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Polymer Additives in Wastewater Treatment Processes: Characterization and Optimization

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ABSTRACT

The polymeric flocculants discussed in this thesis are used to reduce Total Suspended Solids (TSS) concentrations in the final effluent of the Process Wastewater Treatment Plant (PWTP) of Coors Brewing Company in Golden, Colorado. The current strategy for the process control of flocculant addition is based on the level of the solids blanket in the secondary clarifiers and aims to avoid an overflow of solids from the secondary clarifiers rather than to minimize the flocculant dosage. Excess amounts of the flocculant in wastewater lead to a waste of energy and resources, higher costs, the problem of potential toxicity of the residual flocculant in the effluent to aquatic organisms and to the restabilization of suspended solids and increased TSS concentrations in the effluent.

In this thesis the basic design of a flocculant dosage control system and the consequent control parameters were defined. This system was based on the Residual Flocculant Parameter (RFP) to account for possible toxic effects and the Optimum Flocculation Parameter (OFP) to determine the required flocculant dosage. Several analytical methods for the detection of concentrations of residual polymer in wastewater were tested for their applicability but a colloid titration method was successful. In toxicity tests, the toxic effects of the flocculant were evaluated to set concentration limits for residual polymer in the effluent. Furthermore, wastewater parameters were investigated, which can be used as indicators for optimum flocculation (OFP).

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CHAPTER 1
INTRODUCTION

In the introduction to this thesis we will concisely explain the problem definition and the approach to the problem solution. Then we will provide a brief description of the organization of this thesis.

1.1 PROBLEM DEFINITION

The addition of polymeric flocculants is often necessary in some wastewater treatment systems. The polymers discussed in this thesis are used to control Total Suspended Solids (TSS) concentrations in the final effluent of the Process Wastewater Treatment Plant (PWTP) of Coors Brewing Company in Golden, Colorado. Various amounts of flocculant are continuously fed to the secondary clarifiers at the PWTP to improve and expedite the settling of suspended solids.

The required dose of flocculant varies with changes in certain process parameters over time. TSS levels and composition and wastewater flow can strongly affect the demand for polymeric flocculant. In addition, wastewater characteristics such as pH, temperature and conductivity may influence the configuration of the flocculant in wastewater and therefore, its efficiency.

The current strategy for the process control of flocculant addition is based on the observational experience of plant operators. The level of the solids blanket in the secondary clarifiers is checked regularly and the feed rate of the polymer pumps is adjusted correspondingly. Thus the purpose of the actual process control is to prevent the loss of solids from the secondary clarifiers rather than to minimize the flocculant dosage.

While the treated effluent passes TSS limitations, it is usually overdosed with polymer. Excess usage results in higher operating costs and is a waste of energy and resources. In addition, there is also the potential problem of toxicity of the flocculant to aquatic organisms, which can cause failure in toxicity tests required by the NPDES (National Pollutant Discharge Elimination System) discharge permit.

A solution to this problem has been hindered by a lack of knowledge of the chemical composition of the polymer mixture. The polymer flocculant is purchased from specialty

chemical vendors, and consists of a mixture of compounds. Knowledge of the exact composition of the polymer mixture and its physical and chemical characteristics are proprietary, and are held confidential by the manufacturers and vendors in a highly competitive market. Without this information it has proven difficult to develop analytical methods to measure residual polymer concentrations in wastewater samples.

1.2 APPROACH TO THE PROBLEM SOLUTION

The planned approach to resolve the described problems includes the following steps:

- Formulate the design of a flocculant dosage control system and define the required control parameters for the same. Also, determine wastewater characteristics that may influence flocculation mechanisms and overall efficiency.
- Determine and evaluate quantitative, analytical methods for the detection of residual polymer in wastewater and set up the experimental investigation of the same.
- Perform toxicity tests to evaluate potential effects of the flocculant on aquatic organisms and bacteria. Carry out these experiments in the expected concentration ranges of the residual flocculant with different types of wastewater.
- Investigate wastewater parameters that can be used as indicators for optimum flocculation.

The above steps require the characterization of wastewater to investigate to what extent parameters fluctuate in the wastewater of interest, i. e. the mixed liquor. As various wastewater parameters affect the efficiency of the flocculation of suspended solids, their fluctuations over time may have to be taken into account for the design of the flocculant dosage control system.

In addition, the characterization of wastewater requires the determination of retention times for various treatment processes for the following reason. In most cases samples will have to represent the “same” wastewater, but at different points in the process. Thus we have to determine the retention times for these treatment steps such that the samples will correlate in a time-sequenced manner.

1.3 ORGANIZATION OF THE THESIS

In Chapter 1 (Introduction) we have defined the problem of this thesis and have presented our approach to solve this problem. Proceeding to Chapter 2 (Process Control Strategy), we will provide background information necessary to develop a proposed flocculant dosage system, which will be discussed in detail. Then we will describe the characterization of the wastewater in Chapter 3 (Wastewater Characterization) that was required prior to the wastewater sampling. In Chapter 4 (Analytical Methods for Flocculant Determination) we will focus on the investigation of various analytical methods for the quantitative analysis of residual flocculant concentrations in wastewater. Lethal polymer concentrations have been determined in toxicity tests, which are discussed in Chapter 5 (Limits for Residual Flocculant Concentrations – Toxicity Tests). In Chapter 6 (Example for Optimum Flocculation Parameter – Streaming Current Detector) we briefly provide an example of a wastewater parameter that can be used as an indicator for optimum flocculation, the streaming current. Finally, we will present our conclusions in Chapter 7 (Conclusions and Further Investigations), and will suggest further investigations in several fields.

CHAPTER 2
PROCESS CONTROL STRATEGY

In this chapter we will first provide the background information that was necessary to develop a flocculant dosage control system for Coors Process Wastewater Treatment Plant (PWTP). This includes a short description of the wastewater treatment plants, and a presentation of the main principles of flocculation. Later in this chapter, we will suggest a process control system to optimize the flocculant dosage in this system.

2.1 COORS WASTEWATER TREATMENT PLANTS

Adolph Coors founded the Coors Brewing Company (CBC) in 1873. Later the family company was expanded, now including a can and bottle production plant and Coors Ceramics. Today industrial wastewater coming from all production facilities and municipal wastewater from the city of Golden are treated in two independent wastewater treatment plants, the General Wastewater Treatment Plant (GWTP) and the Process Wastewater Treatment Plant (PWTP).

2.1.1 Description of the Treatment Processes

The wastewater coming from the brewery is treated in the PWTP. Various processes in the beer production contribute different hydraulic and organic loads, which are shown in the following table (Table 2.1). The given percentages are approximate (± 5 %), and the hydraulic and organic loads are based on annual averages. It should be noted that on any single day wide fluctuations from these averages can occur. In general, high organic loads and a wide variety of compounds are to be expected in wastewater from any food processing industry.

Table 2.1: Overview of Brewery Wastewater				
Process Area	**Hydraulic load [%]**	**MGD [10^6 gal/day]**	**Organic load [%]**	**TOC/day [lb/day]**
Malting	25	1.5	15	5000
Brewing	20	1.2	20	6600
Fermenting	15	0.9	15	5000
Aging	5	0.3	5	1650
Conditioning	15	0.9	25	8250
Packaging	20	1.2	20	6600
Total	100	6.0	100	33000
Abbreviations: MGD: Million gallons per day (equals ca. 3785 m^3 per day or ca. 158 m^3 per hour) TOC/day: Daily Average of Total Organic Carbon (TOC) content				

(Provided by Coors Brewing Company)

According to the literature (Abwassertechnologie, 1994), the following components can be expected in effluents from the brewing, fermenting, aging, conditioning, and packaging processes (Table 2.2).

Table 2.2: Overview of Wastewater Composition	
Process	**Effluent components**
Brewing	
Mashing	Cellulose, sugars, amino acids, cleaning compounds
Mash filtering	Spent grains, sugars, amino acids, cleaning compounds
Wort boiling	Hops, wort, cleaning compounds
Hop strainer	Spent hops, wort, cleaning compounds
Whirlpool	Sludge, wort, cleaning compounds
Fermentation	Yeast, sludge, beer, cleaning compounds
Lagering / aging	Yeast, protein, beer, cleaning compounds
Beer filtering	Diatomaceous earth, yeast, protein, beer, cleaning compounds
Filling	Beer, glass, crowns, cleaning compounds, lubricants
Bottle washing	Beer, glass, labels, glue, oil, cleaning compounds

(Abwassertechnologie, 1994)

The treatment of brewery wastewater in the PWTP is based on a pure oxygen based activated-sludge process, and includes primary, secondary, and tertiary treatments (Figure 2.1, Figure 2.2, and Figure 2.3). In the preliminary treatment, the wastewater goes through a bar screen, grit removal and equalization basin. Then the primary treatment in the primary clarifiers follows. The primary clarifier effluent is biologically treated in the secondary

treatment process, through aeration trains and secondary clarifiers. Enclosed aeration basins trap the CO_2 by-product of aerobic respiration resulting in a mixed liquor pH below that necessary to a healthy biomass. Lime is added to the primary effluent before it reaches the trains. The addition of lime enhances a more effective aerobic degradation of organic matter, and the amount added varies with many process conditions, principally hydraulic and organic loading rates. After treatment in the aeration trains, the mixed liquor (ML) reaches the splitter box, where it is divided into three equal flows. Polymeric flocculant is added to the ML that enters the secondary clarifiers.

All three secondary clarifiers are identical in size and design. A surface skimmer and bottom scrapers sweep the settled sludge to a pump suction pit. The incoming ML is fed peripherally by dropping through holes in the feed channel. A constant feed is provided around the circumference of each clarifier by increasing the diameter of the holes sequentially while simultaneously decreasing the width of the channel.

The retention time in the secondary clarifiers depends on the flow rate, and varies between about 10 and 13 hours. After settling, the primary and a portion of the secondary sludge go to the sludge processing plant for further treatment and disposal. The other portion of the secondary sludge is recycled to operate the activated sludge system. For that purpose secondary sludge containing flocculant is recycled as return activated sludge (RAS) to the aeration trains. One control of an activated-sludge process is based on regulating the amount of RAS proportional to the organic load.

As shown in Figure 2.3, the secondary effluent leaves the PWTP, flowing through sand filters, which can be bypassed at times, and passes the flow measuring flume at the PWTP outfall. At this point, small amounts of an anti-foaming agent can be added to the effluent to prevent the formation of foam at the final discharge point due to non-degraded surfactants. Finally the effluent of the PWTP passes air strippers, which are operated to reinforce dissolved oxygen and to strip CO_2 from the effluent.

In addition to the PWTP, Coors operates the General Wastewater Treatment Plant (GWTP) to treat the wastewater from the city of Golden and all other Coors facilities. The treatment process of this plant will not be described in further detail, as this is beyond the scope of this thesis. However, it should be mentioned that the final stage in this treatment process is a chlorination step, which is required by law. This is of particular interest to us, because chlorine is highly toxic to aquatic organisms causing great impact at even small overdoses (Szal et al., 1991).

Figure 2.1: Primary Treatment at Coors PWTP (Provided by Coors Brewing Company).

Figure 2.2: Secondary Treatment at Coors PWTP (Provided by Coors Brewing Company).

Figure 2.3: Tertiary Treatment at Coors PWTP (Provided by Coors Brewing Company).

After both treatment processes are completed, the wastewater streams are combined and discharged as Final Commingled Effluent (FCE) into Clear Creek at a point designated as 001.

2.1.2 Operation of Coors Process Wastewater Treatment Plant

Sludge age is the key operating parameter in an activated sludge wastewater treatment plant, as it directly controls nearly all other parameters of interest. Sludge age is the average solids retention time in the process. In the same way that hydraulic retention time equals the volume of water in the aeration basin divided by the hydraulic flow rate, the solids retention time equals the mass of solids in the aeration basin divided by the mass leaving the system each day.

$$R_s = \frac{V \times X_m}{Q_w \times X_w} \qquad \text{(Equation 2.1)}$$

Where R_s = sludge age, [days]

V = aeration basin volume, [L]

X_m = Mixed liquor suspended solids concentration, [mg/L]

Q_w = wasting rate, [L/day]

X_w = Waste suspended solids concentration, [mg/L] (RASS)

The product VX_m is the total mass of solids in the aeration basin and Q_wX_w is the mass of solids wasted each day. As an example, if one tenth of the mass of solids in the aeration basin are wasted each day the mean solids retention time, or sludge age is 10 days. Except at very short sludge ages and low recycle rates the mass of solids in the clarifier is only a small fraction of the total and can be ignored.

The daily wasting of activated sludge solids equals their daily growth if a constant sludge age is to be maintained. Thus if the sludge age is 10 days, the net growth rate of the sludge is one tenth of its mass per day.

In practice, a portion of the daily solids production is lost in the plant effluent. Although the TSS concentration is low, the flow is high and the product cannot be ignored and must be taken into account in calculating the sludge age. Thus:

$$R_s = \frac{V \times X_m}{Q_w \times X_w + Q_e \times X_e}$$

(Equation 2.2)

Where Q_e = effluent flow rate, [L/day]

X_e = effluent TSS, [mg/L]

The Coors PWTP does not use sludge age as a control parameter, but prefers to maintain a constant MLSS or X_m. Thus the equation can be rearranged to give:

$$X_m = \frac{R_s (Q_w \times X_w + Q_e \times X_e)}{V}$$

(Equation 2.3)

Since X_w the waste solids concentration is a function of settling, Q_e, the effluent flow rate, and X_e, the effluent TSS are not directly controllable, it turns out that the wasting rate Q_w, can be used to control either the MLSS or the sludge age.

Besides the control of the solids concentration of the mixed liquor, the operation of the aeration basins at Coors PWTP influences the following treatment steps, flocculation and sedimentation. The composition of the suspended organic material to be removed is strongly dependent on the conditions in the aeration basins.

The Coors PWTP uses pure oxygen to satisfy the respiration requirements of the aerobic organisms. This provides a higher diffusion gradient allowing a more rapid transfer of oxygen and the ability to meet the demands of a higher strength waste. The aeration basins are covered to prevent loss of this oxygen to the atmosphere, and a slight positive pressure of approximately 5 inches of water column is maintained. A negative consequence of pure oxygen with covered basins is the limited ability to vent the CO_2 by-product of aerobic respiration. Since the basins are maintained at a positive pressure, the partial pressure of CO_2 is greatly elevated over that of the atmosphere. Much of this CO_2 dissolves in the mixed liquor forming carbonic acid, which controls its pH. Aeration basin pH's of 6.2 ± 0.3 are typically seen, but would be lower except for the continuous feed of hydrated lime into the plant.

The PWTP is loaded with approximately 32000 pounds of TOC on average every day. The stoichiometry of aerobic metabolism and respiration releases approximately half of this

carbon as CO_2, yielding almost 59000 pounds per day. Basin vents are typically 50% oxygen and 50% carbon dioxide.

2.1.3 Current Strategies for Flocculant Addition and Dosage Control

The polymeric flocculant used at Coors Process Wastewater Treatment Plant, PRAESTOL K280FL, is purchased from Stockhausen Inc. (Greensboro, NC). This polymer mixture is shipped in the form of a viscous, milky white emulsion of high concentration. At the PWTP this emulsion is diluted to a solution strength of approximately 0.5 % (v/v) of the original solution in a mixing tank. We refer to this diluted flocculant emulsion, whenever we use the term "polymer", "flocculant", "polymeric flocculant" or "polyelectrolyte" in this thesis.

From the mixing tank three pumps convey the flocculant emulsion through underground lines to the dosage points at the three secondary clarifiers (numbered 1, 2 and 3). There the polymer is added to the peripherally fed ML. As the concentration of the flocculant emulsion stays fairly constant, the polymer dosage is mainly controlled by the pump rate chosen for each polymer pump. Therefore the flocculant dosage can be controlled for each clarifier independently by changing the pump rate of the corresponding polymer pump. As mentioned earlier, the dosage control strongly depends on the experience of plant operators who set the flow rates of the polymer pumps manually based on the following, two wastewater characteristics in the secondary clarifier.

The first wastewater parameter to be observed is the quality of flocs that are formed shortly after the addition of the flocculant. Experienced plant operators know what size and distribution of flocs are necessary to achieve efficient suspended solids removal in the secondary clarifiers. Further they have knowledge of how the pump rates have to be manipulated to produce the required floc characteristics.

The second wastewater parameter, the so-called "solids blanket", is related to the settling (sedimentation) of suspended solids in the secondary clarifier and will be explained in the following. On the basis of the concentration of suspended solids and their tendency to interact, four types of sedimentation can occur: discrete particle, flocculant, hindered (also called zone), and compression sedimentation (Tchobanoglous, 1991). In the systems under consideration four settling regions can be identified with increasing depths, based on different sedimentation types. On top there is the clear water region, followed by the discrete sedimentation region, where particles settle as individual entities. Once a particle reaches the

flocculant sedimentation region below, it increases in mass and settles at a faster rate. At even further depths, a sedimentation phenomenon called hindered sedimentation takes place. Here the particle concentrations are so high that interparticle forces are sufficient to hinder the settling of neighboring particles. Thus the particles tend to remain in fixed positions with respect to each other, and the mass of particles settles as a unit. A solid-liquid interface, that defines the "solids blanket", develops at the top of the settling mass. In the last settling region, the compression region, the particles are of such high concentration that a structure is formed, and further settling can only occur by compression of this structure due to the high weight of the solids blanket.

To ensure that the limits for TSS required by the discharge permit are not violated, the solids blanket has to be kept at a sufficient depth and should never reach the clear water overflow. Therefore the polymer dosage control is also based on the depth of solids blanket, which is determined by plant operators regularly. For that purpose a transparent acrylic cylinder is slowly lowered through the clear effluent until the liquid/solids interface enters the cylinder. A quick jerk of the chain triggers the spring-loaded end caps to capture the interface. The length of the "wet" chain plus the captured liquid column measures the clear liquid depth. This measurement is subtracted from the known clarifier depth to determine the blanket level. Actually the chain is calibrated in blanket inches.

Although this method provides sufficient information about the position of the solids blanket, there are several drawbacks connected with its use for polymer dosage control. First, this approach does not give us any information about possible flocculant overdosage resulting in high polymer concentrations in the effluent. Thus the same effluent quality concerning TSS might have been reached spending less money and resources, and avoiding possible toxic effects. In addition, predictions of the future development of the solids blanket are not possible, because of lacking information concerning polymer overdosage / underdosage. On the one hand, the blanket level can rise, because too little flocculant was added for the formation of sufficiently large and heavy flocs. On the other hand, polymer overdosing can also result in separation of flocs into smaller ones due to mechanisms that will be subsequently described in greater detail in this chapter (2.2.1 Mechanisms of Flocculation). As these smaller flocs have less mass, the solids blankets will rise and may cause high solids concentrations in the secondary effluent.

Even for experienced plant operators, it is often hard to distinguish between those two mechanisms, as they lead to the same result: a rising solids blanket. In the worst case scenario

a polymer underdosage might be suspected to cause the upward shift of the blanket in a situation where in fact too much polymer has been added. Then an increase of the flow rate of the correlated polymer pump will result in even smaller flocs and in a solids blanket moving up further and possibly reaching the overflow.

An additional disadvantage of this control approach is the fact that it is a feedback rather than a feedforward control mechanism. The flocculant dosage is estimated based on the conditions of the wastewater during the treatment in the secondary clarifier, rather than prior to its treatment. As the retention time in the clarifiers is fairly long, lasting approximately 12 hours, there is little possibility to respond to changes in wastewater conditions of the incoming ML.

2.1.4 Specification of the Flocculant

As mentioned previously the production of polymeric flocculants for wastewater treatment applications is a highly competitive field. Thus the producer of the flocculant emulsion of interest, Stockhausen Inc. (Greensboro, N. C.), did not want to provide detailed information about physical and chemical characteristics of the product used at the Coors PWTP. In addition representatives of Stockhausen, Inc. were not willing to explain information previously given to us. On the certificate of analysis, which is provided for every batch of flocculant delivered, several flocculant parameters like viscosity, residual monomer (%), active substance (%) and cationic charge are specified. However, these results were determined by in-house test methods of Stockhausen, Inc., and were not comprehensible for our use. As a consequence of this lack of knowledge, the search for analytical methods to determine residual flocculant concentrations in wastewater proved to be difficult.

Nevertheless, some information could be found in the Material Safety Data Sheet (MSDS) and by following the C.A.S. (Chemical Abstracts Service) numbers. The polymeric flocculant emulsion, PRAESTOL K280FL, consists of the three components described in the following table (Table 2.3).

Table 2.3: Overview of Ingredients of PRAESTOL K280FL (Serial No. 0343-(1))			
Components	C.A.S. No.	% Wt.	% Wt. Range
Cationic acrylamide copolymer (fluid flocculant)	35429197	50	25-50
Kerosene (petroleum), hydrotreated (hydrocarbon)	64742478	30	25-30
Tergitol NP-35 nonionic (surfactant)	127087870	3	1-3

(Database: SciFinder Scholar)

The product is a viscous, milky white emulsion, and the following physical and chemical properties were given in the MSDS.

Table 2.4: Physical and Chemical Properties of PRAESTOL K280FL	
Boiling Point:	98 °C
Melting Point:	0 °C
Water Solubility:	0.5 – 1 % (due to its viscous nature)
Specific Gravity:	1.02

(Provided by Coors Brewing Company)

According to the information provided in the C.A.S. file, the "hydrocarbon" is a complex mixture having carbon numbers predominantly in the range of C9 through C16. The surfactant is also a polymer with the CA index name "Poly (oxy-1, 2-thanediyl),.alpha.-(4-nonylphenyl)-.omega.-hydroxy-, branched". The cationic acrylamide copolymer is described by the CA index name as "ethanaminium, N, N, N-trimethyl-2-[(2-methyl-1-oxo-2-propenyl)oxy]-, chloride", a "polymer with 2-propenamide (9 Cl)". It is not only the main component of PRAESTOL K280FL but also of a number of other cationic flocculants. This polyacrylic copolymer is denoted as $(C_9H_{18}NO_2.C_3H_5NO.Cl)_x$, and consists of the two monomers, with empirical formulas of $C_9H_{18}NO_2.Cl$ and C_3H_5NO, where the latter is the amide monomer. The other cationic monomer (quat) is possibly the N, N-dimethyl aminoethyl methacrylate methyl chloride quat (DMAEM·MCQ), where the protonated amine group carries the positive charge. Thus the cationic charge of the copolymer is not due to the acrylamide monomer, but the second monomer in the copolymer. As this type is usually more expensive than the amide, polymeric flocculants carrying a higher number of charges, and thus, possibly providing more efficient flocculation, are more expensive.

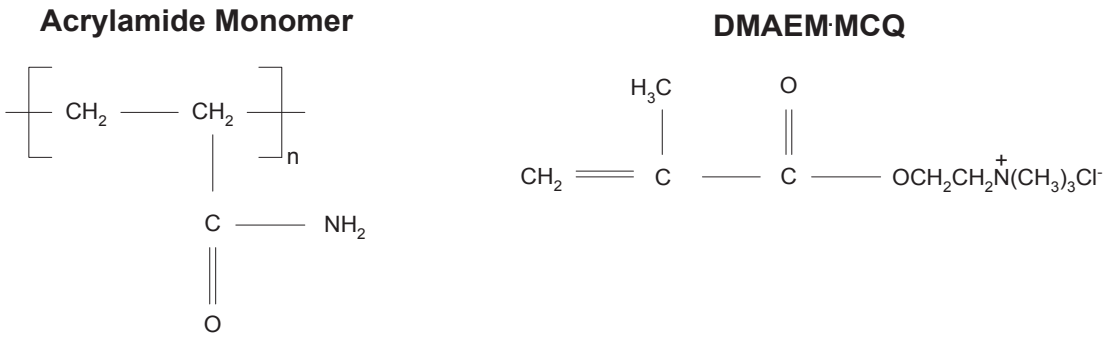

Figure 2.4: Characterization of monomeric compounds of the flocculant (acrylamide monomer and proposed quat) (Encyclopedia of Polymer Science and Engineering, 1987).

We do not have any exact information about the molecular weight or the molecular weight distribution of the copolymer. However, we can assume that it is either high (1 x 10^6 to 5 x 10^6 g/mole) or ultrahigh (> 5 x 10^6 g/mole), because of the fact that the product is sold in form of an emulsion and because of its high viscosity.

In addition, results of the "chemical and biotoxicity tests" performed on selected anoinic and cationic polymers from Stockhausen by Research & Analytical Laboratories, Inc. were available (Table 2.5). For 48 hour-tests on *Ceriodaphnia dubia* the following LC_{50}'s were determined in May 1991. The LC_{50} (lethal concentration – 50 %) is the concentration of the test compound in the test solution that causes lethal effects on 50 percent of the number of test organisms. PRAESTOL K280FL was tested with three different types of surfactant. Due to the lack of knowledge concerning the composition of the polymeric flocculant emulsion, we do not know which surfactant type(s) is (are) contained in the emulsion used at Coors PWTP. In addition, there is no information provided about the test medium. It can probably be assumed, that Natural Synthetic Water (NSW) was used.

Table 2.6: Results of Toxicity Tests by Research & Analytical Laboratories, Inc.

Sample Source	Toxicity / LC_{50} [ppm]
K-280 FL	0.70
K-280 FL (NP 9)	0.38
K-280 FL (Crillet 4)	0.36

(Provided by Coors Brewing Company)

These data indicate that even concentrations less than 1 ppm can cause lethal effects on this test organism. However, this information cannot be directly used for wastewater samples at Coors, because the presence of other compounds in the wastewater may lead to additive, synergistic or antagonistic toxic effects. Therefore toxicity tests had to be performed to investigate possible toxic effects in wastewater samples. These toxicity tests will be described in further detail in sections 2.4 and 3.4.

No uncertainty was connected with the price of the purchased polymeric flocculant. In June 2000 it was reported to be 0.88 cents per pound (1.94 US $ per kilogram). The following tables give an overview of the consumption of flocculant over a time period of several months, and the related cost.

Table 2.7: Consumption of Polymeric Flocculant

Time Period	Amount of Polymer Used in Coors PWTP			
	[gallons]	[lb]	[L]	[kg]
Dec-99	9,000	76,603	34,065	34,746
Jan-00	16,000	136,183	60,560	61,771
Feb-00	7,900	67,240	29,902	30,500
Mar-00	12,500	106,393	47,313	48,259
TOTAL	45,400	386,419	171,839	175,276

(Provided by Coors Brewing Company)

Table 2.8: Cost Estimation for Polymeric Flocculant

Time Period	Cost [US $]	Average Cost/Yr. [US $]	Cost [ATS]	Average Cost/Yr. [ATS]
Dec-99	67,411		1,011,158	
Jan-00	119,841		1,797,614	
Feb-00	59,171		887,572	
Mar-00	93,626		1,404,386	
TOTAL	**340,049**	**1,020,146**	**5,100,730**	**15,302,191**

(Data provided by Coors Brewing Company)

According to this estimation more than one million US $ (15 million Austrian Schillings, based on the exchange rate in August 2000) are spent to purchase the required amounts of polymeric flocculant per annum.

2.2 FLOCCULATION

The process of flocculation is the gathering together or aggregation of small masses, usually in liquid media, into larger masses called flocs (Encyclopedia of Polymer Science and Engineering, 1987). In the past the terms flocculation and coagulation have been used synonymously, but more recent work has attempted to differentiate them. A frequently encountered distinction between the two terms maintains that coagulation is the process whereby the forces holding the solids in suspension are overcome or neutralized; i.e. the suspended solids are destabilized, whereas flocculation is the process whereby destabilized suspended solids are brought together to form larger aggregates. However, more often the terms have been used to distinguish between aggregation caused by simple ions (coagulation) and by polymers (flocculation).

The action of polymeric flocculants is well described (Encyclopedia of Polymer Science and Engineering, 1987; Encyclopedia of Chemical Technology, 1994; Industrial Water Soluble Polymers, 1996). Despite the fact that polymeric flocculants are usually more expensive than inorganic salts, they have numerous advantages including,

- The formation of larger and stronger flocs.
- Faster processing, and a more rapid formation of flocs.
- A smaller volume of generated sludge.
- An overall reduced usage of chemicals.

As polymeric flocculants require smaller dosage, this may compensate for their higher cost in comparison to inorganic salts. Therefore these advantages have led to a more widespread application of polymeric flocculants in the treatment of wastewater from various industries.

2.2.1 Mechanisms of Flocculation

Various mechanisms of flocculation have been suggested (Industrial Water Soluble Polymers, 1996), and a detailed explanation of all would be beyond the scope of this thesis. Therefore we will briefly describe the three main mechanisms of flocculation: charge neutralization, bridging effects, and the electrostatic patch mechanism. The mechanism that occurs in a given situation strongly depends on system parameters and the characteristics of

the applied flocculant. In addition, more than one mechanism of flocculation is often at work for the same treatment process. After the following description of the types of flocculation mechanisms, we will discuss later in this chapter (2.2.2 Influence of Wastewater Parameters on Flocculation) how changes in system parameters may have an impact on the treatment of the Coors wastewater.

Brownian motion prevents suspended particles from settling, and electrostatic repulsion from surface charges prevents an increase in particle size by collision and aggregation. Surface charges are usually due to selective adsorption of ions from solution, ionization of surface groups, or lattice imperfections. According to the electric double layer theory (Stumm and Morgan, 1996), every particle is surrounded by an initial layer of adsorbed ions/molecules (Stern layer) and a diffuse layer of free ions with net opposite charge to the Stern layer (Gouy-Chapman layer). It has been suggested that the potential of the Stern layer must be overcome for aggregation. However, neither the potential of the Stern nor of the Gouy-Chapman layer can be determined directly. Therefore the potential of the shear plane, the Zeta-potential, has been used to approximate the Stern potential and electrostatic repulsion. According to the DLVO (Derjaguin, Landau, Verwey, Overbeek) theory of colloid stability, the total energy of interaction of two colloid particles is given as the sum of the attractive (Van der Waals or hydrogen bonding) and electrostatic repulsive energies, where the latter is due to the Zeta-potential. Attractive forces predominate in short-range distances from the particle center, while repulsive forces predominate at distances greater than the thickness of the electric double layer. At intermediate distances the potential energy is a function of both, and its magnitude depends on these two terms. When the potential energy is greater than the kinetic energy of the particles, the system is stable and no aggregation occurs.

Based on this theory the potential energy barrier can be overcome by either increased kinetic energy of the particles (agitation) or neutralization of the surface charges. The latter can be achieved by double layer compression due to increased ionic strength of the solution (through addition of salts as coagulants) or by adsorption of the flocculant onto the particle surface. Both hydrolyzed metal-flocculants (e. g. based on Al^{+3} or Fe^{+3}) and polymeric flocculants can adsorb to the particle surface and neutralize it.

For polymeric flocculants it is quite common to explain their effects by two mechanisms occuring in parallel, charge neutralization and bridging. The type of flocculation that is dominant depends on the molecular weight (MW) and length of the polymer, and is indicated

by the value of the Zeta-potential at optimum flocculation. Optimum flocculation is reached when no more flocculation can occur on further addition of flocculant.

With increasing amounts of cationic polyelectrolyte, the initially negative Zeta-potential becomes more and more positive. When low MW-polymers are used, charge neutralization predominates and optimum flocculation occurs at a Zeta-potential of around zero. With higher MW, bridging effects become more and more important and optimum flocculation is observed at a more negative Zeta-potential. It is assumed that charge neutralization occurs to some degree in any system, but to what extent depends on current system characteristics. A more negative Zeta-potential at optimum flocculation therefore implies that bridging effects play a stronger role.

Bridging can be described as the attachment of a few segments of the polymer onto the particle surface with unattached segments that extend into the bulk of the solution (Encyclopedia of Polymer Science and Engineering, 1987). This leads to an increased particle-collision diameter, which provides a point of attachment to another particle beyond electrostatic repulsive forces. To ensure that the polymer extensions are sufficiently long, the molecular weight of the polymeric flocculant has to be high enough. In addition, concentration effects have an impact on the numbers of vacant adsorption sites at the point of collision. If the concentration of the polymer is too low, bridging may occur, but not be sufficient for flocculation. On the other hand, if the flocculant concentration is above the optimum, the particle surface may be covered by flocculant to a very large extent. This can lead to problems at particle collision, when there are not enough free surface sites available for the attachment of a polymer chains. Flocculant chains can cover particle surfaces to such a large extent that the bridging of particles is not possible anymore.

Bridging is favored under certain system conditions. First, high solids concentrations (5,000 to 50,000 mg/L) lead to higher probabilities for particle collisions and therefore to increased bridging effects. Second, polymers of high MW and large chain length can adsorb in configurations with polymer loops of greater length extending from the particle surface. In turn, this leads to higher bridging probability. Finally, flocculation due to polymers with no charge or the same charge as the particle surfaces, is mostly related to bridging effects, because of the lack of charge neutralization effects.

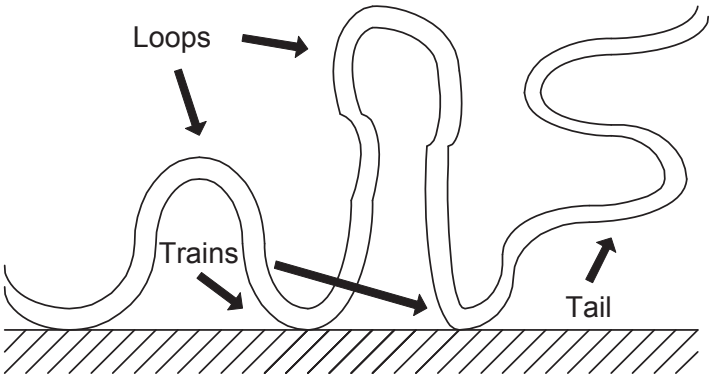

Figure 2.5: Increased particle-collision diameter caused by the attachment of polymer chains to particle surfaces (Encyclopedia of Polymer Science and Engineering, 1987).

In addition to charge neutralization and bridging effects, there exists a cross between the two, called the electrostatic patch mechanism (Encyclopedia of Polymer Science and Engineering, 1987). This mechanism occurs when flocculants of high cationic charge are added to anionic colloidal suspensions. Due to a high attraction between particle surface and polymer, there are fewer loops and trains of the polymer chain formed on the particle surface for bridging, and the polymer becomes completely adsorbed in a flattened configuration. As there is no 1:1 neutralization of the anionic surface charge (which is the case for flocculation due to charge neutralization) positive patches are formed on the surface. These cause electrostatic attraction towards negative patches on other particles and lead to flocculation of particles after collision. The electrostatic patch mechanism predominates if high cationic charge flocculants are added to solutions of low concentrations of colloidal solids.

In systems where the electrostatic patch mechanism is favored, bridging may occur as well. This happens as long as the particle concentration is high enough for collisions to take place on a time scale similar to that required for the polymer to attain a flattened configuration.

Two flocculant characteristics, the molecular weight (MW) and the charge density, strongly influence the dominant mechanism and the efficiency of flocculation. The charge density is the percentage of monomer units bearing a charge, which is usually described in mole percentage. If polymers carrying no charge or like charges as the particles are applied, bridging effects can be assumed to play a dominant role. On the contrary, polymers of

opposite charge as the particles, effect flocculation more by charge neutralization or electrostatic patch mechanisms.

