EVALUATION OF BIOLOGICAL TREATMENT FOR THE DEGRADATION OF PETROLEUM HYDROCARBONS IN A WASTEWATER TREATMENT PLANT

A Thesis

by

PRADIPTA RANJAN BASU

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2005

Major Subject: Civil Engineering

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ABSTRACT

Evaluation of Biological Treatment for the Degradation of Petroleum Hydrocarbons in a Wastewater Treatment Plant. (May 2005)

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Chair of Advisory Committee: Dr. Robin Autenrieth

Biodegradation of petroleum hydrocarbon can be an effective treatment method applied to control oil pollution in both fresh water and marine environments. Hydrocarbon degraders, both indigenous and exogenous, are responsible for utilizing petroleum hydrocarbon as their substrate for growth and energy, thereby degrading them. Biodegradation of hydrocarbons is often enhanced by bioaugmentation and biostimulation depending on the contaminated environment and the competence of the hydrocarbon degraders present. An evaluation of the performance of the biological treatment of petroleum hydrocarbon by the hydrocarbon degrading microbes at the Brayton Fire School's 4 million gallon per day (MGD) wastewater treatment plant was the main research objective. Samples were taken for two seasons, winter (Nov 03 – Jan 03) and summer (Jun 04 – Aug 04), from each of the four treatment units: the inlet tank, equalization tank, aeration tank and the outfall tank. The population of aliphatic hydrocarbon degraders were enumerated and nutrient availability in the system were used to evaluate the effectiveness of on-going bioaugmentation and biostimulation. Monitoring of general effluent parameters was conducted to evaluate the treatment plant's removal efficiency and to determine if effluent discharge was in compliance with the TCEQ permit. The aeration tank is an activated sludge system with no recycling. Hydrocarbon degraders are supplied at a constant rate with additional nutrient supplement. There was a significant decrease in the population of microbes that was originally fed to the system and the quantity resident in the aeration tank. Nutrient levels in the aeration tank were insufficient for the concentration of hydrocarbon degraders, even after the application of dog food as a biostimulant. The use of dog food is not recommended as a nutrient supplement. Adding dog food increases the nitrogen and phosphorus concentration in the aeration tank but the amount of carbon being added with the dog food increases the total chemical oxygen demand (COD) and biochemical oxygen demand (BOD). An increase in the concentration of total COD and BOD further increases the nitrogen and phosphorus requirement in the system. The main objective of supplying adequate nutrients to the hydrocarbon degraders would never be achieved as there would be an additional demand of nutrients to degrade the added carbon source. This research study was conducted to identify the drawbacks in the treatment plant which needs further investigation to improve efficiency.

DEDICATION

To my parents, Mr. Pradip Ranjan Basu and Mrs. Nandita Basu, for their constant support and encouragement through the good time and the bad.

To my friend, Sharmili Datta, for being there for me in my difficult times.

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INTRODUCTION

Pollution on the broader scale is contamination of soil, water, or the atmosphere by the discharge of harmful substances or waste. Industrialization in the 20th century resulted in the production of wastes, some of which are hazardous to the environment and human health. These wastes originate from a variety of sources such as municipal waste discharges, industrial effluents, uncontrolled releases from power plants and refineries, spillages, and runoff from terrestrial sources (Swanell, 1999). Petroleum hydrocarbons and crude oil are significant sources of organic pollutants released. Approximately about 900 millions of gallons of crude oil impact marine environments annually (NRC, 1985). Large spills have resulted from the transport of petroleum products, yet this input represents only 10% of the total input of petroleum to the environment (NRC, 1985). The impacts of these oil spills on marine environment and coastal environments can be devastating. Coastal wetlands and beaches are usually the areas affected by these spills and the damage can be both ecological and economical. Crude oil removal can be done through physical, chemical and biological means. Physical clean-up methods in the wetlands and beaches involve heavy equipment and large work crews and are time consuming. They can also be more destructive than the oil itself. Chemical cleanup methods include the application of dispersants or incineration but the later is not preferred due to other environmental implications.

The style and format of this thesis follows that of Waste Management Journal.

Biological processes or biodegradation is considered the primary natural process for removal of petroleum from the environment (Prince, 1993). Biodegradation of petroleum hydrocarbon has proved to be one of the most effective means to remediate hydrocarbon pollution in both marine and fresh water environments (Atlas, 1995 b; Hinchee *et al.* 1995; Ron and Rosenberg, 2002; Mills *et al.* 2003).

Petroleum hydrocarbons can be categorized into four broad groups: the saturates, the aromatics, the asphaltenes and the resins (Leahy and Colwell, 1990). Susceptibility of the hydrocarbon to biodegradation varies with the molecular structure of the hydrocarbons. Straight-chained alkanes are the most readily degradable followed by branched alkanes, low-molecular-weight aromatics, and cyclic alkanes, with polynuclear aromatics being the least degradable (Atlas, 1984). Various microorganisms, including yeast, fungi, and bacteria, utilize the petroleum hydrocarbon as their carbon source and energy along with other nutrients like nitrogen and phosphorus. Under normal conditions hydrocarbon degraders represent about 1% of the total microbial population, but hydrocarbon influx causes the population to grow to about 10% (Atlas, 1984, 1995 a). Indigenous hydrocarbon-utilizing bacteria were successfully tested in the bioremediation of the *Exxon Valdez* spill with the application of fertilizers (Atlas, 1995 b). Bioaugmentation is done depending on the complexity of the composition of the hydrocarbons to be treated and the suitability of the microorganism to that particular contaminated ecosystem. There are many strains of hydrocarbon degraders available such as Pseudomonas, Acinetobacter, Bacilli, Flavobactria among others (Ron and Rosenberg, 2002). These strains of bacteria can adapt to most natural or artificial environments provided there is adequate supply of substrate, nutrients and oxygen for their metabolism process. Maintaining a healthy population of these hydrocarbon degraders is the key factor for successful biodegradation or bioremediation.

Regulating bodies and agencies (EPA, TCEQ etc.) established in the US monitor the discharges of pollutants to the natural environmental. Incentives were put forward in developing treatment and remediation methods to treat and maintain the statutory discharge parameters. One of the ways to control the pollution of the natural environment from municipal and industrial sources is the construction of water and wastewater treatment plants. Water treatment plants are designed with the protection of human health as its prime objective as they monitored the quality of drinking water. On the other hand, wastewater treatment plants are used in treating wastewater generated from various industrial applications ranging from municipal waste to highly inorganic industrial waste containing heavy metals and other hazardous chemicals before being discharged to natural ecosystems. It is required to provide these services developing a residential community and industrial zones.

The Brayton Fire Training School's wastewater treatment plant is a biological treatment plant where petroleum hydrocarbon is the primary waste generated from a firefighting training field. Biological treatment performance of the plant was monitored for two different seasons. Hydrocarbon degrading microorganisms were identified. Adaptation of the bacteria in this artificial environment, their substrate utilization, rates of nutrient requirements and other limiting factors were investigated.

SITE DESCRIPTION

HISTORY AND LOCATION

The research site chosen for this study is the wastewater treatment plant at Brayton Fire Training Field in College Station, Texas. The Brayton Fire Training School comes under the Emergency Services Training Institute (ESTI) which is one of the many divisions run by Texas Engineering Extension Service (TEEX). Texas Engineering Extension Service or TEEX is a member of the Texas A&M University (TAMU) system. In 1929, the State Firemen's and Fire Marshals' Association of Texas chose TAMU to start a training program for volunteer firefighters. In 1931 Texas Legislature gave TAMU responsibility to create and operate Fire Training School officially in order to provide training for career and volunteer firefighters and fire marshals. ESTI currently trains about 46,000 emergency personnel each year all year from all 50 states in the country and 30 other countries. Classes are held at the world-renowned, 120-acre Brayton Fire Training Field (Figure 1) for municipal, industrial and marine firefighters and also for rescue, hazardous materials and nuclear/chemical/biological terrorism courses.

The wastewater treatment plant forms an important part of the training field. Field operations produce a large volume of petroleum hydrocarbon-water mixture waste, which must be treated efficiently to meet discharge permit requirements. The hydrocarbon waste generated from the treatment plant consists of C9 to C13 aliphatic hydrocarbons. The treatment plant is a small-scale facility and proved to be an ideal site to evaluate the biological degradation of petroleum hydrocarbons. The effect of seasonal climatic variations, substrate fluctuations, and nutrient levels in the system, aeration requirements and other intrinsic variations pertinent to this particular plant were also evaluated.

Figure 1. Aerial View of the Brayton Fire School (Brayton Fire Training Field, 2004)

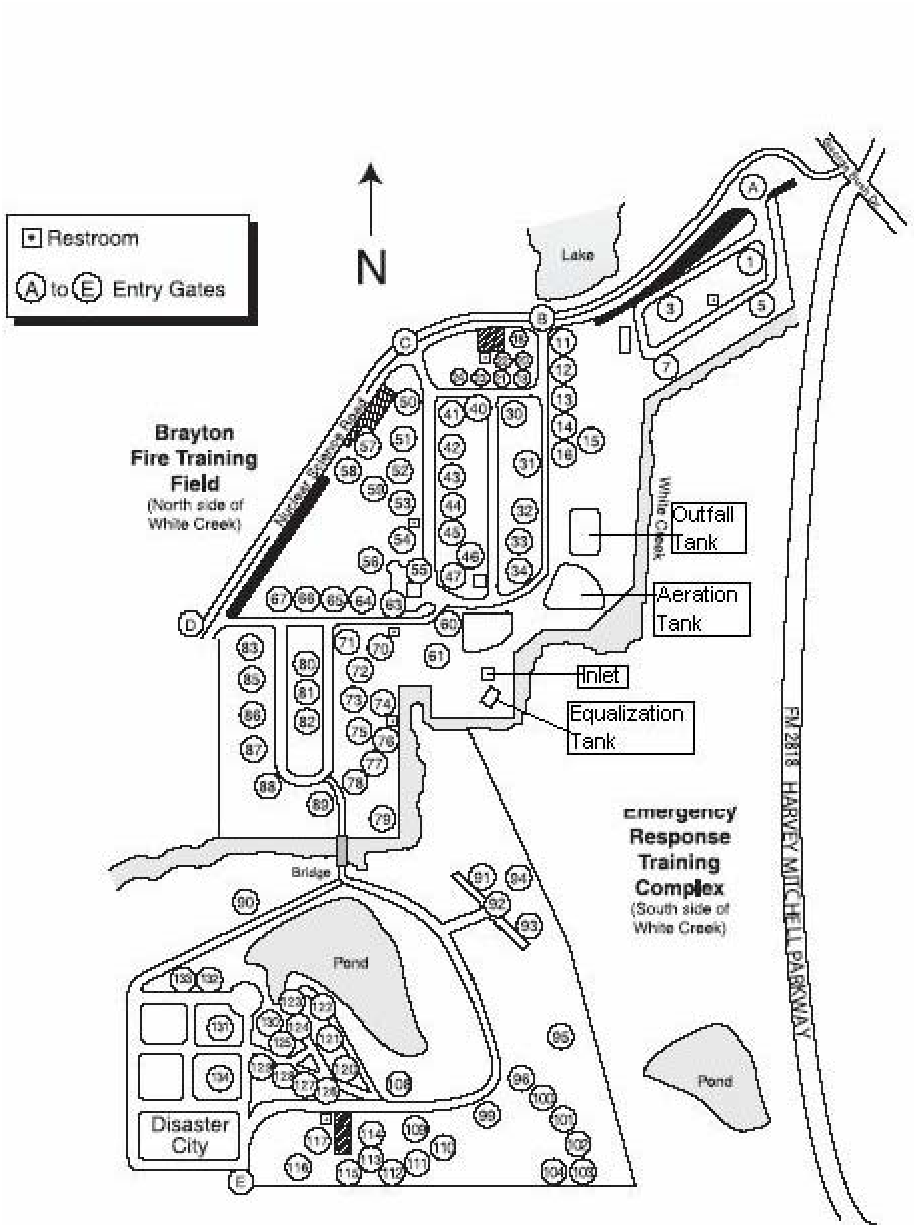

Figure 2. Layout of the Fire Training Field (Map of Brayton Fire Training Field and Disaster City, 2004)

TREATMENT PLANT UNITS

The wastewater treatment plant consists of four basic units, namely: 1) Inlet Tank 2) Equalization Basin 3) Aeration Tank 4) Outfall Tank. The layout of the plant is illustrated in Figure 2.

1) Inlet Tank

The influent of the system comes from the 105-acre firefighting field, which suffers from the periodic influx of petroleum hydrocarbon wastes generated during the day when the firefighting training operation is at its peak. The waste enters the inlet tank, which has a dimension of 10 ft in length, 8 ft 8 inches in width and a depth of 6 ft. The inlet tank (Figure 3) has a volume of about 3890 gallons, which is periodically discharged to the equalization tank. An iron mesh covers the top of the inlet tank, which can be removed for periodic samples and cleaning of the tank.

Figure 3. View of Inlet Tank

2) Equalization Tank

The equalization tank is one of the most important units as it is used to regulate and equalize the fluctuation of the wastewater flow to the system so that the biological degradation unit or the aeration tank does not suffer fluctuating loads of petroleum hydrocarbon in the system. The concrete equalization tank receives wastewater pumped from the inlet tank by a single pump (Figure 4). The primary treatment includes a grit removal chamber (Figure 5) and an oil-water separator (Figure 7) which is preceded by a small skimmer (Figure 6) in the tank. The equalization tank has two different stages of mixing to equalize the flow and the concentration of the wastewater. Before the wastewater goes to the aeration tank for biological treatment, hydrocarbon-degrading bacteria are as the wastewater leaves the equalization tank.

Figure 4. View of the pump

Figure 5. View of the Grit Removal Chamber

Figure 6. View of the Skimmer and Mechanical Mixers

Figure 7. View of Oil -Water Separator

3) Aeration Tank

Biological degradation of petroleum hydrocarbons takes place in the aeration tank (Figure 8) which acts as an activated sludge process without recycle. The aeration tank is essentially a continuous flow completely mixed reactor with three mechanical aerators that provide the oxygen required for the microorganisms. The high-speed mechanical aerators serve the two-fold purpose of supplying adequate oxygen requirements to maintain a healthy population of microbes, as well as maintaining the suspended growth of the microbes. This forms the secondary treatment of the wastewater. The sludge is periodically removed from the tank by mechanical means of scrapping the bottom of the tank. The aeration tank is simply a dugout pond without any protective lining except aggregates. The volume of the tank was calculated by previous studies to be 871,000 gallons (Kramer *et al.*, 2002) and the deepest point in the aeration tanks is 10 feet, near the center where the aerators are placed. The 15 hp aerators in the system are floating units. Seeding of exogenous hydrocarbon degraders is done in the aeration tank on a regular basis along with additional nutrient supplements and algal control. Commercially available dog food called "Clover Brand, Hunter's Special®" from Farmland Industries Inc., Kansas, Missouri was the nutrient supplement provided to microbes. Copper sulfate pentahydrate 99% from ChemOne Ltd. Houston, Texas was used to control the growth of algae. Algal control was necessary to prevent their uptake of the nutrients that are essential for the viability of the petroleum hydrocarbon degraders.

Figure 8. View of the Aeration Tank

Outfall Tank

Tertiary treatment is achieved in the outfall tank (Figure 9) where the wastewater is mixed with well water. The pump station at the side of the tank recycles a portion of water back to the fire-training field. The remaining treated water is discharged to the Brazos River at a daily average of 2.0 million gallons per day with daily maximum flow of effluent limited at 2.4 MGD.

Figure 9. View of Outfall Tank

HYDROCARBON DEGRADERS

The microbial population introduced for hydrocarbon degradation in the treatment plant is a commercially available product called “Micro-Blaze Liquid Spill Control ®” from Verde Environmental (Houston, Texas). Micro-Blaze contains several strains of non-pathogenic, spore forming, facultative bacteria, *Bacillus,* along with a surfactant and nutrients sufficient for biodegradation. The physical characteristics listed for the product (Micro Blaze Spill Control, 2004) are summarized below:

- White, opaque perfumed liquid formulation
- Can handle pH a range from 4.5 to 11.5
- Completely soluble in water
- Bacteria Count approximately 200 billion/gallon
- No mutation – microbial population return to a level consistent with the amount of food and water available for dying off or returning to spore state.
- Type of contaminants on which Micro-Blaze works:-
    - Benzene
    - Petroleum products such as gasoline, diesel, motor oils
    - Aviation fuels such as: JP-8, JP-5, av-gas
    - Glycols (antifreeze compounds)
    - MTBE
    - PAHs, TCE
    - Methanol
    - Toluene, acetone and paint sludge

    - Polyurethane resins wastes
    - Condensate from pipelines
    - Organics such as greases, fats
    - Restaurant greases, oily residues, cellulose waste
    - AFFF wastes
- The microbe solution (3%solution) volume should be calculated as 10% of the total contaminated volume either water or soil.

These hydrocarbon degraders are aerobic heterotrophs which take up hydrocarbons as their carbon source and for use as electron donors, while oxygen molecules are the electron acceptors.

NUTRIENT SUPPLEMENT

Commercially available dog food is used for the nutrient supplement for the exogenous hydrocarbon degrader was from Farmland Industries Inc, Kansas Missouri. Verde Environmental recommended the use of dog food as nutrient supplement for their "Micro-Blaze Liquid Spill Control ®" microbe solution. Three bags of dog food of 40 lbs were put in the aeration tank each week for nutrient supplement. The ingredients of the "Clover Brand Hunters Special" dog food listed on the label are as follows:

Crude Protein not less than –22%
Crude Fat – 8%
Fiber – 5%
Moisture – 12%
Carbohydrates – 50% approximately

This brand of dog food complies with the Association of American Feed Control Officials (AAFCO) dog food nutrient profile. Few required constituents for minimum requirement of maintenance of an adult dog in the current AAFCO dog food nutrient profile (Dietary Requirements in Dogs, 2004) is as follows:

Protein – 18%
Fat – 5%
Phosphorus- 0.5 %(maximum 1.6%)
Calcium – 0.6% (maximum 2.5%)

LITERATURE REVIEW

Microbial biodegradation of petroleum hydrocarbons has been investigated from as early as the 1950's and 1960's. The ability of microorganism to use hydrocarbons as a food source has evolved into their use for the biodegradation of petroleum hydrocarbons (Champagnat, 1964; Champagnat and Llewelyn, 1962). Discovery of metabolic pathways for alkane, cycloalkane and aromatic hydrocarbon utilization set the foundation for bioremediation of petroleum from anthropogenic sources (Atlas and Bartha, 1992). Biodegradation of hydrocarbons by natural microbial populations has been the main means of eliminating hydrocarbon pollutants from the environment (NRC, 1985). The effect of environmental conditions on biodegradation, the elucidation of microbial pathways, and improving the rates of biodegradation are the main topics reviewed.

Biodegradation is basically the microbiological breakdown of petroleum to compounds of lower molecular weight or to more polar compounds (Atlas, 1977). Petroleum hydrocarbons can consist of straight, branched, cyclic alkanes or alkenes to aromatics or could be a complex mixture of all of the above in definitive proportions (Ron and Rosenberg, 2000). The effectiveness of biodegradation is directly related to the chemical structure of the hydrocarbons. Due to structural diversity of the hydrocarbons, some are readily degradable by microorganisms while some are not.

According to Atlas (1984) the basic rules of alkane degradation are defined as:

1) Aliphatic hydrocarbons are assimilated by many microorganisms whereas aromatics, though they are assimilated less efficiently, are oxidized.

3) Long chain n-alkanes are assimilated more easily than short chains.
4) Saturated aliphatic hydrocarbons are more easily degraded that unsaturated ones.
5) Branched-chain compounds are degraded slower than straight-chained ones.

Saturated and low-molecular-weight aromatic fractions of oil are more easily biodegradable than high-molecular-weight aromatic fractions (Atlas and Bartha, 1992). Biodegradations of n-alkanes with molecular weights up to n-C44 have been reported (Haines and Alexander, 1974). Double bonds and branching makes a hydrocarbon more resistant to degradation. N-alkanes from the *Amoco Cadiz* spill degraded about twice as fast as branched alkanes (Atlas *et al.*, 1981). The rate of aromatic hydrocarbons degradation decreases with the increase of condensed aromatic rings. Polynuclear aromatic hydrocarbons (PAH) are more difficult to biodegrade than one- and two-ring aromatics. PAHs are lipophilic chemicals that are formed as by-products of fossil fuel combustion and residues of coal processing. Due to their persistence in the environment and because of their toxic, mutagenic and carcinogenic properties, much research has been performed on biodegradation of PAHs. PAHs with four and five fused benzene rings are more resistant to biodegradation than PAHs with two or three rings. Metabolic pathways of PAH degradation by fungi, algae and bacteria has also been elucidated. The resistance of biodegradation of these high molecular weight PAHs is due to the lack of microorganism that can degrade these highly lipophilic, insoluble compounds.

Metabolism of aliphatic hydrocarbons has been clearly explained in many reviews about microbial biodegradation. Microbial degradation is accomplished by various microorganisms like bacteria, fungi and yeast. There are many enlisted bacteria and yeast which grow on a variety of n-alkanes. Table 1 lists the genera of bacteria and yeast, which act as alkane degraders, of which there are also about 160 listed genera of filamentous bacteria. In natural environments, hydrocarbon degraders constitute less than 0.1% of the microbial community, whereas in oil-polluted environments, the population can constitute up to 100% (Atlas, 1981). The ecosystems can be aquatic (freshwater or saltwater/marine) or soil or sediments. The aquatic ecosystem is one of the largest natural environments consisting of approximately 76% of earth's surface. Considering the great size of the ocean and the diversity of flora and fauna, the microbial population is quiet significant. Total bacterial population can vary by several orders of magnitude from place to place; coastal waters may typically house 10^{11} to 10^{12} organisms/m^3. The sea is not the ideal medium for microbial growth due to lack of utilizable nutrients. The concentration of organic and inorganic nitrogen is typically low due to competition between microbial species. On beaches, oxygen serves as the limiting nutrient instead of nitrogen, but the oxidation/reduction potential of the beach depends on geographical and meteorological conditions. High energy beaches with intense wave action have the highest oxygen concentrations.

Biodegradation is essentially the catalytic reduction of organic compounds by microorganisms. Initial degradation is an oxidative process catalyzed by oxygenases and peroxidases which include activation and incorporation of oxygen in the substrate

Table 1.

Genera of Bacteria and Yeasts that Contain Aliphatic Hydrocarbon –Oxidizing Species. (Britton L.N.,1984)

BACTERIA	YEAST
Achromobacter	candida
acinetobacter	cryptococcus
actinomyces	debaryomyces
aeromonas	endomyces
alcaligenes	hansenula
arthrobacter	mycotorula
Bacillus	pichia
beneckea	rhodotorula
brevibacterium	saccharomyces
corynebacterium	selenotila
flavobacterium	sporidiobolus
methylobacter	sporobolomyces
methylobacterium	torulopsis
methylococcus	trichosporon
methylocystis	
methylomonas	
methylosinus	
Micromonospora	
mycobacterium	
nocardia	
pseudomonas	
spirillum	
vibrio	

(Ron and Rosenberg, 2002). Peripheral degradation pathways convert hydrocarbons into intermediates of the central intermediary metabolism, e.g. tricarboxylic acid cycle. Biosynthesis of cell biomass results from the central precursor metabolites e.g., acetyl-CoA. The general pathway of alkane metabolism follows the pattern: alkane → alcohol → aldehyde → fatty acid → acetate via ß oxidation (Atlas, 1984).

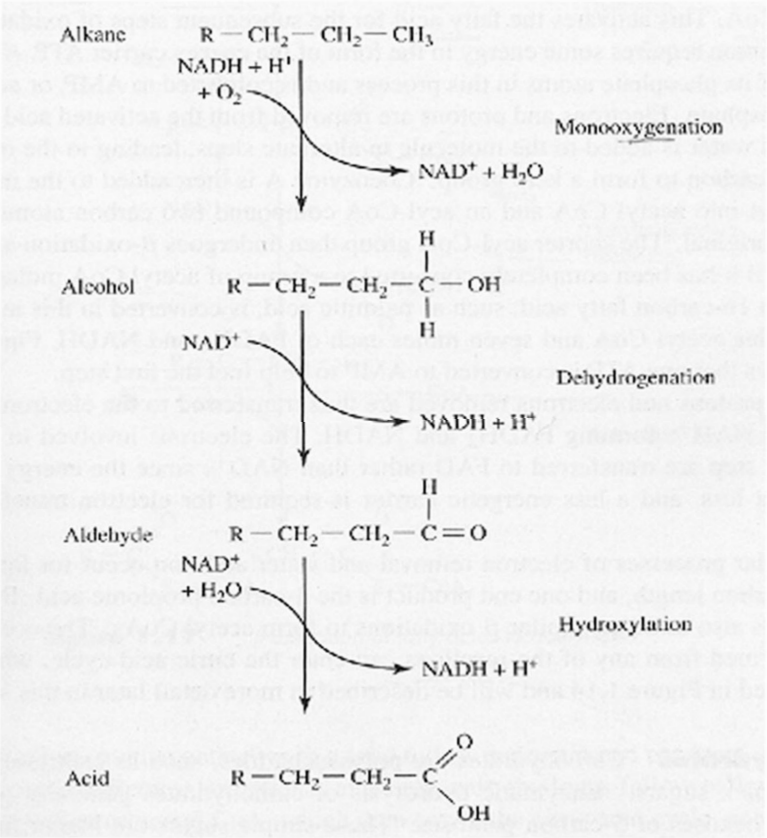

Figure 10. Steps in the Oxidation of an Alkane

(Rittmann B. and McCarty P. (2001).Environmental Biotechnology: Principles and Applications. McGraw-Hill)

Figure 10 represents the general steps of a hydrocarbon oxidation where R represents the alkyl hydrocarbon chain of undefined length. Oxidation involved removal of two atoms of hydrogen and electrons and transfer to electron carrier NADH. Conversation of alkane to an alcohol is carried out by the direct addition of molecular oxygen with the help of oxygenanse enzyme and NADH as cosubstrate. NADH is

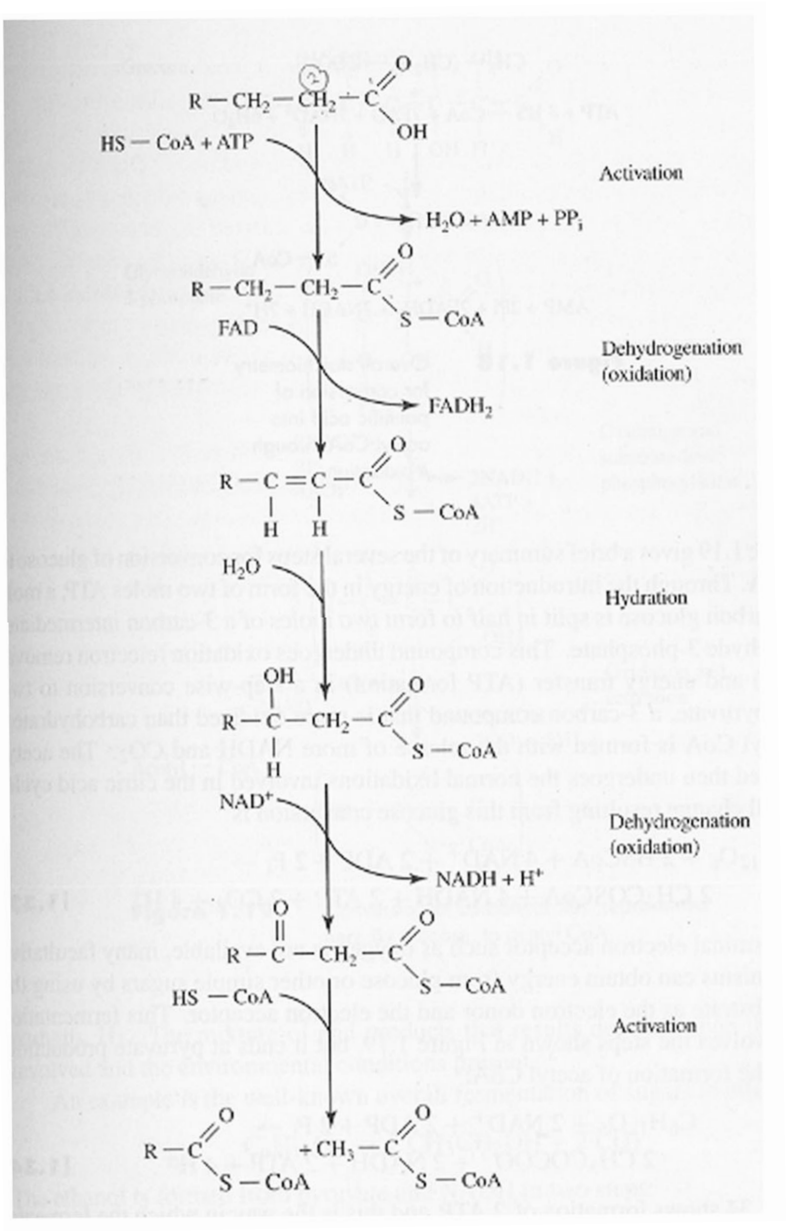

Figure 11. β-Oxidation of Fatty Acids to Acetyl-CoA

(Rittmann B. and McCarty P. (2001).Environmental Biotechnology: Principles and Applications. McGraw-Hill)

generated in the subsequent oxidation steps from an alcohol to an aldehyde and then to an organic acid. Conversion of alcohol to aldehyde takes place by dehydrogenation which is removal of two hydrogen and two electrons. Next step of conversion from aldehyde to acid takes place by removing two hydrogen atoms and two electrons and adding water to aldehyde. This step is called hydroxylation. In the next step acids are oxidized by the process of β-oxidation which is illustrated in Figure 11. The second carbon from the carboxyl carbon of the acid is the β-carbon. Oxidation of this carbon is initiated by the addition of coenzyme A to form acyl coenzyme A. Activation of fatty acids for oxidation requires energy in the form of energy carrier adenosine triphosphate (ATP). ATP in the process gets converted to adenosine monophosphate (AMP) by releasing two of its phosphate atoms. Electron and protons are removed from the acid and water is added which leads to the oxidation of the β-carbon to a keto group. Coenzyme A is added to the molecule to form acetyl coenzyme A and acyl coenzyme A compound which is two carbon atoms shorter. The shorter acyl coenzyme A compound undergoes β-oxidation again.

