MECHANISM AND CONTROL OF CARBON MONOXIDE GENERATION IN AN INDUSTRIAL WASTEWATER TREATMENT PLANT

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Thesis Approved:

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Dedicated to my parents

Krishna Gopal Joshi and Minu Joshi

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CHAPTER I

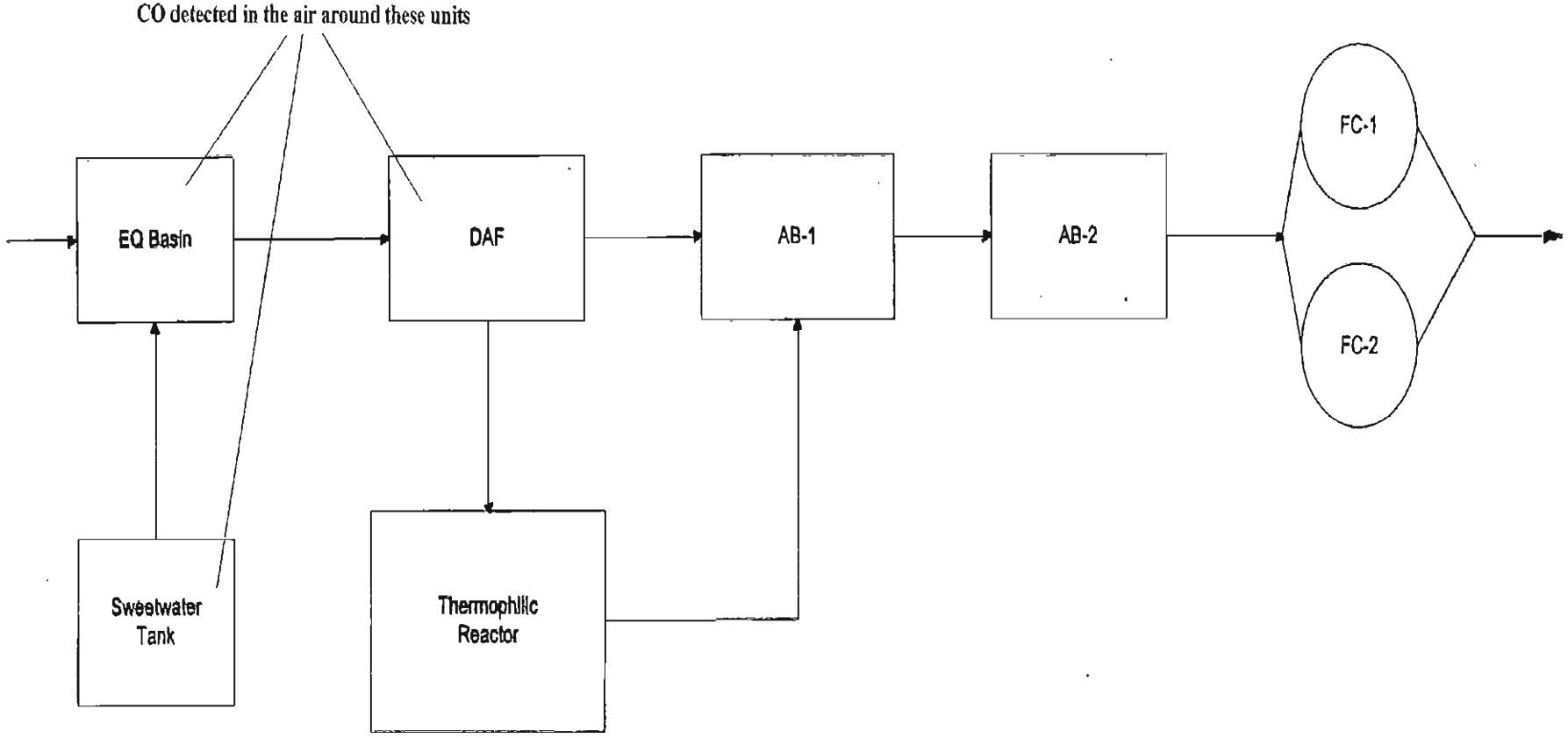
INTRODUCTION

Background

This research addresses the mechanisms and control of carbon monoxide generation in an industrial wastewater treatment plant. A candy manufacturer experiences carbon monoxide (CO) production in equalization tanks utilized for pH adjustment and chemical addition before any treatment. At times, CO evolution reaches levels that raise worker safety concerns. Carbon monoxide has been detected in the air around each waste equalization tank, as well as around a dissolved air flotation (DAF) tank and possibly other downstream treatment units. One waste stream consists of sugar (sucrose) (Stover, personnel communication, 2001a) in wash water from production plant clean-in-place (CIP) operations, in which two cleaning agents are used: one containing potassium hydroxide and potassium hypochlorite, and the other containing phosphoric and organic acids along with anionic surfactants. Sucrose concentrations in this waste stream range between about 30,000 mg/L – 100,000 mg/L as COD (Stover, personnel communication, 2001a). The second waste stream consists of more concentrated sugar syrup, called "sweetwater", which comes from production lines in the plant and which reportedly contain smaller amounts of the cleaning chemicals (Stover, personnel communication, 2001a). COD of this waste is about 100,000 – 300,000 mg/L (Stover, personnel communication, 2001a).

Some of the sucrose is known to ferment to its constituent reducing sugars (glucose + fructose) in the non-aerated equalization basin. Thus, biochemical reactions of this sort may contribute to the problem in the ClP waste stream, but evidence suggests that other mechanisms may also be factors in this situation. First, pH levels are lower and temperatures may also not be as high as previously reported (Nicloux and Nebenzahl, 1929) to cause CO generation by purely chemical mechanisms. Also, the sweetwater waste stream does not contain appreciable amounts of the alkaline cleaning agents which are likely involved in the chemical reactions, yet CO production has been noted in this portion of the waste treatment system. Other chemical reactions or biological processes are possible (direct or indirect) causes. Figure 1 below shows process diagram of the candy manufacturer's wastewater treatment plant. The three lines are showing the three units (CIP EQ tank, sweetwater tank and DAF) at the plants where CO was detected. AB in the diagram denotes aeration basin. Other process flow diagrams of the wastewater treatment plant indicated the aeration basins house an activated sludge system.

Process Diagram of Wastewater Treatment Plant



The Problem

Industrial Wastes

Industrial wastewater must be discharged either to a municipal wastewater system or a receiving stream. Pre-treatment of industrial wastewater is required to meet standards that are established for effluent discharge. With pretreatment, there are always a wide variety of possible treatments to achieve the desired end. A critical examination of the problem almost always indicates one that has clear advantages. A great deal of thought and care should go into schemes for mixed waste treatment to ensure all pitfalls are avoided by choosing proper reagents and proper unit processes.

Chemistry of Carbon Monoxide

Carbon monoxide (CO) is a colorless, odorless, poisonous gas. A product of incomplete burning of hydrocarbon-based fuels, carbon monoxide consists of a carbon atom and an oxygen atom linked together. The natural concentration of carbon monoxide in the atmosphere is around 0.2 parts per million (ppm), an amount that is not harmful to humans (Stokinger and Coffin, 1968). The toxic effects of carbon monoxide on humans are due solely to the interactions of CO with blood hemoglobin (Stokinger and Coffin, 1968). Carbon monoxide enters the bloodstream through the lungs and forms carboxyhemoglobin, a compound that inhibits the blood's capacity to carry oxygen to organs and tissues. Carbon monoxide can affect healthy individuals, impairing exercise capacity, visual perception, manual dexterity, learning functions, and ability to perform complex tasks. The most serious effects of atmospheric CO are expected for individuals

already vulnerable to oxygen deficiencies (Cooper and Alley, 1990). The current standard set by the Occupational Safety and Health Administration (OSHA) limits exposure to 50 parts of carbon monoxide per million parts of air averaged over eight hours. The National Institute for Occupational Safety and Health (NIOSH), which provides research for OSHA, has recommended that the standard be changed to 35 parts per million and that any exposure beyond 200 parts per million be strictly forbidden (CWA, 2000). 50ppm is the safety level as specified by Health and Safety Executive of a campaign website providing CO information (www.carbonmonoxidekills.com/coinformation.htm). The national Ambient Air Quality standard for CO for 8-hour averaging time is 9 ppm and for 1-hour time is 35 ppm (Cooper and Alley, 1990).

The symptoms of CO exposure vary widely based on exposure level, duration and the general health and age of an individual. The recurrent theme that is most significant in the recognition of carbon monoxide poisoning is headache, dizziness and nausea (Medical Effects, 2002). These 'flu-like' symptoms are often mistaken for a real case of the flu and can result in delayed or misdiagnosed treatment. Due to improved insulation and double glazing in household windows, it has become increasingly important to have good ventilation, maintain all appliances regularly and to have absolutely reliable detector alarms installed, giving both a visual and audible alarm immediately upon sensing a buildup of CO to dangerous levels. These precautions are particularly important because of the absence of odor, color, or taste of CO.

Scope of Work

Possible mechanisms for CO evolution from the wastewater treatment system of the manufacturing plant were investigated. Experimental work consisted of chemical characterization of waste samples from the plant and a series of bench-scale screening studies. Bench scale studies were conducted in serum bottles.

This study focused on reproducing operational conditions at the plant and comparing these results with earlier experiments such as those reported by Nicloux and Nebenzahl (1929). Positive results obtained from these experiments would suggest, depending on the operating conditions, if the mechanism(s) of CO production is the same or different as seen the literature, and provide insight into an understanding of the conditions that lead to the production of CO. This knowledge would be used in altering the waste handling process at the plant in order to reduce or eliminate CO production. The analysis was broadened to find the chemical or biological origin of CO production and hence investigate the mechanism(s) responsible for it. Control strategies for the reduction of CO in the plant were investigated in order to recommend an effective solution, which could be implemented at the plant.

Objective of the Study

The objectives are as follows.

- Reproduce conditions reported in the literature to result in CO evolution from reactions between reducing sugars and alkaline chemicals, using the reactants present in the plant's wastewater streams (representative concentrations of glucose and/or fructose and cleaning agents). This is to determine whether conditions prevalent at the plant could result in reported CO generation by abiotic chemical reaction mechanisms.
- Reproduce operating conditions of both equalization basins used at the plant for containment and initial chemical treatment of the CIP and sweetwater waste streams. These reactors will be monitored over a range of reactant concentrations, pH, temperature, etc. to determine conditions under which CO may be produced.
- Investigate other mechanisms that may be responsible for the CO generation at the plant. Positive results from the second set of experiments (range of actual plant operating conditions) will be matched with the results of the first set of experiments (range of reported conditions for abiotic chemical evolution of CO). If CO evolution in the second set is observed only under conditions which match positive tests from the first set, then the mechanism of CO evolution will be presumed to be the same.
- Investigate the role of biological activity in CO evolution, if necessary. If this occurs, another set of experiments would be performed to check the possibility of biological activity contributing to CO production. Conditions from the previous set of experiments (where CO generation was thought to be following biological pathway)

would be duplicated, with one set of reactors run the same as before (potentially biologically active) and another set with bactericidal treatment (biologically inactive negative controls). If possible, positive controls will also be run by inoculating biologically active reactors with known active cultures of organisms capable of metabolically producing CO. This would provide further confirmation that CO production is likely of biological origin under certain conditions and would suggest control strategies based on inhibiting this activity.

- Investigate ways to alter process conditions to insure they will be operated to limit CO production, and make recommendations to be implemented at the plant.

Significance of the Study

Whereas CO is commonly produced from various environments such as industrial plant air exhausts (steel plants, foundries, oil refining, chemical manufacturing and incomplete combustion of carbon-containing fuels (smoking cigarettes, burning of waste, defective heaters, defective stoves and ovens, etc.), there have been also been a few reports of CO arising from reactions of reducing sugars under certain conditions (Air Products Canada, 2001)). This study is one of the first studies to investigate in detail the production of CO caused by reducing sugars under condition experienced at an industrial wastewater treatment facility. Little is known about CO production from reducing sugar and the information were gathered from various sources. This paper is a systematic analysis of mechanism of formation of CO from actual wastewater samples and from the sugar solutions prepared in the laboratory.

CHAPTER II

LITERATURE REVIEW

Reducing Sugars and Non-Reducing Sugars

A reducing sugar is a monosaccharide or disaccharide sugar that can donate electrons to other molecules and can therefore act as a reducing agent. The possession of a free ketone (CO) or aldehyde (-CHO) group enables most monosaccharides and disaccharides to act as reducing sugars (A Dictionary of Science, 2001). Benedict's test can detect reducing sugars. Benedict's reagent is an aqueous solution of copper (II) sulfate, sodium carbonate and sodium citrate. All monosaccharides and most disaccharides will reduce copper (II) sulfate, producing a precipitate of copper (I) oxide on heating, so they are called reducing sugars. The color and density of the precipitate gives an indication of the amount of reducing sugar present, so this test is semi-quantitative. The original pale blue color means no reducing sugar; a green precipitate means relatively little sugar; a brown or red precipitate means progressively more sugar is present. Examples of monosaccharides are glucose, fructose, etc. and those of disaccharides are maltose and lactose, etc. (Gortner, 1949).

A non-reducing sugar is the sugar that cannot donate electrons to other molecules and therefore cannot act as a reducing agent. Sucrose is the most common non-reducing sugar. The linkage between the glucose and fructose units in sucrose, which involves aldehyde and ketone groups, is responsible for the inability of sucrose to act as a reducing

agent. Other examples of non-reducing sugars are trehalose and raffinose (Gortner, 1949).

Chemical Precursors of CO

Reduction of various sugars gives rise to various numbers of alcohols. For example, two isomeric alcohols are formed by the reduction of keto sugars such as fructose. Upon reduction of a ketose, two epimeric alcohols are produced. Thus, d-fructose gives d-mannitol and d-sorbitol (Gortner, 1949). Figure 2 below gives the structure of fructose and its two alcohols.

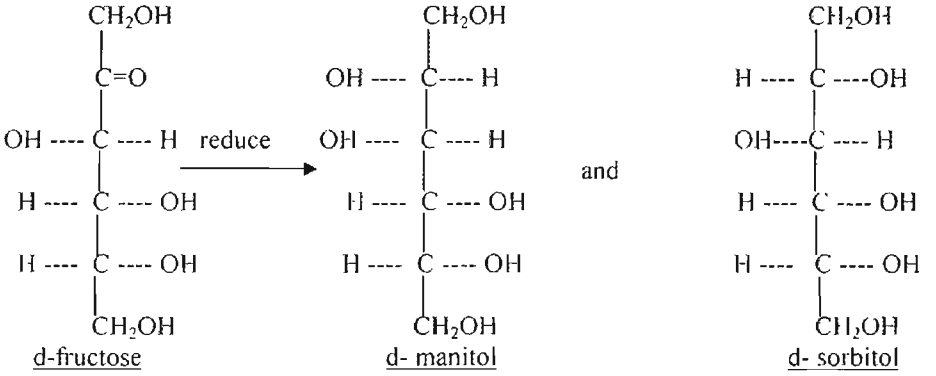


Figure 2: Structure of Fructose (Reducing Sugar)

But an alcohol of a d-series gives rise, upon oxidation, to a keto sugar of the l-series. D-fructose when reduced gives rise to d-sorbital, but oxidation of d- sorbital does not involve carbon-2, which has been reduced; instead the oxidation with *Acetobacter sp.* takes place on what was carbon -5 of fructose. This is because strains of *Acetobacter sp.* are able to transform alcohols into acids (Zigova et al., 2000). Also, if d-glucose is

treated with a solution of N/20 calcium hydroxide, the optical rotation changes to a new equilibrium. Starting with either d-glucose or d-fructose, the same equilibrium is reached and an equilibrium mixture is obtained containing d- glucose, d-fructose, d-mannose, α and β - d- glucose and d - pseudo fructose (Gortner, 1949).

Alkaline Reducing Sugar Hazards

Nicloux and Nebezahl (1929), in an early publication in *Comptes Rendus des Seances de la Societe de Biologie*, clearly showed the production of both CO and CO_2 from reducing sugars under alkaline conditions at temperatures of 81 to 92°C. CO production ranged from about 5 to 8 percent of the oxygen consumed, while CO_2 was produced at about 3.5 to 5 times the amount of CO. The paper did not propose a reaction mechanism or discuss stoichiometry of the reaction.

Aqueous solutions (>2 per cent) of glucose, fructose (laevulose), galactose, arabinose, lactose or maltose at 84°C or above evolved CO in the presence of alkalis or alkaline salts (Nicloux and Nebenzahl, 1929). These conditions occur with < 1 percent to > 5 percent concentrations of NaOH, KOH, $Ca(OH)_2$ or Na-ortho or meta silicates (Nicloux and Nebenzahl, 1929 as cited in Bretherick, 1980). With trisodium orthophosphate, CO evolution occured at 40°C when the pH was as low as 7.4. CO concentrations up to 2000 ppm were detected in closed vessels. Fructose was the reducing sugar studied by Nicloux and Nebenzahl (Bretherick, 1980). Fructose might form in the waste streams as a result of biological activity on the sucrose molecule.

There exists a potential hazard associated with hot alkaline solutions and reducing sugars under such aerobic conditions. There could be a significant and unexpected toxic hazard arising from the use of alkaline cleaning preparations in sugar processing vessels and equipment (Bretherick, 1980). Due to evolution of CO during reaction of >2% aqueous solutions of fructose, galactose, arabinose, lactose, or maltose with <1 to 5 % aqueous alkali or alkaline solutions at 85°C and pH $\geq$ 7.4, care should be taken when using alkaline cleaning compounds in sugar processing vessels (Bretherick, 1980).

Chemical Pathway of Formation of CO

The textbook *Outlines of Biochemistry* (Gortner, 1949) includes several possible chemical pathways. One of the suitable chemical pathways of formation of CO is the oxidation of the hexoses having an aldehyde or ketone group to formic acid, which with the loss of water, produces CO. Evidence also exists that under alkaline conditions, the 3-4 enediol of glucose ruptures at the double bond and yields glyceraldehydes, which, with the loss of water, produce pyruvaldehyde. This compound loses CO to yield acetaldehyde.

Biological Pathway of Formation of CO

CO is produced from CO_2 in the reductive acetyl CoA pathway. The reaction is catalyzed by carbon monoxide dehydrogenase. (Brock, 2001).

$C^*O_2 + H_2 \longrightarrow C^*O + H_2O$

$C^*O + CH_4 \longrightarrow CH_3C^*OOH$ (acetate)

The CO is bound to the enzyme, but it is not clear from the reference whether any is actually released. CO has been found as an obligatory intermediate in anaerobic acetyl-CoA synthesis as cited by Menon and Ragsdale (1996). This article represented the first demonstration of a pathway in which CO was produced and subsequently used as a metabolic intermediate. Different biological reactions were responsible for producing carbon monoxide, which was considered an intercellular signaling molecule and can serve as the carbon and electron source for certain bacteria. The results of studies investigating the biological role for CO showed that CO was produced as an obligatory intermediate during growth of anaerobic bacteria on glucose. CO production is a key step in the Wood-Ljungdahl pathway of acetyl CoA synthesis. The formation of a carbonyl group of acetyl-CoA from the carboxyl group of pyruvate occurs with the following steps (Menon and Ragsdale, 1996):

- Pyruvate undergoes decarboxylation by pyruvate: ferredoxin oxidoreductase to form acetyl-CoA and CO_2,
- CO_2 is reduced to CO by the CO_2 Dehydrogenase (CODH) site of the bifunctional enzyme CO dehydrogenase /acetyl- CoA synthase (CODH/ACS),
- CO generated in situ combines with the anaerobic acetyl-CoA synthesis active site to form a paramagnetic adduct that has been called the NiFeC species, and
- The bound carbonyl group combines with a bound Fe group and CoA to generate acetyl-CoA.

Degradation of Heme in Gram-Negative Bacteria

The heme oxygenase gene from the gram- negative pathogen, *Neisseria meningtidis*, was cloned and expressed in *Escherichia coli* (Zhu, et al., 2000). Expression of the enzyme yielded a solution of catalytically active proteins and caused accumulation of bilverdin within the *E. coli* cells. The purified HemO forms a 1:1 complex with heme and has a heme protein spectrum similar to that previously reported for the purified heme oxygenase (HmuO) from the gram-positive pathogen, *Corynebacterium diphtheriae* and for eukaryotic heme oxygenases. The overall sequence identity between HemO and these heme oxygenases is, however, low. In the presence of ascorbate or the human NADPH cytochrome P 450 reductase sytem, the heme-HemO complex is converted to ferric-biverdin IXα and carbon monoxide as the final products.

CHAPTER III

MATERIALS

Instruments

Materials used for the analysis were those required to conduct bench scale studies in serum bottles and analyzing the samples by gas chromatography.

Gas Chromatograph

A Hewlett Packard (HP) 6890 series Gas Chromatograph (GC) was used to analyze CO. The GC was fitted with a thermal conductivity detector and a syringe injection port.

Detector

A Thermal Conductivity Detector (TCD) was used in the GC. The filament temperature was kept constant, at 150°C with the packed column and 200°C with the capillary column, while alternate streams of reference gas and column effluent (carrier gas plus sample components) passed over it. When the sample was added the power required to keep the filament temperature constant changed. The power differences were measured and recorded. The greatest detector stability resulted when the detector was held at constant temperature and controlled at a temperature slightly above injection port temperature (200°C).

GC Column

A stainless steel packed column (Supelco, HayeSep DB packing) with a porous polymer having 100/120 mesh and capillary column (Supelco, CARBOXEN™ packing 1006 PLOT) of size 30m X 0.53mm were used. The packed column was used when sensitivity was not a major concern and better separations of the peaks were required, while the capillary column was used when sensitive was a major concern. The columns were carefully selected to ensure that the retention times of the components allowed separation of CO from other compounds in the sample. The chromatogram supplied by the manufacturer for the micro packed column is given in Figure 3 below. The detailed methods used for the GC analysis for both columns are contained in Appendix A. Both the packed column and capillary column were operated at an oven temperature of 40°C.

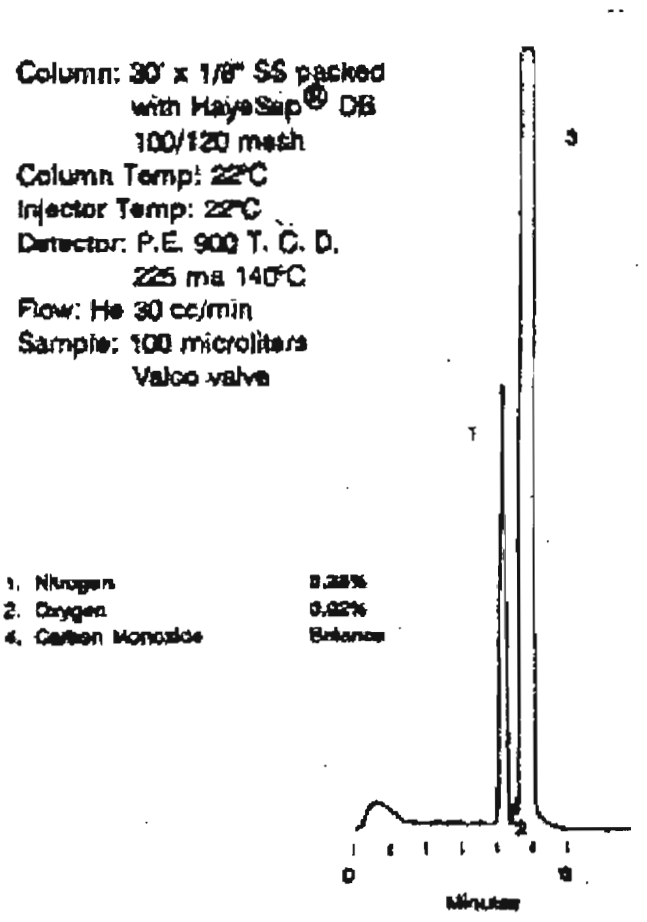


Figure 3: Chromatogram for Micro Packed Column

Glassware

Various glasswares required for the bench scale study and GC analysis of the samples is listed below.

- Serum Bottles (Supelco)(165 ml)
- Rubber septum (Supelco)
- Crimp Top seals (Supelco)
- 25µl, 500µl and 1ml gas tight syringes (Supelco)

- 200ml glass beaker
- 100ml glass graduated cylinder
- General laboratory glassware such as funnels, pipette, test tubes etc.

Reagents

The following reagents, gases and calibration standards were utilized during the study. All the chemicals used were of reagent grade.

Carrier Gas

A cylinder of purified helium with a two-stage regulator was used for GC analysis. Flow rate was verified at the exit of the detector with a soap film flow meter. High purity grade helium (99.9%) was used (Airgas) as the carrier gas for the capillary column. A purified hydrogen (99.9%) cylinder (Airgas) was used while analyzing the samples using the packed column.

Calibration Standards

Standard blends encompassing the concentration range of components in the samples were obtained from a commercial supplier. For this particular experiment, a gas standard having 0.5% CO, 0.5% O_2, and 1% CO_2 balanced in nitrogen was used (Scott Speciality Gases, Supelco, CAT. No. 2-3438). Calibration was further confirmed by diluting pure CO (Airgas) at different concentrations.

Potassium Hydroxide Solution

Four grams of potassium hydroxide (85.8% purity, Fisher Scientific) was added to 1.0 L of distilled water to get a 0.5 N potassium hydroxide solution.

Fructose Solution

Twenty eight and one third (28.3 gm) grams of fructose (crystal, Fisher Scientific) was added to 1.0 L distilled water to achieve a solution with a concentration of 30,000 mg/L of COD. A solution with a concentration of 200,000 mg/L COD was achieved by adding 188.67 gm of fructose to 1.0 L distilled water.

Sucrose Solution

A solution with a concentration of 30,000 mg/L of COD was created by adding twenty six and one eighth (26.8 gm) grams of sucrose (grade II, crystalline, Sigma Chemical Comp.) to 1.0 L distilled water while a solution with a concentration of 200,000 mg/L COD was achieved by adding 178.5 gm to 1.0 L distilled water.

ULTRA Solution

One liter of ULTRA (from the candy manufacturer's wastewater plant) was added to 9.0 L distilled water to create a stock solution.

MANDATE Solution

One liter of MANDATE (from the candy manufacturer's wastewater plant) was added to 9.0 L distilled water to create a stock solution.

Sodium Azide Solution

Dry sodium azide powder (100% purity, Fisher Scientific) of 1.0 gm weight was added to 1.0 L of distilled water to create a stock solution. The molarity of the solution was 0.012 moles/liter.