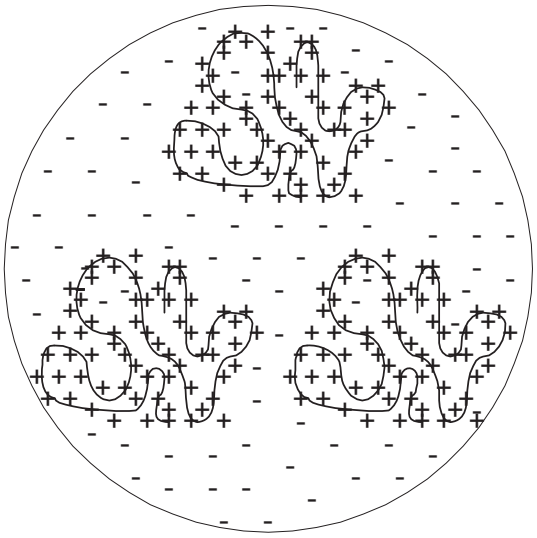

Figure 2.6: Principle of electrostatic patch mechanism (Encyclopedia of Polymer Science and Engineering, 1987).

The MW of the flocculant may also have different effects depending on the dominant mechanism. An increase in MW improves the flocculation by bridging, leading to decreasing amounts of flocculant required for optimum flocculation. However, polymer chains that are too long may hinder effective flocculation due to steric repulsion between polymer molecules. In the case of the electrostatic patch mechanism, fewer polymer chains are attached to the particle surface with increasing MW of the flocculant, while the total weight of attached polymer remains constant. In general we can say that charge neutralization is the main flocculation mechanism at very low MWs. As the MW of the flocculant increases, bridging effects become more and more important.

To sum up, in Coors wastewater with relatively high concentrations of suspended solids, we assume both charge neutralization and bridging to be important mechanisms in the flocculation induced by a cationic, polymeric flocculant. The electrostatic patch mechanism is reported to be favored in solutions of relatively low solids concentrations, and should therefore play a minor role in the treatment of the wastewater of interest. However, the dominant flocculation mechanisms strongly depend on various wastewater parameters. Later in this chapter (2.2.2 Influence of Wastewater Parameters on Flocculation) we will describe in

further detail the impact of various parameters like particle characteristics, ionic strength, pH, etc. on the described flocculation mechanisms.

2.2.2 Influence of Wastewater Parameters on Flocculation

Several wastewater parameters have been reported (Industrial Water Soluble Polymers, 1996; Encyclopedia of Polymer Science and Engineering, 1987; Aquatic Chemistry, 1996; Deng et al., 1996; Gehr and Kalluri, 1983) to influence flocculation. Thus we decided to take a closer look at possible effects of these parameters, and to observe their possible variations in the wastewater of interest over time. Significant changes in wastewater parameters lead to varying demands for flocculant over time to achieve efficient removal of TSS.

The first parameters to consider are the concentration, size, size distribution, and composition of particles. Generally speaking, high concentrations of TSS increase the probability of particle collision and therefore enhance flocculation. The electrostatic patch mechanism is favored at low particle concentrations (< 1 %), while bridging dominates at higher solids concentrations (0.4 to 20 %) (Encyclopedia Of Polymer Science and Engineering, 1987). In addition, it has been reported that there is an inverse relation between the amount of required flocculant dosage and particle size. The composition of the particles also plays an important role, as it might change the surface charge or the hydrophobic character of the solids. At the Coors PWTP, the particles treated in the secondary clarifiers come from the aeration trains, and therefore consist of approximately 85 % organic matter, mainly bacteria. This implies that the particle composition strongly depends on the environment in the aeration trains, as different organisms will be dominant under different conditions.

In typical activated sludge, there is a wide range of particle sizes reported – all the way from single bacteria with dimensions in the approximate range of 0.5 to 5 μm up to large aggregates (flocs) that can reach sizes of more than 1 mm (1000 μm). Activated sludge flocs are made up of two types of components: a biological component consisting of a wide variety of heterotrophic bacteria, fungi, protozoa and some metazoa and a nonbiological component made up of inorganic and organic particulates from the incoming wastewater. In addition extracellular “polymers”, mainly consisting of carbohydrates play a role in the flocculation of activated sludge (Manual on the Causes and Control of Activated Sludge Bulking and Foaming, 1993). More than 400 different species of microorganisms have been identified in

aerobic wastewater treatment processes. However, usually no more than ten species predominate in the aeration trains of a particular treatment plant. The dominant species depend on specific wastewater conditions and may vary over a period of time.

It was decided that the characteristics of solids should not be studied, as a detailed microbiological study would go beyond the scope of this thesis. In addition, it was doubtful whether significant changes in the composition of microorganisms in the aeration trains could be studied over the relatively short time period, during which the project was to be completed.

Besides the characteristics of solids, wastewater parameters like ionic strength (conductivity), pH, and temperature also have an impact on flocculation efficiency. Ionic strength is probably the most important parameter of the above and will be discussed first.

Ionic strength has an effect on flocculation processes in two ways. First, it can influence the electrostatic repulsion between solid particles. Second, it will change the configuration of the polymeric flocculant, and thus the dominant flocculation mechanism. Both effects are explained in further detail below.

As described previously, flocculation can only occur if the potential energy barrier, which is the sum of the electrostatic attraction and repulsion, has been overcome. Increasing ionic strength can effectively screen electrical repulsion, increase the relative importance of Van der Waals attractions, and allow particles to approach each other more closely. This effect is used to enhance flocculation by the addition of salts, which are mostly counter ions that are specifically adsorbing to the particle surface, leading to a decreased or neutralized particle surface charge. Furthermore, the addition of ions that are not specifically adsorbed can also reduce the stability of colloids by double layer compression (Aquatic Chemistry, 1996). In any case, increased ionic strength results in enhanced flocculation, if the flocculation mechanism is mainly based on charge neutralization reactions.

However, ionic strength can have a negative effect on flocculation based on bridging because of changes in the configuration of the polymeric flocculant. First, the adsorption of polyelectrolytes is reported to decrease with increasing salt concentrations (Industrial Water Soluble Polymers, 1996). This can either be a result of the screening of particle surface charges from polyelectrolyte charges in solution, or due to the increased competition for charged surface sites between the ionic segments of the polymer chain and the ions in solution. In addition to the electrostatic attraction between opposite charges on the polymer chain and the particle surface, hydrogen bonding and/or hydrophobic attraction have to be considered as polymer adsorption mechanisms (Industrial Water Soluble Polymers, 1996).

Electrostatic interactions may be the reason why the polymers initially adsorb to the surface, but are not sufficient to keep the chains adsorbed. The other mechanisms mentioned above, are required to achieve that result. Thus the replacement of the adsorbed polymer by other ions in solution may only take place if hydrogen bonding and/or hydrophobic attraction contribute fairly little to the strength of the bonding between polymeric flocculant and particle surface. Based on these considerations we expect the negative effects of increased ionic strength to be less significant for polymeric flocculants of high molecular weight, causing strong hydrophobic attraction.

In addition to the described effects, ionic strength may change the configuration of a charged polymer chain and in turn, the main flocculation mechanism. Charges of the same sign on the polymer chain tend to expand the chain as a result of mutual charge repulsion (Encyclopedia of Polymer Science and Engineering, 1987). As ionic strength increases, these charges are shielded from each other by other ions in solution and allow the polymer to fold and assume a smaller hydrodynamic volume. Thus the screening of repulsive electrostatic interactions results in a coiled rather than an expanded configuration of the polymer. Consequently, during the bridging mechanism the effective particle radius is decreased, because polymer loops and tails are shorter. Generally speaking, we can draw the conclusion that increased ionic strength should have a negative effect on flocculation, if it is mainly based on bridging mechanisms.

As mentioned above, we assume both charge neutralization and bridging to be important mechanisms for the flocculation of suspended solids in Coors wastewater, using a cationic, polymeric flocculant. Increased ionic strength may favor charge neutralization mechanisms and may have a negative effect on bridging. This can result in a change of the dominant flocculation mechanism and in decreased efficiency of the polymer, depending however, on flocculant characteristics.

Therefore we decided to investigate if the ionic strength of the incoming mixed liquor varies over time. As we did not want to analyze each wastewater sample quantitatively for all possible ions in solution, we determined the conductivity of the samples instead of the ionic strength. This decision was based on the assumption that there is a linear relationship between ionic strength and conductivity in the wastewater samples of interest (Water Quality: Characteristics, Modeling, Modification, 1987).

Besides ionic strength, pH and temperature are reported to have an impact on flocculation as well. The pH of the wastewater may have an effect on the charge of both the particle

surfaces and the chains of the polymeric flocculant (Encyclopedia of Polymer Science and Engineering, 1987; Industrial Water Soluble Polymers, 1996). As mentioned previously the cationic charge of the copolymer is due to protonated amine groups of the non-acrylamide monomer component. The hydrolysis of the copolymer can lead to the loss of cationic charge. This loss is caused by oxygen bindings to the nitrogen atoms in the amine groups, and is favored at increasing pH values. For example, for a polyvinylamine flocculant 95 % of the amine groups were protonated at the pH of 3, 13 % at the pH of 9, and only 3 % at the pH of 10 (Encyclopedia of Polymer Science and Engineering, 1987). If the polymeric flocculant applied at the PWTP of Coors Brewing Company behaves similarily, we have to expect the loss of cationic charge with increasing pH values. Less cationic charge may result in less electrostatic attraction between the particle surface and the polymer, and thus smaller amounts of flocculant adsorbed onto this surface. This may have a negative effect on both flocculation mechanisms, charge neutralization as well as bridging. Therefore we would expect a higher flocculant dosage required at increasing pH values to achieve the same removal of suspended solids. To investigate if there are significant variations in pH in the wastewater of interest, the pH of several wastewater samples was analyzed as described in Chapter 3 (3.1.2 Auto Sampler Tests).

The last wastewater parameter to be considered is temperature. Here we found contradictory information in the literature. On one hand, elevated temperatures are reported to cause decreased efficiency of polymeric flocculants due to a decrease in charge of the polymer (Gehr and Kalluri, 1983). In addition, the active volume of polymer coils seems to be reduced, resulting in lower probability of immediate interparticle adsorption and the formation of weaker polymer bridges. On the other hand, the study of adsorption isotherms for copolymers on titanium dioxide surfaces show enhanced sorption at increased temperatures (Deng et al., 1996). In this case it was reported that the colloidal configuration of the polymer at higher temperatures resulted in enhanced flocculation efficiency.

In either case, temperature appears to have an impact on flocculation. Whether the effect is positive or negative will probably depend on the characteristics of suspended solids and of the applied flocculant. Therefore we planned to include the analysis of temperature in our wastewater characterization to see if we had to consider possible variations of this parameter. Later a more detailed investigation of the impacts of temperature on the flocculation process in Coors wastewater could be conducted if necessary.

2.3 SUGGESTED PROCESS CONTROL

As described previously, the current strategy for the process control of flocculant addition is primarily aimed at preventing an overflow of solids from the secondary clarifiers, and not to minimize the dosage of added flocculant. The suggested process control of the polymer addition should ensure that the minimum amount of polymeric flocculant is added to achieve the required removal of suspended solids from the wastewater. It should also control the system in a way that high concentrations of residual flocculant in the effluent are avoided. The latter requirement is necessary for two reasons. First, high residual flocculant concentrations indicate an overdosage of flocculant resulting in a waste of energy, resources and cost (Gehr and Henry, 1983). Second, such concentrations may cause toxic effects on aquatic organisms if they exceed certain limits. Besides the negative impact on the local environment, these effects may also be connected with costly fines.

In the literature, residual flocculant concentration is suggested as a control parameter for flocculant dosage to wastewater (Gehr and Henry, 1983). The use of this parameter, which we have defined as Residual Flocculant Parameter (RFP), has two main advantages.

- Residual flocculant concentration provides information about possible toxic effects caused by overdosage.
- Residual flocculant concentration can be used as an indicator to show how effectively the flocculant was used / consumed during the flocculation process.

We found a relatively good overview of offline methods for the quantitative analysis of polymeric flocculants in solution (Flocculation in Biotechnology and Separation Systems, Process Technology Proceedings, 4, 1987; Taylor and Nasr-El-Din, 1994; Handbook of Water-Soluble Gums and Resins, 1980; Encyclopedia of Industrial Chemical Analysis, 1967; Crummet and Hummel, 1963). However, no information has been found about online applications of these methods and the use of residual polymer concentrations as an online control parameter for flocculant addition. This may be due to the fact that there are also several disadvantages connected with this control parameter including,

- Residual flocculant concentration is usually determined in the effluent from the flocculation process and not in the incoming ML stream. As the retention time of the

wastewater in the clarifiers is relatively long, around 10 to 12 hours, this feedback control may not react fast enough to changing conditions of the incoming wastewater.

- An overdosing of flocculant to the system should lead to an increased residual flocculant concentration in the effluent. However, a low residual flocculant concentration does not necessarily imply that sufficient amounts of flocculant were added. Therefore this parameter can only be used as an indicator of polymer overdosage, but not as an indication for satisfactory removal of suspended solids.

Considering these disadvantages, we suggest the introduction of an additional control parameter to regulate the required amount of flocculant for optimum flocculation, the Optimum Flocculation Parameter (OFP). In this context the term "optimum flocculation" is defined as the point where the chosen optimum flocculation parameter reaches a certain particular value, which is required to reach the target concentration of TSS in the final commingled effluent (FCE). Thus the development of the OFP is related to the development of the TSS concentration in the FCE. Many wastewater and sludge characteristics have been described as useful parameters for this purpose, including sludge viscosity and capillary suction time (Papavasilopoulos, 1997, Hayashi et al., 1990), floc size distribution determined by laser light diffraction (Lartiges et al., 1995), turbidity and particle counting (Wessely, 1995), and streaming current (Abu-Orf, Dentel, 1998).

In this case, the preferred wastewater parameter should be based on the characteristics of the incoming wastewater stream (ML), shortly after flocculant addition, rather than on those of the effluent. The formation of flocs usually occurs immediately after flocculant addition and results in a "clear" wastewater with a high content of flocs in the peripheral feed channel. We assume that this wastewater has similar characteristics as the secondary clarifier effluent (SCE) after the removal of flocs. Therefore it can be used as a sample for the determination of the OFP. To determine the OFP in the incoming ML flocs will probably have to be removed from the "clear" water prior to its analysis. It is assumed that the pretreated sample resembles similar conditions as the wastewater after the treatment in the secondary clarifiers (SCE).

If the online measurement is based on the incoming wastewater after flocculant addition, the control parameter is able to respond rapidly to changing conditions of the wastewater to be treated. This allows a feedforward-like control for the flocculant dosage control system. However, this parameter does not provide any information about possible high flocculant residuals in the effluent and associated toxic effects. Thus the measurement of residual flocculant concentrations is still required as a second control parameter.

A review of the literature for optimum flocculation (see references above) reveals the relationship between a chosen wastewater parameter such as turbidity and the polymer dosage follows a U-shaped curve. The slopes and the shapes of these curves may vary depending on the wastewater parameter and the type of wastewater tested. However, the developments of these curves with increasing polymer dosage stay the same. The importance of this behavior will be discussed based on a qualitative U-shaped curve for turbidity of the effluent as an example indicator for optimum flocculation (Figure 2.10).

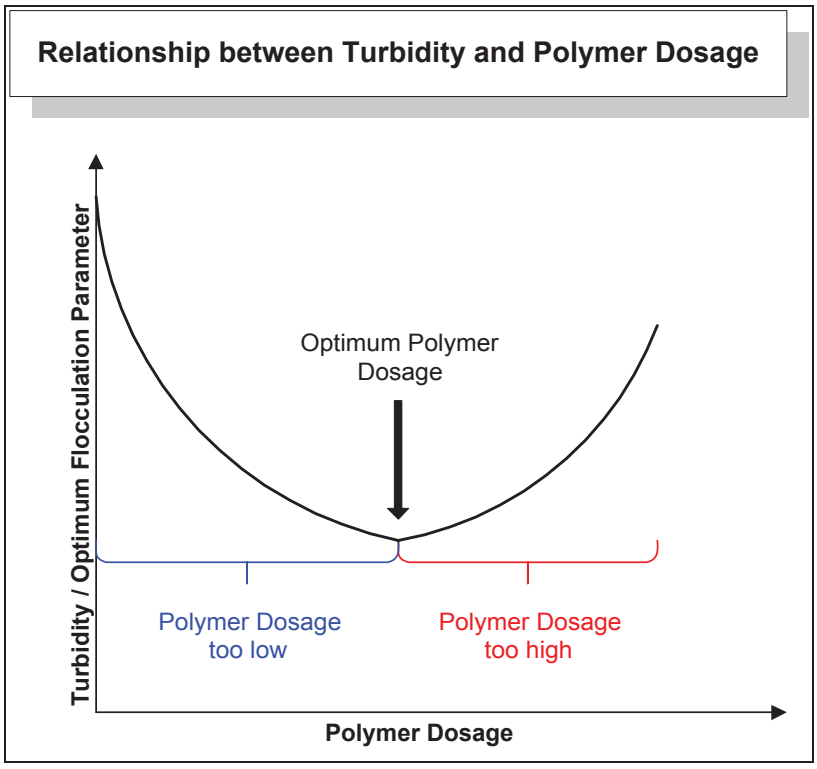

Figure 2.7: Qualtitative relationship between turbidity of the effluent and polymer dosage to the incoming wastewater.

Following this curve, we can see that the turbidity can be decreased and suspended solids can be removed from solution by the addition of polymeric flocculant. Finally after a certain amount of flocculant has been added, the turbidity reaches a minimum, and increases again if the critical amount of flocculant is exceeded. This point is the point of optimum flocculation, and the corresponding polymer dosage is the amount of flocculant required reaching this point. As long as the amount of polymer added to the wastewater is lower than the optimum dosage (to the left of the minimum turbidity) the addition of flocculant results in a decrease of suspended solids in the effluent. However, if we add more than the optimum dosage, the

turbidity of the effluent, and thus the content of suspended solids, will increase again. In addition, we can expect a significant increase in residual flocculant concentration in the effluent in this case. This indicates, that the overdosing of the system will result in a restabilization of flocs, and a possible failure in flocculation, concerning both suspended solids and polymer residual concentrations.

The described response can be explained if we consider the main flocculation mechanisms, charge neutralization and bridging. Too much cationic flocculant added to the wastewater will neutralize particle surface charges to a large extent, minimize electrostatic attraction, and cause steric repulsion between the solids. This may result in the breakage of the polymer bridges connecting particles. Broken bridges cannot be "fixed" as the surface sites required for the adsorption of flocculant to the solids are still occupied by parts of broken polymer chains. Consequently the solids will stay in solution. This shows that an overdosage of flocculant is not only a waste of resources and cost and a hazard to the environment, but may also prevents us from reaching the initial goal of this treatment: the sufficient removal of suspended solids.

Considering the U-shape of the curve shown above, another issue needs to be considered. Because of the shape of the curve, absolute values in turbidity cannot indicate how much flocculant has to be added to the system to reach the point of optimum flocculation. Absolute values in turbidity do not tell us if we have reached the region of overdosage or not, as the same value may be found to the left or to the right of the point of optimum flocculation. Only monitoring the *development* and the *slope* of the curve can give us enough information to decide if the flocculant dosage has to be increased or decreased.

To sum up, the suggested process control for polymeric flocculant addition is based on two parameters: the Residual Flocculant Parameter (RFP), which controls possible toxic effects, and the Optimum Flocculation Parameter (OFP), which ensures sufficient removal of suspended solids.

The RFP is the residual concentration of the polymer in the SCE, preferably measured by an online method. The control value for this parameter will be based on two parameters: EC_{50} concentrations determined in toxicity tests, and the flows of the final effluents from the PWTP and the GWTP to account for dilution effects after the two streams have been commingled. The EC_{50} is the concentration, where the effect, either death or immobilization, is observed for 50 % of the test organisms. The RFP has "failed" whenever the residual flocculant concentration detected in the SCE is higher than the maximum allowable flocculant

concentration. The latter is calculated based on the previously determined toxicity limits (residual flocculant concentration limits) for the FCE and the current flows of the final effluents of the PWTP and the GWTP. Thus the RFP indicates if residual flocculant concentrations are high enough to cause toxic effects.

In addition, increased residual flocculant concentrations (RFP: failed) indicate flocculant overdosage. In this case, the dosed amount of flocculant is not completely "consumed" by suspended solids and stays in solution. A control system for overdosage based on the RFP of the SCE may not react fast enough to changing conditions of the incoming ML stream. Thus an additional RFP measurement of the incoming (pretreated) ML may be required.

Table 2.9: Characteristics of RFP and OFP

	RFP	OFP
Monitoring of:	Residual flocculant conc.	Not defined yet. Possibilities: ▪ Turbidity ▪ Particle counting ▪ Streaming current, etc.
Measurement point:	▪ SCE ▪ Incoming ML (additional)	▪ Incoming ML
Indication for:	▪ Toxicity ▪ Overdosage	▪ Sufficient removal of TSS ▪ Optimum flocculation
"Failed" if:	Residual conc. exceeds limit	Strong increase or decrease
Based on:	▪ Toxicity limits ▪ Current flow rates of final effluents of PWTP and GWTP	▪ Development over time ▪ Current flow rate of incoming ML

Abbreviations:
OFP = Optimum Flocculation Parameter
RFP = Residual Flocculant Parameter
SCE = Secondary clarifier effluent
ML = Mixed liquor
TSS = Total suspended solids
PWTP = Process Wastewater Treatment Plant
GWTP = General Wastewater Treatment Plant

In addition to the RFP the OFP has to be determined. The OFP is a wastewater parameter that is preferably determined in the incoming wastewater stream (ML) shortly after the formation of flocs, as described previously. This parameter is used to determine the required dosage of flocculant to achieve optimum flocculation at current treatment conditions in combination with the wastewater flow rate. As changes in flocculant configuration and flocculation efficiency are hard to predict for varying wastewater conditions (ionic strength

(conductivity), pH, and temperature) this parameter has to be applicable and meaningful even under changing conditions. The OFP is defined as "failed" whenever its development over time shows a strong decrease or increase. The magnitude of changes of the OFP to be taken into account will have to be determined in future experiments (jar tests). An overview of the characteristics of RFP and OFP is given in Table 2.9.

Assuming that the OFP follows a U-shaped curve, and both parameters meet the requirements the following control mechanism may be applied (Figure 2.8).

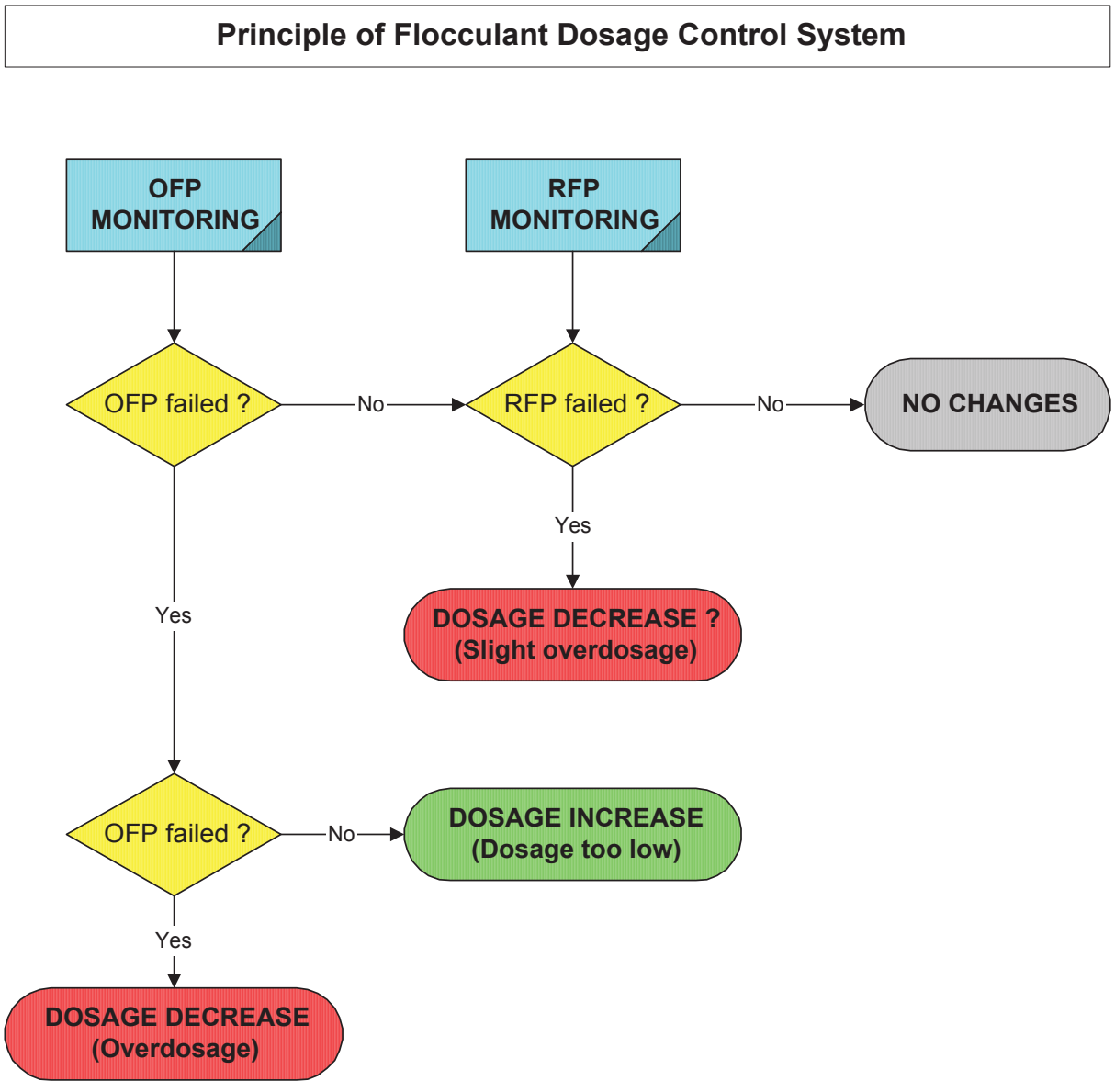

Figure 2.8: Principle of Flocculant Dosage Control System.

According to the proposed control system we can distinguish between four cases, as described in the following.

Case 1: TSS content of ML has increased / dosage too low

Control Parameters:
- OFP: failed
- RFP: passed

Response: Dosage increase

Case 2: TSS content of ML has decreased / overdosage

Control Parameters:
- OFP: failed
- RFP: failed

Response: Dosage decrease

Case 3: System parameters constant

Control Parameters:
- OFP: passed
- RFP: passed

Response: No changes

Case 4: Slight overdosage / other problems

Control Parameters:
- OFP: passed
- RFP: failed

Response: Dosage decrease (?)

In case 4, high residual flocculant concentrations are measured, while the OFP has passed the requirements. This situation does not seem very probable, but may indicate a slight flocculant overdosage or other problems than overdosage in the clarifiers. The plant operators will have to decide if the amount of flocculant added to the system should be decreased or not.

CHAPTER 3
WASTEWATER CHARACTERIZATION

A detailed characterization of certain wastewater streams was performed to get an overview of both, variations of relevant wastewater parameters as well as retention times in the Process Wastewater Treatment Plant (PWTP). This is necessary for two reasons. First, data on retention times are required to ensure that future samples are representative. Second, wastewater conditions strongly affect the configuration of the flocculant in the water and, as a consequence, the dominant flocculation mechanism under these conditions. This, in turn, has an influence on the efficiency of the flocculation process.

To provide this information, two types of tests were set up: tracer tests to determine retention times, and auto sampler tests to characterize temperature, conductivity, and pH of the headworks and mixed liquor (ML) samples. For the ML sample, the Total Suspended Solids (TSS) content was also measured.

The ML is the biologically treated wastewater still containing the active biomass. The biomass is removed as TSS through flocculation and sedimentation in the secondary clarifiers. Its analysis provides data about varying conditions for this process.

The headworks represent the point in the PWTP, where the wastewater enters the treatment plant. Headworks samples were taken for the following reason. Online monitoring data existed for this point at the Coors Brewing Company (CBC). A possible correlation of wastewater characteristics between the headworks and the ML samples could be investigated. If such a relationship were established, wastewater conditions for the ML could be estimated by monitoring the current conditions at the headworks.

3.1 MATERIALS AND METHODS FOR WASTEWATER CHARACTERIZATION

In the following we will describe the experimental setup for the tracer and auto sampler tests performed at Coors PWTP.

3.1.1 Tracer Tests

The purpose of the tracer tests was to determine retention times, which were required to ensure representative samples of wastewater for later toxicity tests. Possible toxic effects on living organisms were to be determined for the mixed liquor (ML), the secondary clarifier effluent (SCE), the General Wastewater Treatment Plant (GWTP) effluent after chlorination, and the final commingled effluent (FCE) at the final discharge point 001. Being aware of the fact that wastewater characteristics can vary significantly with time, we wanted to take samples that represented the same wastewater at different treatment steps of the plant. This would enable true comparisons of toxic effects between different wastewater samples.

The retention time of ML in the secondary clarifiers was calculated based on the average daily flow and the volumes of the clarifiers. Therefore, no additional tracer test was necessary in this case. Tests were required, however, to determine retention times between the secondary clarifier and the final discharge point 001, as well as between the GWTP effluent and the final discharge point.

The tracer used for these experiments was a fluorescent red dye, Rhodamine WT (Formulabs, Inc.). Time was measured using a stopwatch from the moment of the addition of dye tablets to the wastewater to the first visible color change at the checkpoint, the defined endpoint. Usually the color intensity of a tracer follows a certain distribution over time, for example a bell-shaped curve. The peak of this curve is commonly used to estimate the arrival time. Nevertheless, for these experiments the first visible color change was reported rather than the maximum color intensity of red, because it was determined by the naked eye instead of a photometer. Therefore the assumption was made that a smaller error would be involved in the estimation by assessing first color change rather than maximum color change.

3.1.2 Auto Sampler Tests

An ISCO Model 2900 auto sampler was used to collect 24-hour-samples of wastewater at the headworks and the splitter box (ML) for several days of the week. For every day 24 composite samples were taken, each representing wastewater conditions over one hour. An hourly composite sample itself consisted of four samples collected every 15 minutes.

At the beginning, the auto sampler was set up at the headworks, and the starting time of sampling, usually noon, was noted. Later the retention time between headworks and splitter box was calculated based on the current average daily flow and the occupied capacity of the system. The analysis of the samples followed as soon as possible after completion of sampling to ensure representative temperature data. Both types of samples were analyzed for pH (using an Orion pH Meter, Model 520 A), temperature and conductivity (using a YSI Salinity-Conductivity-Temperature-Meter, Model 30/10 FT). In addition, the TSS was determined in ML samples using standard methodology (SM_{18} 2540 D, EPA 160.2 (Smith, 1992)).

After the analysis of several samples it became obvious that, although the sampler was described as well-insulated by the manufacturer, temperature data was strongly dependent on daily air temperatures. Further, the temperature curves determined from these samples were relatively constant and did not follow variations reported by the online monitoring system for the headworks. Therefore this data was not considered to be representative and will not be reported in further detail.

3.2 RESULTS AND DISCUSSION FOR WASTEWATER CHARACTERIZATION

The characterization of wastewater included tracer tests and the analysis of particular wastewater samples for parameters such as pH, conductivity, and total suspended solids (TSS).

3.2.1 Tracer Tests

A detailed description of the set-up for the tracer tests has been given previously (3.1.1 Tracer Tests). The following Table 3.1 provides an overview of the results obtained.

Table 3.1: Overview of Results of Tracer Tests

Date	Average Daily Flow [MGD]	# of Tablets Added [ ]	Wastewater Flow		Retention Time	
			From	To	[min]	[sec]
3/24/00	6.38	12	Secondary Clarifier # 3	PWTP - Effluent	9	50
			PWTP - Effluent	Final Comm. Effl.	30	0
			Secondary Clarifier # 3	**Final Comm. Effl.**	**39**	**50**
3/26/00	6.36	20	Secondary Clarifier # 3	PWTP-Effluent	9	25
			PWTP-Effluent	Aeration Basin	11	55
			Air Stripper	Final Comm. Effl.	19	0
			Secondary Clarifier # 3	**Final Comm. Effl.**	**40**	**20**
3/26/00	3.27	20	**GWTP (after chlorinat.)**	**Final Comm. Effl.**	**53**	**20**

Abbreviations:
MGD = Million gallons per day
PWTP – Effluent = Final Effluent of Process Wastewater Treatment Plant
Final Comm. Effl. = Final Commingled Effluent (of both plants)
GWTP (after chlorination) = Final Effluent of General Wastewater Treatment Plant after Chlorination Treatment

3.2.2 Auto Sampler Tests

Headwork samples taken by the auto sampler were analyzed for pH and conductivity. For ML, the content of TSS was also determined. The results will first be presented for each sample type individually starting with headworks samples. Then comparisons of time-sequenced headwork and ML samples will follow.

The characterizations of pH and conductivity of headwork samples are shown in the following Figures 3.1 and 3.2. Figure 3.1 provides a conductivity overview over several

weekdays for headwork samples, and shows that relatively high variations in conductivity are possible during a single day. Further, it demonstrates that daily patterns can be very different from each other, and that there is obviously no correlation between these trends.

