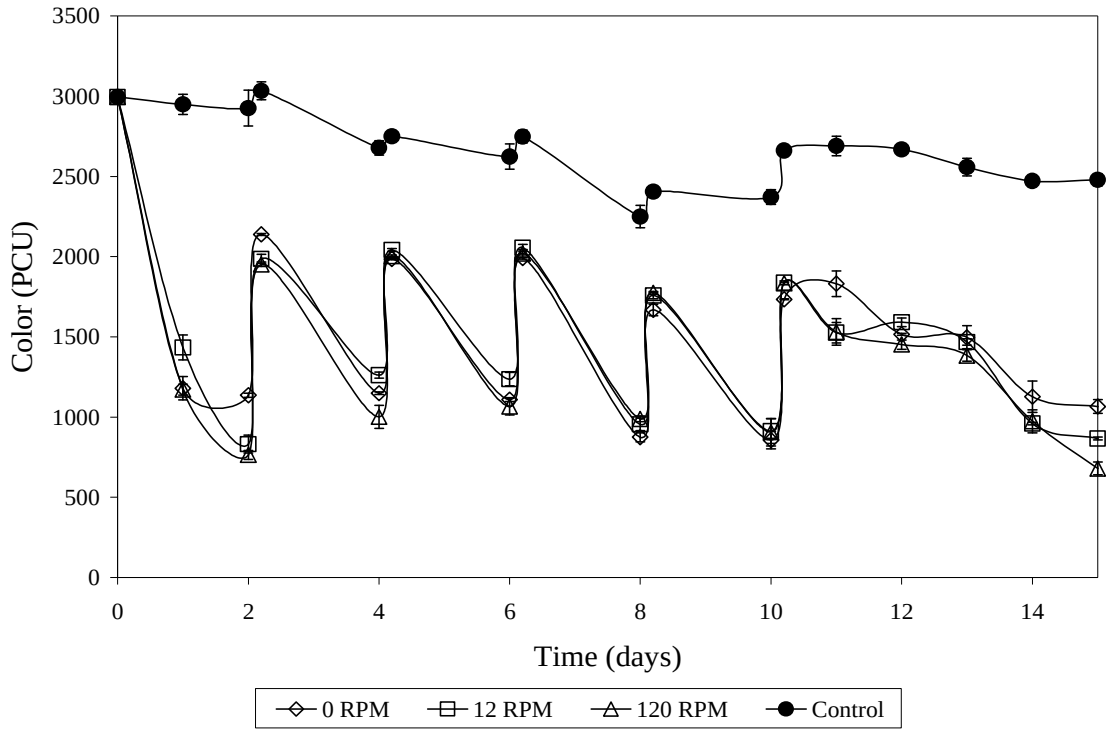


Figure 5.5 Determination of shaking rate effect on decolorization.

Error bars represent ± standard error (n=3).

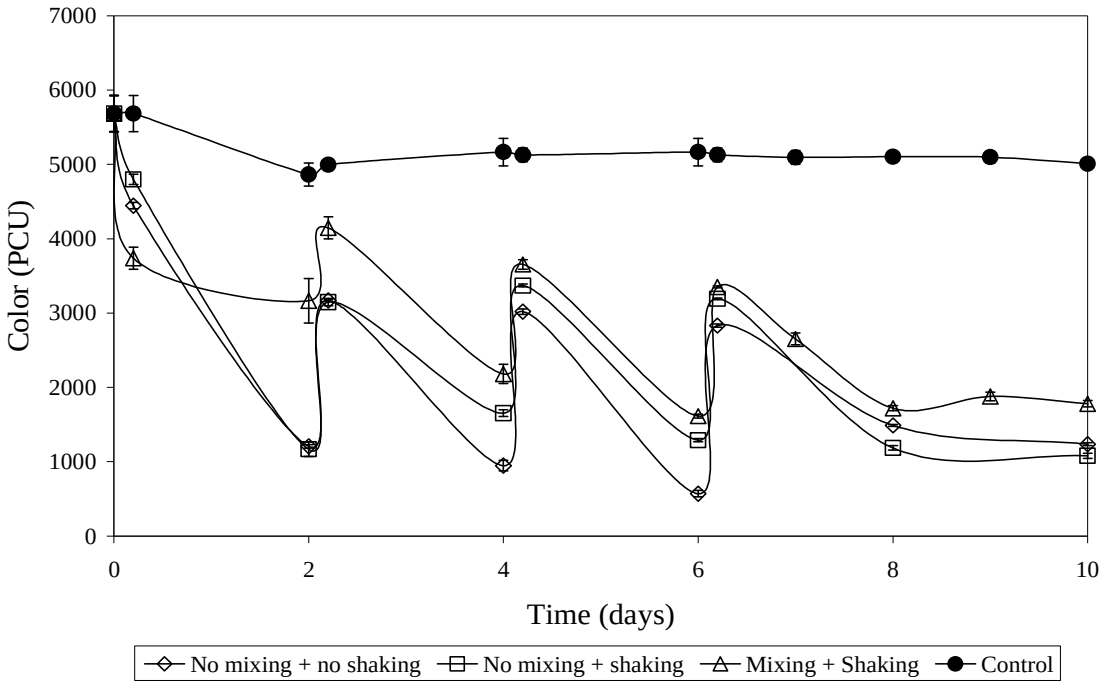


Figure 5.6 Effect of mixing on decolorization.

Error bars represent ± standard error (n=3).

5.3.4. Temperature

The effects of temperature on decolorization are presented in Figure 5.7. Initial color for this wastewater was 5700 PCU. Reactors at 40ºC showed the highest decolorization where color was reduced by 80±0.9% at the end of experiments (final color = 1190 PCU). Reactors at 30ºC and 50ºC had similar color reduction and both were lower than the observed at 40ºC. The final decolorization for reactors at 30ºC was 69±4.9% (final color = 1780 PCU), while for those at 50ºC color was reduced by 67±3.7% (final color = 1870 PCU). Thus, 40ºC appears to be the optimum temperature for operation of the decolorization process. These results are in accordance with literature where it has been documented that methanogenic processes have an optimum temperature around 40ºC (Ahn and Forster 2002; Hensel and Koenig 1988; Wu et al. 1993; Zeikus and Winfrey 1976). Furthermore, the operation between 30ºC and 50ºC still produces a significant degree of color removal. This means that the microbial population is fairly robust with respect to temperature and the process can be operated over a fairly wide range.

5.3.5. Long-Term Operation and Nutrient Limitation

The results of long-term decolorization studies using different nutrient concentrations are presented in Figure 5.8. One general finding of this study is that using the same compost sample for an extended period of decolorization of ESF was feasible. Color reduction exceeding 75% were achieved for the entire 45 day duration in reactors with the largest amount of supplemental nitrogen and phosphorus.

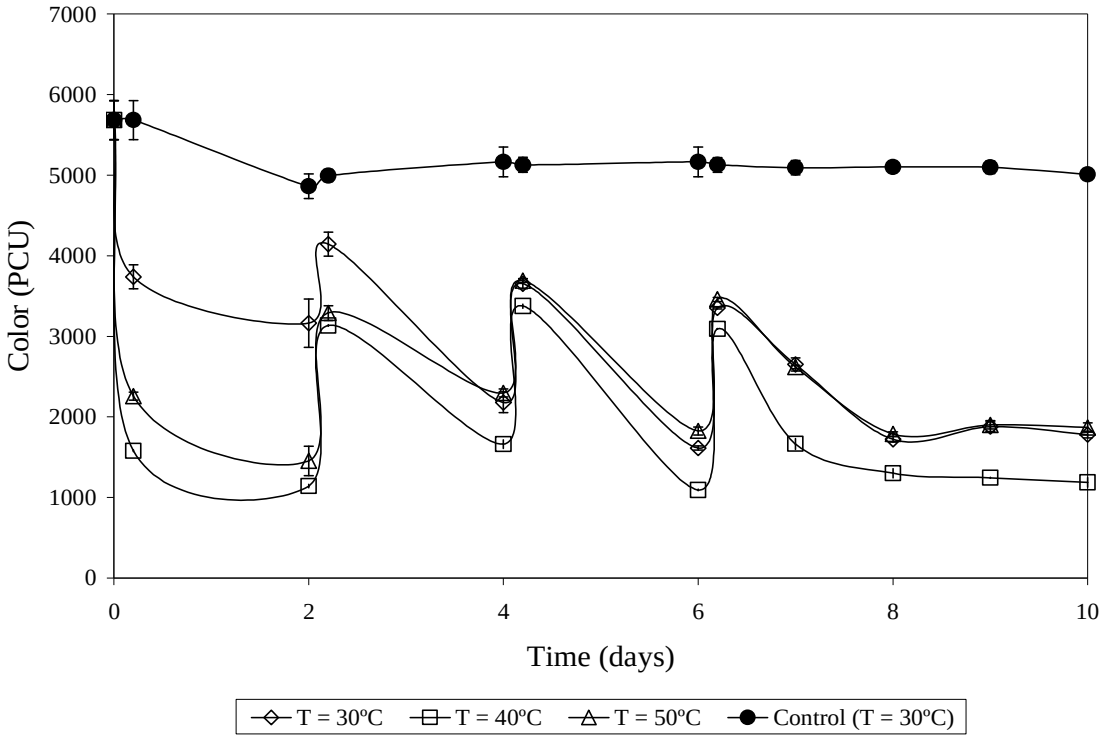


Figure 5.7 Decolorization of ESF at different temperatures.

Error bars represent ± SE (n=3).

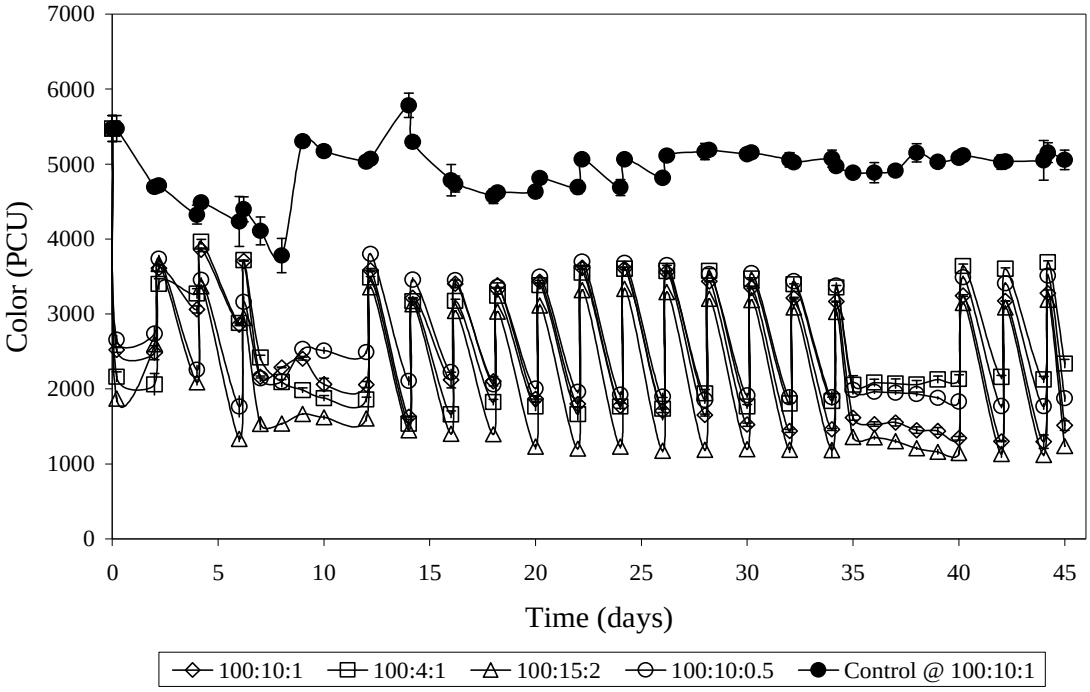


Figure 5.8. Nutrient limitation and long term operation of biodecolorization reactors. Error bars represent ± SE (n=3).

Initially, the operation of the reactors with the lower nitrogen concentration (67% color reduction) was similar to reactors with the higher concentrations of nitrogen (70% color reduction). After longer operation, however, it was observed that the efficiency in decolorization of treatments with lower nitrogen concentration decreased (57% reduction) while color reduction in reactors using other nutrient ratio was not significantly affected. This indicates that low nitrogen concentrations could be a limiting factor in the operation of the reactors for extended period.

