

Practical Applications of Chemical Oxygen Demand Fractionation for Biological Phosphorus
Removal Systems

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ABSTRACT

COLEY, JULIAN HENRY. PRACTICAL APPLICATIONS OF PHYSICOCHEMICAL CHEMICAL OXYGEN DEMAND FRACTIONATION FOR BIOLOGICAL PHOSPHORUS REMOVAL SYSTEMS (UNDER THE DIRECTION OF DR. ANGELLA ALLEN).

Phosphorus removal in wastewater treatment is critical for preventing eutrophication and further degradation of aquatic environments. For phosphorus, a limiting nutrient, just a slight increase in concentration can lead to significant ecological impacts. Wastewater effluent is a substantial contributor to phosphorus loadings in receiving waters. Removal of phosphorus is an operational and economic challenge. With strict permit limits and ever-changing technologies, regulations, and funding, wastewater facilities must optimize treatment processes to achieve the highest removal efficiency in the most cost-effective manner. This study focuses on the practical applications of COD fractionation using physicochemical methods to drive process adjustments and inform design and treatment modeling. Practicality is determined through the data's reproducibility, usefulness through trend formation, and ability to collect and analyze statistically significant data.

Keywords: CHEMICAL OXYGEN DEMAND (COD), READILY BIODEGRADABLE CHEMICAL OXYGEN DEMAND (rbCOD), BIOLOGICAL PHOSPHORUS REMOVAL, ACTIVATED SLUDGE OPTIMIZATION.

BIOGRAPHY

Julian Coley, a North Carolina native, works for Union County Water as a Wastewater Treatment Plant Chief Operator. Before starting his career in water resources, Julian attended UNC Wilmington, where he earned his bachelor's degree in environmental science, focusing on resource conservation. Julian's fascination with the interactions between infrastructure, the environment, and human health began during his time with the NC Coastal Federation as a Coastal Education Outreach Intern. Julian's primary knowledge base centers on maintaining permit compliance through troubleshooting and optimizing nutrient removal processes. Julian is a certified Grade IV Biological Operator, Grade II Wastewater Laboratory Analyst, Pretreatment Specialist Class I, and holds several other NC water and wastewater treatment certifications.

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TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	viii
Chapter 1: Introduction	1
1.1 The Importance of Phosphorus Removal.....	1
1.2 Wastewater Treatment Processes.....	2
1.2a Origins of Activated Sludge.....	3
1.2b Biological Phosphorus Removal.....	4
1.2c Forms of Phosphorus.....	7
1.3 Chemical Oxygen Demand	7
1.3a Method Determination	9
1.4 Study Site.....	11
1.5 Study Objectives	13
Chapter 2: Methods	14
2.1 Sample Collection.....	14
2.2 Sample Preparation	15
2.2a Flocculation & Filtration.....	16
2.3 Sample Analysis.....	17
2.3a Minimizing Interference.....	17
2.3b Quality Control Methods	17
2.4 Data Analysis	18
2.4a Equations	19
2.4.b Statistical Techniques Utilized	20
Chapter 3: Results & Discussion	21
3.1 Interference Minimization	21
3.2 Quality Control	21
3.3 Influent COD Concentrations	22
3.3a Estimated Influent COD Fractions.....	22
3.4 Daily Variations	23
3.5 Reproducibility	26
3.6 Financial Impact	27
3.7 Practical Applications	28
Chapter 4: Conclusion	30
4.1 Summary of Key Findings	30
4.2 Implications & Limitations	31
4.3 Future Considerations	33
4.3a Faster Analysis Methods.....	33
4.3b Expand Sampling Preparation Methods.....	33
4.3c Target Specific Selector Zones	34
4.3d Include Seasonal Analysis	34
References	36

Appendices	38
Appendix A: Analytical Instruments List.....	39
Appendix B: Reagents List.....	40
Appendix C: Materials List	41
Appendix D: Stock Solutions Preparation	42
Appendix E: HACH Method 8000	43
Appendix F: TNTplus [®] 879	51

LIST OF TABLES

Table 3.1	Quality Control Sample Results	22
Table 3.2	COD Concentrations in Plant Influent and Effluent	22
Table 3.3	COD Fraction Percentages in Plant Influent.....	23
Table 3.4	Average Influent COD Concentrations for Days of the Week.....	24
Table 3.5	Ordinary One-Way ANOVA Summary	24
Table 3.6	Method Consumables Cost Estimation	27

LIST OF FIGURES

Figure 1.1	Wastewater Treatment Process Flow Schematic.....	3
Figure 1.2	Anerobic, Anoxic & Aerobic Selector Zones	5
Figure 1.3	Metabolic Pathways of Phosphorus-Accumulating Organisms	6
Figure 1.4	Breakdown of COD Fractions.....	8
Figure 2.1	Sample Preparation Methods for COD Fractions.....	15
Figure 2.2	Process of Flocculation & Filtration Sample Preparation Method.....	16
Figure 3.1	COD Concentrations Compared to Days of the Week.....	25
Figure 3.2	Reproducibility of Sample Preparation Methods using Sample Duplicates	26

CHAPTER 1

Introduction

1.1 The Importance of Phosphorus Removal

Phosphorus and nitrogen are the two most important nutrients for plant growth and ecosystems in both aquatic and terrestrial environments. When investigating water quality, these primary nutrients are of concern when the levels in waterways are in excess, leading to eutrophication and hypoxia, with secondary effects of overall water quality decline, loss of biodiversity, and trophic shifts. Although available phosphorus is below the relative needs for plant growth in the environment, it is also the most important nutrient when looking at the rate of plant growth within aquatic environments, as phosphorus is viewed as the limiting nutrient in waterways (Boyd, 2019). When water bodies have adequate nitrogen levels for algae production, just a small increase in phosphorus can produce an exponential increase in algae growth (Zahed et al., 2022). Nutrients are primarily introduced into waterways through stormwater, agriculture, and wastewater, with wastewater being a large contributor of phosphorus. With no standardized nationwide phosphorus discharge limit, permit limits vary throughout the National Pollutant Discharge Elimination System (NPDES). For some facilities, specifically those of a smaller scale, meeting low discharge limits can be an operational and economic challenge, with significant capital costs involved in expanding treatment systems for phosphorus removal.

As phosphorus limits are included in NPDES permits, utilities are modifying and optimizing current treatment systems for phosphorus removal, which were not designed initially with such criteria. Optimizing current processes provides opportunities for systems to improve their effluent quality as they acquire funding, design, and build larger or more advanced systems.

1.2 Wastewater Treatment Processes

Most commonly, wastewater treatment processes follow the prescribed treatment path summarized in Figure 1.1, starting with the arrival of wastewater from a collection system and into a treatment plant's headworks; this is where primary processes begin. Wastewater passes through screening mechanisms to remove larger debris, including rags, sticks, and other large objects, which may hinder the treatment process; fine screening may follow to remove smaller unwanted objects. Next, grit removal often takes place, where sand, small stones, and other inorganic items that are picked up throughout the collection system are removed. Depending on the facility, wastewater may enter primary clarifiers, where settling takes place to remove organic and inorganic solids. Once the wastewater has been screened and settled, it will make its way into secondary treatment, where the influent is mixed with a carefully cultivated biomass. This biomass is kept alive through the constantly available food sources from the incoming waste and oxygen being supplied from aeration facilities. After nearly all organic matter has been treated through biomass, it will find its way into secondary clarifiers, where solids will sink to the bottom and clear effluent will pass over the top. The effluent produced within this process often meets basic water pollution control standards, but further tertiary treatment is required for many impaired waterways. The wastewater may then go through filtration to remove smaller particles that could not be settled out in the clarifiers, followed by disinfection for pathogenic bacteria reduction. The last step of the process is often re-aeration, where the effluent is then re-aerated before being discharged into a receiving stream. All these processes have many variations in the technology they utilize and opportunities for optimization to ensure peak removal efficiency of harmful pollutants and pathogens. Side-stream processes include the removal of waste-activated sludge that is thickened, digested, and dewatered before disposal through land application or composting.