Improving the efficiency of biodegradation is typically the main objective in remediating a polluted environment. The two basic methods applied in improving the microbial degradation are bioaugmentation and biostimulation. Bioaugmentation or seeding is supplementing the existing microbial population with a competent exogenous population (Venosa and Zhu, 2003). Although hydrocarbon degrading bacteria are abundant in nature, bioaugmentation has been used since the 1970's (Venosa and Zhu, 2003). Bioaugmentation is considered when the indigenous hydrocarbon population is low and seeding helps to start the bioremediation process while increasing the population of the hydrocarbon degraders. In some contaminated areas, the speed of decontamination is the key to successful bioremediation; therefore, bioaugmentation is used to reduce the lag period. There are many commercially available products which claim to enhance microbial biodegradation. Laboratory studies done on Alaska North Slope crude oil with 12 commercially available microbial cultures showed higher biodegradation rates after 38 days (Aldrett *et al.*, 1997). Venosa *et al*. (1991) similarly tested 10 different commercial microbial products on Alaskan crude in flask microcosms. The Alaskan microorganism showed better degradation than the seeded microorganism suggesting that bioaugmentation maybe more effective in controlled environmental conditions than in the field.

The rate-limiting step in biodegradation is not the lack of appropriate microorganisms, but the lack of utilizable sources of nitrogen and phosphorus. Availability of nutrients is often the controlling factor for biodegradation (Ron and Rosenberg, 2002). Biostimulation is another approach that is widely used to overcome these limitations. Biostimulation enhances the growth of microorganisms through the addition of nutrients and other growth stimulating co-substrates (Venosa and Zhu, 2003). Atlas (1984), Ron and Rosenberg (2002) described the role of these limiting factors for microbial activities on petroleum hydrocarbon degradation. The limiting factors were reported to be nutrient requirements (oxygen, nitrogen and phosphate), air, temperature, and pH.

Improving biodegradation involves improving these parameters that affect the efficiency of degradation, such as available oxygen, inorganic nutrient levels, temperature, pH and moisture content. Biostimulation can be done in an environment where the availability of nutrients is restricted. Addition of nutrients to increase the biodegradation rates has been reviewed in various works (Bragg *et al.*, 1994). Nitrogen and phosphorus are the essential nutrients that are incorporated in the microbial biomass and they are elements which comprise the physical structure of cells. The requirement of nutrients for the growth of microbial biomass can be explained from stochiometric equations (Rittmann and McCarty, 2001). The overall stochiometric equation of biodegradation occurring in the wastewater treatment plant at Brayton Fire Training School, are explained in the microbiological analysis part in the paper. Nutrient and oxygen demand are estimated from those stochiometric equations for biodegradation of petroleum hydrocarbons in the system.

The nutrient concentration is maintained at a level high enough to facilitate bacterial growth. Nutrient requirements differ with the complexity in the constituents of the hydrocarbon to be degraded and availability of naturally occurring nutrients in the contaminated environment. Values of the ratios of nutrient to the hydrocarbon degraded are reported in various researches. Atlas (1981) reported values for optimum degradation of 1 mg of oil per liter in seawater ranged from 0.13 to 46 mg of nitrogen and 0.009 to 8.6 mg of phosphorus. Swannell *et al.* (2000), and Swannell and Head (1999) estimates nutrient required for hydrocarbon degradation in marine habitats with typical values of 15% N by weight of oil with a ratio of N to P of 5 and 10:1. Ron and

Rosenberg (2002) suggested approximately 150 mg of nitrogen and 30 mg of phosphorus are utilized to degrade 1 gm of hydrocarbon.

A constant supply of oxygen for the microbes is necessary in aerobic biostimulation. Aerobic conditions are generally considered necessary for extensive degradation of petroleum hydrocarbon as their metabolic pathways involve oxygenases. Conditions of oxygen limitation do not typically occur in the upper levels of the water column in either marine or freshwater environments. Most lakes and rivers are aerobic, but there can be oxygen-limiting conditions in eutrophic waters and near sediments (Cooney, 1984). Oxygen present in 1 L of seawater would degrade 3mg of oil, whereas in freshwater systems 3 to 4 gm of oxygen is required for complete oxidation of 1 gm of alkane (Atlas, 1984). Factors affecting availability of oxygen in the natural environment include wave action, water flow, and the physical state of oil. But in artificial environments, such as, wastewater treatment plants the supply of oxygen can be enhanced by mechanical means to ensure a healthy rate of microbial population.

Other factors that influence biodegradation rates are temperature and pH. Temperature influences the biodegradation of hydrocarbons by affecting the physical and chemical composition and rate of hydrocarbon metabolism by microorganisms (Leahy and Colwell, 1990). The rate of degradation decreases with decreasing temperature due to a reduction in enzymatic activities. Higher temperature tends to increase the rate up to a maximum temperature of 30 to 40 °C (Bossert and Bartha, 1984). Prince *et al.* (2003) conducted bioremediation in the Arctic shorelines where the temperature varies from 3 to 7 C, even though slow release and soluble organic fertilizers were used to enhance the rates. Extensive biodegradation of Metula crude oil by mixed cultures of marine bacteria at 3°C was reported (Colwell and Walker, 1977). Studies indicate that degradation of oil increases with increasing pH and optimum degradation occurs under slightly alkaline conditions (Dibble and Bartha, 1979; Foght and Westlake, 1987). The pH of freshwater can vary widely whereas seawater is slightly alkaline and generally more stable.

Estimating the population of the microbes is necessary to evaluate performance of the activated sludge process. The Fire Training School plant does not have the capability to recycle sludge back to the aeration tank. Recycling makes it possible to maintain a constant healthy population of microorganism in the system depending on the retention time. Absence of recycling in the current system means that constant supplements with fresh input of hydrocarbon degraders be provided. Enumeration of the bacteria is necessary to determine the appropriate dosing per mass of substrate, must be determined. Wrenn and Venosa (1996) developed a most-probable-number (MPN) procedure to enumerate both aliphatic and aromatic hydrocarbon degrading microbes separately using a 96-well microtiter plate method. N-hexadecane is used as a selective growth substrate for alkane degraders and a mixture of phenanthrene, anthracene, flourene and dibenzothiphene are used for aromatic degraders. The incubation periods are 2 weeks and 3 weeks, respectively, for alkane and aromatic degraders. This method provides reliable estimates for the density and composition of microbial population. This method will be followed to estimate the population of the hydrocarbon degraders in the wastewater treatment plant. Briones and Reichardt (1999) showed a computer-assisted method to determine MPN count, error estimates, and confidence limits. This method compliments the MPN procedure stated above and gives a detailed output spreadsheet on the microbial counts which helps in the accurate representation of the MPN count that will be carried on the samples.

The results of the analytical data are compared with the NPDES discharge standards and also the plant's TCEQ discharge permit. TCEQ assigned discharge permit for the treatment plant under provisions of Section 402 of Clean Water Act and Chapter 26 of the Texas Water Code (Table 2). The effluent is discharged to the White Creek which leads to the Brazos River System.

Table 2.

Discharge Limits of the TCEQ Permit for the Wastewater Treatment Plant at Brayton Fire School

Effluent Charateristics	Discharge Limitations				
	Daily Average		Daily Maximum		Single Grab
	lbs/day	mg/l	lbs/day	mg/l	mg/l
Chemical Oxygen Demand	N/A	N/A	3,338	200	200
Total Petroleum Hydrocarbon	200	12	250	15	15
Total Dissolved Solids	N/A	N/A	33,380	2000	2000
Total Suspended Solids	1,502	90	3,338	200	200
Biochemical Oxygen Demand(5-day)	667	40	1,334	80	80
Aluminum,total	13.9	0.835	29.5	1.77	1.77
Dissolved Oxygen	N/A	N/A	N/A	4.0(min)	N/A

1. The daily average flow of effluent shall not exceed 2 MGD.The daily maximum flow of effluent shall not exceed 2.4 MGD
2. The pH shall not be less than 6.0 standard units nor greater than 9.0 standard units.

The average temperature for the Bryan College Station area in the winter and summer months were determined from the National Weather Forecast Office website

(College Station Climate Data, 2004) which provides daily maximum, minimum and average temperature for each and every months. Climatic data of previous years are also available.

MATERIALS AND METHODS

SAMPLING

Grab samples were taken from each of the four units of the wastewater treatment plant namely the inlet tank, equalization tank, aeration tank and outfall tank on a weekly basis. Samples were taken for two seasons, winter (November 2003 to January 2003) and summer (June 2004 to August 2004) to assess the variability in the performance of the plant. Sampling points were chosen based on the accessibility to each unit (Figure12).

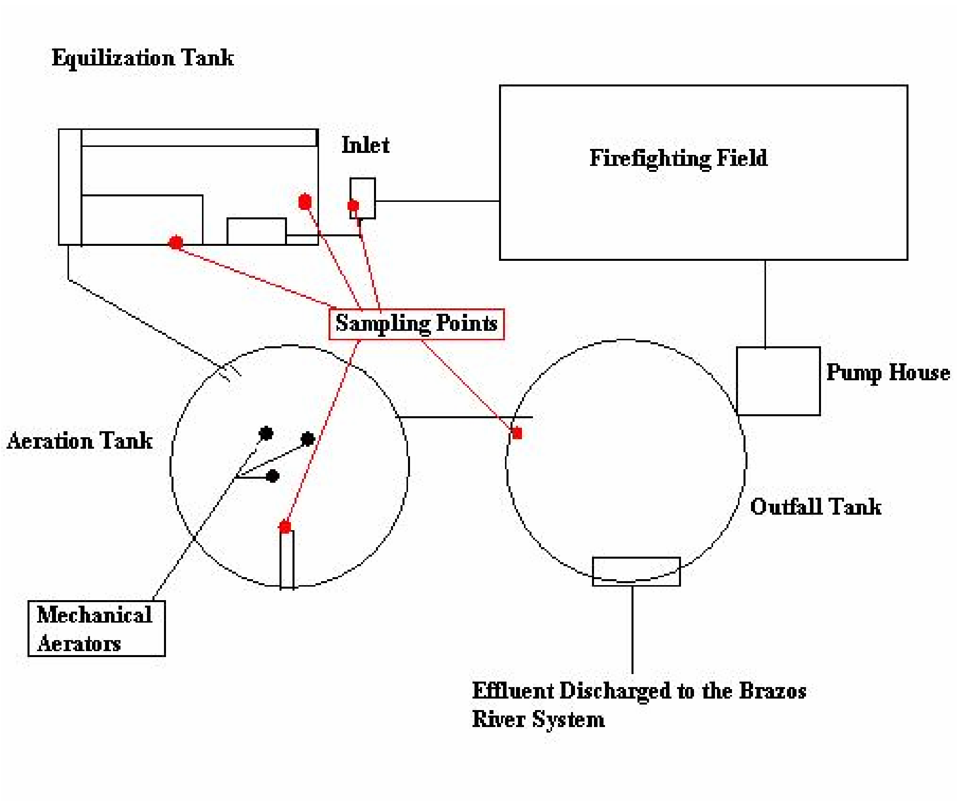

Figure 12. Sampling Locations in the Treatment Plant

Sampling of the aeration tank was difficult because there was no thoroughfare to the centre of the tank where the aerators are located. Ideally, samples should have been taken from where the aerators are located. Samples were taken at the edge of the aeration tank. Wastewater at the inlet tank was collected for sampling approximately 3 feet from the top of the tank; so that the heavy hydrocarbon fractions were captured. In the equalization tank, samples were taken from the part of the tank where the 4 mechanical mixers are located. The samples in the outfall tank were taken at the discharge point to the Brazos River so that the effluent characteristics of the plant could be measured and compared with the TCEQ limits and municipal wastewater discharge limits.

PHYSICAL AND CHEMICAL ANALYSIS

The samples were taken to the analytical laboratory at the Civil Engineering department at TAMU. The analytical tests are conducted on the samples as per the procedures laid down in 20th edition of the *Standard Methods for the Examination of Water and Wastewater* (Clesceri *et al.*,1998) and are as follows:-

- Biological Oxygen Demand (BOD) method 5210
- Chemical Oxygen Demand (COD) method 5220
- Total Organic Carbon (TOC) method 5310
- Dissolved Oxygen method 4500-O
- Alkalinity method 2320
- Aluminum method 3500
- Nitrate method 4500-NO_3
- Nitrite method 4500-NO_2
- Phosphate method 4500-P
- Total Petroleum Hydrocarbon (TPH) Hach Kit Manual, method 10050 Immunoassay method
- Turbidity method 2130
- Conductivity method 2510
- Total Suspended Solids (TSS) method 2540
- Total Dissolved Solids (TDS) method 2540

The methods of these experiments conducted in the laboratory are explained in detail in **Appendix C**, which serves as guidelines for any other follow-up studies conducted with similar sets of equipment. The equipment used for the analyses are state of the art Hach experimental kits and standard laboratory equipments available in the Civil Engineering analytical laboratory at TAMU. These experiments were done to obtain the general profile of plant performance. These analyses were carried out on each sample. The data was used to evaluate the treatment performance from inlet to outfall, calculating the removal efficiency.

Physical analysis of the wastewater was done by measuring total suspended solid, total dissolved solid, turbidity and conductivity. Total suspended solid and dissolved solid gives the estimated amount of solid material present in the wastewater. Turbidity is measured by the dispersion of light when it passes through the wastewater and is reported by comparing the value with the measurement from a standard solution. Scattering of the light is due to the presence of solids in the wastewater. Removal of solids from wastewater will also reduce the turbidity readings.

The 5-day biological oxygen demand (BOD_5) is the most widely used parameter to evaluate the strength of organic wastes in both wastewater and surface water. It is the amount of dissolved oxygen used by the microorganism in the biochemical oxidation of the organic matter and is used to measure the overall efficiency of the treatment facility. It also gives an estimate of the organic matter content in the wastewater along with other methods like chemical oxygen demand (COD) and total organic carbon (TOC). These indicate the presence of organic matter essential to degrade petroleum hydrocarbons. COD indicates the oxygen requirement for chemical oxidation of wastes which are not biodegradable. Total organic carbon (TOC) parameter is analyzed in this system to check the biodegradation of petroleum hydrocarbon and also to measure the amount organic carbon in the system which serves as an indicator of the presence of microbial biomass.

Typical values for the ratio for BOD/COD and BOD/TOC for untreated municipal wastewater are given in Table 3 (Tchobanoglous *et al*., 2003).

Table 3.

Typical Values of Ratio between BOD and COD, BOD and TOC (Tchobanoglous *et al*., 2003).

Type of Wastewater	BOD/COD	BOD/TOC
Untreated	0.3-0.8	1.2-2.0
After primary settling	0.4-0.6	0.8-1.2
Final Effluent	0.1-0.3	0.2-0.5

Generally, if the BOD/COD ratio for untreated wastewater is 0.5 or greater, the waste is easily biodegradable. Below 0.3, the waste contains some toxic components and special kinds of microorganisms are required for biodegradation. These values will be compared with the analytical results to check the biodegradability of the waste in the wastewater treatment plant at Brayton Fire School.

Aluminum is one of the heavy metals analyzed in this study because it is one of the chemicals of concern from the TCEQ discharge permit issued to them. Firefighting operations are carried out by setting life size metal models on fire pertaining to real life situation and then applying water or foam to curb the fire. Metal objects contain a high content of aluminum and after the firefighting operations, burned aluminum get mixed with the wastewater and reaches the treatment plant. The concentration of aluminum is analyzed to compare with the effluent discharge limitations specified in the permit.

MICROBIOLOGICAL ANALYSIS

Maintaining a healthy population of microbes is the key to effective biological treatment in any wastewater treatment plant. A typical activated sludge process is strictly aerobic and consists of an aeration tank, settling tank, solids recycle and a sludge wasting line (Rittmann and McCarty, 2001). The aeration tank is a suspended growth reactor where the microbial mass is kept in suspension by aeration or mechanical means. Normally the treated wastewater along with the microbial flocs is transferred to the settling tank where the heavy flocs are separated out by settling. The flocs are recycled back to the aeration tank to maintain a high concentration of microbes in the reactor.

In the wastewater treatment plant in Brayton Fire School the biological process is essentially an activated sludge unit without any recycle. There are no means to replenish the acclimated microbial mass due to the lack of recycling. The only means of maintaining a constant population of microbes in the system is to daily feed the system with fresh microbes. The microbe solution is introduced into the system at the discharge of the equalization tank to the aeration tank. The microbe solution is directly emptied in the discharge stream at a constant rate of 10ml/min. From the given company specification, 1 gallon of the microbe solution contains 200 billion (2×10^{11} cells/gallon). The number of microbes introduced in the aeration tank is approximately 5.3×10^{7} cells/ml.

Purpose of the microbial analysis was to determine the viability of the microbial population in the aeration tank. Enumeration of the microbial population was performed using selective enumeration of aliphatic hydrocarbon by a MPN procedure. 96-well (12 columns 8 rows) microtiter plates were used in triplicates for the one sample to eliminate any errors and the procedure was repeated for all the samples collected. For alkane degraders, n-hexadecane and Bushnell-Haas broth was used as the specific growth substrate and as growth media respectively. Samples from four units of the wastewater treatment plant were taken to Civil Engineering Laboratory and the experiment was conducted on state of the art Biomek Workstation 1000 robot. Use of this robot ensures precision in regard to filling the wells of the plates accurately with volumes varying from 200 µl to 2 µl and also conducting 10 fold dilutions. The plates were incubated for 14 days at room temperature.

A computer assisted method based on Microsoft Excel spreadsheet and its Solver tool was used to calculate the MPN and confidence limits (Briones and Reichardt, 1998). This method was developed using the MPN formula derived by Halvorson and Ziegler (1933). The equation is as follows:

$$\frac{a_1 p_1}{1-e^{-a_1 x}} + \frac{a_2 p_2}{1-e^{-a_2 x}} + \ldots = a_1 n_1 + a_2 n_2 + \ldots \qquad (1)$$

where a is the volume of dilution used for inoculation, p is the number of tubes showing positive growth; n is the number of replicate tubes used in each dilution level and x is the most probable number. Using the solver tool in Microsoft Excel the value of x is calculated making the right hand side of the equation equal to the left hand side. This procedure is repeated for every plate containing the samples. 95% confidence limits for a particular population were calculated through confidence factor (CF) by multiplying and dividing the MPN by CF.

$$CF = anti\log_{10}((2)(0.58)\sqrt{(\log_{10} dr)/n} \qquad \text{if } dr \geq 10 \tag{2}$$

where dr is the dilution ration and n is the number of replications/dilution level.

Nitrogen and phosphorus are the essential nutrients that are incorporated in the biomass of microbes. Nitrate, nitrite and phosphate are measured to obtain the total concentration of nitrogen and phosphorus available in the system. This data is required to evaluate the biological performance of the wastewater treatment plant.

Growth of hydrocarbon degraders depends on the availability of substrates as population increases with the influx of substrates. Total Petroleum Hydrocarbon (TPH) analyses were performed to monitor the availability of hydrocarbons and also to evaluate the removal efficiency of the same in the system. Results from all these analytical procedures are taken into consideration and are incorporated in various equations of biological models developed for evaluation of biological reactors.

RESULTS AND DISCUSSION

PHYSICAL AND CHEMICAL ANALYSIS

The physical and chemical characteristics of the wastewater plant were evaluated using the monitoring data. The assessment was performed by taking into consideration the performance of all the units from inlet to outfall tank. After the samples were analyzed, the results are represented in a tabulated form. Data taken during the winter months, November 2003 to January 2003, and summer months, June 2004 to August 2004 are listed in **Appendix A**. The removal efficiency for each of the parameters was evaluated for each unit and the values of the measured parameters were represented graphically. In the graphs, values of different parameters are compared against the units of the treatment plant starting from inlet to outfall tank. The inlet, equalization tank, aeration and outfall tank are represented by values 1 to 4 in the graphical representation of the results. Discharge limits for various effluent parameters in the TCEQ permit for the treatment plant are represented in the graphs.

ALKALINITY and pH

Alkalinity is a measure of the buffering capacity of water, or the capacity of bases to neutralize acids. Alkalinity is primarily due to the salts of weak acids, although weak or strong bases may also contribute. The major portion of alkalinity is caused by three major classes of materials namely 1) hydroxides 2) carbonates and 3) bicarbonates. The pH value in the system governs the presence of the above mentioned buffering materials contributing to total alkalinity. Other salts of weak acids such as borates, silicates and phosphates may also contribute to alkalinity. Hydroxide (OH^-) represents most of the alkalinity at a high pH usually well above 10. Above pH 8.3, alkalinity is mostly in the form of carbonate (CO_3^{2-}) and below 8.3; alkalinity is present mostly as bicarbonate (HCO_3^-). Overall alkalinity is represented by the equation 3 (Benjamin, 2002).

$$ALK = \left[HCO_3^-\right] + 2\left[CO_3^{2-}\right] + \left[OH^-\right] + [H_2PO_4] + 2[HPO_4^{2-}] + [HS^-]$$

$$+2\left[S^{2-}\right] - \left[H^+\right] - \left[NH_4^+\right] \tag{3}$$

$$\frac{\left[H^+\right]\left[HCO_3^-\right]}{\left[H_2CO_3\right]} = K_{a1} = 10^{-6.33} \tag{4}$$

$$\frac{\left[H^+\right]\left[HPO_4^{2-}\right]}{\left[H_2PO_4^-\right]} = K_{a2} = 10^{-7.20} \tag{5}$$

$$ALK = \left[HCO_3^-\right] + 2\left[HPO_4^{2-}\right] - \left[H^+\right] \tag{6}$$

Equation 4 and 5 represents equilibrium equations for first ionization of carbonic acid and second ionization of phosphoric acid (Benjamin, 2002). The magnitude of the ionization constant (K) indicates the species of carbonic acid and phosphoric acid dominant at equilibrium in the wastewater. The Brayton wastewater was alkaline with the pH ranging from 7 to over 8 during both seasons (Tables 4 & 5). Winter and summer pH values are presented in Figure 13 with pH 9 being the daily maximum value for the plant (refer to Table 2).Average values of pH in winter and summer months were recorded as 8.1 and 7.57 respectively.