Sodium Nitrate Solution

Approximately 2.0 g of sodium nitrate ($NaNO_3$) (99% purity, Mallinckrodt) were dried at 105°C for 24 hours following method 4110 A from Standard Methods (APHA et al., 1992). Exactly 1.307g of the dried salt were dissolved in distilled water, and diluted to 1.0 L with distilled water in a volumetric flask.

Sodium Nitrite Solution

Approximately 2.0 g of sodium nitrite ($NaNO_2$) (98.3%, J. T. Baker Chemical) were dried to a constant weight for 24 hours in a desiccator containing concentrated H_2SO_4. Exactly 1.4998g of the dried salt were dissolved in distilled water, and diluted to

1.0 L with distilled water in a volumetric flask. The sodium nitrite solution was stored in a sterilized glass bottle in a laboratory refrigerator at 4°C.

Phosphate Stock Solution

Potassium dihydrogen phosphate (98% purity, Fisher Scientific) (KH_2PO_4) of 1.43 g weight was dissolved in distilled water and diluted to 1.0 L with distilled water in a volumetric flask.

Chlorine solution

Clorox of concentration 5.25% was used to make the chlorine solution. A stock of 1:10 dilution was made. The chlorine concentration in the stock solution was 5,250 mg/L.

Wastewater Samples

The wastewater samples (CIP and sweetwater) analyzed for the study were obtained from a candy manufacturer in four different batches. Samples were obtained before Thanksgiving break (5 November, 2001), after Thanksgiving break (11 December, 2001), before Christmas break (20 December, 2001) and after Christmas break (15 January, 2002). First two batches were taken under normal operating conditions of the wastewater plant. The third batch was sampled just before clean up of the equalization basins (prior to shut down during the Christmas break). The last batch was taken immediately after re-start of new wastewater flow following the clean up of the equalization basins. Sampling times coincided with periods of minimal use of cleaners in

plant operations. This ensured that the concentrations of cleaning chemicals could be controlled in each experimental situation throughout the course of this study.

CHAPTER IV

METHODOLOGY

Various methods were applied to conduct the analysis used to determine the mechanism of CO production at the subject wastewater treatment plant.

Concentration of Cleaners Used at The Plant

Two cleaners used in the plant were ULTRA and MANDATE. ULTRA contained 13% potassium hydroxide (KOH) and 3% potassium hypochlorite ($KClO_3$). It was highly alkaline in nature having a pH of approximately 12.1. MANDATE, on the other hand, contained 23 % phosphoric acid (HPO_4), 20-50 % organic acid and an anionic surfactant. It was highly acidic in nature with a pH approximately of 2. This information was obtained from MSDS data provided by ECOLAB food and beverage division.

Based on the information provided by the plant, the average usage of ULTRA was 12 drums (55 gallons) per month and that of MANDATE was 74 gallons per month (Stover, personnel communication, 2001b). The average wastewater flow over a month's time was 1.96 MG. MSDS data also gave the specific gravity of ULTRA as 1.3 at 68°F and that of MANDATE as 1.273 at the same temperature. Since both the cleaners, ULTRA and MANDATE, were very viscous, 1:10 dilution was made so that the stock solution of each cleaner was 1 part cleaner and 9 parts distilled water. Based on this information various dilutions of the ULTRA and MANDATE stock solutions to be utilized in the experiments were calculated and are tabulated in Table 1 below.

Table 1: Concentrations of ULTRA and MANDATE

ULTRA Solution			MANDATE Solution		
Low Concentra-tion	Medium Concentra-tion	High Concentra-tion	Low Concentra-tion	Medium Concentra – tion	High Concentra-tion
0.403 mL per 60 mL sample	0.807 mL per 60 mL sample	1.614 mL per 60 mL sample	0.0465 mL per 60 mL sample	0.093 mL per 60 mL sample	0.186 mL per 60 mL sample

Various data mentioned in Table 1 are the values for the designated concentrations of ULTRA and MANDATE, which are referred to throughout the study. The combination of ULTRA and MANDATE used in the study was a 1:1 ratio, as information on the ratio of the two cleaners used in the plant, or whether they were used in a constant percentage, was not known.

As ULTRA contained chlorine, its chlorine content was calculated and measured. Potassium hypochlorite, $KClO_3$, present in ULTRA provides chlorine to the cleaner. The $KClO_3$ percent in ULTRA is 3 % as obtained from the MSDS. So, the chlorine percent in ULTRA can be calculated as shown below:

$$\%Cl = \frac{MW\ of\ Cl}{MW\ of\ KClO_3} * (3\%) = \frac{35}{1221} * (3\%) = (0.8\%)$$

This is the chlorine content in the raw ULTRA cleaner before preparing the stock solution. Theoretically, the medium concentration of ULTRA solution as mentioned in Table 1 above had a chlorine concentration of 5.23 mg/L. But when it was measured using colorimetric method (APHA et al., 1992), the residual chlorine in a sample consisting of distilled water and medium concentration of ULTRA was 0.1 mg/L after approximately 3 minutes. Within this time, large chlorine concentration in the cleaner seemed to deplete or being used. This indicates about the chlorine demand of ULTRA.

Chemical Characterization Of Samples

The samples were characterized chemically to have a better idea about the primary constituents in them.

Titration

The two sugars reagents (fructose and sucrose) were titrated separately with potassium hydroxide solution (0.067N) to determine the volume of alkaline solution necessary for an alkalinity of N/15 (0.0667N) in the sample. A titration curve was plotted from the different pHs taken at different titrant volumes. The wastewater samples were also titrated with potassium hydroxide solution (N/15 i.e. 0.0667N) to bring about the desired pH of 12. The titration was done to know the amount of titrant (KOH solution of 0.067N) required to be added in the wastewater to get the desired pH.

pH

The pH of raw CIP and sweetwater of the different batches was measured using a Fisher Scientific Model 900 pH meter and the results were recorded. The pH meter was calibrated using a pH 4 buffer. Samples containing the two wastewaters with additions of ULTRA, MANDATE and ULTRA plus MANDATE, were also analyzed for pH and recorded.

Reducing Sugar

The reducing sugar present in CIP and sweetwater was found using Nelson's Method for Colorimetric Determination of reducing sugars (Ramanathan et al., 1968). It was measured and recorded for all four batches of CIP and sweetwater.

COD Determination

The COD of both CIP and sweetwater samples was measured using a spectrophotometer (HACH DR/300 Spectrophotometer). Samples were digested using the standard closed reflux, colorimetric method 5220 D (APHA et al., 1992). The COD was measured in all batches of CIP and sweetwater. HACH high range, up to 1,500 mg/L, COD test tubes were utilized.

Nutrients

Nitrates, nitrites and phosphates were determined by a standard ion chromatography method 4110 A (Standard Methods, 18th Edition, 1992). An approximate retention time was determined for each anion by injecting standards of the sodium nitrate solution (1.307g/L), sodium nitrite solution (1.4998 g/L) and phosphate stock solution (1.43 g/L), whose methods of preparation were mentioned in the previous chapter. Three known concentrations of each anion (NO_3^-, NO_2^- and PO_4^{-2}) were injected and a calibration curve was constructed by plotting peak height against concentration in a

linear plot. Each CIP and sweetwater sample was injected and peak heights and retention time were recorded.

GC Analysis

The carrier flow in GC was checked periodically with a bubble meter. The method adapted for the GC is presented in Appendix A. The method is made on the basis of the values specified for the instrument and the columns used by the manufacturers. After the gas had been flowing for at least three minutes, the thermal conductivity detector was turned on and the current adjusted to the values specified for the instrument by the manufacturer. About thirty minutes was allowed for stabilization. This method was adopted while using both columns (micro packed and capillary column).

Analysis Method(s)

The method used to analyze the analyte in the current study was a gas chromatographic method. In the gas chromatographic method, the sample was directly analyzed in the gas chromatograph (GC) instrument and the concentration of the unknown sample was interpreted from the calibration curve of air standards. The method was developed as recommended by the vendor for the particular column used. The sample was injected as a plug into the carrier gas stream and passed to the gas chromatography column. The samples were swept into the column, which separated CO from the rest of the components. The detector recorded the elution of CO in the sample. Peak areas were used in conjunction with calibration plots for quantitative measurements.

The separation was completed in 8.5 minutes for the packed column and 6.2 minutes for the capillary column.

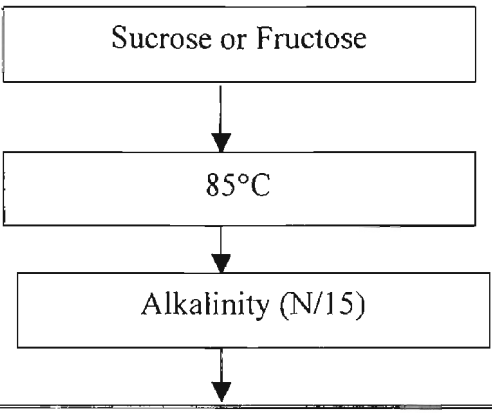
Experimental Design

Specifically, the following experiments were designed to accomplish the above-mentioned objectives.

Reproduce Conditions Reported in the Literature Using Cleaners Used at the Plant

Bench-scale experiments were conducted in serum bottles for the work required to accomplish the first objective of reproducing conditions mentioned in an early report (Nicloux and Nebenzahl, 1929). The experiments were run on two separate reagent chemicals. The first reagent was sucrose, the dominant sugar form in the plant waste stream. The second reagent was fructose. Fructose was selected because it may be formed in the waste stream as a result of biological activity on the sucrose molecule. Each of these two primary substrates, used to represent the plant's waste streams, was tested at 85°C and with a uniform alkalinity of sodium bicarbonate N/15 (0.0667N). Sodium bicarbonate was used to establish alkalinity. The selected temperature and alkalinity are within the optimal range for CO generation as cited by Nicloux and Nebenzahl (1929). Each of the two reagents, sucrose and fructose, was run at two COD concentrations of 30,000 mg/L and 200,000 mg/L to simulate wash water from the production plant CIP and sweetwater. Each of these sugar concentrations was exposed to three concentrations of the cleaners (ULTRA and MANDATE) used at the plant. The

molar (volume) ratio of the two cleaners was held constant at a value of 1:1. The ratio was chosen randomly as what ratio was used at the plant was not known. The pH of these mixtures of sugar reagent and cleaning solutions was monitored and adjusted to a common value (in the range of 11 to 12). In addition, eight serum bottles were run as blanks that contained everything but the reagent chemicals. It was expected that none of these 8 serum bottles would produce CO and thus they would provide quality control for the analytical measurement of CO. At each reagent sugar concentration, a serum bottle was dosed with KOH instead of the ULTRA and MANDATE cleaners and had its pH adjusted to the appropriate range. This would provide information as to whether the caustic is the dominant reagent in the cleaning solutions that impacts the formation of CO i.e. by raising pH alone. Each set of serum bottles for the sucrose and fructose experiments was run in triplicate, to allow statistical evaluation of the results concerning the production of CO under the various conditions assessed. All experiments were run for a constant length of time, approximating the average detention time (4 days) in the equalizations basin at the plant. A graphical illustration of the experiment is shown in Figure 4.



Experiment run in triplicate	
30,000 mg/L COD	200,000 mg/L COD
ULTRA+ MANDATE @ low Concentration	ULTRA + MANDATE @ low Concentration
ULTRA + MANDATE @ medium Concentration.	ULTRA + MANDATE @ medium Concentration.
ULTRA + MANDATE @ high Concentration.	ULTRA + MANDATE @ high Concentration
KOH	KOH
ULTRA + MANDATE (no sugar)	ULTRA + MANDATE (no sugar)
No ULTRA + MANDATE (only sugar)	No ULTRA + MANDATE (only sugar)

Figure 4: Experimental Design to Duplicate the Conditions Reported in Literature and to Apply the Conditions using Reactants used in the Plant.

Reproduce Operating Conditions in the Plant

This set of experiments utilized the two waste streams (one with the concentration of CIP and sweetwater to which no cleaners have been added) from the plant. Each wastewater stream was subjected to three combinations of the cleaners: ULTRA alone, MANDATE alone, and ULTRA + MANDATE (1:1). Each of these cleaner concentrations was run at three different pH values (low ≈ 4.5, medium ≈7.0 and high ≈12.0). Each of the pH values was run at two temperatures (low ≈27°C and high ≈50°C). The reason for choosing this temperature range was because the wastewater treatment plant's normal operating temperature was of 27°C, however after the plant was cleaned with hot water, the temperature went up to 50°C. This design allowed observation of the effects of each of the critical variables in CO formation.

Some of the conditions such as adding KOH as alkaline chemical in the two waste streams were designed to see conditions used to address the first objective of the research plan. So, in one set up of each experiment KOH was added to both the waste samples to repeat the conditions seen earlier in the literature concerning the production of CO from the reaction between a reducing sugar and an alkaline chemical (KOH). The experiment was set-up to monitor for CO, CO_2, and pH. For the combination of variables mentioned above, each waste stream had a total of 108 serum bottles, 36 combinations in triplicate. Statistical information was generated from the experiments run in triplicate. A total of 216 serum bottles were set up for this portion of the experimental plan. The experimental plan is illustrated graphically in Figure 5.

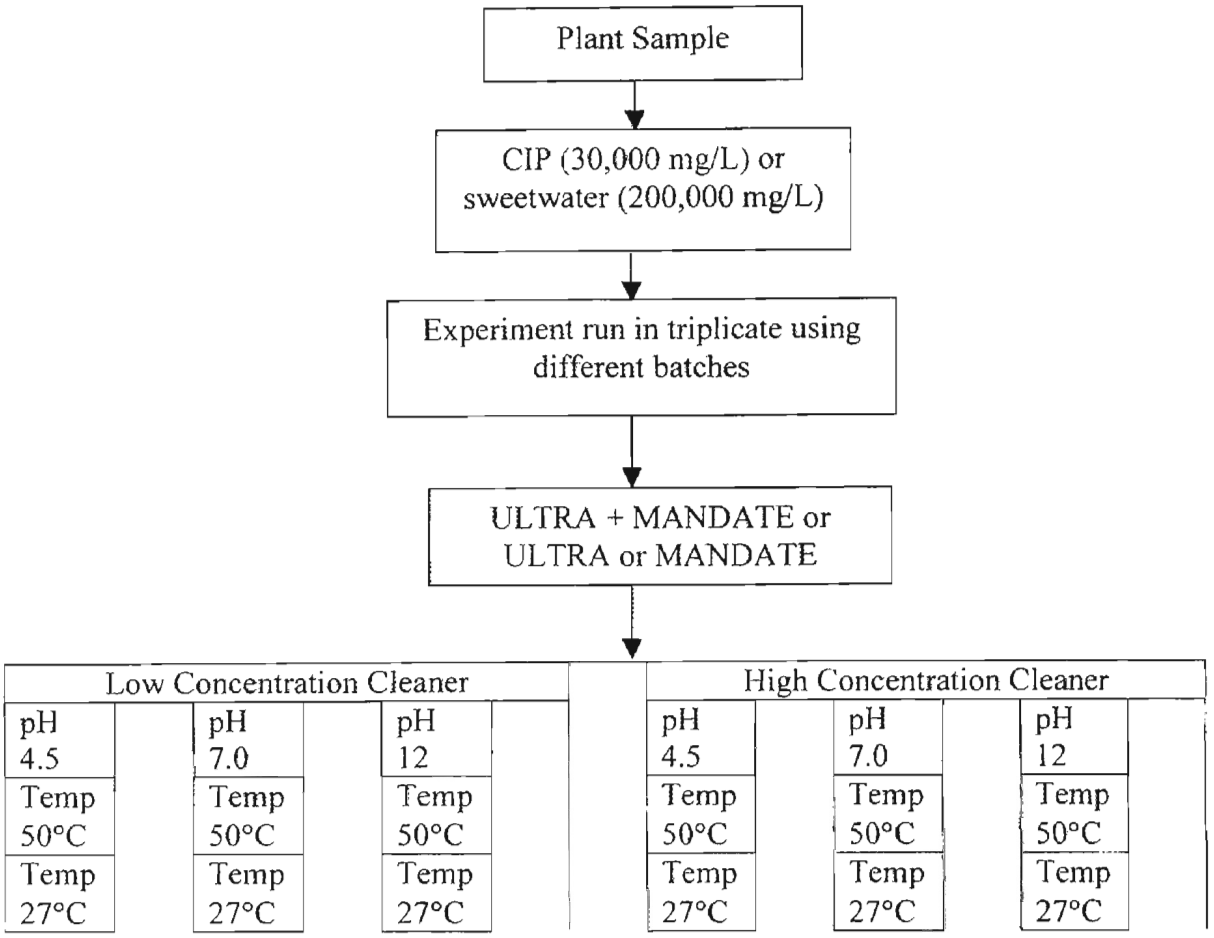
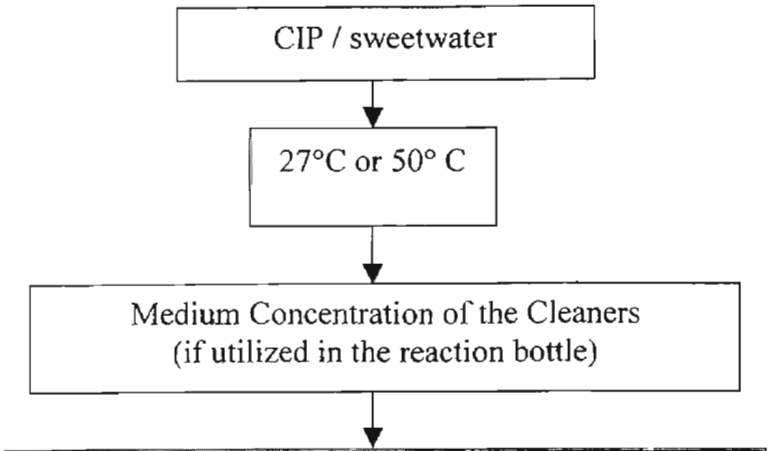


Figure 5: Experimental Design for Reproducing Operating Conditions at Plant

Analysis of Biological Production of CO from the Plant Samples

This set of experiments also utilized the two waste streams (CIP and sweetwater) from the plant. The experimental variable in this set of experiments was the presence or absence of sodium azide. Each type of sample was subjected to three combinations of the cleaners: ULTRA alone, MANDATE alone, and ULTRA plus MANDATE (at a

common ratio) and a-fourth combination with added potassium hydroxide (KOH). Each of these three combinations of the cleaners was carried out at a medium concentration (0.807ml of stock (1:10) solution per 60ml sample of ULTRA and 0.093 ml of stock (1:10) solution per 60 ml of sample of MANDATE). All the experiments were run at two temperatures (low ≈27°C and high ≈ 50°C). As sodium azide is a potent disinfectant, this design was to determine whether the CO production was due to biological activity. A one gm per liter concentration of sodium azide was added in bottle to control bacteria (Fathepure, personnel communication, 2002). For the combinations described above, each waste stream had a total of 24 serum bottles, 8 combinations in triplicate. In addition, 6 serum bottles (2 in triplicate) were run as blanks, which contained only raw samples. The graphical illustration of the experimental set up is shown in Figure 6 below.



Experiment run in triplicate	
With Sodium Azide	Without Sodium Azide
ULTRA	ULTRA
MANDATE	MANDATE
ULTRA + MANDATE	ULTRA + MANDATE
KOH	KOH
Only Sample (no cleaners)	Only Sample (no cleaners)

Figure 6: Experimental Design for Biological Analysis of Production of CO at the Plant

A further experimental set-up was designed to see the effect of CIP (1 mL of CIP in 60 mL of fructose solution) being used as bacterial seed on a fructose solution (30,000 mg/L). The analysis was done at three different temperatures (27°C, 50°C and 85°C). Sodium azide (1 g/L) was added in one of the set-up and they were analyzed at all three temperatures. This would kill the seed bacteria and allow observation of any effect in the production of CO. The cleaners were used at the combination of medium concentration of ULTRA and MANDATE. The experiment was run in duplicate and so a total of 18 serum bottles were required. An illustration of the experimental design is shown in Figure 7.

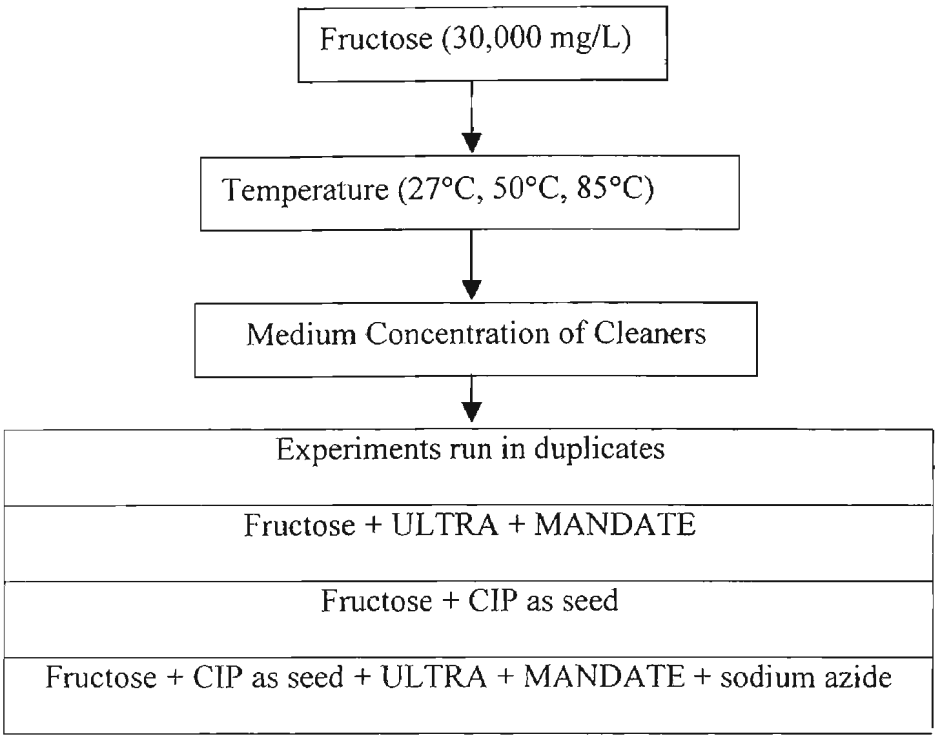


Figure 7: Experimental Design to Analyse Effect of CIP as Seed

Kinetics of CO Production from the Plant Samples

Experiments were conducted in serum bottles for the work required to determine the kinetics of the CO production. Each waste stream samples was exposed to low, high and medium concentrations of the cleaners (ULTRA and MANDATE) used at the plant. The concentration of the cleaners was medium (0.807ml of stock (1:10) solution per 60ml sample of ULTRA and 0.093 ml of stock (1:10) solution per 60 ml of sample of MANDATE). The formation of CO was tested from 3.5 days until 8 days for CIP, and

5.5 days until 8 days for sweetwater. The experiments were run only for 8 days as the estimated retention time of the wastewater in the treatment plant was on the order of 8 days. Kinetic studies were set up based on the operational retention time (actual plant condition) according to the information obtained from the plant (Stover, personnel communication, 2001c). The temperature was either 27°C or 50°C or at both temperatures based on the results obtained from the experiment to reproduce the plant condition as mentioned above. For these combinations, CIP required a total of 24 serum bottles (12 combinations in duplicate) and sweetwater had 18 (9 combinations in duplicate). A graphical illustration of the experimental set up is shown in Figure 8 and 9 below.