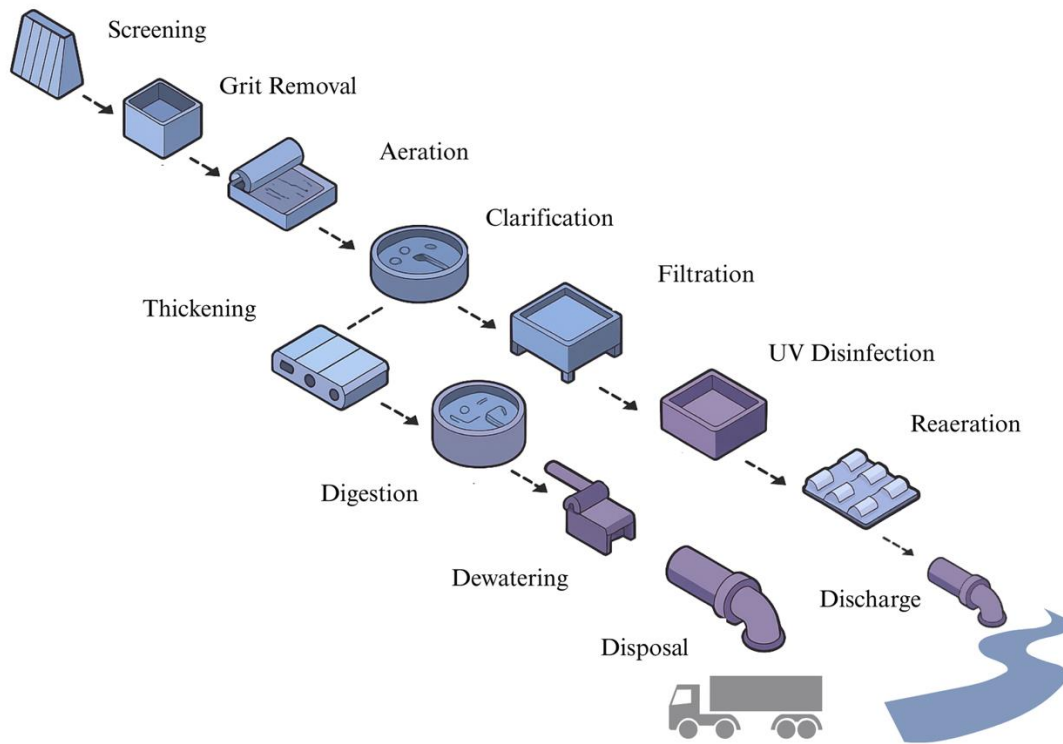


Figure 1.1 *Wastewater Treatment Process Flow Schematic* – Adapted from Hazen & Sawyer’s Twelve Mile Process Evaluation Sampling Report (2025)

1.2a Origins of Activated Sludge Systems

The genesis of the modern-day activated sludge process began at the Lawrence Experiment Station in 1911. However, Arden and Lockett pioneered the process, as their work led to the biomass of activated sludge reactors being an essential control parameter. Arden and Lockett’s work with the suspended-growth microbial community set sewage treatment into the future and was well received by research centers worldwide. Experimental plants and activated sludge facilities adopted the design within a decade of the first publication (Orhon, 2014). In the 1921 bibliography on the activated sludge process of sewage treatment by J. Edward Porter, activated sludge is defined as a flocculent sludge of medium brown color enveloped by masses of aerobic organisms possessing the power of rapidly oxidizing and nitrifying sewage, and

which, though of low specific gravity, settles rapidly. Although the process quickly became well-recognized and utilized, the actual mechanics of how the process functions remained unknown. Today, we recognize that the activated sludge process utilizes microbial species, such as *Nitrospira*, *Candidatus Accumulibacter*, and *Accumulibacter phosphatis*, which promote the degradation of pollutants through optimal conditions for microorganisms to thrive (Song et al., 2023). These optimal conditions require constant monitoring through advanced systems, which now provide consistent conditions designed for the growth of these targeted microbial species.

1.2b Biological Phosphorus Removal Process

Biological phosphorus removal is accomplished through various processes where specific conditions for dissolved oxygen, carbon concentrations, solids retention time (SRT), influent and return flow rates, and pH are utilized to create optimal growing conditions for specific microorganisms to uptake and store phosphorus for removal from the waste system (Zahed et al., 2022). The activated sludge treatment process utilized at the study facility is defined as a Plug-Flow Reactor (PFR) design composed of anaerobic, anoxic, and aerobic regions. A key factor in the proper operation of the specific selector zones (figure 1.2) within the treatment basin is the detention time, which ensures ample time to remove specific pollutants and nutrients. On average, for the study facility, the anaerobic zone's detention time is 0.62 hours, 1.48 hours in the anoxic zone, and 6.03 hours in the aerobic zone, for a cumulative total of 8.13 hours within the treatment train. Although the shortest duration, the time spent within anaerobic conditions is a driving factor in phosphorus removal, ensuring microorganisms are properly pre-conditioned prior to aeration.

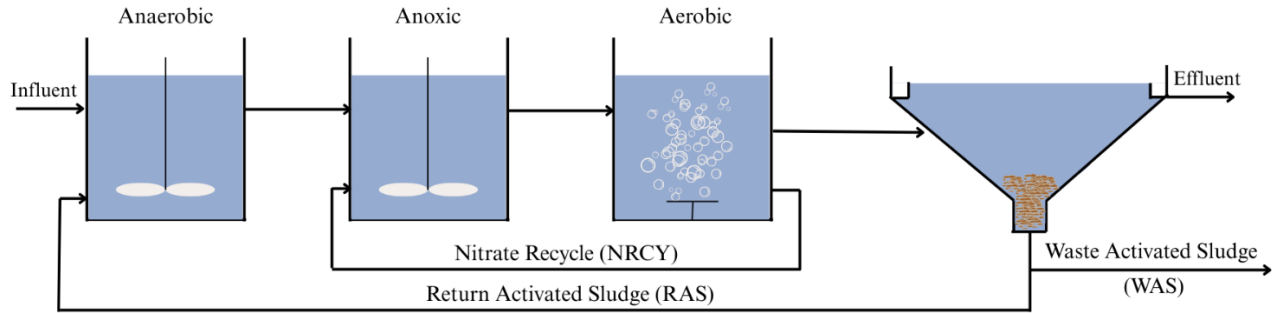


Figure 1.2 *Anerobic, Anoxic & Aerobic Selector Zones*

In the anaerobic zone, heterotrophic phosphorus-accumulating organisms (PAO) store volatile fatty acids (VFAs), the most readily available form of COD, in the form of polyhydroxyalkanoates (PHA) (Dorofeev et al., 2019). Summarized in Figure 1.3, adenosine triphosphate (ATP) is used for energy for the process, along with the reduction of nicotinamide adenine dinucleotide (NADH), which comes from glycolysis or the tricarboxylic acid cycle (TCA) (Zahed et al., 2022). The reason ortho-phosphorus increases within the anaerobic zone is due to the breakdown of the polyphosphate, due to the expansion of energy. Rates of release are measured through the collection and analysis of waste entering and exiting the anaerobic zone. Concentrations of ortho-phosphorus in a well-functioning EBNR system are two to three times the incoming concentration. For this process to work efficiently, there must be sufficient carbon sources, specifically readily available carbon sources in the form of volatile fatty acids. The ratio of incoming soluble carbon to the incoming phosphorus concentration is a limitation for EBNR systems, as the PAOs cannot sequester adequate PHAs for phosphorus accumulation without an appropriate amount of readily available energy sources. (Lin et al., 2015).