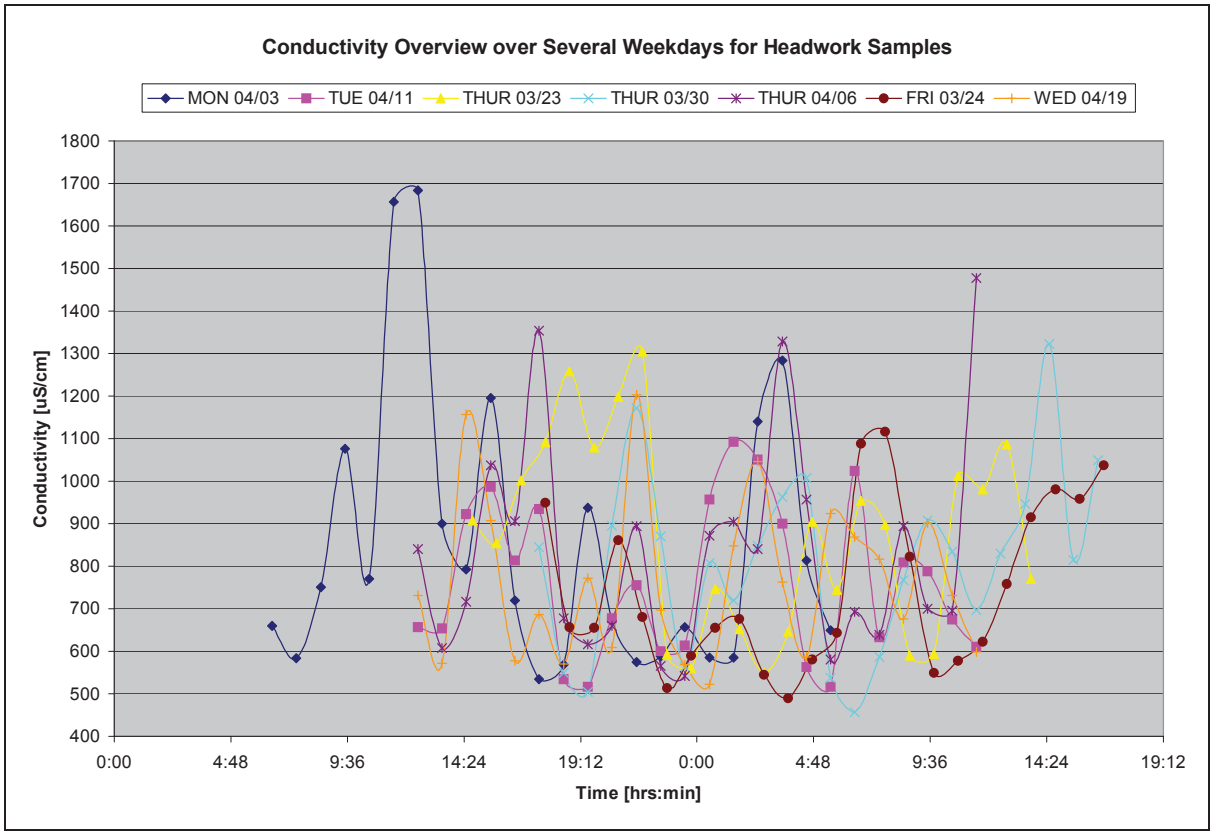

Figure 3.1: Conductivity overview over several weekdays for headwork samples.

In the following Figure 3.2, conductivity data determined on the same weekday (Thursday) are compared. According to these data, there is no correlation or similarity between conductivity data over the same weekday either.

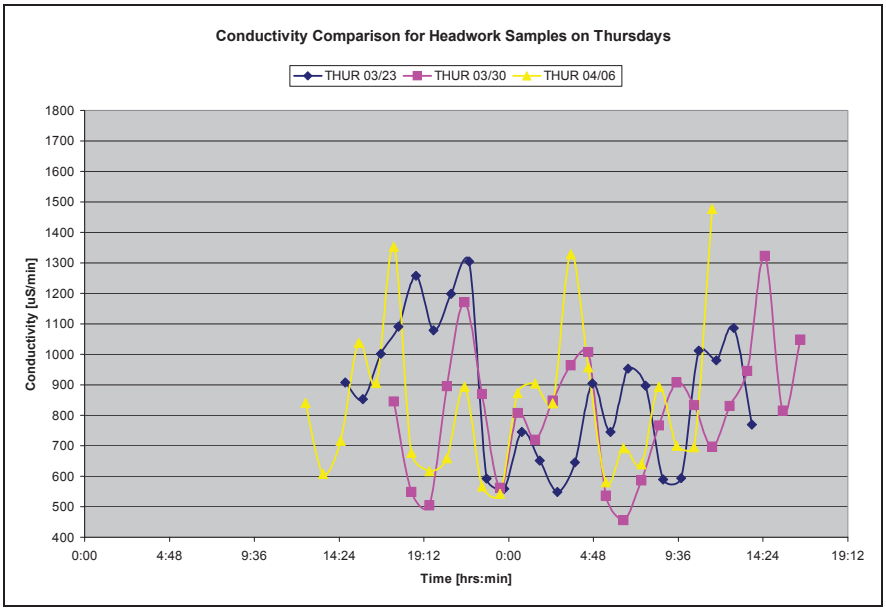

Figure 3.2: Conductivity comparison for headwork samples on Thursdays.

Neglecting isolated peaks, the pH of headwork samples stays relatively constant in the range from approximately 4.5 to 7 (Figure 3.3). Clear trends of pH that change with time, are hard to estimate, although there seems to be similar behavior at some points. For example on several days (Tuesday 04/11, Thursday, 03/23, Thursday 04/06, Friday 03/24, and Wednesday 04/19) a comparable, small peak in pH around 1 a.m. can be found. Similarities like this are probably due to certain stages of the brewing process at certain times creating similar wastewater conditions.

Considering the given pH comparison for headwork samples for the same weekday (Figure 3.4), it can be concluded that the pH varied as much or as little in this case as for different days of the week.

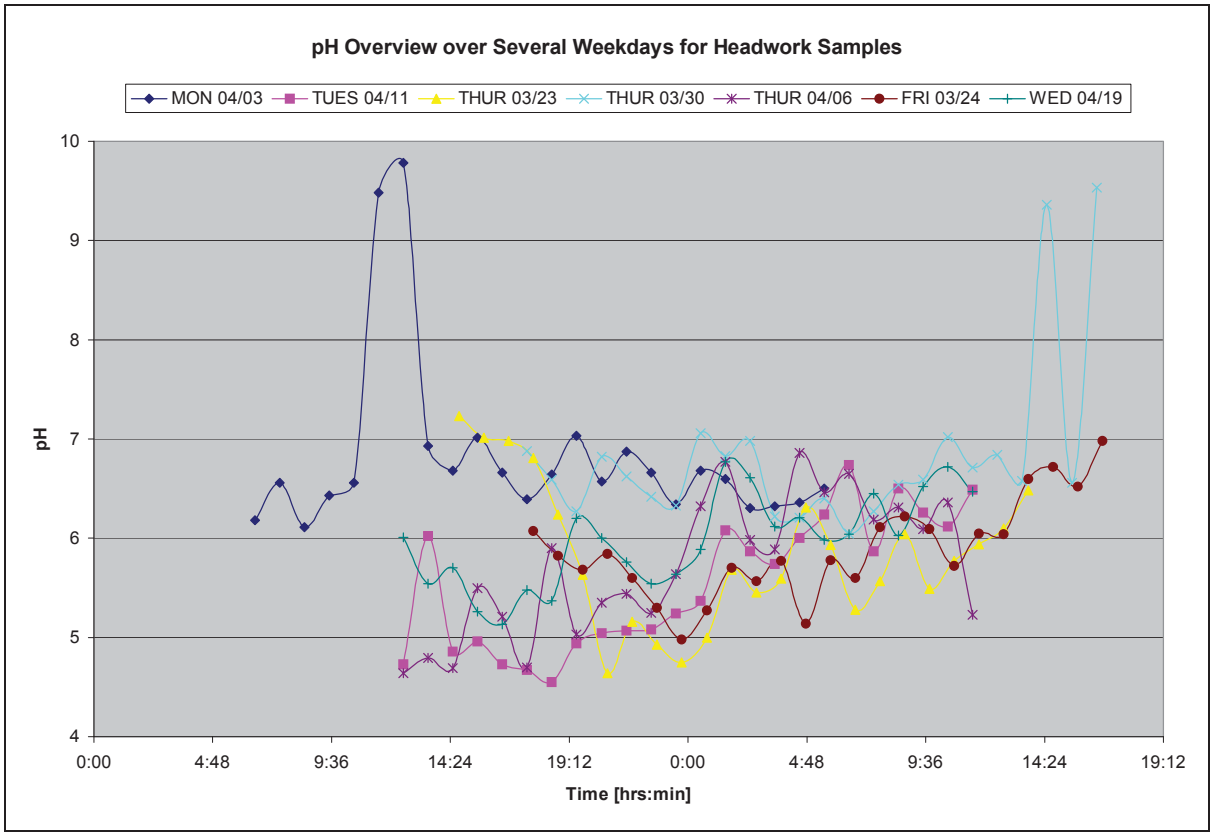

Figure 3.3: pH overview over several weekdays for headwork samples.

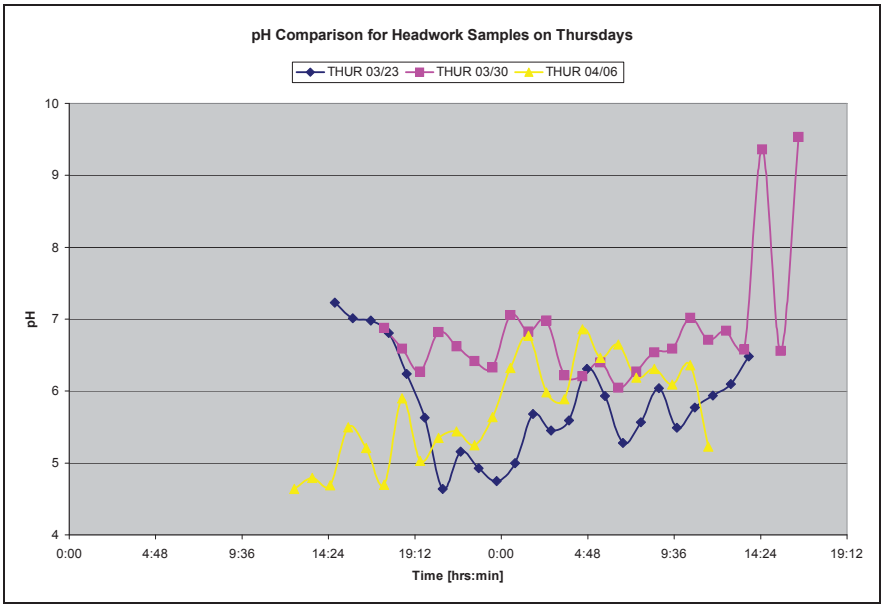

Figure 3.4: pH comparison for headwork samples on Thursdays.

In the following section, pH and conductivity conditions are presented, which were determined by the analysis of the ML samples. Again, it can be seen in the given conductivity overview of several weekdays, that there are no clear trends concerning the variations of this wastewater parameter (Figure 3.5). Further, conductivity comparisons for single weekdays do not indicate a similar behavior either (Figure 3.6 and Figure 3.7).

However, there is an important difference between the conductivity variations in headworks and ML samples: the range, in which the variations of conductivity occur, is significantly smaller for ML than for headworks samples. For this reason, the scale of the Y-axis, which represents this wastewater parameter, was changed to a smaller scale and a shorter range for ML samples.

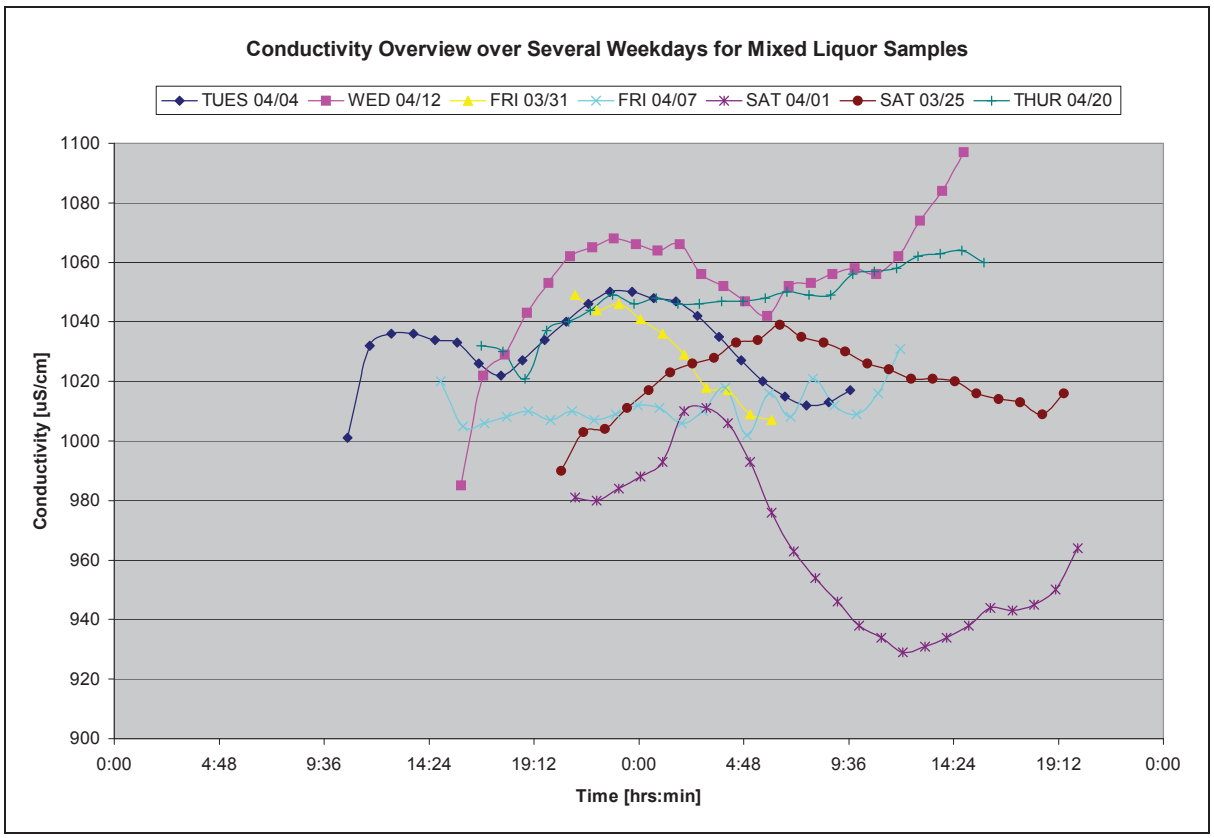

Figure 3.5: Conductivity overview over several weekdays for mixed liquor samples.

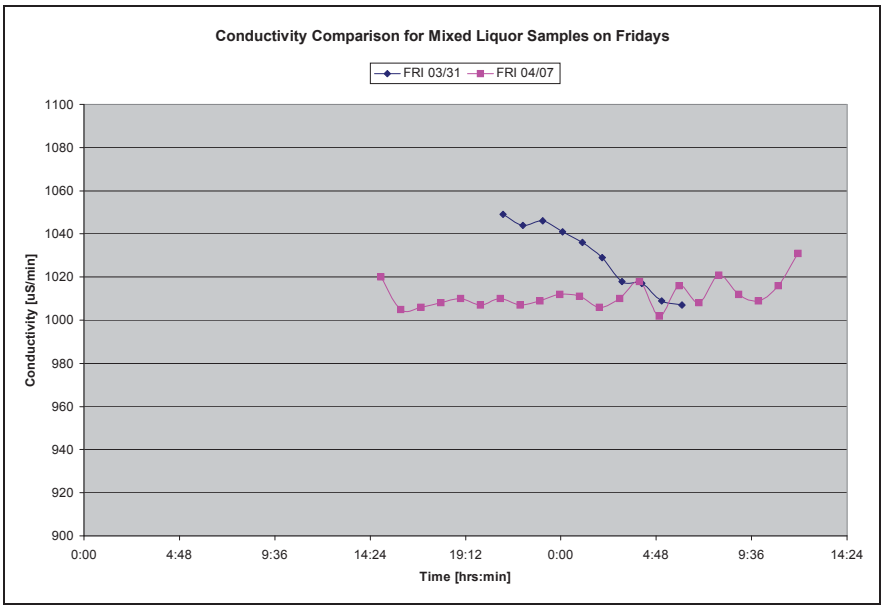

Figure 3.6: Conductivity comparison for mixed liquor samples on Fridays.

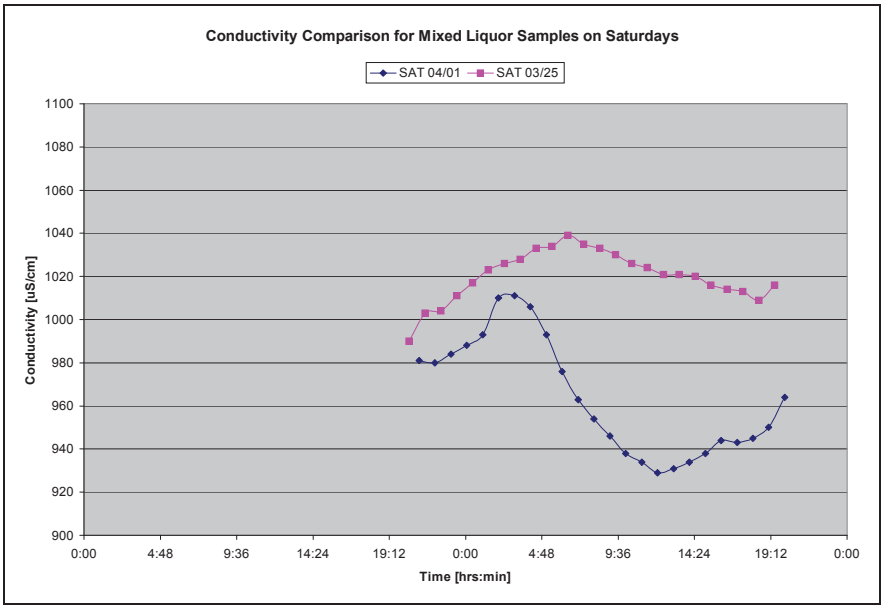

Figure 3.7: Conductivity comparison for mixed liquor samples on Saturdays.

ML samples were also analyzed for pH, and the results are shown Figure 3.8. The range over which the pH values varied in ML samples is quite narrow in comparison to the range observed for headworks samples. Therefore, a different scale was chosen for the Y-axis representing this wastewater parameter. Again, it was hard to determine trends or similarities for these data sets.

As described earlier, the pH of the ML is almost entirely dependent on the concentration of dissolved carbon dioxide, resulting from aerobic respiration. As both, the partial pressure of the headspace and the ML temperature, are relatively constant, only minor changes in pH were expected. The determined data confirmed this assumption (Figure 3.8).

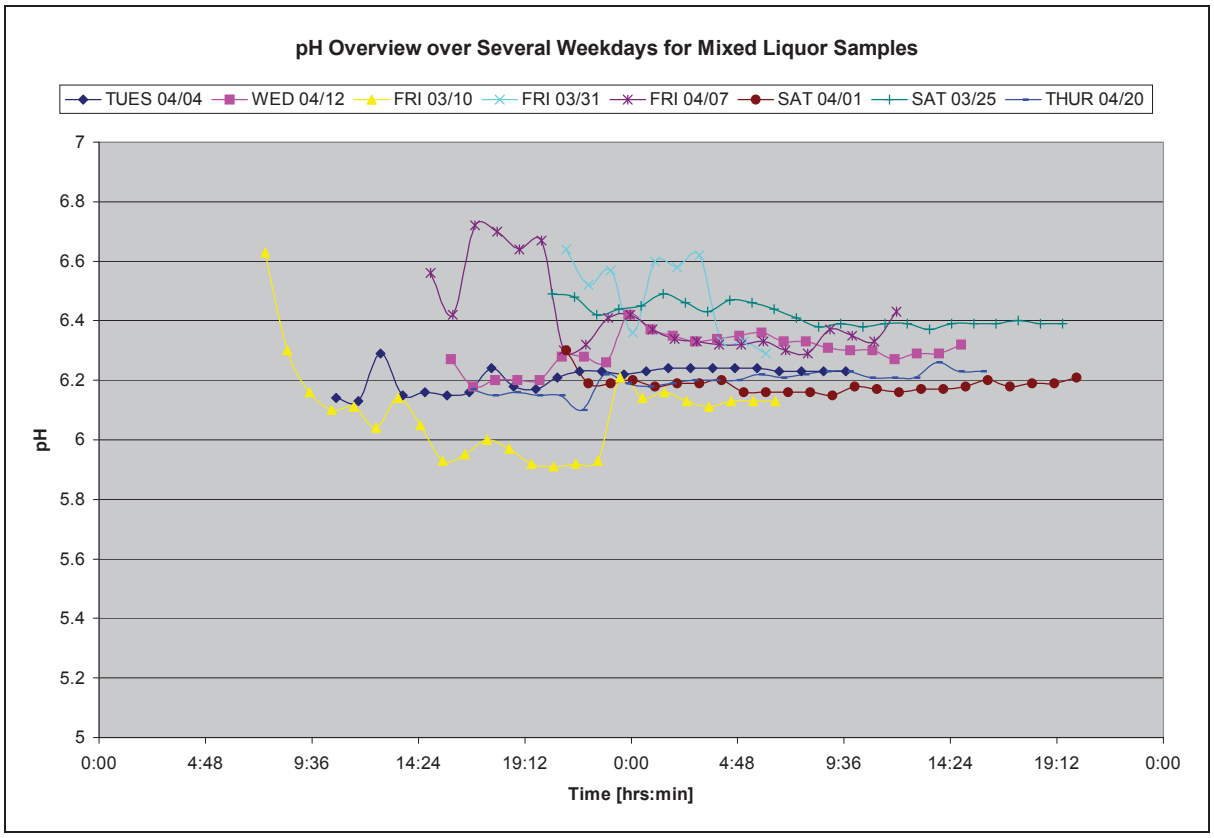

Figure 3.8: pH overview over several weekdays for mixed liquor samples.

In addition to pH and conductivity, the ML samples were analyzed for TSS contents as described previously. This parameter is very important for flocculation, because the purpose of this process is to remove TSS from wastewater. Therefore, the amount of flocculant needed for efficient wastewater treatment strongly depends on the current concentration of suspended solids.

We analyzed each of the 24 hourly samples to be able to describe possible variations in amounts of suspended solids. Figure 3.9 provides an overview of our results. According to this data the TSS can vary from below 5500 mg per liter to almost 7500 mg per liter. No particular trends or repeating developments could be determined. Since problems with the auto sampler occurred during the sampling of ML on Friday 03/31/2000, this data set is

incomplete. Nevertheless, comparing the available numbers with those from Friday 04/07/2000, we can see that there are no similarities for the same weekday either.

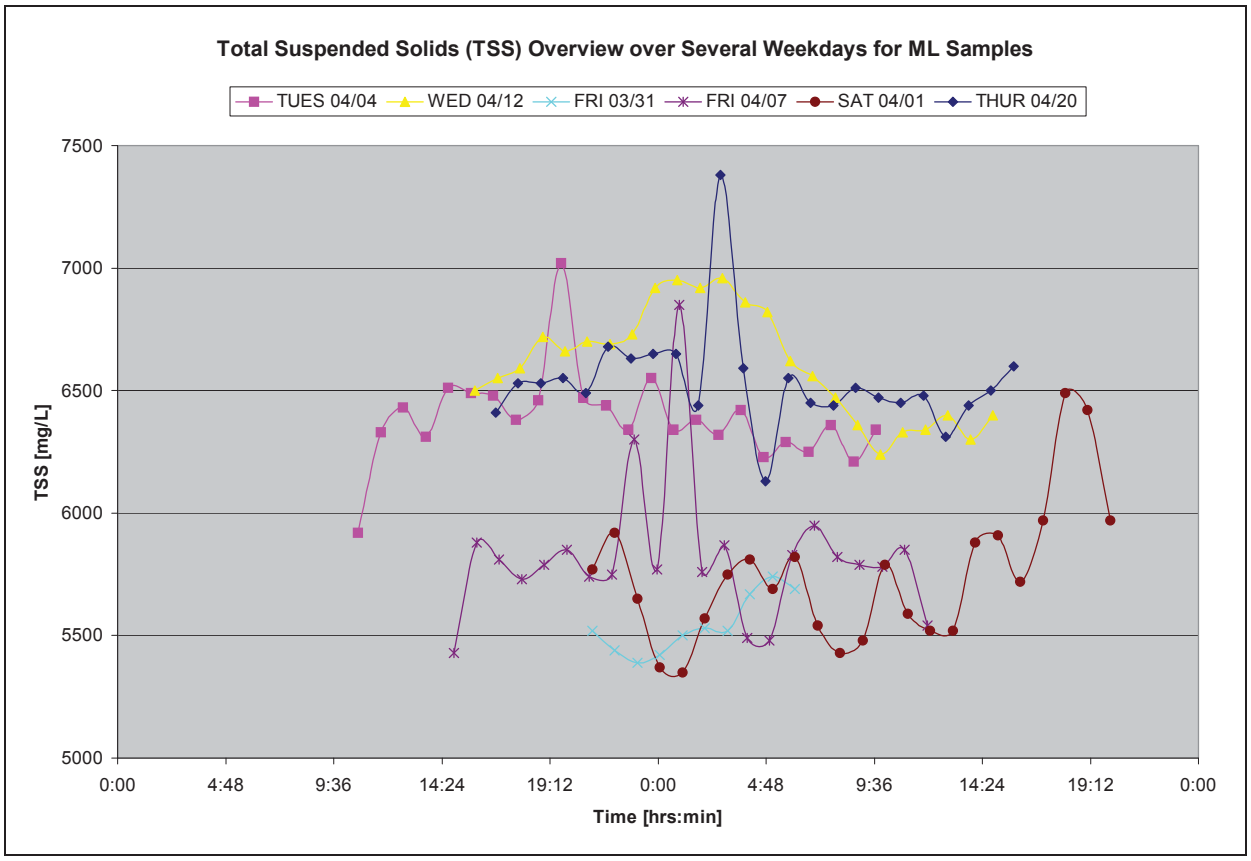

Figure 3.9: Total suspended solids overview over several weekdays for mixed liquor samples.

For five weekdays, the pH and conductivity were determined in headworks samples (taken on Monday, Tuesday, Wednesday, Thursday, and Friday) and the corresponding ML samples (taken on Tuesday, Wednesday, Thursday, Friday, and Saturday). Then the data sets were compared to see if there were correlations between the wastewater streams concerning these parameters. The following Figures 3.10 and 3.11 show the results for conductivity for two weekdays. Additional comparisons can be found in the appendix (Figure 3.12 to 3.14).

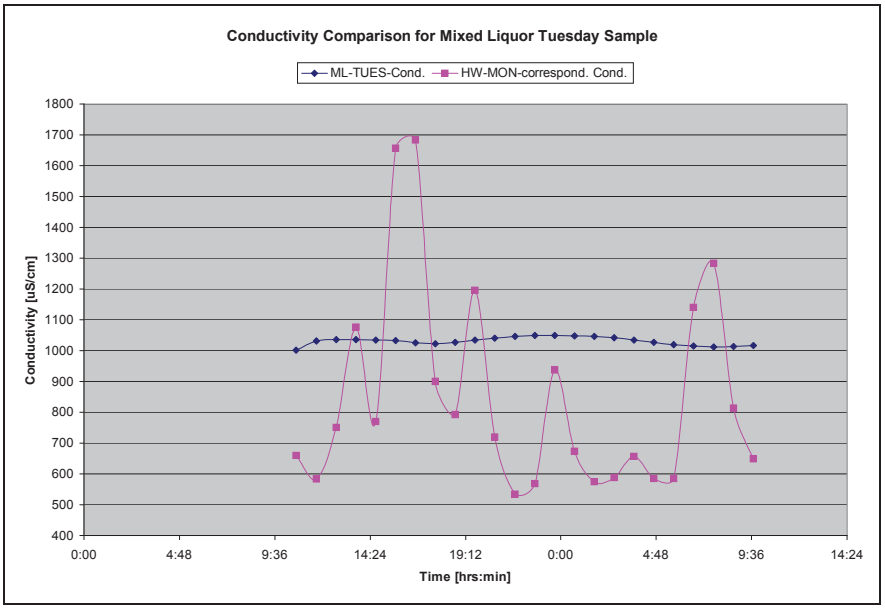

Figure 3.10: Conductivity comparison for mixed liquor Tuesday sample.

Figure 3.11: Conductivity comparison for mixed liquor Saturday sample.

For all days the variations of conductivity for ML samples were much smaller than for headworks samples. Changes in conductivity in the ML, if any, seemed to occur relatively slowly in comparison to headwork samples.

The following Figures 3.15 and 3.16 present the results of pH comparisons between the ML and headwork samples for two weekdays. Additional information for other weekdays can be

found in the appendix (Figure 3.17 to 3.19). Again, pH data for the ML samples are fairly stable and show slow changes in comparison to that of the headwork samples.

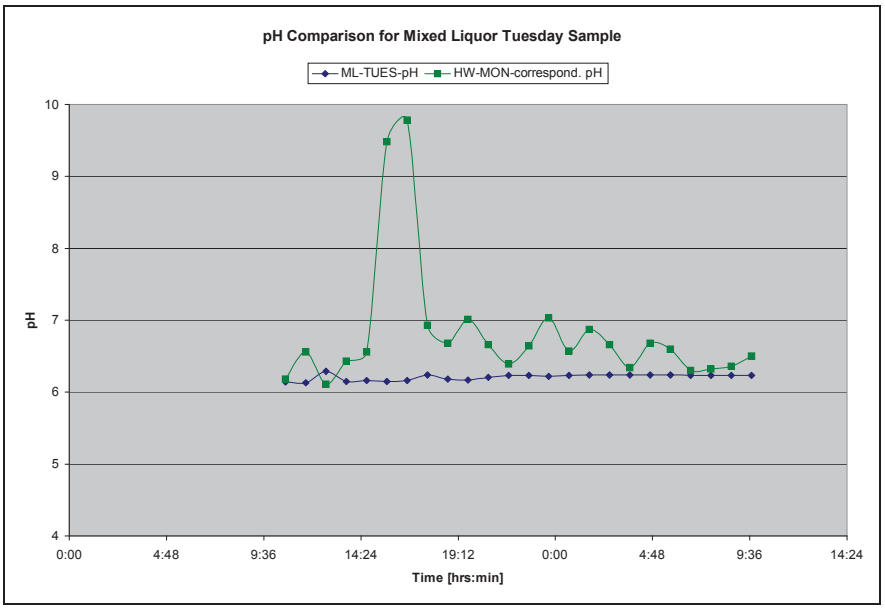

Figure 3.15: pH comparison for mixed liquor Tuesday sample.

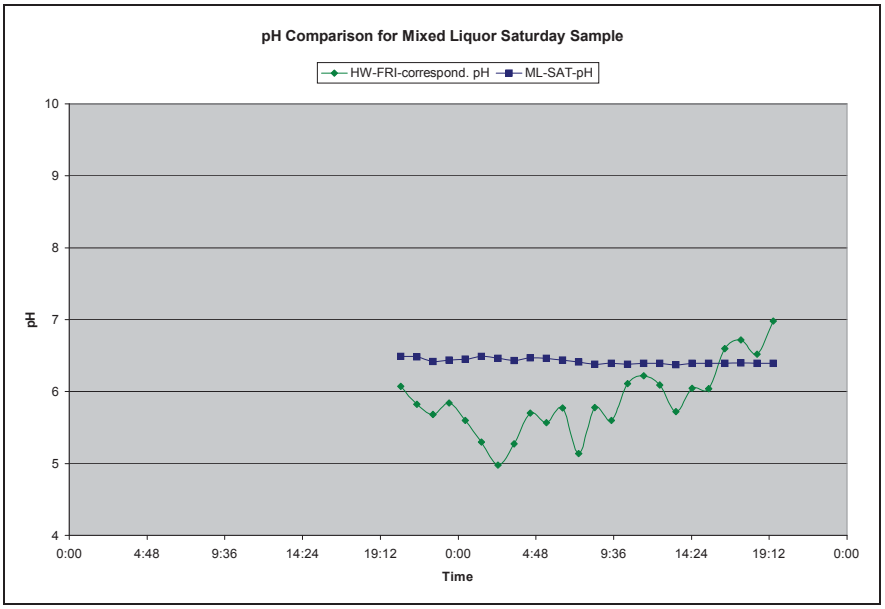

Figure 3.16: pH comparison for mixed liquor Saturday sample.

To sum up, none of the analyzed wastewater parameters in ML or headworks samples followed any definite patterns, which makes predictions of their future behavior impossible. In addition, there is no correlation between the pH and conductivity of headworks and ML

samples. Therefore, online data from the headworks cannot be used to forecast future mixed liquor conditions.

The TSS level in ML is the main determinant of the amount of flocculant to be added. In addition, pH, conductivity and temperature can strongly influence the configuration of the polymer flocculant and consequently its efficiency of flocculation. As these parameters appear to vary independently of each other, it seems impossible to use them for the calculation of the required amount of flocculant. Nevertheless, the changes in pH and conductivity in ML samples were relatively small and slow in comparison to the headworks samples. Therefore, it has to be questioned if the impact of these small variations on the efficiency of the flocculant is negligible or not.

In the past, jar tests were performed by representatives of the flocculant manufacturers using wastewater samples taken over a short period of time. The purpose of these tests was to determine the product that was most suitable for the removal of TSS from the wastewater of interest and the required quantity of this polymer that had to be added to achieve the best flocculation results at the lowest cost. As described above, several wastewater characteristics can vary significantly over time. Therefore, it has to be questioned, if the results determined in a single jar test would be representative of a highly variable process. In addition, the computation of the required flocculant dosage based on current wastewater parameters would be very complicated due to parameter variations over time.

Ideally one selected wastewater parameter should be measured online to ensure that the amount of flocculant added meets the desired requirements. An overview of wastewater parameters useful for this application has been given previously (2.3 Suggested Process Control).

CHAPTER 4
ANALYTICAL METHODS FOR QUANTITATIVE FLOCCULANT ANALYSIS

As described earlier one of the primary goals of this thesis was to find an analytical method for the quantitative determination of the flocculant in the secondary clarifier effluent (SCE). An online method for the same has distinct advantages. However, offline methods were also investigated, as they could be useful to produce reference data.

Besides being online, the measurements involved were required to be fast and simple, as well as inexpensive. Further they should be insensitive to possible interference by other compounds in the wastewater during the analysis. According to wastewater characterization data, several wastewater parameters might change over time. Therefore, in the ideal case, the analysis should neither be influenced by these variations nor should a new calibration curve be required for every new wastewater sample to be analyzed.

Initially, techniques were tested that were based on simple principles, as for example, mass balances for Total Organic Carbon (TOC) and organic nitrogen. As these methods did not seem to meet the defined requirements, new and more complicated ones were investigated. UV detection (Azur Pastel UV®-Analyzer) and viscosity (Brookfield digital viscosimeter) had to be abandoned, as their detection limits were not appropriate.

Due to the lack of detailed knowledge concerning the physical and chemical properties of the flocculant it was difficult to develop a highly specific analytical method. Therefore it was often necessary to compare wastewater samples taken before and after the point of flocculant dosage to the system (mixed liquor and secondary clarifier effluent). Mixed liquor (ML) samples required a pretreatment step to remove high contents of suspended solids prior to its analysis. Thus we had to ensure that the samples were representative and comparable after the sample preparation.

After several methods had been tested in experiments, the application of a colloid titration method finally led to positive results and will be described in detail in the following. Other methods examined will not be discussed further, as this would be beyond the scope of this thesis.

4.0 COLLOID TITRATION

The technique of colloid titration can be used to determine the charge content of colloidal or polymeric matter in solutions (Kam and Greogory, 1999). In addition, the concentrations of large, highly charged compounds in solution can also be determined, if their charge density is known or has been analyzed previously.

Terrayama introduced this technique in 1952. Initially it was applied to the flocculation in the purification of natural waters (Kawawura, 1966). Once determined, the initial raw water colloid charge was used as a parameter for coagulant addition, because it was frequently assumed that the optimum flocculant dosage was close to that required to neutralize the surface charge carried by the particles. Later, colloid titrations were applied for the determination of the charge contents of various compounds, which included proteins (Horn and Heuck, 1982), cell surfaces (Van Damme et al., 1994), and synthetic cationic polyelectrolytes (Gehr and Henry, 1983, Hanasaki et al., 1985, Igarashi et al., 1993, Kam and Gregory, 1999).