In contrast, treatments with the highest concentration of nitrogen (100:15:2) had higher decolorization at the end of the experiments (77% color reduction) than did those with less nitrogen and phosphorus. However, higher concentrations of nitrogen only slightly enhanced decolorization when compared with reactors with 10 ppm of nitrogen that had similar decolorization. For example at the 44^{th} day of operation, decolorization for 100:15:2 treatment was of 79% (±1.9%) while reactors with 100:10:1 color was reduced by 77% (±2.1%).

When comparing excess or deficiencies of phosphorus in Figure 5.8, it can be observed that lower concentrations of phosphorus resulted in lower decolorization. For example, decolorization at day 40 for reactors with 100:10:1 resulted in 76% (±2.2%) color reduction compared to 65% (±3.1%) for reactors with lower concentrations of phosphorus (100:10:0.5). This result shows that phosphorus limitations may adversely affect anaerobic decolorization. Similarly to nitrogen, excess concentrations of phosphorus only had a slight positive effect in decolorization.

5.3.6. Polymer Effects

The decolorization of ESF treated with three different concentrations of a polyelectrolyte flocculent is presented in Figure 5.9. The results show that during operation of the reactors as SBR, decolorization was higher for treatments using 350 ppm than for those that were not pretreated with flocculants (0 ppm). Once operation of the reactors as batch was started, the color reduction in both treatments was similar. Color was reduced in reactors with 350 ppm by 60%, while in reactors without pretreatment the color reduction was of 62%.

When using higher concentrations of flocculent, it was found that decolorization was enhanced. During operation of the reactors the decolorization was as high as 92% for reactors in both treatments. At the end of the batch phase, reduction of color in treatments with 1750 ppm was 81% and those with 3500 ppm the color was reduced by approximately 83%.

In order to corroborate if the color reduction obtained was due to the high concentrations of flocculent or to biodegradation, the color concentration of wastewater pretreated with 1750 and 3500 ppm of flocculent but without anaerobic treatment was measured. Color reduction for wastewater pretreated with 1750 ppm was 62% (±2.7%), while for 3500 ppm there was a 67% (±5.1%) color reduction. This demonstrated that the decolorization of wastewater with high concentrations of flocculent was not only due to the pretreatment of the wastewater, but that anaerobic decolorization was taking place. Possibly, the higher flocculent could be contributing with an additional source of carbon, enhancing the decolorization of the wastewater. Based on these results, we can conclude

that high concentrations of this flocculent are not toxic to biodecolorization process and may actually be slightly synergistic. NOTE: only applies to this type of flocculent.

5.3.7. Pilot-Scale Study

The differences between the operational conditions for the bench-scale and pilot-scale study are presented in Table 5.2. The conditions used for the bench-scale experiments are the same that were used when running decolorization experiments for the first time. Pilot-scale operation was done using optimum conditions determined in previous testing.

Figure 5.10 – 5.11 presents a comparison of the decolorization results obtained for the bench-scale and pilot-scale reactors. The color of the influent wastewater was mostly constant for the bench-scale reactor (average = 5,066 PCU). In contrast, the pilot-scale study presents more variation in the influent color, where values as high as 10,700 PCU were used. Regardless of the initial color levels, the pilot scale was able to achieve more than 90% color reduction during the experiment (see Figure 5.11). In general, the percent of decolorization for the pilot scale study was higher than the obtained during bench-scale testing. The only time where this was not observed was at the start up of the process. Color reduction in the pilot study after 1 day of operation was only 38% compared to 47% for the bench scale study. However, the compost used during the pilot study had not been acclimated, while the compost used during bench studies had been acclimated before starting decolorization experiments. We hypothesized that the larger scale of the reactor lead to higher reducing conditions faster than the bench reactors that were usually aerated when they were refreshed.

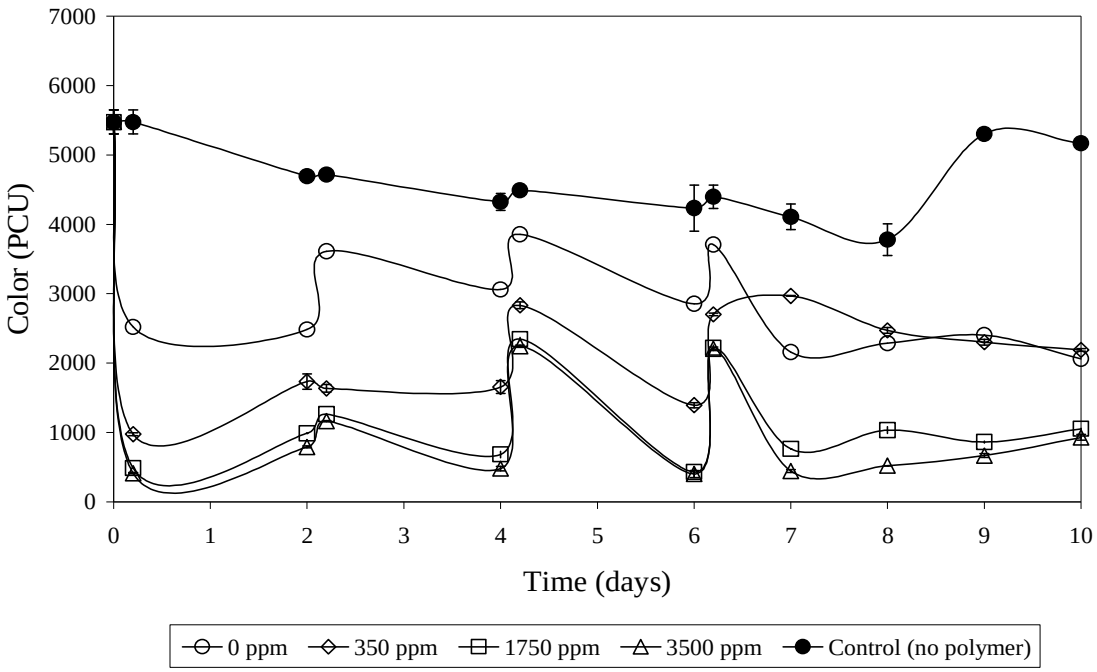


Figure 5.9 Effect of flocculent on decolorization.

Error bars represent ± SE (n=3).

Table 5.2 Decolorization conditions for bench scale and pilot scale study.

Parameter	Bench-Scale	Pilot-Scale
Acclimated Compost	Yes	No
Shaking and Mixing	Yes	No
Nutrients	Amended by liquid solution	Provided by compost
Color Range	4700 – 5400 PCU	2000 – 10700 PCU

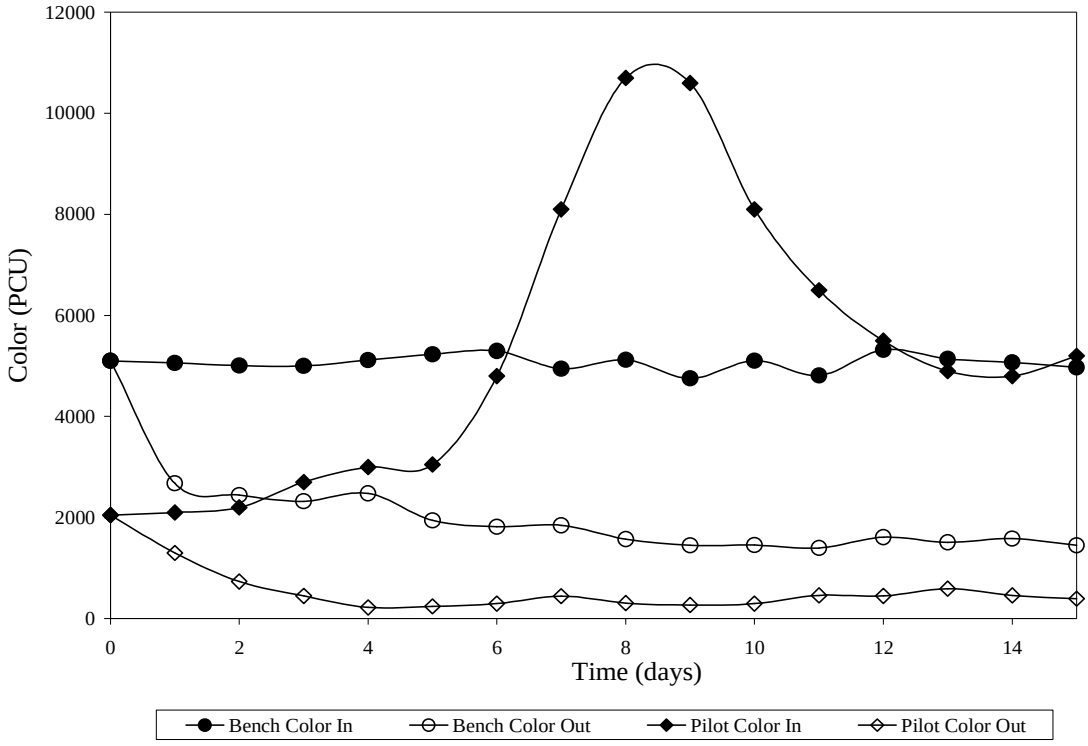


Figure 5.10 Decolorization of ESF using bench-scale and pilot-scale reactors.

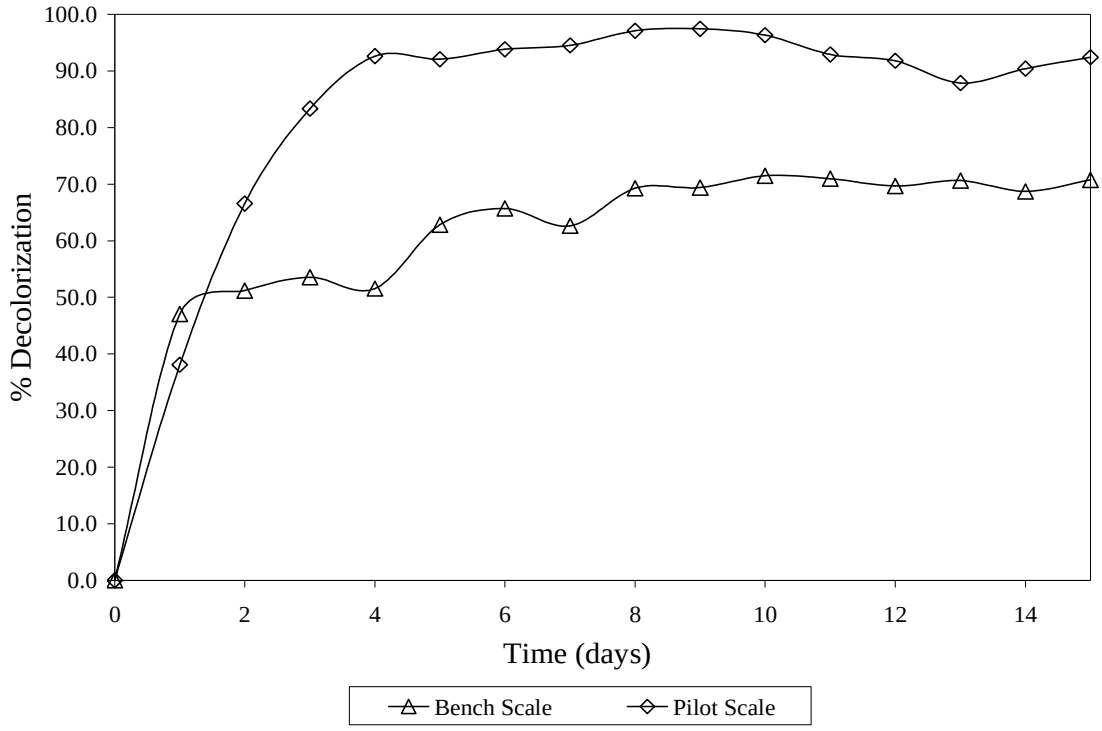


Figure 5.11 Percent of decolorization for bench scale and pilot scale reactors.