Table 4.

pH Value for Winter Months (Nov 03 – Jan 04)

Sampling date	pH INLET	pH EQ. TANK	pH AERATION	pH OUTFALL
11/3/2003	6.7	8.47	8.58	8.3
11/11/2003	8.2	8.28	8.46	8.43
11/18/2003	8.2	8.42	8.38	8.25
11/25/2003	8.39	8.3	8.3	8.46
12/4/2003	8.1	7.98	8.03	8.25
12/10/2003	8.15	8.17	8.2	8.27
12/19/2003	7.85	7.93	8.02	7.7
1/9/2004	7.9	7.5	7.3	8.1
1/15/2004	8.16	8.23	8.1	8
1/21/2004	8.25	8.42	8.22	8.13
1/28/2004	7.85	8	7.5	7.8
Average	**7.98**	**8.15**	**8.10**	**8.15**

Table 5.

pH Value for Summer Months (Jun 04 – Aug 04)

Sampling date	pH	pH	pH	pH
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
6/7/2004	7.65	7.44	7.15	7.33
6/14/2004	7.83	7.25	7.12	7.35
6/22/2004	8.2	7.56	7.34	7.26
6/29/2004	7.92	7.6	7.7	7.75
7/5/2004	7.74	7.44	7.26	7.31
7/12/2004	7.65	7.32	7.3	7.28
7/19/2004	8.15	7.82	7.22	7.15
7/26/2004	8.1	7.95	7.54	7.23
8/9/2004	8.1	7.65	7.24	7.56
8/16/2004	7.58	7.25	7.12	7.25
8/23/2004	8.25	7.4	7.62	8.15
8/27/2004	8.3	7.3	7.8	7.92
Average	**7.96**	**7.50**	**7.37**	**7.46**

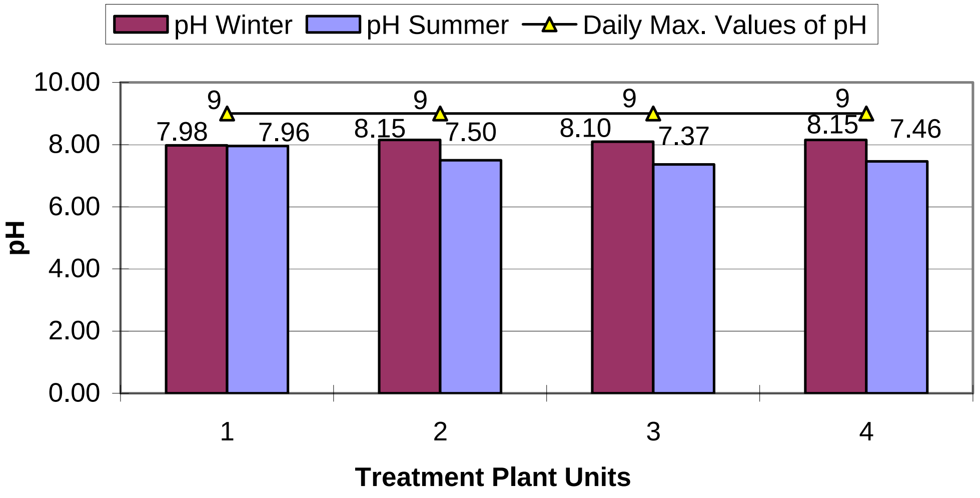

Figure 13. pH Data for Winter and Summer Seasons

The alkalinity data (Tables 6 & 7) in the wastewater treatment plant were constant with a marginal decrease of 3% from the inlet to the outfall in both the seasons and with slight fluctuations noted in equalization and aeration tank in the summer months. The average alkalinity for Nov. 03 – Jan. 04 in all units (384 mg/l as $CaCO_3$) was higher than found for Jun.04 – 04 Aug. (346 mg/l as $CaCO_3$). All trends are depicted in Figure 14. There are no general standards for alkalinity, but 20-200 mg/L are typical of fresh water and from the values measured indicated higher alkalinity values in the treatment plant.

Table 6.

Alkalinity Data in Winter Season (Nov 03 – Jan 04)

Sampling date	ALK mg/l as $CaCo_3$	ALK mg/l as $CaCo_3$	ALK mg/l as $CaCo_3$	ALK mg/l as $CaCo_3$
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
11/3/2003	486.6	473.4	437.1	409.6
11/11/2003	376.3	376.3	395.3	363.3
11/18/2003	388.3	381.3	394.3	376.3
11/25/2003	324.3	336.3	329.3	347.3
12/4/2003	415.4	385.3	403.4	387.3
12/10/2003	379.3	381.3	395.3	366.3
12/19/2003	450.4	426.4	411.4	440.4
1/9/2004	342.1	346.5	365.4	342.1
1/15/2004	339.5	375.5	352.2	339.5
1/21/2004	373.6	412.3	386.2	373.6
1/28/2004	363.8	380.4	382.7	363.8
Average	**385.4**	**388.6**	**386.6**	**373.6**

Table 7.

Alkalinity Data in Summer Season (Jun 04 – Aug 04)

Sampling date	ALK mg/l as $CaCo_3$	ALK mg/l as $CaCo_3$	ALK mg/l as $CaCo_3$	ALK mg/l as $CaCo_3$
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
6/7/2004	382.5	373.3	361.3	362.3
6/14/2004	359.3	364.5	355.3	345.3
6/22/2004	371.3	357.5	363.5	352.3
6/29/2004	338.3	311.3	348.3	327.3
7/5/2004	375.3	363.3	341.3	332.3
7/12/2004	334.3	311.5	326.3	336.3
7/19/2004	369.3	347.3	351.3	375.5
7/26/2004	342.3	325.3	335.3	343.3
8/9/2004	345.3	332.3	382.5	355.5
8/16/2004	361.5	382.3	327.5	312.3
8/23/2004	341.3	339.5	349.3	322.3
8/27/2004	325.3	345.5	370	352.5
Average	**351.2**	**343.7**	**350.1**	**341.4**

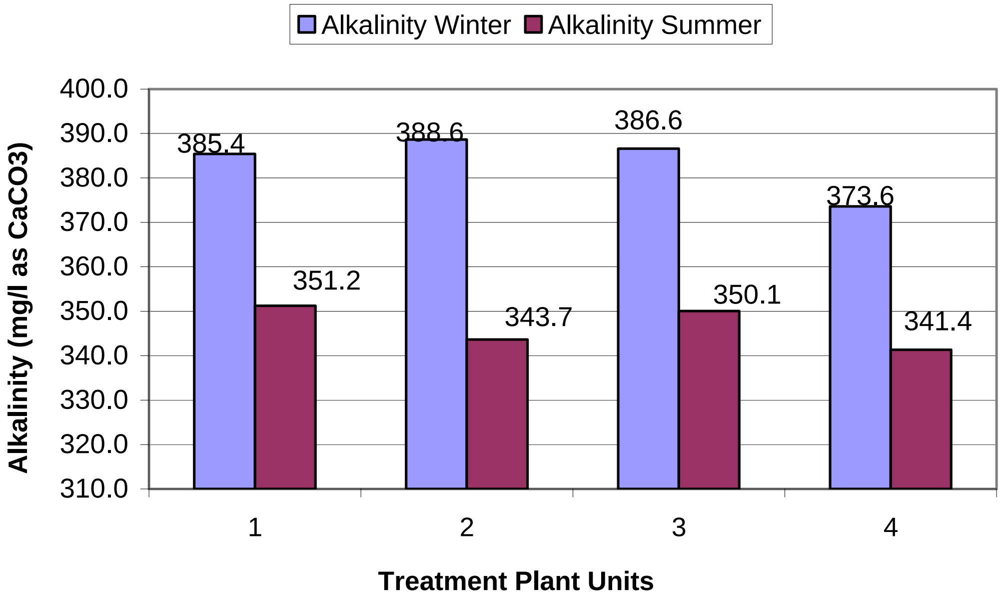

Figure 14. Average Alkalinity Data in Winter and Summer Seasons

CONDUCTIVITY

Conductivity is the ability of a solution to conduct electric current and also a measure of the concentration of inorganic dissolved solids in the system. Conductivity is affected by the presence of inorganic anions such as chloride, nitrate, sulfate, and phosphate and cations such as sodium, magnesium, calcium, iron, and aluminum (Tchobanoglous *et al*., 2003). The basic unit of measurement of conductivity is the mho or siemens. Conductivity is measured in micromhos per centimeter (µmhos/cm) or microsiemens per centimeter (µs/cm). Freshwater has conductivity values ranging from 50 to 1500 µs/cm, while conductivity values for seawater is typically 5000 µs/cm. The conductivity data for winter and summer seasons collected from the current wastewater plant is represented in Tables 8 & 9. Comparison of the two seasons showed a minimal increase of 1.7% in conductivity from inlet to equalization in the winter months whereas the trend was reversed in the summer months with a decrease of 4.2% (Figure 15). These fluctuations were not large enough to be significant. According to EPA, the conductivity of rivers in the United States generally ranges from 50 to 1500 µs/cm and streams supporting fisheries have a range between 150 and 500 µs/cm. Industrial waters can range as high as 10,000 µs/cm (Tchobanoglous *et al*., 2003). Measured values of conductivity in the treatment plant showed a range of 1091 µs/cm to 1124 µs/cm in winter season with an average value of 1105 µs/cm and 1050 µs/cm to 1096 µs/cm in summer season with an average value of 1068, both of which fall within the range of freshwater.

Table 8.

Conductivity Data in Winter Months (Nov 03 – Jan 04)

Sampling date	Conductivity (µS/cm)	Conductivity (µS/cm)	Conductivity (µS/cm)	Conductivity (µS/cm)
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
11/3/2003	1156	1092	1121	1147
11/11/2003	1063	1098	1112	1096
11/18/2003	1083	1106	1120	1076
11/25/2003	1092	1097	1116	1108
12/4/2003	1076	1086	1137	1155
12/10/2003	1098	1121	1126	1086
12/19/2003	1123	1110	1096	1119
1/9/2004	1057	1044	1124	1130
1/15/2004	1104	1125	1158	1146
1/21/2004	1086	1073	1121	1087
1/28/2004	1062	1105	1135	1061
Average	**1091**	**1096**	**1124**	**1110**

Table 9.

Conductivity Data in Summer Months (Jun 04 – Aug 04)

Sampling date	Conductivity (µS/cm)	Conductivity (µS/cm)	Conductivity (µS/cm)	Conductivity (µS/cm)
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
6/7/2004	1154	1126	1110	1085
6/14/2004	1062	1058	1034	1025
6/22/2004	1139	1098	1056	1055
6/29/2004	1086	1033	1010	1026
7/5/2004	1123	1095	1106	1092
7/12/2004	1136	1063	1057	1043
7/19/2004	1082	1035	1043	1064
7/26/2004	1065	1074	1100	1083
8/9/2004	1062	1029	1164	1056
8/16/2004	1134	1100	1041	1011
8/23/2004	1091	1021	1078	1065
8/27/2004	1073	1058	1059	1032
Average	**1096**	**1060**	**1068**	**1050**

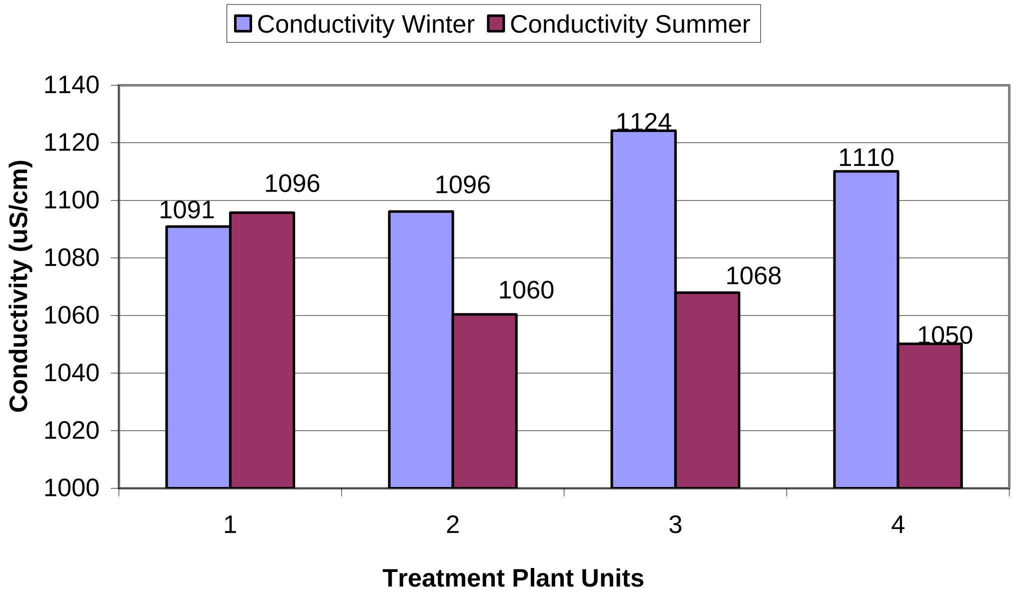

Figure 15. Average Conductivity Data in Winter and Summer Seasons

TOTAL SUSPENDED SOLIDS/TOTAL DISSOLVED SOLIDS/TURBIDITY

Total Suspended Solid (TSS), Total Dissolved Solid (TDS) and Turbidity are interrelated as they all measure the amount of solids present in the wastewater. TSS and TDS are standard parameters used to determine the solids content of wastewater treatment facilities. TDS consist of calcium, chlorides, nitrate, phosphorus, iron, sulfur, and other ions particles that will pass through a filter with pores of around 2 microns (0.002 cm) in size (Tchobanoglous *et al.*, 2003). As discussed earlier, conductivity also represents the dissolved solids present in the wastewater as dissolved solids or ions are responsible for the passage of electric current through the water.

Table 10.

TDS Data for Winter Months (Nov 03 – Jan 04)

Sampling date	TDS (mg/l)	TDS (mg/l)	TDS (mg/l)	TDS (mg/l)
	INLET	EQ. TANK	AERATION	OUTFALL
11/3/2003	640	680	640	640
11/11/2003	830	790	660	660
11/18/2003	710	630	590	590
11/25/2003	770	640	570	570
12/4/2003	680	620	550	520
12/10/2003	740	680	670	630
12/19/2003	650	610	540	550
1/9/2004	730	650	520	490
1/15/2004	710	620	560	520
1/21/2004	770	680	510	500
1/28/2004	680	610	530	480
Average	**719.1**	**655.5**	**576.4**	**559.1**

Table 11.

TDS Data in Summer Season (Jun 04 – Aug 04)

Sampling date	TDS (mg/l)	TDS (mg/l)	TDS (mg/l)	TDS (mg/l)
	INLET	EQ. TANK	AERATION	OUTFALL
6/7/2004	770	580	510	420
6/14/2004	730	610	520	440
6/22/2004	740	630	540	440
6/29/2004	710	590	490	410
7/5/2004	760	590	500	390
7/12/2004	750	620	530	420
7/19/2004	720	610	550	400
7/26/2004	730	580	480	410
8/9/2004	740	610	530	450
8/16/2004	720	690	510	420
8/23/2004	710	670	530	470
8/27/2004	690	620	520	460
Average	**730.8**	**616.7**	**517.5**	**427.5**

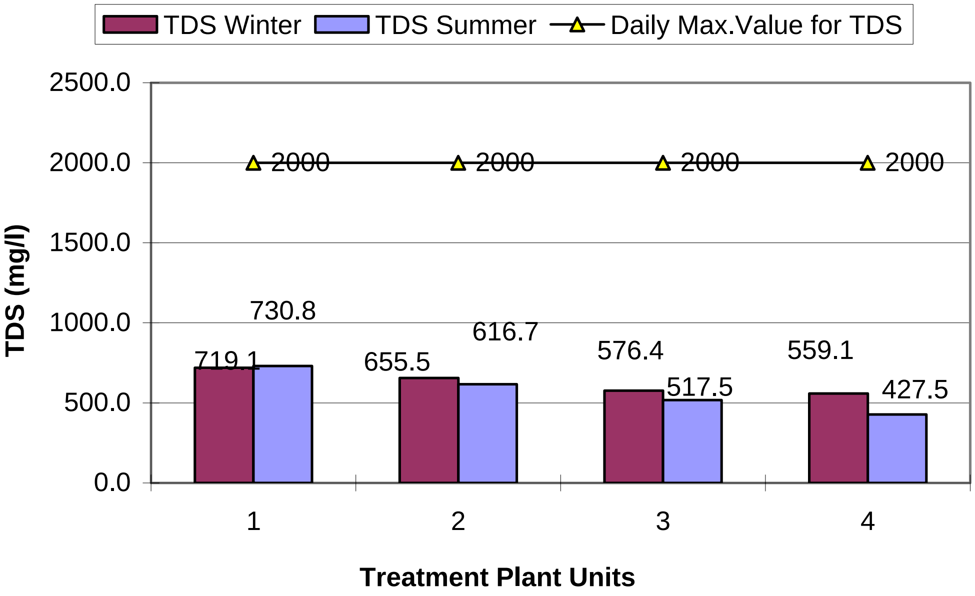

Figure 16. TDS Data for Winter and Summer Months

TDS values were measured for both the seasons in the wastewater treatment plant and are illustrated in Tables 10 & 11 and Figure 16. There is an empirical relationship governing the relationship between TDS concentration and conductivity. TDS can be calculated by multiplying conductivity by a factor between 0.55 and 0.9 and this range signifies consistency of the values measured (Tchobanoglous *et al.*, 2003). The ratio of average values of TDS and conductivity were calculated and the values fell within the range of 0.55 and 0.9 indicating the consistency of the values measured from the samples (Table 12).

Table 12.

Ratio between Average Values of TDS and Conductivity

Units	Conductivity (µS/cm)	TDS (mg/l)	TDS/Conductivity
Inlet (Winter)	1091	719.1	0.66
Inlet (Summer)	1096	730.8	0.67
Eq.Tank (Winter)	1096	655.5	0.60
Eq.Tank (Summer)	1060	616.7	0.58
Aeration (Winter)	1124	576.4	0.51
Aeration (Summer)	1068	517.5	0.48
Outfall (Winter)	1110	559.1	0.50
Outfall (Summer)	1050	427.5	0.41

Table 13.

TSS Data in Winter Season (Nov 03 – Jan 04)

Sampling date	TSS (mg/l)	TSS (mg/l)	TSS (mg/l)	TSS (mg/l)
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
11/3/2003	90	30	20	20
11/11/2003	140	40	30	10
11/18/2003	180	60	40	20
11/25/2003	160	70	50	10
12/4/2003	160	50	40	10
12/10/2003	140	40	20	20
12/19/2003	180	70	40	20
1/9/2004	180	70	40	20
1/15/2004	150	60	30	20
1/21/2004	180	60	20	10
1/28/2004	140	50	20	20
Average	**154.5**	**54.5**	**31.8**	**16.4**

Table 14.

TSS Data in Summer Season (Jun 04 – Aug 04)

Sampling date	TSS (mg/l)	TSS (mg/l)	TSS (mg/l)	TSS (mg/l)
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
6/7/2004	180	75	35	20
6/14/2004	160	65	30	10
6/22/2004	170	70	30	15
6/29/2004	170	60	25	10
7/5/2004	170	60	30	15
7/12/2004	150	70	35	10
7/19/2004	160	60	35	15
7/26/2004	155	60	30	10
8/9/2004	170	65	40	25
8/16/2004	180	70	35	15
8/23/2004	150	70	20	10
8/27/2004	160	60	25	15
Average	**164.6**	**65.4**	**30.8**	**14.2**

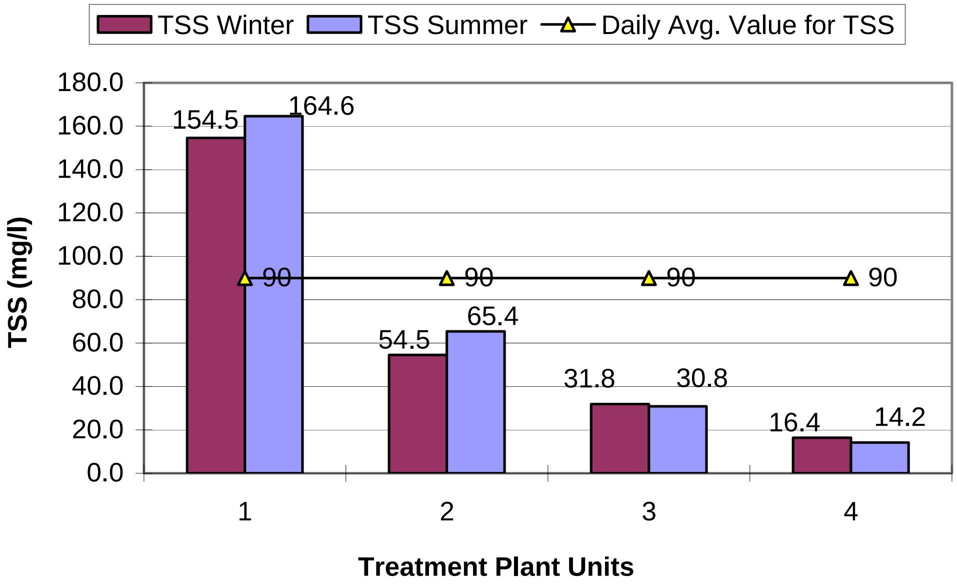

Figure 17. TSS Data for Winter and Summer Months

Turbidity is the measure of light transmittance property of water. The presence of suspended solids, microbial growth and algae in the system decreases the passage of light through the water resulting in high turbidity values. Normally, suspended solids contain clay, silt, sand, algae, microbes, fractions of hydrocarbon in suspension and other substances. TSS values for all the treatment plant units for both seasons showed little variations (Tables 13 &14) and the values were compared in Figure 17. Tables 15 and 16 present the turbidity values in winter and summer seasons and values for both season were compared in Figure 18.

Table 15.

Turbidity Data in Winter Months (Nov 03 – Jan 04)

Sampling date	Turbidity (NTU)	Turbidity (NTU)	Turbidity (NTU)	Turbidity (NTU)
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
11/3/2003	133.8	7.9	12.6	16
11/11/2003	253.8	39.3	34	8.9
11/18/2003	320.5	55.7	38.9	11.2
11/25/2003	178.3	42.9	13.1	6.9
12/4/2003	180.8	41.3	21.6	10.7
12/10/2003	157.8	38	17	15
12/19/2003	172.3	35	19.1	14
1/9/2004	165.2	52.4	16.6	11.6
1/15/2004	189.2	48.6	18.2	14.3
1/21/2004	254.6	65.8	23.1	16.3
1/28/2004	172	43.5	17.2	12.4
Average	**198.0**	**42.8**	**21.0**	**12.5**

Table 16.

Turbidity Data in Summer Season (Jun 04 – Aug 04)

Sampling date	Turbidity (NTU)	Turbidity (NTU)	Turbidity (NTU)	Turbidity (NTU)
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
6/7/2004	198	58.6	22.7	10.4
6/14/2004	194.4	63.4	20.5	11.8
6/22/2004	204.6	61.2	18.3	13.5
6/29/2004	220.5	55.2	17.9	10.6
7/5/2004	212.5	64.2	19.6	12.5
7/12/2004	198.3	62.7	17.3	13.8
7/19/2004	224.8	60.6	21.7	10.6
7/26/2004	205.5	57.8	21.5	12.4
8/9/2004	163.6	55.4	21.8	21.8
8/16/2004	178.6	64.2	19.9	19.9
8/23/2004	194.2	60.5	18.5	18.5
8/27/2004	214.5	58.6	16.5	16.5
Average	**201.0**	**60.3**	**19.4**	**14.7**

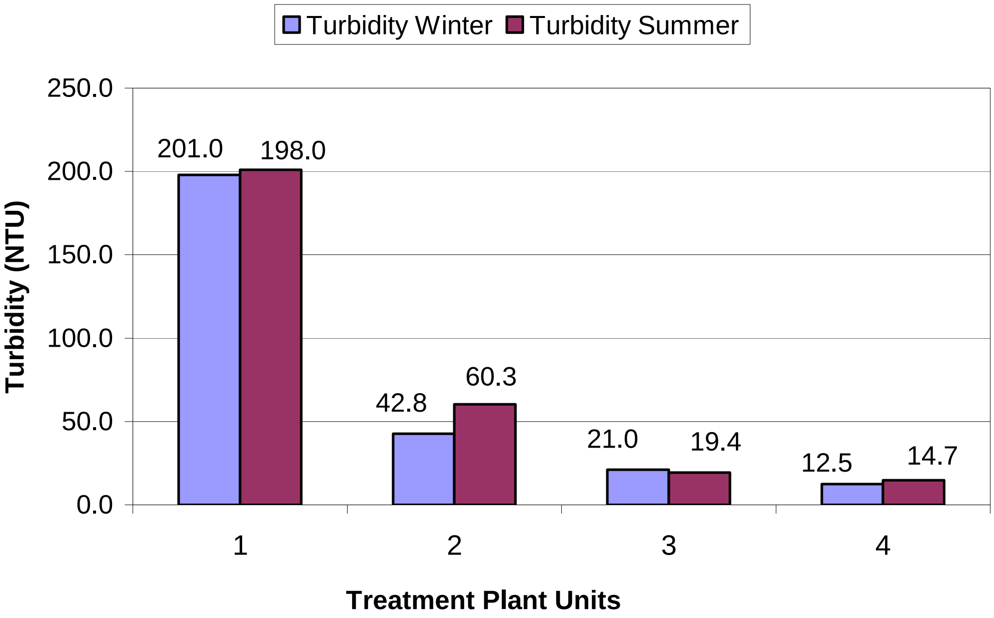

Figure 18. Turbidity Data in Winter and Summer Seasons

Turbidity was highest in the inlet and decreased by 74% in the equalization tank and 92% by the outlet (Figure 16). Turbidity was compared to TSS for winter and summer seasons. Turbidity was found to be directly proportional to the TSS (Figure19) with $R^2 = 0.92$. The microbial activity in the aeration tanks contributes to turbidity due to suspended biomass. For comparison, microbial population numbers were converted to microbial biomass assuming bacterial cell mass of 10 femtogram (1×10^{-15} grams) (Button and Robertson,1999). TSS and microbial biomass did not follow the same seasonal trends (Figure 20 & 21). Therefore, higher microbial number did not directly contribute to higher values of TSS, but are responsible for suspended solids in the aeration tank.