4.0.1 Materials and Methods for Colloid Titration

The principle of direct colloid titration is based on the stoichiometric reaction between the polymer of interest and the titrant, an oppositely charged polyelectrolyte, in the presence of an indicator. The titrant is the anionic polyelectrolyte poly [potassium vinyl sulphate] (PPVS or PVSK), provided by Acros Organics (N.J., U.S.A.) (C.A.S. 26837-42-3). The stock solution is 1.6221 g of PVSK in 1 liter (0.01 N), which is diluted to a standard solution of 0.002 N (Gehr and Henry, 1983). The indicator is the cationic dye Toluidine Blue O (o-Tb or TB-o, 3-amino-7-dimethylamino-2-methyl-phenothiazin-5-ium chloride), purchased from Fisher Scientific (N. J., U.S.A) (C.A.S. 92-31-9). The indicator stock solution is made up with 1 g TB-o in 1 liter. The chemical structures are shown in the following Figure 4.3.

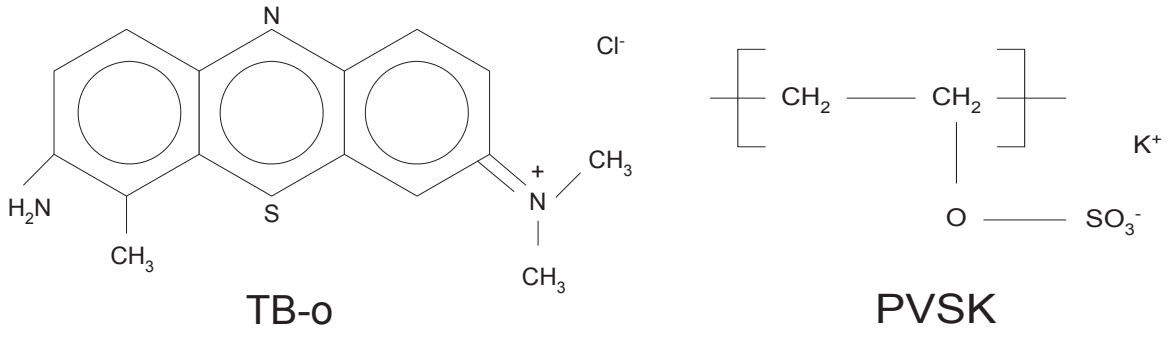

Figure 4.3: Chemical structures of the indicator (TB-o) and the titrant (PVSK) used in colloid titrations (Sjoedin and Oedberg, 1996).

We now proceed to describe the reaction mechanisms during the colloid titration process in further detail. The reaction between cationic polymer and PVSK leads to a mutual neutralization of the charges on their flexible carbon chains, which is assumed to be a 1:1 charge compensation. The bindings between the titrant and the polymer of interest are supposed to be relatively strong, as initial electrostatic bindings are later reinforced by hydrophobic interactions between the carbon chains. Once the neutralization of the cationic polymer in solution has been completed, the positively charged indicator (TB-o) starts to react with the anionic titrant (PVSK) during continuing titration. The interaction between the indicator and PVSK results in a so-called metachromatic band shift of TB-o, where the dye changes its color from blue, when it is free in solution, to red, when it is adsorbed to PVSK. This effect is used to detect the endpoint of the titration using a colorimeter, which is set at the wavelength of 520 nm to sense the increase in red color of the solution (see Figure 4.4).

The overall reaction can be described by the equilibria between the cationic and anionic polymers (P^+ = compound of interest, here the polymeric flocculant, P^- = titrant) and between the anionic titrant and the dye (P^- and D^+):

$$P^+ + P^- \overset{K_1}{\Longleftrightarrow} P^+P^- \qquad K_1 = \frac{[P^+P^-]}{[P^+]\,[P^-]} \qquad \text{(Equation 4.1)}$$

$$D^+ + P^- \overset{K_2}{\Longleftrightarrow} D^+P^- \qquad K_2 = \frac{[D^+P^-]}{[D^+]\,[P^-]} \qquad \text{(Equation 4.2)}$$

$$\frac{K_1}{K_2} = \frac{[P^+P^-]\,[D^+]}{[D^+P^-]\,[P^+]}$$

(Equation 4.3)

In all equations, the concentrations are expressed in charge-equivalent terms. The values for K_1 and K_2 will differ depending on the binding affinities for the titrant-flocculant and titrant-dye interactions. As shown elsewhere (Kam and Gregory, 1999) there is a correlation between the ratio of K_1 to K_2 and the slopes of the titration curves (titrant added plotted against absorbance of red color of the sample solution). As long as this ratio is sufficiently large ($\geq$ 100) the interactions between PVSK and TB-o take place only after the virtual completion of the polyelectrolyte complex formation. Therefore the titration curve shows a constant low initial absorbance of red as long as PVSK reacts only with the cationic polymer of interest and not with the indicator dye. Then there follows a rapid increase in absorbance with a steep slope of the titration curve indicating the start of the reaction between PVSK and TB-o. Finally a higher, constant absorbance is reached, when all neutralization reactions are completed.

For titrations where the ratio of K_1 to K_2 is small (<100), the slope of the absorbance change is less steep. In addition the break point indicating the completed reaction of the titrant with the flocculant and the start of the reactions between titrant and TB-o is not so clearly defined, and the detection of the endpoint becomes more difficult (Kam and Gregory, 1999). K_1 can be assumed to increase with the number of binding sites or charges per polymer chain. Consequently cationic polymers of higher molecular weight and a larger number of monomers are easier to detect using the colloid titration method. The impact of these mechanisms on our experiments will be discussed in further detail later in this chapter (4.5.2 Results and Discussion for Colloid Titration).

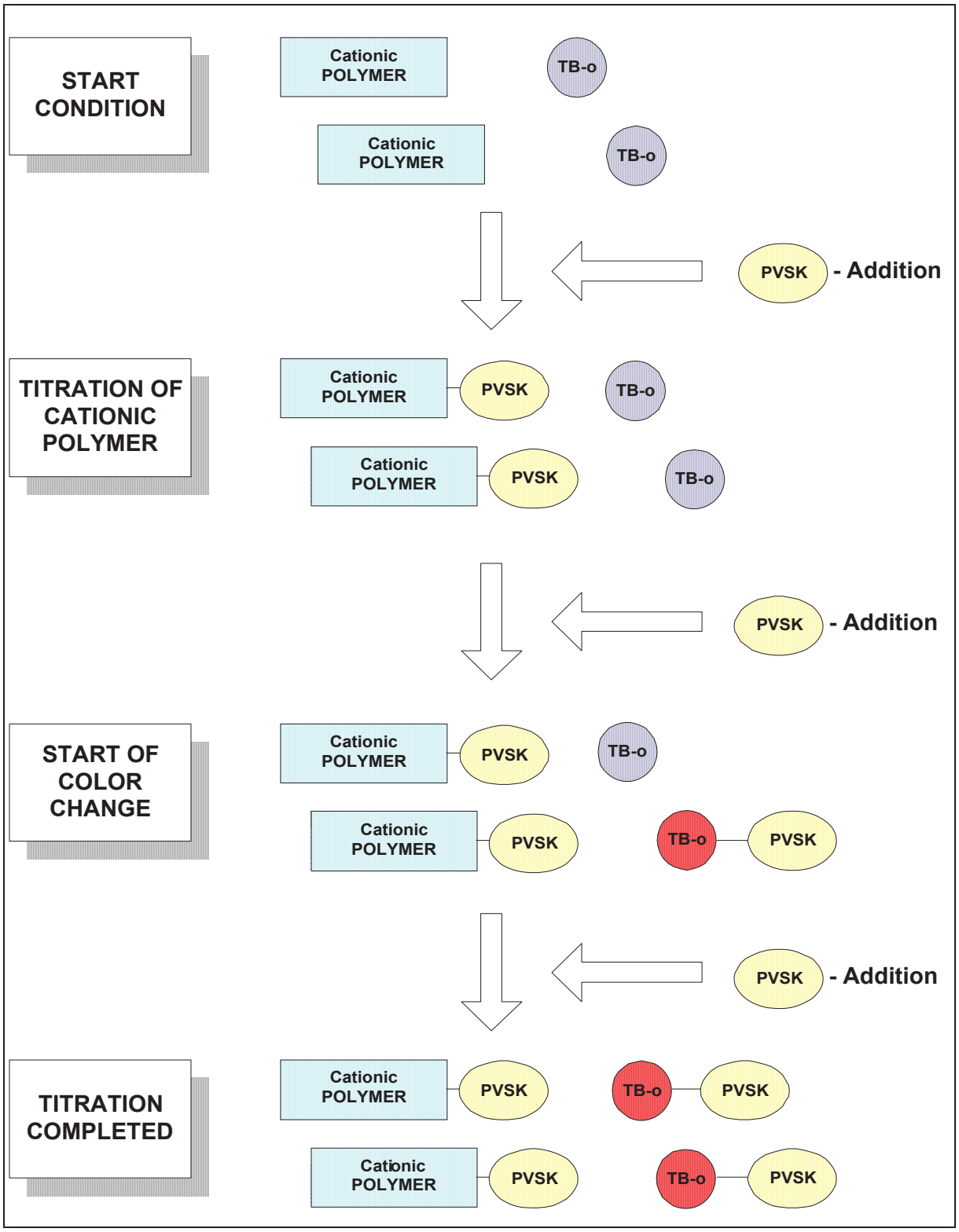

Figure 4.4: Principles of colloid titration.

The theory describing the mechanisms involved with the color change of TB-o are described in detail elsewhere (Ioko et al., 1995; Ortona, et al., 1984). However, the metachromatic color change of the indicator can be explained by the fact that TB-o has different structural configurations when it is free in solution than when bound to the polymeric PVSK. In association with the titrant, the indicator monomers are "stacked" along the titrant polymeric matrix within small distances (< 1nm) which enables interactions between neighboring bound TB-o molecules. As these interactions cannot occur as long as TB-o is free in solution, this leads to the change of its absorbance spectrum and the visible color change from blue to red.

We shall now describe the experimental set-up for colloid titration. The sample was titrated by adding increments of PVSK of 10 μL or more under sufficient mixing conditions, while the change in absorbance was observed using a Brinkmann PC 800 Colorimeter. As low concentrations of the flocculant were expected, a relatively long path length (d = 4 cm) of the sensor cell was chosen. The incoming light was filtered with a 520 nm filter during most of the experiments to measure the increase in red color due to the reactions between TB-o and PVSK. Alternatively, a 640 nm filter (measuring the decrease of blue during the titration) was tested for comparisons with the 520 nm filter titration for one sample. However, as the sensitivity of the measurements was not increased to a great extent, we used the 520 nm filter for the following experiments. The colloid titration procedure for cationic polymers was performed as follows:

- Measure 50 ml of the sample (at room temperature) and add it to a 250 ml Erlenmeyer flask.
- Put the flask on the stirring plate, and add a stirring bar.
- Then place the sensor cell of the colorimeter in the sample solution, and start mixing.
- Add a certain volume of TB-o indicator (usually 60 μl) with a mechanical pipette.
- Then initialize the absorbance measured by the colorimeter to zero.
- Start the titration by adding increments (usually 10 μl) of PVSK with a mechanical pipette, and note the change in absorbance after each addition.
- Complete the titration until no change in absorbance can be observed with the addition of PVSK or until dilution effects become obvious.

In the first set of experiments, the procedure described above was applied to DI water samples spiked with different amounts of polymeric flocculant. This was done to determine if,

in principle, there existed a linear relationship between the concentration of the polymer of interest and the amount of PVSK required to reach the endpoint of the titration. Initially approximately 0.4 ml (8 drops) of TB-o indicator were added to the sample as suggested in the literature (Gehr and Henry, 1983). Soon precipitation occurred, which caused problems in reading a stable and representative absorbance signal. As this effect was also observed in DI water samples that did not contain any flocculant, we assumed that the precipitate was due to interactions between TB-o and PVSK only. Therefore we tested different amounts of added indicator to eliminate analytical problems related to precipitation. The impact of variable quantities of TB-o on titration curves is shown later in this chapter (4.5.2 Results and Discussion for Colloid Titration). Problems caused by precipitation during colloid titrations have previously been reported in the literature, leading to the development of alternate indicators (Tanaka and Sakamoto, 1993a and 1993b). However, we found no information reporting the investigation of possible causes of precipitation in colloid titrations.

Once the appropriate amount of indicator was determined, known amounts of polymeric flocculant were added to DI water samples. These samples were then titrated in triplicate following the procedure described above. In contrast to wastewater, DI water was expected to contain no compounds that would interfere with this method in any way. Consequently this water type should provide an environment where only reactions between polymeric flocculant, TB-o indicator, and PVSK could take place.

Later, wastewater samples (mixed liquor (ML) and secondary clarifier effluent (SCE)) were titrated as well. Here possible interference had to be expected from several other compounds present in the wastewater, like negatively charged organic matter (bacteria) and cationic metal ions (Sjoedin and Oedberg, 1996). Basically any charged matter in wastewater may influence colloid titrations, because of side reactions with the cationic TB-o and flocculant, and the anionic PVSK respectively. Therefore the final endpoint of a titration has to be defined differently for DI water and wastewater samples. In DI water it can be described as the point where both, the cationic flocculant and the TB-o indicator, are completely neutralized by PVSK. In contrast to that, in wastewater, it represents the point where *all* cationic compounds have fully reacted with PVSK. The proposed reactions during colloid titration for both systems are depicted in the following figure (Figure 4.5).

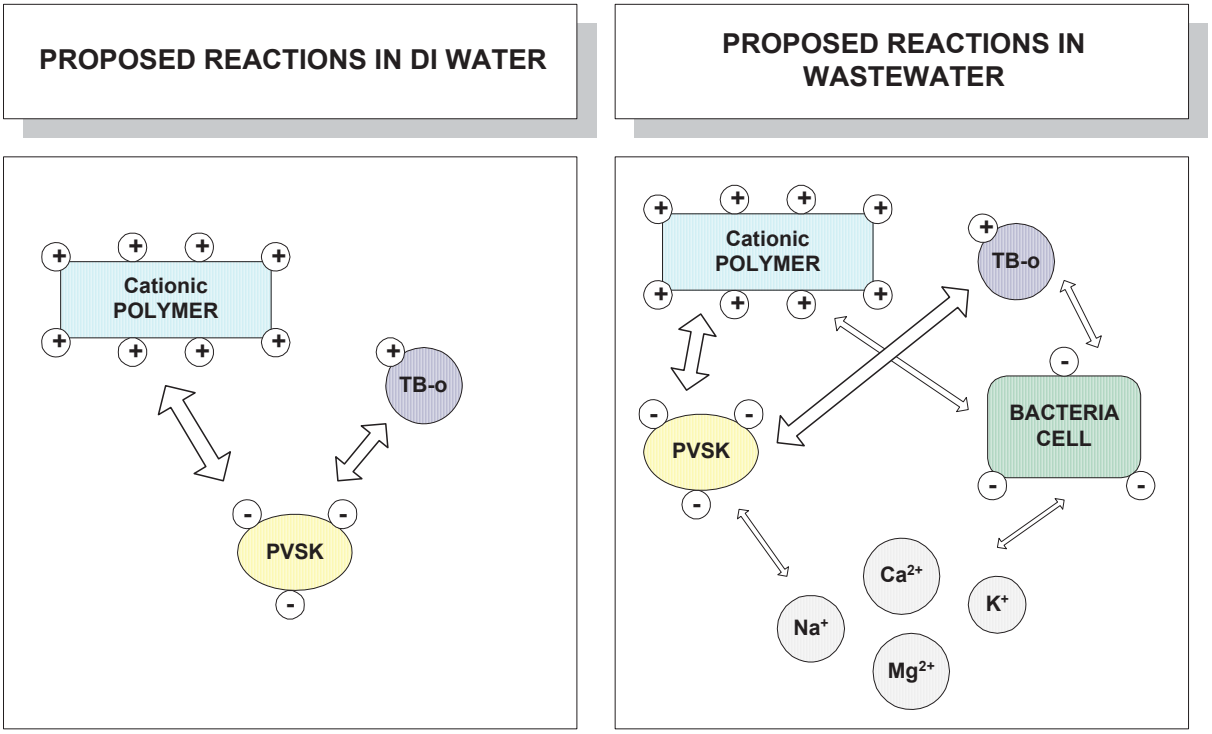

Figure 4.5: Proposed reactions during the colloid titration of DI water and wastewater samples.

To sum up, the PVSK added to DI water samples during titration was only consumed by the cationic flocculant and later on, by TB-o, as long as we neglect interference from carbon dioxide in the atmosphere. In wastewater the titrant may possibly react not only with the TB-o indicator and the flocculant, but also with other metal ions. In addition, negatively charged organic matter may bind to metal ions, the polymer and/or the TB-o. This can cause a color change of TB-o before the indicator actually reacts with the PVSK. Furthermore, less cationic charge of polymeric flocculant may be "available" for binding with PVSK due to interactions between the flocculant chains and organic matter.

As we had to account for all possible interference due to wastewater compounds other than the cationic flocculant, we compared the results of colloid titrations of wastewater samples before and after flocculant addition in the wastewater treatment. The amount of titrant (PVSK) needed for a complete titration of a wastewater sample before flocculant addition was to be used as a background level to be subtracted from a sample containing the residual flocculant. Therefore we assumed that a pretreated ML sample represented the same wastewater conditions as a SCE sample except for the residual polymer concentrations. This

seemed to be a fair assumption, as we did not expect many chemical reactions to occur in the secondary clarifier during the settlement of suspended solids. Small polymer concentrations in the ML due to the recycling of return activated sludge (RAS) containing polymer from the secondary clarifiers to the aeration trains were assumed to be negligible. Further possible differences in CO_2 and oxygen content between ML and SCE samples could be compensated if both sample types were allowed to reach equilibrium with the atmosphere prior to the titration procedure.

To ensure that the contents of suspended solids were comparable in these two samples, a pretreatment of ML was necessary to remove these solids prior to the titrations. Initially, a series of filtration steps were applied to both samples, using filters with the following pore sizes during vacuum filtration: 20-25 μm (Whatman, Grade 4, 12.5 cm diameter), 2.5 μm (Whatman, Grade 5, 12.5 cm diameter), 1.5 μm (Baxter Scientific, S/P glass fiber filter, Grade 394, 4.7 cm diameter), 0.45 μm (Millipore, HA filter, Cat. No. 047 CS), 0.22 μm (Millipore, GS filter), and 0.1 μm (Nuclepore membrane filter, 90 mm diameter). Prior to the first filtration step with filter paper we filtered the sample through paper towel (Kimberly Clark) after washing the towel with DI water. This was done to hasten the first filtration step.

The pore sizes of filters were chosen in this way, because chemical coagulation/flocculation processes were reported to aggregate and remove wastewater constituents in the initial size range from less than 0.1 to about 10 μm (Levin et al., 1985). Therefore it had to be ensured that, prior to a comparison between ML and SCE samples, solids of greater than 0.1 μm particle size had to be removed from both samples in the same way. Unfortunately a TOC analysis of filtered samples indicated that there was carbon leaching from the filter paper for both sample types (data will not be reported in further detail). Therefore the filtration pretreatment was abandoned in favor of a much simpler preparation method, where ML was allowed to settle in a bucket, and its supernatant was used for colloid titration analysis. Here we had to assume that the settlement of suspended solids, without any addition of flocculant, removed nearly the same amount and size fraction of solids as the treatment process in the secondary clarifiers. To confirm this assumption the supernatant of a settled ML sample (dated 05/20/2000) and a corresponding secondary clarifier sample (dated 05/21/2000) were analyzed in a particle counter (Coulter Multisizer SS II). The results of this analysis are shown in figures later in this chapter (4.5.2 Results and Discussion for Colloid Titration).

As there was no calibration data available for the analysis of residual polymer concentrations in SCE samples, we decided to apply the standard addition method. The blank,

accounting for the interference of wastewater compounds other than the flocculant, was the time-sequenced ML sample. Known amounts of flocculant were added to aliquots of the same sample of SCE and analyzed by the colloid titration method. Higher concentrations of polymer in the sample required higher amounts of PVSK to reach the endpoint of titration. From these amounts we subtracted the volume of PVSK required to reach the titration endpoint for the blank. The results could then be used to determine a standard addition curve and thus, the initial unknown polymer concentration in the SCE sample as shown in Figure 4.6. A detailed description of the applied data analysis is provided later in this chapter (4.5.2 Results and Discussion for Colloid Titration).

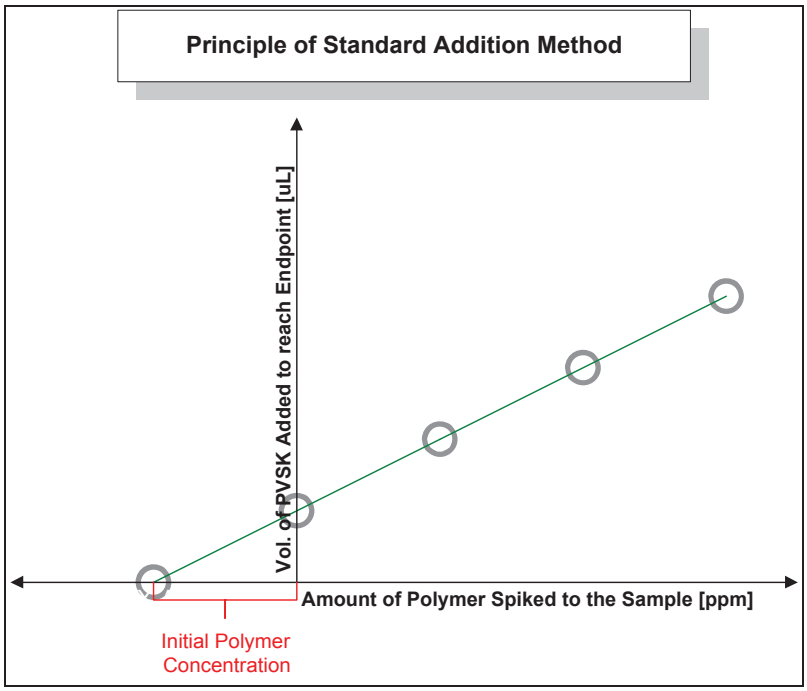

Figure 4.6: Principle of the standard addition method.

It has to be noted that for the appropriate use of the standard addition method several requirements have to be fulfilled. These prerequisites are:

- There exists a linear concentration-response relationship for the compound of interest analyzed by the suggested method in this concentration range.
- Added increments of the compound of interest behave in the same manner as the compound of interest initially present in the sample.

- Additions should be in a concentration range that includes the concentrations anticipated for the original sample.
- The response of the blank to the above analysis is precisely known or can be easily determined.

The linear concentration-response relationship between the polymer concentration and the amount of PVSK required to reach the endpoint of titration had been demonstrated for DI water samples. Therefore, as interfering reactions due to other compounds in wastewater were assumed to be fairly independent of polymer concentrations, a linear behavior was expected in samples of the SCE too. A more detailed description of the analysis of data determined from colloid titrations is provided in the following (4.5.2 Results and Discussion for Colloid Titration).

4.0.2 Results and Discussion for Colloid Titration

In the following, we will present the experimental results obtained during colloid titration experiments analyzing various DI water and wastewater samples. Further, a detailed description of the applied data interpretation method is given.

4.0.2.1 Colloid Titration of DI water samples

As described earlier (4.5.1 Materials and Methods for Colloid Titration) DI water samples were analyzed prior to wastewater samples for several reasons. First, we had to eliminate problems due to precipitation, and to determine the appropriate amount of indicator to be added to the sample to avoid this. In addition, we wanted to demonstrate a linear concentration-response relationship between the polymer concentration in the sample and the amount of PVSK required to reach the final endpoint of titration.

The initial amount of TB-o added, which was recommended in the literature, led to the formation of a precipitate as described earlier. Therefore various, much smaller amounts of added indicator were tested in DI water that contained zero (Figure 4.7) and 1 mg/L (Figure 4.8) polymer respectively. For these tests, only titration curves where no precipitation occurred are reported. All curves represent averages of triplicates. The following figures (Figure 4.7 and Figure 4.8) show the results obtained from these experiments.

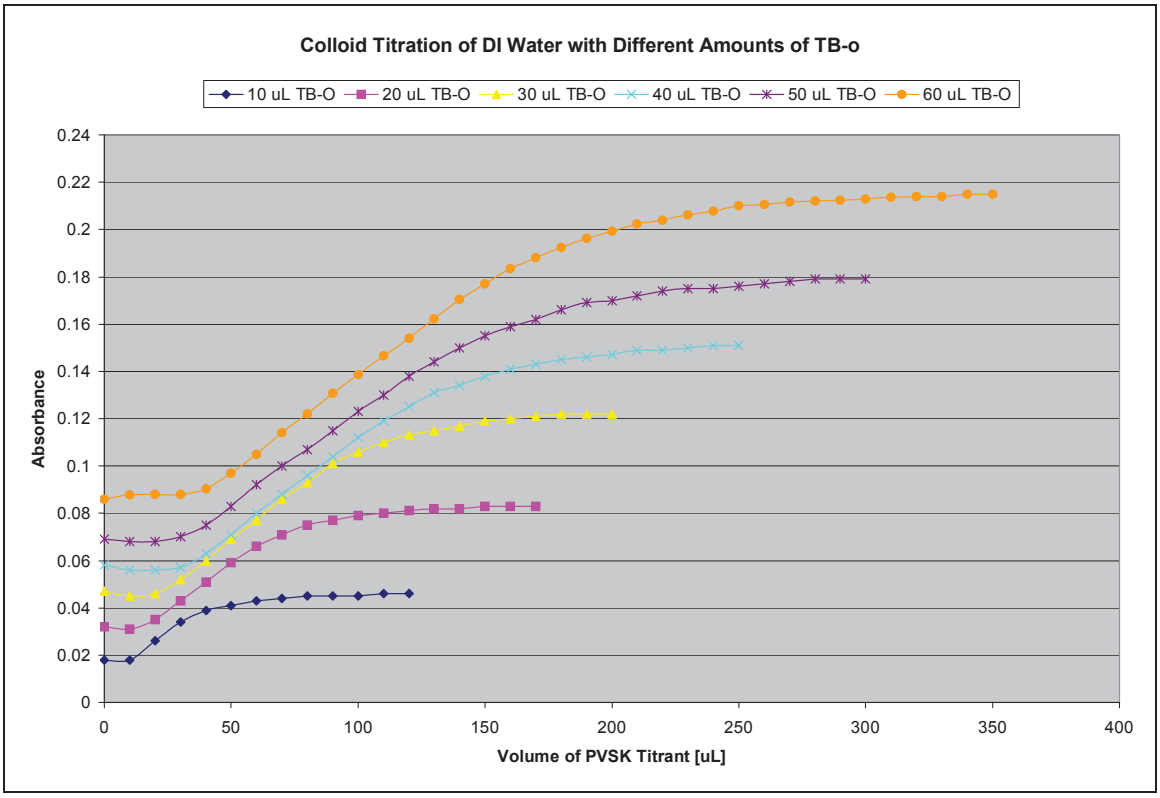

Figure 4.7: Colloid titration of DI water with different amounts of TB-o.

For both DI water samples, the initial absorbance prior to any addition of PVSK titrant was slightly enhanced with increasing volumes of added indicator. This can be explained if we consider the nature of the absorbance measurement of the colorimeter. Due to the chosen filter, it was mainly the increasing absorbance of red that was determined during the titration procedure, but changes in the turbidity of the sample may also have had a slight effect on this parameter. Larger volumes of TB-o added to the sample solutions resulted in higher turbidity and thus, in increased initial absorbance.

At the beginning of the titration procedure for both sample types, different amounts of PVSK had to be added before any changes in absorbance could be observed. Obviously the volume of PVSK required for a first increase in absorbance was dependent on both, the polymer as well as the TB-o concentration in solution. Even for polymer concentrations as low as 1 mg/L of polymer in DI water, a higher volume of PVSK was required for the described color change, in comparison to pure DI water. This indicates that in DI water, the polymeric flocculant actually reacts with PVSK before any interactions between the titrant

and TB-o can occur. It also shows that PVSK has a significantly higher affinity to bind to the flocculant than to the indicator.

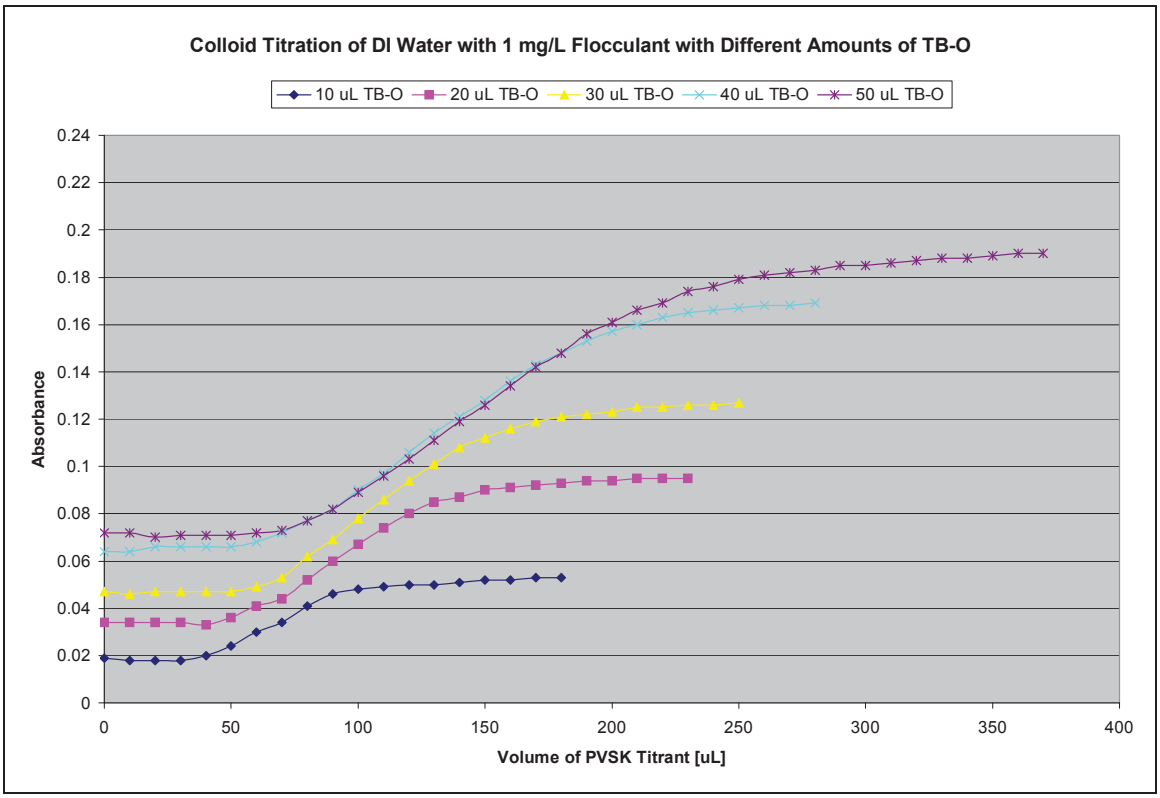

Figure 4.8: Colloid titration of DI water (1 mg/L flocculant) with different amounts of flocculant.

However, the results obtained for samples containing no polymeric flocculant also indicate that there is a "critical" amount of PVSK needed for the titration of these samples before we can see the first color change. This was contrary to our prior expectation to observe an immediate response to the PVSK addition for this type of sample. Therefore we have to assume that even in DI water, there are compounds present that hinder the reactions between the PVSK and TB-o. It is probable that bicarbonates (HCO_3^-), at the determined pH range, are bound to the cationic TB-o and block its reaction with the anionic titrant. A stronger binding effect is observed with higher concentrations of indicator in solution.

Let us now consider the titration results for the DI water samples containing 1 mg/L of the polymeric flocculant. When we compare these results with those presented in Figure 4.7 for DI water, it is clearly evident that a larger amount of PVSK is now required to observe the

first significant absorbance changes. This is true for each comparison between the DI water samples with and without flocculant, containing the same amount of TB-o.

In addition to the higher retardation of the first color change, the slopes of these titration curves are fairly similar to those in Figure 4.7. This indicates that the neutralization of the flocculant must have been almost fully completed before the PVSK started to react with the TB-o molecules. If both reactions took place at the same time, the added increments of PVSK would react simultaneously with the TB-o, and with the polymeric flocculant. This would result in a smaller color change per increment of added titrant, and thus in smaller slopes of titration curves for samples spiked with polymer than for pure DI water. Both observations, similar slopes as well as different starting points of significant color change in the two sets of curves, confirm that the PVSK titrant must have a much higher affinity to the polymer than to the indicator in DI water.

These two sets of curves also clearly show that for both samples, the more TB-o present in solution, the more the PVSK that was required to reach the titration endpoint. The endpoint was defined to be reached with a constant, high absorbance and a slope of approximately zero. The absolute value of the final absorbance is not necessarily a good indicator of the titration endpoint for a particular sample, as the initial addition of TB-o is connected to some error due to the usage of a mechanical pipette.

Once the influence of different amounts of TB-o in solution was determined, we decided to add a volume of 30 μL of indicator prior to further colloid titration of DI water samples. The next step was to demonstrate a linear concentration-response relationship between the polymer concentrations in solution and the required increments of PVSK to reach the endpoint of titration for this sample. For that reason, DI water samples were spiked with different amounts of polymeric flocculant, and analyzed after addition of the same amount of indicator (30 μL). The following figure (Figure 4.9) shows the titration curves determined during these experiments. For all concentrations of the flocculant, the data represents averages of three replicates.

According to this data, the detection limit for this method lies around 1 mg/L of polymer in DI water. For concentrations below 1 mg/L of polymer, the endpoint defined by constant high absorbance, was reached after the addition of approximately the same number of PVSK increments. However, the titration curves for 1, 2, and 4 mg/L of added flocculant were significantly different from each other. The higher the concentration of polymer in solution, the more PVSK it took to complete all neutralizations and to reach the endpoint. Again it

becomes obvious that the value of the absorbance is not a good indicator for the endpoint of the titration. For example, the sample containing 2 mg/L of flocculant reached a higher absorbance at the endpoint than the one spiked with 4 mg/L. This is probably due to the error involved with the addition of TB-o indicator using a mechanical pipette, also leading to a higher initial absorbance for the sample containing 2 mg/L of flocculant. To avoid such an error as much as possible, frequent changes of pipette tips and careful additions are required.