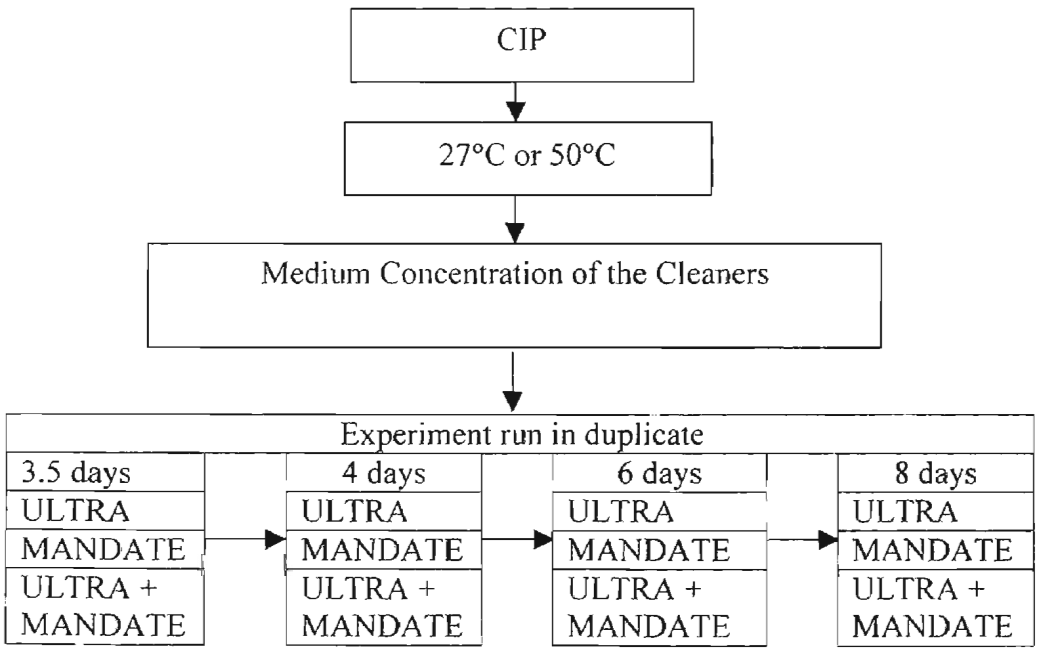


Figure 8: Experimental Design for Kinetics of CO Production in CIP

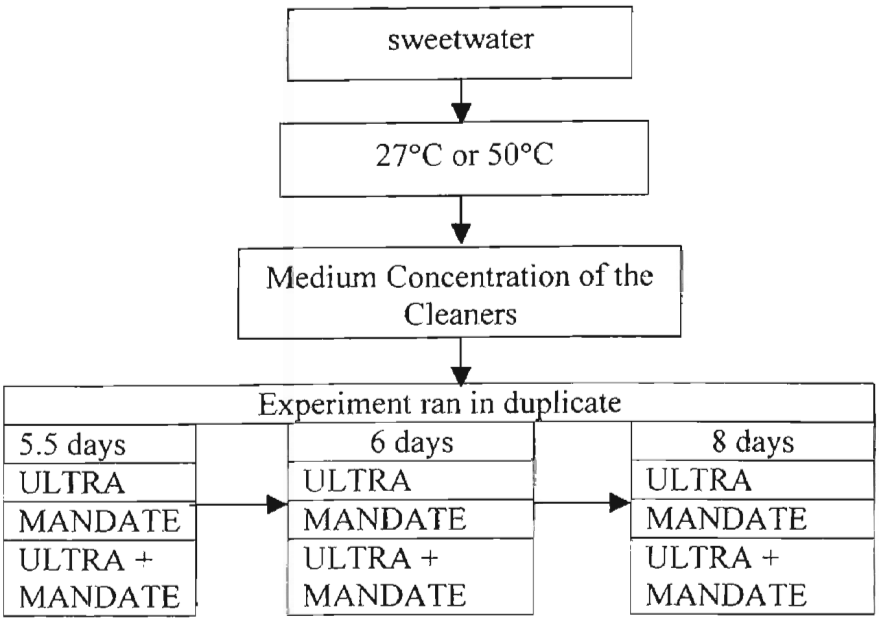
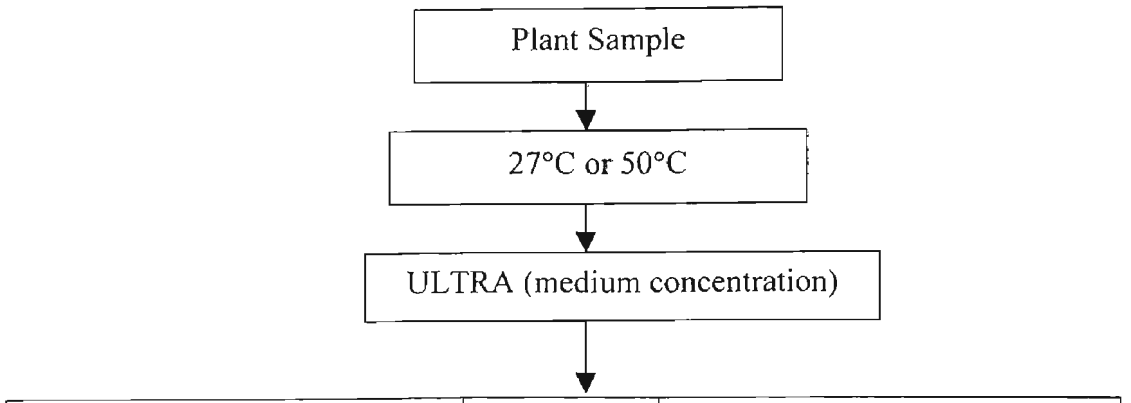


Figure 9: Experimental Design for Kinetics of CO Production in Sweetwater

An experiment to better define the kinetics was based on the need to develop a rate constant for the production of CO. Only one of the cleaners (ULTRA) was added in each of the waste streams. The temperatures used were 27°C or 50°C or at both the temperatures based on the temperatures at which CO was produced in above experiments. Based on the results obtained from the previous set of experiments, further formation rate experiments were run from 3 days until four days at an interval of two-hours for CIP and from 5 days until 6 days for sweetwater also at 2-hour interval. The idea behind this was to identify the rate of production of CO from the waste samples. For this experiment, a total of 26 serum bottles were set up for each waste stream. An illustration of this experimental set-up is shown in Figure 10.



CIP	Sweetwater
3 days	5 days
3 days 2 hrs	5 days 2 hrs
3 days 4 hrs	5 days 4 hrs
3 days 6 hrs	5 days 6 hrs
3 days 8 hrs	5 days 8 hrs
3 days 10 hrs	5 days 10 hrs
3 days 12 hrs	5 days 12 hrs
3 days 14 hrs	5 days 14 hrs
3 days 16 hrs	5 days 16 hrs
3 days 18 hrs	5 days 18 hrs
3 days 20 hrs	5 days 20 hrs
3 days 22 hrs	5 days 22 hrs
3 days 24 hrs	5 days 24 hrs

Figure 10: Experimental Design of Rate of CO Formation at Smaller Range of Time

Control Tests

Chlorine Test

Chlorine was believed to reduce CO production in this wastewater, so a set of experiments was set up using chlorine to reduce CO production from the sample. Each waste stream was treated with one cleaner (ULTRA). Four different doses of chlorine: zero (blank samples), low (5 mg/L), medium (10 mg/L) and high (20 mg/L), were introduced into each waste stream. Each waste stream was subjected to 27°C or 50°C temperatures or was analyzed at both the temperatures depending on the results from

previous experiments. Each set-up of the experiment was run for 4 days (retention time of the plant) for CIP and 5 days for sweetwater. For the combination of the variables mentioned above, 8 serum bottles were set up, 4 combinations in duplicate, for each waste stream. A total of 16 serum bottles were set up for this experimental plan. Figures 11 and 12 illustrate the experimental plan.

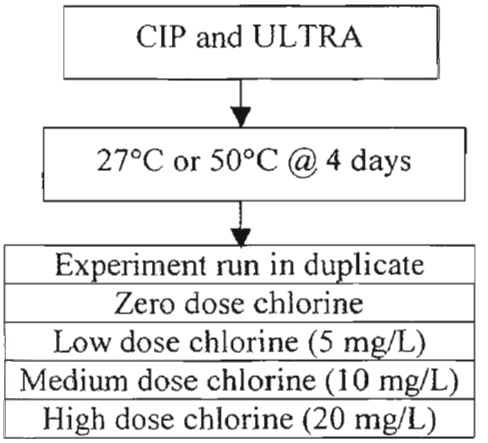


Figure 11: Experimental Design for the Control Test in CIP using Chlorine

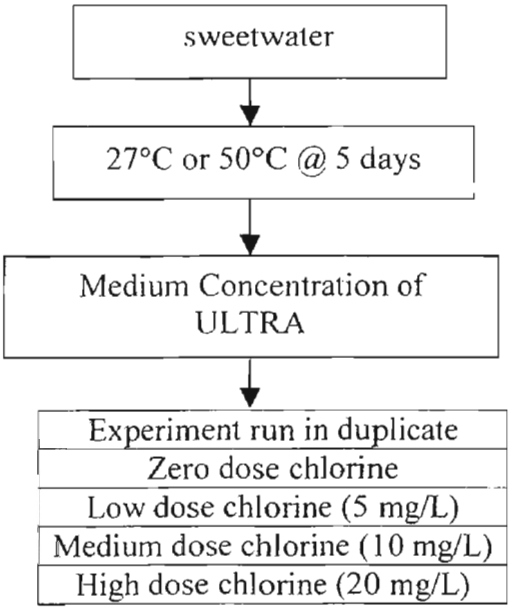


Figure 12: Experimental Design for the Control Test in Sweetwater using Chlorine

A further control test using chorine was set up using fructose having COD concentrations of 30,000 mg/L or 200, 000 mg/L. The experiments were run at 85°C so as to duplicate the conditions mentioned in the literature by Nicloux and Nebenzahl (1929). The purpose of this experiment was to determine whether chlorine would have any effect in reducing CO produced from the reducing sugar at 85°C. Each of these sugar concentrations was subjected to a medium concentration of three combinations of cleaners: ULTRA (0.807 ml of stock (1:10) solution per 60ml sample) alone, MANDATE (0.093 ml of stock (1:10) solution per 60 ml of sample) alone, and ULTRA plus MANDATE together, and also to another combination of KOH alone. Each of these combinations was run with and without adding a chlorine dose at both concentrations of fructose for 4 days. The sugar was subjected to medium dose (10 mg/L) of the chlorine. For this experiment, a total of 16 serum bottles were required. A graphical illustration of this set-up is shown in Figure 13 below.

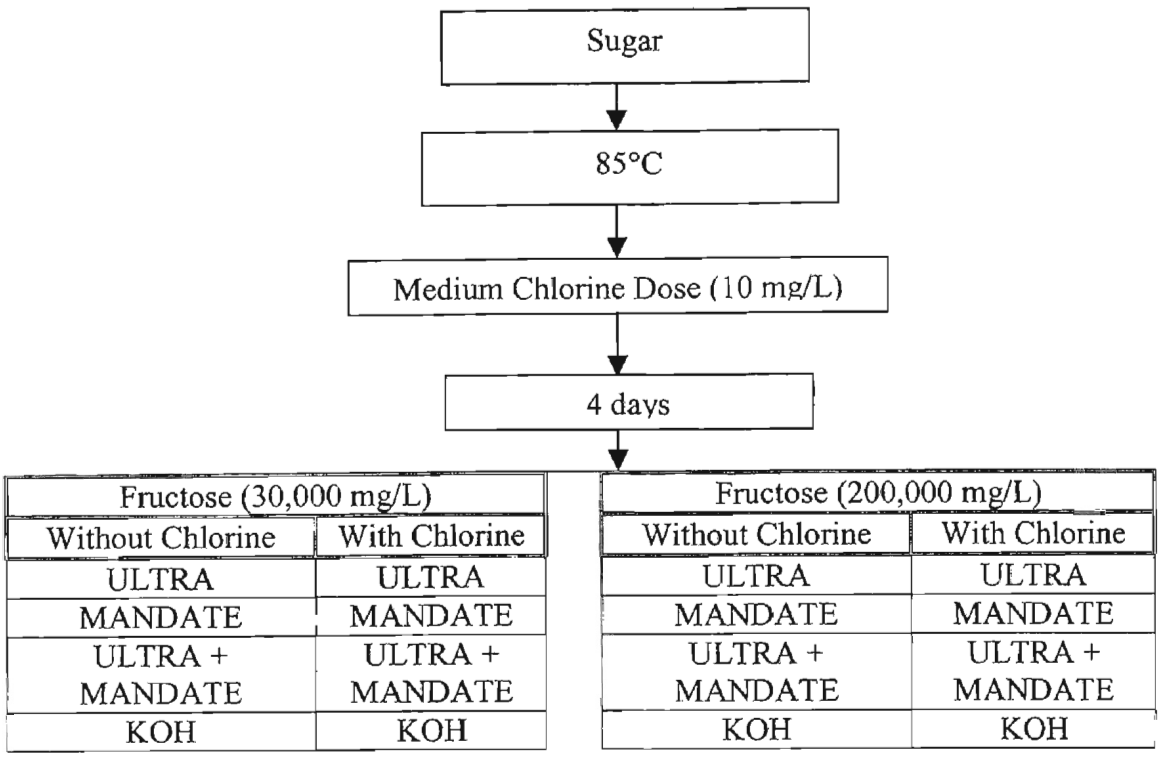


Figure 13: Experimental Design for the Control Test using Chlorine and Fructose

Aeration Test

Based on the observations made at another facility, aeration was thought to be a control strategy to reduce CO formation from the wastewater plant (Stover, personnel communication, 2001d). This experimental set-up utilized the two waste streams from the plant and was run for 4 days for CIP and 6 days for sweetwater. Each waste streams were subjected to the medium concentration of the cleaners used in previous experiments: ULTRA alone, MANDATE alone, and ULTRA plus MANDATE combined. These combinations were run both with and without aeration in each of the waste streams. The DO in the aerated samples was established within the range of 2-4 mg/L. The experiment was run at 27°C or 50°C or analyzed at both the temperatures depending on the result obtained from the experiment reproducing the operating conditions of the plant

(depending on the temperature at which CO was produced in reasonable amounts). A total of 18 serum bottles was required for this set-up, 6 combinations in triplicate for each waste stream. Figures 14 and 15 below further illustrate this set of experiments.

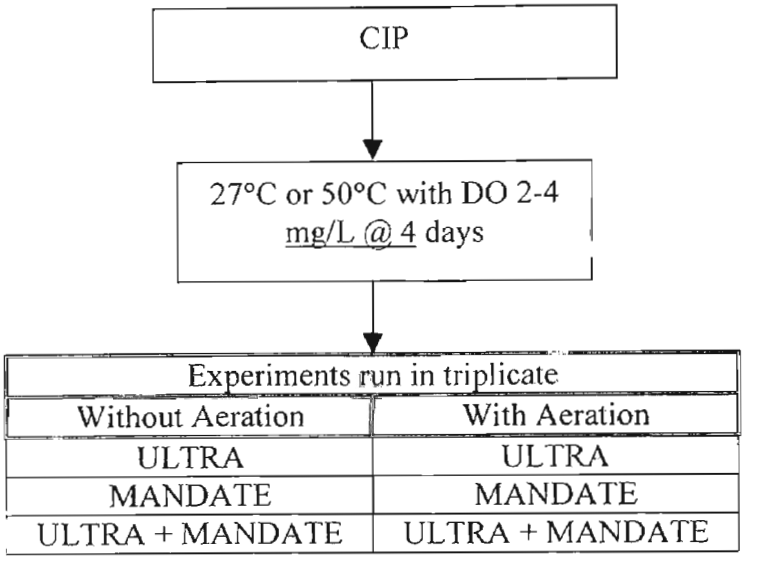


Figure 14: Experimental Design for the Control Test using Aeration in CIP

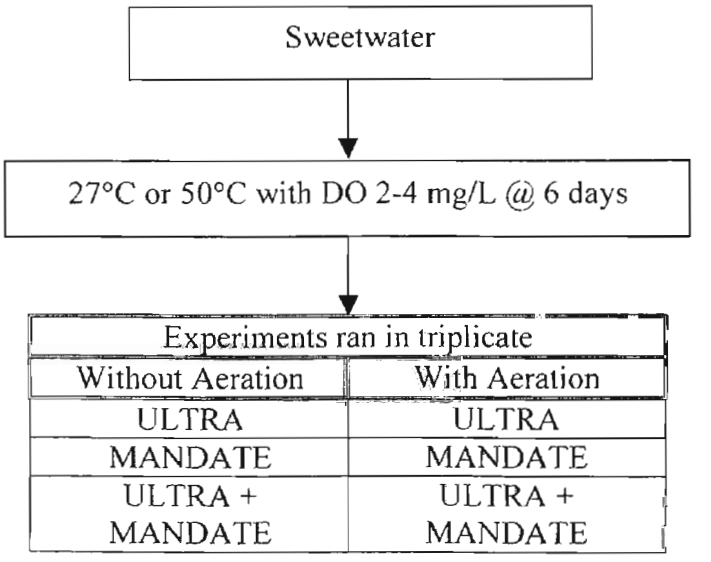


Figure 15: Experimental Design for the Control Test using Aeration in Sweetwater

Sampling Air Standard

A sample of gas standard withdrawn from a glass sampling bomb was used for development of the calibration curve and quickly injected through the inlet into the GC. Different volumes of the samples (10µl to 500µl) were injected and CO peaks were obtained. Area for each volume was recorded.

Sample Analysis

After the sample bottles were kept in an incubator for the required number of days, they were ready to be analyzed by the GC. From the headspace of serum bottles, 25µl of gas was taken and injected in the inlet of the GC. This method was used for both the columns (micro packed and capillary column). All samples, including the blanks, were analyzed in this fashion.

Safety Procedures

CO is an environmental hazard. It was thus required to be handled in a safe environment. The pure CO gas used throughout the experiment and the CO produced from the samples in gas tight serum bottles were handled properly. The following precautionary measures were applied during the experiment.

- The pure CO gas cylinder was kept in an open environment.
- CO and other gases produced in the sample serum bottles were disposed of in an open environment.

- Since unsealing the aluminum crimp and rubber septa from the serum bottles after the analysis produced a small pressure release noise, autoclave gloves and goggles were used by the experimenter as a safety measure. The high concentration of gases which accumulated in some (five) of the bottles resulted in them breaking while unsealing.

CHAPTER V

RESULTS AND DISCUSSION

There were four sample batches of the wastewater received from the plant on different dates (November 5, 2001, December 11, 2001, December 20, 2001 and January 15, 2002). Samples were taken when minimal amount of the cleaners was present in the wastewater, so that the amount present in each of the experiments could be controlled. As per the experimental methodology mentioned in chapter III, various experiments were run. Data analysis of the results was designed to accomplish the study objectives. Results of the various experimental set-ups and discussion of the results obtained are presented in this chapter.

Quality Assurance and Quality Control methods (QA/QC plan) adopted for this study are explained below.

QA/QC Plan

The laboratory capability and routine analysis of QA/QC samples in this study are to document data quality and to demonstrate continued acceptable performance. QA/QC requirements are based on specific performance criteria, or control limits, for data quality indicators, such as accuracy and precision. Typically, control limits for accuracy are based on the historical mean recovery plus or minus three standard deviation units, and control limits for precision are based on the historical standard deviation or coefficient of

variation (or mean relative percent difference for duplicate samples) plus three standard deviation units. QA/QC procedures that are done for this method include instrument calibration and calibration checks; assessment of method detection and quantitation limits; assessment of method accuracy and precision; routine monitoring of contamination considering requirements of continuous use, sensitivity,; appropriate documentation and reporting of data (including QA/QC data) (USEPA, 1991).

Detection Limit

The detection limit was determined by injecting the lowest mass of air standard into the GC, which resulted in a detectable CO concentration. The limit of detection with a hot wire thermal conductivity detector and helium carrier gas expressed, as ppm of a gas, was 50 ppm for the capillary column and 500 ppm for the packed column. This shows that the detection limit of GC was significant enough to detect the concentration that can be a human hazard (provided earlier in document).

Precision and Accuracy

Accuracy is the ability to recover a known amount of some component and establish how close the result is to the true value (Schweitzer, 1994). Accuracy depends upon the availability of accurate calibration standards. These may be obtained with a certificate of analysis from commercial suppliers. Also the linearity of the standard curve

(regression coefficient was approximately 0.99) further confirmed the accuracy of the GC instrument and the air standard. The preparation of reagent blank sample was to determine background interference.

Precision is the ability to repeatedly obtain the same value for a single sample or method (Schweitzer, 1994). Precision is controlled by the mode of sample introduction, primarily. In addition, Henry's constant of CO is 53.6 E-05 atm/mole fraction at 20°C. Higher values for Henry's law constants indicate lower solubility. Comparing the Henry's law constant values of CO in equivalent units at the same temperature with CH_4 (Henry's constant of 37.6 E-05 atm/mole fraction at 20°C), which is very insoluble, the CO seems to be less soluble. So CO produced from the wastewater must be quickly formed in the gas phase. The sample injected from the headspace thus should have the actual concentration of CO formed. Precision is also affected by detector drift, which in turn depends upon the control of carrier gas flow rate and system temperature. Also, duplicate samples were to obtain the precision in the experiment. Figure 16 below shows the concentration of CO produced from different batches of samples (CIP).

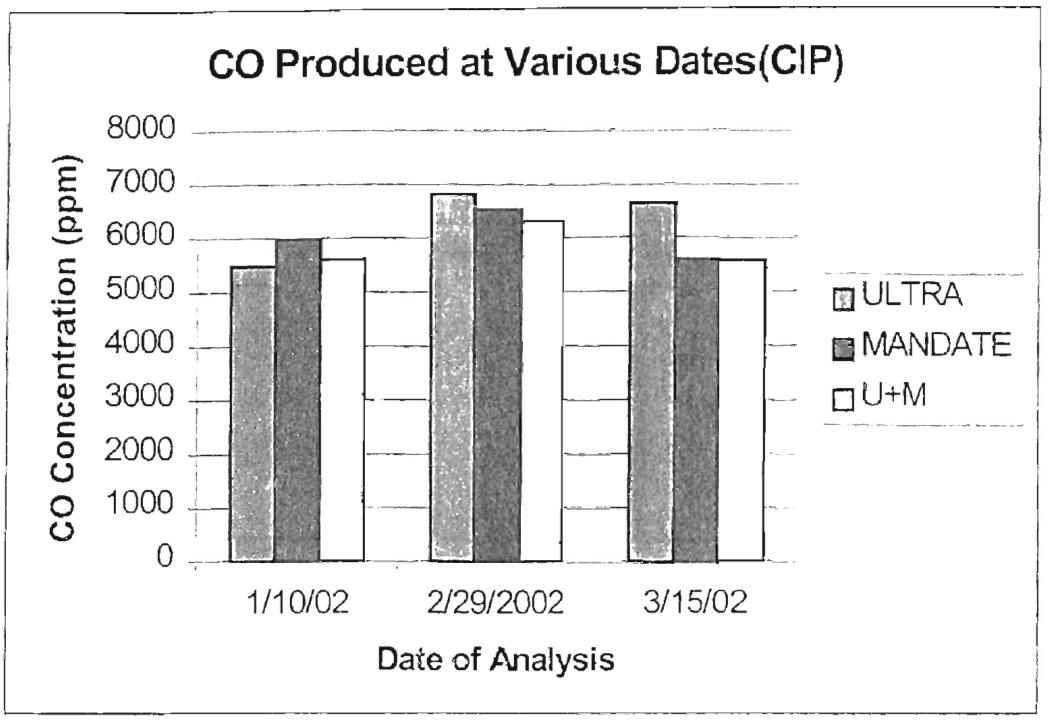


Figure 16: CO Concentration Produced from Different Batches of CIP

Overall, the concentration of CO was relatively consistent for all the batches of CIP. Figure 17 below shows the CO concentration produced at different batches of sweetwater.

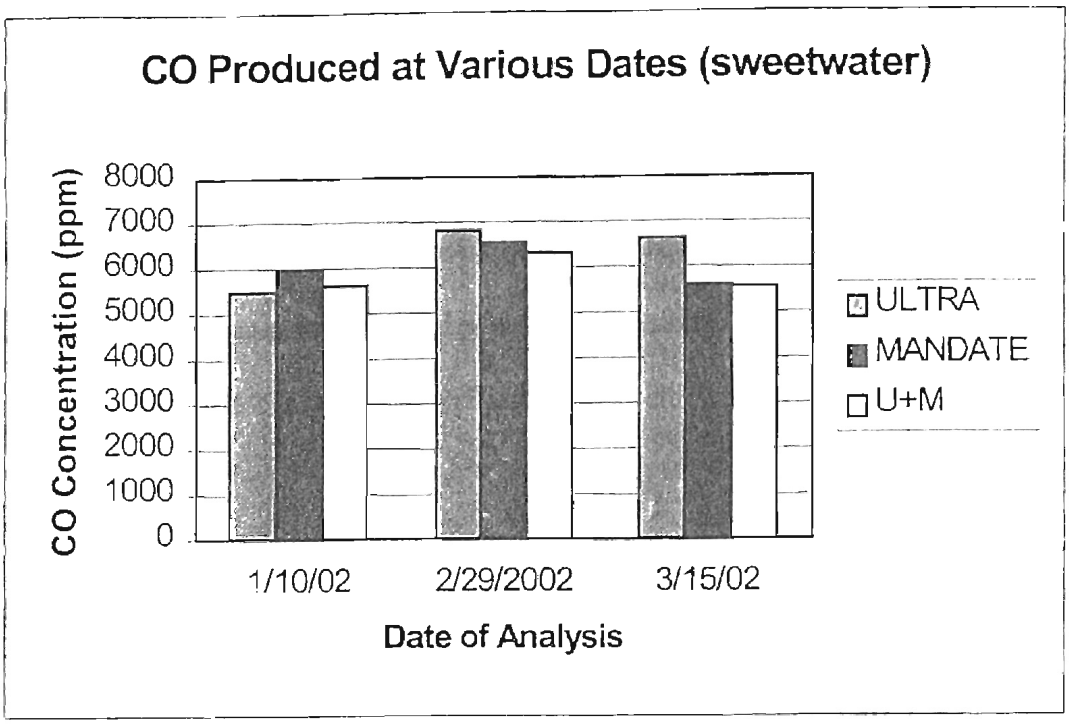


Figure 17: CO Concentration Produced from Different Batches of Sweetwater

Seemingly, values at each of the three experimental conditions were consistent between the three experimental dates in the case of sweetwater.

To gain more confidence in the measurements of CO concentration from the chromatograph, some set-ups of the experiments were analyzed on two GC instruments in two different laboratories. The concentration of a sample injected on the GCs of two laboratories gave same concentration of CO (standard deviation of two wastewaters with same cleaners added was in the range of ± 165 for CO concentration obtained). Also, after the samples were analyzed, two concentrations of the air standard were injected to see if the points closely fit the standard curve prepared at the beginning of the experiment. The points fit on the standard curve and regression coefficient was still very

close to 1. At times, when there was doubt about the formation of the peaks in the chromatograph, assistance of technical representatives at GC's vendor was solicited. The normal volume of sample to inject in the GC was 250µl, but sometimes only 100µl of the sample was required to be analyzed so that very large peaks of adjacent compounds did not dominate the appearance of the peak under study. This was an attempt to acquire more precision and confidence in the result of the chromatograph.

Sample Collection and Preservation

Most reagents were prepared just before the preparation of the samples and the samples were immediately moved to and maintained in an incubator after the preparation. After a specified number of days for various analysis, the samples were taken out of the incubator and immediately injected in the GC. The bottles were not allowed to be outside incubator more than two hours before injection.

Data Quality

For the data to be of defensible quality, a quality assurance procedure called a QA Plan (Stanley and Verner, 1983) should be done. Sample control and documentation procedures, standard operating procedure, calibration procedure, data reduction, validation, etc. are required for a quality experiment. In this experiment, the GC experiment was calibrated every time prior to injecting the samples. Also, the data were taken in duplicate and triplicates to check for any deviation in the two results. The

average of the duplicate results was reported. A blank sample was included in each experiment to see how the data differs from that of the sample with reagent added. An optimized GC procedure for detecting CO production was developed and the same procedure was followed to get precise data. Sample logs, bench sheets and calibration logs were prepared to keep track of the experimental conditions and results. The standard operating procedure was followed for the test by recording a step-by-step instrument calibration procedure, troubleshooting steps for equipment, etc.

Instrument Control Chart

The standard control chart was constructed from the average and standard deviation of the air standards. A control chart was utilized to keep track of the results on a weekly basis. This demonstrated the variation of an established 'standard' or quality control sample when measured by the process (Crosby et al. 1995). A known mass (100 µl volume or 500 µg mass) of a 0.5 % CO air standard was injected into the GC. The peak area should yield the same concentration as the standard curve. The average percentage recovered was taken as the mean of the data from QC samples measured every week, which was 98.7 % in this experiment. The associated standard deviation was approximately 1.71, which was then used to set the action and warning limits on the chart. The warning limits were fixed at ± 2 standard deviations and action limits at ± 3 standard deviations. The control chart showed that none of the points fall outside the

warning limit, which indicate that there was no identifiable problem with the system. The control chart for the system can be located in Appendix G.