5.4. Summary and Conclusions

Decolorization efficiency can be increased by identifying and manipulating key operational variables affecting the color removal process. In this research, we studied some of these operational parameters. When studying the feed-to-compost ratio, we found that 1:2 and 1:3 (kg/L) had similar effects in the degree of decolorization. The ratio of 1:2 was selected for the rest of the experiments since it allowed the treatment of more wastewater in the same reactor when compared with the other ratio. The results also showed that mixing of the compost was not required, as decolorization degree was similar in reactors that were not mixed. Optimal decolorization was found for reactors operating at 40ºC which means that reactors may need heating during winter season. No adverse effects on decolorization were found when pre-treating the wastewater with high concentrations of polyelectrolyte polymer. Long-term operation of the reactors was feasible and reduction of degree of biodecolorization was only observed in nitrogen limited reactors. A pilot-scale study resulted in efficient decolorization results as higher than 90% of color reduction was obtained for most of the operation of the reactors. The results obtained in this study are promising since it was demonstrated that the optimization conditions obtained in previous experiments were adequate for the scale up of the process.

CHAPTER 6. ISOLATION AND CHARACTERIZATION OF MICROORGANISMS IN ANAEROBIC COMPOST DECOLORIZING PAPER MILL WASTEWATERS

The following chapter studies the isolation and identification of decolorizing microorganisms in the anaerobic compost. First, the interaction between different anaerobic microbial communities is studied. Afterwards, an unknown culture was isolated and its 16S rRNA gene was sequenced for identification. The final part of this chapter presents decolorization experiments performed with the isolated unknown.

6.1. Introduction

Around 60 m^3 of water are required to produce a ton of paper resulting in the generation of large volumes of wastewater. Wastewaters from paper mills are characterized by their high color. Some of the byproducts obtained from the conversion of wood pulp into paper are compounds such as residual lignin and lignin derivates along with polymerized tannins that impart color to the water (Crooks and Sikes 1990; Sarkanen et al. 1971). In addition, the color present can be attributed to the formation of chromophores created from the degradation of lignin (Jha et al. 2002; Kemeny and Banerjee 1997; Kringstad and Lindstroem 1984; Selvam et al. 2002; Tarlan et al. 2002).

While color is mostly considered an aesthetical problem, other adverse effect are associated to it. Photosynthesis is one of natural process that is inhibited when dark colored wastewaters are discharged into aquatic environments. Particles in solution producing color will scatter and absorb light, reducing the photosynthetically available radiation (Kirk 1994). In addition, brown color wastewaters increase water temperature leading to decreased levels of dissolved oxygen (Kringstad and Lindstroem 1984; Selvam et al. 2002). Therefore, the colored substances in aquatic systems have been associated with changes in primary productivity (Del Giorgio and Peters 1994; Ilmavirta and Huttunen 1989). Currently, there are no specific restrictions regarding the color limits in pulp and paper mill wastewaters. The recent recognition of the adverse effect this problem has in the ecosystem has lead to the promulgation of laws in different parts of the world limiting the levels of color in wastewaters.

In an effort to reduce color in paper mill wastewaters, researchers are working on developing environmentally friendly and economical approaches to reduce color. One of the decolorization approaches being researched consists of the use of microorganisms in an anaerobic composting process. Recent experiments conducted in our laboratories demonstrated the feasibility of using anaerobic composting for the treatment of wastewater with different characteristics (Lange and Mendez-Sanchez 2007; Méndez-Sánchez et al. 2007). Results of these experiments shows that a large degree of color reduction could be achieved in these reactors, ranging from 75% for a wastewater with initial color of 89,500 PCU to 88% for a wastewater of 3,900 PCU. Strong reducing conditions were required for the successful decolorization of the wastewater.

Within the textile industry the use of anaerobic processes for the reduction of color has shown substantial results. Complete decolorization of textile wastewaters under anaerobic conditions has been reported by several authors (An et al. 1996; Beydilli et al. 1998; Brown and Laboureur 1983; Fontenot et al. 2002; Lee et al. 2005; Quan et al. 1991; Razo-Flores et al. 1997; Yoo et al. 2001). However, the pulp and paper industry does not follow the same trend. Some researchers argue that bleached wastewaters are not suitable for anaerobic decolorization due to the low biodegradability and presence of toxic compounds that can affect methanogens (Pokhrel and Viraraghavan 2004). Most literature regarding biological decolorization states that lignin degradation does not take place under anaerobic conditions (Feijoo et al. 1995; Kirk and Farrell 1987). Therefore, anaerobic decolorization of wastewaters containing lignin chromophores is not expected.

As part of the understanding of this anaerobic method of decolorization, it is important to identify microorganisms present in the paper mill compost that are actively removing color. We hypothesized that different anaerobic microorganisms perform the oxidation of compost to produce the reducing equivalents necessary for decolorization. From the high concentration of methane and hydrogen sulfide generated by this process, we believe that methanogens and sulfur reducing bacteria (SRBs) are among the different communities of microorganisms that are possibly responsible for the oxidation of compost and decolorization of the wastewater.

In the first part of the research presented in this paper, we studied the interaction between methanogens, SRBs, and other anaerobic microorganisms in order to determine their impact to the biodecolorization process. This was done by performing decolorization experiments with chemical inhibitors for methanogens and SRBs. The

second part of this research focused on the isolation and identification of three unknown bacteria growing in paper mill anaerobic compost, which may be involve in the decolorization process. To achieve this objective, a study was conducted using a selective enrichment media for microbial growth and isolation, DNA isolation for microbial community analysis, and testing of the phenotypic diversity of cultured isolates to compare with its closest relative. Finally, the extent and rate of decolorization using the isolated bacteria was tested using reactors containing paper mill wastewater without compost.

6.2. Materials and Methods

6.2.1. Sample Description

6.2.1.1.Wastewater

Samples from the Pulp Mill Upset Tank (PMU) were used in this study. The measured color for PMU averaged 5,300 PCU. The redox for this sample averaged approximately - 255 mV which means the waste is devoid of oxygen and will sustain highly anaerobic reactions such as sulfate reduction. The PMU contained a large amount of organic carbon, with COD and BOD averaging 1250 mg/L and 960 mg/L, respectively.

6.2.1.2.Compost

Samples were taken from anaerobic compost pits (10 Acres * 20-25 ft depth). Characterization of the compost shows that the average ORP for the compost was -250 mV and pH was 6.8. Solid content varied from 12% (thick liquid) to 23% (solid/firm).

The concentration of sulfide was as high as 66 mg/kg representing conditions characteristic of anoxic sulfur reduction. Total phosphate and nitrogen average for the compost was P = 0.50% and TKN = 1.12%. The ratio of nutrients measured on the samples is within the range of nutrient concentration suitable for composting of mixed paper mill sludge (Haug 1980) and would help provide N & P for degradation of organic carbon in the studied wastewater. Lime concentration was high enough (2.35%) as to provide a good buffering capacity to the compost. The organic content for the surface samples was 79%.

6.2.2. Inhibition Experiments

The compost was mixed with reject fibers in a 2:1 ratio (w/w) in order to improve the porosity and hydraulic characteristics of the compost. A 1:2 ratio of wastewater to compost was added to serum bottles that were used as reactors for testing the decolorization. Mineral solution (100mL) was added in order to satisfy nutritional requirements. The solution contained the following salts in (g/L): KH_2PO_4 (0.053), K_2HPO_4 (0.1068), NH_4Cl (1.00), Na_2SO_4 (2.00), KNO_3 (2.00), $CaCl_2$ (0.735), $MgSO_4 \cdot 7H_2O$ (0.20). The different inhibition treatments applied to the reactors are included in Table 6.1. Samples were taken at 0, 0.5, 2, 4, 7, 10, 14, and 21 days and analyzed for color, pH, and ORP.

6.2.3. Media Preparation for Isolation

Wilkins Chalgren agar (WCA) was used to isolate anaerobes from our environmental sample. Agar plates were prepared by adding 2.2 g of WCA in 100 ml of

Table 6.1 Design Matrix for inhibition experiment.

Inhibitor	Function	Microbial community inhibit
CaO_2	Study color reduction using SRB as main decolorizing community	Methanogens
Na_2MoO_4	Study color reduction using methanogens as main decolorizing community	SRB
No inhibitor	Positive control – study color reduction without bacteria inhibition	None
CaO_2, Na_2MoO_4, NaN_3	Negative control – study abiotic removal of color	"Complete" microbial inhibition

distilled water. This concentration of WCA was half of the actual concentration recommended (Difco Laboratories, Detroit, Michigan). This mixture was then autoclaved 30 minutes and kept at 55°C for 10-30 minutes until poured in agar plates. Serial dilutions (10^{-2} – 10^{-4}) were done by adding 1 g of compost samples to a 99 ml water bottle and vigorously mixing the samples after each dilution. 100 µl from each of the dilutions was plated into WCA plates and incubated at STP in an anaerobic jar. After three days of incubation, a colony was selected and restreaked into TSA agar plates for isolation of the unknown bacteria. The plates were incubated under anaerobic conditions.

6.2.4. Genomic DNA Isolation, PCR Amplification, and Sequencing

Genomic DNA was isolated using an Ultra Clean™ Microbial Isolation Kit (MO BIO Laboratories Inc.). The isolated genomic DNA from the pure microbial culture was used as a template in PCR. The master mix for PCR amplification included the primer sets 27F and 1492R (universal Bacteria) or 338F-GC and 518R. After PCR amplification, DNA was purified using the Wizard® SV Gel and PCR Clean-Up System (Promega Corporation). The microcentrifuge tube containing the eluted DNA was stored at -20°C. Sequencing of purified DNA was performed via BigDye Sequencing Chemistry with primer 27F used to amplify the 5' region of the 16S rRNA genes.

6.2.5. Phylogenetic Analysis

The 16S rDNA sequence was compared to the database of non-redundant sequences using the BLASTn algorithm with default parameters within the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) to identify related 16S rDNA sequences. Multiple alignment was created using Clustal X (version 1.81)

program (Thompson 1997). Neighbor Joining Tree and bootstrap was created using Clustal X. A total of 2000 bootstrap trials were used for this method. Maximum Parsimony Tree and Bootstrap (200 bootstrap replication) was created using PAUP 1.0b. The resulting trees were drawn using TreeView.

6.2.6. Biochemical Characteristics

Some of the biochemical characteristics examined to assist in identification of the unknown isolate included carbon source degradation, as well as enzymatic and antibiotic properties testing. Within the carbon source tested were glucose, maltose, cellulose, citrate, and indole. Cells samples were collected from the TSA plates used for storage and suspended in test tubes containing water. 20% sterile filter solution was prepared for glucose and maltose and added to test tubes. Growth for these test tubes was determined by measuring optical density (OD) after three days of incubation. Cellulose degradation was tested by adding 0.5 g of cellulose directly to the test tubes. Controls were test tubes for cellulose degradation were prepared to compare changes in OD due to abiotic factors. The unknown isolate was stab and then streak up the slant in test tubes used to test citrate and indole degradation. All test tubes used to study different carbon sources degradation were incubated at standard temperature and pressure. Enzymatic testing included catalase, oxidase, urease, as well as the hydrolysis of esculin, starch, lipids and blood. Catalase was tested by adding 1 – 2 drops of 3% hydrogen peroxide into a glass slide. A wooden stick was used to transfer the isolate into the slide and mix. The oxidase test was performed by taking a cotton swab sample of the isolate and adding 1 – 2 drops of oxidase reagent into the swab. Color development was observed after 30 seconds. 2 – 3

colonies were inoculated into the surface of test tube for urease and esculin testing. The test tube was incubated for 48 hours with the cap loose. Changes in color of the medium were recorded after incubation. Agar plates for starch hydrolysis, lipid hydrolysis, and blood lysis were inoculated using isolation techniques. The agar plates were incubated until good growth was obtained. Results in color change were observed at the end of the incubation period. Other physiological characteristics examined included oxygen requirements, temperature growth range, and susceptibility/resistance to antibiotic. TSA plates were used to test the growth of the isolated bacteria when incubated at different temperatures under aerobic and anaerobic conditions. The temperatures used for testing were: room temperature (20ºC – 25ºC), 37ºC, and 55ºC. Antibiotic susceptibility was tested following the Kirby-Bauer procedure. A defined inoculum equivalent to McFarland 0.5 OD standard was streaked as a lawn onto TSA plates. The antibiotic discs and concentrations used were Ampicillin (10 μg), Cephalothin (30 μg), Ciprofloxacin (5 μg), Erythromycin (15 μg), Oxacillin (1 μg), and Gentamicin (10 μg).