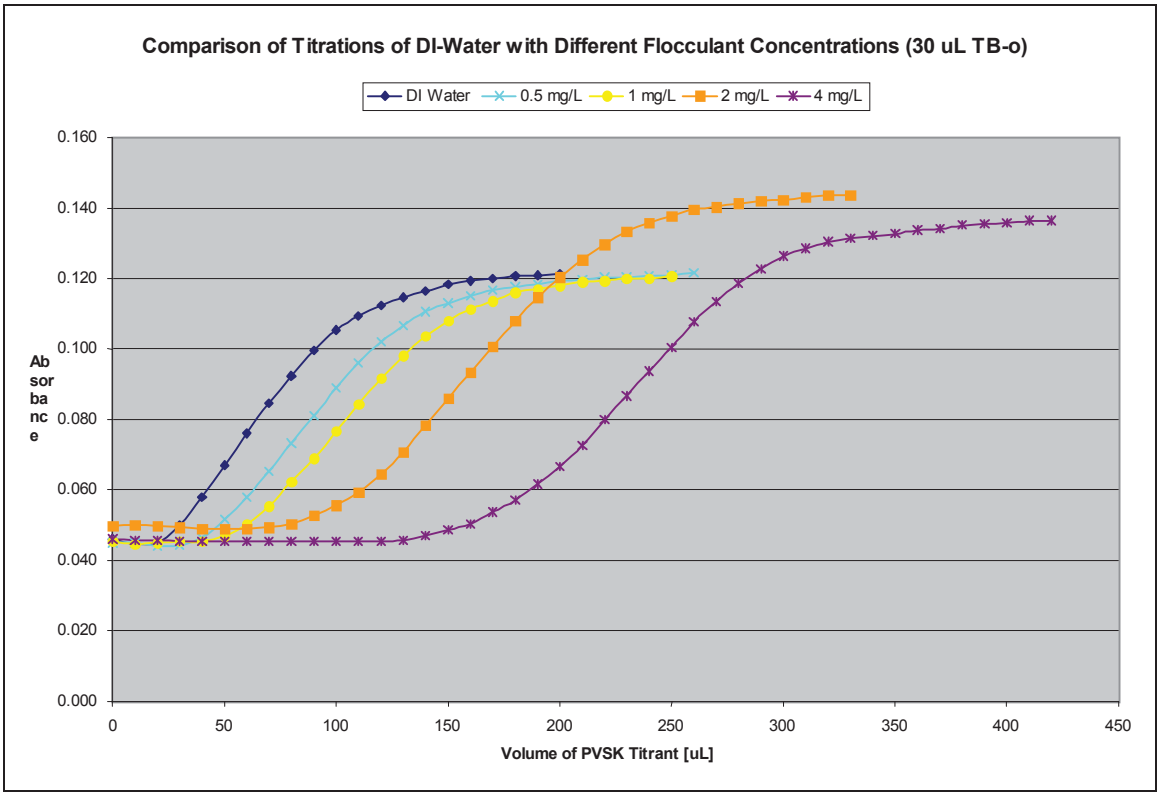

Figure 4.9: Comparison of titrations of DI water with different flocculant concentrations (30 µL TB-o).

Usually the inflection point of a titration curve is used to determine the endpoint of titration. Therefore, we computed the slopes of the titration curves using the average of the three replicates of the DI water blank and for all samples spiked with different concentrations of polymeric flocculant emulsion. Then we plotted the slopes of these average titration curves against the volume of titrant (PVSK) added to the sample during the titrations (Figure 4.10). Polynomial trend lines of the same order were applied to the slope curves of each concentration to make the determination of the inflection points by eyesight simpler and more accurate.

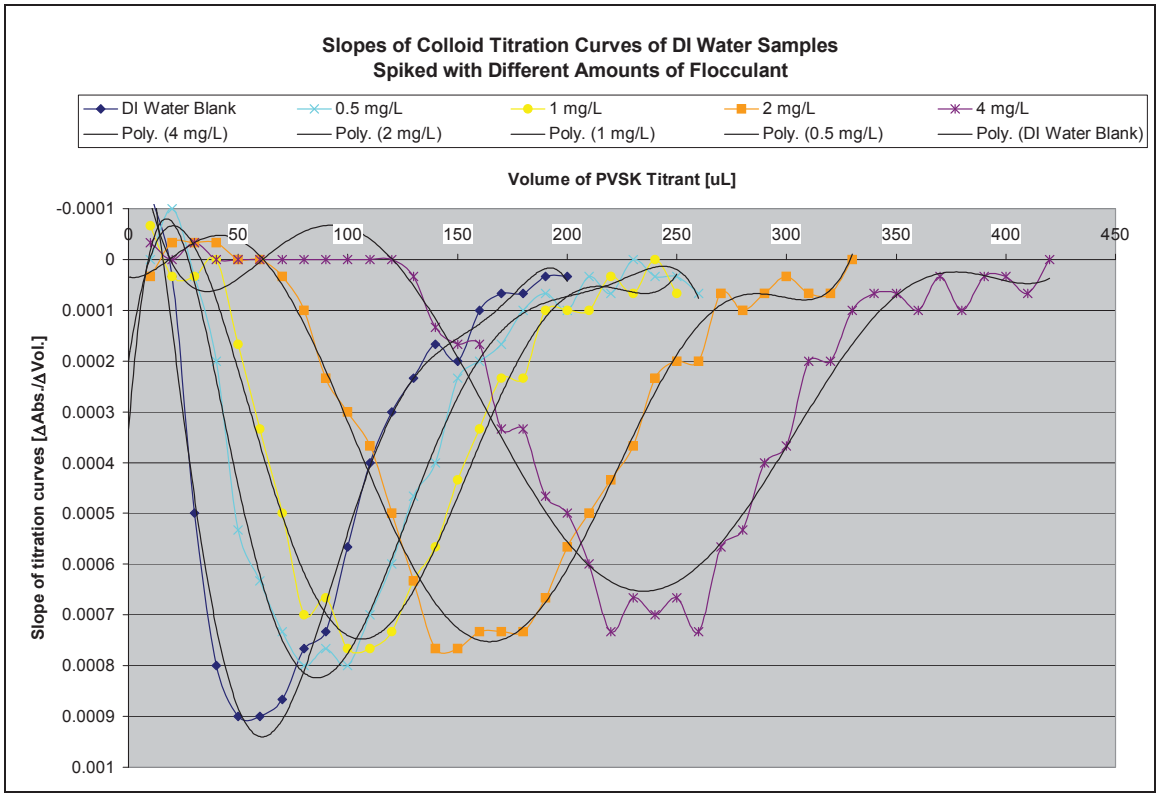

Figure 4.10: Slopes of colloid titration curves of DI water samples spiked with different amounts of flocculant.

In addition, we calculated the three-point averages of the slopes of the titration curves and plotted them as well over the volume of titrant (Figure 4.11). As the slope curves were much smoother than before, trend lines were not longer necessary to support the determination of the inflection points by eyesight.

The inflection points obtained from the curves of the three-point average slopes are mostly the same as those determined from the curves of the "real" slope (Table 4.14). This indicates that both methods of data interpretation determine almost the same endpoints of titration, and hence, almost the same concentration-response relationship. Therefore only the data based on the curves of the "real" slope were plotted to show a linear concentration-response relationship between the amount of polymer added to the sample and the required amount of PVSK to reach the endpoint (Figure 4.12).

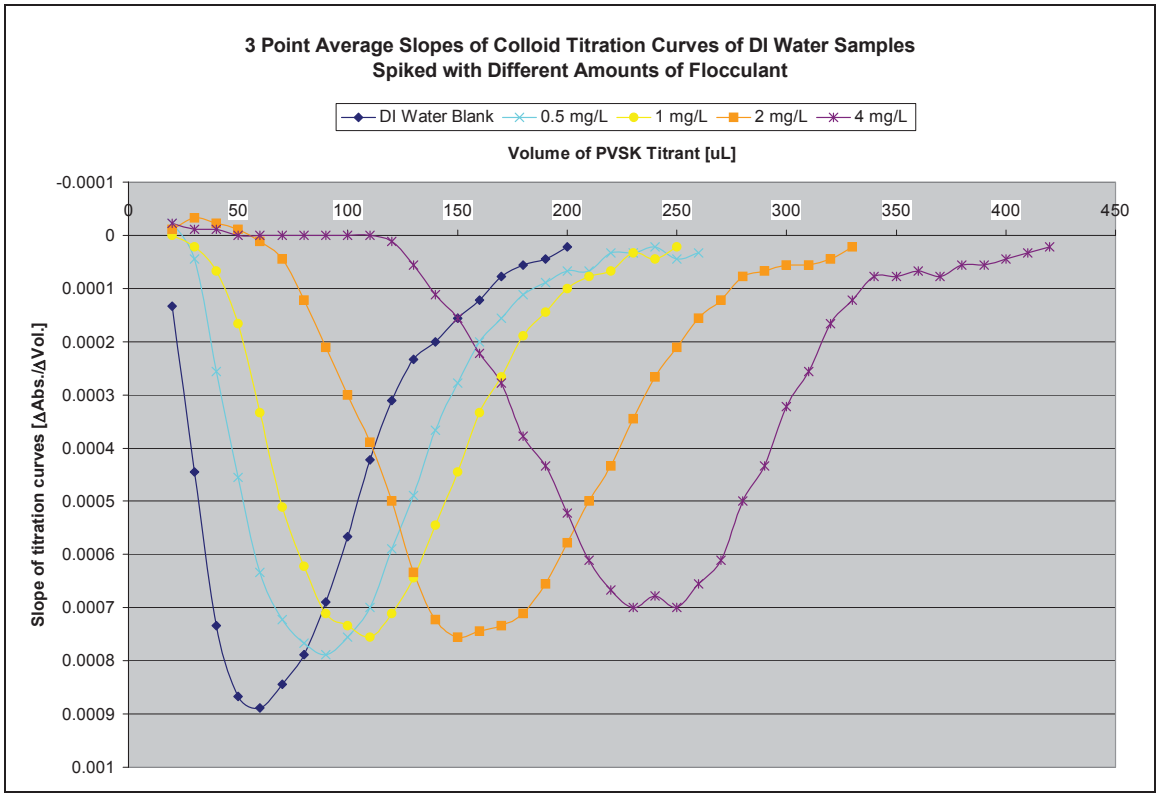

Figure 4.11: 3 point average slopes of colloid titration curves of DI water samples spike with different amounts of flocculant.

Table 4.14: Comparison of Determined Inflection Points		
Amount of Spiked Polymer	Vol. of added PVSK to reach inflection point	
	Real Slope (Trendline)	3 Point Aver. Of Slope
[mg/L]	[uL]	[uL]
0	60	60
0.5	90	90
1	110	110
2	170	150
4	240	240

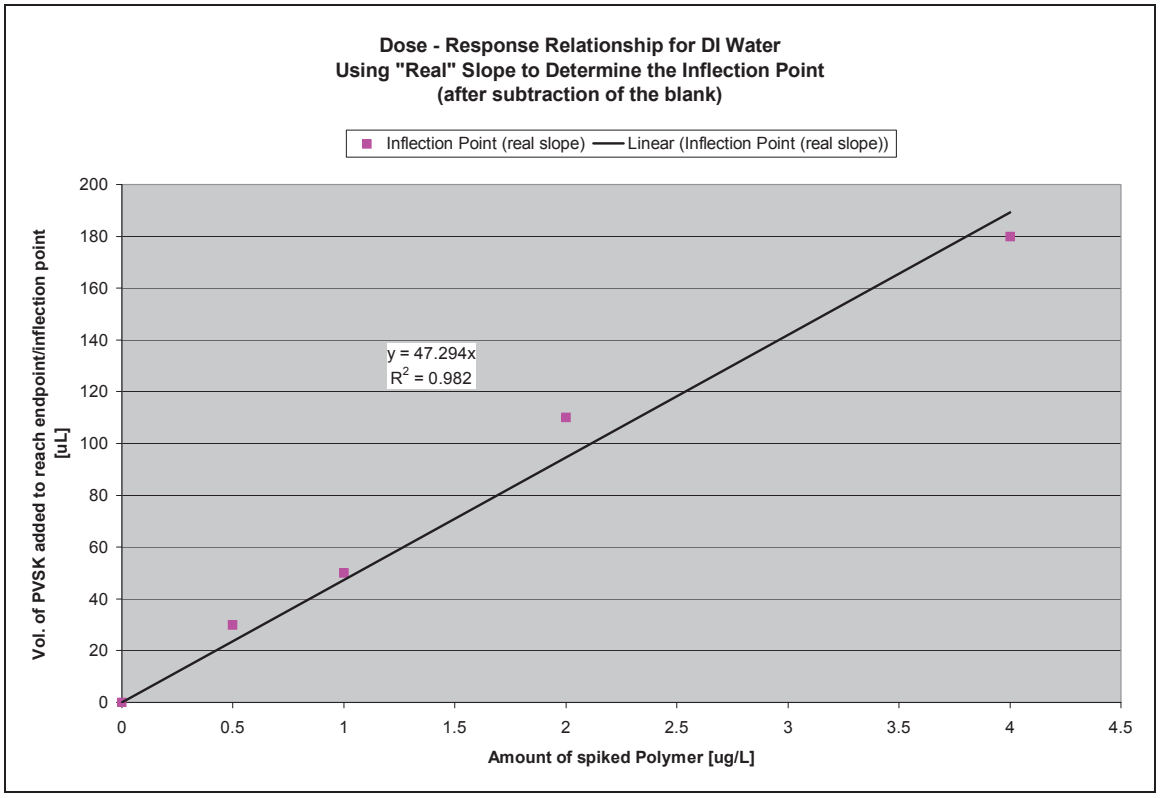

Figure 4.12: Dose-response relationship for DI water using "real" slope to determine the inflection point.

4.0.2.2 Data Interpretation based on the Standard Deviation of Slopes

As we will see in the following (4.5.2.3 Colloid Titration of Wastewater Samples), the titration curves determined from the analysis of wastewater samples look quite different from those of the DI water samples due to the numerous interfering reactions in wastewater. Probably the most significant difference is the absence of inflection points in the titration curves determined from wastewater samples. Thus the titration endpoint has to be defined in a new way that allows us to interpret titration curves without using inflection points. However, the results of this new method should preferably be comparable to the results obtained using the inflection point method. For this reason, we developed a method for the interpretation of titration data based on the standard deviation of slopes, and tested it against results determined from the same DI water samples based on the inflection point method. The new method is described in further detail below.

As mentioned earlier, a constant high absorbance value at the end of the titration procedure indicates that all the charged compounds present in the sample have been neutralized. This is

based on the assumption that the TB-o indicator has a lower or equal affinity to the titrant (PVSK) in comparison to all other wastewater compounds. Therefore, we will see absorbance changes as long as PVSK reacts with charged wastewater compounds, and a constant value in absorbance after all these possible reactions have terminated. We first determined the amount of PVSK required to reach the titration endpoint for a blank sample (pretreated ML sample) containing only the charged interfering wastewater compounds, but no flocculant. We then subtracted this amount from the amounts of PVSK required to reach the endpoint for all the other wastewater samples with varying flocculant concentrations. The resulting amounts can now be used to compare the titrations of the wastewater samples with varying amounts of flocculant in relative terms. Increasing amounts of PVSK are required to reach the endpoint for samples with correspondingly higher concentrations of flocculant.

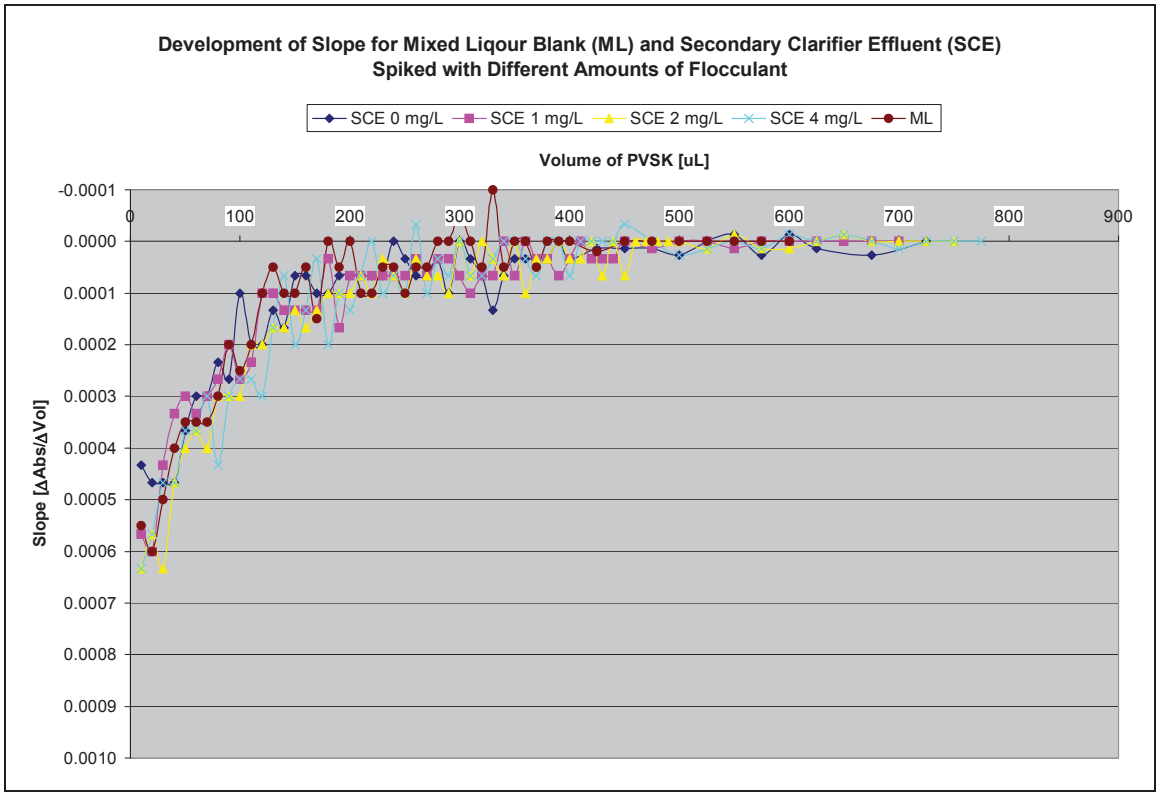

Figure 4.13: Development of slope for ML and SCE spiked with different amounts of flocculant.

Considering the development of slopes for DI water samples shown previously (Figure 4.10), we will have to deal with varying slopes in all regions of the titration curve. For wastewater these variations in slope seem to be even higher (Figure 4.13). As we assumed

that a lot of the variations in the slopes were either due to measurement errors or calculation inaccurancies, we wanted to interpret our data in a way that we could neglect values of the slope that were not significantly different from zero, to be able to determine the endpoint.

Initially we considered the smallest slope that could be measured during the titration experiment as an indicator of our endpoint (the cut-off criteria). As the lowest absorbance number that could be read on the display of the colorimeter was 0.001 and increments of 10 μL of PVSK were constantly added to DI water, the smallest slope that could actually be determined in the experiment, was 0.0001 ΔAbs/ΔμL. However, this method to determine the endpoint became more complicated when applied to titration curves for wastewater samples. As the titrations were more time-consuming for wastewater, the titrant was not necessarily added in the same increments over the entire experiment. In addition, the point where we switched to different, mostly larger, increments varied for samples of different polymer concentrations. Therefore, the choice of the cut-off criterion would have been rather arbitrary.

Considering these difficulties, we sought a more objective way to determine a cut-off criterion to distinguish between slopes that were significantly larger than zero, and slopes that were not. Thus we decided to use statistical criteria to judge the extent to which the observed variations in slope were primarily due to measurement/calculation errors. For this purpose, we analyzed our data using the following procedure for each type of wastewater sample:

- For each replicate, determine the point-to-point slopes (X_i) over the entire titration curve.
- Calculate mean slopes (X_{mean}) among all replicates, always comparing the developments of the curves after equal amounts of PVSK addition.
- Use the mean slopes at each point to calculate $(X_i - X_{mean})^2$ at each point of every replicate.
- Using these values, calculate the overall variance and standard deviation of slopes for each replicate.
- The cut-off criterion for each sample type with a certain flocculant concentration, is set as the mean of the standard deviations of all replicates of that sample.
- Then apply this cut-off criterion to the interpretation of titration data: Set all the slopes smaller than the mean standard deviation of all replicates for a particular sample type to zero.

Following this procedure, we decided that all numbers smaller than the mean standard deviation of slopes for all replicates of the same sample type were not significantly larger than zero. Therefore, these values are plotted as zero using an appropriate conditional clause in EXCEL.

To prove that this data interpretation method is also correlated with a linear concentration-response relationship, we applied it to the titration curves of DI water samples. However, using this method, it became obvious that for most DI water sample types, the slope did not reach zero at the end of the titration (no data reported in further detail). Obviously the DI water samples were not titrated long enough, which can also be clearly seen in Figure 4.11. At this stage of our research, we did not yet know, that the results determined from the titration of the wastewater samples did not contain inflection points in the curves. Therefore, the then main purpose of the DI water titrations was to obtain curves utilizable for endpoint determination using inflection point analysis, and not using a final slope of zero as that determinant. However, a general comparison between the two interpretation methods was still possible, if we made our cut-off criterion less sensitive by applying twice the determined standard deviations.

Table 4.15: Modified Cut Off Criteria for DI Water

Sample Type	2 x Standard Deviation
DI Water Blank	6.53E-05
0.5 mg/L	8.54E-05
1 mg/L	9.52E-05
2 mg/L	8.84E-05
4 mg/L	9.89E-05

Using these criteria, the slope developments for the titrations of DI water samples were determined again and are shown in the following figure (Figure 4.14).

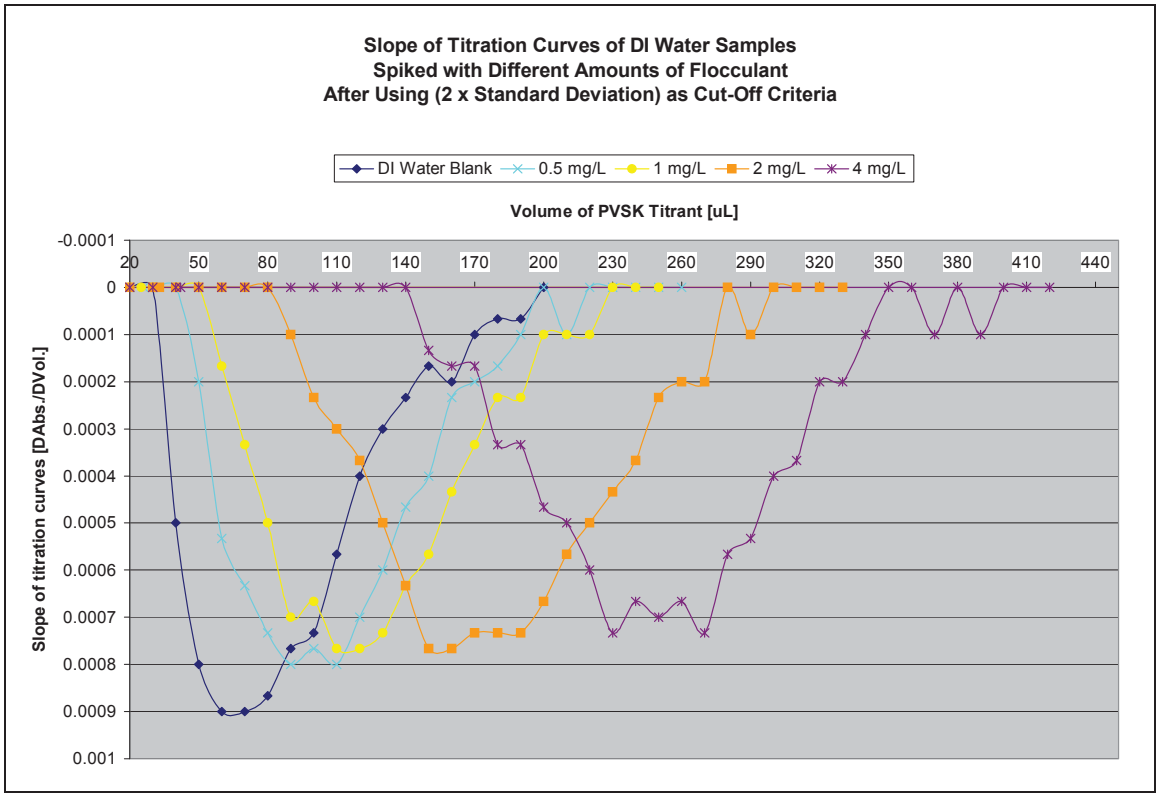

Figure 4.14 Slope of titration curves of DI water samples spiked with different amounts of flocculant after using (2 x standard deviation) as cut-off criteria.

Based on these results, we determined the amount of PVSK that had to be added to reach the endpoint, defined as the first point of zero slope, for each sample type. Then we used these volumes of PVSK to show a linear concentration-response relationship for this method, and to compare it with that of the inflection point interpretation (Figure 4.15).

According to our results, both data interpretation methods lead to a linear concentration-response relationship between the volume of titrant required to reach the endpoint of the titration and the polymer concentration in the sample. There is a significant shift along the Y-axis for the two relationships, because they use two different criteria to determine the endpoints from the titration curves. The amount of PVSK required to reach the endpoint will always be higher when the point of zero slope as opposed to the inflection point, is used as a determinant for the endpoint. However, the slopes of the linear concentration-response relationships are quite similar (51.5 for the standard deviation method and 44.5 for the inflection point method).

The standard deviation method is thus validated for the titration data of the DI water samples, and can therefore be used for the interpretation of titration data from wastewater samples as well.

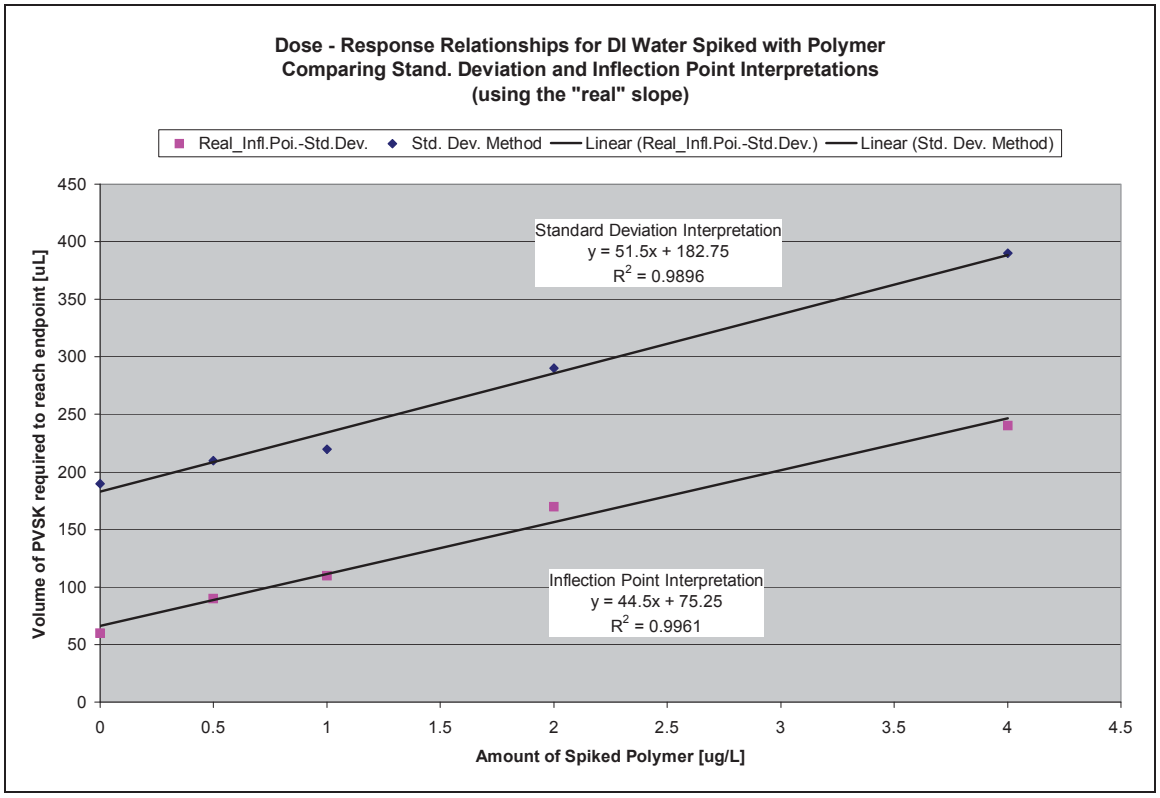

Figure 4.15: Dose-response relationships for DI water spiked with polymer comparing standard deviation and inflection point interpretations.

4.0.2.3 Colloid Titration of Wastewater Samples

As described earlier in this chapter (4.5.1 Materials and Methods for Colloid Titration) the quantitative analysis of the flocculant in secondary clarifier effluent (SCE) samples by colloid titration is based on the standard addition method. Therefore parts of the same sample of SCE were spiked with different, known amounts of polymer and titrated with PVSK. In addition, a time-sequenced mixed liquor (ML) sample was analyzed as a blank following the same procedure.

For the use of the ML sample as a blank we had to ensure that it represented the same composition as the SCE prior to flocculant addition. In the chosen type of pretreatment, we allowed the ML to settle in a bucket and used the supernatant for the titration procedure. Prior to that, we analyzed the supernatant of the ML and the untreated correlated SCE sample in a

particle counter to ensure that the size range of suspended solids was comparable in both samples. The similarity of the results of the particle counter analysis, which are shown in the following figures (Figure 4.16 and Figure 4.17), encouraged us to apply this simpler pretreatment method.

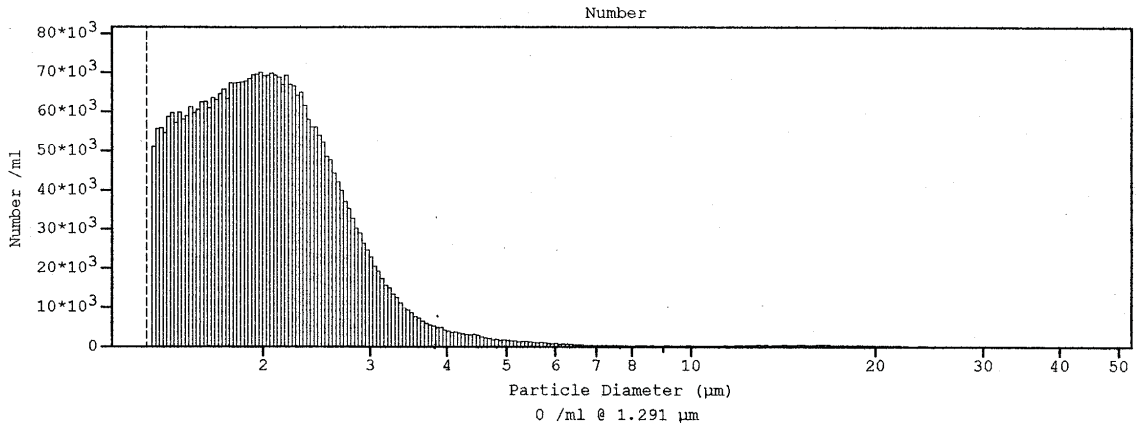

Figure 4.16: Results of the particle counter analysis for the supernatant of the mixed liquor sample.

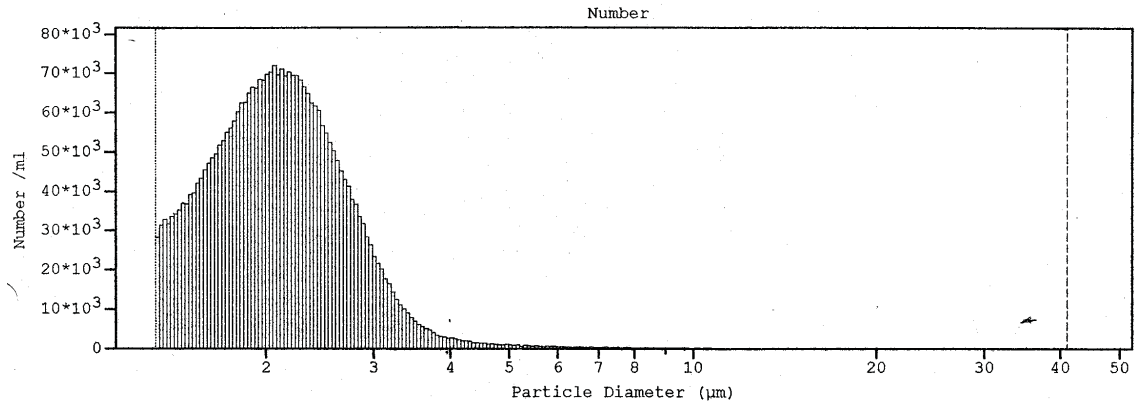

Figure 4.17: Results of the particle counter analysis for the secondary clarifier effluent sample.

Then, all the samples were analyzed using the colloid titration method described earlier. However, the initial turbidity of the wastewater samples was too high to initialize the absorbance in the colorimeter to zero. Therefore, we first prepared SCE samples of different polymer concentrations, and diluted it afterwards by 50 % using DI water. The sample concentrations reported are therefore the concentrations after the dilutions with DI water.

All titrations were performed at least in triplicates after the addition of 60 µL of TB-o. Whenever problems occurred during the titrations, for example the formation of bubbles on the mirror of the sensor of the colorimeter during the titration, more replicates were analyzed. The average titration curves of replicates for all SCE samples and the ML sample are shown in Figure 4.18. The curves in this figure indicate that the titration curve of SCE with 4 mg/L polymer added is anomalous, which was also manifested in later data interpretations. As this sample did not follow the concentration-response pattern observed in the titrations of all other samples, it was discarded as an anomaly. We attributed this discrepancy to human error.

During some titrations of the ML sample, bubbles formed and attached to the mirror of the colorimeter sensor, which resulted in an increased measured absorbance number. As soon as this interference was detected, the bubbles were removed and the absorbance continued to follow the usual pattern for this sample. However, this problem occurred for two out of four replicates (Rep. 2 and 4) in approximately the same region. Therefore the average curve based on the titrations of only two replicates (Rep. 1 and 3) for the ML sample, where no such problems were observed, was plotted in the same figure (Figure 4.18).