Assuming proper operation of the anaerobic zone, with now depleted PAOs, the biomass will enter the aerobic zone, where the pre-conditioned PAOs perform luxury phosphorus uptake to compensate for their energy deficiencies. The luxury uptake is accomplished by uptaking

must be present so that the PAOs can begin storing the carbon, expanding their energy, and releasing the stored phosphorus.

1.2c *Forms of Phosphorus*

Phosphorus in waste treatment systems can be measured in many forms, with total phosphorus encompassing all types. Within total phosphorus, there are two main forms: soluble phosphorus and particulate phosphorus. Soluble phosphorus can be further broken down into soluble reactive phosphorus and soluble unreactive phosphorus. Different forms are measured in the treatment system for various process applications, with total phosphorus being the chosen parameter for measuring effluent quality and for NPDES permit compliance. Discussed within the study is orthophosphate, also known as soluble reactive phosphorus. This is the preferred method of analysis within the system, due to the fast reaction time required during analysis.

1.3 Chemical Oxygen Demand

To measure the carbon loading, a frequently utilized water quality parameter is Biochemical Oxygen Demand (BOD₅), which measures the amount of oxygen utilized by microorganisms to break down decomposable organic material over five days. It is calculated as mg of oxygen per liter of water. COD is the measure of the amount of dissolved oxygen required to oxidize chemical organic materials. COD and BOD₅ are similar in that they are indirect measures of pollutants in a sample, which is important in water quality monitoring, for the understanding of potential pollutant loadings. COD provides a broader measurement, including both biodegradable and non-biodegradable substances. COD as the primary parameter for the determination of carbon loading in wastewater has gained acceptance, as the ability to fractionate the samples allows for better understanding of the biodegradation and assists in the design and further optimization of treatment facilities (Myszograj, 2016). It is found that bCOD and tCOD of the influent of a wastewater treatment plant can provide more information regarding the

treatment system's possibilities compared to more traditional parameters, such as suspended solids or BOD₅ (Roeleveld & van Loosdrecht, 2002). The relationships of these fractions to tCOD are visually portrayed in Figure 1.4, with the dashed blue line representing the biodegradable portion of tCOD. This fraction is of most interest for this study due to its influence on the PAOs.

To understand how effective biological treatment systems are, it is important to understand the portion of the waste that is non-biodegradable, which can be of most difficulty to treat, and does not provide the added value that readily available carbon does for EBNR. The removal of phosphorus and nitrogen is influenced by the dynamics of the system, including the oxygen demand, sludge age, and kinetic parameters, which are all influenced by the carbon loadings of the facility (Myszograj, 2016).

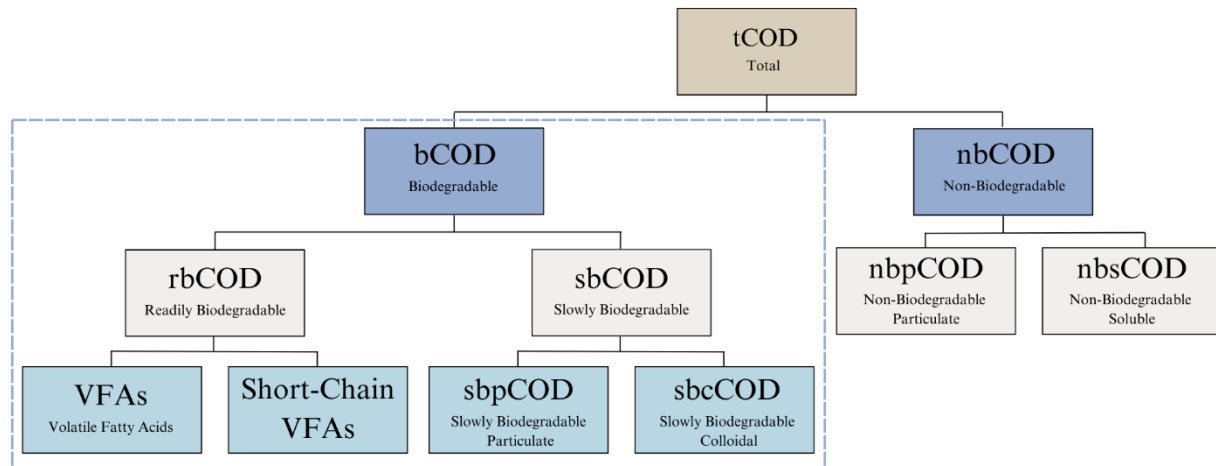


Figure 1.4 Breakdown of COD fractions

Total COD is a measurement utilized in treatment system design and operation to understand the complete amount of oxygen required to oxidize the chemical organic matter. Of all portions of COD, the biodegradable soluble portion is of most interest, as heterotrophic bacteria can assimilate it and are vital for bioreactors' efficient operation, as well as its ability to

inform mathematical process modeling. (Myszograj, 2016). For the PAOs, which prefer readily available carbon sources, such as the VFAs, this fraction is vital for ensuring adequate food sources are available. Supplemental sources can be implemented in systems where the level of readily available carbon limits treatment efficiencies. Dosing supplemental chemicals can be costly, requiring the process operator to thoroughly understand the current available carbon in order to calculate the necessary supplemental amount needed. To find the various fraction concentrations, an accurate and practical sample preparation method is required before analysis.

1.3a Method Determination

A major difference between COD and BOD₅ testing is the length of time required to analyze a sample. COD requires approximately 3 hours to complete, whereas BOD₅ requires at least 5 days. For samples that require time-sensitive analysis, COD is often the chosen parameter. BOD is useful for understanding the potential impacts on aquatic life from effluents, whereas COD is most useful for assessing overall water quality and monitoring treatment processes. A method of testing had to be determined to evaluate the practical application of COD fractionation as a process control parameter. Two primary methods for fractionation are possible: physiochemical and respirometry. Various methods can be utilized to determine the same share of fractions with varying degrees of accuracy. However, none are devoid of errors (Myszograj, 2019).

Physiochemical methods utilize the physical particle size and coagulation and flocculation properties of the wastewater to determine soluble and slowly biodegradable portions of the total COD of the waste stream. The concentration of the target COD portion is then tested using colorimetric testing, which uses sample digestion and spectrophotometry to determine the milligrams of oxygen consumed per liter of sample. A disadvantage of using a physicochemical method is the lack of a formal definition of colloidal organic matter. (Drewnowski, 2019).