6.2.7. Decolorization Assays using Isolated Bacteria

6.2.7.1. Isolate Maintenance

Isolated colonies were transferred to a nutrient solution consisting of (g/L): 1.33 KH_2PO_4, 2.67 K_2HPO_4, 1.0 NH_4Cl, 2.0 Na_2SO_4, 2.0 KNO_3, 0.05 $FeSO_4 \cdot 7H_2O$, and 0.2 $MgSO_4 \cdot 7H_2O$. The solution also contained 0.5 g/L of sterilized compost, one milliliter of a trace metal solution, and one milliliter of a vitamin solution. The composition of the trace metal solution was (g/L): 3.7 $CaCl_2 \cdot 2H_2O$, 2.5 H_3BO_3, 0.87 $MnCl_2$, 0.65 $FeCl_3$, 0.44 $ZnCl_2$, 0.29 $Na_2Mo_4 \cdot 2H_2O$, 0.01 $CoCl_2$, and 0.001 $CuCl_2$. The vitamin solution

consisted of (mg/l): 20.0 biotin, 20.0 folic acid, 100.0 pyridoxine-HCl, 50.0 thiamine-HCl, 50.0 riboflavin, 50.0 nicotinic acid, 50.0 panthoteic acid, 50.0 PABA, 50.0 cyanocobalamin, 50.0 lipoic acid, and 100.0 coenzyme M. The isolated consortium was maintained in the nutrient solution for further biodegradation experiments by doing weekly transfers of 25% of volume to 200mL of fresh nutrient solution under anaerobic conditions. The flasks were kept in a mechanical shaker at 28±2 °C and 150 rpm.

6.2.7.2. Decolorization Assays

Serum bottles were used as reactor for these experiments. They were prepared by adding 100mL of paper mill wastewater and 40mL of nutrient solution. One milliliter of bacteria (5×10^7 CFU/L) was used to inoculate the serum bottles. The bacteria were washed prior to inoculation with a saline solution containing (in g/L): 0.34 KH_2PO_4, 1.21 K_2HPO_4, and 8.0 NaCl. All the reactors were placed in a mechanical shaker at 28±2 °C and 150 rpm. Samples were for color, pH, ORP, and VSS were taken every day for a week.

6.2.8. Analytical Methods

All samples were analyzed for pH and ORP using a Thermo Orion Model 720Aplus meter (Thermo Electron Corporation). Samples were prepare for color testing following standard methods (NCASI 1999). Absorbance was measured using Hach DR/2500 Spectrophotometer (Hach Company). The spectrophotometer was operated and calibrated following manufacturer's guidelines. Volatile suspended solids (VSS) was measured following Standard Method 2540 – E (American Public Health Association et al. 1998).

6.3. Results and Discussion

6.3.1. Inhibition Test

To determine the relative contributions of the methanogenic and sulfur reducers populations to decolorization, selective inhibitors were utilized. The results are presented in Figure 6.1. Kinetic analysis of these results is presented in Figure 6.2. The slope of the lines in Figure 6.2, indicate the rate of color removal. In addition, a summary of the final percentage of decolorization as well as reaction rate constants are presented in Table 6.2.

The abiotic control reactor was treated with sodium azide to reduce anaerobic activity. There was little reduction in color (6%) over the 20 day test period, indicating that biological activity was required for decolorization. The non-inhibited control produced a significant rate and degree of color removal, achieving a 66% color reduction after 20 days. The reactor treated with calcium peroxide, which inhibits the methanogenic population, had a slightly lower rate of color production and achieved only 43 percent color reduction after 20 days. This indicates that methanogenic bacteria likely play a role in anaerobic color reduction. The molybdate treated reactor had the highest rate and degree of color removal. Approximately 84 percent of the color was removed after 20 days. This demonstrates that the sulfur reducing bacteria have a detrimental affect on color removal. Other studies (Esty 2005) indicate that high concentrations of sulfide can increase the apparent color of pulp mill wastewater. In Esty's results it was found that sulfide exposure yielded as great as 100% color increase in some cases; whereas, in other tests, the results suggested sulfide had relatively no effect on color reversion.

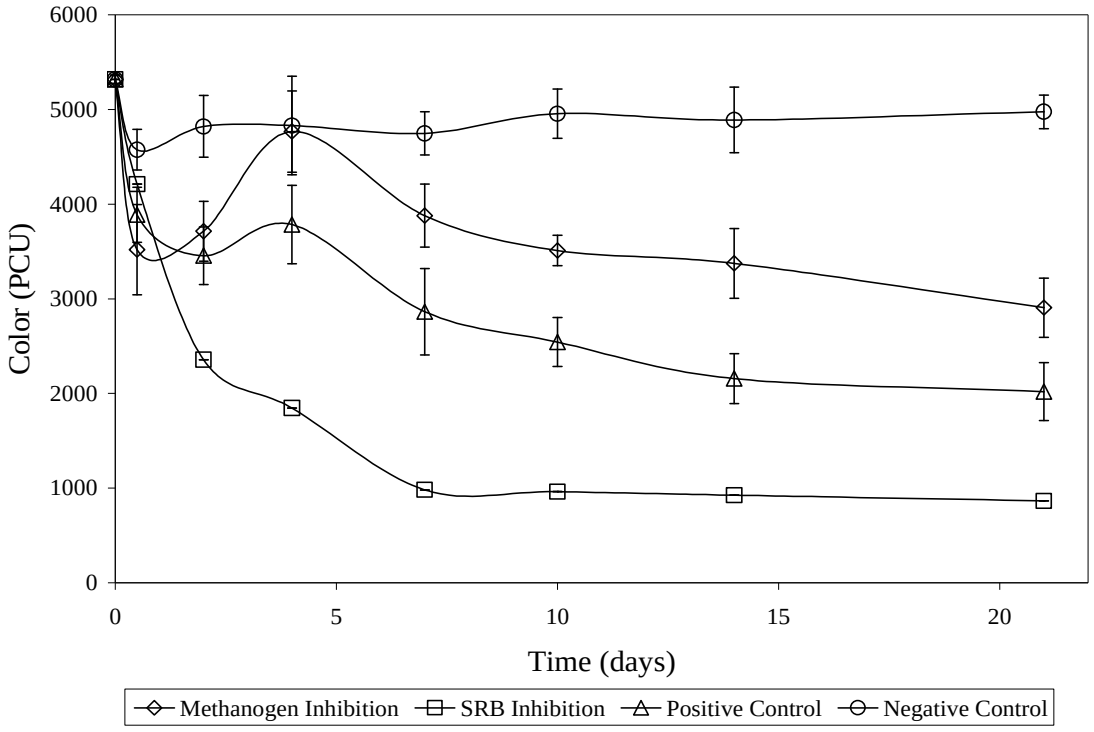


Figure 6.1 Decolorization of PMU wastewater using inhibitors for methanogens and SRB. Error bars represent ± SE (n=4).

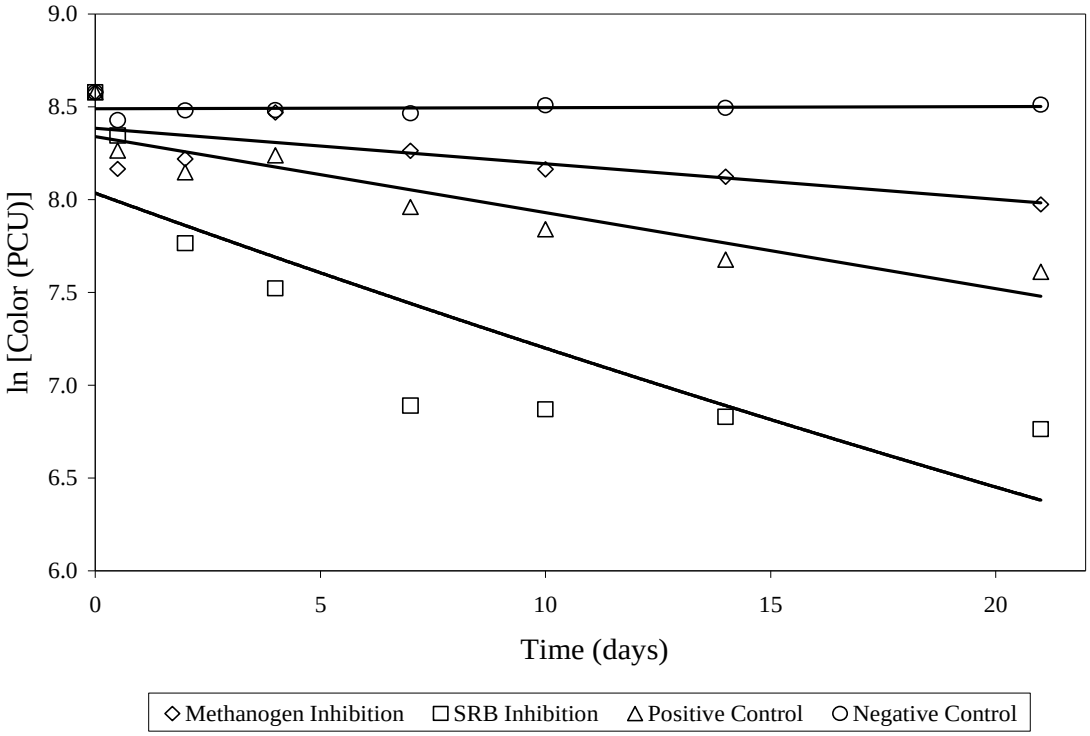


Figure 6.2 Kinetic analysis of PMU wastewater decolorization using inhibitors for methanogens and SRB. Error bars represent ± SE (n=4).

Table 6.2 Percent of decolorization and reaction rate for inhibition test

Treatment	% Decolorization	Reaction Rate Constant ($days^{-1}$)	R^2
Methanogen Inhibition	45 (±3.5)†	0.024	0.76
SRB Inhibition	84 (±6.8)	0.084	0.75
Positive Control	62 (±2.9)	0.041	0.84
Negative Control	6(±0.9)	0.001	0.035

†Values in parenthesis represent standard error (n=4).

6.4. Isolation and Identification

The inoculated anaerobic compost sample showed satisfactory growth in WCA and TSA plates. The number of colonies formed in TSA plates after three day of incubation under anaerobic conditions was approximately 2.02×10^6 ($\pm 3.5 \times 10^5$). Initial identification was done using staining techniques. Results shows that cells were Gram-negative bacillus shaped bacteria. In addition, the cultured bacteria showed no spore formation or presence of mycolic acid.

6.4.1. Phylogenetic Analysis

Table 6.3 shows the 405 base pair sequence obtained for the Unknown Isolate 1 colony PCR. The Neighbor Joining phylogenetic tree constructed using ClustalX for our isolated is presented in Figure 6.3. The results show that the closest relative to the cultured bacteria isolated from the paper mill anaerobic compost was *Aeromonas punctata*. When comparing this sequence with those available within the National Center for Biotechnology Information, it was found that our cultured unknown had a 99.6% identity with *Aeromonas sp*. and a 82.1% identity with *Aeromonas punctata.* In addition, other of the isolates cultured in our group (Unknown Isolate 2 and Unknown Isolate 3 in Figure 6.3) shows that *Aeromonas sp*. are dominant among cultured bacteria from the paper mill compost environment from which the sample was collected. The high similarity obtained based on 16S rRNA gene is an excellent starting point for finding the true identity of the cultured unknown. Biochemical tests and antibiotic sensibility present an important tool for the identification of our cultured unknown. In addition, Figure 6.3 presents other bacteria that have been documented in literature as able to degrade lignin

and lignin derivatives (El-Hanafy et al. 2007; Kuhnigk and König 1997; Maia et al. 2001). Comparison of the 16S rRNA gene for these bacteria and our isolated unknowns shows significant evolution between each other.

6.4.2. Biochemical Characteristics

Biochemical tests and antibiotic sensibility present an important tool for the identification of our cultured unknown. The results for favorable growth conditions of the unknown isolate are summarized in Table 6.4. These results are compared with the biochemical characteristics of *Aeromonas punctata* (Abbott et al. 2003; Burke et al. 1982; Gilardi 1967). Study of carbon sources reveals that similarly to *A. punctata,* the cultured unknown had the ability to grow using glucose, maltose, citrate, and indole. While no information regarding cellulose degradation by *A. punctata* was found in literature, the lack of growth indicates that the isolated unknown will not be able to metabolize cellulose present in the compost sample.