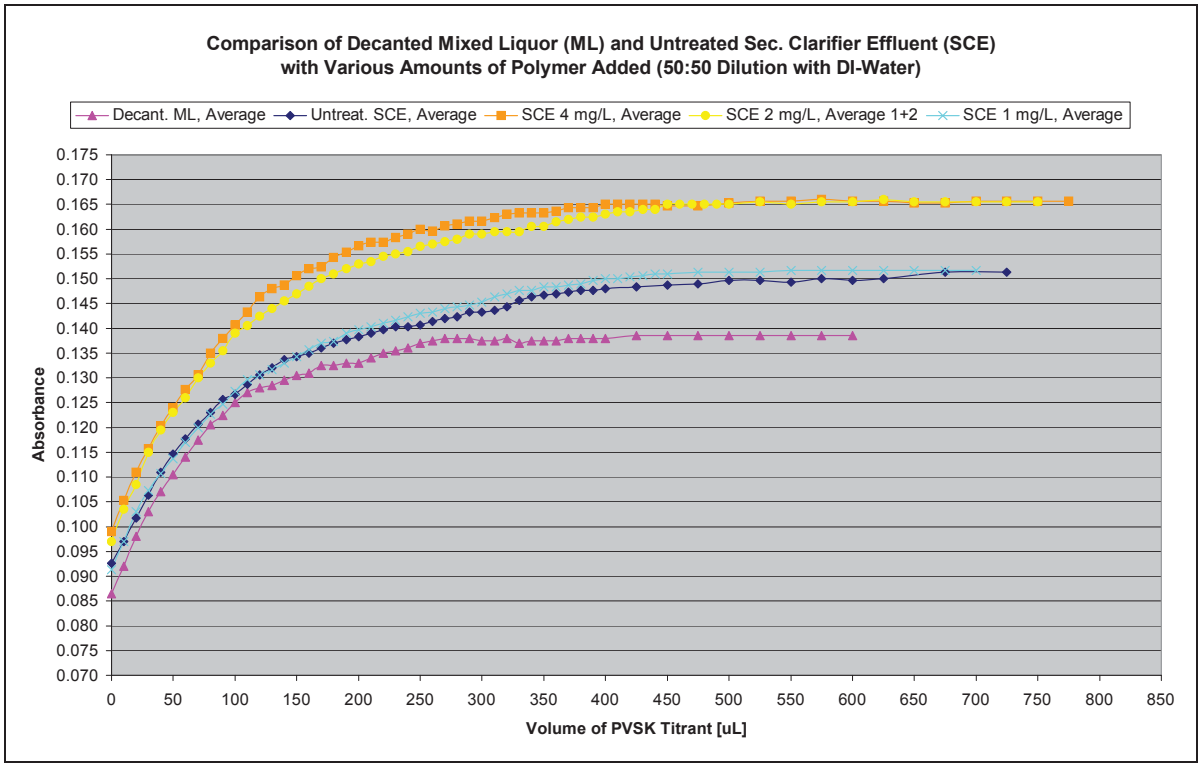

Figure 4.18: Comparison of the colloid titration of decanted mixed liquor and untreated secondary clarifier samples with various amounts of polymer added.

The average titration curves for the SCE samples spiked with 2 mg/l flocculant is based on the replicate numbers 1 and 2 (out of three). This selection has been made to exclude an outlier for the following reason. For this sample, the determined absorbance curve for replicate number 3 showed significantly higher absorbance values than for the other two replicates. We observed a very fine precipitate attached to the mirror of the sensor cell of the colorimeter. Thus, assuming that this precipitate led to the shift in absorbance, we neglected this replicate.

As mentioned earlier, there are some differences between the titration curves of DI water and wastewater samples. First, for wastewater samples, there is no range of constant absorbance at the beginning of the titration indicating that the differences in affinity for the PVSK titrant between TB-o and flocculant are not as great as in DI water. Furthermore, the shapes of the curves are different. Most of them show no inflection point and all of them reach a much lower final value in absorbance compared with DI water samples. We assume that all these differences are due to interfering reactions with other wastewater compounds. These reactions have already been described earlier in this chapter (4.5.1 Materials and Methods for Colloid Titration) and will not be repeated here.

Because of the lack of inflection points for titration curves of wastewater samples, we applied the previously tested interpretation method based on the standard deviation of slopes. The titration of wastewater samples was usually continued long enough to reach the “real” slope of zero. Therefore, only the mean standard deviation (MSD) of the slopes for each sample type (and not two times the MSD) could be applied as a cut-off criterion to neglect slopes that were not significantly different from zero. The following MSD’s were determined for the titrated wastewater samples (Table 4.16).

Table 4.16: Data for Standard Addition Curve

Sample type	Polymer additon [mg/L]	Standard deviation (cut-off criteria)	Replicates used	Vol. PVSK to reach endpoint [uL]	Vol. PVSK to reach endpoint minus blank [uL]
ML Blank	0	5.7858E-05	1,2,3,4	260	0
SCE	0	5.1761E-05	1,2,3	350	90
	1	4.3660E-05	1,2,3	400	140
	2	4.3570E-05	1,2	460	200
	4	4.7519E-05	1,2,3	Not Used	Not Used

The calculation for the SCE sample spiked with 2 mg/l flocculant is again based on replicates number 1 and 2 (out of three). For the interpretation of the ML sample the MSD of the slopes was computed based on all four replicates. However, the part of the titration where the bubbles occurred, was neglected in this calculation. Then the determined cut-off criterion was applied to the entire average titration curve of only the replicates 1 and 3. The use of the average of slopes in all replicates after neglecting the region of interference was not possible, as the first point of zero slope was to be found in this region.

Based on the cut-off criteria using the MSD method, we determined the volume of PVSK that had to be added to the sample to reach the endpoint (Table 4.16). The volume of PVSK required for the titration of the ML sample was subtracted from the other wastewater samples as a blank. The standard addition curve is obtained by plotting the volumes of PVSK after blank subtraction, for each polymer concentration in SCE samples (Figure 4.19).

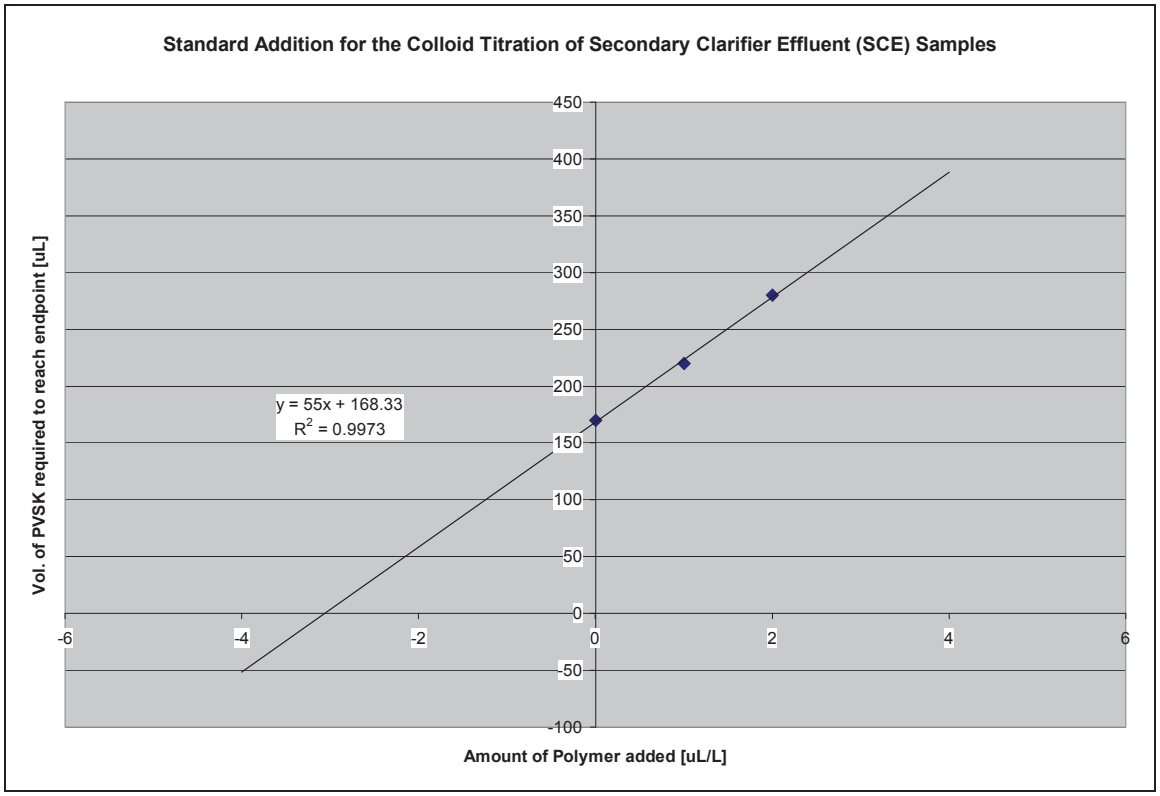

Figure 4.19: Standard addition for the colloid titration of secondary clarifier (SCE) samples.

After extrapolating the curve, we can determine the initial polymer concentration in the SCE sample.

Table 4.17: Calculation of Initial Polymer Concentration		
Standard additon curve y = 55x + 168.33		**Initial polymer conc.** [mg/L]
y	x	
0	-3.06	**3.1**

According to our results, the initial concentration of polymeric flocculant in our SCE samples was 3.1 mg/L. This concentration was in the concentration range we had expected it to be. It also falls in the concentration range of the polymer spiked to the sample. Therefore, we meet all the requirements for the application of the standard addition method as described earlier in this chapter (4.5.1 Materials and Methods for Colloid Titration).

To conclude, the applied colloid titration method proved to be useful for the quantitative analysis of polymeric flocculant concentrations in the SCE. The titration of the ML sample, as a blank, was necessary to account for numerous interfering reactions caused by wastewater compounds other than the flocculant. The endpoints of the titrations, defined as the points where the slope of the curve first reached zero, was determined using cut-off criteria based on the standard deviations of slopes. A standard addition curve was established, showing the relationship between increasing amounts of polymer added to the samples and the volume of titrant (PVSK) required to reach the endpoint. Based on this standard addition curve, the initial polymer concentration of the secondary clarifier sample under consideration was calculated to be 3.1 mg/L.

This method can thus be used for the offline measurement of residual flocculant concentrations in the SCE.

CHAPTER 5
LIMITS FOR RESIDUAL FLOCCULANT CONCENTRATION – TOXICITY TESTS

The purpose of the toxicity tests in this study was to investigate possible toxic effects of the polymeric flocculant used at the Coors Process Wastewater Treatment Plant (PWTP). This was necessary to provide a basis for future concentration limits for the residual flocculant in the secondary clarifier effluent (SCE). The Residual Flocculant Parameter (RFP) of the Polymer Dosage Control System will be defined as "passed" for flocculant concentrations below the established limit and ass "failed" for concentrations above.

First, the singular effects of several wastewater samples were tested by the dilution with natural synthetic water (NSW) (United States Environmental Protection Agency, 1993). We wanted to know if any wastewater types were toxic themselves, and if toxic effects changed during the treatment of the wastewater. Further, these experiments were expected to help us determine the response of aquatic organisms to the exposure of wastewater samples taken before and after flocculant dosage.

For these reasons four wastewater samples were taken during regular treatment conditions on two days, and analyzed in toxicity tests. The sampling is further described in Table 5.1 and Table 5.2, and subsequently.

Table 5.1: Sampling for Microtox Tests (regular treatment conditions)

Sample ID	Date	Time	Point	Chlorine Analysis
ML	03/03/2000	22:15	Splitter Box	No
SCE	03/04/2000	8:49	Clarifier # 3	No
FCE	03/04/2000	9:14	Discharge Point 001	Yes
GWTP-E	03/04/2000	8:29	GWTP	No

List of abbreviations:
ML: Mixed liquor
SCE: Secondary clarifier effluent
FCE: Final commingled effluent
GWTP: General Wastewater Treatment Plant
GWTP-E: Effluent of GWTP prior to chlorination

Table 5.2: Sampling for WET Tests (regular treatment conditions)				
Sample ID	**Date**	**Time**	**Point**	**Chlorine Analysis**
ML	03/30/2000	20:20	Splitter Box	No
SCE	03/31/2000	6:49	Clarifier # 3	No
FCE	03/31/2000	7:14	Discharge Point 001	Yes
GWTP-Cl	03/31/2000	6:29	GWTP	Yes

List of abbreviations:
WET: Whole Effluent Toxicity
ML: Mixed liquor
SCE: Secondary clarifier effluent
FCE: Final commingled effluent
GWTP: General Wastewater Treatment Plant
GWTP-Cl: Effluent of GWTP after chlorination

All samples were placed in new containers to prevent any kind of sample contamination. The mixed liquor (ML) sample was always allowed to settle first in a bucket or container to remove high amounts of suspended solids. Then the supernatant was decanted and used for the toxicity tests. The sample of the final commingled effluent (FCE) was analyzed for chlorine using a Wallace & Tierman Amperometric Titrator right after the sampling to ensure that we collected only those samples where the chlorine concentration was not too high. Very high chlorine concentrations caused by overdosing during the chlorination of the effluent of the General Wastewater Treatment Plant (GWTP-E) would result in a high toxicity of the sample (Szal et al., 1991). However, this toxicity would primarily be due to the chlorine, and the sample would not be representative for standard conditions.

As all samples should represent the same wastewater, but at different treatment steps of the plant, time-sequenced samples were taken based on retention times for each treatment step. All retention times – except for that of the secondary clarifier – had been determined in previous tracer tests during the wastewater characterization. The time required for the treatment in the secondary clarifiers was computed from the average daily flow of incoming ML and the volume of the clarifier basins.

In addition to the samples representing regular treatment conditions, sequenced wastewater samples were taken during the breakdown of one of the three secondary clarifiers. This breakdown led to modified treatment conditions, for example to a decrease in retention time for the secondary clarification step, assuming a constant flow. In a situation like this, it is much more difficult, even for an experienced plant operator, to choose the appropriate amount

of flocculant dosage to be added. Therefore there is a higher probability for mistakes in the polymer addition and for increased concentrations of residual flocculant in the SCE. Thus, a situation like this may lead to the enhancement of toxic effects of the discharged wastewater. We investigated these possible effects by testing dilutions of three sequenced wastewater samples in toxicity tests (Table 5.3).

Table 5.3: Sampling for Microtox and WET Tests (irregular treatment cond.)

Sample ID	Date	Time	Point	Chlorine Analysis
ML	02/26/2000	5:50	Splitter Box	No
SCE	02/26/2000	13:08	Clarifier # 3	No
FCE	02/26/2000	13:29	Discharge Point 001	Yes

List of abbreviations:
WET: Whole Effluent Toxicity
ML: Mixed liquor
SCE: Secondary clarifier effluent
FCE: Final commingled effluent

The FCE was analyzed for chlorine right after the sampling, this time using a free and total chlorine test kit (Hach, model CN 66, Cat. No. 2231).

The wastewater at all treatment steps of the PWTP is of a complex but varying composition. Therefore higher residual concentrations of polymeric flocculant can lead to synergistic, antagonistic or additive toxicity in combination with other compounds in the wastewater. To show that higher flocculant concentrations in the samples lead to increased toxic effects, we tested those of different amounts of flocculant spiked in various water types. In Whole Effluent Toxicity (WET) tests, which will be described in detail later, fresh NSW, SCE, and FCE were used. The Natural Synthetic Water (NSW) (United States Environmental Protection Agency, 1993) was prepared by the addition of the following salts to approximately 5 gallons of DI water on 03/22/2000.

Table 5.4: Composition of Natural Synthetic Water (NSW)

Salt	Added to Five Gallons of DI Water [g]
$NaHCO_3$	1.824
$CaSO_4$	1.140
$MgSO_4$	1.140
KCl	0.076

In addition to the NSW, two wastewater samples were tested in WET tests for flocculant addition. Sequenced samples of SCE and FCE were taken in the same way as described earlier on 04/03/2000, where the chlorine content of FCE was checked using the Hach test kit. The flocculant emulsion used for spiking the samples was taken directly from the mixing tank at the PWTP on 02/26/2000. Therefore the tested flocculant had the same concentration (0.5 % v/v) as that dosed in the ML at the treatment plant.

Toxicity tests where the flocculant had been added to the samples, were performed not only by following the WET procedures, but also by carrying out Microtox® tests, which will be described in detail later. For the latter, the same sample of polymer emulsion was tested with certain flocculant concentrations in DI water. Then these samples were further diluted with Microtox Diluent following the recommended test protocol.

In all, two types of samples were analyzed in toxicity tests: wastewater samples of different levels of dilutions with NSW, and wastewater or DI water samples spiked with varying amounts of polymeric polymer. In both cases two kinds of toxicity tests, Microtox tests and the WET tests were preformed. The Microtox test determines the response of bioluminescent bacteria (*Vibrio fischeri*) to the toxicant. It follows a relatively simple and fast procedure, though expensive in materials. The second type, the WET test is more time-consuming and labor-intensive, though inexpensive. Several kinds of test organisms can be used to perform WET tests, including *Ceriodaphnia dubia* (freshwater flea) and *Pimephales promelas* (fathead minnow). We decided on *Daphnia magna*, a water flea that is relatively easy to breed in lab cultures. The test organisms are neonates of *Daphnia magna*, not older than 24 hours. To ensure that enough neonates are "available" at the start of the test, certain precautions have to be taken prior to an experiment. Therefore experiments have to be carefully planned and are hard to start "spontaneously". For that reason we could not simultaneously test more than three types of wastewater samples during toxicity tests associated with the breakdown of a clarifier. For the same reason, we could only use three replicates instead of four for each sample concentration for several samples in WET test II and III.

The principles and experimental setup for both tests will now be described in further detail.

5.1 MICROTOX® TESTS

The Microtox® test developed by the Microbics Corporation (now AZUR Environmental) has been applied to determine wastewater toxicity in various situations (Chen, 1999, Ince and Erdogdu, 1998, Aruldoss and Viraraghavan, 1998, Hao et al., 1996).

The test is based on the following principles of operation (Microtox Manual, 1992). During the test, organisms are exposed to samples, and toxic effects on the organisms are measured. The Microtox Reagent contains the test organims, living bioluminescent bacteria that have been grown under optimal conditions, harvested, and then lyophilized (freeze-dried). The lyophilized bacteria are rehydrated with Microtox Reconstitution Solution prior to starting the test to provide a ready-to-use cell suspension.

The Microtox Test System measures the light output of the luminescent bacteria after they have been challenged by a sample of unknown toxicity, and compares it to the light output of a control group (reagent blank). The difference in light output is related to the toxic effect of the sample on the organisms. The degree of light loss is an indication of metabolic inhibition in the test organisms, and indicates the degree of toxicity of the sample. Various toxic materials require different time periods to complete their effect on the test organisms. Therefore the percentage of light loss is usually measured after 5 and 15 minutes of exposure.

The Microtox system also includes software to determine the concentration-response curve for the toxic effect of the sample. Based on this curve, the effective concentration (EC_{XX}, e. g. EC_{50}) that causes a particular percentage of light loss is calculated.

A big advantage of this system is its high precision. Unlike most bioassays, the Microtox test uses standardized test organisms, in statistically significant numbers. Each test cuvette contains roughly a million individual test organisms that are challenged by the same test sample. The toxic effect of the sample is measured by a single parameter, the simultaneous light output of all organisms in one test cuvette. Therefore variations among individual organisms become statistically insignificant.

5.1.1 Materials and Methods for Microtox® Tests

To perform Microtox tests, we used the Microtox Analyzer M 500 connected with a PC to interpret the data electronically by Microtox software (Microtox Data Collection and

Reduction Software, Version 7.9). During the analysis the cuvettes were placed in a matrix-like arrangement of wells. Rows are indicated by letters like A, B etc., and particular positions in a row by numbers like A1, B2 etc.

Besides the analyzer connected to the PC, Microtox testing requires several special items in addition to those commonly found in testing laboratories (Microtox Manual, 1992).

- **Microtox Reagent**

  Microtox Reagent is a freeze-dried culture of a specially developed strain of the marine bacterium, *Vibrio fischeri*. The sensitivity of the reagent is essentially unchanged for 1 – 2 hours after reconstitution, but may significantly decrease after that time.

- **Microtox Reconstitution Solution**

  This solution is distilled water, specially prepared to ensure that it is free of toxic material.

- **Microtox Diluent**

  The diluent is a specially prepared 2 % sodium chloride solution, free of toxicity.

- **Microtox Osmotic Adjustment Solution (MOAS)**

  The marine bacterium in the reagent requires osmotic protection. Therefore the Microtox test is usually run in 2 % NaCl. MOAS is a specially prepared 22 % sodium chloride solution (toxicity free) that is used to adjust the osmotic pressure of the samples to approximately 2 %.

As mentioned above, two different kinds of toxicity tests had to be performed. At first, the testing of dilutions of wastewater samples during regular treatment conditions and during the breakdown of one clarifier was performed. Then DI water samples spiked with different amounts of flocculant were tested. Therefore two different Microtox test protocols were followed during the toxicity tests of our samples. The 100 % Test was applied for the analysis of diluted wastewater samples, and the Basic Test for the testing of samples spiked with flocculant. The condensed protocols can be found in the appendix. All tests were performed in triplicate.

After the performance of each test series had been completed the data interpretation followed for both 5 and 15-minute testing periods using the Microtox software. Based on the light intensities emitted by the bacteria during the experiment in the control group, and in samples of various concentrations, the dose-response relations and EC_{50} values were computed by the program. For that purpose the I_0, the I_5 and the I_{15} were measured.

5.1.2 Results and Discussion for Microtox® Tests

The first samples to be tested using Microtox® tests were those from mixed liquor (ML), secondary clarifier effluent (SCE), and final commingled effluent (FCE), all three of which were taken during the breakdown of one of the clarifiers. The toxic effects of these solutions on *Vibrio fischeri* were determined following the Microtox 100 % test protocol as described previously. According to the procedure, wastewater samples of concentrations 90, 45, 22.5, and 11.25 percent were tested for their toxic effects after 5 and 15 minutes of exposure.

According to the computed results none of the samples caused any metabolic inhibition in the test organisms resulting in light loss. In addition, the Microtox data interpretation reported, that for all replicates, the EC_{50} was greater than the highest sample concentration (90 %). Therefore, we conclude that none of these wastewater samples, taken during the breakdown of one clarifier, had toxic effects on the tested bacteria.

Despite these results, we also tested the toxicities of the same wastewater samples taken under regular treatment conditions. In addition, a sample of the effluent of the GWTP prior to chlorination (GWTP-E) was also tested.

Again, the data interpretation programs reported that for all samples, except one, the EC_{50} was greater than the highest concentration. For the second replicate of the ML sample during the 5-minute exposure, a negative slope was observed. This was probably due to an error connected with the transfer of small volumes of the sample during the preparation of the dilution series. However, none of the tested wastewater samples had significant toxic effects on the tested bacteria.

We then tried to determine if the flocculant was toxic to the bacteria *Vibrio fischeri*. For this purpose, we first performed a range finding test, starting with relatively high polymer concentrations in DI water. Based on the results of this test, we investigated the toxic effects of lower flocculant concentrations in DI water on bacteria. Both tests followed the Basic Test protocol described previously, and determined the possible toxic effects after 5 and 15 minutes. Each sample was prepared using a flocculant sample (0.5 % v/v) taken directly from the mixing tank, and tested in triplicate. The results of these two tests are presented in the following tables (Table 5.5 and Table 5.6). These tests also determine the difference in light emitted at the beginning of the test (I_0) and after the testing period (I_t) for the control group. Based on these changes in light intensities of the control group, a correction factor (CR) is

calculated by dividing I_t by I_0. This correction factor ensures that the determined effects of the samples are significantly different from the control group. Therefore it is used to calculate the toxic effect in percent based on the following equations. With the determined effects for known concentrations, a dose-response curve is established and the EC_{50} calculated.

$$\text{Effect [\%]} = \frac{CR \times I_0 - I_t}{CR \times I_0} = \frac{\Gamma}{CR} \times \frac{I_t}{I_0}$$

where, (Equation 5.1)

$$\Gamma = \frac{CR \times I_0 - I_t}{I_t}$$

(Equation 5.2)

Based on the calculations described above, the Microtox software determined the EC_{50} concentrations for all replicates, except the first one, where the 95 % confidence range exceeded the limits.

Table 5.5: Flocculant Range Finding Test: 5 – Minute Exposure

Replicate No.	EC_{50} [µL/L]
1	NA
2	348.0
3	260.8
Average	304.4

Table 5.6: Flocculant Range Finding Test: 15 – Minute Exposure

Replicate No.	EC_{50} [µL/L]
1	NA
2	219.7
3	245.3
Average	232.5

The determined average EC_{50} after an exposure of 15 minutes is a little less than after an exposure of 5 minutes. This indicates that stronger metabolic inhibition of the test organisms occurs after a longer time of exposure. Variances in the observed inhibition effects may be due to technical problems during the tests. As the flocculant is a highly viscous emulsion, the transfer of sample containing high concentrations of polymer is relatively difficult. However,

this first range finding test gave us a good idea about the concentration range of the EC_{50} values for the tested bacteria to be expected. Based on these results, we performed another Microtox Basic Test (flocculant toxicity test) starting with lower concentrations in the flocculant sample. This time the dilution factor was chosen to be 2, instead of 10, as in the previous experiment (Table 5.7 and Table 5.8).

Table 5.7: Flocculant Toxicity Test: 5 – Minute Exposure

Replicate No.	EC_{50} [µL/L]
1	NA
2	317.5
3	136.6
Average	227.05

For replicate number 1 the 95 % confidence range exceeded the limits again. Therefore no EC_{50} value was reported for this replicate.

Table 5.8: Flocculant Toxicity Test: 15 – Minute Exposure

Replicate No.	EC_{50} [µL/L]
1	NA
2	NA
3	191.5
Average	191.5

For replicate number 1 for the 15 – minute exposures, the confidence limits were exceeded again. For replicate number 2, a negative slope was reported. In both cases, no EC_{50} could be calculated. We assume that most of the problems during the data interpretation were due to difficulties concerning the handling of the flocculant samples. As mentioned above, the sample has a high viscosity and is very sticky.

However, both tests indicated that the EC_{50} values range from 190 to 305 µL/L (ppm) depending on the exposure time. As this concentration range is much higher than any flocculant concentration added to the mixed liquor during the treatment at the Process Wastewater Treatment Plant, we conclude that most residual flocculant concentrations in the secondary clarifier effluent do not have *acute* toxic effects on bacteria. This statement is based on the assumption that other bacteria species react similar to the tested *Vibrio fischeri* on the exposure to the flocculant.

5.2 WHOLE EFFLUENT TOXICITY (WET) TESTS

In the past, the programs of the U. S. Environmental Protection Agency (EPA) for the control of toxic discharges were based largely on the effluent limitations for individual chemicals. This required that the water quality criteria for many pollutants be established based on comprehensive testing and evaluation. Then this information could be used to limit the discharge of evaluated toxicants. However, data on the toxicity of substances to aquatic organisms were and are available only for a limited number of elements and compounds. Therefore it is hard to predict possible toxic effects of effluents, if the compounds in them have not been comprehensively tested, or their chemical composition is not known or is a matter of variations in effluent quality. In addition, possible additive, synergistic, or antagonistic effects cannot be described.

For these reasons, Whole Effluent Toxicity (WET) has been introduced as a pollutant parameter itself, which can be used to limit the effluent's toxicity without exact information about the toxicants creating that toxicity. WET is a term used to describe the aggregate toxic effect of an aqueous sample, e.g. whole effluent wastewater discharge, as measured according to an organism's response upon the exposure to the sample (http://www.epa.gov/owm/wettest.htm). The response of the test organisms may be defined differently depending on the demands of their application. Possible endpoints are lethality, impaired growth or reproduction. WET tests try to replicate to the greatest extent possible, the total effect and actual environmental exposure of aquatic life to effluent toxicants. At the same time, there is no specific information required about the identification of specific toxicants.

The organisms tested in WET tests are indicators or surrogates for the aquatic community to be protected. For the freshwater toxicity tests, *Ceriodaphnia dubia* or similar daphnid cladocerans, and the test organism *Pimephales promelas* (fathead minnow) are used. They are exposed to toxicants in two different basic types of WET tests: an acute test or a chronic test. The acute test lasts 96 hours or less and uses mortality as an endpoint. The chronic test is a 7-day life cycle test choosing one of various possible endpoints like growth, reproduction, and mortality. Several examples for applications of chronic WET tests are given in the literature (Versteeg and Woltering, 1990, Kosmala et al., 1999). All the WET tests we performed were acute tests.

As WET tests are designed to predict the impact and toxicity of effluents discharged from point sources into waters of the U.S., WET limits are included in NPDES (National Pollutant Discharge Elimination System) permits. Alternatively, WET monitoring requirements are often included in permits. According to the recent CDPS (Colorado Discharge Permit System) Permit No. CO-0001163 for Coors Brewing Company, chronic WET tests are required for the final discharge point 001 (Part I, A, p. ii, amendment No. 2 – Rationale). The monitoring requirements and the consequences connected with a test failure are described in the same permit in detail (Part I, B, p. xix) and will be summarized briefly below.

Chronic WET testing with *Ceriodaphnia dubia* and fathead minnow (*Pimephales promelas*) has to be done in accordance with EPA guidelines on a quarterly frequency. The results are reported to the Division (Colorado Department of Health) and the U.S. EPA. A test is defined as "failed" when a statistically significant difference in lethality between the control and any effluent concentration less than or equal to the instream concentration, has been observed. A test failure has the following consequences. First, a written notification of the failure of a WET test has to be sent to the Division of Water Quality, which is part of the Colorado Department of Health and Environment (CDPHE), within 21 days of the demonstration. Second, "Accelerated Testing" and/or a "Preliminary Toxicity Investigation" (PTI) in combination with a "Toxicity Identification Evaluation" (TIE) are performed (EPA/600/6-91/005F, May 1992; EPA/600/6-91/003, Feb. 1991; EPA/600/3-88/035, Feb. 1989; EPA/600/3-88/035, Feb. 1989). In Accelerated Testing, the single test organism that was found to be more sensitive is tested to deduce a "Pattern of Toxicity" or "No Pattern of Toxicity". The testing is done at least once a week every two weeks for up to five tests until either:

- Two consecutive tests fail or three of five tests fail indicating a "Pattern of Toxicity".
- Two consecutive tests pass or three of five tests pass, which implies, that there is "No Pattern of Toxicity".

If no Pattern of Toxicity is found, but a significant level of erratic toxicity remains, the Division may require an increased frequency of routine monitoring or some other modified approach. But if a pattern was derived, then the PTI and TIE follow. The PTI is an optional brief search for possible sources of the WET. This approach is advocated if a certain incident may be responsible for the toxicity problems. With appropriate corrective actions, this method is more cost effective than a formal TIE. However, if the PTI allows no identification of the toxicity source, the TIE has to be conducted within 120 days following the EPA guidelines

(EPA/600/6-91/005F, May 1992; EPA/600/6-91/003, Feb. 1991; EPA/600/3-88/035, Feb. 1989; EPA/600/3-88/035, Feb. 1989). The procedure of the TIE is described in detail in the EPA guidelines we referred to earlier. As indicated by the title, this procedure is intended to investigate which compound in the wastewater is the toxicant. It is usually conducted by private laboratories.

5.2.1 Materials and Methods for WET Tests

One of the targets of our control system for flocculant dosage was to prevent toxicity of the final commingled effluent (FCE) caused by high residual flocculant concentrations. For this purpose, we performed toxicity tests to determine effective concentrations of the flocculant in various wastewater samples, which can be used as control limits for the Residual Flocculant Parameter (RFP) (2.3 Suggested Process Control).

The WET tests to investigate possible toxic effects of the flocculant on aquatic organisms were performed following published EPA guidelines (EPA-600/4-90-027F, Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 1993). We decided to use a static acute test for our purposes. This test type lasts for 96 hours or less, where the test organisms are exposed to a static environment and the sample solution is changed every 48 hours during the test. We shall now describe the testing procedure and the applied materials and instruments in a step-wise manner.

- **Sampling**

  Detailed information about the sampling, including sampling dates, times, and points, has been provided earlier. According to the EPA guidelines mentioned above, samples have to be used in a WET test within 36 hours after sampling, and to be refrigerated prior to the test.

- **Sample preparation**

  If the sample has been cooled during the storage period, it has to be ensured that it reaches room temperature prior to any other treatment. Then several water quality parameters of the sample are checked. According to EPA requirements several water quality variables have to be monitored during the WET test to ensure that the observed toxic effects are truly due to the chemical composition of the sample solution. For example, if the pH is not kept within an appropriate range for the test organisms until the test is completed, we will not know if the lethality was caused by the toxicity of the

sample or by pH conditions inappropriate for the organisms. Using *Daphnia magna* (freshwater flea) as the test organism the following water parameters had to be kept within the required ranges:

- pH: 6.0 – 9.0
- Dissolved Oxygen: > 4 mg/L for warm water species
  > 6 mg/L for cold water species
- Temperature: 20 °C ± 1 °C or 25 °C ± 10 °C
- Conductivity / salinity: To be monitored

Daphnia are reported to be "eurythermal", able to survive in broad temperature ranges (Peters and de Bernardi, 1987). They will do best in the range between 15 and 25 °C, but they can also be maintained at constant temperatures from 2 to about 30 °C. However, either of the limits for dissolved oxygen (DO) contents should be applicable.

We checked several water parameters including the total residual chlorine and the DO content of the original samples prior to the preparation of various sample dilutions or flocculant concentrations. For these and later measurements, we used an analog pH meter (Orion Research, model 301), a dissolved oxygen meter (VWR Scientific, model 4000, Cat. No. 34105-052) to determine dissolved oxygen and temperature, and a salinity-conductivity-temperature meter (YSI, model 30/10FT, SN: 96E50244) or conductance meter (YSI, model 35) to analyze the sample for conductivity. The latter instrument used for the conductivity measurement was connected to a smaller electrode, and thus it was easier to handle without affecting the test organisms in solution.

In our samples, the total residual chlorine concentration was never higher than the detection limits of the applied methods. However, the dissolved oxygen level of the original samples was sometimes below the requirements. In these cases, the samples were aerated with an air pump (Cole-Parmer Masterflex, Model No. 7553-71) prior to any other preparations.

Then the required sample dilutions or flocculant concentrations in wastewater were prepared using NSW as diluent or the flocculant emulsion (0.5 % v/v) to spike the samples.

- **Preparation of the testing chambers**

  During all of our WET tests, except for the first one, where we tested samples taken during the clarifier breakdown, we used new, translucent, disposable plastic beakers (Solo, P325, 3 ¼ oz. (96.1 mL)) as testing chambers. For the first test we reused plastic beakers (4 oz. ~ 120 mL), which had been acid-washed, and rinsed with DI and NSW prior to the test. The beakers were labeled, preparing three or four replicates per sample concentration. Then a volume of 50 mL of the prepared sample solution was added to the corresponding sampling chamber. In addition, one or more control groups of four or three replicates were prepared containing 50 mL of NSW or non-spiked wastewater samples. Less than four replicates for control or test solutions were only used when we were not able to "produce" enough neonates for the testing of various samples in parallel.

  Finally, DO, pH, temperature, and conductivity were determined and noted for the starting time of the test (t = 0). The measurements were performed for one replicate of each sample dilution/concentration and for one replicate of the control group.

- **Transfer of test organisms and arrangement of test chambers**

  According to the EPA guidelines, three types of freshwater fleas may be used as test organisms for acute toxicity tests: *Ceriodaphnia dubia*, *Daphnia pulex*, and *Daphnia magna* (*D. magna*). We decided to use *D. magna* in all of our WET toxicity tests. The tested neonates were cultivated in NSW in our own lab cultures. At least two hours prior to the start of the test, the neonates were provided with *additional* food according to the EPA manual.

  The test organisms were randomly placed into the test chambers using a small pipette. Usually five *Daphnia* were added per test chamber containing the sample solution and 10 of them per replicate of the control group, except when the number of "available" neonates was limited by the "production capacity" of our lab cultures. During the pipetting of the *Daphnia*, we tried to transfer as little NSW as possible to minimize dilution effects of the samples, but enough to prevent any damage to the animals. In addition, we paid attention to the position of the pipette when the *Daphnia* were released to the sample solution. It had to be ensured that the pipette tip was placed below the water surface to avoid the introduction of air bubbles. These bubbles could affect the air bladders of the organisms (air bubbles disease), which would force them to float on the surface.