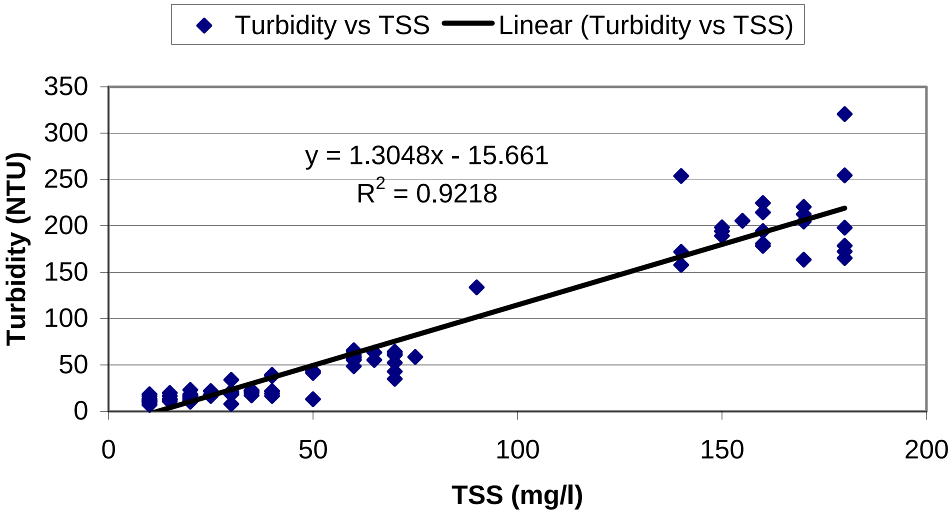

Figure 19. Comparison between Turbidity and TSS values

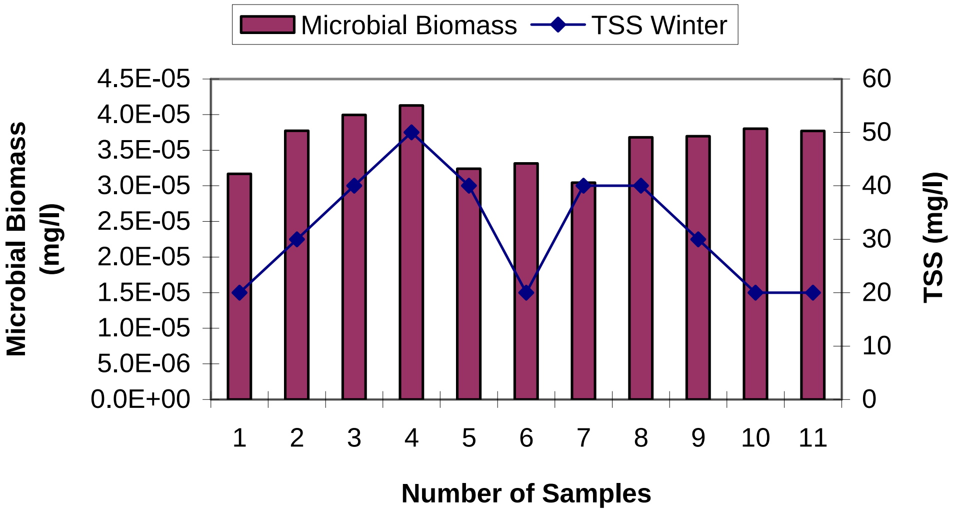

Figure 20. Comparison between Microbial Biomass and TSS in Winter Months

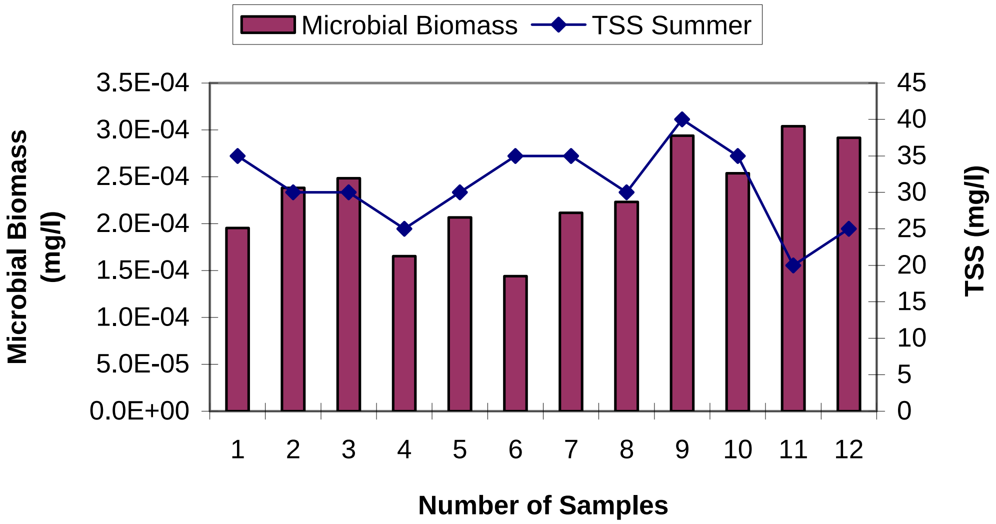

Figure 21. **Comparison between Microbial Population and TSS in Summer Months**

Primary treatment uses a grit chamber and skimmer to remove the lighter floating petroleum hydrocarbons in the equalization tank. It was responsible for removing 60% of TSS, with an overall removal of 90% in the system from inlet to outfall in both seasons. There was significant decrease in turbidity values from inlet tank to the outfall with an overall decrease of about 92% – 93% in both seasons with a decrease of about 75% removal from in the equalization. There was also a gradual removal of dissolved solids of about 22% overall.

DISSOLVED OXYGEN/BIOCHEMICAL OXYGEN DEMAND/CHEMICAL OXYGEN DEMAND

Dissolved Oxygen (DO) is the amount of oxygen present in the system. In an aerobic treatment process, oxygen is the terminal electron acceptor and it is required by the microorganism to oxidize the substrate available in the system. In the current wastewater treatment plant, hydrocarbon degraders utilize oxygen to degrade petroleum hydrocarbons and a measure of DO indicates the availability of oxygen in the current system. It also indicates the functioning of the mechanical aerators in the aeration tank which provides adequate oxygen required for biodegradation. DO levels increased from the inlet to the outfall (Tables 17 & 18) because of mechanical aerators and also both aeration and outfall provided more surface area of water in contact with atmospheric oxygen. The overall values of DO in the winter were lower than those in the summer (Figure 22) which was expected due to the higher temperatures. An average value of about 7 mg/l was measured in the aeration tank, but this value could have increased if the DO sampling was taken closer to the aerators. Due to accessibility problems in the aeration tank, this could not be done during sampling.

BOD is a measure of the cumulative oxygen consumption by the microorganism to biodegrade the waste. BOD is used to indicate the amount of waste in any system, natural or artificial. In this wastewater treatment plant, aliphatic hydrocarbons are the chemicals of concern and BOD indicated the amount of waste coming into the system and also the performance of the treatment plant overall. The amount of BOD present in each unit was used to evaluate the efficiency of the treatment plant. For the aeration

Table 17.

DO Data for Winter Months (Nov 03 – Jan 04)

Sampling date	**DO (mg/l)**	**DO (mg/l)**	**DO (mg/l)**	**DO (mg/l)**
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
11/3/2003	4.6	5.6	6.2	6
11/11/2003	4.8	5.4	6.5	6.5
11/18/2003	4.7	5.6	6.4	6.3
11/25/2003	4.6	5.5	6.7	6.6
12/4/2003	4.5	5.8	6.8	6.4
12/10/2003	4.6	5.7	7	6.2
12/19/2003	4.8	5.6	7.2	6..4
1/9/2004	4	6.4	6.8	6.8
1/15/2004	4.4	6.2	7	6.5
1/21/2004	4.9	6.2	7.2	6.6
1/28/2004	4.5	6.5	7	6.9
Average	**4.6**	**5.9**	**6.8**	**6.5**

Table 18.

DO Data for Summer Months (Jun 04 – Aug 04)

Sampling date	**DO (mg/l)**	**DO (mg/l)**	**DO (mg/l)**	**DO (mg/l)**
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
6/7/2004	4.5	6.2	7.5	6.8
6/14/2004	4.2	6.5	7.4	6.5
6/22/2004	3.8	6	7.2	6.6
6/29/2004	4.2	6.4	7.1	6.4
7/5/2004	4.4	6.3	7.8	6.9
7/12/2004	3.9	6.5	7.6	6.4
7/19/2004	4.1	6.4	7.2	6.1
7/26/2004	4.2	6.1	7.3	6.2
8/9/2004	4.2	5.8	7.4	7
8/16/2004	4	6.2	7.2	7.2
8/23/2004	4.3	6.4	7	6.8
8/27/2004	4.2	6.3	7	6.6
Average	**4.2**	**6.3**	**7.3**	**6.6**

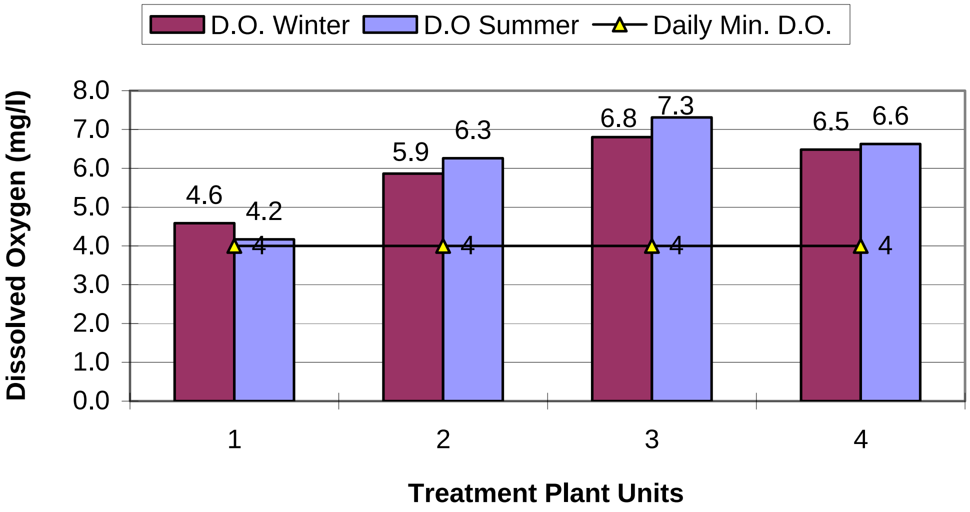

Figure 22. DO Data for Winter and Summer Seasons

tank, BOD indicates the available substrate for the hydrocarbon-degrading microorganism. Temperature corrections for the BOD were applied to the measured values to get the actual BOD reading. The BOD reaction rate $k_{(T)}$ is directly related to the temperature and the relationship is based on Van't Hoff's law (Eckenfelder, Jr., 1980) as stated in Equation 7.

$$k_{(T)} = k_{(20)}\theta^{(T-20)} \tag{7}$$

Values of θ are 1.056 at 20°C to 30°C and 1.135 at 4°C to 20°C (Schroepfer *et al.*,1964) and value of $k_{(20)}$ for untreated wastewater range from 0.12 to 0.46 d^{-1},with a typical value of 0.23 d^{-1} (Tchobanoglous *et al.*, 2003). Even though water temperature was not available, air temperatures for both winter and summer months were taken into consideration to get the approximate values for $k_{(T)}$. Average value of $k_{(20)}$ was taken as 0.23 d^{-1} to calculate values of $k_{(T)}$ (Table 19).

Table 19.

Values of $k_{(T)}$ corresponding to Different Temperature

Month	Celsius	k(T)
November	17.2	0.16
December	11.4	0.07
January	11.7	0.08
June	26.7	0.33
July	28.2	0.36
August	27.9	0.35

BOD_5 values measured in the laboratory from the samples taken from all the units were corrected for the temperature using the corresponding values of $k_{(T)}$. BOD_5 values were measured at temperature of 20° and BOD_5 values were corrected for actual temperature onsite to evaluate the BOD for the treatment plant (Tables 20 & 21). The following equation (Equation 8) was used to calculate the BOD_5 at different temperatures:

$$BOD_5 = BOD_u(1 - e^{-k_{(T)}t}) \tag{8}$$

where BOD_u is the total or ultimate carbonaceous BOD, mg/l and t is time, d.

Influent BOD is higher in summer months than in winter months (Table 20&21) due to intensive training at Brayton Fire Training School. Between the months of June to August, firefighters from all over Texas and from other part of the country take part in the annual firefighting training operations. Fire training operation continued on weekdays from morning till evening, which generated high amount of hydrocarbon waste for the treatment plant. Higher BOD also indicated a higher degradable component of the waste in the summer months. The BOD removal efficiency in the treatment plant for both the seasons was 85% and the effluent BOD for both seasons was below the effluent standard of 40mg/l specified in the TCEQ permit (Figure 23).

Table 20.

BOD Data for Winter Months (Nov 03 – Jan 04)

Sampling date	BOD (mg/L)	BOD (mg/L)	BOD (mg/L)	BOD (mg/L)
	INLET	EQ. TANK	AERATION	OUTFALL
11/3/2003	76.5	29.8	20.4	13.1
11/11/2003	131.2	30.3	23.6	14.7
11/18/2003	108.6	35.3	24.7	13.4
11/25/2003	51.5	24.3	15.4	7.7
12/4/2003	43.1	18.1	18.2	9.8
12/10/2003	39.3	19.7	16.9	8.9
12/19/2003	39.2	17.5	17.9	8.9
1/9/2004	37.9	18.4	12.8	6.9
1/15/2004	40.1	16.9	10.8	7.5
1/21/2004	45.1	19.6	11.2	6.0
1/28/2004	42.6	16.2	12.3	6.5
Average	**59.6**	**22.4**	**16.8**	**9.4**

Table 21.

BOD Data for Summer Months (Jun 04 – Aug 04)

Sampling date	BOD (mg/L)	BOD (mg/L)	BOD (mg/L)	BOD (mg/L)
	INLET	EQ. TANK	AERATION	OUTFALL
6/7/2004	96.4	43.9	29.4	14.5
6/14/2004	111.3	41.6	26.4	16.5
6/22/2004	109.1	44.9	28.3	14.3
6/29/2004	107.0	45.0	26.2	14.2
7/5/2004	143.6	52.3	33.8	16.8
7/12/2004	138.3	54.4	33.2	19.3
7/19/2004	139.5	57.7	31.0	18.7
7/26/2004	130.4	54.4	32.5	19.9
8/9/2004	114.9	49.0	36.3	20.8
8/16/2004	120.5	53.2	31.6	18.3
8/23/2004	132.0	58.2	33.3	18.7
8/27/2004	131.5	54.2	33.8	19.0
Average	**122.9**	**50.7**	**31.3**	**17.6**

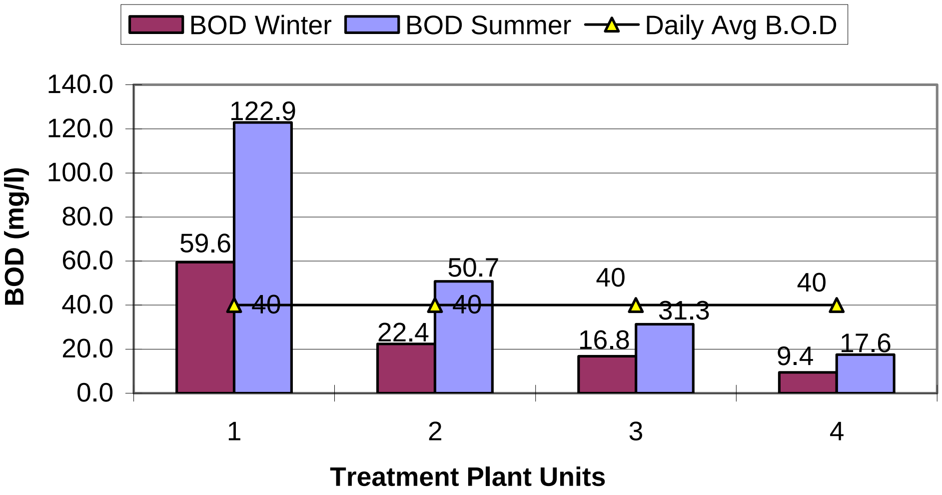

Figure 23. Corrected BOD Data for Winter and Summer Seasons

COD was higher in the inlet tank and dropped significantly after the equalization tank because of the removal of petroleum hydrocarbons in the equalization tank (Table

22 & 23). COD removal in the system overall was 95% with 75% removed in the equalization tank (Figure 24). COD values were comparable between seasons which indicated a constant load to the wastewater facility.

Table 22.

COD Data for Winter Months (Nov 03 – Jan 04)

Sampling date	**COD (mg/L)**	**COD (mg/L)**	**COD (mg/L)**	**COD (mg/L)**
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
11/3/2003	1051.5	196	77	32.5
11/11/2003	1100	317.5	148.5	40
11/18/2003	969.3	245	89	33.75
11/25/2003	991	226	87	32
12/4/2003	146	47	24	33
12/10/2003	137	44	29	30
12/19/2003	123	51	26	37
1/9/2004	815	175	74	34
1/15/2004	910	183	82	29
1/21/2004	977	240	81	31
1/28/2004	859	196	78	33
Average	**734.4**	**174.6**	**72.3**	**33.2**

Table 23.

COD Data for Summer Months (Jun 04 – Aug 04)

Sampling date	COD (mg/L)	COD (mg/L)	COD (mg/L)	COD (mg/L)
	INLET	EQ. TANK	AERATION	OUTFALL
6/7/2004	774	187	72	38
6/14/2004	825	215	74	35
6/22/2004	814	226	83	32
6/29/2004	796	199	75	33
7/5/2004	845	204	73	36
7/12/2004	862	215	71	31
7/19/2004	795	228	76	32
7/26/2004	813	210	74	30
8/9/2004	835	172	80	36
8/16/2004	810	195	73	26
8/23/2004	832	210	76	29
8/27/2004	828	178	84	41
Average	**819.1**	**203.3**	**75.9**	**33.3**

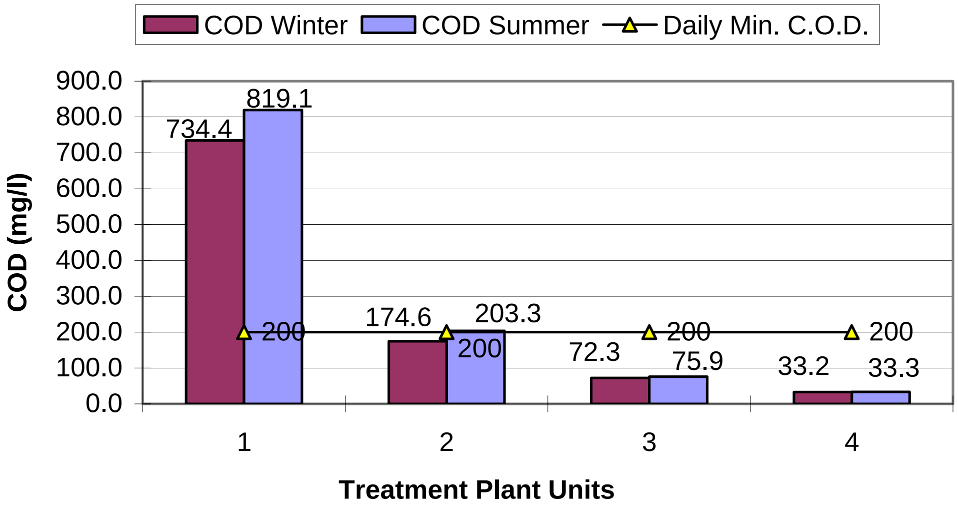

Figure 24. COD Data for Winter and Summer Seasons

The ratio of BOD to COD (Table 24) indicated the amount of oxygen required to biodegrade the waste to the amount of oxygen required to completely oxidize all the waste. The ratio of effluent BOD to COD in the current wastewater treatment plant in winter and summer months were 0.28 and 0.53, respectively, as compared to 0.1–0.3 range in the municipal treatment plant (Tchobanoglous *et al*., 2003). The significantly higher quantity of COD indicated the fraction of waste that cannot be consumed by the microorganisms. Higher BOD values in the summer than winter were responsible for higher BOD to COD ratio and also indicate presence of residual nonbiodegradable waste in the effluent. Higher ratios also indicate that the system is not efficiently removing the wastes. The plant is not performing at a desirable level of efficiency.

Table 24.

Ratios between BOD, COD and TOC

Treatment Plant Unit	BOD (mg/L)	COD (mg/L)	TOC (mg/l)	BOD/COD	BOD/TOC	COD/TOC
Winter						
INLET	59.6	734.4	147.5	0.08	0.40	4.98
EQ. TANK	22.4	174.6	54.7	0.13	0.41	3.19
AERATION	16.8	72.3	43.0	0.23	0.39	1.68
OUTFALL	9.4	33.2	24.3	0.28	0.39	1.37
Summer						
INLET	122.9	819.1	190.8	0.15	0.64	4.29
EQ. TANK	50.7	203.3	72.1	0.25	0.70	2.82
AERATION	31.3	75.9	47.4	0.41	0.66	1.60
OUTFALL	17.6	33.3	21.3	0.53	0.82	1.56

Due to the presence of petroleum hydrocarbon in the wastewater entering the treatment plant, TOC is also an indicator of the waste removal in the system. Organic carbon content in the system indicates the presence of hydrocarbons and also includes the amount of biomass in the aeration tank. The TOC removal efficiency was 88% overall (Tables 25& 26) and the trend is shown in Figure 25. The ratio of BOD: TOC (Table 24) represents the ratio of oxygen required for biodegradation to the amount of organic carbon present in the system. The ratio of BOD: TOC in the effluent in winter and summer months were 0.39 and 0.82 respectively compared to 0.2 – 0.5 range in municipal treatment plant. The higher ratios of BOD: TOC in the summer months is due to high BOD influx because of intensive firefighting training. Assuming that TOC

Table 25.

TOC Data for Winter Months (Nov 03 – Jan 04)

Sampling date	**TOC (mg/l)**	**TOC (mg/l)**	**TOC (mg/l)**	**TOC (mg/l)**
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
11/3/2003	132	52	35	22
11/11/2003	220	50	40	25
11/18/2003	179	62	41	23
11/25/2003	89	41	27	13
12/4/2003	134	58	57	31
12/10/2003	121	62	54	28
12/19/2003	125	55.6	56	28
1/9/2004	154	68	38	22
1/15/2004	138	50	45	24
1/21/2004	192	62	39	28
1/28/2004	139	41	41	23
Average	**147.5**	**54.7**	**43.0**	**24.3**

Table 26.

TOC Data for Summer Months (Jun 04 – Aug 04)

Sampling date	TOC (mg/l)	TOC (mg/l)	TOC (mg/l)	TOC (mg/l)
	INLET	EQ. TANK	AERATION	OUTFALL
6/7/2004	185	68	48	24
6/14/2004	192	70	52	19
6/22/2004	182	74	46	20
6/29/2004	194	76	50	18
7/5/2004	193	71	45	23
7/12/2004	186	69	42	24
7/19/2004	190	75	51	20
7/26/2004	182	70	47	21
8/9/2004	215	78	45	23
8/16/2004	186	71	43	21
8/23/2004	195	69	48	24
8/27/2004	189	74	52	19
Average	**190.8**	**72.1**	**47.4**	**21.3**

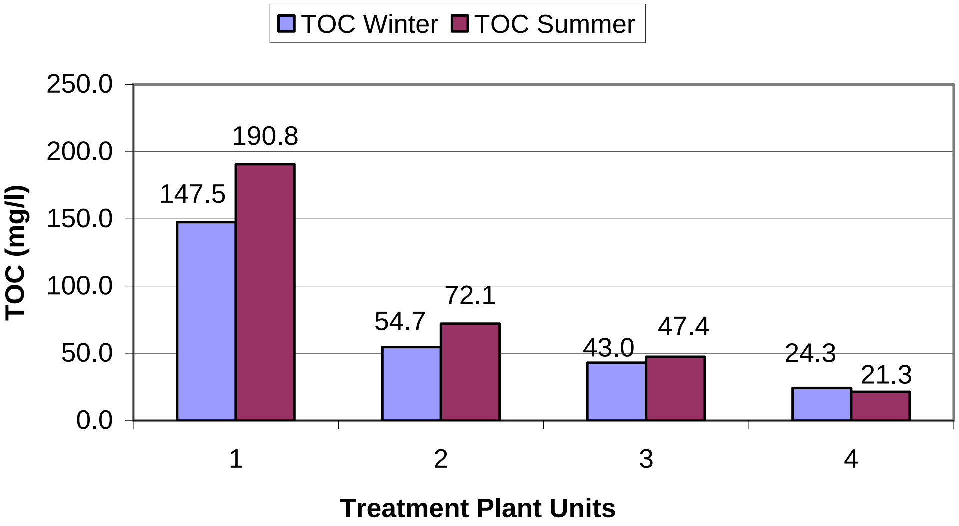

Figure 25. TOC Data for Winter and Summer Seasons

represented the amount of substrate present in the system, higher BOD values indicate that there are other sources contributing to the total BOD in the system. The higher ratios in the Brayton effluent indicate the presence of biodegradable waste as well as residual biomass from the aeration tank in the outfall tank. Again, degradable organic matter is being released in the outfall.

A reduction in the COD to TOC ratio (Table 26) was observed in the system. Intermediate compounds may be formed during biological process without any significant conversion of organic matter to carbon dioxide which may result in reduction in COD without any reduction in TOC (Eckenfelder, Jr., 1980). A high COD to TOC ratio in the inlet tank indicates the presence of wastes that exert a chemical oxygen demand other than pure hydrocarbons that are biodegraded. The ratio decreases both due to the decrease in COD and a comparative increase of TOC with the presence of hydrocarbon waste and microbial biomass. Eckenfelder Jr. (1980) reported COD: TOC ratio of 2.70 and 1.85 for raw waste and treated effluent for petrochemical waste through biological treatment. Values obtained from the Brayton wastewater treatment plant were compared. Higher ratios in the inlet tank were observed in the wastewater treatment plant because of the heavier fractions of hydrocarbons present in greater quantities in the petrochemical waste than that in the waste of the treatment plant. Petrochemical waste would have a higher TOC value resulting in the low ratio of COD: TOC in the raw waste. Effluent ratios in the treatment plant are below the ratio indicated in the petrochemical waste which indicates fairly good effluent removal in the system, but still more improvement in the treatment performance necessary.

ALUMINUM

Al data in the winter and summer months were highly variable (Tables 27 & 28). In the winter months, the concentration of Al increased from the inlet to outlet by 92%. To account for this unusual trend of concentration of Al in the winter more investigation is required. The inlet and outfall concentration were below the discharge limitation required by the TCEQ permit (Figure 26). In the summer months, the concentration decreased from inlet to outfall by 30%. No alum ($Al_2(SO_4)_3.14H_2O$) is added to the treatment plant; therefore the concentration of aluminum in the treatment plant comes in from the firefighting training. In the Brayton Fire Training School, firefighting operations are carried out by lighting up the metallic models which contains aluminum, to simulate real life situations with the help of petroleum hydrocarbons. Intense heat of the firefighting operation melts the aluminum at 660°C and thereby it flows into the inlet of the wastewater treatment plant as a waste. Aluminum was measured for it was one of the chemicals of concern in the TCEQ permit for the wastewater treatment plant.

Aluminum is not a toxic substance as reported by Agency for Toxic Substances and Disease Registry (ATSDR-ToxFAQs^{TM}, 2004). EPA has not classified aluminum as a carcinogen and only higher levels of aluminum results in respiratory problems in humans. Spills or accidental releases of 5,000 pounds or more of aluminum sulfate ($Al_2(SO_4)_3$) should be reported as per EPA guidelines. EPA recommends that the concentration of aluminum in drinking water not exceed 0.2 mg/l because of taste and odor problems whereas TCEQ permit limits the daily average concentration of the aluminum in the wastewater plant to 0.835 mg/l. Even though the concentration of aluminum in the effluent is below the specified TCEQ permit limit, it is still above the concentration fit for drinking water. Aluminum enters the wastewater as in the metallic ionic form Al^{3+} and forms various species with hydroxide ions. Visual MINTEQ® software was used to compute the various species of aluminum occurring in wastewater. Concentration of aluminum, hydroxide, carbonate, phosphate ions at different measured pH values were fed into the software and concentrations and activity of the various species are checked every time for different pH. The species computed by the Visual MINTEQ® software were $Al(OH)_2^+$, $Al(OH)_3$, $Al(OH)_4^-$, $Al_2(OH)_2^{4+}$, $Al_3(OH)_4^{5+}$, $Al(OH)^{2+}$, $Al_2PO_4^{3+}$ and $AlHPO_4$+. Aluminum sulfate and aluminum phosphide (AlP) are possible toxic species of aluminum. These species are not among the possible list of species of aluminum that may exist in the wastewater in the Brayton Fire Training School.

Table 27.

Aluminum Data in Winter Months (Nov 03 – Jan 04)

Sampling date	AL (mg/l)	AL (mg/l)	AL (mg/l)	AL (mg/l)
	INLET	EQ. TANK	AERATION	OUTFALL
11/3/2003	0.1307	0.1478	0.3015	0.31
11/11/2003	0.1421	0.1766	0.324	0.3346
11/18/2003	0.1349	0.1702	0.2964	0.3157
11/25/2003	0.1227	0.1856	0.3271	0.3567
12/4/2003	0.1467	0.1892	0.2064	0.2374
12/10/2003	0.1356	0.1689	0.2684	0.3024
12/19/2003	0.1481	0.1741	0.3146	0.3257
1/9/2004	0.1495	0.1865	0.2135	0.223
1/15/2004	0.1458	0.1786	0.1962	0.1856
1/21/2004	0.1385	0.1754	0.2247	0.2034
1/28/2004	0.1416	0.181	0.173	0.1624
Average	**0.140**	**0.176**	**0.259**	**0.269**

Table 28.