Table 6.3 DNA Sequence obtained from PCR product from colony PCR using universal bacterial primers.

1	TCGAGCGGCAGCGGGAAAGTAGCTT
26	GCTACTTTTGCCGGCGAGCGGCGGA
51	CGGGTGAGTAATGCCTGGGAAATTG
76	CCCAGTCGAGGGGGATAACAGTTGG
101	AAACGACTGCTAATACCGCATACGC
126	CCTACGGGGGAAAGCAGGGGACCTT
151	CGGGCCTTGCGCGATTGGATATGCC
176	CAGGTGGGATTAGCTAGTTGGTGAG
201	GTAATGGCTCACCAAGGCGACGATC
226	CCTAGCTGGTCTGAGAGGATGATCA
251	GCCACACTGGAACTGAGACACGGTC
276	CAGACTCCTACGGGAGGCAGCAGTG
301	GGGAATATTGCACAATGGGGGAAAC
326	CCTGATGCAGCCATGCCGCGTGTGT
351	GAAGAAGGCCTTCGGGTTGTAAAGC
376	ACTTTCAGCGAGGAGGAAAGGTCGG
401	TAGCT

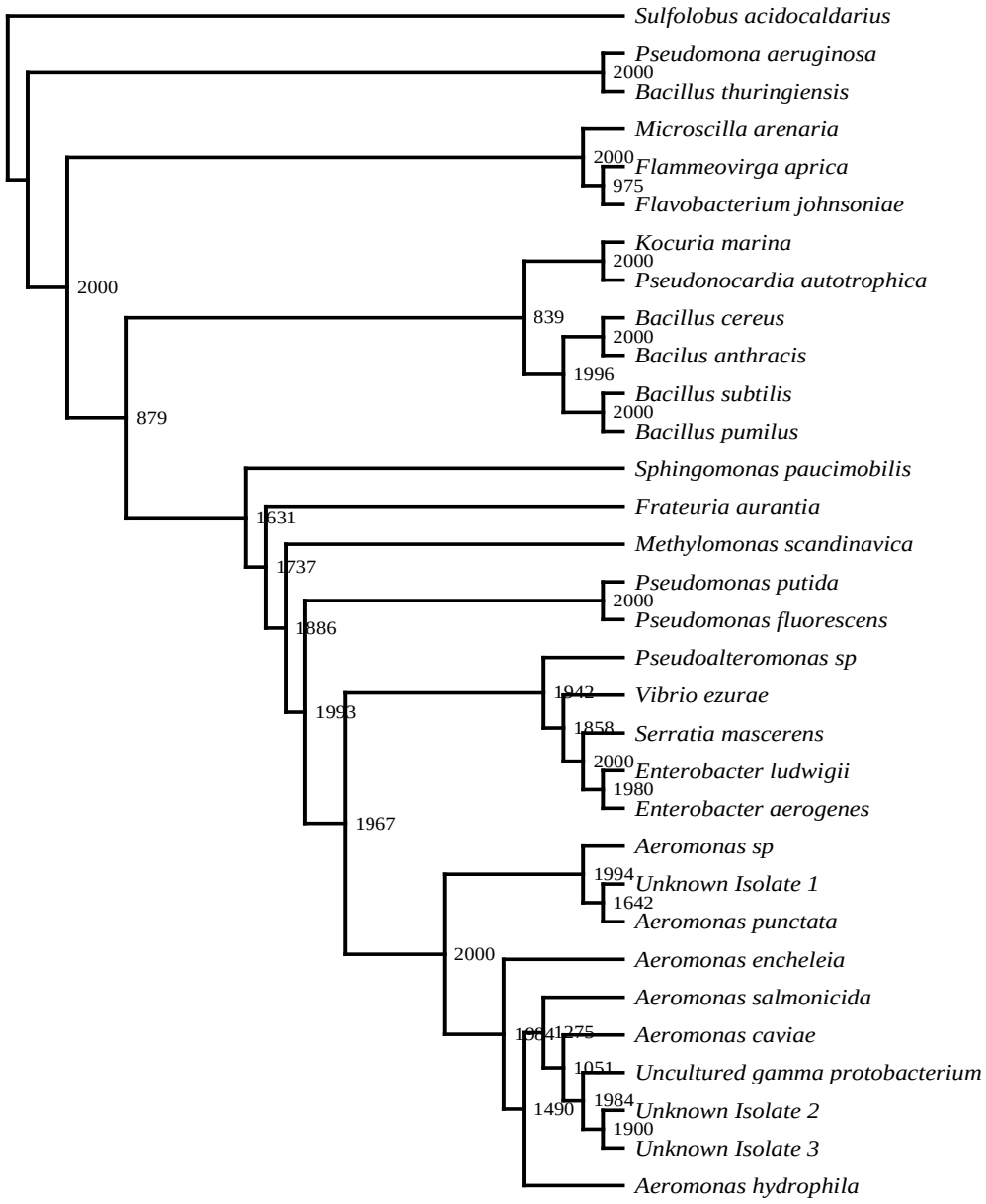


Figure 6.3 Neighbor Joining Phylogenetic Tree by ClustalX based on 16S rRNA gene.

Table 6.4 Results of stain, biochemical test, and antibiotic tests.

Test	Cultured unknown	*Aeromonas punctata*
Shape	Bacillus	Bacillus
Staining		
• Gram reaction	–	–
• Endospore-forming	–	–
• Acid fast	–	–
Carbon Source		
• Glucose	+	+
• Maltose	+	+
• Cellulose	–	**N/A**
• Citrate	+	+
• Indole	+	+
Enzymatic Testing		
• Catalase	+	+
• Oxidase	+	+
• Urease	–	–
• Esculin hydrolysis	+	+
• Starch hydrolysis	+	**N/A**
• Lipids hydrolysis	–	**N/A**
• Blood lysis	+ (β hemolysis)	+ (β hemolysis)
Aerobic Conditions @ Temperature =		
• 20 - 25ºC	+	+
• 30ºC	+	+
• 55º	–	**N/A**
Anaerobic Conditions @ Temperature =		
• 20 - 25ºC	+	+
• 30ºC	+	+
• 55º	–	**N/A**
Antibiotic Susceptibility		
• Ampicillin (AM)	**Resistant**	**N/A**
• Cephalotin (CF)	**Resistant**	**N/A**
• Ciprofloxacin (CIP)	**Susceptible**	**N/A**
• Erythromycin (ER)	**Resistant**	**Resistant**
• Oxacillin (OX)	**Resistant**	**N/A**
• Gentamicin (GM)	**Resistant**	**N/A**

+ = Positive, **–** = Negative, **N/A** = information not available

In terms of enzymatic properties, both *A. punctata* and our isolate showed similar results. Enzymatic testing was positive for all the enzymes tested except for urease and lipids hydrolysis. The study of optimal temperature and oxygen conditions indicates that both the cultured unknown and *A. punctata* are facultative microorganism that can grow in anaerobic and aerobic condition and mesophilic bacteria. Antibiotic susceptibility was only observed for growth of the unknown isolate in Ciprofloxacin. Literature shows little research regarding the susceptibility of *A. punctata* to the antibiotic tested so no clear conclusions can be made with this testing. However, similar to the culture unknown, other *Aeromonas sp.* are also resistant to the other antibiotic tested (Fosse et al. 2003; Popoff and Veron 1976).

6.4.3. Decolorization Assays using Isolated Bacteria

While the phylogenetic analysis for the identification of the cultured unknown was successful, our original quest would not be accomplished without testing the decolorizing activity of the isolate. The results for those decolorization assays using our isolated colony (*A. punctata*) are presented in Figure 6.4. In addition, this figure presents decolorization assay for another isolated colony from our compost that is yet to be identified. Kinetic analysis of the results is presented in Figure 6.5. This isolated colony (*Slime*) was characterized by the formation of extracellular polysaccharide. It can be observed in the figure that both isolated bacteria had the ability to reduce PMU color. The average final decolorization for both isolates was 55%, while only 7% color reduction was obtained for control reactors. Notice that the degree of decolorization in this experiment is lower than for the inhibition experiments. However, reactors used for this

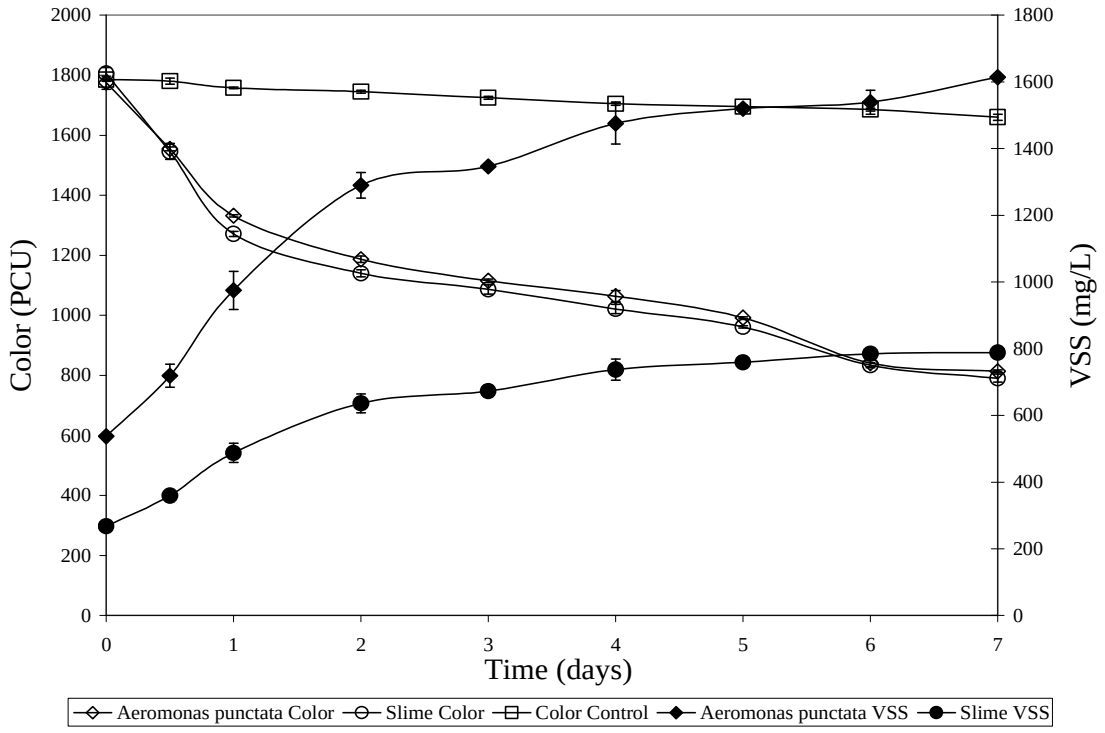


Figure 6.4 Decolorization of PMU using isolated colonies of *A. punctata* and *Slime*.

Error bars represent ± SE (n=3).