After the transfer of the test organisms had been completed, the test chambers were randomly arranged under a table lamp connected to an automatic timer. The timer was set in a way to provide cycles of 16 hours of light and 8 hours of darkness, an optimum environment for *Daphnia*. Then the test chambers were loosely covered with plastic sheets to avoid significant losses of the sample solution by evaporation.

Figure 5.1: Female *Daphnia magna*.

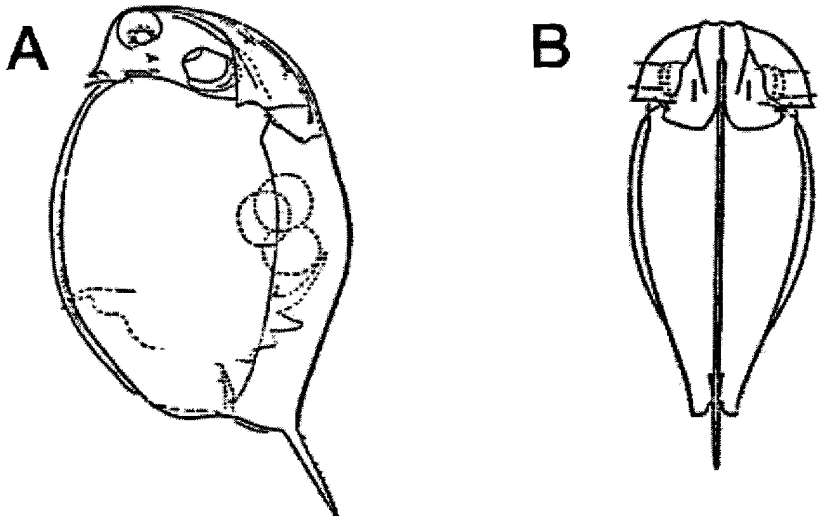

Figure 5.2: Female *D. magna*. A: Lateral view, B: Dorsal view (EPA-600/4-90-027F).

- **Ongoing testing over 48 or 96 hours**

  Every 24 hours (± 1 hour), the required water quality parameters, including dissolved oxygen, pH, temperature and conductivity, were measured and noted for one replicate of each sample concentration and the control group using the instruments described previously. In addition, we counted the number of living organisms exactly after every 24 hours in each testing chamber. This was repeated until the testing period of 48 hours was over, or until all test organisms in one testing chamber reached the endpoint (lethality).

In Table 5.9 we provide an overview of the experimental setups for all three performed WET tests. For WET test I, wastewater samples were taken during the clarifier breakdown, and for WET test II, under regular treatment conditions. In both cases, the wastewater samples were tested in dilutions. Finally, in WET test III we spiked various wastewater samples with different concentrations of flocculant emulsion.

Table 5.9: Experimental Setups for WET Tests

WET Test I: Wastewater Toxicity under Irregular Treatment Conditions	
TESTING	
Starting date and time:	02/27/00, 17:48
SAMPLE	
Sampling date(s):	02/26/00
Sample identification:	Mixed liquor (decanted) Secondary clarifier effluent Final commingled effluent
Sample dilutions / concentration:	100 %, 50 %, 25 %, 12.5 %, 6.25 %
No. of replicates per dilution / conc.:	4
No. of organisms per testing chamber:	5
CONTROL GROUP	
No. of control groups:	2
Liquid medium of control group:	Natural Synthetic Water
No. of replicates per control group:	4
No. of organisms per testing chamber:	10

Table 5.9 (continued): Experimental Setups for WET Tests	
WET Test II: Wastewater Toxicity under Regular Treatment Conditions	
TESTING	
Starting date and time:	03/31/00, 1:05
SAMPLE	
Sampling date(s):	03/30/00 and 03/31/00
Sample identification:	Mixed liquor (decanted) Secondary clarifier effluent Final commingled effluent Effluent of GWTP after chlorination
Sample dilutions / concentrations:	100 %, 50 %, 25 %, 12.5 %, 6.25 %
No. of replicates per dilution / conc.:	Mixed liquor: 4; all other samples: 3
No. of organisms per testing chamber:	5
CONTROL GROUP	
No. of control groups:	3
Liquid medium of control group:	Natural Synthetic Water
No. of replicates per control group:	Groups I and III: 4; group II: 3
No. of organisms per testing chamber:	10; except outlier in Rep. 4 of group I: 8
WET Test III: Flocculant Addition (samples taken under regular conditions)	
TESTING	
Starting date and time:	04/04/00, 18:00
SAMPLE	
Sampling date(s):	04/03/00
Sample identification:	Natural Synthetic Water Secondary clarifier effluent Final commingled effluent
Sample dilutions / concentrations:	4, 2, 1, 0.5, 0.1 μL/L of flocculant added
No. of replicates per dilution / conc.:	3
No. of organisms per testing chamber:	5
CONTROL GROUP	
No. of control groups:	3: Non-spiked samples of: Natural Synthetic Water Secondary clarifier effluent Final commingled effluent
Liquid medium of control group:	Natural Synthetic Water Secondary clarifier effluent Final commingled effluent
No. of replicates per control group:	4
No. of organisms per testing chamber:	10; except outlier in rep. 1 of Natural Synthetic Water control: 12

5.2.2 Results and Discussion for WET Tests

In this chapter, we will sum up and discuss the results obtained from the WET tests described previously (5.2.1 Materials and Methods for WET Tests). The experimental results for the three test setups can be found in the appendix (Table 5.10 to Table 5.12). For the interpretation of the observed toxicity data, we followed the guidelines of the EPA (United States Environmental Protection Agency, EPA-600/4-90-027F, Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 1993). We will provide an overview of the applied data interpretation methods in Figure 5.1 and explain which statistical models were applied to which samples. The applied statistical methods are explained in detail in the appendix.

The results of our data interpretation will be described qualitatively, and reported with computed EC_{50} values. In our conclusions, we will try to answer questions concerning the toxicity of various wastewater samples and the toxic effects of the flocculant.

The following flow chart (Figure 5.1) provides a good overview on how to decide on the statistical model for the data interpretation. The term “partial mortality” stands for a response, which is not an “all or nothing response”. In other words, partial mortalities are observed for concentrations, when they cause neither 0 nor 100 % effects.

Prior to the interpretation of the determined WET test data, we downloaded the statistical programs from the EPA homepage mentioned earlier. Depending on several parameters, different statistical models had to be applied to different sets of samples. For example, the Probit Method was not appropriate for some data where we determined more than one partial mortality, but the proportion mortalities did not bracket 0.5. The requirement, that the observed percent mortalities bracket the 50 %, must be fulfilled for all of the described statistical methods. Thus in a case like this, none of the described statistical models could be applied to compute an EC_{50}. However, despite the limited statistical information that could be derived, we were able to qualitatively interpret the results.

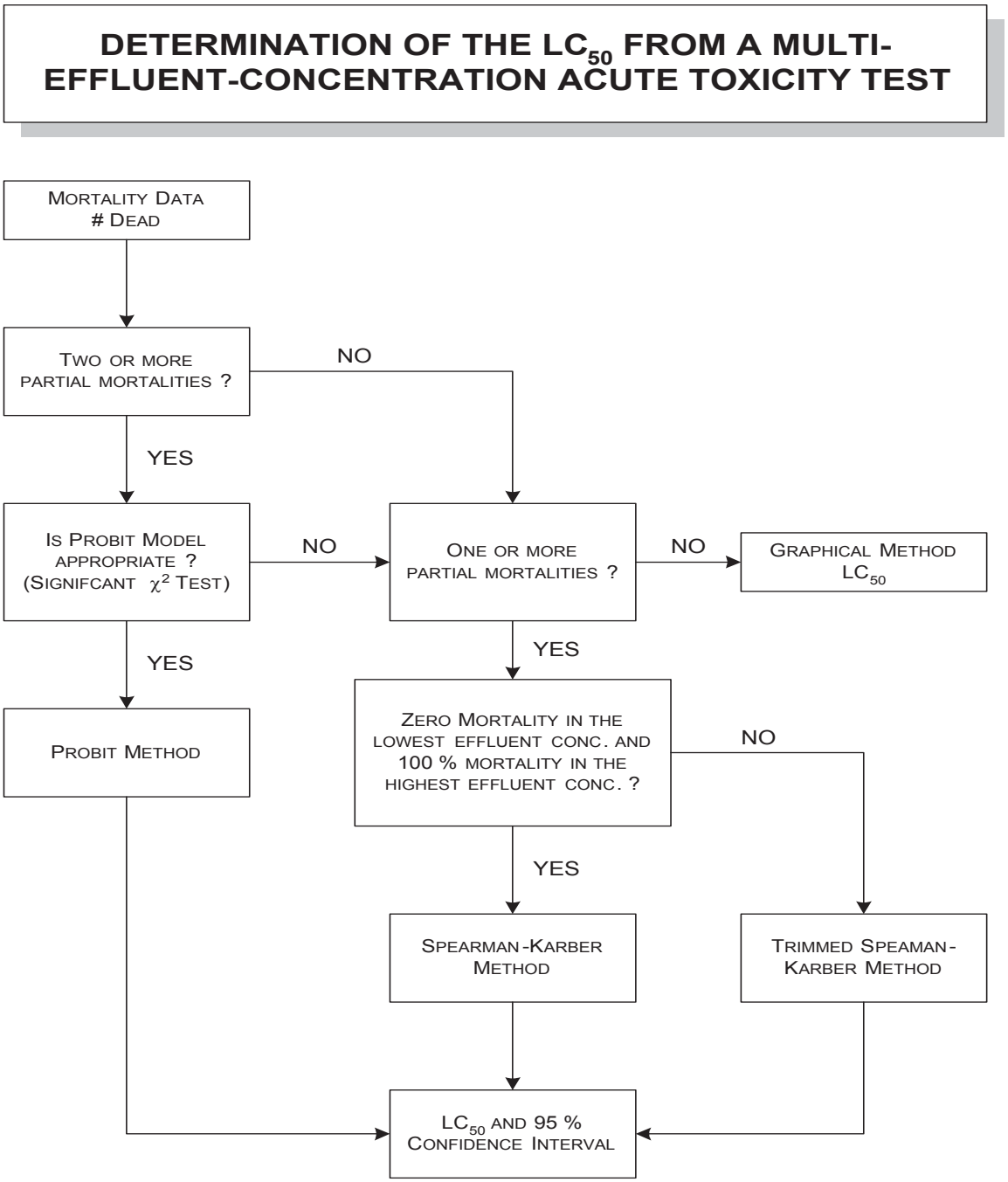

Figure 5.1: Determination of the LC50 from a multi-effluent-concentration acute toxicity test (EPA-600/4-90-027F).

For the interpretation of the data, it had to be ensured that the requirements concerning the control groups were fulfilled. Thus, if the survival of the control was less than 90 %, the WET test was not valid, and the results determined from the data interpretation were not significant.

The following table (Table 5.13) provides information about which of the statistical models was appropriate for the data interpretation of each set of mortality data after 48 hours of exposure.

Table 5.13: Overview of Statistical Models Applied for Data Interpretation

Test	Sample Identification	Appropr. Statistical Method
WET I	Mixed liquor	None
	Sec. clarifier effluent	Probit Method
	Final commin. effluent	None
WET II	Mixed liquor	None
	Sec. clarifier effluent	None
	Final commin. effluent	None
	GWTP Effl. after chlorination	Spearman-Karber
WET III	Flocculant in Natural Synthetic Water	None
	Flocculant in sec. clarifier effluent	Spearman-Karber
	Flocculant in final commin. effluent	Spearman-Karber

In WET test I, three wastewater samples were tested for their toxicity: mixed liquor (ML), secondary clarifier effluent (SCE), and final commingled effluent (FCE). The data sets determined from the ML and SCE samples could not be interpreted using any of the described statistical models, because the observed proportional mortalities did not bracket the 0.5. However, on reviewing these two data sets, it seems that neither of them shows mortalities significantly different from the control groups. Therefore the samples of ML and FCE taken during the breakdown of one of the clarifiers do not cause toxic effects on *Daphnia magna*.

In contrast, the data set for the secondary clarifier effluent sample can be interpreted based on the Probit Method, and an EC_{50} value of 66.8 % was computed (Table 5.14). As the SCE taken under regular treatment conditions did not show any toxic effects (see later data interpretation), we assumed that the toxicity of the SCE in WET test I was due to residual flocculant concentrations. This may indicate that the recent flocculant dosage control may not be able to respond effectively to modified treatment conditions, and may lead to flocculant overdosing. However, due to the dilution effects corresponding to the combination of the PWTP and GWTP effluents, possible toxic effects of the final commingled effluent can be decreased. This may explain the reason why toxicity was observed when testing the SCE, but not in case of the FCE.

For the WET test II, four wastewater samples – ML, SCE, FCE, and effluent of the General Wastewater Treatment Plant after chlorination (GWTP-Cl) – were taken under regular treatment conditions. The total residual chlorine contents in the FCE and the GWTP-Cl were determined immediately after the sampling, and were reported to be less than 0.05 mg/L and 1.05 mg/L respectively.

On reviewing the data, it becomes obvious that for all data sets except the GWTP-Cl, the observed percent mortalities do not bracket the 50 %, and therefore none of the described statistical models is applicable. However, none of these three samples showed any significant mortalities, and the ML and the FCE samples did not show any mortality at all. On the contrary, *Daphnia magna* exposed to these wastewater samples increased body weight faster and showed higher mobility during the test than in the control groups, where ML had the most positive effects. We assumed that this was due to a different amount of nutrition available for the test organisms during the testing. Especially in the ML, we may still find a high content of suspended organic material. This may increase the amount of available nutrition in comparison to the control groups where no nutrition was added. However, although this effect may be less important for the SCE and FCE samples, none of the samples except the GWTP-Cl caused toxic effects.

The data of the GWTP-Cl sample of WET test II was first interpreted using the Probit Method. As the application did not show any convergence in 25 iterations, this implied that the probit model was not appropriate to analyze this concentration-response data. Therefore the Spearman-Karber Method was used to determine an EC_{50} of 21.3 % and the 95 % confidence interval values (Table 5.14). Considering the fact that the chlorine content of this sample was rather high (1.05 mg/l), we assumed that the toxicity was mainly caused by this compound. Therefore, we computed an EC_{50} of 230 µg/L chlorine for *Daphnia magna* under the assumption that the toxicity of this sample was *only* due to the total residual chlorine. This seems to fit relatively well with the literature data, where effective concentrations in a similar range were reported for *Pimephales promelas* (fathead minnows) (Szal, G. M., 1991). The literature provides further information on how the sensitivities of these two organisms can be compared (Kaiser, 1993).

Finally, in WET test III, we tested the toxic effects of various flocculant concentrations in three water types, NSW, SCE, and FCE. For each sample type, the corresponding control group represented a sample of the same, but untreated water. As reported previously, the control group of NSW did not meet the required survival rate of 90 % for 48 hours of

exposure. Therefore, this test has to be designated as "failed" and the results should not be used in any other interpretations. However, based on this data set *Daphnia* would strongly show response to various concentrations of flocculant in NSW with an EC_{50} below 1 µL/L ass shown in the appendix (Table 5.12). The application of the statistical model of the Graphic Method did not allow us to calculate an EC_{50} value, as the smoothed proportion mortalities did not bracket 0.5. Nevertheless the results of this interpretation implied that the EC_{50} should be expected to be less than 0.65 µL/L. Although all these results confirm our interpretation of a strong toxic effect of flocculant in NSW on *Daphnia magna*, they should be used with appropriate caution, as we did not meet the requirement of 90 % survival in the control group.

For both, SCE as well as FCE samples, no problems occurred concerning the survival of *Daphnia* in the control groups. For the SCE sample we determined only one partial mortality for the data of 48 hour-exposure. Therefore the Spearman-Karber rather than the Probit Method was applied for the interpretation of these data. The computed EC_{50} for the 48 hour exposure period is 1.62 µL/L.

Table 5.14: Overview of Results of WET Test Toxicity Data Interpretation

Test	Sample Identification	Toxic Effect	95 % Confidence	
			Lower	Upper
WET I	Mixed liquor	None	--	--
	Sec. clarifier effluent	EC_{50} = 66.8 %	39.2 %	203.2 %
	Final commin. effluent	None	--	--
WET II	Mixed liquor	None	--	--
	Sec. clarifier effluent	None	--	--
	Final commin. effluent	None	--	--
	GWTP effluent after chlorination	EC_{50} = 21.3 %	18.0 %	25.2 %
WET III	Flocculant in Natural Synthetic Water	NA	--	--
	Flocculant in sec. clarifier effluent	EC_{50} = 1.62 µL/L	1.41 µL/L	1.87 µL/L
	Flocculant in final commin. effluent	EC_{50} = 1.23 µL/L	1.07 µL/L	1.42 µL/L

For the FCE sample the determined data reported no more than one partial mortality, and thus the Spearman-Karber Method was applied. The computed EC_{50} for the 48 hour exposure is 1.23 µL/L.

According to our results we can assume that high concentrations of flocculant in all water types result in toxic effects on *Daphnia magna*. For the tested wastewater samples, SCE and FCE, the EC_{50} values ranged between one and two µL/L for 48 hours of exposure. Although the effective concentrations for natural synthetic water could not be computed, the data still strongly implied that the flocculant had toxic effects on the test organisms, probably with even a lower EC_{50} value.

For all water types we computed the effective concentrations based on the flocculant concentrations which were *added* to the samples. In case of the wastewater samples unknown, residual concentrations of the polyelectrolyte may have been present in the samples prior to the spiking. Though at this point of our research we have not been able to determine small polymer concentrations in wastewater samples. Therefore we assumed that the residual concentrations were relatively low in these samples. This consideration was based on the fact that the test organisms showed a strong response even at the addition of small concentrations, while the control groups showed very little mortality.

It is essential to test the toxicity of the flocculant in wastewater samples where increased concentrations may be expected, to investigate possible synergistic, antagonistic, or additive toxic effects due to the presence of other wastewater compounds. Therefore the problem of possible unknown background concentrations of flocculant can not be avoided but we could compensate for it with the future quantitative analysis of the original samples for cationic polyelectrolyte prior to the toxicity tests.

Despite this problems the determined results correlate well with the ones from Research & Analytical Laboratories, Inc. reported in Chapter 1 (1.2.3 Specification of Flocculant). Here EC_{50} values equal to and below 0.7 ppm had been computed dependent on the type of the surfactant in the flocculant emulsion. In addition, the literature data for various cationic flocculants present very similar LC_{50} values in the same range of concentrations (reported in ppm or mg/L) as ours (in µL/L). For example the acute LC_{50} for 48 hours of exposure was reported to be < 0.78 ppm for a polyacrylamide polymer (Godwin-Saad et al. 1994).

However, the tested polymers were usually poorly characterized in literature, possibly due to the fact that a lot of the information is proprietary (Fort and Stover, 1995; Godwin-Saad et al. 1994; Biesinger and Stokes, 1986; Takigami et al., 1998; Beim and Beim, 1993; Hall and Mirenda, 1991). This leads to problems concerning the applicability of the determined data, as toxicity of flocculants strongly depends on the chemical composition of all compounds in the flocculant emulsion. For example, while numerous data reported in the literature indicate

toxic effects of cationic polyelectrolytes on invertebrates (see above), contradictory results have been published as well. For example, tests of the flocculant PERCOL 757, which - according to the Chemical Abstracts (CAS number: 35429-19-7) - contains the same cationic acrylamide copolymer as the flocculant we tested, reported it to be nontoxic (LC_{50} > 100 mg/L) or slightly toxic (LC_{50} < 100 mg/L) (Cowgill and Millazzo, 1991). Therefore we cannot predict toxic effects of polyelectrolytes based on tests of similar emulsions, lacking sufficient knowledge about the exact chemical composition of all compounds in the emulsion.

Several possible mechanisms of toxicity have been suggested in the literature. Some of them could be identified during our toxicity experiments, and will be described in further detail.

First, we observed during the WET test III, that *Daphnia magna* were obviously immobilized in solutions of high flocculant concentrations. Several test organisms happened to “stick together” and could not move apart from each other. At the end of the test, we tried to separate these daphnids using a small pipette, without succeeding. However, this physical entrapment of test organisms seemed to be a result of the strong flocculating abilities of the flocculant. Once the cationic polymeric chains connected one or more organisms, the daphnids were “flocculated” and immobilized. This explanation is supported by the fact that *Daphnia magna* consists largely of chitin, a compound that also carries negative charges.

In addition, we could see this effect occurring more strongly in samples with smaller solid concentrations. The observed physical entrapment was higher in NSW than in any wastewater type, and higher in the FCE than in the SCE sample. This also corresponds to literature reports that differences in flocculant toxicity in various wastewater samples appears to be primarily due to differences in suspended solids levels (Fort and Stover, 1995). Here higher contents of suspended solids or other negatively charged compounds in the samples will provide more available binding sites other than those on the daphnids for the present flocculant. Therefore, less flocculant will be bound to membranes of *Daphnia magna* and fewer test organisms will be physically immobilized. It could be shown that fish survived in a suspension of cationic polyelectrolyte as long as particles were present to adsorb the polymer (Biesinger and Stokes, 1986). Based on these considerations, it was suggested that clay particles be added to wastewater for detoxification prior to discharging. These particles would provide additional negative binding sites for the neutralization of the flocculant and decrease possible toxic effects.

Some literature also reports that lethal effects of the toxicant are mainly contributed by the molecular weight fraction of the flocculant emulsion greater than 100,000 (Takigami, et al., 1998). Considering that bridging effects causing flocculation are enhanced with higher chain length and a higher molecular weight of the polymers, this also seems to indicate that the proposed toxicity mechanism is reasonable. Others suggested that increasing positive charges of polymers result in stronger toxic effects (Beim and Beim, 1993). It is possibe that more positive charges on the polymer chain may lead to increased adsorption. Thus nonionic and anionic polyelectrolytes tested by Biesinger (Biesinger and Stokes, 1986) were not acutely toxic at 100 mg/L to four species of aquatic animals with the exception of one experimental anionic polymer. In contrast, of the 15 cationic polyelectrolytes tested by the same scientists, only two were not toxic at 100 mg/L.

In addition to physical entrapment, polymer chains adsorbed to the test organisms may have other negative impacts. Excess polymer molecules can adsorb to the gill surfaces leading to the formation of a film on gills of test fish (Biesinger and Stokes, 1986). This "membranotropic mode of action" (Beim and Beim, 1993) may finally lead to death from suffocation. Furthermore, the mechanical action of the cationic polyelectrolyte characterized by flocculant sorption to body surfaces may also inhibit other vital functions such as feeding, digestion and reproduction (Beim and Beim 1993). In any case, the toxic effects are increased with higher concentrations of flocculant adsorbed to body surfaces of the organisms.

Besides the toxicity mechanisms based on the adsorption of flocculant to the body surfaces of organisms, the chemical composition of the polyacrylamide copolymer may have an impact as well. To be more precise, residual acrylamide monomer concentrations in the flocculant emulsion due to the water-in-oil polymerization process may cause toxic effects, if they exceed certain levels. Acrylamide is a potent neurotoxin to man and animals and is also classified as a probable carcinogen to humans. Acrylamide may cause neurological disorders in humans and experimental animals by affecting the mitochondrial metabolism of certain neuro-filaments in distal nerve endings (Brown et al., 1980). According to the limited data provided by Stockhausen, Inc., the manufacturer of the applied flocculant, residual monomer can range from 0 to 0.686 % in the flocculant emulsion.

The LC_{50} value for lethal effects of acrylamide on daphnids was reported to be 160 mg/L after 48 hours of exposure in acute toxicity tests (Krautter, 1986). This value is much higher than the residual acrylamide concentrations we would expect in our wastewater samples due to the residual polyacrylamide copolymer. Environmental degradation of polyacrylamides

was investigated to determine if this was a possible source of higher monomer concentrations (Smith et al. 1996 and 1997). The determined data implied that, even if there was biodegradation of polyacrylamide occurring it would probably take too long to result in an increase of monomer concentrations in wastewater treated in a relatively short process. Therefore, this implies that the contribution of acrylamide to the toxicity of the flocculant emulsion is probably minor in comparison to the physical effects of adsorption discussed earlier.

It is interesting that we were able to determine toxic effects of the flocculant emulsion on the invertebrate, *Daphnia magna,* but not on the bacteria tested in the Microtox tests. Considering the two possible toxicity mechanisms, adsorption of the flocculant to the test organisms and acrylamide monomer reacting as a neurotoxin, the differences in response may be explained. First, bacteria do not have a neural system that could be inhibited by a toxic compound like acrylamide. Second, the adsorption of flocculant on bacterial surfaces may have less negative effects than in the case of invertebrates, because bacterial respiration does not depend on gills. In addition, smaller organisms like bacteria may be less affected by physical immobilization than larger ones like daphnids. However, the literature reports responses of bacteria other than death, to cationic flocculants. On testing possible toxic effects of cationic flocculants on *Bacillus subtilis*, eight out of ten flocculants caused DNA damage with LC_{50} values between 0.1 and 10 mg/L (Takigami et al., 1998). The detected genotoxicity seemed to be due to the combined effects of various compounds of the tested flocculant emulsions, such as polymers, oligomers, monomers and additives.

Our toxicity tests did not target any response other than mortality. Nevertheless it whould be of interest to determine if the applied flocculant shows genotoxicity. If this was the case it may have an impact on the bacteria in the aeration trains, which are exposed to flocculant due to the recycling of return activated sludge (RAS).

CHAPTER 6
EXAMPLE FOR OPTIMUM FLOCCULATION PARAMETER – STREAMING CURRENT DETECTOR

The process control suggested in Chapter 2 (2.3 Suggested Process Control) is based on two parameters, the Residual Flocculant Parameter (RFP) and the Optimum Flocculation Parameter (OFP). The former provides us with a measure to control possible toxic effects from the secondary clarifier effluent (SCE) and the final commingled effluent (FCE), while the latter is a measure of the removal of suspended solids from the mixed liquor (ML). In Chapter 2, we discussed possible wastewater parameters that could be used as OFP's for an optimization of the flocculation process. We will now describe how we tried using one of these parameters as an OFP. However, the description of this parameter, streaming current, its principles and possible applications will be described briefly, as a detailed discussion of this complex subject would go beyond the scope of this thesis.

6.1 MATERIALS AND METHODS FOR STREAMING CURRENT DETECTOR

The streaming current detector was chosen as an example for an OFP because of promising results presented in the literature (Abu-Orf and Dentel, 1998). An evaluation of streaming current detectors in November 1988, sponsored by the AWWA (American Waterworks Association) Research Foundation, reported an average reduction of flocculant usage by 12 percent under stable conditions, and 23 percent under changing raw water conditions (http://www.awwarf.com/exsums/90536.htm).

The principles of the streaming current detector are based on the following assumption. The point of optimum flocculation occurs at a certain value of the surface charge of suspended solids, or at a particular electrical potential at the surface of shear between the stationary and mobile portions of the electric double layer (2.2.1 Mechanisms of Flocculation), which is the Zeta-potential. The streaming current is generally proportional to the average particle Zeta-potential and its value can therefore be used as a determinant of optimum flocculation. Depending on the main mechanism of flocculation and on system characteristics, the streaming current may be more or less negative at the point of optimum flocculation. If the flocculation is mainly due to charge neutralizations the current will be very close to zero,

while it becomes more and more negative with increasing influence of bridging effects (2.2.1 Mechanisms of Flocculation).

The sensor of the streaming current detector (SCD) consists of a reciprocating piston in a dead-end cylinder. Water containing the particles to be characterized, flows through this cylinder. Electrically charged colloids in the fluid sample momentarily attach to the piston and the cylinder surfaces, where colloids attached to the piston travel with the piston velocity, while those on the cylinder walls remain stationary. As the overall electrical charge in this system must be neutral, the negative charge density must be balanced by a layer of counter-ions in the water contained in the annulus, the space between the piston and the water. The two layers of the oppositely charged ions form the electro-double layer separated by the shear plane. As the piston moves up and down, the fluid in the annulus moves at a much greater velocity than that of the piston and it also transports the counter-ions located beyond the shearing plane. Thus the two layers are moving relative to each other at the shear plane, and this provides a measurable current, the streaming current. The streaming current (SC) is detected by electrodes at opposite ends of the flow paths and are sampled and amplified by the electronic components of the SCD to give a digital output. Because this SC is related to the electrical charge of the colloids, it may provide an indication of charge-related particle destabilization. More details about the functioning of this detector can be found in various sources (Abu-Orf and Dentel 1997).

The streaming current value related to the point of optimum flocculation may be dependent on several system characteristics, like the composition of the treated wastewater and the main mechanisms of flocculation. The latter is a function of the physical and chemical characteristics of the flocculant, including its configuration, which in turn is strongly dependent on wastewater parameters like pH, conductivity, etc. Therefore, the streaming current, as a parameter for process control of flocculant addition at the secondary clarifier, may require a new setting in case of dramatically changed wastewater conditions.

However, first we wanted to test whether a decrease or an increase in flocculant dosage would result in a change of the streaming current signal. We expected that different amounts of flocculant added to the system may provide more or less charge neutralization of the particle surfaces, assuming higher rates of polymer adsorption at higher concentrations. To test the response of the streaming current to this effect, a streaming current detector provided by Chemtrac Systems, Inc. (Model SCM 2000 XR Monitor) was employed.

The original plan was to set up the instrument close to the point of flocculant addition at the secondary clarifier. The installation of the detector close to the dosage point should provide a short lag time between the dosage and response, resulting in a more sensitive measurement. However, it had to be ensured that the sample flow, containing flocs, did not clog the sensor and thus stop the experiment. For this reason and due to the fact that the sample tubing would have interfered with the movement of the skimmer, the streaming current detector was first set up further downstream at the outfall of the PWTP. The streaming current detector was initialized to zero and connected to a chart recorder to monitor the changes in streaming current over a period of several days. Later on, the determined streaming current data were to be correlated with the changing amounts of flocculant dosed to that particular clarifier. As higher or lower flocculant dosages should lead to changes in the surface charges of suspended particles, we expected to see varying values of streaming current in these cases. However, it was soon determined that there was no correlation between the relatively stable streaming current numbers and variable amounts of flocculant dosed to the system. Therefore we assumed that the lag time between dosage and response was too long, and that the sampling point was not appropriate for our purposes.

We decided to move the sampling point further upstream, on the peripheral feed in a secondary clarifier, approximately 33 feet (ten meters) away from the point of polymer addition. The potential problem associated with the interference of the movement of the skimmer was overcome with the addition of a relatively simple steel construction protecting the sample tubing.

The results obtained from this experimental set-up will be discussed qualitatively in the following section.

6.2 RESULTS AND DISCUSSION FOR STREAMING CURRENT DETECTOR

As described in the previous section the final setup of the streaming current detector was at one of the secondary clarifiers close to the point of flocculant addition. With this setup the lag time between dosage and response was minimized and corresponding changes of the streaming current signal were expected with variable flocculant dosages.

However, the monitoring of streaming current data using a chart recorder over several days showed very little variations in the current signal over time despite the fact that the flocculant dosage for this clarifier had been changed several times. First, we assumed that the detection

of the streaming current was inhibited by solids clogging the sensor cell. As this would probably lead to both, a non-representative signal as well as a decrease in sensitivity of the detector, the response of the current to changing flocculant dosages would not be possible anymore. Thus we cleaned the sensor cell and the sampling tubing, and set up the experiment again.

However, there was still no improvement observed concerning the response of the current to changing polymer addition. Thus, we assumed that there were problems connected with the data output on the chart recorder. Later, after we had ensured that the recorder performed properly, we suspected that the measured streaming current signal was not related to changing flocculant concentrations in the wastewater at all.

This was verified, when on one particular occasion, the plant operator in charge decided that the flocculant dosage had to be increased for the secondary clarifier, where we had set up the streaming current detector. For that purpose the pump rate of the polymer pump was raised by approximately 20 %. However, even a change in flocculant dosage of this magnitude did not lead to any corresponding change in streaming current signal. Therefore we abandoned further experiments with this instrument.

The failure of this method cannot be fully explained at this stage of our research. Assuming that the streaming current signals were representative, a relatively constant signal at various flocculant concentrations could be interpreted as follows. A constant signal implies that no change in the surface charge of suspended particles occurred. This can be attributed to two possible mechanisms. First, in the case of a permanent overdosing of the system, the surface charges would be so close to complete neutralization, that the changes made to the flocculant dosage, could not produce a significantly sufficient change in the streaming current to be detected by the instrument. However, considering the U-shaped dosage response curve obtained during the flocculation of suspended solids, a high permanent overdosing should lead to the restabilization of particles and result in high contents of suspended solids in the effluent. In that case, we would not expect sufficient removal of solids in the secondary clarifiers, which was not observed to be the case.

A second possible explanation for a relatively constant streaming current signal is related to bridging as a main flocculation mechanism. In the case of high bridging effects, charge neutralization may play a minor role in flocculation. Therefore, the changes in surface charge may not be detectable anymore, while flocculation may still remove sufficient amounts of suspended solids. However, even with strong bridging effects some charge neutralization has

to occur due to the binding of the cationic flocculant to the solids surface. If this change in surface charge was not detectable by the instrument, its sensitivity is probably not high enough for this application. However, we had no possibility to test an instrument based on similar principles, but with higher sensitivity. Therefore, at this stage of our research we cannot fully explain the failure of the streaming current detector for this application.

We hope that the above discussion provides a starting point for further investigations in the application of streaming current as an OFP for flocculant dosage control in the system under consideration. In addition, the above experiments provide a demonstration of how other wastewater parameters can be validated as potential OFP's for this process. We also suggest that further investigations in this regard should include not only online measurements, but also batch experiments in the form of jar tests. A wide variety of process conditions can then be simulated in these batch experiments.

CHAPTER 7
CONCLUSIONS AND FURTHER INVESTIGATIONS

The brewery wastewater produced by the Coors Brewing Company is treated in the Process Wastewater Treatment Plant (PWTP), which includes primary, secondary, and tertiary treatment steps. During the secondary treatment, suspended solids are removed by settling in three secondary clarifiers. To increase the efficiency of solids removal, and to hasten the treatment process, a polyelectrolyte is dosed to the incoming mixed liquor at the clarifiers. The applied flocculant is a polyacrylamide copolymer purchased in the form of an emulsion, which contains additional compounds to a minor extent. Until now, the variations in flocculant dosage were exclusively based on the personal experience of plant operators. Depending on their judgement of the wastewater conditions and of the solids blanket level in each clarifier, the pump rates of the polymer pumps are adjusted individually.

However, there are several reasons why the personal experience of the plant operators should be supported by a flocculant dosage control mechanism based on an objective analysis of the treatment conditions. The target of the suggested process control for flocculant addition is to minimize the amount of flocculant dosed to the system while meeting the limits for Total Suspended Solids (TSS) and Whole Effluent Toxicity (WET) required by the NPDES (National Pollutant Discharge Elimination System) discharge permit. Overdosing wastewater with polyelectrolytes should be avoided for several reasons.