As per the experimental methodology mentioned in Chapter III, various set-ups of the experiments were run. Data were measured and recorded. The data analysis of the results was done to accomplish the objectives of the study. Below are mentioned results of the various experimental set-ups and also the discussion of the results obtained.

Chemical Characterization of Wastewater Samples (CIP and sweetwater)

The wastewater samples (CIP and sweetwater) were characterized chemically in terms of pH, COD, nutrients, reducing sugar and titration.

pH

The range of pH values taken before and after analyzing the samples of CIP and sweetwater at each of the different conditions, both with and without the cleaning agents, are tabulated in Table 2 below. The data summarized in the table can be located in Appendix B.

Table 2: Range of pH of Samples Before and After the Analysis for each Experimental Condition

Sample	pH Reading (before analysing)	pH Reading (after analyzing)
CIP	3.7- 4.5	3.1 - 3.2
CIP and ULTRA	5.2 - 6	4.4 - 4.6
CIP and MANDATE	3.7- 4.6	3.5 - 3.8
CIP, ULTRA, and MANDATE	4.8 - 5.3	4.5 - 4.6
sweetwater	3.3 - 4.2	3.5 - 4.1
sweetwater and ULTRA	4.3 – 5.5	4.3 - 4.4
sweetwater and MANDATE	3.2 – 4.3	3.1 - 3.3
sweetwater, ULTRA, and MANDATE	4 – 4.65	4.2 - 4.3

All samples (both adding ULTRA and MANDATE and without adding them) were found to be acidic. Also, it can be observed from Table 2 that the pH of samples taken before and after analyzing the samples didn't differ much though it decreased slightly in most of the cases after analyzing.

COD

The COD of samples was analyzed according to the methods outlined in Chapter IV. Table 3 below shows the average of six COD readings measured in the wastewater from the plant.

Table 3: COD of Samples from Plant

Sample	Average Measured COD (mg/L)	Std. Deviation of COD Measured	Expected COD (mg/L)
CIP	27,148	180	30,000 –100,000
Sweet Water	281,923	104	100,000 -300,000

The average sugar concentrations in both the waste streams came within the expected range of mg/L COD as reported by the plant. The standard deviation shows the COD value did not deviate much from the mean value.

Reducing Sugar Analysis

Results from the colorimetric determination of reducing sugar in the two samples are tabulated in Table 4. Colorimetric analysis gave the value of reducing sugar in terms of sugar concentration (mg/L). The concentration value (mg/L) was converted to theoretical COD and then compared the CODs to get percentage. According to the information obtained from the plant, reducing sugars in the CIP is less than 1 brix and

that of sweetwater is more than 1 brix. The brix unit is used for measuring reducing sugar and is same as the percent measurement of the reducing sugar.

Table 4: Reducing Sugar of Samples from Plant

Sample	Measured Average Reducing Sugar (mg/L) of four batches of samples	Measured Average Reducing Sugar (%)
CIP	1,770	0.177
sweetwater	26,130	2.613

As expected, the average reducing sugar analyzed for different batches of each wastewater sample (CIP and sweetwater) showed that the CIP contained a lower percent of sugar than the sweetwater. The reducing sugar obtained from the colorimetric determination was used to find the theoretical BOD of the wastewater as seen in Table 5 below. The stoichiometric equation for fructose used to find the relationship was:

$$C6H12O2 + 6O6 \rightarrow 6CO2 + 6H2O$$

Table 5: Reducing Sugar Percent of Total Wastewater

Sample	COD (mg/L) measured	Average reducing sugar (%) of COD	Theoretical COD based on reducing sugar measured	Ratio of theoretical COD and measured COD	Reducing sugar (%) of total wastewater
CIP	21,467	0.177	1,888	$\frac{1888}{21467} \approx 10\%$	10 %
sweetwater	277,397	2.613	27,914	$\frac{27914}{277397} \approx 10\%$	10%

The results show that the reducing sugar percentage of the CIP is approximately 10% of the wastewaters COD. Similarly, the reducing sugar present in sweetwater is also approximately 10% in terms of a COD ratio. This shows that the reducing sugar present in the wastewaters is low and there are other constituents in addition to the reducing sugars in the wastewaters. Analysis of the relationship between reducing sugar and COD of the sample was done to determine how consistent the presence of reducing sugar was compared to the COD. Figures 18 and 19 below show the consistency of the relationship between COD and amount of reducing sugar of the wastewater. The straight line equation shown in the figure was automatically obtained from the linearly fitting operation of MS EXCEL.

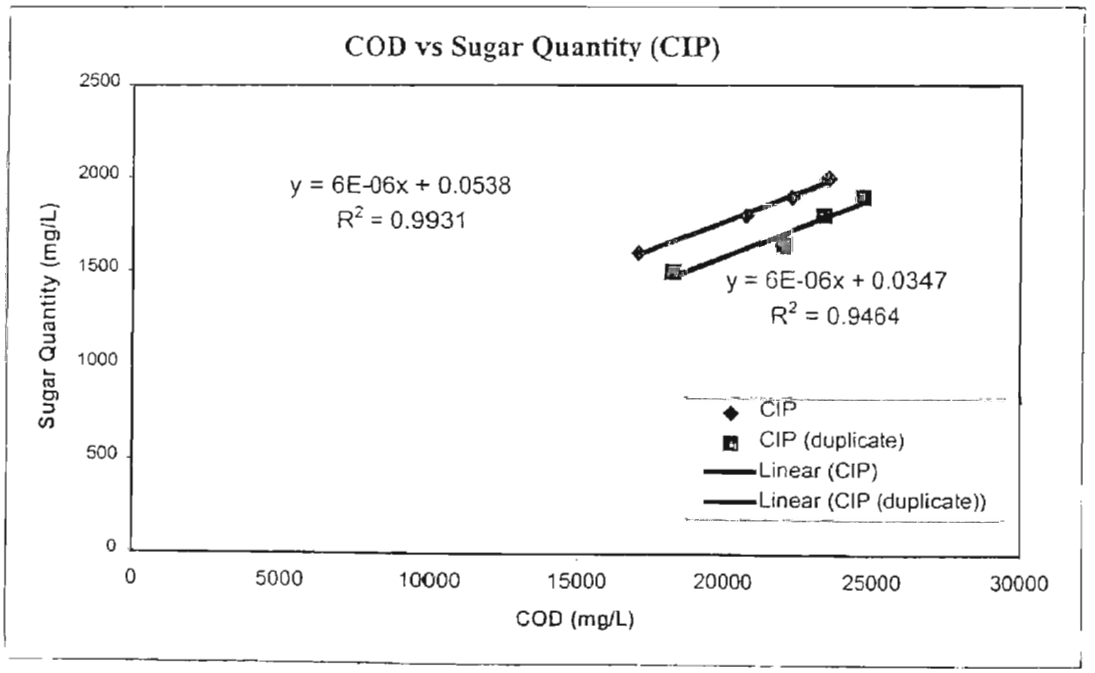


Figure 18: Reducing Sugar in CIP

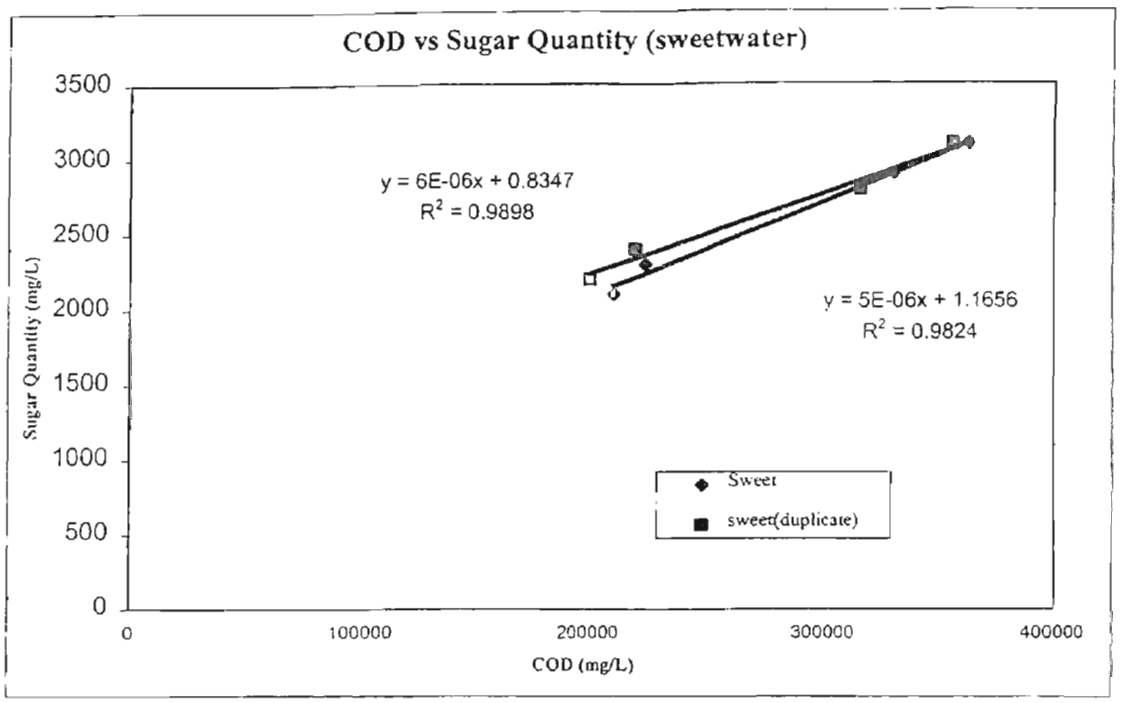


Figure 19: Reducing Sugar in Sweetwater

Figures 18 and 19 show the duplicate runs produced similar results in each of the waste streams. Figure 20 shows the correlation between reducing sugar (combining all the data from Figures 18 and 19) and COD, mg/L.

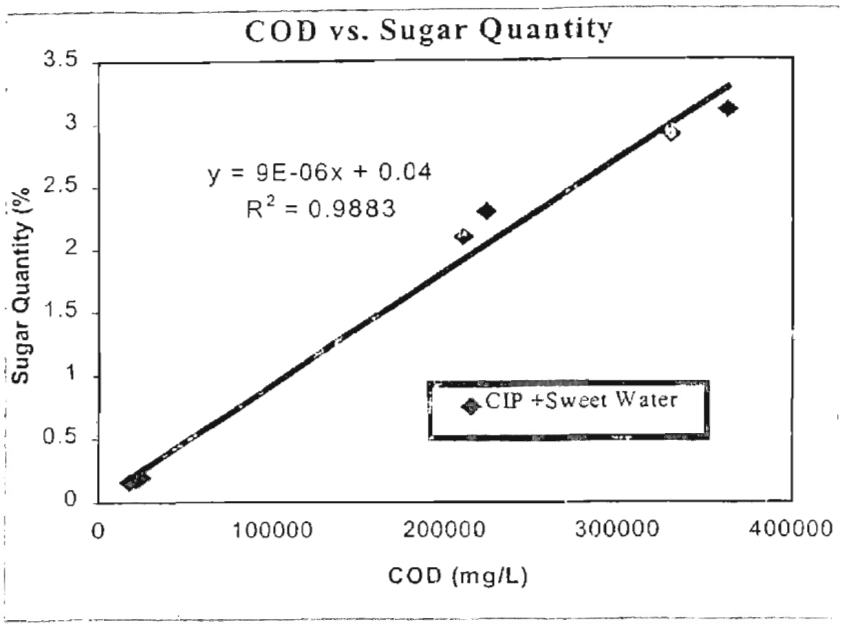


Figure 20: Relationship Between Reducing Sugar and COD in the CIP Wastewater

Figure 20 shows that that the reducing sugar present in wastewater streams directly correlates with the corresponding concentration as COD. The lower data in Figure 20 are those of CIP, i.e. less than 100,000 mg/L COD, while the upper data are those of sweetwater, i.e. more than 200,000 mg/L COD. Thus all the data of both the wastewaters were well fitted to the same line.

Titration of CIP and Sweetwater

As seen in Table 2 above, both CIP and sweetwater are acidic in nature. The experimental design of this next analysis was to vary the pH from 4.5 up to 12 as shown in Figure 5. Both the wastewater samples of volume 200 mL were titrated with KOH until the maximum pH (12) seen at the plant was achieved. The titration curve plotted for both the CIP and sweetwater can be observed in Figure 21 below.

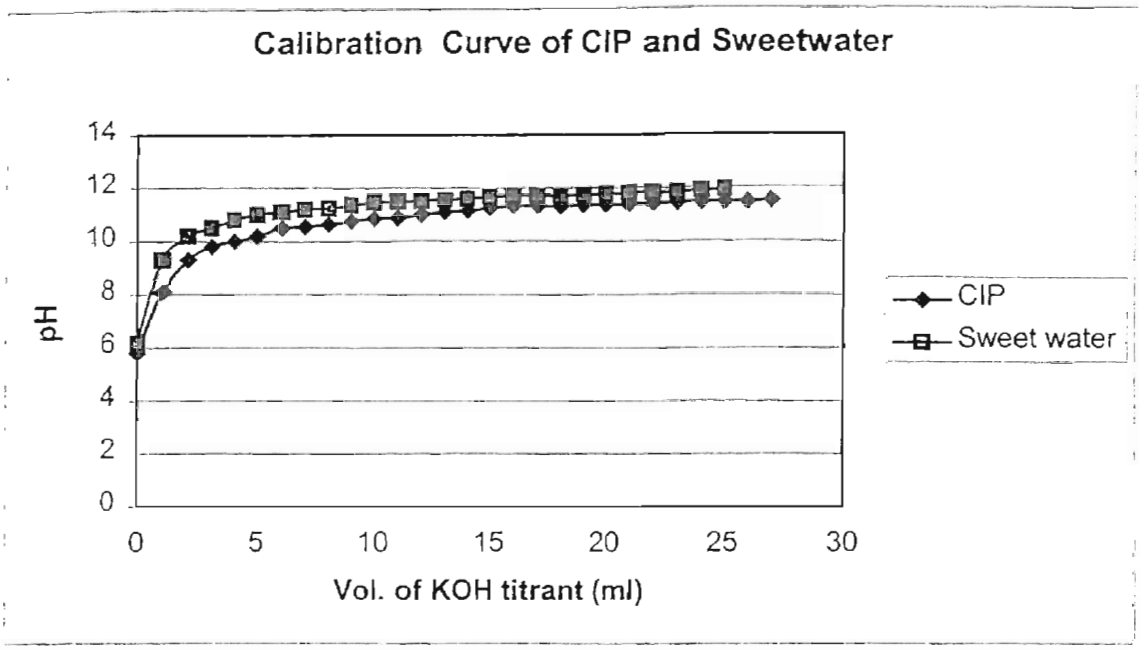


Figure 21: Titration Curve for CIP and Sweetwater

It can be noted from Figure 21 that the stoichiometric end point (point in the curve from where it starts flattening) lies approximately at pH 9. The plot was useful as a reference for pH adjustment of the wastewater samples (CIP and sweetwater).

Nutrients

Anions (nitrates, nitrites and phosphates) in both of the waste solutions were determined using ion chromatography as described in the Chapter IV. The calibration curves that were developed were used to determine the concentration (amount) of each anion in the wastewater samples in mg/L. Table 6 below shows the average (of two samples) concentration of nutrients in the wastewater samples.

Table 6: Nutrients in Wastewater Samples

Component Name	Concentration, mg/L	
	CIP	Sweetwater
Nitrate	76.61	29.75
Nitrite	1.05	-
Phosphate	7.22	2.05

From Table 6, it is apparent that the nitrate concentration was high and, phosphate and nitrites were either not present or at a minimal quantity in both the wastewaters.

Standard Curve and Calibration

A standard curve of peak area vs. mass (micrograms) of a known standard injected into a GC was prepared for carbon monoxide by analyzing the calibration standards. Most of the standard curves prepared before each set of experiments were linear with R^2 values ranging from 0.97-0.99. Except for a few erroneous CO peak areas due to instrumental flaws, most values were acceptable (i.e. the retention time of the CO peak were similar to the recommended retention time, the peak areas were distinctive, and the GC could detect the area). As mentioned above in Chapter III, both capillary column and packed column were used during study. The reason was with the packed column, the peaks could be well separated and would offer more confidence about the

peaks obtained while the capillary column was needed when more sensitivity was required in the analysis. The detection limit of GC using packed column was 500ppm while that of capillary column was 50ppm. The capillary column was useful mainly during biological analysis of the wastewater samples and control tests to reduce CO, where very small peak formed would be a matter of concern during the analysis. Figure 22 below illustrates one of the standard curves taken during sampling of waste stream samples using packed column. The peak area would correspond to the mass of gas injected into the GC for a given volume of the sample. Most of the masses of the CO injected into the GC using this standard curve were approximately in the middle range of the mass in the y-axis of the curve. Thus, the calibration curve was in the range close to what was being used during the analysis.

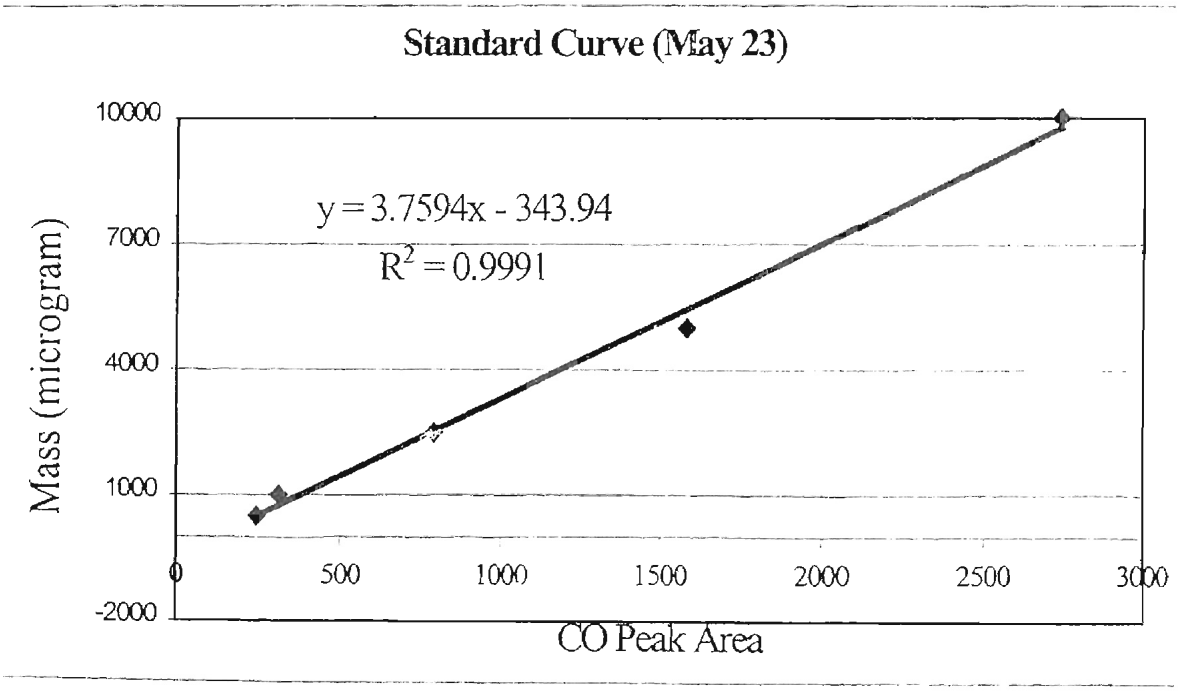


Figure 22: Standard Curve for GC Analysis using Packed Column

Figure 23 below shows a standard curve obtained using the capillary column for more sensitive analysis. It can be seen that the CO concentrations that can be obtained using this standard curve are lower than those in Figure 22. The mid range of the calibration curve was close to the masses of the samples obtained from the sample analysis.

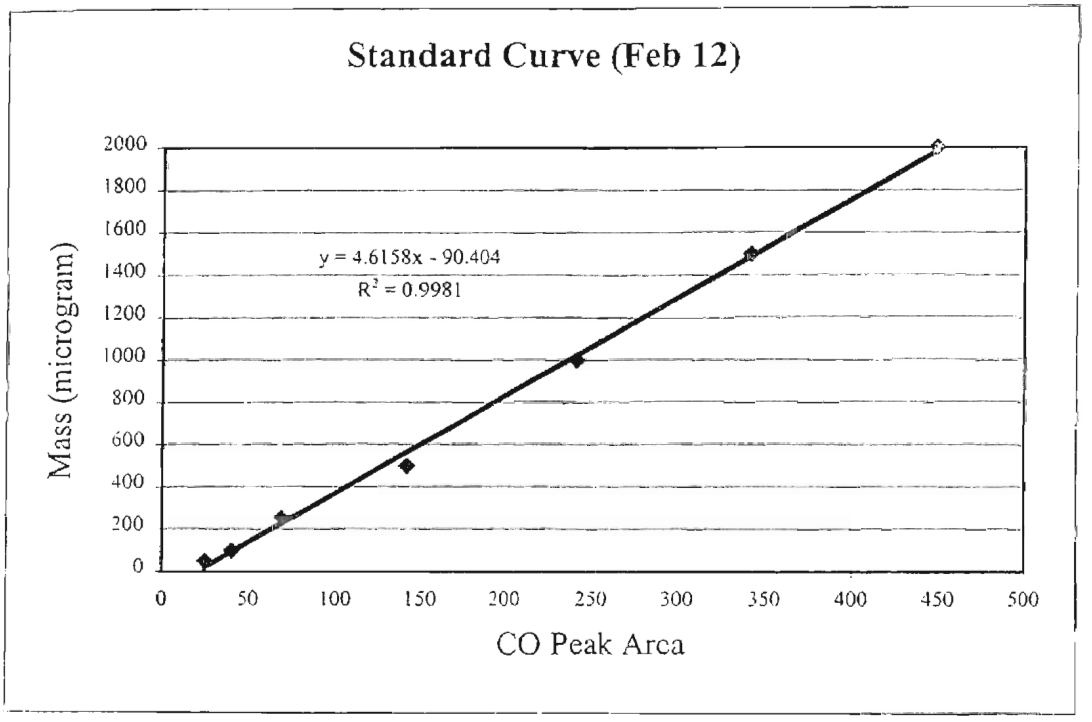


Figure 23 : Standard Curve of GC Analysis using Capillary Column

Duplicated Analysis of CO Production

Some chromatograms produced to duplicate analysis of the earlier literature i.e. at 85°C (Nicloux and Nebenzahl, 1929) are presented in Figures 24 and 25 below. The results in Table 7 are based on the experiments conducted with reducing sugar at 85°C.

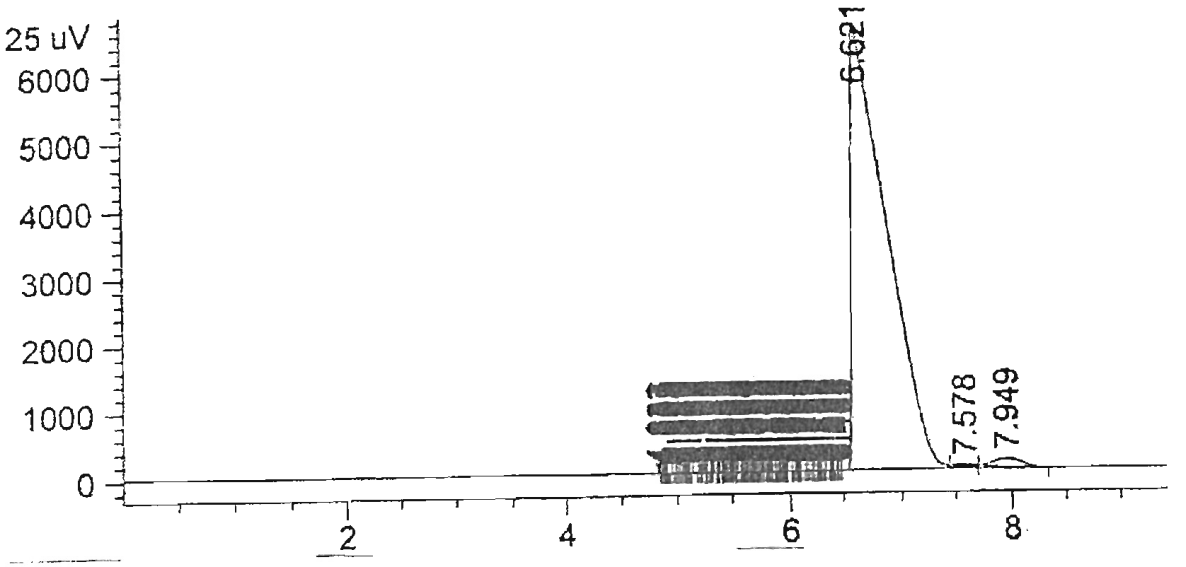


Figure 24 : Chromatogram (Sugar + ULTRA)

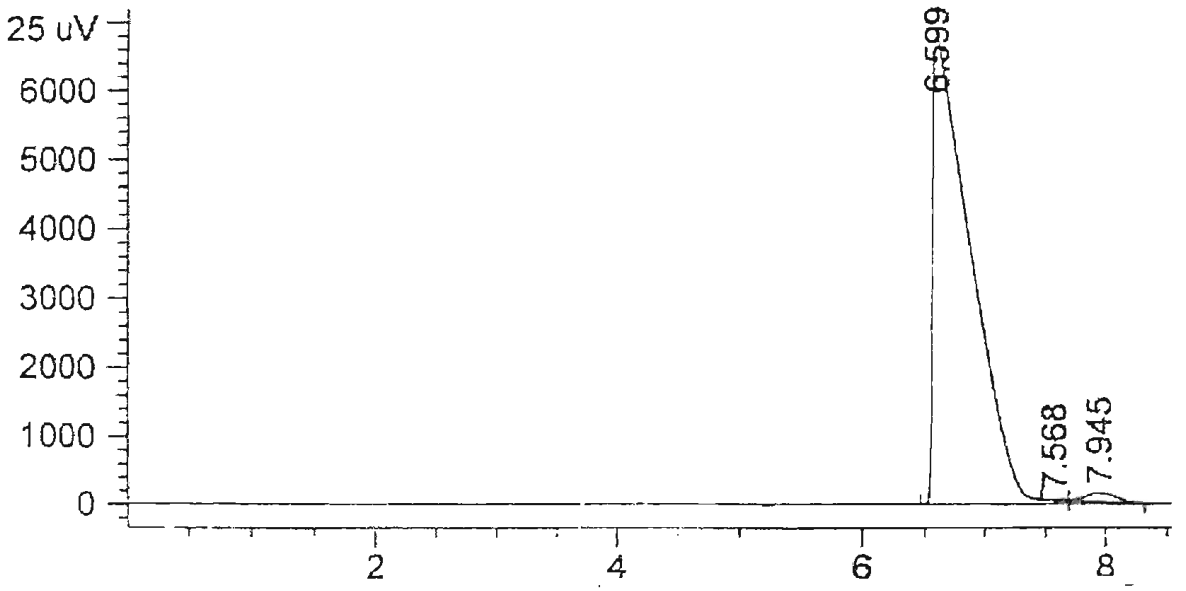


Figure 25: Chromatogram (Sugar + KOH)

The packed column was used for analyze in this experiment. In the above chromatograms, 6.6 minutes was the retention time for nitrogen, 7.5 minutes for oxygen, and 7.9 minutes for carbon monoxide. The CO peak seems sharp enough to confirm that the peak was of the analyte under study. The retention time of CO in above chromatograph was very close to what was found from the chromatograph of air standard. Figure 25 confirms the conclusion derived from the earlier French literature (Nicloux and Nebenzahl, 1929) that mentioned CO production due to reactions of reducing sugar and alkaline chemical. Figure 24 illustrates that the conditions prevalent at the plant could result in CO generation by abiotic chemical reaction mechanisms. The concentration of the CO produced is shown in Table 7 below.