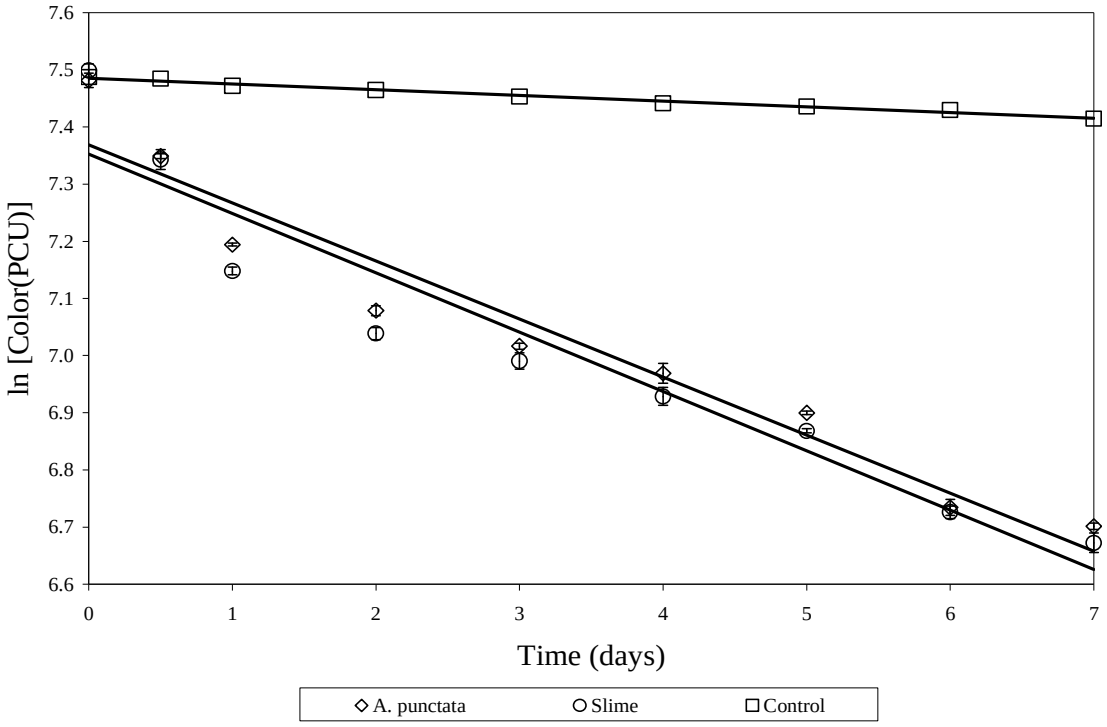


Figure 6.5 Kinetic analysis of PMU decolorization using isolated colonies of *A. punctata* and *Slime*. Error bars represent ± SE (n=3).

experiment were constructed without adding compost or any other source of substrate for bacteria. Kinetic analysis of the decolorization shows no significant difference between the reaction rates (*A. punctata* = 0.104 day^{-1}, *Slime* = 0.102 day^{-1}).

6.5. Conclusions

Interactions between anaerobic microbial communities were tested by using selective inhibitors for methanogens ands SRB. The results of these experiments show that inhibition of SRB resulted in higher biodecolorization (84%) indicating the methanogenic bacteria play an important role in anaerobic color reduction.

The isolation of anaerobic microorganism yielded an isolate that after analysis of its 16S rRNA had 99.6% identity with *Aeromonas sp*. and 82.1% identity with *Aeromonas punctata.* Biochemical tests and antibiotic sensibility experiments were performed and served to further confirm our findings.

The decolorization ability of the isolate *A. punctata* was tested along with another unknown isolated colony (Slime) from the compost. Color reduction for both isolates was 55%. While these results are lower than the obtained during the inhibition experiments, the testing conditions were different as no compost or any other substrate was added to the reactors. In addition, this shows the importance of anaerobic microbial communities interaction in reducing color.

CHAPTER 7. CHANGES IN THE SIZE DISTRIBUTION OF CHROMOPHORES IN EFFLUENTS FROM PAPER MILLS DURING ANAEROBIC DECOLORIZATION

This chapter studies the fate of chromophores in paper mill effluents through the use of size exclusion chromatography in combination with UV-VIS spectrophotometry. Effluent samples from two different sources within a paper mill before and after anaerobic biodecolorization were analyzed and the resulting size distribution of color bodies was compared.

7.1. Introduction

Color in paper and pulp mils is associated with the thermal, mechanical, and chemical conversion of wood into pulp, as well as to some of the byproducts obtained such as residual lignin and lignin derivates along with polymerized tannins (Crooks and Sikes 1990; Sarkanen et al. 1971). In addition, chromophores are created from the degradation of lignin (Jha et al. 2002; Kemeny and Banerjee 1997; Kringstad and Lindstroem 1984; Mounteer et al. 2005; Selvam et al. 2002; Tarlan et al. 2002).

During the processing of the pulp, delignification and brightening is performed in different bleaching stages. Bleaching of the pulp also contributes to the formation of colored compounds. Bleaching chemicals react with lignin and other components of the

pulp (Eriksson et al. 1985; Kringstad et al. 1985; Yin 1989) resulting in the formation of chlorinated organics that further contribute to wastewater color (Bajpai et al. 1999). such as residual lignin and lignin derivates along with polymerized tannins (Crooks and Sikes 1990; Sarkanen et al. 1971).

Falkehag et al. (1966) identified CH=CH double bonds conjugated with aromatic ring, quinomethides and quinones, chalcone structures, and metal complexes with catechol as potential contributors to color. Sarkanen and Lundwing (1971) also identified compounds with double bonds conjugated within their aromatic ring, quinone methides, and quinone groups as possible sources of color in paper mill wastewaters solution. Sjostrom (1981) partially corroborated these findings and identified other possible color sources in pulp and paper mills. In addition to the structures identified by Falkehag, Sjostrom stated that leucochromophores (colorless chromophores) could be converted to chromophores by air oxidation. Kringstrad and Lindstroem (1984) identified high molecular weight chlorinated organics as carriers of chromophoric structures. Recently, Agarwal and Atalla (2000) and Zawadzki et al. (2000a; 2000b) studied the chromophores in lignin. Similarly to results from previous studies, they found that the main contributors to color were the C=C bonds conjugated with aromatic rings and C=O bonds such as in quinones molecules.

The problem of color from paper mill wastewaters and the negative effects associated with highly colored effluents have created special interest during the last few years (Dilek and Bese 2001). Strict limitations of color levels are already in effect in Eastern European countries, Scandinavia as well as Japan and Canada (Davies and Wilson 1990). In the United States, EPA does not establish effluent limitations guidelines

and standards for color because it is a concern more appropriately addressed in individual permits based on applicable water quality standards and believes that the limits should be a case-by-case basis through individual NPDES permits or, when appropriate, through local limits (USEPA 1998; USEPA 2002).

In order to meet the discharge regulations, researchers are focused on finding efficient ways for color removal. The process of anaerobic composting offers a novel approach for the decolorization. It is typically believed that decolorization does not take place under anaerobic conditions (Feijoo et al. 1995; Kirk and Farrell 1987). However, research performed in our laboratory has shown the potential of using anaerobic biodegradation for the removal of color from paper mill effluents (Lange 2004; Lange and Mendez-Sanchez 2008; Méndez-Sánchez et al. 2007). As part of the development of this process, studies were performed in order to select operational variables that will optimize the biodecolorization of paper mill effluents as well as the identification and isolation of anaerobic microorganism driving the decolorization process. The goal in this paper is to study the fate of chromophores during the anaerobic decolorization of Pulp Mill Upset Tank (PMU) as well as E Stage Filtrate (ESF). These effluents are characterized as having dissimilar characteristics in term of color and oxidation reduction potential (ORP).

7.2. Materials and Methods

7.2.1. Wastewater Characterization

Wastewaters from two different sources in the pulp mill were studied in this experiment. Pulp Mill Upset Tank (PMU) was characterized as having color levels of

5,680 PCU, was slightly basic with pH = 10.1, and had a highly reductive potential with an average ORP of -210 mV. The E Stage Filtrate had color levels of 2,970 PCU, the pH was very basic at 11.2, slightly reductive ORP of -115 mV. Previous decolorization experiments showed that the level of decolorization for PMU and ESF was 79% and 81%, respectively (Méndez-Sánchez et al. 2007).

7.2.2. Size Distribution of Chromophores

The size distribution of color bodies in PMU and ESF, before and after biological treatment was determined by size exclusion chromatography. The samples were afterwards analyzed using UV-VIS spectrophotometry. Experiments to study the changes in size distribution were conducted using 2.54 cm water-jacketed chromatography column packed with Sephadex 100 size exclusion media. A media depth of 5 cm and a flow of 25 mL/ minute (1 bed volume/ min) were used. Dextran molecular weight markers, ranging from 1K amu to 150K amu, were purchased from Sigma Scientific and were used to determine the mass corresponding to various fractions. Color intensity of the color bodies were quantified using a Hewlett Packard Diode Array spectrophotometer at fixed wavelengths of 254, 280, 455, and 480 nm. The lower wavelengths (254 and 280 nm) are indicative of UV sorbing compounds such as aromatics, while the higher two wavelengths (455 and 480) are visible colors in the yellow-brown range (Fessenden et al. 1998).

7.3. Results and Discussion

The color for various size fractions of PMU and ESF before anaerobic treatment is presented in Figure 7.1 and Figure 7.2. It can be observed in both figures that most of

the visible color (455 nm and 480 nm) is present in the fractions with larger sizes. The sizes for the visible color ranged between 30,000 and 150,000 amu. A gel of larger size color bodies was observed in the top of the size exclusion column. These color bodies could not pass through the gel media, as it exceeded the 150K amu cut-off of the packing media. This high molecular way gel was passed through an ultrafiltration membrane with a 300K cutoff. The large fractions also contained significant UV color, indicating the presence of large amounts of aromatics and other materials possessing double bonds. In contrast, smaller molecular weight fractions contained had large amounts of color in the UV range and little color in the visible range. This distribution of color is typical for small aromatic molecules. Further analysis of the small molecular weight fractions showed the presence of methoxyphenolic monomers and dimmers of lignan, which are typical byproducts from lignin depolymerization (Albinsson et al. 1999; Dittmar and Lara 2001; Pareek et al. 2001; Raj et al. 2007).

Results for the size distribution of color bodies of biological treated PMU and ESF is presented in Figure 7.3 and 7.4. As can be observed, the distribution of compounds in the UV color range (254 and 280nm) with low molecular weight (< 30K amu) was reduced significantly. This indicates that biological activity resulted in the polymerization and/or biodegradation of smaller molecular weight chromophores such as lignin monomers. The larger size fraction had a small increase in both the visible and UV region. This may be the result of chemical changes due to biological transformation but was not confirmed. For example, the release of hemicellulose during composting can contribute to the formation of color as it absorbs light within the visible region (Beyer et al. 2006).

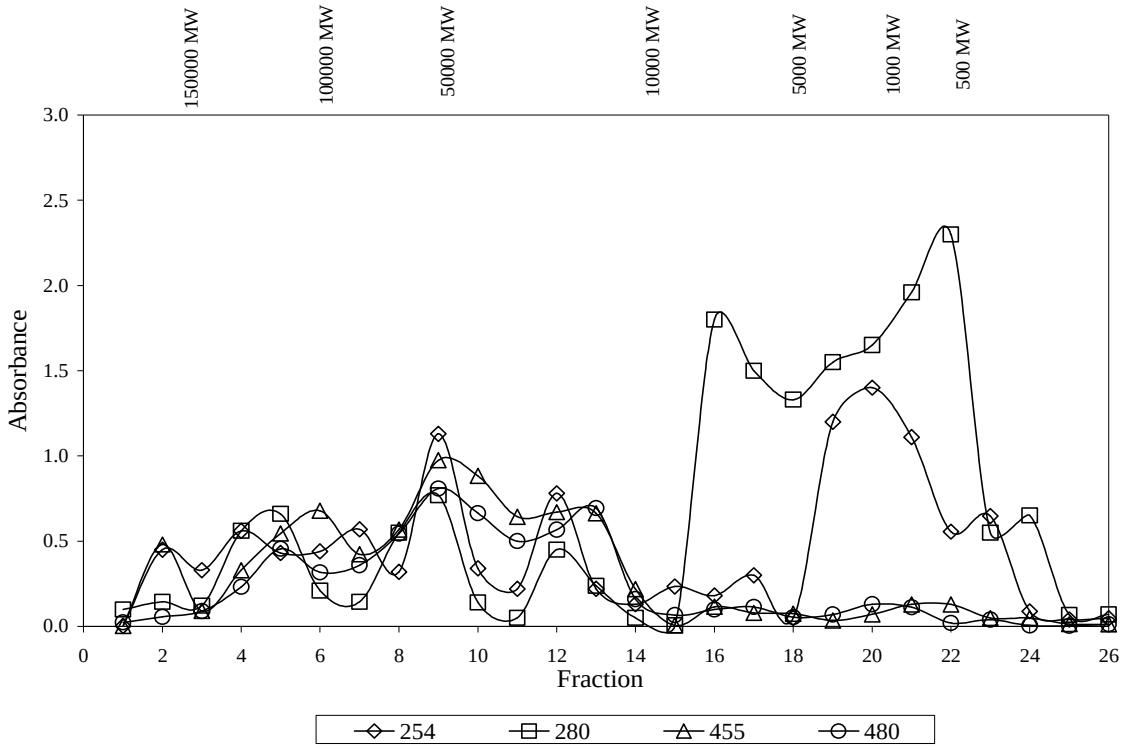


Figure 7.1 Absorbance for various size fraction of PMU before anaerobic treatment at fixed wavelengths (254, 280, 455, and 480 nm).