Aluminum Data in Summer Months (Jun 04 – Aug 04)

Sampling date	AL (mg/l)	AL (mg/l)	AL (mg/l)	AL (mg/l)
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
6/7/2004	0.2845	0.2412	0.2235	0.1845
6/14/2004	0.2556	0.2213	0.211	0.1765
6/22/2004	0.2754	0.2354	0.225	0.1687
6/29/2004	0.2853	0.2325	0.1934	0.1612
7/5/2004	0.2421	0.2351	0.2014	0.2014
7/12/2004	0.2611	0.2236	0.2115	0.2115
7/19/2004	0.257	0.2152	0.2071	0.2071
7/26/2004	0.2576	0.229	0.1932	0.1932
8/9/2004	0.2511	0.2336	0.2235	0.195
8/16/2004	0.2833	0.2586	0.1962	0.1856
8/23/2004	0.2685	0.2574	0.2247	0.2034
8/27/2004	0.324	0.241	0.173	0.1624
Average	**0.270**	**0.235**	**0.207**	**0.188**

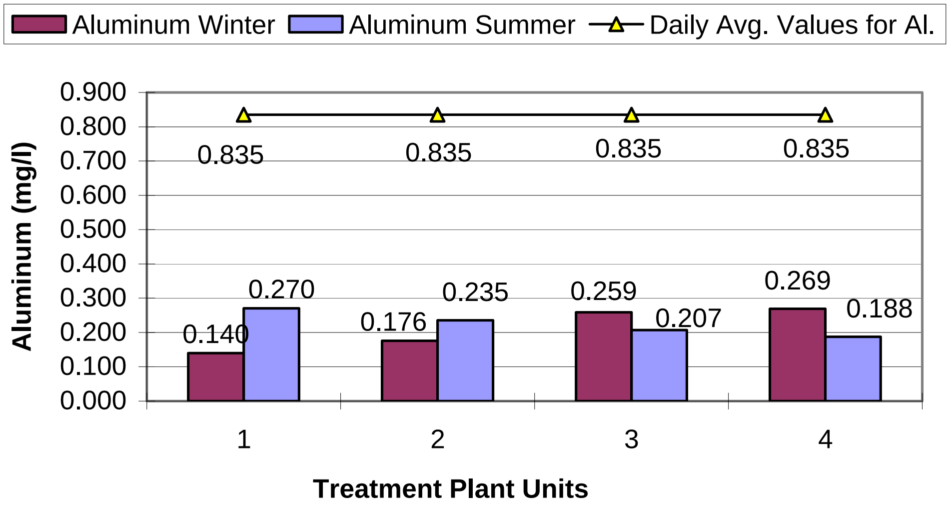

Figure 26. Aluminum Data in Winter and Summer Seasons

MICROBIOLOGICAL ANALYSIS

MOST PROBABLE NUMBER COUNT (MPN COUNT)

Microbial biodegradation of hydrocarbons in the Brayton wastewater plant is enhanced by bioaugmentation and biostimulation. Bioaugumentation is practiced by adding a solution called “Micro-Blaze” (Verde Environmental, Houston, Texas) to the effluent at a constant rate of 10 ml/min of the equalization tank prior to entering the aeration basin. The consortia of microorganisms consist primarily of Bacillus, which are known hydrocarbon degraders (Micro Blaze Spill Control, 2004). MPN data for aliphatic degraders were collected from the aeration tank, the primary site of petroleum hydrocarbon biodegradation. The microbial population in the outfall tank was also assessed to compare the population difference in the aeration basin and at the outfall. MPN data was collected for the aeration tank and the outfall during the winter and summer seasons and are illustrated in Figures 27 & 28 and Figures 29 & 30, respectively **(Appendix B).**

During the winter months, the population of the hydrocarbon degraders in the aeration tank ranged from 3×10^3 cells/ml to 4×10^3 cells/ml. In the summer months, the values ranged from 1.5×10^4 cells/ml to 3×10^4 cells/ml, which were nearly ten times the values recorded in winter months. However, considerable error is inherent in the MPN technique which prevents this trend from being statistically significant. This trend is shown in the comparison graphs for both winter and summer months (Figure 31 & 32). In the outfall, the population of hydrocarbon degraders was recorded for both seasons.

The values ranged from 1.5 ×10^3 cells/ml to 2×10^3 cells/ml in winter and 6 ×10^3 cells/ml to 7.5×10^3 cells/ml in summer.

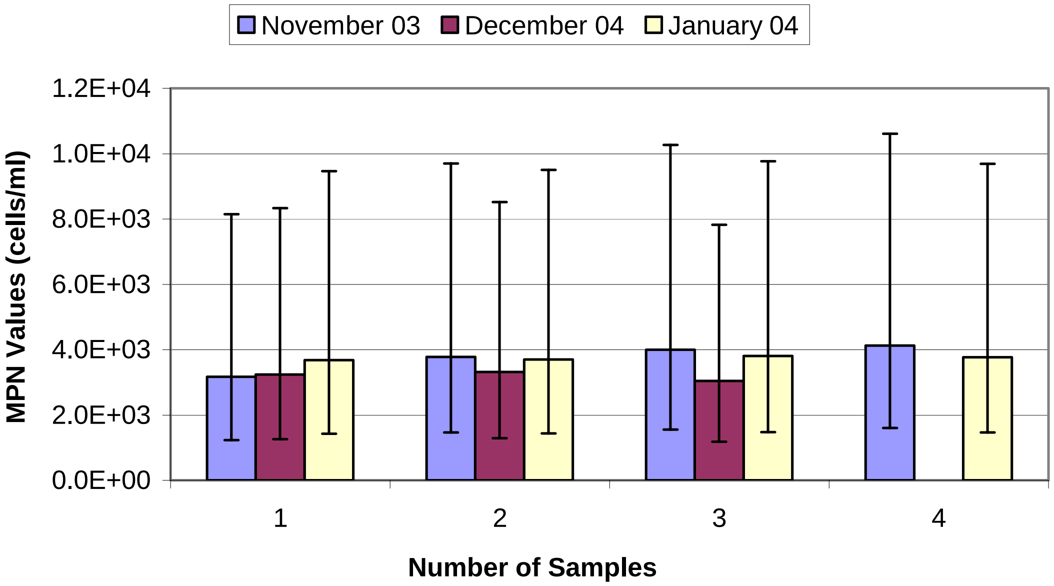

Figure 27. MPN Data of Aliphatic Degraders in Aeration Tank for Winter Season (Nov 03 – Jan 04) (Error bars represent upper and lower 95% confidence intervals for MPN Data)

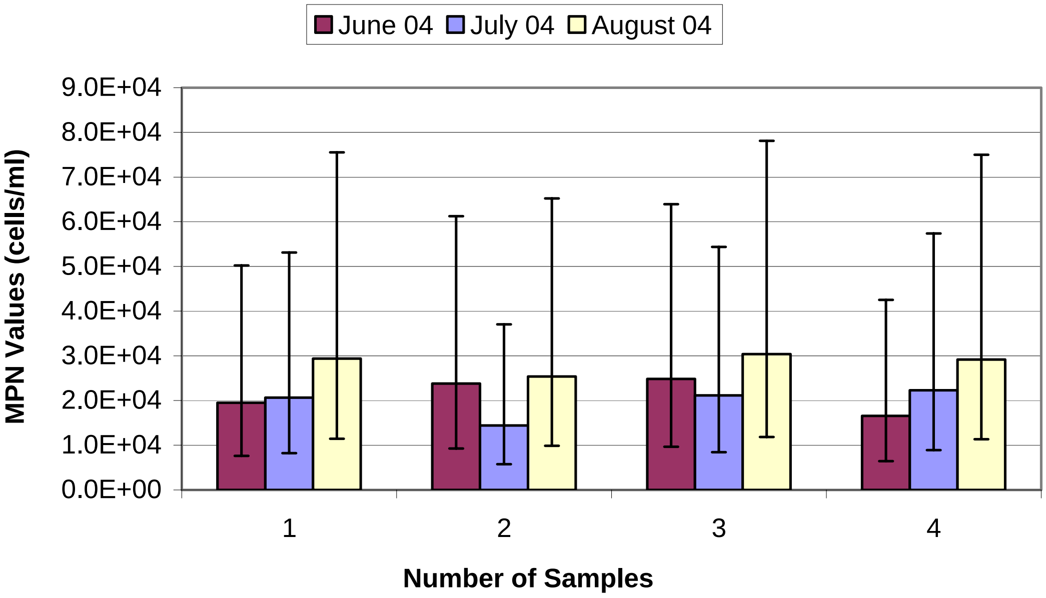

Figure 28. MPN Data of Aliphatic Degraders in Aeration Tank for Summer Season (Error bars represent upper and lower 95% confidence intervals for MPN Data)

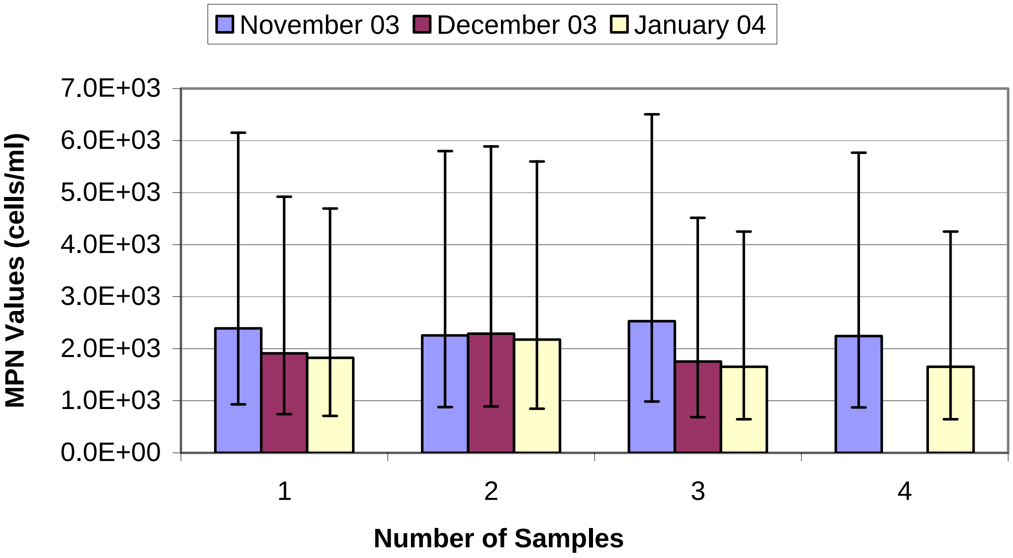

Figure 29. MPN Data of Aliphatic Degraders in Outfall Tank for Winter Season (Nov 03 – Jan 04) (Error bars represent upper and lower 95% confidence intervals for MPN Data)

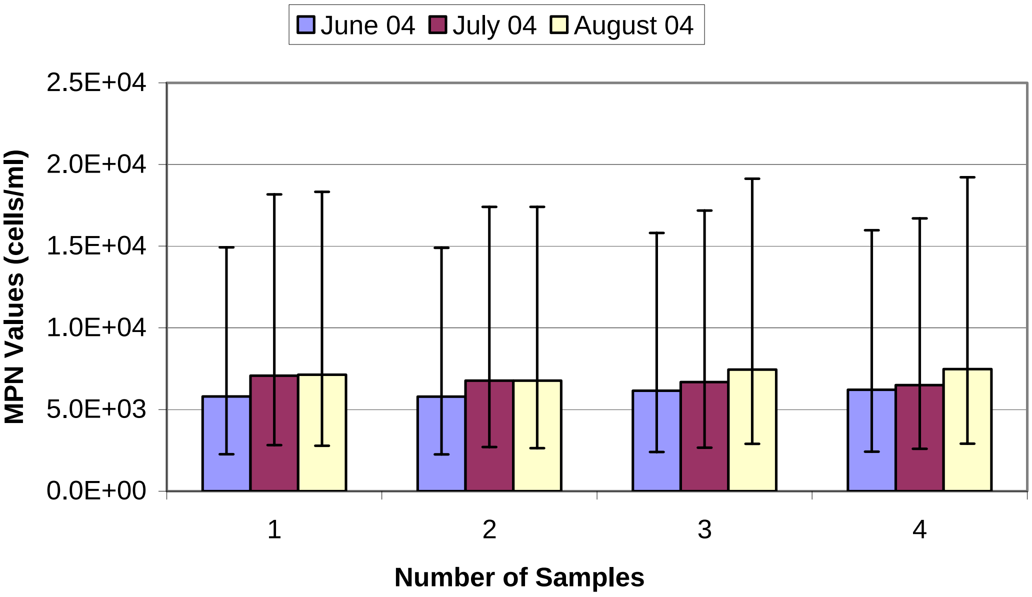

Figure 30. MPN Data of Aliphatic Degraders in Outfall Tank for Summer Season (Jun 04 – Aug 04) (Error bars represent upper and lower 95% confidence intervals for MPN Data)

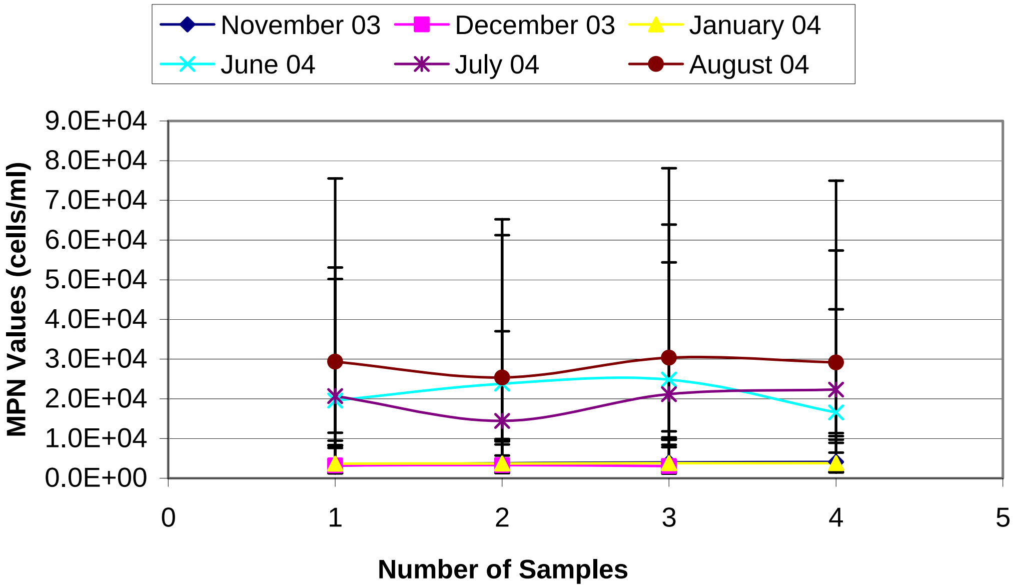

Figure 31. Comparison of MPN Values of Aliphatic Degraders in Aeration Tank for Winter and Summer Seasons (Error bars represent upper and lower 95% confidence intervals for MPN Data)

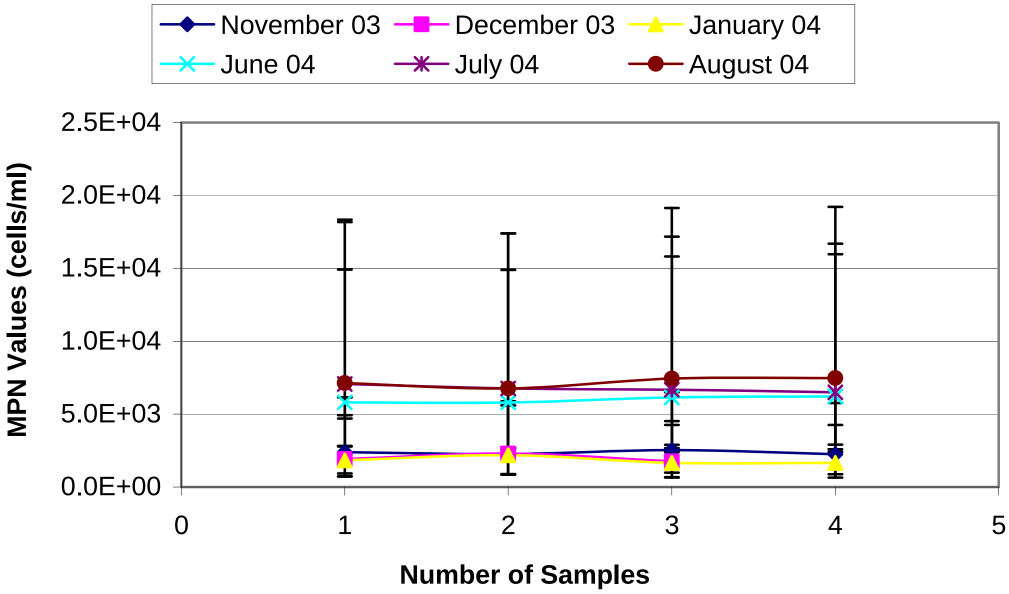

Figure 32. Comparison of MPN Values of Aliphatic Degraders in Outfall Tank for Winter and Summer Seasons (Error bars represent upper and lower 95% confidence intervals for MPN Data)

Therefore, microbial biomass is flowing out of the aeration tank into the outfall in both seasons. This phenomenon could be attributed to an earlier study where short circuiting was noticed in the aeration tank (Kramer *et al.*, 2002). The calculated mean cell residence time of the aeration tank is approximately 21 hours but the tracer study conducted in the study suggested that waste load entering the aeration basin begins to exit it after 2 hours (Kramer *et al.*, 2002). Therefore, along with the waste, suspended biomass was carried out of the aeration tank to the outfall.

The variation of microbial population in winter and summer months was due to higher biological activity in the aeration tank. Intense firefighting during summer months resulted in higher BOD concentration in summer than in winter. There was small increment in COD and TOC values from winter to summer, but comparable values in both winter and summer months indicated a constant supply of organic loading in the aeration. Therefore, the variation in the microbial population could not be solely attributed to the intense firefighting in summer.

The "Micro-Blaze" solution contains a microbial population of 2×10^{11}cells per gallon of the solution. At a constant rate of 10ml/min into the aeration tank, the microbial population should be on the order of 5.3×10^{7} cells/ml allowing for dilution effects. The average microbial population in the aeration tank was 3.5×10^{3} cells/ml in winter months and 2.3×10^{4} cells/ml in summer months. There was a 99% decrease in the population density from the initial feed and that measured in the tank. This decrease in population could be attributed to the nutrient and substrate availability in the system which is evaluated in the next section. Another reason is the short circuiting of the flow in the aeration tank which is responsible for the flow of suspended biomass from the aeration tank to the outfall, thereby decreasing the microbial population in the tank.

TOTAL PETROLEUM HYDROCARBONS (TPH) AND NUTRIENTS

Petroleum hydrocarbon biodegradation requires a balance of environmental factors such as nutrients, oxygen, temperature, pH, commensurate with the amount of organinc carbon. Since saturated n-alkanes ranging from C10 to C26 are the most readily degradable components in a petroleum mixture (Zobell 1946; Atlas 1981; 1995), and the hydrocarbon source to the plant ranges from C9 to C13, these hydrocarbons should be degraded easily. If the high organic load is not balanced by essential nutrients, biodegradation will be limited.

Total Petroleum Hydrocarbon method (TPH) values estimate the amount of petroleum hydrocarbon waste coming into the system. This was one of the important parameters that was measured to check the removal efficiency of the treatment plant and also to estimate the amount of substrate in the aeration tank for the hydrocarbon degraders. In both seasons, the system showed an overall hydrocarbon waste removal efficiency of 99% with about 87% removal occurring in the equalization tank (Tables 29 & 30). Evaporation losses of the lighter fraction of hydrocarbons and removal by the skimmer in the equalization tank accounts for the high percent removal of hydrocarbons. Effluent TPH values of 5.6 mg/l in winter and 5.1mg/l in summer were well below the TCEQ discharge limit of 12 mg/l (Figure 33). The average TPH value measured in the aeration tank was 32.1 mg/l in winter and 37.3 mg/l in summer months. Substrate required by the hydrocarbon degraders was estimated from the measured TPH values.

Table 29.

TPH Value for Winter Months (Nov 03 – Jan 04)

Sampling date	TPH (mg/l)	TPH (mg/l)	TPH (mg/l)	TPH (mg/l)
	INLET	EQ. TANK	AERATION	OUTFALL
11/3/2003	952	125	38	7
11/11/2003	1015	139	41	6
11/18/2003	926	120	35	5
11/25/2003	974	133	39	5
12/4/2003	1023	128	24	5
12/10/2003	957	121	26	6
12/19/2003	925	130	31	5
1/9/2004	914	138	28	5
1/15/2004	895	125	34	7
1/21/2004	1104	129	32	6
1/28/2004	1084	133	25	5
Average	**979.0**	**129.2**	**32.1**	**5.6**

Table 30.

TPH Value for Summer Months (Jun 04 – Aug 04)

Sampling date	TPH (mg/l)	TPH (mg/l)	TPH (mg/l)	TPH (mg/l)
	INLET	EQ. TANK	AERATION	OUTFALL
6/7/2004	1115	132	36	5
6/14/2004	1096	125	43	6
6/22/2004	1148	126	38	5
6/29/2004	1153	115	35	5
7/5/2004	1127	141	35	5
7/12/2004	1058	136	32	5
7/19/2004	1025	140	38	6
7/26/2004	1136	129	35	5
8/9/2004	1059	113	42	6
8/16/2004	1123	139	36	4
8/23/2004	1050	127	40	5
8/27/2004	986	107	38	4
Average	**1089.7**	**127.5**	**37.3**	**5.1**

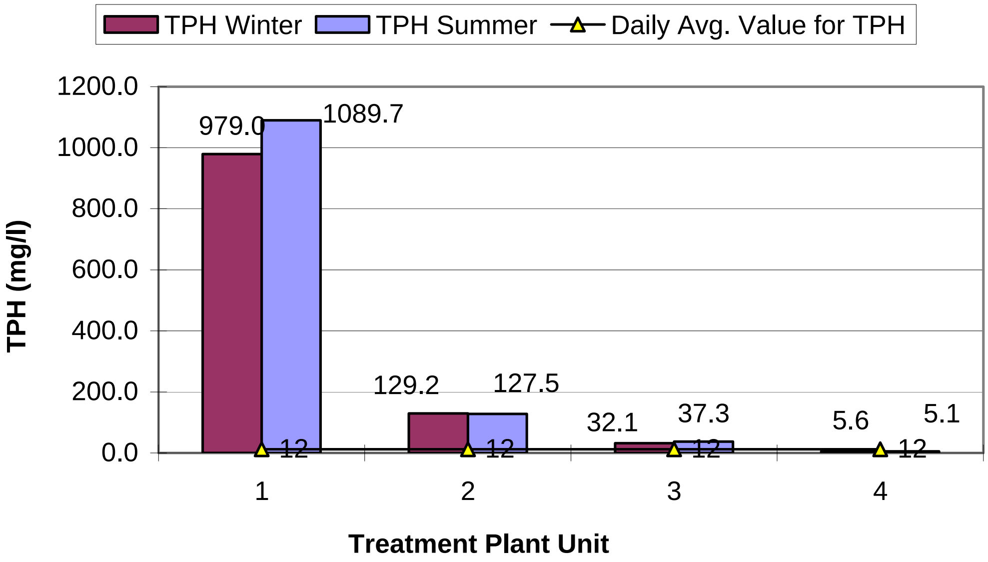

Figure 33. TPH Data for Winter and Summer Seasons

Stochiometric relationships can be used to estimate the minimal nutrients necessary to biodegrade a known quantity of organic material. Typical growth reactions for aerobic heterotrophic bacteria are as follows (Rittmann and McCarty, 2001).

Cell Synthesis Equation (R_c) with ammonia as nitrogen source

$$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O \qquad (9)$$

Electron Acceptor Equations (R_a) with oxygen as electron acceptor (aerobic)

$$\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O \qquad (10)$$

Suitable organic half-reactions to describe the degradation of aliphatic hydrocarbons stochiometrically can only be estimated due to the complexity of the mixture. Organic half-reactions for electron donors can be estimated from the custom organic half reaction such as (Rittmann and McCarty, 2001):

Custom Organic Half Reaction (R_d) for electron donor

$$\frac{(n-c)}{d}CO_2+\frac{c}{d}NH_4++\frac{c}{d}HCO_3^-+H^++e^-=\frac{1}{d}C_nH_aO_bN_c+\frac{(2n-b+c)}{d}H_2O$$

where $d=(4n+a-2b-3c)$ (11)

Brayton wastewater consists of C9 to C13 aliphatic straight chain hydrocarbons with molecular formulas ranging from C_9H_{20} to $C_{13}H_{28}$. Donor half reactions can be used to estimate the stochiometry and determine the quantity of nutrients needed. Donor equation pertaining to C_9H_{20} and $C_{13}H_{28}$ are :

Electron Donor Half Reaction (R_d) for C_9H_{20}

$$\frac{9}{56}CO_2+H^++e^-=\frac{1}{56}C_5H_{20}+\frac{18}{56}H_2O \tag{12}$$

Electron Donor Half Reaction (R_d) for $C_{13}H_{28}$

$$\frac{1}{80}CO_2+H^++e^-=\frac{1}{80}C_{13}H_{28}+\frac{26}{80}H_2O \tag{13}$$

When microorganisms use an electron donor substrate for synthesis, C9 to C13 aliphatic hydrocarbons in this case, a portion of electrons (f_e^0) is transferred to the electron acceptor to provide energy and with the remaining available electrons (f_s^0) available for cell synthesis. A standard value for f_s^0 is 0.6 and 0.4 for f_e^0 are stated for aerobic heterotrops utilizing substrates other than carbohydrates as electron donors and oxygen as electron acceptors (Rittmann and McCarty, 2001). The overall reaction (R) for biological growth is derived from the equation stated below:

$$R = f_e^{\,0} R_a + f_s^{\,0} R_c - R_d \tag{14}$$

The overall equation of microbial degradation of hydrocarbons C9 to C13 was derived. For C9 aliphatic hydrocarbon the overall reaction for biodegradation is

$$0.03NH_4^+ + 0.1O_2 + 0.03HCO_3^- + 0.017C_9H_{20} = 0.03C_5H_7O_2N + 0.4CO_2 + 0.15H_2O \tag{15}$$

and for C13 aliphatic hydrocarbons

$$\begin{aligned} 0.03NH_4^+ + 0.1O_2 + 0.03HCO_3^- + 0.0125C_{13}H_{28} = 0.03C_5H_7O_2N + 0.107CO_2 \\ +0.14H_2O \end{aligned} \tag{16}$$

Similar equations were derived for the other hydrocarbon fractions present in the waste.

Nitrogen and oxygen required by a given microbial biomass to biodegrade hydrocarbons were estimated. More accurate estimation based on the percent composition of specific hydrocarbons in the waste requires extensive characterization. Assuming C9 and C13 are the lightest and heaviest fraction of hydrocarbons, estimated values of the nitrogen and oxygen required to degrade those fractions will provide an estimate of the theoretical demand. Approximate theoretical average values of nitrogen and oxygen to degrade 1 gm of hydrocarbon from C9 to C13 were calculated from the overall stoichiometric equation and were found to be 0.19 gm of nitrogen and 1.43 gm of oxygen respectively. Assuming that nitrate and nitrite comes from ammonia consumption, the amount of ammonium in the system was calculated from the data collected for nitrate and nitrite in winter and summer season. The main source of nitrogen available to the microorganisms is through the ammonium present in the system. Therefore, total nitrogen (NH_4^+- N mg/l) present in every unit was calculated from the ammonium concentration. Phosphorus present in the system was quantified as orthophosphate.