Data on CO evolution from reactions between reducing sugars and cleaning agents used in the plant (and with KOH) are shown in Table 7 below. The results are the average CO concentration produced from triplicate sample set-up. One set-up of sucrose and two set-up of fructose were run as triplicates.

Table 7: CO Concentration Obtained from the GC Analysis of Sucrose and Fructose

Sample	Average Concentration of CO (ppm) ± std. deviation	
	30,000 mg/L as COD	200,000 mg/L as COD
Sucrose / Fructose +ULTRA +MANDATE (low concentration)	2506 ± 295	1893 ± 106
Sucrose / Fructose + ULTRA +MANDATE (medium concentration)	2232 ± 147	4362 ± 425
Sucrose / Fructose + ULTRA + MANDATE (high concentration)	3162 ± 41	4117 ± 353
Sucrose / Fructose + KOH (N/15)	1750 ± 172	2667 ± 139
ULTRA + MANDATE (no sugar)	0	0
No ULTRA + MANDATE (only sugar)	0	0

The results in Table 7 show that CO is produced in each case except when sugar is not added or only when sugar is in the reaction vessel. The CO concentration is large enough to be considered a serious health hazard. The pathway of production of CO in this case was found to be from a chemical reaction, as cited by Nicloux and Nebenzahl (1929), because sodium azide did not have any effect on the samples having fructose and KOH and analyzed at 85°C and CO was still produced. Standard deviations were based on the three samples under study as mentioned in Chapter IV.

Reproduced Analysis of Operating Conditions

Samples incubated at conditions similar to the plant operating conditions were subjected to GC analysis, and the quantities of CO were determined from the standard curves. The duplicate and triplicate samples were compared to see the precision of the experiment. Also a blank sample was analyzed as a control. CO was found to be produced in CIP after 4 days and after 6 days in sweetwater. Some of the chromatograms produced during the analysis of waste stream samples are presented in Figures 26 and 27 below. The column used to analyze this set of experiments was the packed column.

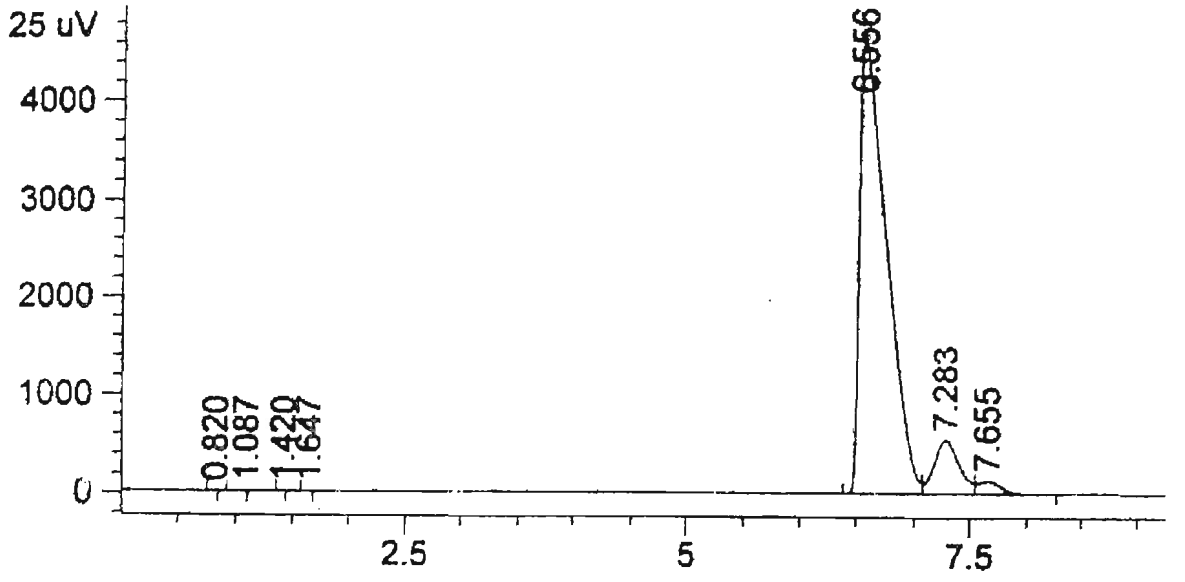


Figure 26: Chromatogram (sweetwater at pH 12)

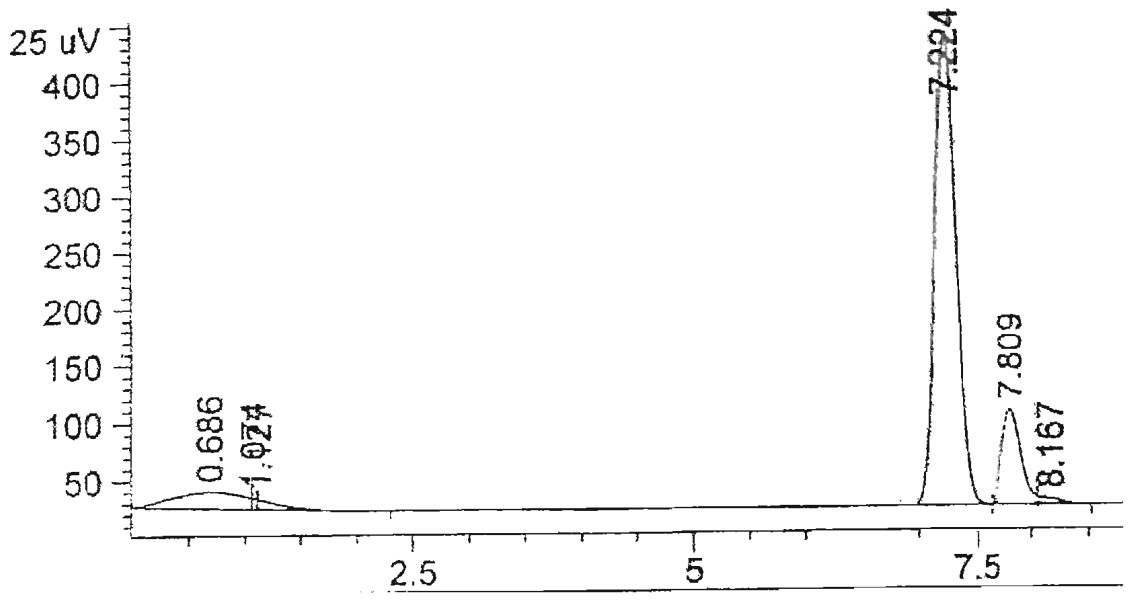


Figure 27: Chromatogram (CIP at pH 7)

The chromatograms in both Figures 26 and 27 show that the CO peak (retention time 7.6 minutes in Figure 26 and 8.1 minutes in Figure 27) are distinctive (as determined by HP ChemStation software). The sample condition and concentration results for the CO peak in the chromatograms are shown in Table 8 below. The results were the average result of the analysis in triplicate.

Table 8: Concentration of CO Produced at Operational Condition of the Plant

Sample	Temp (°C)	pH	CO Concentration (ppm) ± std. deviation	
			Low concentration of cleaner	High concentration of cleaner
CIP +ULTRA	27	4.5	6032 ± 897	6277 ± 795
	27	7	6093 ± 1160	5851 ± 540
	27	12	5784 ± 827	6809 ± 413
CIP+MANDATE	27	4.5	8794 ± 734	8377 ± 210
	27	7	6569 ± 1035	9540 ± 1181
	27	12	7987 ± 570	6911 ± 955
CIP+ULTRA+MANDATE	27	4.5	9003 ± 755	5230 ± 701
	27	7	8214 ± 1452	5927 ± 582
	27	12	6594 ± 958	8033 ± 586
Sweetwater +ULTRA	27	4.5	3866 ± 414	5416 ± 664
	27	7	4862 ± 235	7036 ± 488
	27	12	6880 ± 299	7828 ± 414
Sweetwater +MANDATE	27	4.5	5774 ± 606	8211 ± 681
	27	7	7246 ± 636	6837 ± 490
	27	12	7999 ± 834	7780 ± 156
Sweetwater +ULTRA+MANDATE	27	4.5	590 ± 38	659 ± 4
	27	7	705 ± 82	751 ± 29
	27	12	779 ± 28	919 ± 95

Table 8 shows that the highest CO produced is 9540 ppm and the lowest is 590 ppm. Even the lowest CO produced is high enough to be considered a concern. CO is produced in both acidic and alkaline samples. The experiment was also conducted at a 50°C temperature (the upper range of the plant operating conditions) and the CO production detected was negligible (data not shown in the table). Thus, it was speculated that under these experimental conditions, the CO evolution was temperature dependent and was produced only at the plant's lower temperature range (27°C) in case of wastewater and was produced at 85°C in case of a reducing sugar solution. But no obvious effect of pH and different cleaner concentrations could be observed in producing CO. Having CO produced only at the lower temperature range was inconsistent with the observations at the plant. Therefore, it was speculated that the CO production from this set of experiments was due to some biological mechanisms, with the bacteria being inactivated at the higher temperature. The control test, with no cleaners did not give any CO production as expected. Standard deviation of the results was based on triplicate samples.

Controls for this set-up of the experiment were the raw wastewater samples, i.e. CIP and sweetwater, alone, without adding any concentration of the cleaners. The samples did not produce any CO, detectable by GC using packed column. Also, Table 7 showed that the cleaners alone did not produce any CO. The question then was what did the cleaners do to produce CO. Also, ULTRA was alkaline in nature while MANDATE was acidic in nature, but seemingly no significant difference could be found in terms of

CO production with the addition of these two different cleaners in CIP. How could either cleaners alone or the combination stimulate very similar concentrations of CO production in CIP? A potential answer could be that the reaction between the wastewater sample and the cleaners might have modified the matrices of the wastewater so that it was easier to produce CO. Analysis was also carried out just adding CIP and sweetwater with no additional cleaners. Co was not produced in this case. This would strengthen the case that the cleaners somehow modify the wastewater matrices. This could be another control. Also, sugar alone, with no cleaners, did not produce CO.

Biological Analysis of CO Production from the Plant

All the second set of experiments (in the range of actual plant operating conditions) did not agree with the results of the previous set of experiments (in the range of reported conditions for abiotic chemical evolution of CO). The reducing sugar in reaction with cleaners gave CO production at 85°C while the wastewater sample in reaction with cleaners produced CO at a lower temperature (27°C). Figures 28 and 29 are the chromatograms for CIP without and with adding sodium azide, respectively. Figure 28 and Figure 29 are the chromatograms for sweetwater without and with sodium azide respectively. Sodium azide was added in one set of samples because sodium azide has the ability to kill bacteria if present in the wastewater.

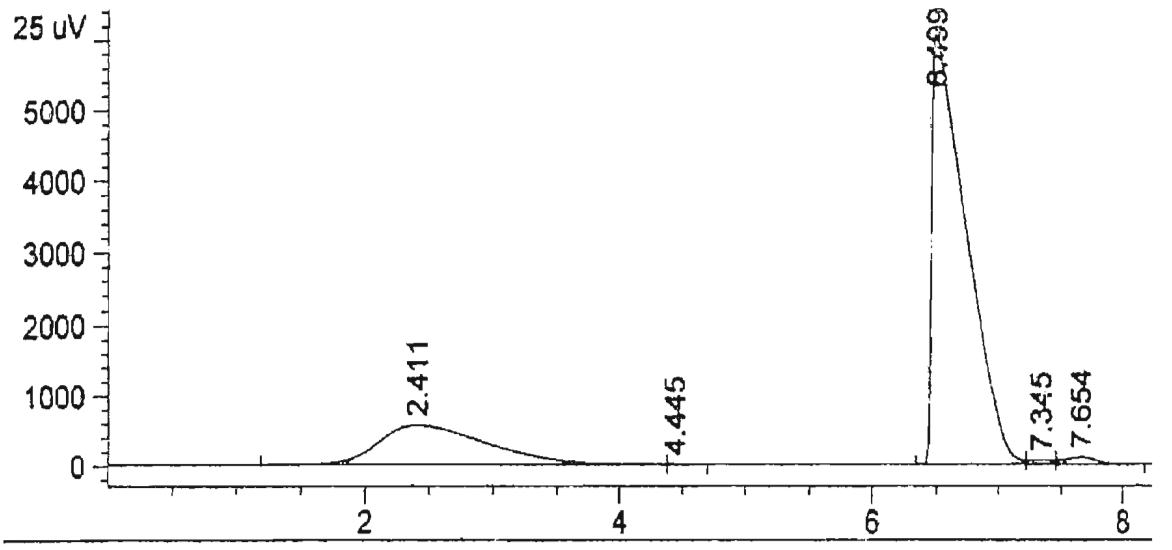


Figure 28: Chromatogram for CIP without Sodium Azide

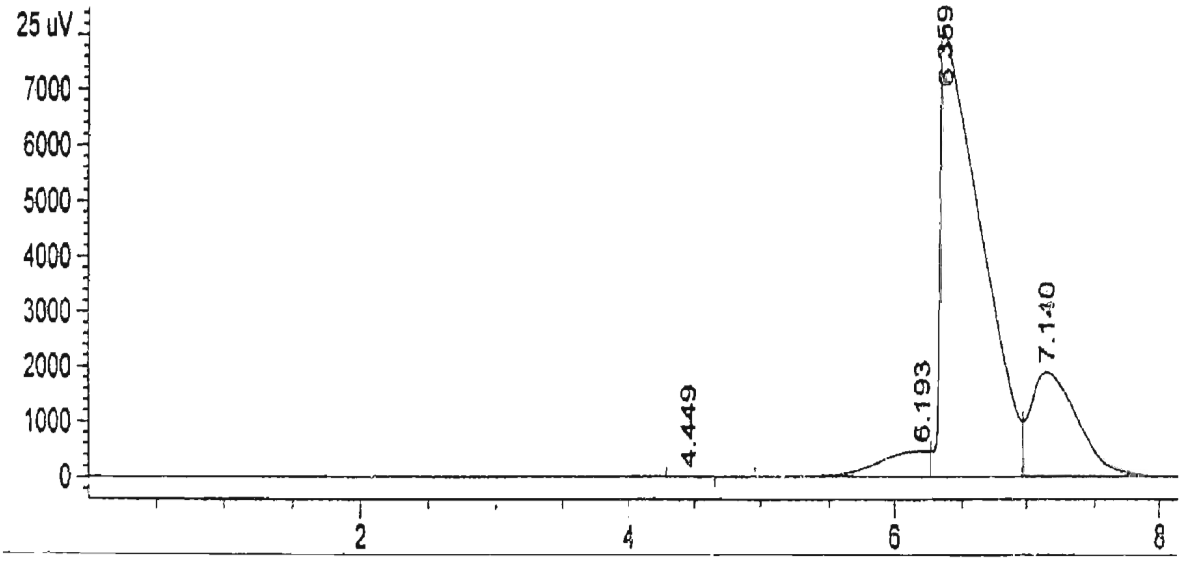


Figure 29: Chromatogram for CIP with Sodium Azide

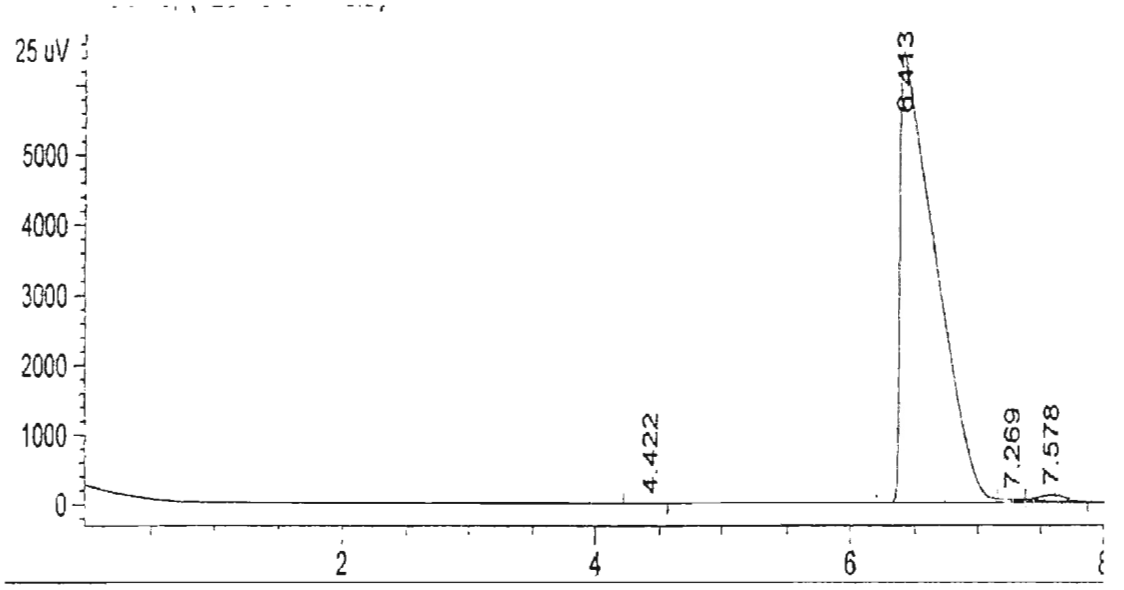


Figure 30: Chromatogram for Sweetwater without adding Sodium Azide

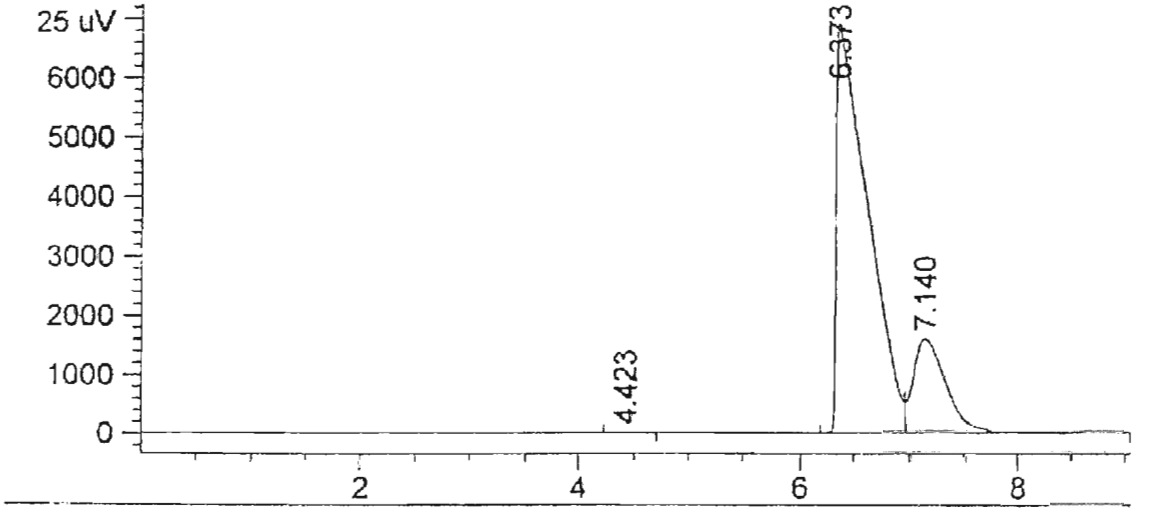


Figure 31: Chromatogram for Sweetwater with Sodium Azide

Chromatograms in Figures 28 and 29 show the difference between CO peaks while adding sodium azide in CIP. The CO peak (retention time 7.6 minutes) did not appear in the chromatogram of the sample with sodium azide (Figure 29), while the peak

is sharp in the chromatogram of the sample where no sodium azide was added (Figure 28). This indicates that the CO is produced by a biological reaction. This was further confirmed by the large oxygen peak (retention time 7.14 minutes in Figure 29) found in the chromatogram with sodium azide addition (Figure 29) in comparison to Figure 28. The oxygen is likely present because bacteria have been killed by the addition of sodium azide, and hence not consuming the oxygen in the reactor. Similar differences were found in the case of chromatograms of sweetwater with and without addition of sodium azide (Figures 30 and 31, respectively). It conflicts with the results produced from first set of experiment in that the literature mentioned the pathway of formation of CO to be chemical whereas this data indicates the pathway to be biological in case of wastewater.

Data analysis of CO produced under the above conditions is shown in Table 9. This experiment was done in triplicate on three different wastewater batches. The values in Table 9 are the average of these three different sets of experiments. The standard deviation of the data is also presented in the table.

Table 9: Concentration of CO with and without adding Sodium Azide, after Incubation at 27°C

Sample	CO Concentration (ppm) ± std. deviation	
	Without sodium azide	With sodium azide
CIP +ULTRA	7573 ± 380	Undetected
CIP +MANDATE	7718 ± 0.81	Undetected
CIP + ULTRA +MANDATE	7854 ± 6.22	Undetected
CIP + KOH	8402 ± 1.26	Undetected
Sweetwater +ULTRA	2799 ± 98.88	Undetected
Sweetwater +MANDATE	1553 ± 153	Undetected
Sweetwater + ULTRA +MANDATE	812 ± 62	Undetected
Sweetwater + KOH	6593 ± 497	Undetected

Table 9 gives the concentration of CO both with and without adding sodium azide. No CO was detected in samples where sodium azide was added. It can be seen, in case of sweetwater, addition of medium concentration of ULTRA and MANDATE, inhibited CO production in comparison to other conditions. The reason could be because of the structure of chemicals that act on the organics (matrices) such that the product formed will be acceptable for microorganisms in the sample.

Figures 32 and 33 show the standard deviation of the results from the triplicates. The graphs were plotted using the mean method of WinSTAT (version 2001.1). The plots

help to know about the standard deviation of data in each bar diagram. The top of the bar diagram indicates the mean value of the concentration of CO produced and the upper end and lower end of the error lines indicates ± Std. Deviation.

Means (CIP)

	N	Mean	95% Conf. (±)	Std.Error	Std.Dev.
ULTRA(U)	3	7574	770	179	310
MANDATE(M)	3	7718	156	36	63
U+M	3	7855	1214	282	489

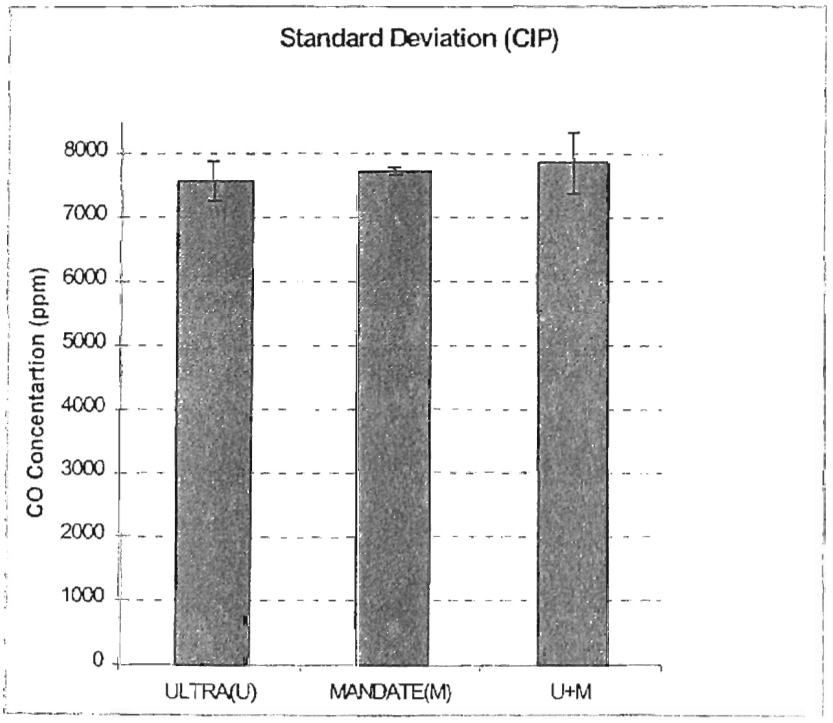


Figure 32: Means Method for Triplicate Results of CO Production from CIP

Means (sweetwater)

	N	Mean	95% Conf. (±)	Std.Error	Std.Dev.
ULTRA(U)	3	2800	246	57	99
MANDATE(M)	3	1553	380	88	153
U+M	3	812	154	36	62

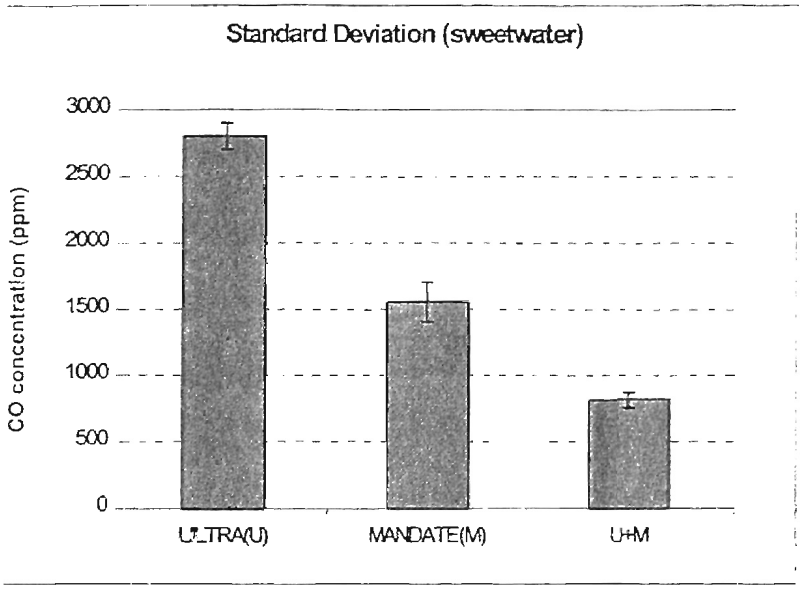


Figure 33: Means Method for Triplicate Results of CO Production from Sweetwater

The results of the experiments designed to see the effect of CIP bacterial seed at various temperatures are shown in Table 10 below.