First, any kind of overdosing is a waste of energy, resources, and costs. As estimated in a previous chapter, about 1 million U$ (about 15 million ATS) are spent on the flocculant at Coors PWTP anually. This number indicates that polyacrylamide flocculants are costly compounds, which should be handled with care to avoid the waste of monetary resources.

Second, overdosing leads to higher residual concentrations of flocculant in the secondary clarifier effluent (SCE), and in turn also in the final commingled effluent (FCE). As shown in a previous chapter, the applied flocculant has the potential to cause toxic effects on aquatic organisms. Besides the impacts on the local ecosystems and the environment, this may also cause failures in WET tests required by the discharge permit, resulting in penalties.

In addition, the excessive overdosing of wastewater with polyelectrolytes may prevent us from reaching the main target of the treatment process: the sufficient removal of suspended solids during the secondary clarification. As discussed previously, the concentration-response

relationship between the wastewater parameter used as an indicator for optimum flocculation and the polymer dosage usually follows a U-shaped curve. This development indicates that flocculant overdosing may lead to the restabilization of previously flocculated particles resulting in an increase in suspended solids in the effluent. To avoid these negative impacts, we proposed a process control mechanism for flocculant dosage at the secondary clarifiers.

The suggested process control for flocculant addition is based on two parameters, the Residual Flocculant Parameter (RFP), which controls possible toxic effects, and the Optimum Flocculation Parameter (OFP), which indicates sufficient removal of suspended solids. Both of them should be determined by online measurements to minimize the lag time of the control system. The OFP should be measured at the incoming wastewater to the secondary clarifiers and the RFP at the secondary clarifier effluent. This will enable us to estimate the flocculant dosage based on the OFP and to check possible residual flocculant concentrations in the effluent with the RFP measurement.

In this thesis, we focused on the quantitative analysis of the flocculant, as it was initially assumed that the process control for flocculant dosage would only be based on the RFP. The search for an analytical method, used for flocculant determination, was aggravated by the lack of knowledge concerning the characteristics of the flocculant. The manufacturer of the flocculant, Stockhausen, Inc., did not provide us with supportive information concerning the chemical composition or physical characteristics of its product. In addition, information about their in-house analytical methods to determine residual monomer concentrations and several physical parameters was not supplied. This led to the investigation of numerous analytical methods before we succeeded in a method for the determination of the residual polymer.

To make sure that we used representative wastewater samples in our experiments, we conducted a wastewater characterization prior to sampling. Then, several analytical methods were investigated, including spectrophotometry, density, viscosity, and mass balances for organic carbon and nitrogen. Finally, colloid titration proved to be useful for our purposes. This method is based on the titrations of the cationic flocculant with an anionic polymer, where the endpoint was defined as the point of completed charge neutralization.

In this field we recommend further investigations including the following.

- We suggest that the positive results obtained by the colloid titration should be repeated. Further simplifying modifications of this method, i.e. the application of an automatic titrator, have to be considered. However, this method was very time-

consuming, required intensive data interpretation, and is only applicable in a laboratory setting.

- Thus, we suggest further investigations concerning a more specific method for the quantitative measurement of flocculant in wastewater. We recommend the development of a two-step method, where in the first step, the sample is pretreated to provide a more concentrated sample for the following detection step. For the first step we suggest the application of a cationic exchange resin, for the second size exclusion chromatography in combination with a UV-detection.
- Future investigations should also include considerations about possible online applications of the analytical method chosen.

Using residual flocculant concentrations as a control parameter for toxicity, we will have to establish concentration limits, which are not to be exceeded to avoid toxic effects of the FCE on aquatic organisms. For that purpose, we performed toxicity tests using bacteria (*Vibrio fischeri*) and *Daphnia magna* as test organisms.

First, we investigated possible toxic effects of several wastewater samples taken under regular treatment conditions and during the breakdown of one of the three clarifiers. We assumed that the appropriate flocculant dosage during the breakdown would be difficult to control, even for experienced plant operators. Thus, the probability of overdosage connected with toxic effects was expected to be much higher than under regular conditions. The secondary clarifier sample taken during the breakdown was toxic to *Daphnia magna*, confirming our concerns. However, dilution by the combination of the final effluents of the PWTP and the General Wastewater Treatment Plant (GWTP) prevented the final commingled effluent from being toxic.

In further toxicity tests we investigated the effects of various flocculant concentrations added to two wastewater samples and to natural synthetic water (NSW). The results indicated that the flocculant had severe toxic effects on *Daphnia magna* in all water types. The computed EC_{50} values agree with previously published values and could be used as basis for the RFP.

Microtox® tests based on the response of bacteria indicated that none of the tested wastewater samples showed acute toxicity to these test organisms. The flocculant had toxic effects on these bacteria only in a concentration range much higher than the amounts of flocculant added during the wastewater treatment. However, the literature reports possible

genotoxic effects of similar compounds on bacteria. The present study did not assess genotoxic effects.

We proposed two possible mechanisms of toxicity from the tested flocculant. First, the adsorption of the cationic polyelectrolyte to membranes of *Daphnia magna* may lead to physical entrapment, and the development of thin films of polymer on their gills, causing death by suffocation. In addition, the adsorption of the toxicant may result in the malfunctioning of other vital functions as such as feeding, digestion and reproduction. It was proposed that the addition of negatively charged particles could decrease flocculant toxicity by providing additional binding sites for its adsorption.

The second toxicity mechanism proposed, is based on the assumption that residual acrylamide monomer in the emulsion acts as a neurotoxin. However, the reported EC_{50} values for the toxic effects of acrylamide on daphnids were much higher than the concentrations we would expect in our test solutions. Therefore, we suggest that the effects connected with the adsorption of the flocculant to body surfaces are the main mechanism for the toxicity of the flocculant.

Based on our results and conclusions, we recommend the following further investigations concerning toxicity of the flocculant.

- First, we suggest that the mechanisms of toxicity on *Daphnia magna* and bacteria should be analyzed in greater detail. The toxic effects may be mainly due to the adsorption of the flocculant to the body surfaces of the test organisms or due to the acrylamide monomer acting as a neurotoxin. The testing of various size/weight fractions of the flocculant emulsion on *Daphnia magna* provides information to determine which of the proposed mechanisms is dominant. Fractions of high molecular weight cause toxic effects by adsorption, as longer polymer chains cover a higher area of body surface. Fractions of low molecular also contain acrylamide monomer, and can be used to test the possible toxic effects of this compound.
- If it is possible to show that the toxicity of the flocculant is mainly based on its adsorption to body surfaces, the influence of the cationic charge of the polymer on toxic effects should be tested. Anionic or non-ionic polyelectrolytes have been reported to be less toxic or non-toxic in comparison to the cationic ones in the literature.
- Further investigations should also target the addition of negatively charged particles to the test solutions as a possible way to decrease the toxic effects of the flocculant on

invertebrates. If this proves to be the case, clay or similar compounds could be used for detoxification of the PWTP effluent in emergency cases after the determination of high residual flocculant concentrations.

- Concerning the flocculant toxicity to bacteria, studies should examine whether the flocculant has genotoxic effects on bacteria similar to those in the aeration trains. The organisms in the aeration trains are exposed to small flocculant concentrations due to the recycling of Return Activated Sludge (RAS) containing the flocculant from the treatment in the secondary clarifiers.

In addition to the investigations connected with the RFP, wastewater parameters were studied, which could possibly be used as the OFP. An example of such a parameter, the streaming current, was tested. However, the online tests of the streaming current detector indicated that this instrument could not be used for our purposes.

- Thus, we recommend the testing of other wastewater parameters for their application as OFP's. These tests should be performed as online measurements and in the form of jar tests, where various wastewater conditions could be simulated.

Reviewing the results of our work, we strongly recommend further steps towards the installation of an automated dosage control system for the flocculant addition based on the proposed control parameters. The lack of information about the required flocculant dosage and the resulting residual flocculant concentrations can lead to several problems. The removal of suspended solids from the wastewater may not be sufficient due to the restabilization of particles, and residual flocculant concentrations may increase the toxicity of the FCE. In addition, we should always keep in mind, that flocculant overdosing is a costly matter.

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APPENDIX

APPENDIX FOR CHAPTER 3

WASTEWATER CHARACTERIZATION

3.2 RESULTS AND DISCUSSION FOR WASTEWATER CHARACTERIZATION

3.2.2 Auto Sampler Tests

Conductivity Comparisons between Mixed Liquor and Headworks Samples

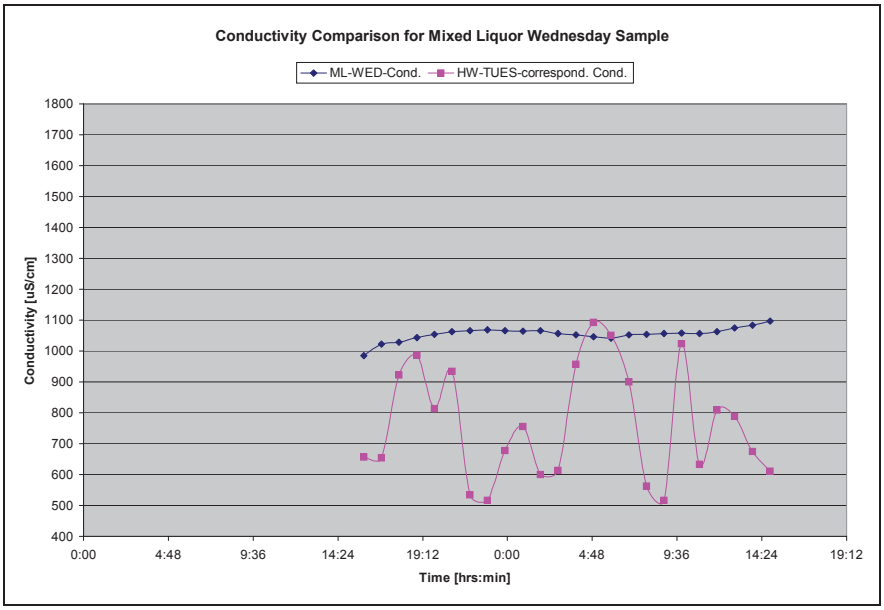

Figure 3.12: Conductivity comparison for mixed liquor Wednesday sample.

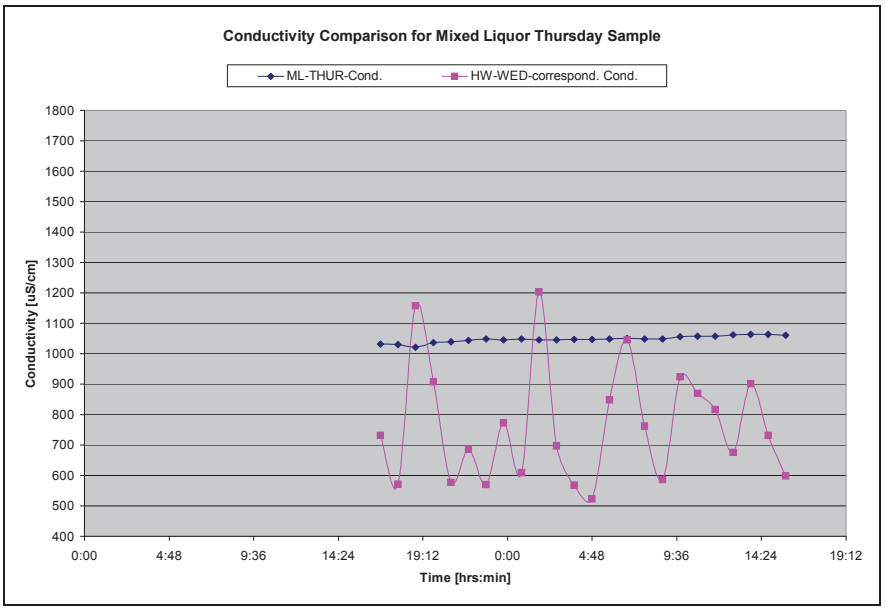

Figure 3.13: Conductivity comparison for mixed liquor Thursday sample.

Figure 3.14: Conductivity comparison for mixed liquor Friday sample.

pH Comparisons between Mixed Liquor and Headworks Samples

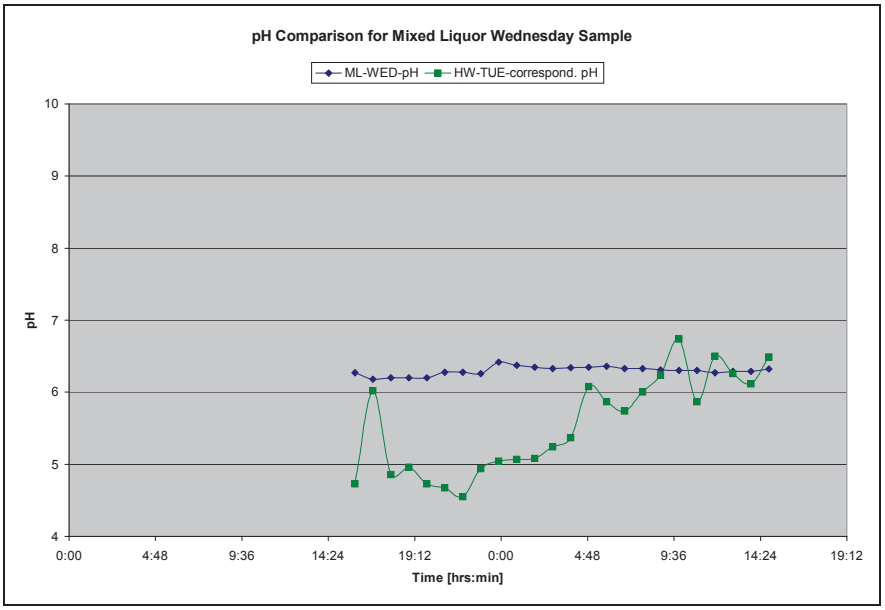

Figure 3.17: pH comparison for mixed liquor Wednesday sample.

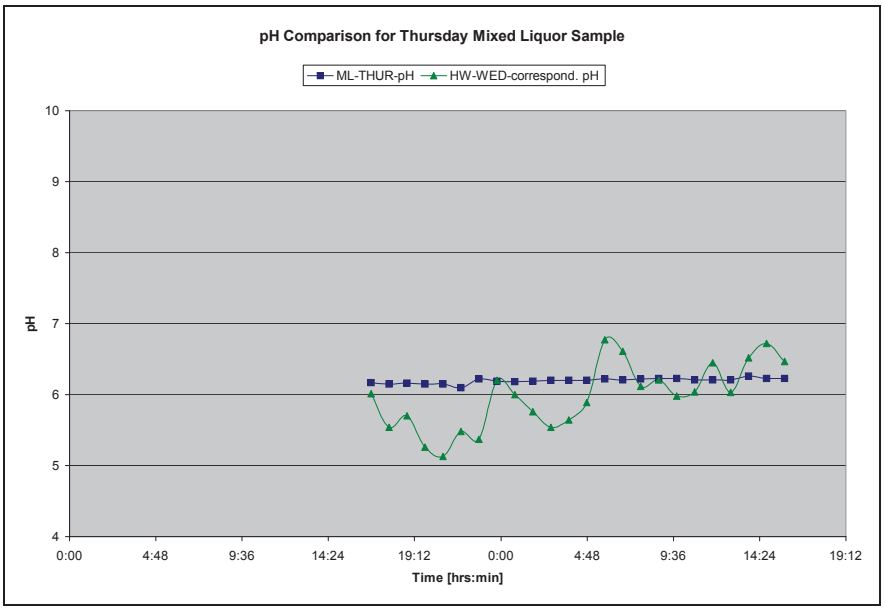

Figure 3.18: pH comparison for mixed liquor Thursday sample.

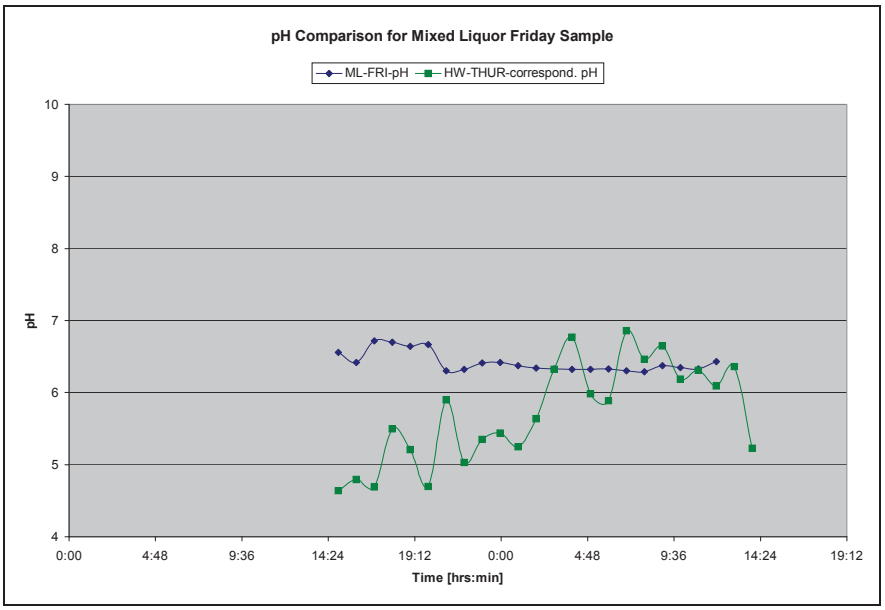

Figure 3.19: pH comparison for mixed liquor Friday sample.

APPENDIX FOR CHAPTER 5

LIMITS FOR RESIDUAL FLOCCULATION CONCENTRATION – TOXICITY TESTS

5.1 MICROTOX® TEST PROTOCOLS

100 % Test Protocol: (Microtox Manual, 1992)

Analyzer Preparation:

- Place cuvettes in incubator row A and the reagent well.
- Add 1000 μL reconstitution solution to the reagent well.
- Add 1000 μL diluent to cuvettes in A1 through A4.

Sample Preparation – Osmotic Adjustment:

- Add 250 μL MOAS to A5.
- Add 2500 μL sample to A5, and mix.
- Make 1:2 serial dilutions by transferring 1000 μL from A5 to A4, A4 to A3, A3 to A2, with mixing after each sample transfer.
- Discard 1000 μL from cuvette A2 and 750 μL from cuvette A5.
- Wait 5 minutes.

Reagent Preparation:

- Reconstitute a vial of reagent.
- Mix reagent with 500 μL pipettor 20 times.

Computer Preparation:

- Enter % (100 %) at the Master Menu prompt.
- Set the number of tests: 1.
- Set current Test Parameters: the number of controls and dilutions, duplicate (yes/no), the initial concentration, dilution factor, etc.

Test Protocol

- Hit the computer space bar.
- Transfer 10 μL reagent to A1 through A5.
- Mix cuvettes with 250 μL pipettor or by shaking for A1 through A5.
- Hit the computer space bar.
- When the timer sounds, place the A1 cuvette in the READ well. Press the SET button.

- Read the light levels (I_t) at times t = 5, 15 minutes as prompted by "ENTER" on the computer screen.
- Reduce the data of the data report at the prompt.

Basic Test Protocol: (Microtox Manual, 1992)

Analyzer Preparation:

- Place the cuvettes in incubator rows A & B and the reagent well.
- Add 1000 μL reconstitution solution to the reagent well.
- Add 500 μL diluent to B1 through B5.
- Add 1000 μL diluent to A1 through A4.

Sample Preparation – Osmotic Adjustment:

- Add 250 μL MOAS to A5.
- Add 2500 μL sample to A5, and mix.
- Make 1:2 serial dilutions by transferring 1000 μL from A5 to A4, A4 to A3, A3 to A2, with mixing after each sample transfer.
- Discard 1000 μL from cuvette A2 and 750 μL from cuvette A5.
- Wait 5 minutes.

Reagent Preparation:

- Reconstitute a vial of reagent.
- Mix reagent with 500 μL pipettor 20 times.
- Transfer 10 μL reagent to B1 through B5.
- Mix cuvettes with 250 μL pipettor or by shaking for B1 through B5.
- Wait 15 minutes.

Computer Preparation:

- Enter B (Basic) at the Master Menu prompt.
- Set the number of tests: 1.
- Set current Test Parameters: the number of controls and dilutions, duplicate (yes/no), the initial concentration, the dilution factor, etc.

Test Protocol

- Place the B1 cuvette in the READ well. Press the SET button.
- Hit the computer space bar.
- Read light levels I_0 at time t = 0 as prompted by the computer screen.

- Then immediately make the following 500 μL transfers, with mixing after each transfer: A1 to B1, A2 to B2, A3 to B3, A4 to B4, A5 to B5.
- Hit the computer space bar.
- When the timer sounds, read I_t at times t = 5, 15 minutes for light levels as prompted by "ENTER" on the computer screen.
- Reduce the data of the data report.

5.2 WHOLE EFFLUENT TOXICITY (WET) TESTS

5.2.1 Data Obtained from WET Tests

Table 5.10: Test Results Overview of WET Test I (Irregular treatment conditions)

Control Group

No. I: Natural Synthetic Water			No. II: Natural Synthetic Water		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	40	100.0	0	40	100.0
24	39	97.5	24	40	100.0
48	39	97.5	48	37	92.5

Mixed Liquor, Decanted **Use of Control Group I.**

100 % ML Solution			50 % ML Solution			25 % ML Solution			12.5 % ML Solution			6.25 % ML Solution		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	20	100.0	0	20	100.0	0	20	100.0	0	20	100.0	0	20	100.0
24	20	100.0	24	19	95.0	24	20	100.0	24	20	100.0	24	19	95.0
48	19	95.0	48	19	95.0	48	20	100.0	48	19	95.0	48	17	85.0

Secondary Clarifier Effluent **Use of Control Group I.**

100 % SCE Solution			50 % SCE Solution			25 % SCE Solution			12.5 % SCE Solution			6.25 % SCE Solution		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	20	100.0	0	20	100.0	0	20	100.0	0	20	100.0	0	20	100.0
24	20	100.0	24	20	100.0	24	19	95.0	24	20	100.0	24	20	100.0
48	7	35.0	48	12	60.0	48	14	70.0	48	17	85.0	48	17	85.0

Final Commingled Effluent **Use of Control Group II.**

100 % FCE Solution			50 % FCE Solution			25 % FCE Solution			12.5 % FCE Solution			6.25 % FCE Solution		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	20	100.0	0	20	100.0	0	20	100.0	0	20	100.0	0	20	100.0
24	20	100.0	24	20	100.0	24	20	100.0	24	20	100.0	24	19	95.0
48	17	85.0	48	17	85.0	48	18	90.0	48	19	95.0	48	19	95.0

Table 5.11: Test Results Overview of WET Test II (Regular treatment conditions)

Control Group

No. I: Natural Synthetic Water			No. II: Natural Synthetic Water			No. III: Natural Synthetic Water		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	38	100.0	0	30	100.0	0	40	100.0
24	38	100.0	24	30	100.0	24	40	100.0
48	35	92.1	48	28	93.3	48	37	92.5

Mixed Liquor, Decanted — **Use of Control Group I.**

100 % ML Solution			50 % ML Solution			25 % ML Solution			12.5 % ML Solution			6.25 % ML Solution		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	20	100.0	0	20	100.0	0	20	100.0	0	20	100.0	0	20	100.0
24	20	100.0	24	20	100.0	24	20	100.0	24	20	100.0	24	20	100.0
48	20	100.0	48	20	100.0	48	20	100.0	48	20	100.0	48	20	100.0

Secondary Clarifier Effluent — **Use of Control Group II.**

100 % SCE Solution			50 % SCE Solution			25 % SCE Solution			12.5 % SCE Solution			6.25 % SCE Solution		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0
24	15	100.0	24	15	100.0	24	15	100.0	24	15	100.0	24	15	100.0
48	15	100.0	48	15	100.0	48	15	100.0	48	15	100.0	48	14	93.3

Final Commingled Effluent — **Use of Control Group II.**

100 % FCE Solution			50 % FCE Solution			25 % FCE Solution			12.5 % FCE Solution			6.25 % FCE Solution		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0
24	15	100.0	24	15	100.0	24	15	100.0	24	15	100.0	24	15	100.0
48	15	100.0	48	15	100.0	48	15	100.0	48	15	100.0	48	15	100.0

GWTP Effluent, After Chlorination — **Use of Control Group III.** — **Cl = 1.05 mg/L**

100 % GWTP Solution			50 % GWTP Solution			25 % GWTP Solution			12.5 % GWTP Solution			6.25 % GWTP Solution		
Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival	Time	# of Live Organisms	Survival
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0
24	0	0.0	24	0	0.0	24	8	53.3	24	15	100.0	24	15	100.0
48	0	0.0	48	0	0.0	48	4	26.7	48	14	93.3	48	15	100.0

Table 5.12: Test Results Overview of WET Test III (Flocculant Addition)

Control Group

No. I: Natural Synthetic Water			No. II: Sec. Clarifier Effl.			No. III: Final Commin. Effl.		
Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival
	Organisms			Organisms			Organisms	
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	42	100.0	0	40	100.0	0	40	100.0
24	42	100.0	24	40	100.0	24	40	100.0
48	36	85.7	48	40	100.0	48	40	100.0

Natural Synthetic Water — **Use of Control Group I.**

4.0 ppm Flocculant Solution			2.0 ppm Flocculant Solution			1.0 ppm Flocculant Solution			0.5 ppm Flocculant Solution			0.1 ppm Flocculant Solution		
Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival
	Organisms			Organisms			Organisms			Organisms			Organisms	
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0
24	4	26.7	24	9	60.0	24	12	80.0	24	15	100.0	24	6	40.0
48	0	0.0	48	0	0.0	48	0	0.0	48	6	40.0	48	3	20.0

Secondary Clarifier Effluent — **Use of Control Group II.**

4.0 ppm Flocculant Solution			2.0 ppm Flocculant Solution			1.0 ppm Flocculant Solution			0.5 ppm Flocculant Solution			0.1 ppm Flocculant Solution		
Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival
	Organisms			Organisms			Organisms			Organisms			Organisms	
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0
24	6	40.0	24	13	86.7	24	15	100.0	24	15	100.0	24	15	100.0
48	0	0.0	48	3	20.0	48	15	100.0	48	15	100.0	48	15	100.0

Final Commingled Effluent — **Use of Control Group III.**

4.0 ppm Flocculant Solution			2.0 ppm Flocculant Solution			1.0 ppm Flocculant Solution			0.5 ppm Flocculant Solution			0.1 ppm Flocculant Solution		
Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival	Time	# of Live	Survival
	Organisms			Organisms			Organisms			Organisms			Organisms	
[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]	[hrs]	[ ]	[%]
0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0	0	15	100.0
24	10	66.7	24	15	100.0	24	15	100.0	24	15	100.0	24	15	100.0
48	0	0.0	48	0	0.0	48	12	80.0	48	15	100.0	48	15	100.0

5.2.2 Statistical Methods for the Data Interpretation of WET Tests (EPA-600/4-90-027F)

The Probit Method

- Description

  The Probit Method is a parametric statistical procedure for estimating the LC_{50} and the associated 95 % confidence interval. As concentration-response data are often normally distributed a conversion of the percent response into units of deviation from the mean or normal equivalent deviates (NED) were suggested. The NED for a 50 % response is zero and that for an 84.1 % response is +1. Later the value of 5 was added to the NED to eliminate negative numbers. The converted units of NED plus 5 were called probits.

  The probit analysis consists in transforming the observed proportion mortalities with a probit transformation, and transforming the effluent concentrations to base 10 logarithms (log_{10}). Given the assumption of normality for the log_{10} of the tolerances, the relationship between the transformed variables mentioned above is approximately linear. This relationship allows estimation of linear regression parameters, using an iterative approach. The estimated LC_{50} and associated confidence interval are calculated from the estimated linear regression parameters.

- Requirements

  To obtain a reasonably precise estimate of the LC_{50} with the Probit Method, the observed proportion mortalities must bracket 0.5. The log_{10} value of the tolerance is assumed normally distributed. To calculate the LC_{50} estimate and associated 95 % confidence interval, two or more of the observed proportion mortalities must be between zero and one.

- General Procedure

  Due to the intensive nature of the calculations for the estimated LC_{50} and associated 95 % confidence interval using the Probit Method, it is recommended that the data be analyzed by a computer program. For that reason the EPA made probit software available on the internet (http://www.epa.gov/nerleerd/stat2.htm).

The Spearman-Karber Method

- Description

  The Spearman-Karber Method is a nonparametric statistical procedure for estimating the LC_{50} and the associated 95 % confidence interval. This procedure estimates the mean

of the distribution of the $\log_{10}$ of the tolerance. If the log tolerance distribution is symmetric, this estimate of the mean is the equivalent to an estimate of the median of the log tolerance distribution. If the response proportions are not monotonically non-decreasing with increasing concentration (constant or steadily increasing with concentration), the data are smoothed. Abbott's procedure is used to "adjust" the test results for mortality occuring in the control. Use of the Spearman-Karber Method is recommended when partial mortalities occur in the test solutions, but the data do *not* fit the Probit Model.

- Requirements

To calculate the LC_{50} estimate, the following must be true:

a.) The smoothed adjusted proportion mortality for the lowest effluent concentration (not including the control) must be zero.

b.) The smoothed adjusted proportion mortality for the highest effluent concentration must be one.

To calculate the 95 % confidence interval for the LC_{50} estimate, one or more of the smoothed adjusted proportion mortalities must be between zero and one.

- General Procedure

The first step in the estimation of the LC_{50} by the Spearman-Karber Method is to smooth the observed response proportions, p_i, if they do not satisfy $p_k \geq \ldots \geq p_0$, where p_0, p_1, …, p_k denote the observed proportion mortalities for the control and the k effluent concentrations. The smoothing replaces any adjacent p_i's that do not conform to the requirement stated above, with their average. For example, if p_i is less than p_{i-1}, then:

$$p_{i-1}{}^s = p_i{}^s = \frac{p_{i-1} + p_i}{2} \qquad \text{(Equation 5.3)}$$

where: $p_i{}^s$ = the smoothed observed proportion mortality for effluent concentration i.

Then adjust the smoothed observed proportion mortality in each effluent concentration for mortality in the control group using the following formula.

$$p_i{}^a = \frac{p_i{}^s - p_0{}^s}{1 - p_0{}^s} \qquad \text{(Equation 5.4)}$$

where: $p_0{}^s$ = the smoothed observed proportion mortality for the control.

Plot the smoothed adjusted data on 2-cycle semi-log graph paper with the logarithmic axis (the y axis) used for percent effluent concentration and the linear axis (the x axis) used for observed percent mortality. Then calculate the $\log_{10}$ of the estimated LC_{50}, m, as follows:

$$m = \sum_{i=1}^{k-1} \frac{(p_{i+1}^{a} - p_i^{a})(X_i + X_{i+2})}{2} \qquad \text{(Equation 5.5)}$$

where: p_i^a = the smoothed adjusted proportion mortality at concentration i.

X_i = the $\log_{10}$ of concentration i.

k = the number of effluent concentrations tested, not including the control.

Then calculate the estimated variance of m as follows:

$$V(m) = \sum_{i=2}^{k-1} \frac{p_i^{a} \quad (1 - p_i^{a}) \quad (X_{i+1} + X_{i-1})^2}{2} \qquad \text{(Equation 5.6)}$$

where: p_i^a = the smoothed adjusted proportion mortality at effluent concentration i.

X_i = the $\log_{10}$ of concentration i.

k = the number of effluent concentrations tested, not including the control.

n_i = the number of organisms tested at effluent concentration i.

The next step is to compute the 95 % confidence interval for m: $m \pm \sqrt{V(m)}$

The estimated LC_{50} and a 95 % confidence interval for the estimated LC_{50} can be found by taking $base_{10}$ antilogs of the above values. With the exclusion of the plot of the smoothed adjusted data on 2-cycle semi-log graph paper, the above calculations can be carried out using the Trimmed Spearman-Karber computer program available at EPA's homepage (http://www.epa.gov/nerleerd/stat2.htm).

The Trimmed Spearman-Karber Method

- Description

The Trimmed Spearman-Karber Method is a modification of the Spearman-Karber nonparametic statistical procedure for estimating the LC_{50} and the associated 95 % confidence interval. This procedure estimates the trimmed mean of the distribution of the $\log_{10}$ of the tolerance. If the log tolerance distribution is symmetric, this estimate of the trimmed mean is equivalent to an estimate of the median of the log tolerance distribution.

Use of the Trimmed Spearman-Karber Method is *only* appropriate when the requirements for the Probit Method and the Spearman-Karber Method are not met.

- Requirements

  To calculate the LC_{50} estimate with the Trimmed Spearman-Karber Method, the smoothed, adjusted, observed proportion mortalities must bracket 0.5. To calculate a confidence interval for the LC_{50} estimate, one or more of the smoothed, adjusted, observed proportion mortalities must be between zero and one.

- General Procedure

  Smooth the observed proportion mortalities as described for the Spearman-Karber Method previously. Adjust the smoothed observed proportion mortality in each effluent concentration for mortality in the control group using the formula given earlier. Then plot the smoothed, adjusted data as described in the same way as for the Spearman-Karber Method. Calculate the amount of trim to use in the estimation of the LC50 as follows:

(Equation 5.7)

$$\text{Trim} = \max (p_1^a, 1 - p_k^a)$$

where: p_1^a = the smoothed, adjusted proportion mortality for the lowest effluent concentration, exclusive of the control.

p_k^a = the smoothed, adjusted proportion mortality for the highest effluent concentration.

k = the number of effluent concentrations tested, not including the control.

Due to the intensive nature of the calculation for the estimated LC_{50} and the calculation for the associated 95 % confidence interval using the Trimmed Spearman-Karber Method, it is recommended that the data be analyzed by computer. A program, which has been made available by EPA (http://www.epa.gov/nerleerd/stat2.htm) automatically performs the smoothing, the adjustment for mortality in the control, and the calculations of the trim, the LC_{50}, and the associated 95 % confidence interval.

The Graphical Method

- Description

  The Graphical Method is a mathematical procedure for calculating the LC_{50}. The procedure estimates the LC_{50} by the linear interpolation between points of a plot of observed percent mortality versus the $\log_{10}$ of percent effluent concentration. It does not

provide a confidence interval for the LC_{50} estimate. The use of the Graphical Method is *only* recommended when there are no partial mortalities and other statistical models cannot be applied.

- Requirements

  The only requirement for the Graphical Method is that the observed percent mortalities bracket 50 %.

- General Procedure

  The smoothing of the observed proportion mortalities is used to fulfill the same requirements and follows the same principles as described for the Spearman-Karber Method earlier. The same applies to the adjustment for mortality in the control group of the smoothed observed proportion mortality in each effluent concentration. Then the smoothed, adjusted data are plotted on 2-cycle semi-log graph paper with the logarithmic axis (the y axis) used for percent effluent concentration and the linear axis (the x axis) used for observed percent mortality. From this graph the two points on the graph which bracket the 50 % mortality are located and connected with a straight line. On the scale for percent effluent concentration, read the value for the point where the plotted line and the 50 % mortality line intersect. This value is the estimated LC_{50} expressed as a percent effluent concentration.