Table 31.

Nitrate and Nitrite Value for Winter Months (Nov 03 – Jan 04)

Sampling date	Nitrate (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)
	INLET	**INLET**	**EQ. TANK**	**EQ. TANK**	**AERATION**	**AERATION**	**OUTFALL**	**OUTFALL**
11/3/2003	11.3	0.014	6.2	0.014	5.7	0.015	5.3	0.006
11/11/2003	21.9	0.083	5.6	0.004	4.7	0.007	4	0.008
11/18/2003	16.3	0.016	6.3	0.014	6.1	0.015	5.2	0.011
11/25/2003	8	0.017	6.2	0.015	6	0.021	5.9	0.039
12/4/2003	22.3	0.072	8.2	0.007	4.9	0.009	4.2	0.008
12/10/2003	25.7	0.081	6.7	0.014	5.3	0.014	4.7	0.007
12/19/2003	31.2	0.092	10.8	0.006	6	0.016	10	0.009
1/9/2004	18.5	0.084	11.4	0.013	5.1	0.008	4.5	0.006
1/15/2004	22.6	0.095	9.2	0.011	5.6	0.001	4.9	0.008
1/21/2004	15.5	0.073	8.7	0.008	5.2	0.013	4.1	0.011
1/28/2004	24.1	0.092	7.1	0.007	4.8	0.011	4.4	0.01
Average	**19.8**	**0.06**	**7.9**	**0.01**	**5.4**	**0.01**	**5.2**	**0.01**
Ammonia	**5.96**		**2.29**		**1.62**		**1.56**	
Total Nitrogen	**4.6**		**1.76**		**1.24**		**1.2**	

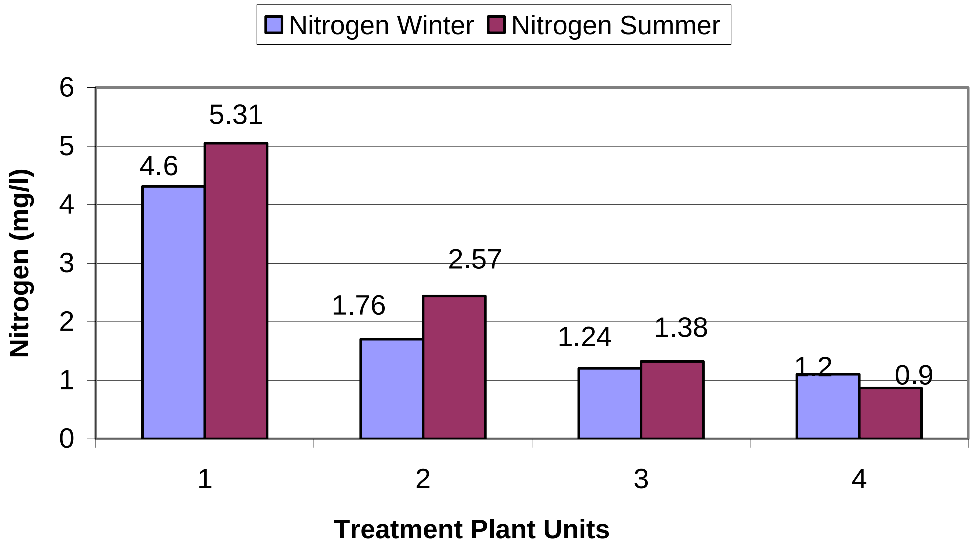

Figure 34. Total Nitrogen Data for Winter and Summer Seasons

Table 32.

Nitrate and Nitrite Value for Summer Months (Jun 04 – Aug 04)

Sampling date	Nitrate (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)
	INLET	INLET	EQ. TANK	EQ. TANK	AERATION	AERATION	OUTFALL	OUTFALL
6/7/2004	26.4	0.081	11.8	0.011	5.8	0.009	3.6	0.009
6/14/2004	25.8	0.078	10.5	0.012	6.1	0.011	3.9	0.01
6/22/2004	22.5	0.08	10.2	0.014	6.5	0.012	4	0.013
6/29/2004	18.3	0.074	11.2	0.015	6.8	0.014	4.1	0.012
7/5/2004	25.8	0.082	11.5	0.009	6.6	0.01	3.5	0.009
7/12/2004	26.4	0.079	11.8	0.012	6.3	0.013	3.4	0.011
7/19/2004	24.2	0.08	10.3	0.01	6.4	0.011	3.6	0.01
7/26/2004	22.3	0.085	10.4	0.01	6.7	0.013	3.3	0.008
8/9/2004	19.5	0.085	12.8	0.014	4.8	0.009	4.4	0.007
8/16/2004	24.5	0.09	11.5	0.013	5.6	0.005	4.7	0.009
8/23/2004	17.6	0.088	9.4	0.012	5.4	0.012	4.2	0.011
8/27/2004	21.4	0.094	11.6	0.014	5.1	0.013	4.5	0.01
Average	**22.9**	**0.08**	**11.1**	**0.01**	**6.0**	**0.01**	**3.9**	**0.01**
Ammonia	**6.9**		**3.34**		**1.8**		**1.17**	
TOTAL Nitrogen	**5.31**		**2.57**		**1.38**		**0.9**	

Ammonium values present in the treatment plant were calculated from the nitrate and nitrite values collected in both winter and summer (Tables 31 & 32). Total nitrogen values (NH_4^+-N mg/l) were estimated from the ammonium values. The average nitrogen concentration in the aeration tank was 1.24 mg/l in winter and 1.38 mg/l in summer (Figure 34). The average concentration of phosphorus in the aeration tank was 0.22mg/l in winter (Table 33) and 0.7 mg/l in summer (Table 34) and the trend was illustrated in Figure 35.

Table 33.

Phosphorus Value for Winter Months (Nov 03 – Jan 04)

Sampling date	Phosphate (mg/l)	Phosphate (mg/l)	Phosphate (mg/l)	Phosphate (mg/l)
	INLET	EQ. TANK	AERATION	OUTFALL
11/3/2003	2.24	1.07	0.79	0.71
11/11/2003	1.98	0.79	0.75	0.74
11/18/2003	2.03	0.77	0.73	0.7
11/25/2003	2.11	0.72	0.71	0.76
12/4/2003	1.74	0.71	0.67	0.74
12/10/2003	2.09	0.65	0.7	0.77
12/19/2003	1.18	0.48	0.39	0.86
1/9/2004	2.1	0.92	0.78	0.75
1/15/2004	2.24	0.84	0.72	0.81
1/21/2004	1.68	0.71	0.65	0.61
1/28/2004	1.89	0.75	0.61	0.68
Average	**1.93**	**0.76**	**0.68**	**0.74**
Phosphorus	**0.62**	**0.25**	**0.22**	**0.24**

Table 34.

Phosphorus Value for Summer Months (Jun 04 – Aug 04)

Sampling date	Phosphate (mg/l)	Phosphate (mg/l)	Phosphate (mg/l)	Phosphate (mg/l)
	INLET	**EQ. TANK**	**AERATION**	**OUTFALL**
6/7/2004	3.61	1.86	2.05	0.78
6/14/2004	3.52	1.93	2.11	0.89
6/22/2004	3.31	2.12	2.32	0.91
6/29/2004	3.22	1.98	2.15	0.75
7/5/2004	3.57	1.76	2.15	0.75
7/12/2004	3.62	1.73	2.26	0.82
7/19/2004	3.45	1.92	2.14	0.93
7/26/2004	3.15	1.84	2.03	0.8
8/9/2004	3.5	1.93	2.2	0.78
8/16/2004	3.86	1.86	2.51	0.89
8/23/2004	3.42	1.88	2.16	0.91
8/27/2004	2.88	2.14	2.35	0.75
Average	**3.43**	**1.91**	**2.20**	**0.83**
Phosphorus	**1.1**	**0.61**	**0.7**	**0.26**

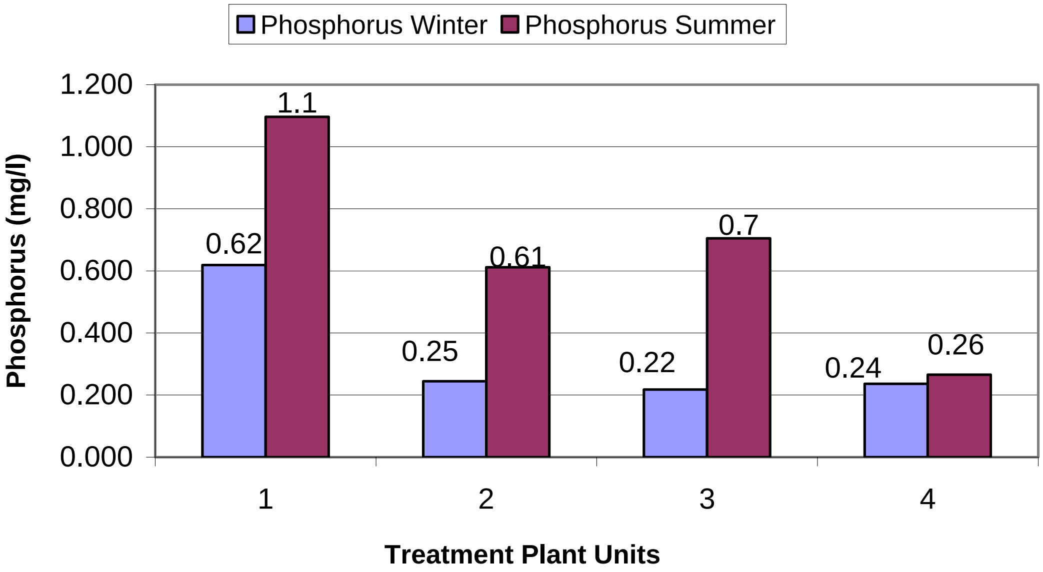

Figure 35. Total Phosphorus Data for Winter and Summer Seasons

Nutrient consumption in the treatment plant was estimated by first calculating the rate of nutrient consumption followed by the effluent concentration of nutrient at the aeration tank. This procedure is illustrated by Rittmann and McCarty (2001) and nutrient deficiency in the system was checked through this procedure.

Rate of substrate utilization (r_{ut}) is calculated by

$$r_{ut} = -\frac{(S^0 - S)}{\theta_x} \tag{17}$$

where S^0 and S are initial and final substrate concentration, and θ_x is the means cell residence time or sludge age.

Rate of nutrient consumption (r_n) is estimated by:

$$r_n = \gamma_n Y r_{ut} \frac{1 + (1 - f_d) b\theta_x}{1 + b\theta_x} \tag{18}$$

where r_n = rate of nutrient consumption,

γ_n = the stochiometic ratio of nutrient mass to Volatile Suspended Solids (VSS) for biomass

f_d = fraction of active biomass that is biodegradable

b = endogenous decay coefficient.

An overall mass balance on nutrient consumption is given by:

$$C_n = C_n^{\ 0} + r_n \theta_x \tag{19}$$

where C_n^0 and C_n are the influent and effluent nutrient concentrations.

Some assumptions were necessary to calculate the nutrient consumption in the aeration tank. The initial and final substrate concentrations in the aeration tank were assumed based on the TPH values measured in both winter and summer months. Influent TPH values in the aeration were 129.2 mg/l and 127.5 mg/l in winter and summer months, respectively. These values were assumed as initial substrate concentration. Final substrate concentrations in the aeration tank were 32.1 mg/l and 37.3 mg/l for winter and summer months, respectively. Mean cell residence time (θ_x) is assumed to be 21 hours or 0.875 d^{-1} which is documented in a previous project done at the Brayton wastewater treatment plant (Kramer *et al.*, 2002). For the empirical formula for bacterial VSS, $C_5H_7O_2N$, γ_n was calculated to be 14 g N / 133 g VSS or 0.124 g N/ g VSS (Rittmann and McCarty, 2001). Phosphorus requirement was assumed to be 20% of the nitrogen requirement and γ_p was calculated to be 0.025 g P / 133 g VSS (Rittmann and McCarty, 2001). The endogenous decay rate (b) varies with the species of microorganisms, but for aerobic heterotrophs b values range from 0.1 to 0.3/d (Rittmann and McCarty, 2001). For the nutrient consumption calculation, values of b are assumed to be 0.15/d. Generally fraction of active biomass that is biodegradable (f_d) is assumed be 0.8 or 80% (Rittmann and McCarty, 2001). The influent nutrient concentrations used for the calculation were the measured average values of nitrogen and phosphorus in both winter and summer months. Influent concentrations in the aeration tank for nitrogen were 1.24 mg/l in winter and 1.38 mg/l in summer months. Similarly, the measured average values for phosphorus in the aeration tank were 0.22 mg/l and 0.7 mg/l.

Nitrogen consumption rates for winter and summer months were calculated. Nitrogen consumption rates of 5.24 mg N/l-d and 4.87 mg N/l-d were calculated for winter and summer months, respectively. Corresponding of phosphorus consumption rates for winter and summer month were calculated to be 1.05 mg P/l-d and 0.97 mg P/l-d, respectively. Effluent nitrogen and phosphorus in both seasons were calculated from the mass balance equation. The effluent concentrations of nitrogen were -3.34 mg N/l and -2.88 mg N/l for winter and summer months. Similarly, effluent concentrations for phosphorus were calculated to be – 0.7 mg P/l and -0.15 mg P/l for winter and summer months. The negative values of effluent concentration signify that there was deficiency in the concentration of nitrogen and phosphorus in the aeration tank Therefore, in the winter season (Nov 03 – Jan 04), there was deficiency in the concentration of nitrogen and phosphorus by 3.34 mg N/l and 0.7 mg P/l, whereas in summer months the deficiency was by 2.88 mg N/l and 0.15 mg P/l, respectively.

Biostimulation was recommended by the manufacturer of the microbial consortium. They recommended the addition of dog food to the aeration basin. A commercially available dog food “Clover Brand Hunters Special” (Farmland Industries Inc, Kansas Missouri) was used as a nutrient supplement. It contains 22% crude protein which provides a source of nitrogen and phosphorus ranging from 0.5% to 1.6%. Crude protein contains 16% of nitrogen. According to the specification provided, a 40 lb bag of dog food was added to in the aeration tank three times a week. Therefore, 120 lbs of dog food was added to the aeration tank whose volume is 871,000 gallons (3292380 liters). Assuming complete dissolution the concentration of nitrogen added from dog food is 0.58 mg N/l (0.19 mg N/l per bag) and phosphorus is 0.16 mg P/l (0.05 mg P/l per bag) assuming 0.8% (average value) content. Therefore, addition of current quantity of dog food (3 bags) was insufficient in meeting the nutrient deficiency in the aeration tank.

Complying with microbial consortium manufacturer's recommendation of adding dog food as additional nutrient source, it was evident that more dog food was required. There was a deficiency of 3.34 mg N/l and 2.88 mg N/l in nitrogen concentration for winter and summer seasons. Since each bag of dog food contributes to 0.19 mg/l of nitrogen, about 16 bags of dog food has to be added to the aeration tank. However, approximately 50% of this dog food is carbohydrates. For simplicity, it is assumed that glucose represents the carbohydrates in the dog food. For 16 bags of dog food, approximately 44 mg/l of carbohydrate would be added in the aeration tank. Assuming that all carbohydrates are in the form of glucose, the overall stochiometric equations are derived to estimate the additional oxygen and nutrient demand. Overall stochiometric equation for glucose (carbohydrate) removal is as follows:

$$0.041C_6H_{12}O_6 + 0.075O_2 + 0.035HCO_3^- + 0.035NH_4^+ = 0.035C_5H_7O_2N + 0.11CO_2 + 0.215H_2O \qquad (20)$$

From the above equation, 66 mg of nitrogen and 0.32 gm of oxygen is required to remove 1 gm of glucose by the microbes. Removal of 44 mg/l of carbohydrates from the aeration tank would require 2.9mg/l of nitrogen and 0.58 mg/l of phosphorus. The added COD, BOD and TOC concentrations would be 14 mg/l, 9.7 mg/l and 17.6 mg/l, respectively. The constituents of carbohydrates in the dog food were not given and longer chains of carbohydrates are likely to exist. However, using glucose provides a means to estimate the new load and nutrient requirement which is illustrated in Table 35. Therefore increasing the number of dog food would increase the BOD loading as well as nutrient requirements in the system. The objective of nutrient supplement for the hydrocarbon degraders would never be achieved with the application of dog food.

Table 35.

Final Nutrient Conditions in the Aeration Tank.

Final Nutrient Conditions in the Aeration tank	Winter mg N/l	Summer mg N/l
Nitrogen deficit for hydrocarbons	3.34	2.88
Additional Nitrogen demand for dog food	2.9	2.9
	mg P/l	mg P/l
Phosphorus deficit for hydrocarbons	0.7	0.15
Additional Phosphorus demand for dog food	0.58	0.58

OXYGEN REQUIREMENT

The other limiting factor controlling aerobic biodegradation for high organic loads is the availability of the oxygen in the system. In the current system three high speed mechanical floating aerators of 15 hp each is supplying oxygen to the aeration tank. In the field condition the typical transfer rate of oxygen of these kinds of aerators have a range of 1.2-2.0 lb oxygen per hp per hr (Tchobanoglous *et al.*, 2003). Assuming the average transfer rate of 1.6 lb oxygen per hp per hr and total power input of 45 hp, the amount of oxygen concentration in the aeration tank was calculated as 238mg/l. Therefore, the rate of supply of oxygen by the aerators in the aeration tank was calculated to be 8.95×10^5 g O_2/d. Oxygen concentration in the current system is sufficient to degrade the amount of hydrocarbon in the aeration tank. The ratio of oxygen to hydrocarbon in aeration tank in winter and summer months range from 6:1 to 7:1 which is better than documented ratio of 3:1 in freshwater systems (Hughes and Mckenzie, 1975). Ratio of theoretical oxygen demand to hydrocarbon degraded is calculated from overall degradation reaction. Approximately 1.57 gm of oxygen is required to degrade 1 gm of hydrocarbons. Theoretical oxygen demand in the aeration tank was calculated from the measured TPH values assuming that all TPH was exerted simultaneously (Table 36). This was done to check the oxygen demand in the aeration tank to the oxygen supplied by the aerators.

Table 36.

Theoretical Oxygen Demand Corresponding to TPH Values in Aeration Tank.

Sampling date	TPH (mg/l)	Theoretical Oxygen Demand (mg/l)
	AERATION	
11/3/2003	38	59.7
11/11/2003	41	64.4
11/18/2003	35	55.0
11/25/2003	39	61.2
12/4/2003	24	37.7
12/10/2003	26	40.8
12/19/2003	31	48.7
1/9/2004	28	44.0
1/15/2004	34	53.4
1/21/2004	32	50.2
1/28/2004	25	39.3
6/7/2004	36	56.5
6/14/2004	43	67.5
6/22/2004	38	59.7
6/29/2004	35	55.0
7/5/2004	35	55.0
7/12/2004	32	50.2
7/19/2004	38	59.7
7/26/2004	35	55.0
8/9/2004	42	65.9
8/16/2004	36	56.5
8/23/2004	40	62.8
8/27/2004	38	59.7
Average	**34.8**	**54.7**

TEMPERATURE

Temperature data of the local area Bryan College Station was sourced in from National Weather Forecast Office website and the average temperature of winter months and summer months is complied and tabulated below (Table 37).

Table 37.

Average Monthly Temperature in Bryan College Station for Winter and Summer Seasons (College Station Climate Data, 2004)

Month	Fahrenheit	Celsius
November	63.1	17.2
December	52.6	11.4
January	53.1	11.7
June	80.2	26.7
July	82.9	28.2
August	82.3	27.9

From the temperature data, summer temperatures provided favorable conditions for biodegradation, as temperature of 20 to 25 °C is perfect for optimum biodegradation year round (Bossert and Bartha, 1984). Colder temperature in the winter months may have reduced the biodegradation rate in the system.

RECOMMENDATIONS

To improve the biological degradation of petroleum hydrocarbons in the Brayton wastewater treatment plant and to increase the population of hydrocarbon degraders in the system more nutrients are essential. Nutrients are supplied through the addition of dog-food, but do not meet the required minimum nitrogen and phosphorus for the quantity of hydrocarbons being loaded into the plant. Use of dog food was recommended by the manufacturer of the microbial consortium as a nutrient supplement. Current quantity of 3 bags of dog food that is put in the aeration tank is insufficient to meet the demands of nitrogen and phosphorus. Nutrient deficiency in the aeration tank was calculated and an additional 16 bags of dog food would be sufficient to meet the demands for the hydrocarbons present. However, the dog food increases the BOD, TOC and TOC loading in the system as well as nutrient requirements. The objective of nutrient supplement for hydrocarbon degraders would never be achieved. Dog food would also increase TDS, TSS and turbidity in the aeration tank. The BOD loading from dog food was in the form of carbohydrate, which would result in substrate deficiency for the petroleum degraders resulting in the decrease of their population. Application of dog food for nutrient supplement will not improve hydrocarbon degradation. No amount of dog food will meet nutrient deficiency in the aeration tank because of the organic load this food adds. Other options of nutrient supplement have to be considered for the biodegradation of petroleum hydrocarbon without affecting the BOD loading and other parameters in the system.

More detailed investigation is required to come up with accurate recommendation in the plant as very less is known about the various types and species of microbes present in the system which is affecting the growth of hydrocarbon degraders in the system. Recycle of the active biomass in the system will increase population numbers which should improve performance. These are some of the important aspects that are to be investigated more to come up with accurate recommendation of improving the biological processes in the plant.

CONCLUSION

An evaluation of the performance of the biological treatment of petroleum hydrocarbon by the hydrocarbon degrading microbes at the Brayton Fire School's wastewater treatment plant was the main research objective. After each firefighting operation, water-hydrocarbon mixture flows to the treatment plant where they are biologically treated under aerobic conditions to reduce the hydrocarbon concentration before they are discharged to White Creek, which flows to the Brazos River. The research was organized as follows:-

1. Identification of the hydrocarbon degrading microorganism and their metabolism of hydrocarbons.
2. Evaluation of the biological processes evaluating a correlation between the bacterial count and other parameters such as nutrient requirements, aeration, temperature, pH.
3. Comparison of seasonal wastewater treatment performance parameters by taking analytical data in both winter (Nov 2003 – Jan 2004) and summer months (Jun 2004 – Aug 2004) and to evaluate the removal efficiency.

The wastewater treatment plant is an activated sludge system consisting of four basic units. Aeration tank is the unit where biological degradation of hydrocarbon waste generated from the firefighting takes place. Biological degradation of C9 to C13 hydrocarbons was studied along with application of bioaugmentation and biostimulation in a wastewater treatment plant where petroleum hydrocarbon is the main contaminant.

Bioaugmentation is carried out in the wastewater treatment plant at the Brayton Fire School is the introduction of exogenous hydrocarbon degraders. The hydrocarbon degraders used is a commercially available product called “Micro-Blaze Liquid Spill Control” from Verde Environmental (Houston, Texas). Micro-Blaze contains several strains of non-pathogenic, spore forming, facultative bacteria, *Bacillus*. *Bacillus* strain microbes are introduced in the system as a microbial solution at a constant rate of 10ml/min which introduces about 5.3×10^{7} cells/ml. The microbial population in the system was enumerated by calculating MPN data for alkane degraders for aeration and outfall. There was a significant decrease in the population of microbes that was originally fed to the system and that found in the aeration tank. The average microbial population during the winter months was 3.5×10^{3} cells/ml in the winter months and summer months was 2.3×10^{4} cells/ml.

Biostimulation in this system was achieved by adding dog food as a nutrient supplement. The concentration of nitrogen and phosphorus was evaluated to check the nutrient levels and the levels was found lower than what is desired for effective biodegradation of the hydrocarbons. Application of dog food as a biostimulant was analyzed and additional amount of dog food required to compensate for the nutrient deficiency in the system was calculated. Dog food is not the best option for biostimulation in the system as the application of dog food increases the organic loading, oxygen and nutrient demand, TSS, TDS, turbidity. Dog food will result in the decrease of the population of hydrocarbon degraders due to substrate limitation due to organic loading other than hydrocarbons. Aeration provided in the tank by the mechanical aerators was adequate for the current concentration of hydrocarbon but increased application of dog food would increase the oxygen demand. The measured pH range in the system from upwards of 7 to just above 8 is ideal for the Bacillus strain hydrocarbon degraders. Another aspect of the research was to monitor the general performance of the wastewater treatment plant and measure the effluent parameters for two seasons. None of the effluent parameters measured in the treatment plant violated the effluent standards in the TCEQ permit.

This research is a small pilot project for the wastewater treatment plant at Brayton Fire Training School regarding biological evaluation. There has been one prior project conducted in evaluating the general performance of the plant (Kramer *et al.*, 2002) but no studies have been conducted in evaluating the biological processes in the plant. From that research valuable information regarding the retention time of the aeration is obtained. The calculated mean cell residence time of the aeration tank is approximately 21 hrs but the tracer study conducted on the previous study suggested that waste load entering the aeration basin begins to exit it after 2 hours. This means there is short circuiting of the flow in the aeration tank which affects the performance of the treatment plant overall. This reduces contact time of the waste with the microbial population which results in the less efficient removal of BOD in the system. This research was necessary to determine the microbial population in the system and to determine the parameters affecting biodegradation. Further extensive research is required to evaluate the diurnal variation in the population of the microbes and the available nutrients in the system. Such an effort is necessary to identify specific changes to improve system performance.