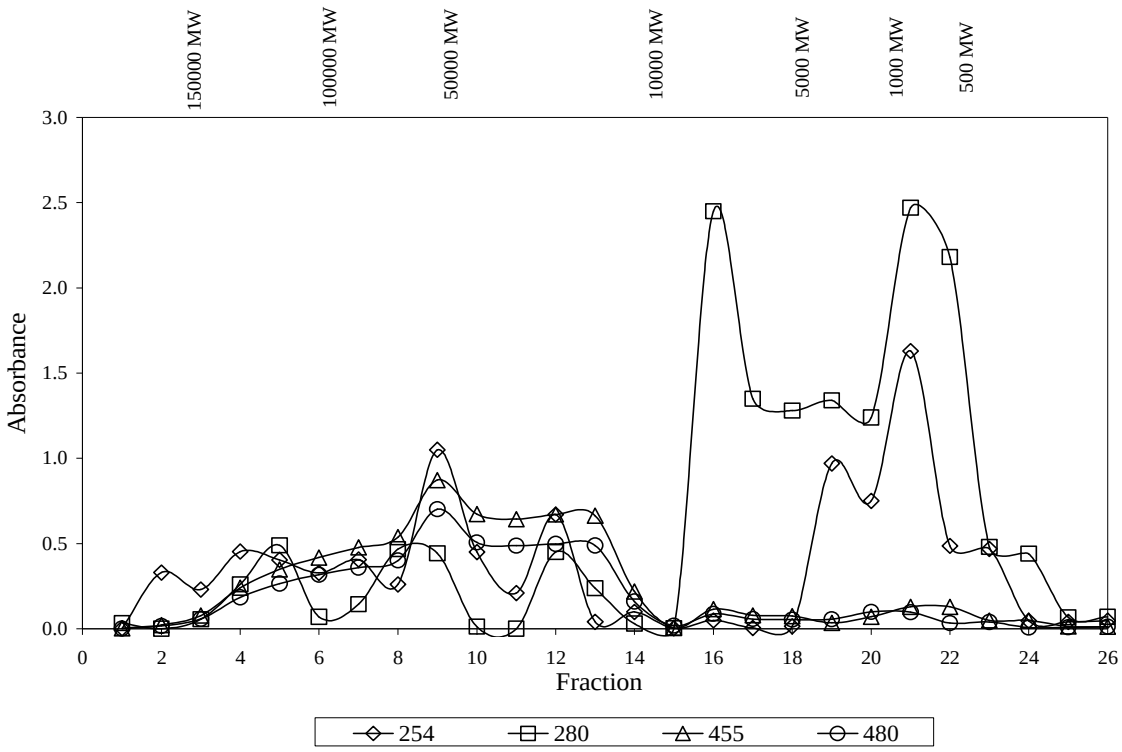


Figure 7.2 Absorbance for various size fraction of ESF before anaerobic treatment at fixed wavelengths (254, 280, 455, and 480 nm).

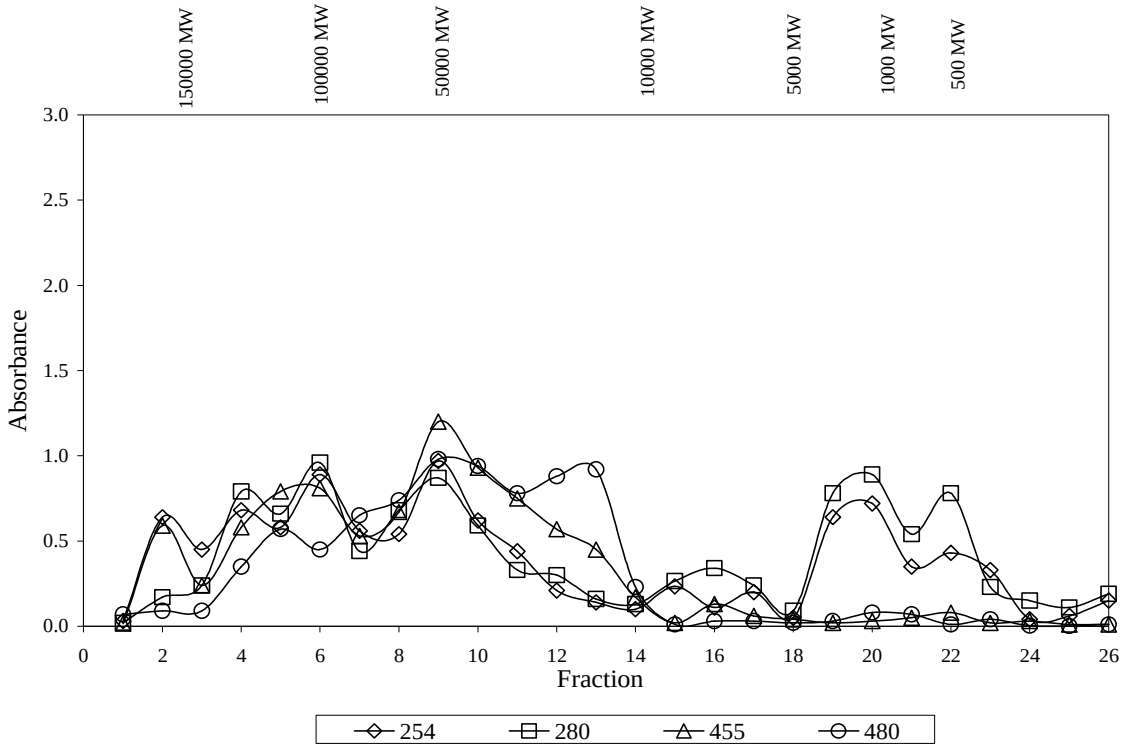


Figure 7.3 Absorbance for various size fraction of PMU after anaerobic treatment at fixed wavelengths (254, 280, 455, and 480 nm).

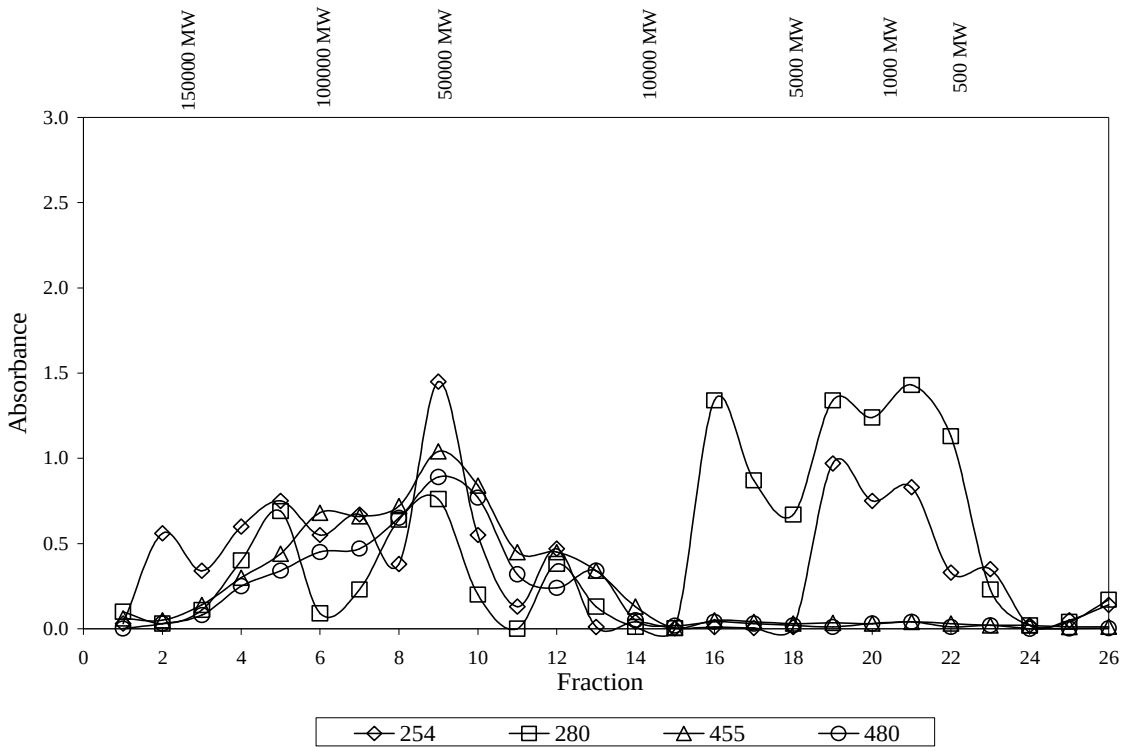


Figure 7.4 Absorbance for various size fraction of ESF after anaerobic treatment at fixed wavelengths (254, 280, 455, and 480 nm).

The observed degradation of aromatic organics is significant for the color reduction process. The degradation of both aromatic organics with C=C bonds and functional groups such as quinones and quinomethides would lead to a permanent color removal. In the case of quinones, their reduction (due to changes in ORP) to hydroquinones will result in removal of color. However, this type of oxidation-reduction reaction is reversible once exposed to an oxidant such as oxygen and color reversion will take place (Paulsson et al. 1996; Pew and Connors 1971; Zollinger 1987). Experiments performed in our lab shows that there was no significant increase of color after 48 hrs of aeration at DO levels above 2 mg/L (Méndez-Sánchez et al. 2007). This further corroborates that the observed reduction of aromatic organics was due to degradation and not just to changes in redox potential. In addition, it was observed that during aeration of two of the effluents studied (PHLA and PHLB), the levels of color was reduced by as much as 42%. These two effluents are characterized by having a high concentration of sugars such as hemicellulose. This indicates that the release of color during the anaerobic biological treatment could easily be reduced during aerobic degradation.

Figure 7.5 shows the changes size distribution of visible color bodies (460 nm) during treatment of PMU. The results show that as time increased the absorbance of chromophores with size fractions larger than 50K amu increased A similar trend was observed for small molecular weight fractions (<500 amu). This is likely due removal and accumulation of lignin monomers. In contrast, the medium sized fractions (1K – 50K) resulted in decrease of absorbance as time increased. This indicates that either selective biodegradation or polymerization occurs during treatment. In the case of biodegradation, this would explain the increase of smaller molecular fractions with time. The same can be said about the polymerization of medium size fractions regarding the increment of absorbance with time.

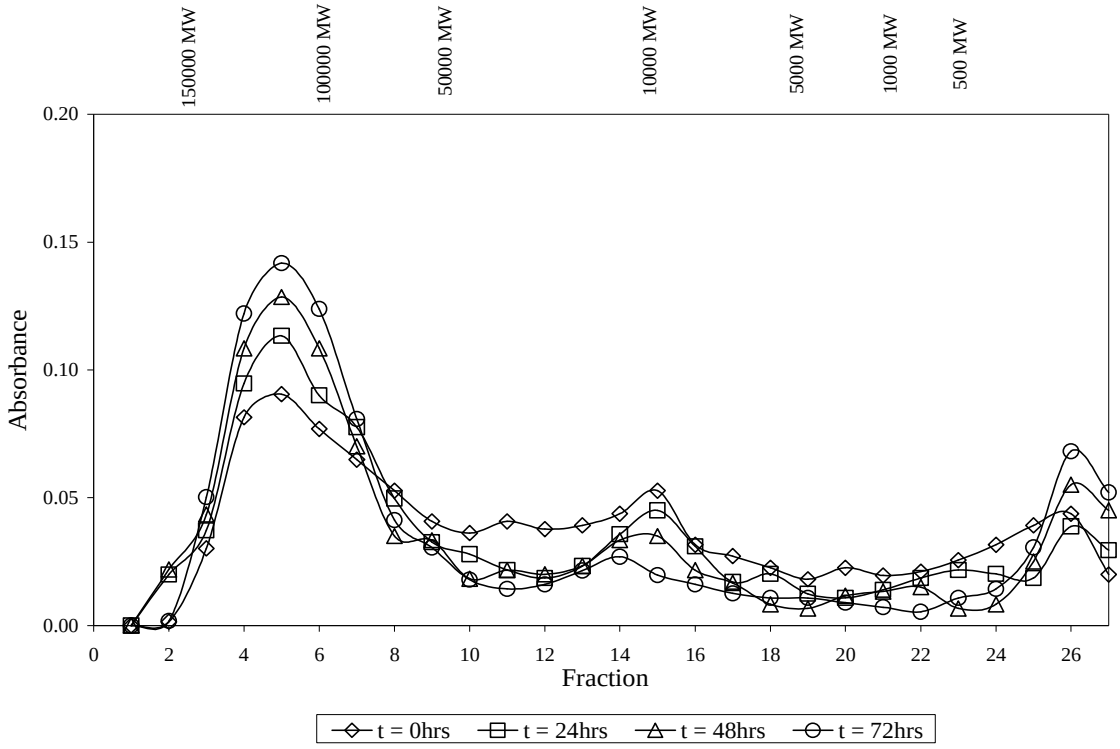


Figure 7.5 Variation of absorbance with time for various size fraction of PMU during anaerobic treatment at a fixed wavelength of 460 nm.