Although physicochemical methods are relatively simple, results can show significant differences depending on the filter pore size utilized. It has been shown that utilizing a pre-flocculation step before filtration reduces the importance of filter pore sizes, which is the method utilized within this study (Choubert et al., 2013). This future consideration will be discussed further regarding the standardization of the physicochemical method. It was found in a 2019 study by Pluciennik-Koropczuk & Myszograj, that the particle sizes found most often in raw wastewater were between 0.22 to 1.72 μm , and 0.16 to 0.30 μm in the filtered wastewater. The particle size distribution of less than 0.45 μm confirms that using the 0.45 μm pore size is appropriate for the fraction separation. In addition, the minimal cost and readily available supplies for the physicochemical testing procedures make it a promising method for process control.

Respirometry testing is based on the principle of measuring the Oxygen Uptake Rate (OUR) as a function of time within a closed, stirred, and aerated vessel to understand how microorganisms utilize oxygen for the degradation of organic materials. Respirometry is a powerful method that can determine “true” rbCOD values while tracking advanced oxidation protocols and understanding nitrification and denitrification processes. (Alloway, n.d.). Respirometry can be utilized in waste treatment as a fast method for determining multiple kinetic parameters with one method, which allows for a better understanding of the microbial community (Mainardis et al., 2021). A drawback is the lack of respirometry protocols with varying degrees of accuracy. The lack of formalized protocols can be difficult for process control laboratories focusing on traditional pollutants and system performance parameters. An area of future development for respirometry methods is the innovative approach of combining the data with microbial community analysis, allowing the connection of system performance to the

community structure and diversity (Mainardis et al., 2021). Respirometers are known to be heavily concentrated in the research field, with academic and research institutions utilizing them for small bench-top testing. Few respirometers on the market are advertised to plant operations and process control laboratories.

Both methods have been shown to have benefits and drawbacks, with the steep upfront investment of the respirometry method influencing the studies' chosen method and the access to supplies for the physicochemical method. The study seeks to understand if the physicochemical method's low entry barrier also translates into accurate and usable data for process control monitoring and optimization efforts.

1.4 Study Site

The study took place at a water reclamation facility rated for 7.5 million gallons per day, providing advanced secondary and tertiary treatment using an enhanced biological nutrient removal process (EBNR), followed by disk membrane filtration and ultraviolet disinfection. The facility is unique because it services only domestic waste with no industrial waste. The facility is subjected to diurnal flow variations and extreme flow variations during wet weather events, primarily due to inflow and infiltration (I&I) into the collection system. An NPDES permit governs the facility and discharges its effluent into a nearby receiving stream, which is classified as Class C waters and part of a vulnerable river basin. Class C water criteria state that there should be no increase in nutrients over the background levels unless of natural variation and do not endanger human health, safety, or welfare. These waters are protected for aquatic life, for their propagation and survival, as well as for agricultural and recreational uses. (North Carolina Department of Environmental Quality, n.d.). Secondary recreational uses are those that do involve human body contact with the water, which may be activities such as wading or kayaking.

For the study facility, total phosphorus remains the most challenging nutrient removal limit to meet. For the facility, total phosphorus is reported as a loading rate over a 12-month rolling average, with a maximum average of 20.85 pounds/day, as well as a monthly average of 41.70 pounds/day. Historically, the plant has achieved, on average, a 12-month rolling average of 11.54 pounds/day. Although within compliance, this provides no room for process error, especially as treatment plant flows increase.

System owners have commissioned many process evaluation studies for consulting firms to evaluate the efficacy of the treatment process, with process changes being proposed, capital improvement schedules developed, and treatment modeling using advanced models to determine optimal system set points. Among operations staff, it has been a long-debated topic of whether the plant is carbon-limited due to the inconsistent phosphorus release and removal. Many theories have been developed for why the system can intermittently perform well and then quickly underperform. To stabilize plant conditions, solids retention time (SRT) was optimized to ensure wasting rates for the facility were allowing for a consistent 8.5 days SRT. Previous theories were disproven that there was a lack of PAO population through Microbial Community Analysis (MCA) and quantitative Polymerase Chain Reaction (qPCR) testing. Collection system samples are analyzed as part of a system-wide monitoring plan to ensure that inhibiting amounts of specific contaminants are not preventing the PAO populations from reproducing and uptaking phosphorus, including heavy metals. Jar testing was completed to optimize chemical dosages, and a heavy amount of trending was done to ensure consistent flow rates. It was discovered by plant staff through daily COD and cBOD₅ analysis that the plant experienced large swings in influent COD concentrations and loading through the weekdays. The discovery of such wide variation led to the desire to understand the COD fractions better and to learn what the readily

biodegradable portion of rbCOD, as well as the soluble portion of sbCOD, is. It was also desired to understand why the rbCOD were experiencing greater swings compared to the nbsCOD concentrations.

Understanding the various portions of available carbon will assist operations staff in determining process improvements that can be completed at a low cost and would have the greatest impact on phosphorus release and uptake. Due to the facility's unique configuration of being a 7.5 MGD facility that receives no industrial waste and does not have the required infrastructure to complete onsite fermentation, supplemental carbon feeds are the most promising solution. Supplemental carbon feeds can be costly, so proper usage and optimization of this feed are topics that need to be better understood to ensure low-cost implementation. Suppose a physicochemical COD fractionation method is found to be a practical method for rbCOD estimations through the study. In that case, the implementation of wide-scale uses throughout the facility's process control testing can be utilized in chemical feed design and optimization models. Objectives were set to guide the study and set the defining criteria for practicality.

1.5 Study Objectives

1. Compare the average rbCOD value for the experiment with literature values previously used in process evaluations utilizing Activated Sludge Models (ASM).
2. Validate field observations through statistical analysis between the influent COD fractions and the days of the week.
3. Determine if fractionation of COD utilizing physicochemical methods is a practical process control parameter for daily operations, focusing on the reproducibility of the data.

CHAPTER 2

Methods

2.1 Sample Collection

Sample collection was performed at two sample points (as shown in Figure 1.1): the treatment plant's influent and effluent. The influent sampling point is located at the headwork of the facility, post-screening, and pH stabilization, but pre-grit removal or activated sludge treatment processes. Effluent sample collection took place post-ultraviolet disinfection but pre-post aeration. Each sample was collected using an ISCO 5800 composite sampler, using a flow pace sampling program. Samplers were programmed to complete one 150 ml sample pull every 17-32 pulses, with each pulse equally 1500 gallons (influent) and 2500 gallons (effluent). The composite sample was stored in a low-density polyethylene 20-liter carboy. The sampler temperature remained between 0 to 6 °C, with the sample temperature at time of collection ranging from 5.5-8.5 °C. Samples were collected daily during the fall season, for a total number of 62 sampling days, with one sample collection missed due to mechanical malfunction. The sample period yielded 61 samples per sample site.

Total composite sample times were 24hrs \pm 1 hr. Samples were collected by certified wastewater operators in 500 ml High-density polyethylene, level 3 bottles, with 18m sulfuric acid (H₂SO₄) for preservation. Samples were returned to the laboratory within 15 minutes of sample collection. Sample collections were originally bottled with and without a preservative. If samples were not analyzed within 3 hours, they were subjected to H₂SO₄ preservative to a pH of less than two and stored at 4° \pm 2°C. The maximum hold time for a preserved sample was 28 days. Samples were accompanied by a time-flow sheet, tracking the start, end, collection, and re-start time of all composite samplers. Duplicate samples intended for ensuring proper analysis were sent to 3rd party labs, accompanied by a chain of custody, sealed in plastic sample bags, and

transported on wet ice in a sealed cooler for analysis. These samples were subjected to sample preparation onsite before delivery to the third-party lab for analysis only. Samples stayed in the possession of the operators in a secure lab fridge until relinquished through the chain of custody to the sample courier.