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APPENDIX A

MONITORING RESULTS FOR THE WINTER (NOV 03 – JAN 04) AND SUMMER MONTHS

Monitoring Results for the Wastewater Treatment Plant at Brayton Fire Training School (November 2003)

Inlet tank

Sampling date	ALK mg/l as CaCo$_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
11/3/2003	486.6	4.6	95.1	1051.5	1156	6.7	133.8	90	640	11.3	0.014	2.24	132	0.1307	952
11/11/2003	376.3	4.8	163.2	1100	1063	8.2	253.8	140	830	21.9	0.083	1.98	220	0.1421	1015
11/18/2003	388.3	4.7	135	969.3	1083	8.2	320.5	180	710	16.3	0.016	2.03	179	0.1349	926
11/25/2003	324.3	4.6	64.1	991	1092	8.39	178.3	160	770	8	0.017	2.11	89	0.1227	974

Equalization

Sampling date	ALK mg/l as CaCo$_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
11/3/2003	473.4	5.6	37	196	1092	8.47	7.9	30	680	6.2	0.014	1.07	52	0.1478	125
11/11/2003	376.3	5.4	37.7	317.5	1098	8.28	39.3	40	790	5.6	0.004	0.79	50	0.1766	139
11/18/2003	381.3	5.6	43.9	245	1106	8.42	55.7	60	630	6.3	0.014	0.77	62	0.1702	120
11/25/2003	336.3	5.5	30.2	226	1097	8.3	42.9	70	640	6.2	0.015	0.72	41	0.1856	133

Aeration

Sampling date	ALK mg/l as CaCo$_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
11/3/2003	437.1	6.2	25.4	77	1121	8.58	12.6	20	640	5.7	0.015	0.79	35	0.3015	38
11/11/2003	395.3	6.5	29.4	148.5	1112	8.46	34	30	660	4.7	0.007	0.75	40	0.324	41
11/18/2003	394.3	6.4	30.7	89	1120	8.38	38.9	40	590	6.1	0.015	0.73	41	0.2964	35
11/25/2003	329.3	6.7	19.2	87	1116	8.3	13.1	50	570	6	0.021	0.71	27	0.3271	39

Outfall

Sampling date	ALK mg/l as CaCo$_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
11/3/2003	409.6	6	16.3	32.5	1147	8.3	16	20	630	5.3	0.006	0.71	22	0.31	7
11/11/2003	363.3	6.5	18.3	40	1096	8.43	8.9	10	550	4	0.008	0.74	25	0.3346	6
11/18/2003	376.3	6.3	16.7	33.75	1076	8.25	11.2	20	460	5.2	0.011	0.7	23	0.3157	5
11/25/2003	347.3	6.6	9.6	32	1108	8.46	6.9	10	420	5.9	0.039	0.76	13	0.3567	5

TPDES PERMIT AT DISCHARGE

Daily Max/Min	NA	4.00	80	200	NA	9.00/6.00	NA	200	2000	NA	NA	NA	NA	1.77	15
Daily Avg	NA	NA	40	NA	NA		NA	90	NA	NA	NA	NA	NA	0.835	12

TableA1 - Monitoring Result for themonth of November 2003

Monitoring Results for the Wastewater Treatment Plant at Brayton Fire Training School (December 2003)

Inlet tank

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
12/4/2003	415.4	4.5	98.3	146	1076	8.1	180.8	160	680	22.3	0.072	1.74	134	0.1467	1023
12/10/2003	379.3	4.6	89.6	137	1098	8.15	157.8	140	740	25.7	0.081	2.09	121	0.1356	957
12/19/2003	450.4	4.8	89.3	123	1123	7.85	172.3	180	650	31.2	0.092	1.18	125	0.1481	925

Equalization

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
12/4/2003	385.3	5.8	41.3	47	1086	7.98	41.3	50	620	8.2	0.007	0.71	58	0.1892	128
12/10/2003	381.3	5.7	44.9	44	1121	8.17	38	40	680	6.7	0.014	0.65	62	0.1689	121
12/19/2003	426.4	5.6	40	51	1110	7.93	35	70	610	10.8	0.006	0.48	55.6	0.1741	130

Aeration

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
12/4/2003	403.4	6.8	41.4	24	1137	8.03	21.6	40	550	4.9	0.009	0.67	57	0.2064	24
12/10/2003	395.3	7	38.6	29	1126	8.2	17	20	670	5.3	0.014	0.7	54	0.2684	26
12/19/2003	411.4	7.2	40.9	26	1096	8.02	19.1	40	540	6	0.016	0.39	56	0.3146	31

Outfall

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
12/4/2003	387.3	6.4	22.4	33	1155	8.25	10.7	10	520	4.2	0.008	0.74	31	0.2374	5
12/10/2003	366.3	6.2	20.3	30	1086	8.27	15	20	630	4.7	0.007	0.77	28	0.3024	6
12/19/2003	440.4	6..4	20.4	37	1119	7.7	14	20	550	10	0.009	0.86	28	0.3257	5

TPDES PERMIT AT DISCHARGE

Daily Max/Min	NA	4.00	80	200	NA	9.00/6.00	NA	200	2000	NA	NA	NA	NA	1.77	15
Daily Avg	NA	NA	40	NA	NA		NA	90	NA	NA	NA	NA	NA	0.835	12

Table A2– Monitoring Result for the month of December 2003

Monitoring Results for the Wastewater Treatment Plant at Brayton Fire Training School (January 2004)

Inlet tank

Sampling date	ALK mg/l as CaCo$_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
1/9/2004	342.1	4	78.5	815	1057	7.9	165.2	180	730	18.5	0.084	2.1	154	0.1495	914
1/15/2004	339.5	4.4	83.2	910	1104	8.16	189.2	150	710	22.6	0.095	2.24	138	0.1458	895
1/21/2004	373.6	4.9	93.5	977	1086	8.25	254.6	180	770	15.5	0.073	1.68	192	0.1385	1104
1/28/2004	363.8	4.5	88.4	859	1062	7.85	172	140	680	24.1	0.092	1.89	139	0.1416	1084

Equalization

Sampling date	ALK mg/l as CaCo$_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
1/9/2004	346.5	6.4	38.2	175	1044	7.5	52.4	70	650	11.4	0.013	0.92	68	0.1865	138
1/15/2004	375.5	6.2	35.1	183	1125	8.23	48.6	60	620	9.2	0.011	0.84	50	0.1786	125
1/21/2004	412.3	6.2	40.6	240	1073	8.42	65.8	60	680	8.7	0.008	0.71	62	0.1754	129
1/28/2004	380.4	6.5	33.6	196	1105	8	43.5	50	610	7.1	0.007	0.75	41	0.181	133

Aeration

Sampling date	ALK mg/l as CaCo$_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
1/9/2004	365.4	6.8	26.5	74	1124	7.3	16.6	40	520	5.1	0.008	0.78	38	0.2135	28
1/15/2004	352.2	7	22.4	82	1158	8.1	18.2	30	560	5.6	0.001	0.72	45	0.1962	34
1/21/2004	386.2	7.2	23.2	81	1121	8.22	23.1	20	510	5.2	0.013	0.65	39	0.2247	32
1/28/2004	382.7	7	25.5	78	1135	7.5	17.2	20	530	4.8	0.011	0.61	41	0.173	25

Outfall

Sampling date	ALK mg/l as CaCo$_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
1/9/2004	342.1	6.8	14.2	34	1130	8.1	11.6	20	490	4.5	0.006	0.75	22	0.223	5
1/15/2004	339.5	6.5	15.6	29	1146	8	14.3	20	520	4.9	0.008	0.81	24	0.1856	7
1/21/2004	373.6	6.6	12.5	31	1087	8.13	16.3	10	500	4.1	0.011	0.61	28	0.2034	6
1/28/2004	363.8	6.9	13.5	33	1061	7.8	12.4	20	480	4.4	0.01	0.68	23	0.1624	5

TPDES PERMIT AT DISCHARGE

Daily Max/Min	NA	4.00	80	200	NA	9.00/6.00	NA	200	2000	NA	NA	NA	NA	1.77	15
Daily Avg	NA	NA	40	NA	NA		NA	90	NA	NA	NA	NA	NA	0.835	12

Table A3– Monitoring Results for the month of January 2004

Monitoring Results for the Wastewater Treatment Plant at Brayton Fire Training School (June 2004)

Inlet tank

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
6/7/2004	382.5	4.5	88.5	774	1154	7.65	198	180	770	26.4	0.081	3.61	185	0.2845	1115
6/14/2004	359.3	4.2	82.4	825	1062	7.83	194.4	160	730	25.8	0.078	3.52	192	0.2556	1096
6/22/2004	371.3	3.8	95.2	814	1139	8.2	204.6	170	740	22.5	0.08	3.31	182	0.2754	1148
6/29/2004	338.3	4.2	93.3	796	1086	7.92	220.5	170	710	18.3	0.074	3.22	194	0.2853	1153

Equalization

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
6/7/2004	373.3	6.2	35.2	187	1126	7.44	58.6	75	580	11.8	0.011	1.86	68	0.2412	132
6/14/2004	364.5	6.5	37.5	215	1058	7.25	63.4	65	610	10.5	0.012	1.93	70	0.2213	125
6/22/2004	357.5	6	35.6	226	1098	7.56	61.2	70	630	10.2	0.014	2.12	74	0.2354	126
6/29/2004	311.3	6.4	38.4	199	1033	7.6	55.2	60	590	11.2	0.015	1.98	76	0.2325	115

Aeration

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
6/7/2004	361.3	7.5	24.5	72	1110	7.15	22.7	35	510	5.8	0.009	2.05	48	0.2235	36
6/14/2004	355.3	7.4	25.1	74	1034	7.12	20.5	30	520	6.1	0.011	2.11	52	0.211	43
6/22/2004	363.5	7.2	22.6	83	1056	7.34	18.3	30	540	6.5	0.012	2.32	46	0.225	38
6/29/2004	348.3	7.1	24.2	75	1010	7.7	17.9	25	490	6.8	0.014	2.15	50	0.1934	35

Outfall

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
6/7/2004	362.3	6.8	14.8	38	1085	7.33	10.4	20	420	3.6	0.009	0.78	24	0.1845	5
6/14/2004	345.3	6.5	12.4	35	1025	7.35	11.8	10	440	3.9	0.01	0.89	19	0.1765	6
6/22/2004	352.3	6.6	14.1	32	1055	7.26	13.5	15	440	4	0.013	0.91	20	0.1687	5
6/29/2004	327.3	6.4	12.2	33	1026	7.75	10.6	10	410	4.1	0.012	0.75	18	0.1612	5

TPDES PERMIT AT DISCHARGE

Daily Max/Min	NA	4.00	80	200	NA	9.00/6.00	NA	200	2000	NA	NA	NA	NA	1.77	15
Daily Avg	NA	NA	40	NA	NA		NA	90	NA	NA	NA	NA	NA	0.835	12

Table A4 – Monitoring Results for the month of June 2004

Monitoring Results for the Wastewater Treatment Plant at Brayton Fire Training School (July 2004)

Inlet tank

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
7/5/2004	375.3	4.4	91.5	845	1123	7.74	212.5	170	760	25.8	0.082	3.57	193	0.2421	1127
7/12/2004	334.3	3.9	98.2	862	1136	7.65	198.3	150	750	26.4	0.079	3.62	186	0.2611	1058
7/19/2004	369.3	4.1	94.6	795	1082	8.15	224.8	160	720	24.2	0.08	3.45	190	0.257	1025
7/26/2004	342.3	4.2	95.4	813	1065	8.1	205.5	155	730	22.3	0.085	3.15	182	0.2576	1136

Equalization

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
7/5/2004	363.3	6.3	38.5	204	1095	7.44	64.2	60	590	11.5	0.009	1.76	71	0.2351	141
7/12/2004	311.5	6.5	35.8	215	1063	7.32	62.7	70	620	11.8	0.012	1.73	69	0.2236	136
7/19/2004	347.3	6.4	37.2	228	1035	7.82	60.6	60	610	10.3	0.01	1.92	75	0.2152	140
7/26/2004	325.3	6.1	39.5	210	1074	7.95	57.8	60	580	10.4	0.01	1.84	70	0.229	129

Aeration

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
7/5/2004	341.3	7.8	22.4	73	1106	7.26	19.6	30	500	6.6	0.01	2.15	45	0.2014	35
7/12/2004	326.3	7.6	23.1	71	1057	7.3	17.3	35	530	6.3	0.013	2.26	42	0.2115	32
7/19/2004	351.3	7.2	22.7	76	1043	7.22	21.7	35	550	6.4	0.011	2.14	51	0.2071	38
7/26/2004	335.3	7.3	21.2	74	1100	7.54	21.5	30	480	6.7	0.013	2.03	47	0.1932	35

Outfall

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
7/5/2004	332.3	6.9	12.1	36	1092	7.31	12.5	15	390	3.5	0.009	0.75	23	0.1723	5
7/12/2004	336.3	6.4	11.5	31	1043	7.28	13.8	10	420	3.4	0.011	0.82	24	0.1653	5
7/19/2004	375.5	6.1	13.2	32	1064	7.15	10.6	15	400	3.6	0.01	0.93	20	0.155	6
7/26/2004	343.3	6.2	12.8	30	1083	7.23	12.4	10	410	3.3	0.008	0.8	21	0.1605	5

TPDES PERMIT AT DISCHARGE

Daily Max/Min	NA	4.00	80	200	NA	9.00/6.00	NA	200	2000	NA	NA	NA	NA	1.77	15
Daily Avg	NA	NA	40	NA	NA		NA	90	NA	NA	NA	NA	NA	0.835	12

Table A5– Monitoring Results for the month of July 2004

Monitoring Results for the Wastewater Treatment Plant at Brayton Fire Training School (August 2004)

Inlet tank

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
8/9/2004	345.3	4.2	89.2	835	1062	8.1	163.6	170	740	19.5	0.085	3.5	215	0.2511	1059
8/16/2004	361.5	4	78.6	810	1134	7.58	178.6	180	720	24.5	0.09	3.86	186	0.2833	1123
8/23/2004	341.3	4.3	82.4	832	1091	8.25	194.2	150	710	17.6	0.088	3.42	195	0.2685	1050
8/27/2004	325.3	4.2	90.3	828	1073	8.3	214.5	160	690	21.4	0.094	2.88	189	0.324	986

Equalization

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
8/9/2004	332.3	5.8	37.2	172	1029	7.65	55.4	65	610	12.8	0.014	1.93	78	0.2336	113
8/16/2004	382.3	6.2	33.5	195	1100	7.25	64.2	70	690	11.5	0.013	1.86	71	0.2586	139
8/23/2004	339.5	6.4	36.4	210	1021	7.4	60.5	70	670	9.4	0.012	1.88	69	0.2574	127
8/27/2004	345.5	6.3	39.8	178	1058	7.3	58.6	60	620	11.6	0.014	2.14	74	0.241	107

Aeration

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
8/9/2004	382.5	7.4	22.2	80	1164	7.24	21.8	40	530	4.8	0.009	2.2	45	0.2235	42
8/16/2004	327.5	7.2	24.8	73	1041	7.12	19.9	35	510	5.6	0.005	2.51	43	0.1962	36
8/23/2004	349.3	7	21.6	76	1078	7.62	18.5	20	530	5.4	0.012	2.16	48	0.2247	40
8/27/2004	370	7	22.8	84	1059	7.8	16.5	25	520	5.1	0.013	2.35	52	0.173	38

Outfall

Sampling date	ALK mg/l as $CaCo_3$	DO (mg/l)	BOD (mg/L)	COD (mg/l)	Conductivity (µS/cm)	pH	Turbidity (NTU)	TSS (mg/l)	TDS (mg/l)	Nitrate (mg/l)	Nitrite (mg/l)	Phosphorous (mg/l)	TOC (mg/l)	AL (mg/l)	TPH (mg/l)
8/9/2004	355.5	7	13.6	36	1056	7.56	12.6	25	450	4.4	0.007	0.78	23	0.195	6
8/16/2004	312.3	7.2	14.2	26	1011	7.25	14.8	15	420	4.7	0.009	0.89	21	0.1856	4
8/23/2004	322.3	6.8	12.5	29	1065	8.15	11.5	10	470	4.2	0.011	0.91	24	0.2034	5
8/27/2004	352.5	6.6	12.8	41	1032	7.92	12.4	15	460	4.5	0.01	0.75	19	0.1624	4

TPDES PERMIT AT DISCHARGE

Daily Max/Min	NA	4.00	80	200	NA	9.00/6.00	NA	200	2000	NA	NA	NA	NA	1.77	15
Daily Avg	NA	NA	40	NA	NA		NA	90	NA	NA	NA	NA	NA	0.835	12

Table A6– Monitoring Results for the month of August 2004

APPENDIX B

MPN DATA FOR WINTER (NOV 03 – JAN 04) AND SUMMER MONTHS

Most Probable Number Count for Aliphatic Hydrocarbon Degrading Microorganism at the Wastewater Treatment Plant.

MPN-Aliphatic Hydrocarbon				Positive wells in each column															95% Confidence Intervals		
Sample Name	Run Date	Plate Type	Actual Count Date	1	2	3	4	5	6	7	8	9	10	11	#	MPN (mL^{-1})	L	R	Confidence Factor	Upper Confidence Limit	Lower Confidence Limit
AERATION	11/6/2003	C16	11/20/03		8	8	8	2	1	1						2.705E+03	1.77778	1.77778	2.571117552	6.955E+03	1.052E+03
		C16	11/20/03		8	8	8	2	2	2						3.935E+03	1.77778	1.77778	2.571117552	1.012E+04	1.531E+03
		C16	11/20/03		8	8	8	3	2	1						2.862E+03	1.77850	1.77778	2.571117552	7.359E+03	1.113E+03
OUTFALL	11/6/2003	C16	11/20/03		8	8	8	4								3.068E+03	1.77778	1.77778	2.571117552	7.888E+03	1.193E+03
		C16	11/20/03		8	8	8	2								1.796E+03	1.77778	1.77778	2.571117552	4.617E+03	6.984E+02
		C16	11/20/03		8	8	8	3								2.311E+03	1.77778	1.77778	2.571117552	5.943E+03	8.990E+02
AERATION	11/14/2003	C16	11/28/03		8	8	8	3	2	1						4.247E+03	1.77778	1.77778	2.571117552	1.092E+04	1.652E+03
		C16	11/28/03		8	8	8	3	2							3.545E+03	1.77778	1.77778	2.571117552	9.115E+03	1.379E+03
		C16	11/28/03		8	8	8	3	1	1						3.523E+03	1.77778	1.77778	2.571117552	9.058E+03	1.370E+03
OUTFALL	11/14/2003	C16	11/28/03		8	8	8	3								2.312E+03	1.77778	1.77778	2.571117552	5.944E+03	8.991E+02
		C16	11/28/03		8	8	8	1	1							1.737E+03	1.77778	1.77778	2.571117552	4.465E+03	6.754E+02
		C16	11/28/03		8	8	8	2	2							2.717E+03	1.77778	1.77778	2.571117552	6.985E+03	1.057E+03
AERATION	11/19/2003	C16	12/04/03		8	8	8	3	2							3.545E+03	1.77778	1.77778	2.571117552	9.114E+03	1.379E+03
		C16	12/04/03		8	8	8	4	1							3.813E+03	1.77778	1.77778	2.571117552	9.804E+03	1.483E+03
		C16	12/04/03		8	8	8	4	2							4.628E+03	1.77778	1.77778	2.571117552	1.190E+04	1.800E+03
OUTFALL	11/19/2003	C16	12/04/03		8	8	8	4								3.067E+03	1.77778	1.77778	2.571117552	7.885E+03	1.193E+03
		C16	12/04/03		8	8	8	3								2.312E+03	1.77778	1.77778	2.571117552	5.944E+03	8.991E+02
		C16	12/04/03		8	8	8	2	1							2.208E+03	1.77778	1.77778	2.571117552	5.677E+03	8.587E+02
AERATION	11/26/2003	C16	12/10/03		8	8	8	3	2	1						4.247E+03	1.77778	1.77778	2.571117552	1.092E+04	1.652E+03
		C16	12/10/03		8	8	8	3	2							3.545E+03	1.77778	1.77778	2.571117552	9.115E+03	1.379E+03
		C16	12/10/03		8	8	8	4	1	1						4.592E+03	1.77778	1.77778	2.571117552	1.181E+04	1.786E+03
OUTFALL	11/26/2003	C16	12/10/03		8	8	8	3								2.311E+03	1.77778	1.77778	2.571117552	5.943E+03	8.990E+02
		C16	12/10/03		8	8	8	2	1							2.208E+03	1.77778	1.77778	2.571117552	5.677E+03	8.587E+02
		C16	12/10/03		8	8	8	2	1							2.208E+03	1.77778	1.77778	2.571117552	5.677E+03	8.587E+02

Table B1 – MPN Results for the month of November 2003

Most Probable Number Count for Aliphatic Hydrocarbon Degrading Microorganism at the Wastewater Treatment Plant.

MPN-Aliphatic Hydrocarbon				Positive wells in each column															95% Confidence Intervals		
Sample Name	Run Date	Plate Type	Actual Count Date	1	2	3	4	5	6	7	8	9	10	11	#	MPN (mL^{-1})	L	R	Confidence Factor	Upper Confidence Limit	Lower Confidence Limit
AERATION	12/6/2003	C16	12/19/03		8	8	8	3	2							**3.542E+03**	1.77778	1.77778	2.571117552	**9.106E+03**	**1.377E+03**
		C16	12/19/03		8	8	8	3	1							**2.878E+03**	1.77778	1.77778	2.571117552	**7.399E+03**	**1.119E+03**
		C16	12/19/03		8	8	8	2	2	1						**3.300E+03**	1.77778	1.77778	2.571117552	**8.485E+03**	**1.284E+03**
OUTFALL	12/6/2003	C16	12/19/03		8	8	8	2	1							**2.207E+03**	1.77778	1.77778	2.571117552	**5.674E+03**	**8.583E+02**
		C16	12/19/03		8	8	8	1	1							**1.737E+03**	1.77778	1.77778	2.571117552	**4.466E+03**	**6.755E+02**
		C16	12/19/03		8	8	8	2								**1.795E+03**	1.77778	1.77778	2.571117552	**4.616E+03**	**6.983E+02**
AERATION	12/12/2003	C16	12/24/03		8	8	8	3	1							**2.878E+03**	1.77778	1.77778	2.571117552	**7.399E+03**	**1.119E+03**
		C16	12/24/03		8	8	8	3	1	1						**3.520E+03**	1.77778	1.77778	2.571117552	**9.049E+03**	**1.369E+03**
		C16	12/24/03		8	8	8	3	2							**3.544E+03**	1.77778	1.77778	2.571117552	**9.111E+03**	**1.378E+03**
OUTFALL	12/12/2003	C16	12/24/03		8	8	8	2	1							**2.207E+03**	1.77778	1.77778	2.571117552	**5.674E+03**	**8.583E+02**
		C16	12/24/03		8	8	8	2								**1.796E+03**	1.77778	1.77778	2.571117552	**4.617E+03**	**6.984E+02**
		C16	12/24/03		8	8	8	2	1							**2.207E+03**	1.77778	1.77778	2.571117552	**5.673E+03**	**8.582E+02**
AERATION	12/20/2003	C16	01/03/04		8	8	8	3	2							**3.544E+03**	1.77778	1.77778	2.571117552	**9.111E+03**	**1.378E+03**
		C16	01/03/04		8	8	8	3	1							**2.878E+03**	1.77778	1.77778	2.571117552	**7.400E+03**	**1.119E+03**
		C16	01/03/04		8	8	8	2	1	1						**2.706E+03**	1.77778	1.77778	2.571117552	**6.957E+03**	**1.052E+03**
OUTFALL	12/20/2003	C16	01/03/04		8	8	8	1	1							**1.736E+03**	1.77778	1.77778	2.571117552	**4.464E+03**	**6.753E+02**
		C16	01/03/04		8	8	8	2								**1.795E+03**	1.77778	1.77778	2.571117552	**4.616E+03**	**6.983E+02**
		C16	01/03/04		8	8	8	1	1							**1.737E+03**	1.77778	1.77778	2.571117552	**4.466E+03**	**6.756E+02**

Table B2 – MPN Results for the month of December 2003

Most Probable Number Count for Aliphatic Hydrocarbon Degrading Microorganism at the Wastewater Treatment Plant.

MPN-Aliphatic Hydrocarbon				**Positive wells in each column**															**95% Confidence Intervals**		
Sample Name	Run Date	Plate Type	Actual Count Date	1	2	3	4	5	6	7	8	9	10	11	#	**MPN (mL^{-1})**	**L**	**R**	**Confidence Factor**	**Upper Confidence Limit**	**Lower Confidence Limit**
AERATION	1/10/2004	C16	01/24/04		8	8	8	2	2	1						**3.302E+03**	1.77778	1.77778	2.571117552	**8.490E+03**	**1.284E+03**
		C16	01/24/04		8	8	8	3	1	2						**4.218E+03**	1.77778	1.77778	2.571117552	**1.085E+04**	**1.641E+03**
		C16	01/24/04		8	8	8	3	1	1						**3.522E+03**	1.77778	1.77778	2.571117552	**9.056E+03**	**1.370E+03**
OUTFALL	1/10/2004	C16	01/24/04		8	8	8	3								**2.311E+03**	1.77778	1.77778	2.571117552	**5.943E+03**	**8.990E+02**
		C16	01/24/04		8	8	8	1								**1.429E+03**	1.77778	1.77778	2.571117552	**3.673E+03**	**5.556E+02**
		C16	01/24/04		8	8	8	1	1							**1.736E+03**	1.77778	1.77778	2.571117552	**4.464E+03**	**6.753E+02**
AERATION	1/16/2004	C16	01/30/04		8	8	8	3	2							**3.544E+03**	1.77778	1.77778	2.571117552	**9.111E+03**	**1.378E+03**
		C16	01/30/04		8	8	8	3	2	1						**4.248E+03**	1.77778	1.77778	2.571117552	**1.092E+04**	**1.652E+03**
		C16	01/30/04		8	8	8	2	2	1						**3.301E+03**	1.77778	1.77778	2.571117552	**8.488E+03**	**1.284E+03**
OUTFALL	1/16/2004	C16	01/30/04		8	8	8	2	1							**2.207E+03**	1.77778	1.77778	2.571117552	**5.674E+03**	**8.583E+02**
		C16	01/30/04		8	8	8	1	2							**2.117E+03**	1.77778	1.77778	2.571117552	**5.444E+03**	**8.235E+02**
		C16	01/30/04		8	8	8	2	1							**2.207E+03**	1.77778	1.77778	2.571117552	**5.673E+03**	**8.582E+02**
AERATION	1/23/2004	C16	02/06/04		8	8	8	4	1							**3.813E+03**	1.77778	1.77778	2.571117552	**9.803E+03**	**1.483E+03**
		C16	02/06/04		8	8	8	3	3							**4.275E+03**	1.77778	1.77778	2.571117552	**1.099E+04**	**1.663E+03**
		C16	02/06/04		8	8	8	2	3							**3.319E+03**	1.77778	1.77778	2.571117552	**8.532E+03**	**1.291E+03**
OUTFALL	1/23/2004	C16	02/06/04		8	8	8	2								**1.795E+03**	1.77778	1.77778	2.571117552	**4.615E+03**	**6.982E+02**
		C16	02/06/04		8	8	8	1	1							**1.736E+03**	1.77778	1.77778	2.571117552	**4.464E+03**	**6.753E+02**
		C16	02/06/04		8	8	8	1								**1.428E+03**	1.77778	1.77778	2.571117552	**3.673E+03**	**5.556E+02**
AERATION	1/29/2004	C16	02/12/04		8	8	8	3	1	1						**3.520E+03**	1.77778	1.77778	2.571117552	**9.049E+03**	**1.369E+03**
		C16	02/12/04		8	8	8	3	2							**3.544E+03**	1.77778	1.77778	2.571117552	**9.113E+03**	**1.378E+03**
		C16	02/12/04		8	8	8	3	2	1						**4.245E+03**	1.77778	1.77778	2.571117552	**1.091E+04**	**1.651E+03**
OUTFALL	1/29/2004	C16	02/12/04		8	8	8	1	1							**1.736E+03**	1.77778	1.77778	2.571117552	**4.464E+03**	**6.753E+02**
		C16	02/12/04		8	8	8	1								**1.428E+03**	1.77778	1.77778	2.571117552	**3.673E+03**	**5.556E+02**
		C16	02/12/04		8	8	8	2								**1.795E+03**	1.77778	1.77778	2.571117552	**4.615E+03**	**6.981E+02**

Table B3 – MPN Result for the month of January 2004

Most Probable Number Count for Aliphatic Hydrocarbon Degrading Microorganism at the Wastewater Treatment Plant.