Table 10: Effect of Using CIP as seed on CO Production at different Temperature

Sample	Average CO Concentration (ppm)			pH
	27°C	50°C	85°C	
Fructose (30,000 mg/L) + ULTRA + MANDATE	Undetected	Undetected	2,316	5.1
Fructose (30,000 mg/L)+ CIP as seed (1 mL/60 mL)	1,052	Undetected	Undetected	4.2
Fructose (30,000 mg/L)+ CIP as seed (1 mL/60 mL) + ULTRA + MANDATE + sodium azide (1 g/L)	Undetected	Undetected	2,238	4.3

Table 10 shows that the reaction between reducing sugar and cleaners do not produce CO at 27°C and 50°C. The concentration could be seen at 85°C, which was the condition duplicating the experiment done by Nicloux and Nebenzahl (1929) as analyzed previously. As mentioned in their literature, the mechanism of production of CO at 85°C was speculated to be a chemical pathway. So it becomes apparent that the CO formation from a reaction between reducing sugar and alkaline chemicals is only at the higher temperature and hence nothing was seen at lower temperature, i.e. at 27°C and 50°C. The chemical pathway of the formation of CO at the higher temperature while the biological pathway occurred at the lower temperature was further supported by the evidence of CO being produced at only 27°C from the combination of sucrose and CIP as seed. However, the concentration of CO produced (1,052 ppm) was smaller than what was produced at

85°C alone with sugar and cleaner. As can be seen in Table 10, in the set of analysis where sodium azide was added to kill the bacteria present in CIP used as seed, CO could not be detected at 27°C while it was observed at 85°C. The reason could be the bacteria were killed by sodium azide and hence was observed at 27°C while the condition for the chemical mechanism of formation of CO still held true at 85°C and thus CO was produced. It further supports the idea of two pathways, biological pathway at the lower temperature and chemical pathway at the higher temperature. Nothing being observed at intermediate temperature within this range (50°C) could be speculated as it being a dead zone, where organisms can neither withstand the high temperature nor the condition becomes favorable enough for chemical phenomenon to occur (slow rate of kinetics). The intermediate temperature range (50°C) is slightly above the upper range of mesophilic organisms, i.e. temperatures slightly above 34-47°C, where these organisms are frequently kill by inactivating critical enzymes (Frobisher et al., 1974). This is possibly the reason why nothing was seen at 50°C.

Kinetics of CO Production

Further work was done on the kinetics of CO production for CIP and sweetwater in order to capture rising leg of the CO production from each waste stream. All the previous experimental data showed the CO peak first appeared at 3.5 days for CIP and

5.5 days for sweetwater. Table 11 below shows the average concentration of CO produced from the duplicate experiment at different incubation time.

Table 11: Kinetics of CO Produced from the Wastewater Samples

Samples	CO Concentration (ppm) ± std. deviation				
	3.5 days	**4 days**	**5.5 days**	**6 days**	**8 days**
CIP + ULTRA	7436 ± 185	7510 ± 110		7378 ± 224	6851 ± 115
CIP +MANDATE	8300 ± 27	8226 ± 100		7129 ± 215	8376 ± 143
CIP +ULTRA + MANDATE	7636 ± 80	7332 ± 12		7240 ± 104	7267 ± 11
sweetwater + ULTRA			2099 ± 11	2360 ± 8	3059 ± 76
sweetwater + MANDATE			1403 ± 3	1428 ± 9	1693 ± 41
sweetwater + ULTRA + MANDATE			444 ± 32	522 ± 7	545 ± 15

It can be observed from Table 11 that CO production from CIP started at 3.5 days and the concentration observed was pretty consistent through 8 days (duplicate samples

do not vary much). In the case of sweetwater, it started at 5.5 days and increased through 8 days.

The time frame was further narrowed down between 3 days and 4 days at an interval of 6 hours for CIP, and between 5 days and 6 days at the same interval for sweetwater. The idea was to determine the time frame when CO really starts evolving and at that point checking the amount of CO produced. There appears to be a lag phase between zero day and 3.25 days in case of CIP and zero days to 5.25 days for sweetwater. The rate of CO generation from 3.25 days to 4 days for CIP and 5.25 days to 6 days for sweetwater was thought to be helpful in showing the rate of formation of CO over a smaller interval of time. However, data from the previous experiment showed that CIP had produced essentially its maximum CO by 3.5 days and in order to find the true rate constants, shorter sampling time intervals would be needed. The next kinetic experiment had sampling intervals of 2 hours. The reason was to find out whether the CO generation was gradual or not. Table 12 below shows the concentrations of CO produced at these intervals.

Table 12: Initial CO Production Kinetics at Short Time Interval

Time (days)	CIP (ULTRA)	Time (days)	Sweetwater (ULTRA)
0 days	0	0	0
3 days 6 hrs	1587	5 days 6 hrs	575
3 days 8 hrs	4567	5 days 8 hrs	1243
3 days 10 hrs	6845	5 days 10 hrs	1642
3 days 12 hrs	7126	5 days 12 hrs	1972
3 days 14 hrs	7168	5 days 14 hrs	2152
3 days 16 hrs	7277	5 days 16 hrs	2046
3 days 18 hrs	7336	5 days 18 hrs	2531
3 days 20 hrs	7458	5 days 20 hrs	2886
3 days 22 hrs	7468	5 days 22 hrs	3038
4 days	7474	6 days	3092

The results for CIP show gradual production of CO through 3 days and 18 hours and then remain almost constant thereafter. Figure. 34 further illustrates this observation.

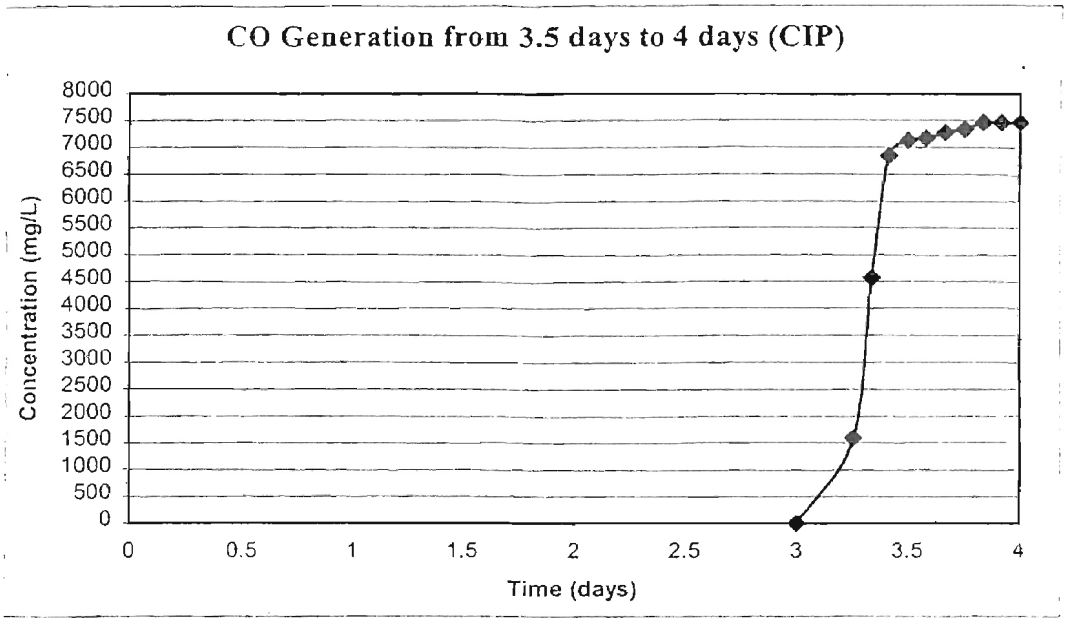


Figure 34: Kinetics of CO Generation from CIP

In case of sweetwater, CO production rises from the onset through 5 days 22 hours after which the production begins to level off. Figure 35 below further illustrates this pattern.

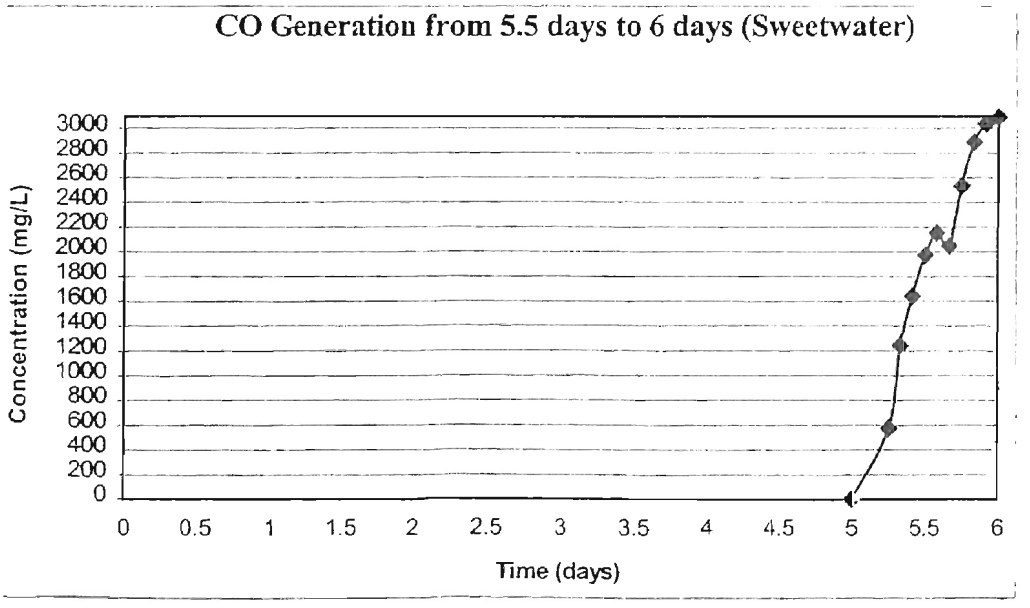


Figure 35: Kinetics of CO Generation from Sweetwater

Both the kinetic analysis shown in Figures 34 and 35 is used the capillary column, which had a 50ppm detection limit. So zero concentration in both the figures represent the undetected portion of the chromatograph or the concentration below 50ppm. Thus, at 27°C, the end of lag phase is seen at 3.25 days in CIP and 5.25 for sweetwater. A first order reaction (lnC vs. t) best fitted the kinetics data of both the wastewater samples (regression coefficient was greater than 0.9). Figures 36 and 37 below show the plots of first order reaction for CIP and sweetwater respectively.

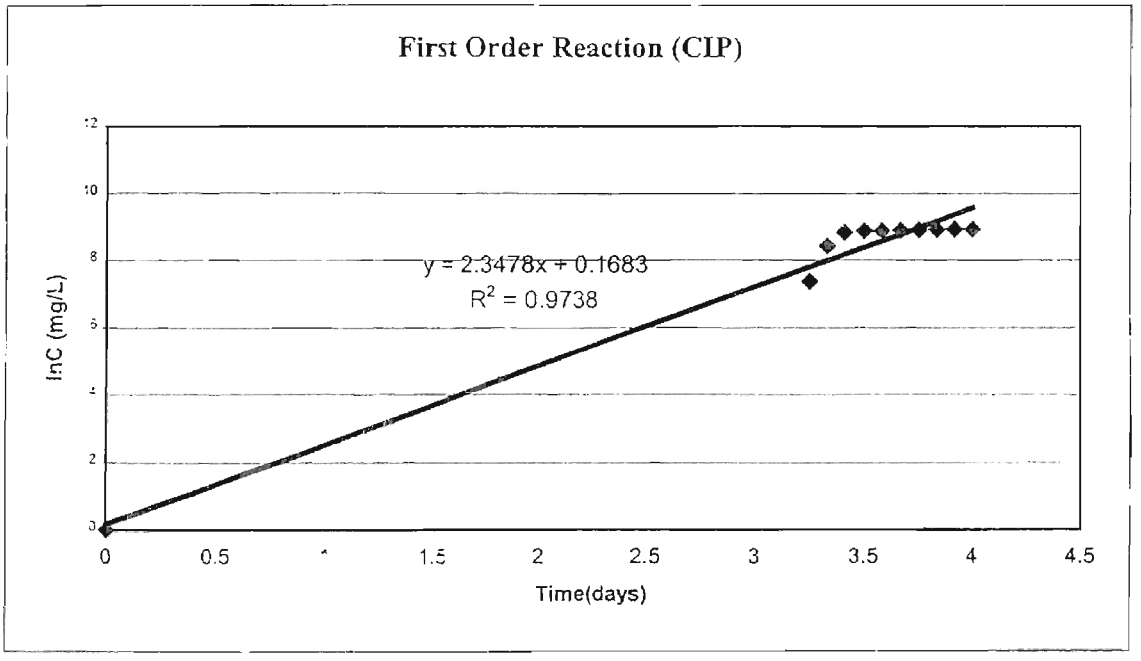


Figure 36: First Order Reaction Plot for CIP

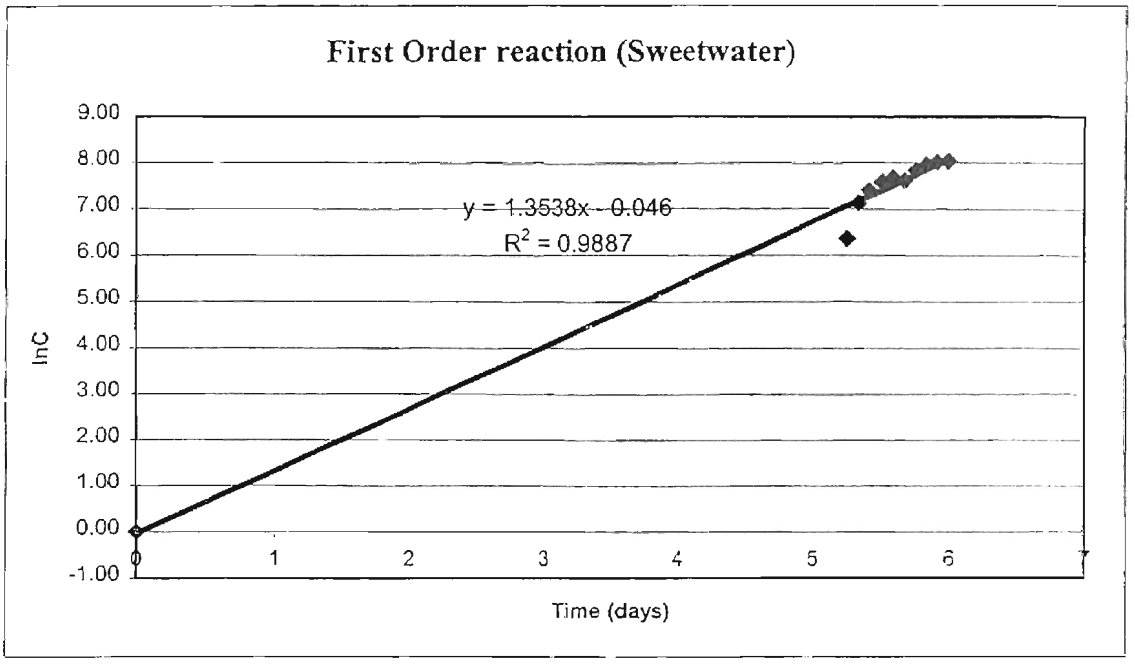


Figure 37: First Order Reaction Plot (Sweetwater)

Figure 36 and 37 shows that the rate constant for CIP was 2.3 while that of sweetwater was 1.3

Figures 38 and 39 below show the relationship between the concentration of CO produced due to additions of different combinations of the cleaning agents, ULTRA and MANDATE, in a batch of wastewater samples at different days in the production of CO.

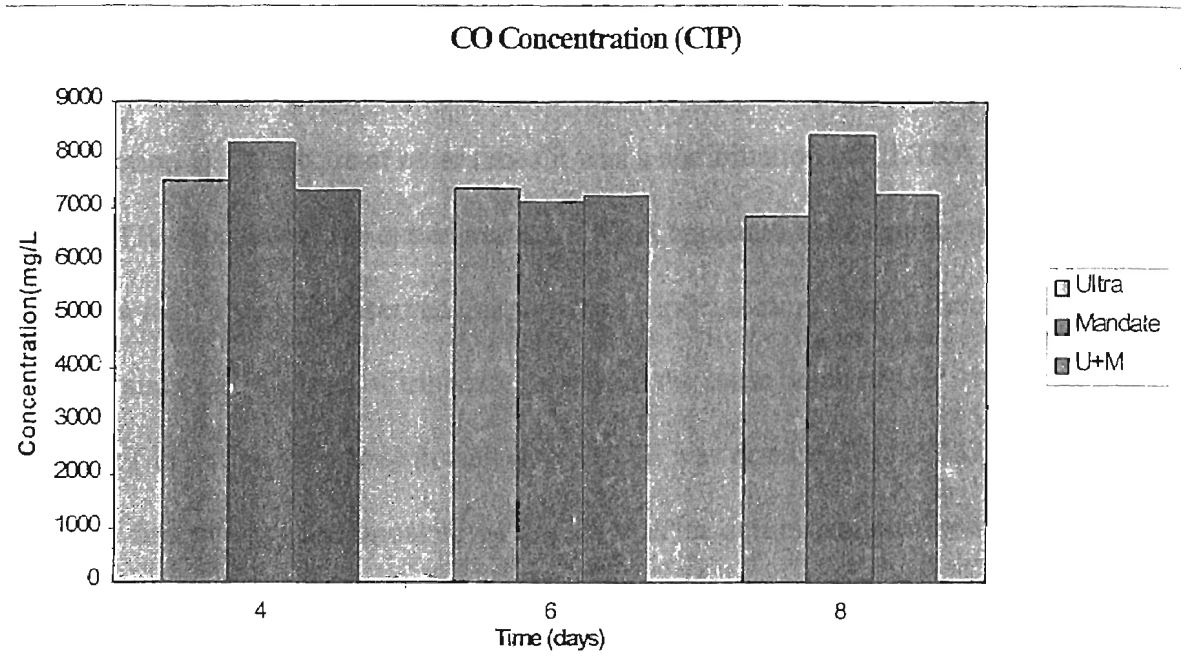


Figure 38: Effect of ULTRA and MANDATE on CO Generation from CIP at Different Incubation Times

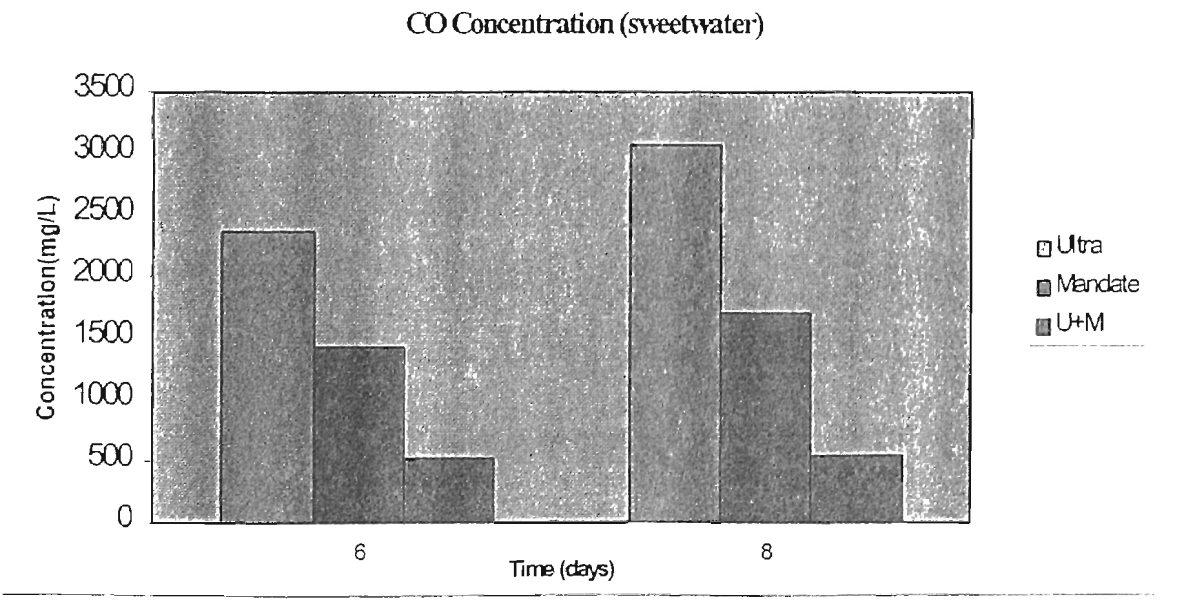


Figure 39: Effect of ULTRA and MANDATE on CO Generation from Sweetwater at Different Incubation Times

The above relationships show the concentration of CO produced seemed to fluctuate in the case of CIP (Figure 38) as can seen where sometimes MANDATE was dominating the production of CO, while at other times it was a combination of ULTRA and MANDATE. In a few of the experiments, ULTRA appeared to be dominating the CO production too. This was observed mainly when triplicate runs from different batches of CIP were analyzed. In case of triplicate runs from the same batch of CIP, the result was consistent (in last two batches) in that MANDATE was dominating the CO production and in the first two batches, most of the time, it was the combination of both the cleaners dominating the production. A potential reason that can be given for this observation was that different batches of CIP appeared to have different colors and viscosity, which may indicate differences in the wastewater nature between different batches that led to fluctuations in reactions with the different cleaners.

CHAPTER VI

TESTS OF CONTROL CARBON MONOXIDE STARTEGIES

Various control stategies to inhibit CO production were evaluated and tested as described below.

Chlorine Test

Clorox having 5.25 % chlorine concentration was used for this test. A stock solution of 1:10 dilution was made. Various doses of the stock solution: low (5 ppm), medium (10 ppm) and high (20 ppm), were added in 60mL of CIP and sweetwater samples. The chromatograms from GC analysis are shown in Figures 39 and 40.

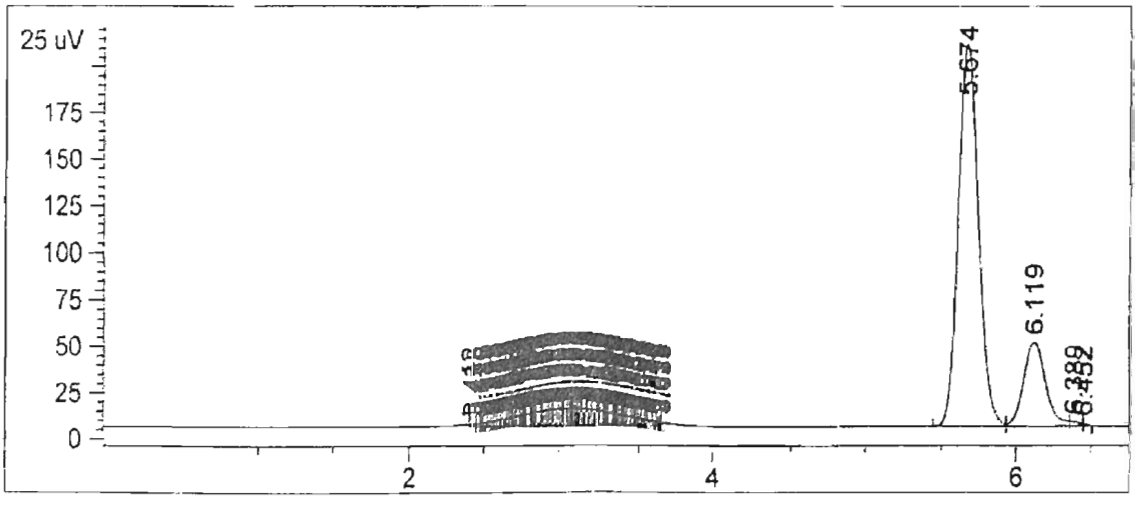


Figure 40: Chromatogram of CIP with High Dose of Chlorine Added

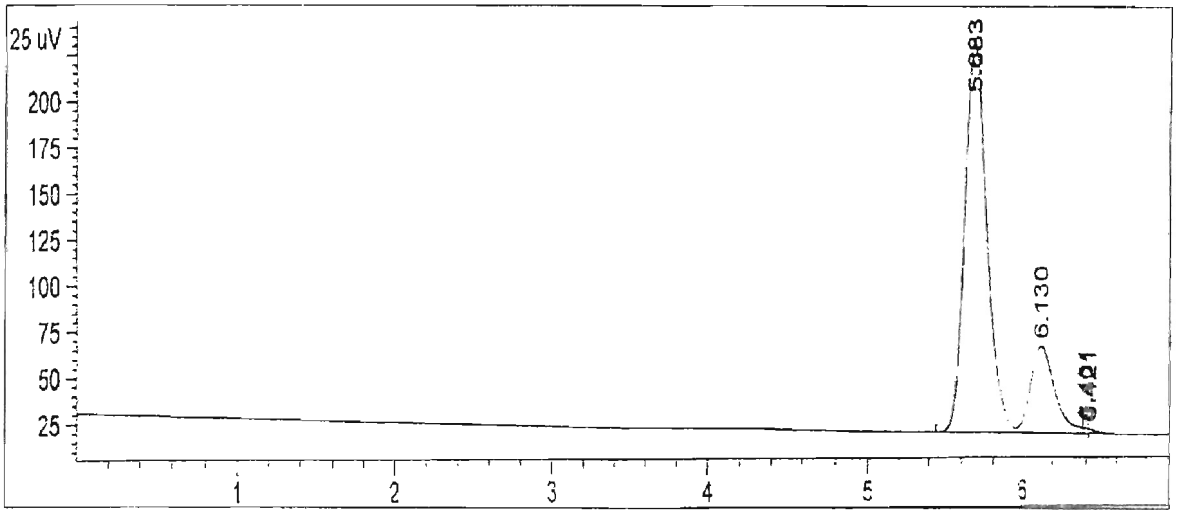


Figure 41: Chromatograph of Sweetwater with Low Dose of Chlorine Added

The CO retention time shown in Figures 39 and 40 is 6.4 minutes. Adding chlorine to the wastewater samples reduced the CO concentration to almost negligible or at least below the worker's safety limit. Table 13 below shows the difference between samples with chlorine addition and those without chlorine addition.