2.2 Sample Preparation

For fractionation of the samples, different physicochemical sample preparation steps were taken to isolate a specific portion of the sample for analysis. The various fractions include tCOD, sCOD, and ffCOD. Preparation method one was used for total COD¹ determination; the sample was thoroughly homogenized and ensured that solids remained in suspension during transfer into the testing vials. Preparation method two was used to determine soluble portions, as well as the slowly biodegradable colloidal portions² (Figure 2.1). The sample was filtered through a 0.45 μm nylon luer lock syringe filter prior to transfer into the testing tube.

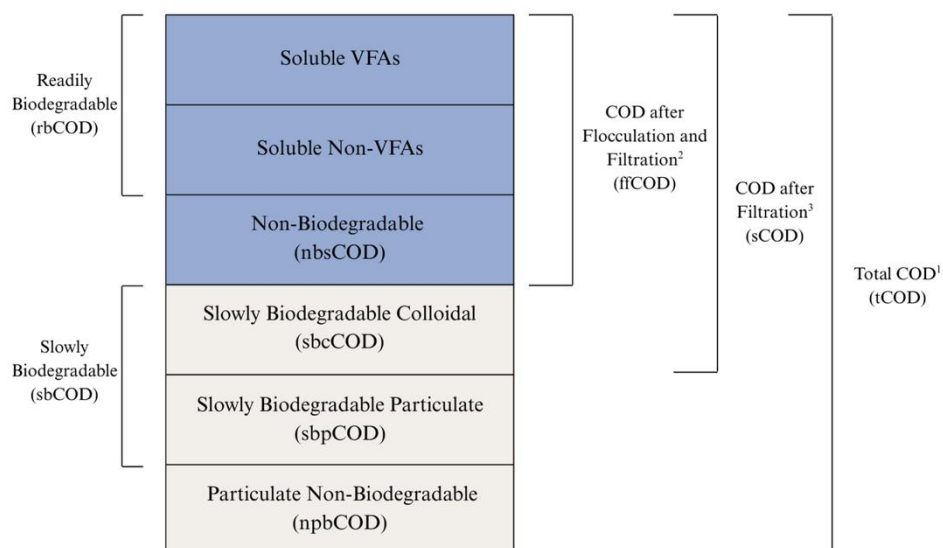


Figure 2.1 Sample preparation methods for COD fractions.

2.2a Flocculation and Filtration

The most extensive sample preparation was method three, which involved the flocculation and filtration of the sample. The purpose of method three is to utilize flocculation for the removal of colloidal and particulate materials from the analysis of the soluble portions, allowing for only the soluble portions to be brought into the estimation equation for rbCOD³. As summarized in figure 2.2, the method involved, 200 ml of sample volume was transferred into a clean 200 ml beaker with a ½ in magnetic stir bar and placed at a medium stirring speed to ensure complete mixing and suspension of all material during the addition of solutions. The samples were allowed to come to full suspension prior to the addition of 2mls of 100 mg/l Zinc sulfate (ZnSO₄). The sample was allowed to stir on the stir plate for 1 minute before the pH was adjusted to 10.5 with a 2-M solution of Sodium Hydroxide (NaOH). The sample was then allowed to stir for one additional minute before being removed from the stir plate and allowed to settle for five minutes. 10 ml of clear supernatant was then removed from the top using a 10 ml syringe, and the sample was filtered through a 0.45 µm filter into a clean, 50 ml beaker. The filtrate was then transferred into the testing tube for analysis.

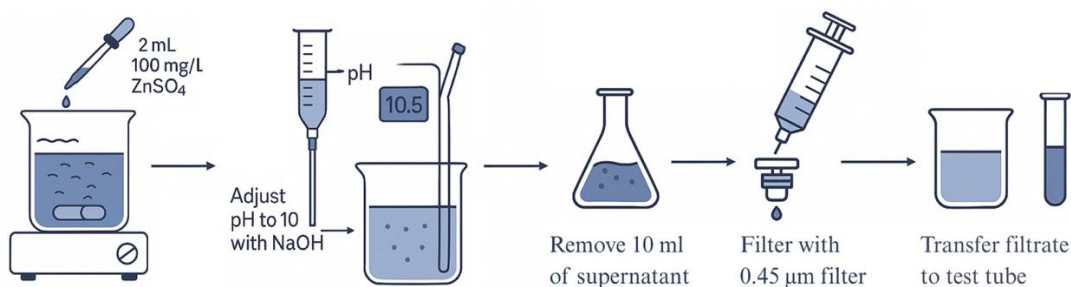


Figure 2.2 Process of Flocculation & Filtration Sample Preparation Method

2.3 Sample Analysis

After the final preparation of samples was complete, samples were analyzed for COD, using Hach TNTplus[®] 821 and 822. Hach Method 8000 is a USEPA-approved analysis method, as approved in 40 CFR 136.3(a) (Appendix E). Method 8000 is summarized by the heating of the sample for 2 hours at 150°C in the presence of H₂SO₄ and potassium dichromate (K₂Cr₂O₇). The oxidizable organic compounds react with the K₂Cr₂O₇ and reduce the dichromate ions (Cr₂O₇²⁻) to green chromic ions (Cr³⁺). Utilizing the colorimetric method, the remaining Cr⁶⁺ is measured. Care was taken to follow the prescribed method to minimize variation between analysts, and to ensure appropriate testing ranges were utilized for the required concentrations. No dilutions were required for any of the samples.

2.3a Minimizing Interference

A concern with COD analysis is chloride interference, as the chloride ions can be oxidized and consume the dichromate with the organic matter in the sample. The consumption of dichromate with the organics will result in an overestimated COD value. To minimize the possibility of interference, vials containing mercuric sulfate (HgSO₄) were utilized. Use of testing methods with mercuric sulfate (HgSO₄) was important to mitigation of possible interferences, however the method does generate mercury laden waste. Special consideration was given to ensure mercury-contaminated waste was mitigated. The minimization plans included disposing of all COD vials in a waste recycling receptacle and shipping them to a waste recycling facility in compliance with all local, state, and federal hazardous waste regulations.

2.3b Quality Control Methods

To ensure proper operation of the composite samplers, samplers were serviced at regular intervals, which included the change of suction line and pump tubing, sample strainers and counterweights, and carboys. Samplers were calibrated weekly or after each service to

ensure sample volume was $150\text{ml} \pm 15\text{ml}$. Sampler thermometers measuring ambient sampler temperature, as well as sample thermometers for measuring sample temperature at collection, are maintained by a third-party laboratory, with yearly calibrations against a NIST-approved thermometer. Operators who completed sampling wore clean nitrile gloves at each sample site, ensured the sample location was clean and free of possible sources of contamination, and only sampled either influent or effluent. In the rare event an operator needed to sample influent and effluent in the same sampling period, the operator would work from clean to dirty sample sites, beginning at the effluent. Samples were grouped in batches for ease of analysis, with all batches receiving a field blank, field duplicate, laboratory blank, and laboratory duplicate.

Laboratory duplicates were analyzed within the process control laboratory, as well as with a third-party laboratory to ensure adherence to Method 8000. Field blanks were utilized to ensure a lack of contamination from sampling techniques, with no significant findings of contamination during field collection. Laboratory blanks were utilized to look for contamination within the sample preparation or analysis procedure, specifically cross-contamination between glassware or analyzers. Of the 55 samples, 8 were accompanied by laboratory blanks.