MPN-Aliphatic Hydrocarbon				Positive wells in each column															95% Confidence Intervals		
Sample Name	Run Date	Plate Type	Actual Count Date	1	2	3	4	5	6	7	8	9	10	11	#	MPN (mL^{-1})	L	R	Confidence Factor	Upper Confidence Limit	Lower Confidence Limit
AERATION	6/7/2004	C16	06/21/04		8	8	8	6	5	3	2	1				**2.172E+04**	1.77778	1.77778	2.571117552	**5.584E+04**	**8.446E+03**
		C16	06/21/04		8	8	8	7	2	2	2					**1.860E+04**	1.77778	1.77778	2.571117552	**4.783E+04**	**7.235E+03**
		C16	06/21/04		8	8	8	6	5	2	1	1				**1.828E+04**	1.77778	1.77778	2.571117552	**4.699E+04**	**7.109E+03**
OUTFALL	6/7/2004	C16	06/21/04		8	8	8	3	3	1	1					**5.751E+03**	1.77778	1.77778	2.571117552	**1.479E+04**	**2.237E+03**
		C16	06/21/04		8	8	8	4	2	1	1					**6.265E+03**	1.77778	1.77778	2.571117552	**1.611E+04**	**2.437E+03**
		C16	06/21/04		8	8	8	4	1	1	1					**5.396E+03**	1.77778	1.77778	2.571117552	**1.387E+04**	**2.099E+03**
AERATION	6/14/2004	C16	06/28/04		8	8	8	7	4	2	2	1				**2.742E+04**	1.77778	1.77778	2.571117552	**7.051E+04**	**1.067E+04**
		C16	06/28/04		8	8	8	6	5	3	1	1				**1.998E+04**	1.77778	1.77778	2.571117552	**5.136E+04**	**7.769E+03**
		C16	06/28/04		8	8	8	7	3	2	2	1				**2.403E+04**	1.77778	1.77778	2.571117552	**6.179E+04**	**9.348E+03**
OUTFALL	6/14/2004	C16	06/28/04		8	8	8	4	2	2	1					**7.116E+03**	1.77778	1.77778	2.571117552	**1.830E+04**	**2.768E+03**
		C16	06/28/04		8	8	8	3	2	1	1					**4.971E+03**	1.77778	1.77778	2.571117552	**1.278E+04**	**1.934E+03**
		C16	06/28/04		8	8	8	2	3	2	1					**5.293E+03**	1.77778	1.77778	2.571117552	**1.361E+04**	**2.059E+03**
AERATION	6/22/2004	C16	07/06/04		8	8	8	7	5	2	2					**2.821E+04**	1.77778	1.77778	2.571117552	**7.254E+04**	**1.097E+04**
		C16	07/06/04		8	8	8	7	4	1	2	1				**2.464E+04**	1.77778	1.77778	2.571117552	**6.335E+04**	**9.583E+03**
		C16	07/06/04		8	8	8	6	5	3	2	1				**2.172E+04**	1.77778	1.77778	2.571117552	**5.583E+04**	**8.446E+03**
OUTFALL	6/22/2004	C16	07/06/04		8	8	8	4	2	1	1					**6.268E+03**	1.77778	1.77778	2.571117552	**1.612E+04**	**2.438E+03**
		C16	07/06/04		8	8	8	4	3	1	1					**7.172E+03**	1.77778	1.77778	2.571117552	**1.844E+04**	**2.789E+03**
		C16	07/06/04		8	8	8	3	3	1						**5.008E+03**	1.77778	1.77778	2.571117552	**1.288E+04**	**1.948E+03**
AERATION	6/29/2004	C16	07/13/04		8	8	8	7	4	3	1	1				**2.750E+04**	1.77778	1.77778	2.571117552	**7.069E+04**	**1.069E+04**
		C16	07/13/04		8	8	8	7	4	2	2					**2.472E+04**	1.77778	1.77778	2.571117552	**6.357E+04**	**9.616E+03**
		C16	07/13/04		8	8	8	7	5	3	1					**2.842E+04**	1.77778	1.77778	2.571117552	**7.307E+04**	**1.105E+04**
OUTFALL	6/29/2004	C16	07/13/04		8	8	8	4	3	1	1					**7.172E+03**	1.77778	1.77778	2.571117552	**1.844E+04**	**2.789E+03**
		C16	07/13/04		8	8	8	3	2	2	1					**5.711E+03**	1.77778	1.77778	2.571117552	**1.468E+04**	**2.221E+03**
		C16	07/13/04		8	8	8	3	3	1	1					**5.751E+03**	1.77778	1.77778	2.571117552	**1.479E+04**	**2.237E+03**

Table B4 – MPN Result for the month of June 2004

Most Probable Number Count for Aliphatic Hydrocarbon Degrading Microorganism at the Wastewater Treatment Plant.

MPN-Aliphatic Hydrocarbon				Positive wells in each column															95% Confidence Intervals		
Sample Name	Run Date	Plate Type	Actual Count Date	1	2	3	4	5	6	7	8	9	10	11	#	**MPN (mL^{-1})**	**L**	**R**	**Confidence Factor**	**Upper Confidence Limit**	**Lower Confidence Limit**
AERATION	7/5/2004	C16	07/19/04		8	8	8	6	5	3	2	1				**2.172E+04**	1.77778	1.77778	2.571117552	**5.585E+04**	**8.448E+03**
		C16	07/19/04		8	8	8	6	6	2	1	1				**2.030E+04**	1.77778	1.77778	2.571117552	**5.220E+04**	**7.896E+03**
		C16	07/19/04		8	8	8	6	5	2	2	1				**1.994E+04**	1.77778	1.77778	2.571117552	**5.126E+04**	**7.754E+03**
OUTFALL	7/5/2004	C16	07/19/04		8	8	8	4	3	1	1					**7.169E+03**	1.77778	1.77778	2.571117552	**1.843E+04**	**2.788E+03**
		C16	07/19/04		8	8	8	4	2	2	1					**7.116E+03**	1.77778	1.77778	2.571117552	**1.830E+04**	**2.768E+03**
		C16	07/19/04		8	8	8	5	1	1	1					**6.919E+03**	1.77778	1.77778	2.571117552	**1.779E+04**	**2.691E+03**
AERATION	7/12/2004	C16	07/26/04		8	8	8	7	4	2	2	1				**2.742E+04**	1.77778	1.77778	2.571117552	**7.051E+04**	**1.067E+04**
		C16	07/26/04		8	8	8	6	5	3	2	1				**2.169E+04**	1.77778	1.77778	2.571117552	**5.578E+04**	**8.437E+03**
		C16	07/26/04		8	8	8	6	5	2	2	1				**1.991E+04**	1.77778	1.77778	2.571117552	**5.120E+04**	**7.745E+03**
OUTFALL	7/12/2004	C16	07/26/04		8	8	8	4	3	2	1					**8.042E+03**	1.77778	1.77778	2.571117552	**2.068E+04**	**3.128E+03**
		C16	07/26/04		8	8	8	3	3	1	1					**5.752E+03**	1.77778	1.77778	2.571117552	**1.479E+04**	**2.237E+03**
		C16	07/26/04		8	8	8	3	3	2	1					**6.507E+03**	1.77778	1.77778	2.571117552	**1.673E+04**	**2.531E+03**
AERATION	7/19/2004	C16	08/02/04		8	8	8	6	5	2	2	1				**1.994E+04**	1.77778	1.77778	2.571117552	**5.127E+04**	**7.756E+03**
		C16	08/02/04		8	8	8	7	3	2	2	1				**2.393E+04**	1.77778	1.77778	2.571117552	**6.153E+04**	**9.307E+03**
		C16	08/02/04		8	8	8	6	4	3	2	1				**1.958E+04**	1.77778	1.77778	2.571117552	**5.034E+04**	**7.616E+03**
OUTFALL	7/19/2004	C16	08/02/04		8	8	8	4	2	2	1					**7.116E+03**	1.77778	1.77778	2.571117552	**1.830E+04**	**2.768E+03**
		C16	08/02/04		8	8	8	4	3	1	1					**7.172E+03**	1.77778	1.77778	2.571117552	**1.844E+04**	**2.789E+03**
		C16	08/02/04		8	8	8	3	3	1	1					**5.751E+03**	1.77778	1.77778	2.571117552	**1.479E+04**	**2.237E+03**
AERATION	7/26/2004	C16	08/09/04		8	8	8	6	4	3	2	1				**1.960E+04**	1.77778	1.77778	2.571117552	**5.040E+04**	**7.625E+03**
		C16	08/09/04		8	8	8	7	4	2	2	1				**2.737E+04**	1.77778	1.77778	2.571117552	**7.036E+04**	**1.064E+04**
		C16	08/09/04		8	8	8	6	5	3	1	1				**1.998E+04**	1.77778	1.77778	2.571117552	**5.137E+04**	**7.771E+03**
OUTFALL	7/26/2004	C16	08/09/04		8	8	8	3	3	3	1					**7.264E+03**	1.77778	1.77778	2.571117552	**1.868E+04**	**2.825E+03**
		C16	08/09/04		8	8	8	3	2	2	1					**5.711E+03**	1.77778	1.77778	2.571117552	**1.468E+04**	**2.221E+03**
		C16	08/09/04		8	8	8	3	3	2	1					**6.507E+03**	1.77778	1.77778	2.571117552	**1.673E+04**	**2.531E+03**

Table B5 – MPN Results for the month of July 2004

Most Probable Number Count for Aliphatic Hydrocarbon Degrading Microorganism at the Wastewater Treatment Plant.

MPN-Aliphatic Hydrocarbon				**Positive wells in each column**															**95% Confidence Intervals**		
Sample Name	Run Date	Plate Type	Actual Count Date	1	2	3	4	5	6	7	8	9	10	11	12	**MPN (mL^{-1})**	**L**	**R**	**Confidence Factor**	**Upper Confidence Limit**	**Lower Confidence Limit**
AERATION	8/9/2004	C16	08/23/04		8	8	8	7	3	3	3	1				**2.939E+04**	1.77778	1.77778	2.571117552	**7.556E+04**	**1.143E+04**
		C16	08/23/04		8	8	8	7	4	2	2	1				**2.747E+04**	1.77778	1.77778	2.571117552	**7.063E+04**	**1.068E+04**
		C16	08/23/04		8	8	8	7	5	2	2	1				**3.127E+04**	1.77778	1.77778	2.571117552	**8.041E+04**	**1.216E+04**
OUTFALL	8/9/2004	C16	08/23/04		8	8	8	4	2	2	1					**7.108E+03**	1.77778	1.77778	2.571117552	**1.827E+04**	**2.764E+03**
		C16	08/23/04		8	8	8	4	2	2	1					**7.116E+03**	1.77778	1.77778	2.571117552	**1.830E+04**	**2.768E+03**
		C16	08/23/04		8	8	8	4	3	1	1					**7.168E+03**	1.77778	1.77778	2.571117552	**1.843E+04**	**2.788E+03**
AERATION	8/16/2004	C16	08/30/04		8	8	8	6	5	4	2	1				**2.345E+04**	1.77778	1.77778	2.571117552	**6.030E+04**	**9.122E+03**
		C16	08/30/04		8	8	8	6	5	4	3	1				**2.526E+04**	1.77778	1.77778	2.571117552	**6.494E+04**	**9.824E+03**
		C16	08/30/04		8	8	8	7	4	2	2	1				**2.740E+04**	1.77778	1.77778	2.571117552	**7.046E+04**	**1.066E+04**
OUTFALL	8/16/2004	C16	08/30/04		8	8	8	4	3	2	1					**8.042E+03**	1.77778	1.77778	2.571117552	**2.068E+04**	**3.128E+03**
		C16	08/30/04		8	8	8	3	3	1	1					**5.752E+03**	1.77778	1.77778	2.571117552	**1.479E+04**	**2.237E+03**
		C16	08/30/04		8	8	8	3	3	2	1					**6.507E+03**	1.77778	1.77778	2.571117552	**1.673E+04**	**2.531E+03**
AERATION	8/23/2004	C16	09/06/04		8	8	8	7	5	2	2	1				**3.128E+04**	1.77778	1.77778	2.571117552	**8.043E+04**	**1.217E+04**
		C16	09/06/04		8	8	8	7	3	3	3	1				**2.943E+04**	1.77778	1.77778	2.571117552	**7.568E+04**	**1.145E+04**
		C16	09/06/04		8	8	8	7	4	3	2	1				**3.042E+04**	1.77778	1.77778	2.571117552	**7.821E+04**	**1.183E+04**
OUTFALL	8/23/2004	C16	09/06/04		8	8	8	4	2	2	1					**7.116E+03**	1.77778	1.77778	2.571117552	**1.830E+04**	**2.768E+03**
		C16	09/06/04		8	8	8	4	3	1	1					**7.172E+03**	1.77778	1.77778	2.571117552	**1.844E+04**	**2.789E+03**
		C16	09/06/04		8	8	8	4	3	2	1					**8.041E+03**	1.77778	1.77778	2.571117552	**2.067E+04**	**3.127E+03**
AERATION	8/27/2004	C16	09/13/04		8	8	8	6	6	4	2	1				**2.585E+04**	1.77778	1.77778	2.571117552	**6.647E+04**	**1.006E+04**
		C16	09/13/04		8	8	8	7	4	3	2	1				**3.029E+04**	1.77778	1.77778	2.571117552	**7.788E+04**	**1.178E+04**
		C16	09/13/04		8	8	8	7	5	3	1	1				**3.134E+04**	1.77778	1.77778	2.571117552	**8.059E+04**	**1.219E+04**
OUTFALL	8/27/2004	C16	09/13/04		8	8	8	3	3	3	1					**7.264E+03**	1.77778	1.77778	2.571117552	**1.868E+04**	**2.825E+03**
		C16	09/13/04		8	8	8	4	2	2	1					**7.117E+03**	1.77778	1.77778	2.571117552	**1.830E+04**	**2.768E+03**
		C16	09/13/04		8	8	8	4	3	2	1					**8.042E+03**	1.77778	1.77778	2.571117552	**2.068E+04**	**3.128E+03**

Table B6 – MPN Results for the month of August 2004

APPENDIX C

PROCEDURE OF THE EXPERIMENTS

PROCEDURE OF THE EXPERIMENTS

A. Alkalinity

1. Prepare reagents: Standard titrant – 0.02N H_2SO_4, Stock alkalinity control standard - dissolve 4.24 gm of Na_2CO_3, (dried at 250° C for two hours and cooled in a desiccator) in reagent water and dilute to one liter in a volumetric flask. Use this solution to prepare control standards by dilution with volumetric labware.
2. Calibrate pH meter.
3. Titrate standard and thereafter 100ml of sample with the 0.02N sulfuric acid to pH 4.5 using moderately stirring action near the end of the titration.
4. Take down the reading.
5. Calculation: Total Alkalinity as $CaCO_3$ in mg/L = (ml of titrant) X 10

Reference: Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 2-26

B. BOD

1. Prepare reagents: Glucose-glutamic acid solution
2. Prepare dilution water: Use HACH nutrient pillow. Put one pillow into 6L deionized water.
3. Prepare seed solution: Place the contents of one PolySeed capsule into 500mls of dilution water, and aerate it for 60 minutes.
4. Sample pretreatment: Neutralize samples to ph 6.5-7.5 with a solution of H_2SO4 or NaOH of such strength that the quantity of reagent does not dilute the sample by more than 0.5%. Bring the samples to 19~21°C before making dilutions.
5. Seed control factor (SCF): Allow the PolySeed solution to settle for 5 to 15 minutes before preparing the seed control samples. Prepare the seed control using 10, 20 mls. Of PolySeed solution. Run the PolySeed SCF sample for the full 5-day test period.
6. Glucose-Glutamic Acid Standard: After the glucose-glutamic acid standard solution is prepared, use 4 mls. Of PolySeed solution for each BOD_5 bottle.

7. Dilution water blank: Use a dilution water blank as a rough check on quality of unseeded dilution water and cleanliness of incubation bottles.
8. Take each sample for 5ml and 10ml into BOD bottles, and add 4 mls. of PolySeed solution into those bottles. Determine the initial DO for every BOD bottles.
9. Incubate at 19~21°C BOD bottles containing desired dilutions, seed controls, dilution water blanks, and glucose-glutamic acid checks.
10. Determine the final DO after 5-day incubation.

Reference: Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 5-3

C. COD (Using the HACH apparatus, Manganese III Digestion Method)

1. Press – Hach Program "PRGM"
2. Press: "18", "ENTER" – COD
3. Homogenize 100ml of sample for 30 seconds in a blender.
4. Pipet 0.5ml of sample into Mn III COD vial. Cap and invert several times to mix.
5. Prepare Blank: Prepare a blank by substituting 0.5ml of deionized water for the sample.
6. Place the vials in the COD reactor that is preheated to 150° C. Digest for 1 hour.
7. Remove the vials and wipe with kimwipe.
8. Place the blank in the sample cell adapter.
9. Tightly cover the sample cell with the instrument cap. Press "ZERO"
10. Place the sample in the adapter, and cover the sample cell with the cap. Press "READ"
11. Write down the readings.

Reference: 1. Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 5-13

2. HACH apparatus manual

D. TOC (Using the HACH apparatus)

1. Turn the COD reactor. Heat to 103-105°C.
2. Add 10ml of sample to a Erlenmeyer flask containing a stir bar.
3. Add 0.4ml of Buffer Solution, pH 2.0
4. Place the flask on a stir plate and stir at a moderate speed for 10 minutes.
5. Using a funnel, add the content of one TOC Persulfate Powder Pillow to each Acid Digestion vial.
6. Add 0.3ml of deionized water to the reagent blank vial and 0.3ml of prepared sample to the sample vial.
7. Rinse the blue Indicator Ampules with deionized water and wipe them with kimwipe.
8. Lower one unopened ampule into each Acid Digestion vial. Snap the top off the ampule and cap the vial tightly.
9. Place the vials in the COD reactor for 2 hours at 103-105° C.
10. Press the HACH botton“PRGM”,”115”,”ENTER”.
11. Put the reagent blank vial into the adapter, and cover the vial with the cap. Press “ZERO”
12. Put the sample vial into the adapter, and cover the vial with the cap. Press “READ”
13. Write down the readings.

Reference: 1. Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 5-18

2. HACH apparatus manual

E. Nitrate (Using the HACH apparatus)

1. Press “PRGM”, “51”, ”ENTER”
2. Fill a sample cell with 10ml of sample.
3. Add the contents of Nitra Ver 5 Nitrate Reagent Power Pillow to the sample cell. Cap the sample cell.
4. Press “TIMER”, “ENTER”
5. After the timer beeps, the display will show: 5:00 TIMER 2. Press “ENTER”

6. Fill another cell with 10ml of sample (The blank).
7. Place the blank into the cell holder. Tightly cover the sample cell with the cap. Press “ZERO”.
8. Place the sample into the cell holder. Tightly cover the sample cell with the cap. Press “READ”
9. Write down the readings.

Reference: 1. Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 4-114

2. HACH apparatus manual

F. Nitrite (Using the HACH apparatus)

1. Press “PRGM”, “60”, ”ENTER”
2. Fill a sample cell with 10ml of sample.
3. Add the contents of Nitra Ver 3 Nitrate Reagent Power Pillow to the sample cell. Cap the sample cell and shake to dissolve.
4. Press “TIMER”, “ENTER”, A 15-minutes reaction period will begin.
5. Fill another cell with 10ml of sample (The blank).
6. Place the blank into the cell holder. Tightly cover the sample cell with the cap. Press “ZERO”.
7. Place the sample into the cell holder. Tightly cover the sample cell with the cap. Press “READ”
8. Write down the readings.

Reference: 1. Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 4-112

2. HACH apparatus manual

G. Phosphate (Using the HACH apparatus)

1. Press “PRGM”, “79”,”ENTER”.
2. Fill a sample cell with 10ml of sample.
3. Add the contents of Phos Ver 3 Phosphate Power Pillow to the sample cell. Cap the sample cell and shake 15 seconds.
4. Press “TIMER”, “ENTER”, a 2-minutes reaction period will begin.

5. Fill another cell with 10ml of sample (The blank).
6. Place the blank into the cell holder. Tightly cover the sample cell with the cap. Press “ZERO”.
7. Place the sample into the cell holder. Tightly cover the sample cell with the cap. Press “READ”
8. Write down the readings.

Reference: 1. Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 4-139

2. HACH apparatus manual

H. Turbidity (HACH 2100 AN Turbidimeter)

1. Turn on the meter.
2. Press “Cal” to calibrate the turbidimeter.
3. Put 6 different stablcal formazin standards to calibrate.
4. Press “Cal”, the meter will be ready to analyze
5. Put sample into the meter, then press “enter”
6. Check the first reading
7. Write down and record it.

Reference: Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 2-8

I. Conductivity (Corning Conductivity meter 441)

1. Turn on the meter.
2. Press “Cal”, to calibrate the meter. Here use 2-points calibration
3. Rinse the sensor with deionized water and prepare to measure sample.
4. Place the sensor in the sample and press “read” to start measurement.
5. Write down the readings.

Reference: 1. Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 2-44

2. CORNING Instruction Manual

J. PH (Accumet PH Meter 925)

1. Press” Multi point Cal”
2. Choose 3 points calibration, Press “Enter”

3. Calibrate the meter by using pH 4,7,10 buffer solution
4. After calibration, measure each sample and write down the readings.

Reference: Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 4-86

K. Total Suspended Solids

1. Prepare the evaporation dish: Heat clean dish to 103~105°C for 1 hour.
2. Select 10ml to be the sample volume.
3. Place the 0.7μm filter on the base and clamp on funnel and apply vacuum. Filter with a small volume of deionized water to seal the filter.
4. Shake the sample strongly and put 10 ml into the funnel through the filter. Remove all traces of water by continuing to apply vacuum after sample has passed through.
5. Rinse the funnel onto the filter with some deionized water. Remove all traces of water by continuing to apply vacuum after sample has passed through.
6. Remove the filter from the base. Dry one hour at the temperature 103~105°C. Let it cool and then check the weight.

Reference: Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 2-57

L. Total Dissolved Solids

1. Prepare the evaporation dish: Heat clean dish to 180°C for 1 hour.
2. Select 10ml to be the sample volume.
3. Place the 0.7μm filter on the base and clamp on funnel and apply vacuum. Filter with a small volume of deionized water to seal the filter.
4. Shake the sample strongly and put 10 ml into the funnel through the filter. Remove all traces of water by continuing to apply vacuum after sample has passed through.
5. Rinse the funnel onto the filter with some deionized water. Remove all traces of water by continuing to apply vacuum after sample has passed through.
6. Remove the filtered sample from the flask. Dry at least one hour at the temperature 180°C. Let it cool and check the weight.

Reference: Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 2-56

M. Dissolved Oxygen

1. Use DO meter
2. Calibrate the DO meter
3. Turn to the “ZERO” mode, and adjust it with the zero knob.
4. Turn to “Full Scale” mode, and adjust it with the Full Scale knob.
5. Turn to “Cal” mode, and adjust it with the with the Calibrate knob.
6. Put the probe into sample, and then set the temperature.
7. Turn to “Read”
8. Write down the readings.

Reference: Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 4-129

N. Aluminum (Using Flame Atomic Absorption Spectrometry)

The instrument used is the state of the art SOLAAR ATOMIC ABSORPTION SPECTROMETER

Flame type- Nitrous oxide/acetylene.

Primary Wavelength of absorption- 309.3nm

1. Prepare standard solutions of metal concentrations 5mg/l, 10mg/l, 15mg/l, 20 mg/l which will be used to calibrate the readings which is expected to bracket the expected metal concentration of the samples.
2. The samples are filtered and stored after acidifying with conc. nitric acid to drop the pH below 2.0.
3. The samples have to be digested to get rid of any organic matter which may cause interference during the analysis.
4. Digestion process requires boiling 100ml of the sample after being acidified with 5ml conc. nitric acid, to the lowest volume possible.
5. Dilute the concentrated samples to 100 ml and portions of this solution are taken for metal determination.
6. Mix 2ml potassium chloride solution into 100ml sample.

7. Aspirate the blank consisting of a deionised water containing 1.5ml containing 1.5ml nitric acid and adjust the rate between 3 and 5ml/min.
8. The instrument is zeroed and the metal standard solutions are aspirated to calibrate the instrument.
9. The instrument is then ready to run the samples.

Reference: Lenore S. Clesceri, Arnold E. Greenberg, Andrew D.Eaton, Standard Methods for the water and wastewater, 20th edition Page 3-56

O. Most Probable Number Count. (Using Biomek Workstation 1000)

This method was adopted to enumerate the bacterial population in the system and it was to only aliphatic degraders as the fuel used in the firefighting operation are C9-C13 Isoalkanes which are a mixture of aliphatic hydrocarbons. The method is carried out on a 96 well (12 columns×8 rows) microtiter plates where the bacterial culture takes place. Beckman Biomek Workstation 1000 is a laboratory robot which fills the wells with medium and performs ten fold dilutions of the sample.

1. The growth medium i.e. Bushnell-Haas broth is prepared and then the robot added 180 µl to each 11 wells, leaving the first row empty.
2. 200 µl of undiluted sample is transferred in the first row and then ten fold dilutions were carried out except the last row, which served as a sterile control.
3. 2 µl hexadecane was added to each well, which is the representative growth substrate for aliphatic degrading bacteria.
4. The plates were sealed in plastic bags and incubated for 14days at 20˚C.

Reference: 1. Brain A. Wrenn and Albert D. Venosa, Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most probable number procedure, 1996, Can. J. Microbiol. 42: Pages 252-258.

2. J.R.Haines, B.A.Wrenn, E.LHolder, K.L.Strohmeier, R.T.Herrington, and A.D.Venosa, Measurement of hydrocarbon degrading microbial populations by a 96-well plate most probable number procedure, 1995,Journal of Industrial Microbiology 16: Pages 36-41.

P) Total Petroleum Hydrocarbon (Using the HACH apparatus)

1. Touch single wavelength button, then press λ and put in 450 nm and then press ok.

2. Lebel antibody cuvette for each calibrator and samples.
3. Pipet 0.5 ml of diluent solution into each calibrator cuvette.
4. Pipet 0.5 ml of samples from all four units into appropriate cuvette.
5. Using a Wiretol pipet transfer 50 µl of each calibrator in the calibrator cuvettes.
6. Using a Wiretol pipet transfer 50 µl of methanol in sample cuvette and 0.5 ml of TPH Enzyme into calibrator and sample cuvette.
7. In the timer, 10 mins is set for reaction period and after that empty all contents of the cuvettes.
8. Wash each cuvettes forcefully with deinonised water.
9. Put 0.5 ml of Color Developing Solution in each cuvette.
10. Set timer for 10 min reaction time.
11. Put 0.5 ml of Stop solution.
12. Fill a Zeroing Cuvette with deionized water and put in the cell holder and press zero.
13. Place calibrator cuvette in cell holder and measure the concentration of TPH with reference to that.

Reference: 1. HACH apparatus manual method 10050 Immunoassay method.

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