7.4. Conclusions

The fate of chromophores in the decolorization process shows that most of the visible color initially present in the effluent was due to molecules with sizes between 30K and 150K. Molecules with lower molecular size showed high absorbance in the UV region of the spectrum. Biological treatment of the effluents resulted in a small increase in the absorbance of molecules with high molecular sizes within the visible color region, while the absorbance for molecules within the UV region was significantly reduced. This indicates that both polymerization and degradation of chromophores took place. An example of this is presented in the release of hemicellulose which in turn increases the levels of color in the visible range. The observed degradation of aromatic organics is significant as they usually lead to permanent removal of color from the effluents. While the color reduction could be due to oxidation-reduction reactions, it was shown in previous experiments that after aeration of the sample there was no color reversion. This corroborates that the reduction of aromatic organics was due to degradation and not just to a changes in redox potential.

CHAPTER 8. SUMMARY AND CONCLUSIONS, IMPLICATIONS, AND SUGGESTION FOR FUTURE WORK

8.1. Summary and Conclusions

The development of methods to remove color is a subject currently being research by many scientists in the area of pulp and paper processing. This phenomenon is fueled by the strict color limitations imposed in discharging effluents of pulp-mill and paper-mill by environmental regulating agencies around the world.

The overall goal for this research was to determine the mechanism by which color was removed in the compost pits. Through the different tasks presented in this work, we were able to obtain valuable information about the color removal mechanism involved in this anaerobic process. It was found in this study that removal of color through adsoption played a small role. In contrast, anaerobic degradation and polymerarization were vital in the mechanism of color removal.

The innovative biodecolorization process studied in this research presents a feasible, economical, and sustainable alternative to achieve those color levels. In addition, this process offers the advantage of reusing materials that otherwise would be regarded as waste. In order to provide an efficient design, a series of interrelated tasks were performed. The results obtained are summarized below.

In Chapter 3, the wastewater and compost used in this anaerobic decolorization process were characterized. Also, adsorption and kinetic experiments were performed to determine the mechanism by which color was being removed from paper mill effluents using anaerobic composting. The experiments were performed using two different effluents, PMU and ESF. Adsorption experiments performed using active and gamma-sterilized compost showed that the adsorption capacity for both wastewaters using active compost was at least nine times higher than for those reactors using gamma-sterilizedcompost. Adsorption experiments testing the effect of pH showed that biological color removal appears to be robust with respect to pH and is largely unaffected by pH over a range from pH 5 to pH 10. The effect of aging in sorption was tested and results showed that virgin compost could establish a microbial population in less than 50 days which resulted in compost with sorption capacity similar to aged compost. Kinetic tests also showed that color degradation rates were higher when using active compost than when using gamma-sterilized compost. The final decolorization for SPE and EBF was 91% and 83%, respectively, while only 33% was the highest decolorization achieved with gamma sterilized compost.

Chapter 4 presented experiments to assess the applicability of this decolorization process to four different wastewater streams within a paper mill having dissimilar characteristics. The sustainability of color removal and the degree and rate of decolorization was tested using anaerobic sequential batch reactors. The reactors were prepared by combining anaerobic compost and effluents with dissimilar characteristics. In addition, experiments were conducted to study the color reversion and to confirm that color reduction was due to anaerobic biodegradation. Results of these experiments shows

that color reduction in these reactors varied from 75% for an effluent with initial color of 89,500 PCU to 88% for an effluent of 3,900 PCU. Reaction constants for the effluents studied varied from 0.0083 to 0.0164/hr. Aeration of the effluents showed little color reversion, which indicates that color was degraded under anaerobic conditions.

Results for the optimization of the decolorization process are presented in Chapter 5. Some of the parameters studied included the feed-to-compost ratio, mixing and agitation requirements, optimal decolorization temperature, effect of pretreatment of the effluent with polyelectrolytes, and long-term operation of biodecolorization reactors. The optimized parameters were then used to treat effluent in a pilot-scale reactor. Results showed that 1:2 feed-to-compost ratios had similar results had similar degree of biodecolorization to the 1:3 ratios. Since a 1:2 ratio will allow more treatment of effluents, this ratio was selected for the construction of reactors in the remaining experiments. In terms of mixing, there was no significant difference in terms of color reduction between the reactors that were not mixed and those that were mixed. No adverse effects on the degree of biodecolorization were found when pre-treating the wastewater with high concentrations of polyelectrolyte polymer. Long-term of the process only showed reduction in the decolorization when there were nitrogen limitations in the reactors. The pilot test was able to achieve more than 90% color reduction during the experiment, which was higher than the obtained during the bench-scale testing. The results obtained in this experiment are promising since it was demonstrated that the optimization conditions obtained in previous experiments were adequate for the scale-up of the process.

Chapter 6 presents experiments done to study the interactions between different microorganisms in the process of biodecolorization. Results showed a positive effect in decolorization by methanogenic bacteria, while sulfur reducing bacteria resulted in lower decolorization. Unknown cultures were isolated from paper mill compost used for wastewater decolorization. Growth occurred on both TSA and Wilkin-Chalgren Anaerobic agar with number of cells above 10^6 CFU/L. Analysis of the 16S rRNA gene sequence (~405 bp) for one of the isolated colonies showed 99.6% similarity with *Aeromonas species* and 82.1% similarity with *Aeromonas punctata*. These results were further confirmed with biochemical and antibiotic sensibility experiments. The final part of this chapter tested the decolorization capability of the identified isolate as well as other unknown colonies obtained from the compost. Both cases showed similar results with 55% reduction of color. While the results were lower than obtained during the inhibition experiments, the testing conditions differed as no compost or any other substrate was added to the reactors. This shows the ability of the isolates to use the chromophores as source of carbon, but also elucidates the need of interaction with other anaerobic microorganisms in order to produce higher decolorization.

The fate of chromophores in the decolorization process was assessed in Chapter 7. This was performed by studying the changes in the distribution of size of chromophores for PMU and ESF. The results show that most of the visible color initially present in the effluent was due to molecules with sizes between 30K and 150K. Molecules with lower molecular size showed high absorbance in the UV region of the spectrum. Biological treatment of the effluents resulted in a small increase in the absorbance of molecules with high molecular sizes within the visible color region, while the absorbance for molecules

within the UV region was significantly reduced. The results indicate that both polymerization and degradation of chromophores was taking place. The observed degradation of aromatic organics is significant as they usually lead to permanent removal of color from the effluents. While the color reduction could be due to oxidation-reduction reactions, it was shown in previous experiments that after aeration of the sample there was no color reversion. This corroborates that the reduction of aromatic organics was due to degradation and not just to a changes in redox potential.

8.2. Implications

This dissertation studied the process of decolorization using anaerobic composting. Results from treatability test help develop a process that is currently being used for the removal of color in a paper mill in Georgia. This decolorization scheme presents an economical method of treating effluents as it involves the use of material that otherwise would be regarded as waste. Reject fibers which are not used in the pulp making process and that then were composted serves as the main material for the removal of color from effluents. Therefore, the development of this process presents a feasible solution for reducing the amount of solid waste generated as well as treating the problem of color in the effluents. The fact that this innovative process was applied successfully in other paper mills shows a promising approach in preventing the contamination of the aquatic ecosystem receiving discharges from pulp and paper mills.

In addition, this work shows the ability of reducing color under anaerobic conditions. Anaerobic processes have been used for the pretreatment of pulp and paper effluents, but the problem of color has mostly been target using aerobic treatment. The findings of this study are remarkable as to this day literature and research has not

presented an anaerobic system that can reduce color as significantly as the results presented in this work. While it may be thought that the decolorization observed is possibly due to the change in oxidation-reduction potential of the effluents, it has been demonstrated on this work that degradation of chromophores took place as insignificant amount of color reversion occurred upon aeration of the treated wastewater. In turn, this implies that the biodecolorization process offers a way of permanently removing the wastewater color.

8.3. Suggestions for Future Work

This research demonstrates the ability of color removal for pulp and paper mills using anaerobic composting. While most of the operational parameters involved in the process have been optimized, more research is needed to control or reduce the concentration of hydrogen sulfide generated. In addition, the possibility of recovering methane should also be assessed. Results from the studies focused on studying the interactions between anaerobic microorganisms in the compost showed that sulfidogenic bacteria have a detrimental affect on color removal while methanogens played an important role in decolorization. Taking this in consideration, the possibility of completely inhibiting SRB or creating environments within the process where methanogens are favored should be study.

Additional research is also suggested in order to study the possibility of using this decolorization process for the treatment of effluents from industries processing sugar, wine, and olives. As with the pulp and paper mill processing, these industries also face similar color problems in their effluents. In addition, there is significant resemblance

between the chromophores found in the effluents of these three industries and those in pulp and paper mill (Ipek et al. 2001; Jaouani et al. 2006; Paraskeva and Diamadopoulos 2006; Vrhovsek et al. 2002). The use of this anaerobic decolorization process could present an alternative for the reduction of color in those industries.

In addition to using anaerobic composting for the reduction of color, the applicability this process for the remediation of other contamination problems should be assessed as well. Generally speaking, anaerobic composting has high remediation potential in areas where strong reducing conditions are required (Robinson 2003). An example of this includes the feasibility of using anaerobic composting for the biodegradation of chlorinated compounds and the reduction of selenate and selenite in petroleum contaminated soils.

Chlorinated solvents are one of the most common classes of contaminants in the world. Aerobic processes have been effective in the biodegradation of TCE; however, PCE is highly persistent in aquifers under aerobic conditions. Other chlorinated compounds such as 2,4-dichlorophenol, 2,6-dichlorophenol, and pentachlorophenol are characterized as being toxic and recalcitrant to degradation. In addition, they are been listed by the US EPA as priority pollutants (Sittig 1991). Most of the literature regarding PCE biodegradation reveal that bioremediation of PCE takes place under anaerobic conditions where partial or complete microbial dechlorination has been reported (Fennell et al. 1997; Hopkins et al. 1993). Literature research for the chlorophenols and the other chlorinated compounds shows that anaerobic degradation of these contaminants can also follow reductive dechlorination (Haggblom et al. 1988; Juteau et al. 1995; Sun et al.

2000; Woods et al. 1989). Conditions found in the anaerobic composting process are very favorable for the type of reactions involved in reductive dechlorination.

While free selenium is nontoxic (Hettiarachchi and Gupta 2008; Kopsell and Kopsell 2007), many of its compounds are extremely toxic having modes of action similar to arsenic. Contamination of water bodies with Se usually arises from industrial discharges in the areas of coal, metal mining, and petroleum refining (Hamilton 2004; Lawson and Macy 1995). The biological reduction of selenium can be directly related to the reduction of sulfur-containing compounds. In the periodic table, selenium is located directly below sulfur. As a consequence, many of selenium's properties are analogous to sulfur. An example of this can be observed in the similarities found between the biogeochemical cycle of sulfur and selenium. Just like sulfur reducing bacteria, a number of microorganisms have been identified as capable of reducing selenium oxyanions through respiratory reduction processes (Stolz and Oremland 1999). Due to the strong reductive conditions in our process, it is very possible that the remediation of selenate and selenite from oil refinery wastewater could be achieved using this anaerobic composting process.

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