2.4 Data Analysis

The results are displayed in mg/l of COD, defined as the milligrams of oxygen consumed per liter of sample. To analyze the feasibility objectives, readily available Excel functions were utilized to compare cost and time. Reproducibility was analyzed using a paired t-test for the paired ($P < 0.10$) duplicates that accompanied each batch. The paired t-test evaluated whether there was a significant difference between the two sets of measurements, and the correlation coefficient (<0.7) was calculated to determine the effectiveness of the pairing and the strength of their linear relationship.

2.4a Equations

For the determination of the fractions, equations were adapted from Drewnowski (2019), and primarily Myszograj (2016). Utilizing the known values of ffCOD, tCOD, nbsCOD, and sCOD from sample preparation methods 1 through 3 and the utilization of Method 8000 for concentration determination, the prescribed equations could be used to find the unknowns.

Calculations for rbCOD were conducted in Excel, and the difference between the influent's ffCOD value and the nbsCOD was calculated. An effluent sample filtered at 0.45 μm represents the nbsCOD, as shown in Figure 2.1. However, the study's scope did not allow for the implementation of sample preparation method two to be applied to the collected effluent samples. To account for the variation between the effluent's tCOD and nbsCOD in the calculation, a 0.95 correction factor was applied, as this value has been utilized across other literature studies to account for the observed difference. After the study's data collection period, further sample analysis for the effluent was conducted to verify the percentage of tCOD that is nbsCOD, and 0.95 was validated.

$$rbCOD = ffCOD - nbsCOD_{(0.95)} \quad (1)$$

Calculations for soluble Slowly Biodegradable Colloidal (sbcCOD) were conducted in Excel and consisted of taking the difference between the flocculated and filtered influent COD (ffCOD) value and the filtered COD (sCOD) value of the influent.

$$sbcCOD = sCOD - ffCOD \quad (2)$$

Calculations for the particulate portion, Slowly Biodegradable particulates (sbpCOD), and Particulate Non-Biodegradable (nbpCOD) were conducted in Excel and consisted of taking the difference between the influent sCOD value and the influent tCOD.

$$nbpCOD + sbpCOD = tCOD - sCOD \quad (3)$$

2.4b Statistical Techniques Utilized

To determine the statistically significant relationship between rbCOD estimates and the performance of the biological phosphorus removal system, analysis was completed using GraphPad Prism™ by Dotmatics. Due to the exploratory nature of this study and to maintain a desire to utilize findings to identify future study areas, a higher significance level value was selected to avoid a false negative. A significance level of 0.10 was chosen for analyzing the significance of the COD concentration among the varying days of the week and to account for the sample size as well as the exploratory nature of the study.

To better understand how the concentrations of carbon fluctuate through the days of the week, each day of the week was assigned a numerical value, with Monday as day 1, and so forth. Utilizing the daily concentration values for tCOD, sCOD, rbCOD, and sbpCOD, distribution plots were created, with violin plots chosen to provide greater detail of the distribution plot and reveal the spread and multimodal tendencies of the dataset. The violin plot lends itself well to understanding any clusters within the dataset. An ordinary one-way ANOVA test was also completed on the same data set as the violin plots, and F, P, and R values were generated. Utilizing the established P (< 0.10) & R (<0.7) values, the level of significance was evaluated for how the effects of the day of the week and the concentration of the specific COD fraction.

CHAPTER 3

Results & Discussion

3.1 Interference Minimization

Along with ensuring reproducibility, it is important to verify that interference will not prevent the method from producing accurate results. Historically, the study plant's influent has shown low to non-detectable levels of chloride, so chloride interference testing was completed three times throughout the project. Testing in the first, second and fifth week of sample collection was completed using Hach TNT[®] 879 vials (Appendix F). The chloride interference testing resulted in an average value of 45.2 mg/l, below the 2000 m/l maximum threshold for chloride interference in the ultra-low range and low-range testing methods, as reported in Method 8000. It is found that chloride was not a determining factor in the practical use of flocculation and filtration methods, however, it should continue to be a consideration in plants with historically high levels of chloride.

3.2 Quality Control

The results of the laboratory blanks are summarized in Table 2.1. Some results exceeded the method detection limit of < 25. The sample concentrations were adjusted to account for the background contamination within the glassware or sample preparation steps to ensure that the values were accounted for. The same was completed for the field blanks, which had results that exceeded the method detection limit. The average results for the field blanks were lower (26.84 ± 0.89) than the level in the laboratory blanks.

Table 3.1 *Quality Control Sample Results*

Sample Type	Value	Result	Absorbance	Unit
Field Blank	Minimum	< 25	0.05	mg/l
	Maximum	33.5	0.674	mg/l
	Average	26.84 ± 0.89	0.352	mg/l
Laboratory Blank	Minimum	< 25	0.676	mg/l
	Maximum	37.2	0.052	mg/l
	Average	28.41 ± 1.53	0.056	mg/l

3.3 Influent COD Concentrations

Using the prescribed equations, the concentrations of each portion of COD were calculated, and descriptive statistics were formulated for the individual portions (table 3.2). On average, the soluble COD, determined using the sample preparation method two, had a concentration of 199 ± 2.87 mg/l. ffCOD, which was prepared using sample preparation method three and comprised of the rbCOD and nbsCOD portions, had an average value of 166 ± 3.12 mg/l. Utilizing equation one, the rbCOD value was calculated, which comprises of the Soluble VFAs and non-VFAs. On average, the rbCOD was 139 ± 3.09 mg/l.

Table 3.2 *COD Concentrations in Plant Influent & Effluent*

Sample Type	Value	tCOD	sCOD	ffCOD	rbCOD
Influent	Minimum	415	159	126	100
	Maximum	922	257	236	209
	Average	658 ± 12.57	199 ± 2.87	166 ± 3.12	139 ± 3.09
Effluent	Minimum	21.6			
	Maximum	32.3	-	-	-
	Average	27.41 ± 0.26			

3.3a Estimated Influent COD Fractions

The estimated rbCOD value for this study was 21.64 ± 0.47 and is presented as an average percentage of rbCOD within the total COD concentration of the influent sample.

Referenced material has shown a wide range of rbCOD estimations within treatment facilities, with estimations ranging from 10 to 61.7 %. The estimated rbCOD percentage is within a 10% difference of the referenced literature, which aligned their testing method with the ATV-A131 method. The average rbCOD fraction identified in the literature, using the ATV-131 method, was 24.2 ± 4.3 (Myszograj, 2016). To align with the scope of this study, the estimated rbCOD value was also compared to a study where similar flocculation and filtration methods were utilized to estimate the rbCOD value. It was found using the coagulation-flocculation method identified in Mamais et al. (1993), the average % of COD classified as rbCOD was 22.8 (Drewnowski, 2019).

Table 3.3 *COD Fraction Percentages in Plant Influent*

Sample Type	Value	rbCOD	sbcCOD	ffCOD	sCOD	s/npbCOD	nbsCOD
Influent	Minimum	14%	0%	17%	23%	55%	3%
	Maximum	38%	45%	77%	6%	0%	0%
	Average	21%	5%	26%	31%	69%	4%
	Std. Dev	4%	3%	4%	4%	4%	1%

A key difference between Drewnowski's (2019) estimation method and the tested method is the incorporation of the heterotrophic biomass into the fractionation calculation. Due to the various constraints, such as time and cost, the heterotopic biomass was not included.