Table 13: CO Concentration of Wastewater Samples with Different Doses of Chlorine

Sample	CO Concentration (ppm)			
	Low (5mg/L)	Medium (10 mg/L)	High (20 mg/L)	Zero dose
CIP + ULTRA + MANDATE	< 50	< 50	< 50	6029
Sweetwater + ULTRA +MANDATE	< 50	< 50	< 50	6765

The results reported in Table 13 show that no CO was detected in the CIP and sweetwater samples. The results are after incubation at 27°C, as in the biologically active samples reported previously. The samples were incubated at 27°C for 4 days. At low, medium

and high doses, the CO production was less than 50ppm, which was the detection limit of the capillary column used to analyze this experiment. The residual test for chlorine was done before and after the analysis and is tabulated in Table 14.

Table 14: Residual Chlorine for Wastewater

Samples	Residual chlorine before test (mg/L)	Residual chlorine after test (mg/L)
CIP + ULTRA & MANDATE @ medium concentration+ chlorine	5	2.3
	10	6.1
	20	12.7
Sweetwater + ULTRA & MANDATE @ medium concentration + chlorine	5	1.9
	10	5.3
	20	13.5

It can see in Table 14, that the residual chlorine after the analysis in both the wastewaters was reduced to almost half the initial concentrations. Since a reasonable chlorine residual remained in the sample at the end of the 4 days reaction period, it was able to provide disinfecting capabilities to the sample. As noted previously, the chlorine contained in ULTRA was used almost immediately to meet a chlorine demand in the sample, leaving minimal residual chlorine to act as a disinfectant. Further it supports the evidence of chlorine acting as disinfectant to kill the microorganisms in the wastewater undergoing biological phenomenon.

Further analysis was done to confirm if the reduction of CO evolution due to addition of chlorine also holds for the conditions mentioned in research done by Nicloux and Nebenzahl (1929). In their article, the authors have mentioned the pathway of formation of CO to be chemical. The results are shown in Table 15. The chlorine dose used was medium concentration (10 mg/L) and the temperature for this analysis was 27°C with retention time of 4 days. The cleaners concentration used was medium.

Table 15: Chlorine Test on Fructose

Sample	CO Concentration (ppm)		Residual Chlorine left after 4 days
	Without Chlorine	With Chlorine	
Fructose (30,000 mg/L) +ULTRA	2895	2793	8.8 mg/L
Fructose (30,000 mg/L) + MANDATE	2882	3749	9.3 mg/L
Fructose (30,000 mg/L) +ULTRA +MANDATE	2638	2366	9.4 mg/L
Fructose (30,000 mg/L) + KOH	1531	1613	9.1 mg/L
Fructose (200,000 mg/L) +ULTRA	2214	1965	8.4 mg/L
Fructose (200,000 mg/L) + MANDATE	2531	2382	8.9 mg/L
Fructose (200,000 mg/L) +ULTRA +MANDATE	2264	1921	9.4 mg/L
Fructose (200,000 mg/L) + KOH	1961	1988	8.5 mg/L

When following the method of Nicloux and Nebenzahl (1929), chlorine addition did not reduce the CO production in either fructose solutions at 30,000 mg/L and 200,000 mg/L as COD. This indicates that chlorine had no effect on the chemical mechanism of

CO production at high temperature, but will inhibit biological CO production at lower temperature (27°C). CO formation seems to be following chemical and biological pathways at higher temperature and lower temperature respectively. Because CO was formed at 27°C in the wastewater and the mechanism appeared to be biological, while at 85°C, as cited by Nicloux and Nebenzahl (1929), the mechanism of formation of CO is chemical. Hence addition of chlorine at this condition didn't have its effect in reducing CO. Also, it was found that the residual chlorine in these samples after the analysis did not change significantly from the initial chlorine residual (Table 15). This indicates that the chlorine has not being used to undergo any kind of reactions within the samples. This leads to the conclusion that, at conditions similar to the operating condition of the plant, CO is being produced via a biological pathway because chlorine, a disinfectant, reduced the CO production. The wastewater should contain an adequate culture that is undergoing some type of biological reactions and with the addition of the cleaners; the matrix is modified sufficiently providing the microorganisms in it the opportunity to produce CO.

Aeration Test

The amount of CO formation was thought to be reduced with aeration in the wastewater samples (CIP and sweetwater). An aeration test was carried out as a control test to reduce CO production from the candy manufacturing plant wastewaters. Dissolved Oxygen (DO) was measured with a DO probe in BOD bottles containing CIP and sweetwater. DO values were found to be 1.4 mg/L and 1.2 mg/L, respectively before

aeration. Cylinder compressed air was bubbled into each BOD bottle containing CIP and sweetwater to increase the DO concentrations. After the samples were aerated for 3 minutes, DO was measured in both the bottles. DO in CIP was found to be 4.8 mg/L while that in sweetwater was 4.5mg/L. The samples were incubated for four days at 27°C in serum bottles and analyzed. Chromatograms obtained for both CIP and sweetwater both, with aeration and without aeration, are shown in Figures 41, 42, 43 and 44, respectively.

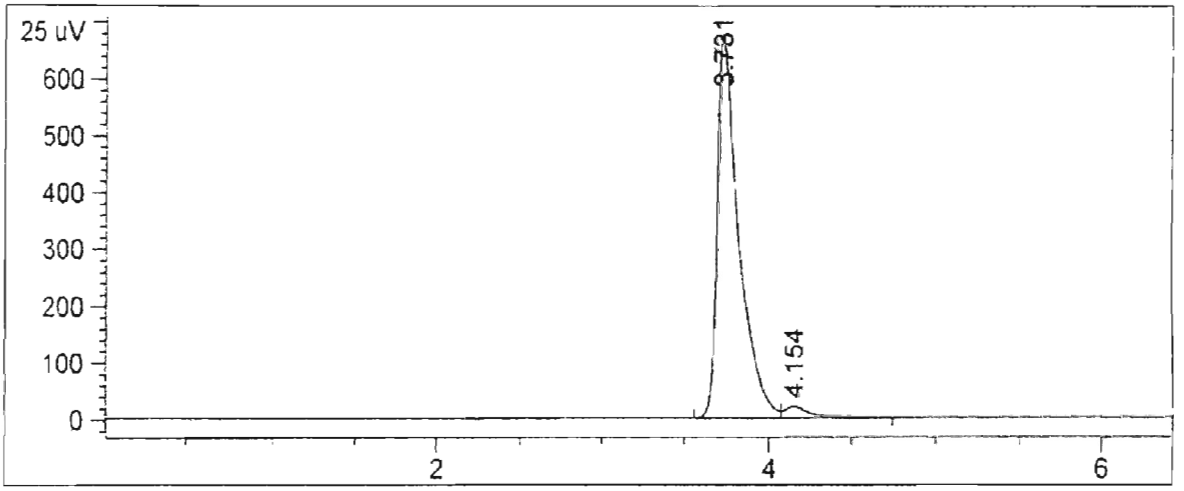


Figure 42: Chromatograph of CO Production from CIP with Aeration

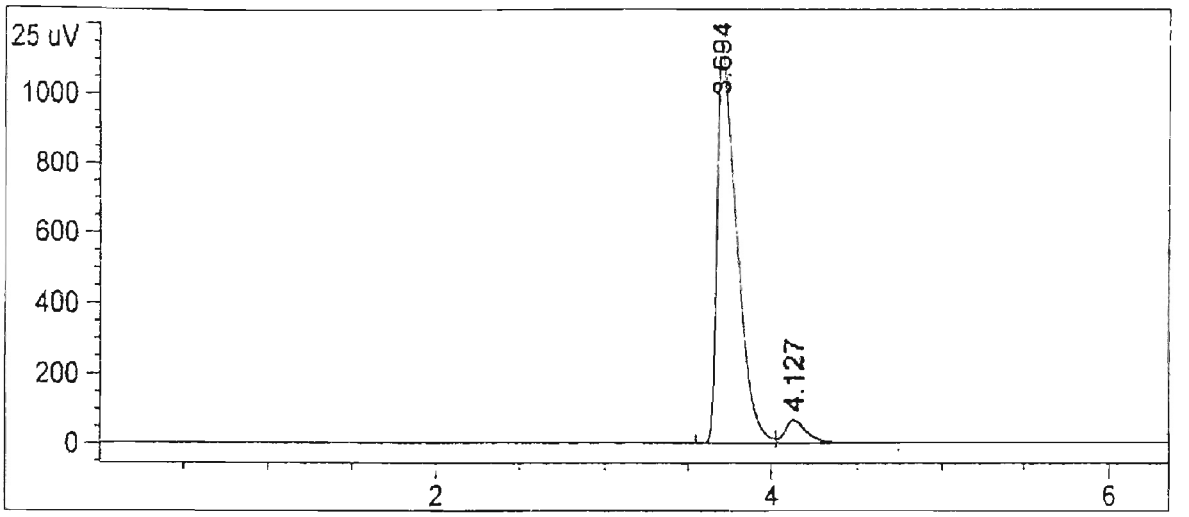


Figure 43: Chromatograph of CO Production from CIP without Aeration

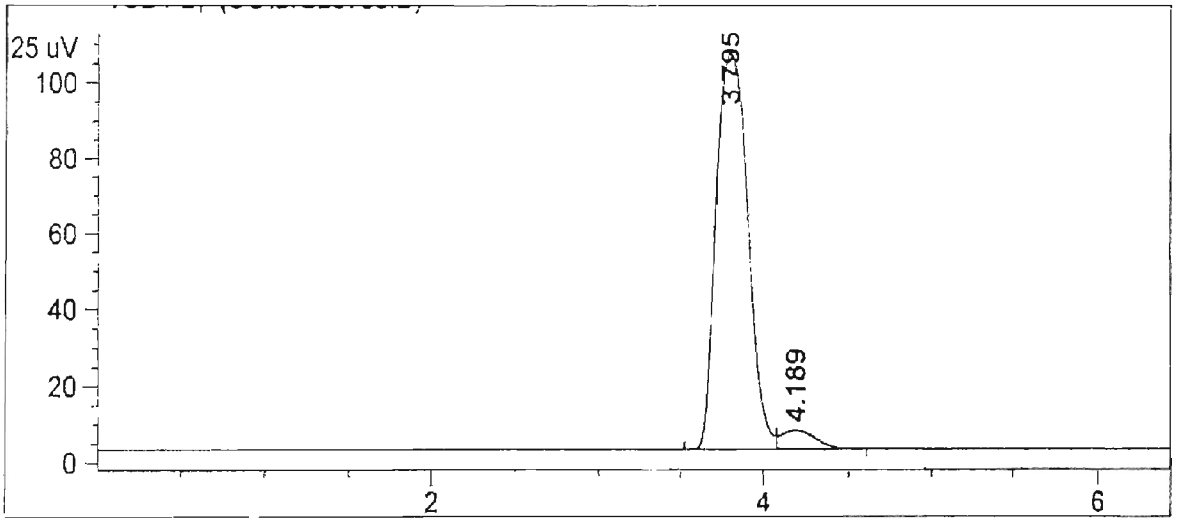


Figure 44: Chromatograph of CO Production from Sweetwater with Aeration

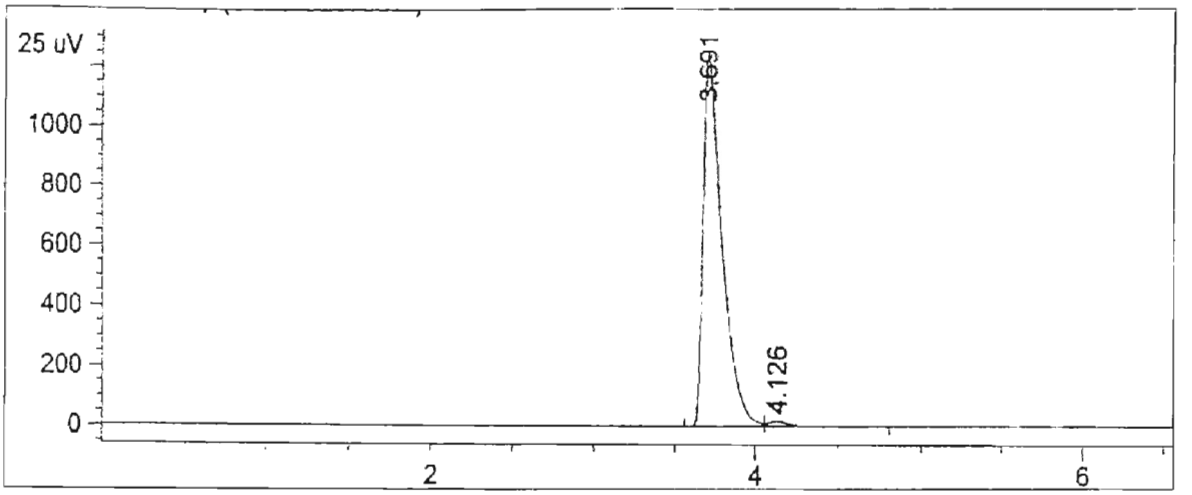


Figure 45: Chromatograph of CO Production from Sweetwater without Aeration

The CO retention time in this analysis was 4.2 minutes. The reduction of retention time of CO compared to previous experiments could be due to wear on the column with time. CO concentration produced from samples with and without aeration is shown in Table 16. The results are the average value of concentration of CO produced from the samples.

Table 16: CO Production from Wastewater with and without Aeration

Sample	CO Concentration (mg/L)	
	Without Aeration	With Aeration
CIP + ULTRA	6218	10497
CIP + MANDATE	7366	13845
CIP + ULTRA + MANDATE	7519	11521
sweetwater + ULTRA	3259	5843
sweetwater + MANDATE	12481	23540
sweetwater + ULTRA + MANDATE	1064	2528

As can be observed in the table, aerating the samples had almost doubled the CO production. Thus aerating the samples provides air for growth of microorganisms in the sample, which aids in production of more CO. Probably, facultative microorganisms mediated the reaction. A positive correlation between aeration and generation of CO was observed by laboratory experiments on biodegradation of compost biomass (Hellebrand and Kalk, 1999). They saw more CO with increased aeration. There is reasonable similarity between the two systems in that they are both high carbon system with limited oxygen available. This supports the results of Table 16, which indicates the CO concentration was stimulated by the availability of air in the samples. This further indicates a biological pathway of production of CO in CIP and sweetwater at the lower temperature studied in these experiments.

CHAPTER VII

CONCLUSIONS

The present study demonstrated potentials mechanisms of CO generation in an industrial wastewater treatment plant and gives a control methodology for the reduction of CO produced. Some of the important findings are:

1. Sugar concentrations in the waste streams were found to be 27,148 mg/L of CIP and 281,923 mg/L of sweetwater which were within the expected ranges i.e. 30,000 mg/L – 100,000 mg/L as COD for CIP and 100,000–300,000 mg/L as COD for sweetwater.
2. pH levels of raw wastewater as received, were within the range of 3.5- 4.5.
3. Reducing sugar of CIP was found to be 0.177% of the total COD, while that of sweetwater was 2.613%. It appears from chemical characterization of the samples that only 10% (approximately) of total waste streams of CODsample is reducing sugar. The results from this study show that reducing sugars might not be the only component of the wastewater likely producing CO.
4. Earlier studies of similar experiments were duplicated as per the conditions given in the literature. The amount of CO produced (high of 4363 mg/L and low of 1750 mg/L) was high enough to be considered a health hazard. The pathway of CO formation was chemical as mentioned in the literature under higher temperature conditions (85°C) in preliminary experiments.

5. The range of operational condition of the plant was reproduced by adjusting pH in the range of 4.5 to 12, temperatures at 27°C and 50°C, and representative reactant concentrations. CO was produced at all the pH ranges and all reactant concentrations. Surprisingly, CO production seemed temperature dependent as it was observed only at 27°C. Under all the conditions at 27°C, the concentration of CO produced was large enough to be considered a risk. The concentration was in the range of 590 mg/L to 9540 mg/L. Co was not produced at 50°C.
6. Since the CO evolution from the operational conditions of the plant was not observed at high temperature (50°C) while in the literature by Nicloux and Nebenzahl (1929), CO was mentioned to be produced at higher temperature (85°C), the mechanism of CO evolution was presumed not to be the same.
7. Biological pathway being followed at 27°C based on azide experiments and physical at 85°C for the production of CO, the intermediate temperature 50°C seemed to be a dead zone as nothing was produced at this temperature. It was speculated as the temperature being high enough for bacterial survival that produces CO and low enough for the chemical phenomenon to occur.
8. A kinetic study of the production of CO was carried out to determine the rate of CO production. CO was found to be produced from 4 days till 8 days in CIP while that in sweetwater from 6 days till 8 days. A further detail kinetic study showed CO starting to produce from 3.25 days and 5.25 days in CIP and sweetwater respectively. There was lag time of CO production between zero day and 3.2 days in cae of CIP and zero days and 5.2 days in case of sweetwater. The

rate of production of CO was found to be increasing gradually starting from 3.25 days and 5.25 days in CIP and sweetwater respectively.

9. Chlorine was found to be an effective control for reduction of CO production. Chlorine is a disinfectant able to kill microorganisms in the wastewater responsible for producing CO. Aerating the waste samples stimulated the production of CO, which was thought to be due to air speeding up the bacteria producing CO.

CHAPTER VIII

RECOMMENDATIONS

Following are recommendations for future studies:

1. With the addition of chlorine to both the waste streams, CO was not detected. The samples were taken before any treatment mechanism was applied in the plant. It would thus be a good idea to add chlorine before treating the wastewater if it is followed closely by a biological treatment unit. The required optimum dose of the chlorine should be investigated as this would most probably be the case for CO emissions from wastewater. Also, the treatment plant seemed to be an activated sludge system.
2. Waste characterization of the sample shows that there are a lot other constituents than reducing sugar in the wastewater. It is recommended to conduct the study to analyze all the constituents of the wastewater and thereby further investigate to find the compounds in it responsible for CO production.
3. CO production in the wastewater was of biological origin. Further analysis can be done by inoculating biologically active reactors with known active cultures of organisms capable of metabolically producing CO.
4. Radioactive isotopes can be used to trace precisely where reducing sugars are being transported from and to, as well as measuring the rate of transport. A radiotracer is a carbon compound that has been labeled with ^{14} C (a radioactive isotope of carbon) for the purpose of tracing the degradation pathway of the labeled compound. The radioactivity can be traced using photographic film (an

autoradiograph) or a GM tube. This techniques can be used to trace reducing sugars effectively.

5. As nothing could be seen at the intermediate temperature 50°C, further investigation could be done with the reducing sugar and alkaline chemicals to see if analyzing the experiments for a longer time period, say 7 days until 10 days, would produce CO at this temperature. It was speculated as 50°C, being lower temperature than 85°C, may take longer time to form the matrices at the lower temperature that would produce CO (i.e. slower kinetics).

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APPENDIX

APPENDIX A

GC METHODS IN USING PACKED COLUMN AND CAPILLARY COLUMN

Capillary Column

The instrument used for the gas chromatography is Hewlett Packard 6890 series, GC system. The column used for the experiment is Carboxen^{TM} 1006 PLOT capillary column of size 30m X 0.53mm. The recommended retention times of the peak are as follows:

1. Nitrogen:	3.33 min
2. Carbon monoxide:	3.82 min
3. Methane:	6.87 min
4. Carbon dioxide:	17.86 min

The air standard used as the sample is the mixture of analyzed gases having balance of Nitrogen in:

Carbon dioxide:	0.5%
Carbon monoxide:	0.5%
Hydrogen:	0.504%
Oxygen:	0.505%
Accuracy of analysis:	=/- 2%

The sampling was done through 500µl gas tight syringe and the volume of sample injected in the instrument was 200µl of the air standard.

The details of the methods employed for running the gas chromatography is attached in the next page.

INLET (back)

Mode:	splitless
Gas:	He
Heater:	50°C
Pressure:	2.21 psi
Total flow:	150 ml/min
Purge flow:	split vent = 145ml/min @o.75 min

COLUMNS

Mode:	Constant flow
Inlet :	Back
Detector:	Back
Pressure:	2.21 psi
Flow:	3ml/min
Avg.Velocity:	22cm/sec

OVEN

Setpoint:	40°C
Oven Configuration:	maximum is 225°C
	Equilibration minimum is 1.00
Oven Ramp:	ramp 1 at 24°C/min
	Next ramp at 210°C
	Hold at 10min

DETECTOR (back)

Heater:	200°C
Reference flow:	9ml/min
Make up flow:	11ml/min
Filament:	on

SIGNALS

Signal 1:	Detector is back
	Data Rate is 20 Hz
	Minimum peak width is 0.01min
Signal 2:	Detector is back
	Data Rate is 20 Hz
	Minimum peak width is 0.01min

Packed column

The instrument used for the gas chromatography is Hewlett Packard 6890 series, GC system. The column used for the experiment is Hayesep DB stainless steel packed column having 100/120 mesh. The recommended retention times of the peak are as follows:

1. Nitrogen:	7.1min
2. Carbon monoxide:	8.0 min
3. Oxygen:	7.5 min

The air standard used as the sample is the mixture of analyzed gases having balance of Nitrogen in:

Carbon dioxide:	0.5%
Carbon monoxide:	0.5%
Hydrogen:	0.504%
Oxygen:	0.505%
Accuracy of analysis:	=/- 2%

The sampling was done through 500µl gas tight syringe and the volume of sample injected in the instrument was 200µl of the air standard (except some samples, where volume need to be increased or decreased do that bigger peaks wont dominate smaller ones)

The details of the methods employed for running the gas chromatography is attached in the next page.

INLET (front)

Gas:	H_2
Heater:	100°C
Pressure:	3.31 psi
Total flow:	100 ml/min

COLUMNS

Mode:	Constant flow
Inlet :	Front
Detector:	Back
Pressure:	3.31 psi
Flow:	12 ml/min

OVEN

Setpoint:	40°C
Oven Configuration:	maximum is 290°C
Oven Ramp:	ramp 1 at 24°C/min
	Next ramp at 250°C
	Hold at 10min

DETECTOR (back)

Heater:	150°C
Reference flow:	9 ml/min
Make up flow:	13ml/min
Filament:	on

SIGNALS

Signal 1:	Detector is back
	Data Rate is 20 Hz
	Minimum peak width is 0.01min
Signal 2:	Detector is back
	Data Rate is 20 Hz
	Minimum peak width is 0.01min

APPENDIX B

Sample Point GC sampling (

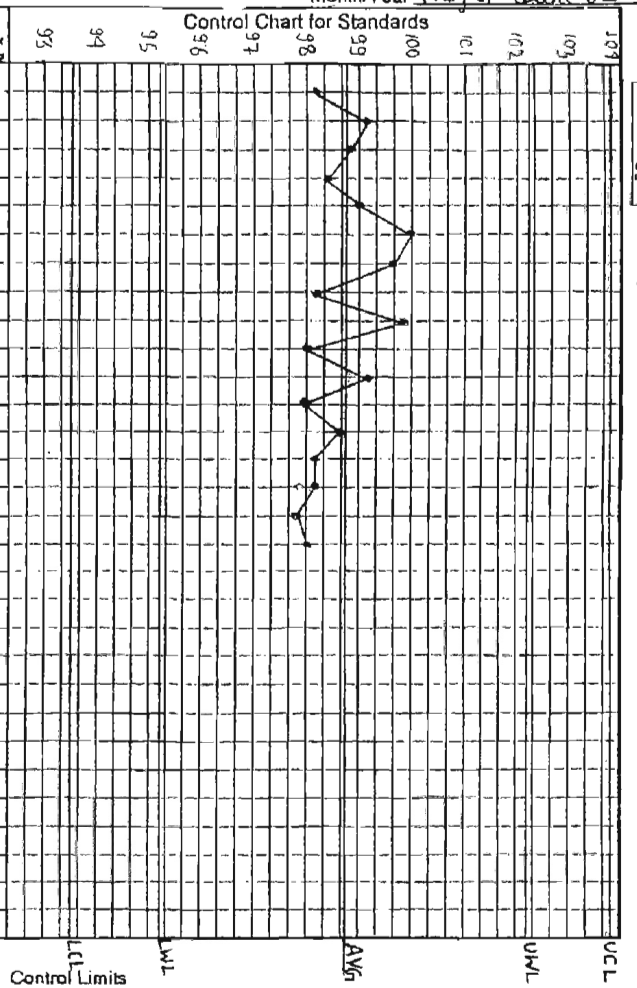
Analysis CO Analysis

Month/Year May 01 - June 02

Analytical Results

Sample #	True Value	Test Value	% Recovery	% Recovery Squared	Lab Tech	Out of Control
1	500	491	98.2	9693	KJ	
2	500	496	99.2	9841	KJ	
3	500	494	98.8	9761	KJ	
4	500	492	98.4	9683	KJ	
5	500	505	99	9801	KJ	
6	500	500	100	10000	KJ	
7	500	502	99.6	9920	KJ	
8	500	491	98.2	9693	KJ	
9	500	499	99.8	9960	KJ	
10	500	510	98	9604	KJ	
11	500	504	99.2	9890	KJ	
12	500	490	98	9604	KJ	
13	500	507	98.6	9721	KJ	
14	500	504	98.2	9693	KJ	
15	500	491	98.2	9693	KJ	
16	500	489	97.8	9564	KJ	
17	500	510	98	9604	KJ	
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
Total		1677	16547	8.2		

Average 98.7



$$S = \sqrt{\frac{\sum X^2 - \frac{(\sum X)^2}{N}}{N-1}}$$

$$S = \sqrt{\frac{165478 - \frac{(1677)^2}{17}}{17-1}}$$

$$= 1.71$$

UWL = Average % Recovered + (2 X S) = 102.12 LWL = Average % Recovered - (2 X S) = 95.2

UCL = Average % Recovered + (3 X S) = 103.83 LCL = Average % Recovered - (3 X S) = 93.5