3.4 Daily Variations

To understand how physicochemical rbCOD analysis can be a practical method for COD fractionation, it is important to determine if the data produced using the method is accurate and can be used to identify trends in carbon loadings in the treatment system. The identification of trends in the carbon loadings can inform supplemental carbon addition, ensuring appropriate carbon loads for efficient phosphorus removal. Early in the process of the investigation, the facility operators noted a variation in treatment efficiency throughout the week. Based on the

carbon loadings and concentrations, it was speculated that the higher soluble portions of COD on specific days could be positively affecting phosphorus removal. Various COD fractions were compared to the day of the week for trends, with average values for influent concentration shown in mg/l in Table 3.4.

Table 3.4 Average Influent COD Concentrations for Days of the Week

Value	Sample Type	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Average	tCOD	725	648	640	643	640	633	669
	sCOD	228	203	189	183	187	198	204
	rbCOD	149	135	135	137	132	136	146
	sbcCOD	52	35	26	25	28	31	30

It was found using an ordinary one-way ANOVA and other descriptive statistics and data visualization (figure 3.1) that there is a significant difference among the means of the groups (days of the week). sCOD, tCOD, rbCOD, and sbcCOD p-values were all below $P < 0.10$, signifying that, based on the evidence, the observed differences between the sample groups are unlikely to have occurred by random chance (Table 3.5). sCOD was the most statistically significant among the days of the week ($P < 0.0001$). The second highest significant difference is sbcCOD ($P < 0.025$), followed by tCOD and rbCOD.

Table 3.5 Ordinary One-Way ANOVA Summary

Sample Type	sCOD	tCOD	rbCOD	sbcCOD
F	6.362	0.9516	0.573	2.654
P value	<0.0001	0.4665	0.75	0.025
P value summary	****	*	*	*
SD ($P < 0.10$)?	Yes	Yes	Yes	Yes
R squared	0.4141	0.09562	0.05985	0.2277

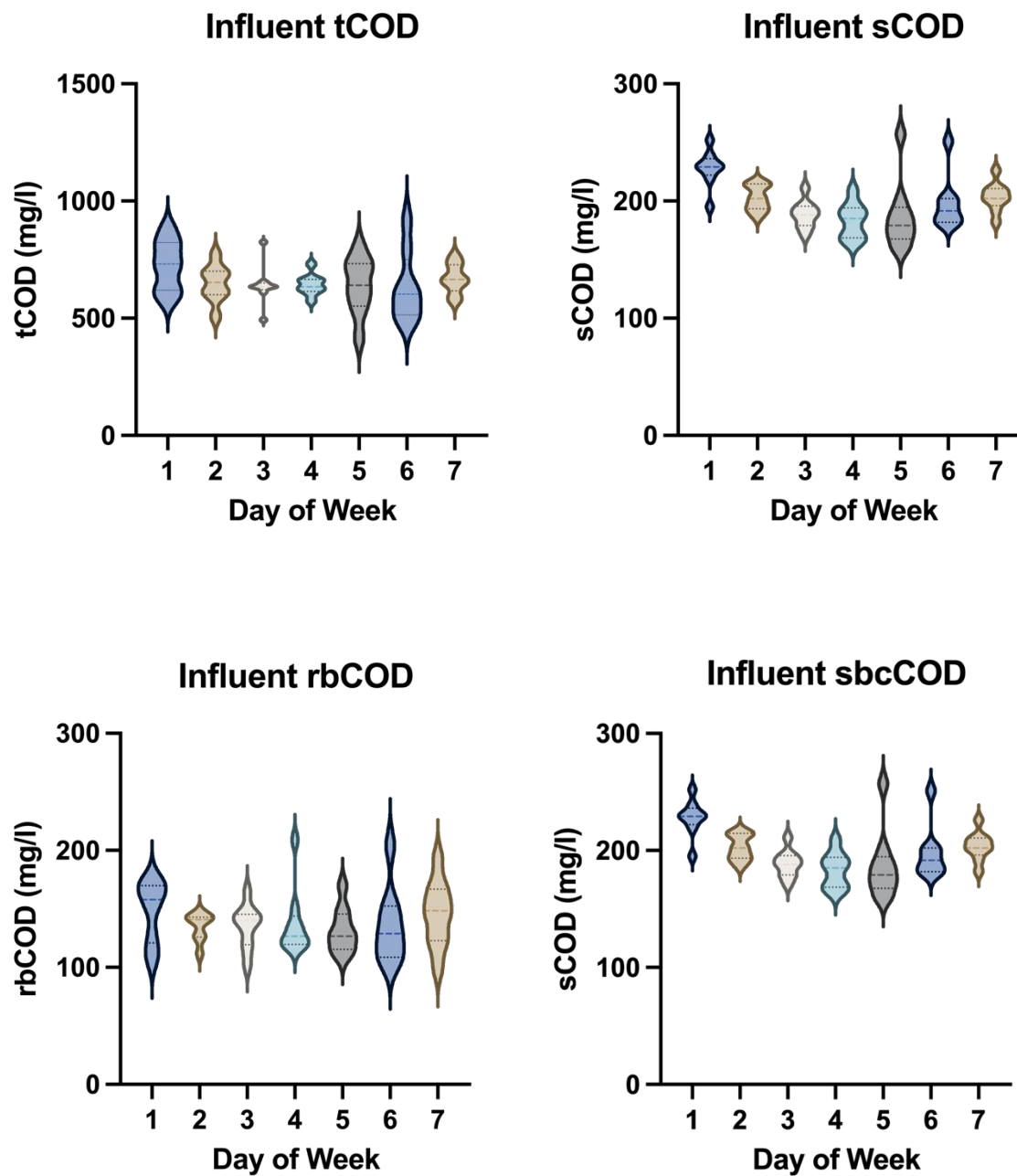


Figure 3.1 COD concentrations compared to days of the week. i.e., 1-7 is Monday through Sunday, respectively.

3.5 Reproducibility

To determine if the sample preparation method of flocculation and filtration will reproduce consistent results for the estimation of rbCOD values, each sample batch had one sample paired with a laboratory duplicate subjected to the same procedure. There was no statistically significant difference between the two groups. The two-tailed t-test ($p < 0.05$) produced a p-value of 0.678. With a strong positive correlation, $r = 0.7990$, the relationship is significant between duplicate 1 and duplicate 2, as defined by the study's set R value of < 0.7 . This meets the study's expectations. The mean difference between duplicate 1 and duplicate 2 is 1.287, with duplicate 2 being, on average, higher than duplicate 1 (Figure 3.2).

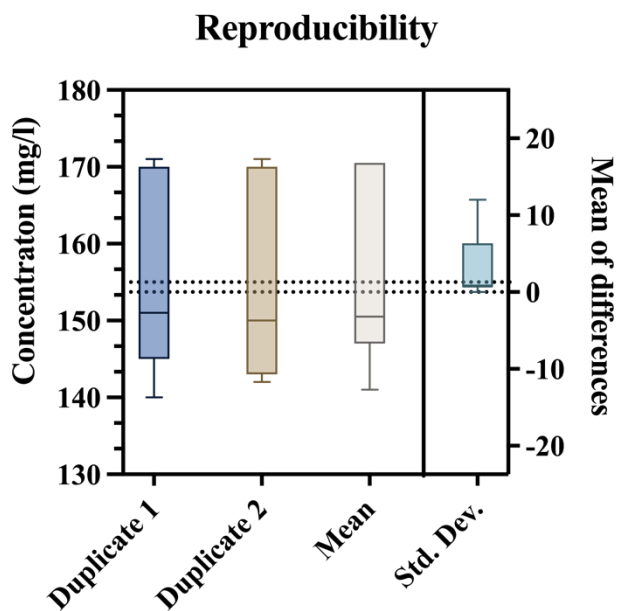


Figure 3.2 - *Reproducibility of sample preparation methods using sample duplicates.*

With a standard deviation of 7.804, the duplicate samples lacked considerable variability, indicating the sample preparation method can consistently flocculate particulate material and filter out all parts other than the soluble portions. A high level of reproducibility is essential, as this concentration value is utilized in equation 1, for determining the rbCOD concentration. Failure to produce a consistent value could result in a rbCOD value, which includes portions of sbpCOD or npbCOD. Flocculation and filtration rely on the kinetics of the particulate material to respond to the chemical treatment of Zinc Sulfate and proper pH adjustment, which is shown to be consistent through the high level of reproducibility.