APPENDIX C

CHEMICAL CHARACTERIZATION OF SAMPLES

pH of different batches of waste water

Sample	Batch	pH reading	Average
CIP	First batch	4.5	4.23
	Second Batch	3.7	
	Third Batch	4.4	
	Fourth Batch	4.3	
CIP+Ultra (after keeping at 27 C, and for 4 days)	First batch	6	5.6
	Second Batch	5.2	
	Third Batch	5.8	
	Fourth Batch	5.4	
CIP +Mandate (after keeping at 27 C, and for 4 days)	First batch	4.6	3.99
	Second Batch	3.7	
	Third Batch	3.9	
	Fourth Batch	3.75	
CIP + Ultra + Mandate (after keeping at 27 C, and for 4 days)	First batch	5.2	5.05
	Second Batch	4.8	
	Third Batch	4.9	
	Fourth Batch	5.3	
Sweet	First batch	4.2	3.9
	Second Batch	3.3	
	Third Batch	4	
	Fourth Batch	4.1	
Sweet+Ultra (after keeping at 27 C, and for 4 days)	First batch	5.3	4.9
	Second Batch	4.5	
	Third Batch	5.5	
	Fourth Batch	4.3	
Sweet +Mandate (after keeping at 27 C, and for 4 days)	First batch	4.1	3.78
	Second Batch	3.2	
	Third Batch	4.3	
	Fourth Batch	3.5	
Sweet + Ultra + Mandate (after keeping at 27 C, and for 4 days)	First batch	4.65	4.4
	Second Batch	4.2	
	Third Batch	4.8	
	Fourth Batch	4	

Note:

First Batch = under normal condition

Second batch = sample after thanx giving

Third batch = sample before Christmas break

Fourth batch = Sample after Christmas break

Titration Data

Sweetwater, Dilution 1:10, Titrant = KOH (0.5N)

Vol of Titrant	pH
0	6.2
1	9.3
2	10.2
3	10.5
4	10.8
5	11
6	11.1
7	11.2
8	11.25
9	11.35
10	11.45
11	11.48
12	11.5
13	11.55
14	11.6
15	11.65
16	11.7
17	11.7
18	11.7
19	11.75
20	11.77
21	11.79
22	11.8
23	11.83
24	11.87
25	11.9

Titration Data

CIP, Dilution 1:10, titrant = KOH (0.5N)

pH	Vol of Titrant
5.8	0
8.1	1
9.3	2
9.8	3
10	4
10.2	5
10.5	6
10.55	7
10.65	8
10.75	9
10.85	10
10.89	11
11	12
11.1	13
11.15	14
11.25	15
11.3	16
11.3	17
11.3	18
11.35	19
11.37	20
11.38	21
11.4	22
11.45	23
11.45	24
11.45	25
11.45	26
11.5	27

COD of various batches of waste water

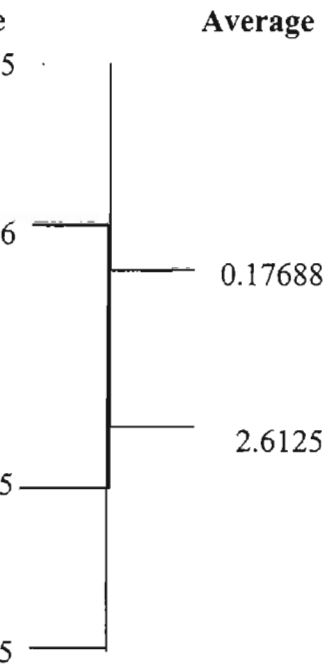
Sample	Batch	Dilution	Spect. Reading	COD	COD Average	Remark
CIP	First	1:30	997	29910	27147.5	using 15,00 mg/l test tube
	Second	1:25	110	27500		using 15000 mg/l test tube
	Third	1:30	815	24450		using 15,00 mg/l test tube
	Fourth	1:30	891	26730		using 15,00 mg/l test tube
Sweet	First	1:30	7033	210990	281922.5	using 15,00 mg/l test tube
	Second	1:25	1450	362500		using 15000 mg/l test tube
	Third	1: 200	1650	330000		using 15,00 mg/l test tube
	Fourth	1: 950	236	224200		using 15,00 mg/l test tube

First Batch = sample before Thanks giving
Second batch = sample after thanx giving
Third batch = sample before Christmas break
Fourth batch = Sample after Christmas break

Reducing Sugar Vs. Reducing Sugar

	Sample Description	COD (mg/L)	Amount of Sugar (%)	Average
CIP	before Thanks giving	23460	0.2	0.1825
	after Thanks giving	22250	0.19	
	before Christmas break	17100	0.16	
	after Christmas break	20700	0.18	
Sweet Water	before Thanks giving	210990	2.1	2.6
	after Thanks giving	362500	3.1	
	before Christmas break	330000	2.9	
	after Christmas break	224200	2.3	

	Sample Description	COD (mg/L)	Amount of Sugar (%)	
CIP(duplicate)	before Thanks giving	24640	0.19	0.17125
	after Thanks giving	23321	0.18	
	before Christmas break	18260	0.15	
	after Christmas break	22012	0.165	
Sweetwater(duplicate)	before Thanks giving	200256	2.2	2.625
	after Thanks giving	355629	3.1	
	before Christmas break	315600	2.8	
	after Christmas break	220000	2.4	



Note: At 30,000mg/L and 200, 000mg/L respectively , from plot of CIP+Sweet water, Reducing sugar for CIP = 0.31 and for sweet = 1.84

APPENDIX D

DUPLICATED STUDY OF EARLIER EXPERIMENTS

Duplicated Study of Earlier Experiments

Sugar

↓

85°C

↓

Alkalinity (N/15)

↓

Experiment No. 1 (sucrose)			
30,000 mg/L COD		200,000 mg/L COD	
	CO Conc. (ppm)		CO Conc. (ppm)
Ultra + Mandate Low conc.	2189.25	Ultra + Mandate Low conc.	1770.68
Ultra + Mandate medium conc.	2303.4	Ultra + Mandate medium conc.	4383.58
Ultra + Mandate high conc.	3191.28	Ultra + Mandate high conc.	3952.32

→

Experiment No. 2 (fructose)			
30,000 mg/L COD		200,000 mg/L COD	
	CO Conc. (ppm)		CO Conc. (ppm)
Ultra + Mandate Low conc.	2555.8	Ultra + Mandate Low conc.	1948
Ultra + Mandate medium conc.	2062.41	Ultra + Mandate medium conc.	4777
Ultra + Mandate high conc.	3115.18	Ultra + Mandate high conc.	3876

→

Experiment No. 2 (sucrose)			
30,000 mg/L COD		200,000 mg/L COD	
	CO Conc. (ppm)		CO Conc. (ppm)
Ultra + Mandate Low conc.	2773	Ultra + Mandate Low conc.	1960.94
Ultra + Mandate medium conc.	2329	Ultra + Mandate medium conc.	3926.96
Ultra + Mandate high conc.	3179	Ultra + Mandate high conc.	4523.1

120

KOH	1643.84	KOH	2747.34
Ultra + Mandate (No sugar)	0	Ultra + Mandate (No sugar)	0
No Ultra + Mandate (only sugar)	0	No Ultra + Mandate (only sugar)	0

KOH	1948.25	KOH	2747
Ultra + Mandate (No sugar)	0	Ultra + Mandate (No sugar)	0
No Ultra + Mandate (only sugar)	0	No Ultra + Mandate (only sugar)	0

KOH	1657	KOH	2506.35
Ultra + Mandate (No sugar)	0	Ultra + Mandate (No sugar)	0
No Ultra + Mandate (only sugar)	0	No Ultra + Mandate (only sugar)	0

30,000		**200,000**	
Average	std dev	Average	std dev
2505.92	294.91	1893.3	106.37
2231.528	147.01	4362.4	425.31
3161.688	40.77	4117.2	353.56
1749.536	172.21	2667.0	139.14

Biological Analysis of Earlier Experiments

Fructose (30,000 mg/L), 85°C, 4 days, Medium Concentration

	W/o Sodium Azide		With Sodium Azide	
	Peak Ar.	**Concentration (mg/L)**	**Peak Area**	**Concentration (mg/L)**
ULTRA	176	1908.04	155	1663.6
MANDATE	198	2164.12	289	3223.36
U+M	221	2431.84	185	2012.8
KOH	131	1384.24	112	1163.08
Fructose only	-	-	-	-

APPENDIX E

REPRODUCED STUDY OF THE OPERATING CONDITION OF THE PLANT

Reproduced Study of Operating Condition of The Plant

CIP 30,000 mg/L COD

Ultra + Mandate or Ultra and Mandate

Experiment No. 1

	Low Concentration					High Concentration				
	pH 4.5		pH 7		pH 12	pH 4.5		pH 7		pH 12
	CIP only		-		-	-		-		-
	Temp 120°F		Temp 50°C		Temp 50°C	Temp 50°C		Temp 50°C		Temp 50°C
	-		-		-	-		-		-
	Temp 27°C		Temp 27°C		Temp 27°C	Temp 27°C		Temp 27°C		Temp 27°C
U	5431.69		6305.46		4964.15	5447.02		5316.72		7271.20
M	9616.57		6864.97		7915.02	8137.30		10482.67		7608.44
U+M	8704.48		8205.51		6979.94	4373.98		5539.00		7769.40

U= ULTRA
M = MANDATE

Experiment No. 2

	Low Concentration						High Concentration				
	pH 4.5		pH 7		pH 12		pH 4.5		pH 7		pH 12
	CIP only		-		-		-		-		-
	Temp 120°F		Temp 50°C		Temp 50°C		Temp 50°C		Temp 50°C		Temp 50°C
	-		-		-		-		-		-
	-		-		-		-		-		-
			Temp 27°C		Temp 27°C		Temp 27°C		Temp 27°C		Temp 27°C
U	7064.25		7133.23		6619.71		6351.45		5837.92		6681.02
M	8206.28		7838.38		8589.51		8466.88		9923.15		7301.86
U+M	8443.88		9670.22		6895.63		5745.94		5646.30		8704.48

Experiment No. 3

	Low Concentration						High Concentration				
	pH 4.5		pH 7		pH 12		pH 4.5		pH 7		pH 12
	CIP only		-		-		-		-		-
	Temp 50°C		Temp 50°C		Temp 50°C		Temp 50°C		Temp 50°C		Temp 50°C
	-		-		-		-		-		-
	Temp 27°C		Temp 27°C		Temp 27°C		Temp 27°C		Temp 27°C		Temp 27°C
U	5600.31		4841.52		5768.94		7033.60		6397.43		6474.08
M	8558.85		5768.94		7455.15		8528.19		8213.94		5822.59
U+M	9861.83		6765.33		5906.90		4803.20		6596.71		7623.77

Sweetwater 200,000 mg/L COD

Ultra + Mandate or Ultra and Mandate

Experiment No. 1

	Low Concentration						High Concentration				
	pH 4.5		pH 7		pH 12		pH 4.5		pH 7		pH 12
	sweetwater only		-		-		-		-		-
	Temp 50°C		Temp 50°C		Temp 50°C		Temp 50°C		Temp 50°C		Temp 50°C
	-		-		-		-		-		-
	Temp 27°C		Temp 27°C		Temp 27°C		Temp 27°C		Temp 27°C		Temp 27°C
U	3392.91		4603.92		6535.40		5132.77		7163.89		8229.27
M	6382.10		7386.17		7915.02		7930.35		7171.56		7915.02
U+M	633.65		794.61		809.94		664.31		717.96		1024.55

Experiment No. 2

	Low Concentration					High Concentration				
	pH 4.5		pH 7		pH 12	pH 4.5		pH 7		pH 12
	sweetwater only		-		-	-		-		-
	Temp 50°C		Temp 50°C		Temp 50°C	Temp 50°C		Temp 50°C		Temp 50°C
	-		-		-	-		-		-
	Temp 27°C		Temp 27°C		Temp 27°C	Temp 27°C		Temp 27°C		Temp 27°C
U	4167.03		5063.79		7056.59	6175.16		6497.07		7401.50
M	5171.10		6550.73		7209.88	7715.74		6274.80		7815.38
U+M	572.34		687.31		771.62	656.65		763.95		840.60

Experiment No. 3

	Low Concentration					High Concentration				
	pH 4.5		pH 7		pH 12	pH 4.5		pH 7		pH 12
	sweetwater only		-		-	-		-		-
	Temp 50°C		Temp 50°C		Temp 50°C	Temp 50°C		Temp 50°C		Temp 50°C
	-		-		-	-		-		-
	Temp 27°C		Temp 27°C		Temp 27°C	Temp 27°C		Temp 27°C		Temp 80°F
U	4036.74		4918.17		7048.92	4941.16		7447.48		7853.71
M	5768.94		7800.06		8871.63	8988.07		7064.25		7608.44
U+M	564.67		633.65		756.29	656.65		771.62		894.25

CIP (27°C)

	Average	St Dev	Average	St Dev	Average	St Dev	Average	St Dev	Average	St Dev	Average	St Dev
U	6032.09	898	6093.40	1160.5	5784.27	827.88	6277.35	795.88	5850.69	540.47	6808.77	413.63
M	8793.90	734	6824.10	1035.3	7986.56	570.55	8377.45	210.23	9539.92	1181.92	6910.96	954.94
U+M	9003.40	755	8213.69	1452.5	6594.16	958.48	4974.37	701.82	5927.34	582.17	8032.55	586.45

Sweetwater (27°C)

	Average	St Dev	Average	St Dev	Average	St Dev	Average	St Dev	Average	St Dev	Average	St Dev
U	3866	414	4862	235	6880	299	5416	664	7036	488	7828	414
M	5774	606	7246	636	7999	834	8211	681	6837	490	7780	156
U+M	590	38	705	82	779	28	659	4	751	29	919	95

APPENDIX F

BIOLOGICAL ANALYSIS OF WASTEWATER

Biological Analysis of Wastewater

I(01/10/02)

Sample (4 days, 86°C)	Ultra			Mandate			Ultra+Mandate			KOH(NaN_3)			KOH(No NaN_3)		
	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark
CIP	1992	7147		1899	7780	CH_4 P	1857	7605				CH_4 Peak	1746	8332	
Sweet	957	2697		175	1381		315	822.9	CH_4 Pk.				1495	7111	CH_4 Peak
CIP only	-			-			-			-			-		
sweet							-			-			-		

II (02/29/02)

Sample (4 days, 86°C)	Ultra			Mandate			Ultra+Mandate			KOH(NaN_3)			KOH(No NaN_3)		
	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark
CIP	1890	7702		1873	7632	CH_4 P	1797	8537				CH_4 Peak	1800	8552	
Sweet	901	2809		194	1605		414	745.6	CH_4 Pk.				1385	6549	CH_4 Peak

III(03/15/02)

Sample (4 days, 86°C)	Ultra			Mandate			Ultra+Mandate			KOH(NaN_3)			KOH(No NaN_3)		
	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Remark
CIP	1945	7873		1913	7743	CH_4 P	1834	7422				CH_4 Peak	1763	8322	
Sweet	821	2894		154	1673		465	868.4	CH_4 Pk.				1298	6119	CH_4 Peak

		I	II	III	Avg	STd dev
CIP	**U**	7147	7702	7873	**7574**	379.8
	M	7780	7632	7743	**7718**	0.81
	U+M	7605	8537	7422	**7855**	6.22
	KOH	8332	8552	8322	**8402**	1.26

		I	II	III	Avg	STd dev
Sweet	**U**	2697	2809.28	2894	**2800**	98.88
	M	1381	1604.83	1673	**1553**	152.96
	U+M	822.9	745.595	868	**812**	62.11
	KOH	7111	6549.39	6119	**6593**	497.69

Note: The data here are those without adding sodium azide only as we didn't see any peak area in those samples where we added sodium azide)

Sweet= sweetwater

APPENDIX G
KINETICS STUDY

Kinetics Study

First set

Sweet	**Ultra**												
	27°							**50°**					
	5.5 days			**6 days**		**8 days**		**4 days**		**6 days**		**8 days**	
	Peak Ar.	**Conc. (mg/l)**		**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**
	399	2110		442	2368	1093	3135						
	Mandate												
	27°							**50°**					
	5.5 days			**6 days**		**8 days**		**4 days**		**6 days**		**8 days**	
	Peak Ar.	**Conc. (mg/l)**		**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**
	985	1406		1006	1437	1204	1734						
	Ultra + Mandate												
	27°							**50°**					
	4 days			**6 days**		**8 days**		**4 days**		**6 days**		**8 days**	
	Peak Ar.	**Conc. (mg/l)**		**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**	**Peak Ar.**	**Conc. (mg/l)**
	116	413.7		133	515.6	114	561.6						
	sweetwater only			-	-	-	-	-	-	-	-	-	-

CIP	Ultra														
	27°								50°						
	3.5 days		4 days		6 days		8 days		4 days		6 days		8 days		
	Peak Area	conc. (mg/l)	Peak Area	conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	
	1298	7500	1318	7620	1315	7602	1209	6966							
	Mandate														
	27°								50°						
	3.5 days		4 days		6 days		8 days		4 days		6 days		8 days		
	Peak Area	conc. (mg/l)	Peak Area	conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	
	1427	8273	1436	8327	1272	7344	1468	8519							
	Ultra + Mandate														
	27°								50°						
	3.5 days		4 days		6 days		8 days		4 days		6 days		8 days		
	Peak Area	conc. (mg/l)	Peak Area	conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar. (new)	Conc. (mg/l)	
	1334	7716	1341	7758	1272	7344	1261	7278							
	CIP only	-	-		-		-		-		-				

Second set

Sweet	Ultra												
	27°							50°					
	5.5 days			6 days		8 days		4 days		6 days		8 days	
	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)
	412	2089		456	2353	561	2983						
	Mandate												
	27°							50°					
	5.5 days			6 days		8 days		4 days		6 days		8 days	
	Peak Ar.	Conc. (mg/l)	Remark	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar. (old)	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)
	297	1400		537	1419	615	1653						
	Ultra + Mandate												
	27°							50°					
	5.5 days			6 days		8 days		4 days		6 days		8 days	
	Peak Ar. (old)	Conc. (mg/l)	Remark	Peak Ar. (old)	Conc. (mg/l)	Peak Ar. (new)	Conc. (mg/l)	Peak Ar. (new)	Conc. (mg/l)	Peak Ar. (new)	Conc. (mg/l)	Peak Ar. (new)	Conc. (mg/l)
	143	476.6		123	356.7	152	530.6						

CIP															
	Ultra														
	27°								50°						
	3.5 days		4 days		6 days		8 days		4 days		6 days		8 days		
	Peak Area	conc. (mg/l)	Peak Area	conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	
	1273	7251	1298	7401	1257	7155	1187	6736							
	Mandate														
	27°								50°						
	3.5 days		4 days		6 days		8 days		4 days		6 days		8 days		
	Peak Area	conc. (mg/l)	Peak Area	conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	
	1398	8001	1419	8126	1217	6915	1437	8234							
	Ultra + Mandate														
	27°								50°						
	3.5 days		4 days		6 days		8 days		4 days		6 days		8 days		
	Peak Area	conc. (mg/l)	Peak Area	conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	Peak Ar.	Conc. (mg/l)	
	1324	7557	1268	7320	1254	7137	1274	7257							

CIP

	3.5 days				4 days				6 days				8 days			
	I	**II**	**Avg**	**Stddev**	**I**	**II**	**Avg**	**Stddev**	**I**	**II**	**Avg**	**Stddev**	**I**	**II**	**Avg**	**Stddev**
U	7251	7620	7436	184.50	7620	7401	7511	109.50	7602	7155	7379	223.50	6966	6736	6851	115.00
M	8273	8327	8300	27.00	8327	8126	8227	100.50	7344	6915	7130	214.50	8519	8234	8377	142.50
U+M	7716	7557	7637	79.50	7320	7344	7332	12.00	7344	7137	7241	103.50	7278	7257	7268	10.50

Sweet

	5.5 days				6 days				8 days			
	I	**II**	**Avg**	**Stddev**	**I**	**II**	**Avg**	**Stddev**	**I**	**II**	**Avg**	**Stddev**
U	2110	2089	2100	10.50	2368	2353	2361	7.50	3135	2983	3059	76.00
M	1406	1400	1403	3.00	1437	1419	1428	9.00	1734	1653	1694	40.50
U+M	413	476	444.5	31.50	515	528	521.5	6.50	561	530.6	546	15.20

Kinetics at Short Interval

Kinetics Data

	Time (days)	CIP(with ULTRA)			
		Pk. Area	Conc (mg/L)	lnC	1/C
0 days	0		0	0	
3 days 6 hrs	3.25	149	1587	7.37	0.00063012
3 days 8 hrs	3.33	302	4567	8.43	0.00021896
3 days 10 hrs	3.41	412	6845	8.83	0.00014609
3 days 12 hrs	3.5	577	7126.70	8.87	0.00014032
3 days 14 hrs	3.58	648	7168.37	8.88	0.00013950
3 days 16 hrs	3.666	680	7277.86	8.89	0.00013740
3 days 18 hrs	3.75	685	7336.45	8.90	0.00013631
3 days 20 hrs	3.833	719	7458.62	8.92	0.00013407
3 days 22 hrs	3.916	734	7468.09	8.92	0.00013390
3 days 24 hrs	4	744	7474.18	8.92	0.00013379

	Time (days)	Sweetwater(with ULTRA)			
		Pk. Area	Conc (mg/L)	lnC	1/C
0 days	0	0	0	0.00	
5 days 6 hrs	5.25	48	574	6.35	0.00174216
5 days 8 hrs	5.33	85	1243	7.13	0.00080451
5days 10 hrs	5.41	198	1642	7.40	0.00060901
5 days 12 hrs	5.5	214.8	1972.45	7.59	0.00050698
5 days 14 hrs	5.58	263	2152.25	7.67	0.00046463
5 days 16 hrs	5.666	253	2046.79	7.62	0.00048857
5 days 18 hrs	5.75	267	2531.92	7.84	0.00039496
5 days 20 hrs	5.833	283	2886.12	7.97	0.00034649
5 days 22 hrs	5.916	299	3038.30	8.02	0.00032913
5 days 24 hrs	6	401	3092.27	8.04	0.00032339

U = ULTRA

APPENDIX H
CONTROL TESTS

Chlorine test

First Set

Sample		Concentartion (ppm)							
	Low(5 mg/L)		Medium (10 mg/L)		High (20 mg/L)		Zero dose	
	Peak Ar.	Conc.	Peak Ar.	Conc.	Peak Ar.	Conc.	Peak Ar.	Conc.
CIP + ULTRA + MANDATE	5.3	-11.4264	4	-30.76	7	13.856	424	6215.48
Sweetwater + ULTRA +MANDA	6.9	12.3688	6	-1.016	4	-30.76	406	5947.78

Note: Low, Medium, high is as per the amount of chlorine added.

Second Set

Sample		Concentartion (ppm)							
	Low(5 mg/L)		Medium (10 mg/L)		High (20 mg/L)		Zero dose	
	Peak Ar.	Conc.	Peak Ar.	Conc.	Peak Ar.	Conc.	Peak Ar.	Conc.
CIP + ULTRA + MANDATE	1.9	-61.9912	5.9	-2.5032	2.2	-57.53	399	5843.68
Sweetwater + ULTRA +MANDA	2.1	-59.0168	UD	UD	3.1	-44.145	516	7583.70

UD = Undetectable

Average

Sample	Concentartion (ppm)			
	Low(5 mg/L)	Medium (10 mg/L)	High (20 mg/L)	Zero dose
	Conc.	Conc.	Conc.	Conc.
CIP + ULTRA + MANDATE	-36.7088	-16.6316	-21.8368	6029.58
Sweetwater + ULTRA +MANDA	-23.324	-1.016	-37.4524	6765.744

Chlorine Test (Duplicating Condition of French article)

		Without Chlorine		With Chlorine	
		CO Peak Area	Conc.(mg/L)	CO Peak Area	Conc.(mg/L)
Fructose(30,000)	U	974	2895.21	942	2793.96
	M	970	2882.55	1244	3749.46
	U+M	893	2638.93	807	2366.84
	KOH	543	1531.57	569	1613.83
Fructose (200,000)	U	759	2214.97	680	1965.02
	M	859	2531.36	812	2382.66
	U+M	560	2264.79	484	1921.28
	KOH	493	1961.96	436	1988.38

Chlorine dose was medium i.e. 10 mg/L
Temperature was 85 degree centigrade

U = ULTRA
M = MANDATE

	Cleaners added	chlorine added	Residual Chlorine left
Fructose (30,000 mg/L)	U	10 mg/L	8.8 mg/L
	M	10 mg/L	9.3 mg/L
	U+M	10 mg/L	9.4 mg/L
	KOH	10 mg/L	9.1 mg/L
Fructose (200,000 mg/L)	U	10 mg/L	8.4 mg/L
	M	10 mg/L	8.9 mg/L
	U+M	10 mg/L	9.4 mg/L
	KOH	10 mg/L	8.5 mg/L

Aeration test

Duplicate samples

		without aeration					with aeration				
		Pk. Area	**Conc(ppm)**	**Pk. Area**	**Conc(ppm)**	**Avg.**	**Pk. Area**	**Conc(ppm)**	**Pk. Area**	**Conc(ppm)**	**Avg.**
CIP	U	441	6293.63	463	6597.97	6445.8	167.00	10013.00	152	9182.99	9598
	M	555	7870.65	536	7607.81	7739.2	190.00	11285.68	189	11230.35	11258
	U+M	563	7981.32	561	7953.65	7967.5	198.00	11728.35	213	12558.36	12143
	U	523	3713.99	504	3582.57	3648.3	221.00	6500.52	224	6583.52	6542
Sweet	M	659	12412.44	679	12781.34	12597	199.00	23567.37	214	25227.39	24397
water	U+M	182	1084.30	158	951.50	1017.9	65.00	2184.47	70	2322.80	2254

Third sample

		without aeration		with aeration	
		Pk. Area	**Conc(ppm)**	**Pk. Area**	**Conc(ppm)**
CIP	U	419	5989.29	162.00	9736.33
	M	618	6993.73	183.00	15546.40
	U+M	625	7071.19	267.00	10898.34
	U	401	2870.14	172.00	5144.83
Sweet	M	433	12365.92	232.00	22682.85
water	U+M	267	1110.46	138.00	2802.77

		Avg. conc(w/o aeration)		Avg. conc (aeration)	
CIP	U	6218		10497	
	M	7366		13845	
	U+M	7519		11521	
Sweet	U	3259		5843	
	M	12481		23540	
	U+M	1064		2528	

VITA #2

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