3.6 Financial Impact

A major difference between utilizing a respirometer and physicochemical methods is the large capital investment for a respirometer compared to a colorimetric method. Physicochemical sample preparation and colorimetric methods utilize readily available laboratory equipment, such as stir plates, spectrophotometers, and available reagents. Method 8000 for colorimetric analysis requires consumables for each test, including reagents, filters, and testing vials. The average cost of the applied physicochemical sample preparation and colorimetric analysis is \$3.95 per test. The testing vial is the costliest consumable utilized in this study. The reagents for flocculation and the 0.45 μm filters used to remove solids and particulate matter do not pose a significant burden.

Table 3.6 *Method Consumables Cost Estimation*

Reagent	Cost per Unit	Cost per Sample
TNTplus [®] Vials (qty. 25)	\$86.2	\$3.448
Zinc Sulfate (qty. 500g)	\$133.6	\$0.053
Sodium Hydroxide (qty. 500g)	\$126.13	\$0.062
0.45 μm Filters (qty. 100)	\$38.99	\$0.389
		\$3.954

A facility's determining factor for running a physicochemical method, could be based on the available budget for the facility's process control testing. For the study site, Method 8000 is already in use daily for monitoring tCOD concentrations, so the addition of reagents for the flocculation step does not cause a significant cost increase for the facility. Utilizing the physicochemical method would allow labs to utilize equipment for COD analysis that would be consistent with EPA-approved methods, enabling them to perform testing for permit compliance. In contrast, a respirometer only provides process control data, with no approved methods of analysis to maintain discharge permit compliance. A facility that does not currently support respirometry methods could incur costs upwards of \$30,000 to set up a respirometer. It should be noted that the cost of consumables is minimal for respirometers, and there is a lack of hazardous waste generated from potassium dichromate and mercuric sulfate.

3.7 Practical Applications

Operations staff and laboratory personnel are already burdened with the vast number of samples required to be analyzed daily to ensure NPDES permit requirements are met, so the testing method must be able to be performed consistently and accurately, with close alignment to current methods. For the determination of practicality, the reproducibility and usefulness of the data were examined. A high level of reproducibility is required to ensure the data collected is defensible and can be used to inform plant process models for accurate plant optimization. The usefulness is focused on the applications of the data for plant operations staff and whether the rbCOD estimates serve as a process control parameter that can be referenced daily.

For the study site, daily or more frequent utilization of COD fractionation techniques can assist plant operations in determining the required dosage of supplemental carbon sources for phosphorus removal and assist in deciding flow management plans, including the use of equalization tanks and diversion capabilities to minimize plant loadings and flow. An important

process control parameter for treatment plants is the F to M ratio, which represents the amount of biodegradable organic matter (food) that is available to the microbial biomass in the system.

Consistent monitoring of both aspects of the equation can assist in understanding the daily ratio and better inform the required wasting rates to manage the appropriate ratios. Developing F/M trends can show when a high ratio is causing fast degradation of organic matter, leading to poor settling, or if a low F/M ratio is indicating efficient and stable treatment. Long-term trends, over periods of months to years, can inform the operation staff of future process upsets. If staff know that historically the rbCOD value is lowest during the spring season, it will allow for staff to begin adjustments of process parameters, such as Sludge Retention Time (SRT), Return Activated Sludge (RAS) rates, Hydraulic Retention Time (HRT), or the start-up of supplemental carbon feeds ahead of the anticipated process upset.

CHAPTER 4

Conclusion

4.1 Summary of Key Findings

To understand how physicochemical methods can be practically used in the fractionation of COD, the average values for the fractions were first calculated, including rbCOD, sbcCOD, ffCOD, sCOD, s/npbCOD, and nbsCOD. The percentage of rbCOD was compared to literature values, including those previously used in process evaluations utilizing Activated Sludge Models (ASM) to determine the degree of variation. The estimated rbCOD value for this study was 21.64 ± 0.47 , with reference material values ranging from 10 to 61.7 %. Literature value estimations that were used to compare in this study were, of 24.2 ± 4.3 (Myszograj, 2016) and 22.8 (Drewnowski, 2019). The study's estimated rbCOD percentage is within 10% difference of the referenced literature. This is consistent with the study facilities' generally accepted degree of variance.

To understand how the data can be practically utilized, a field observation was compared to the data derived from the sampling, which explored whether there is a statistically significant relationship between the COD fractions and the days of the week, finding that there is a statically significant difference between the days of the week. Using an ordinary one-way ANOVA a significant difference among the means of the groups (days of the week) was observed. sCOD, tCOD, rbCOD and sbcCOD p-values were all below $P < 0.10$, signifying that the observed differences between the sample groups are unlikely to have occurred by random chance (Table 3.5). This validation of field observations provides direction for future studies that would compare the average COD fractions to other process variables to determine if one fraction influences phosphorus removal more than others.

To determine if fractionation of COD utilizing physiochemical methods is a practical process control parameter for daily operations, the cost related to the method was calculated, the reproducibility and quality of the data was examined, and the ability to utilize the data to validate field observations were all compiled. It is found that physicochemical COD fractionation is a practical method of fractionating COD for process control activities, including phosphorus removal system optimization, due to the low start-up cost and the ability to utilize established colorimetric methods for the analysis after successful sample preparation, such as flocculation and filtration.

4.2 Implications & Limitations

With the understanding that this method is practical and produces defensible data, it could be applied to a large process evaluation or process monitoring plan, allowing for long-term trending of the specific COD fraction values and further feeding process models. The optimization of treatment models will allow for increased removal rates of phosphorus and a reduction in phosphorus discharged into the receiving streams, leading to improved ecosystem health.

Verifying the field observation regarding fluctuations in daily COD values among the various fractions provides new insight into the influent characteristics of the facility. This information can be used to optimize supplemental carbon feeds, which are utilized to provide additional rbCOD for the PAO populations. Due to the cost, feed rates of supplemental carbon must be carefully monitored so as not to increase the cost per gallon of treatment. Through the understanding that specific days of the week see higher concentrations of rbCOD, the feed rates of supplemental carbon could be reduced during those periods. With additional procedure development and sampling plans, the fractionation of COD could be expanded throughout the

treatment process to target specific zones and produce data that could be utilized in process modeling and developing a deeper understanding of the phosphorus removal kinetics and the amount of available rbCOD for PAO populations. Process optimization studies are often utilized with modeling software, such as BioWin from EnviroSim, which allows for the use of biological modeling, with supplemented process modeling to troubleshoot and optimize treatment processes. The models require operating inputs to simulate the process, so through the collection and analysis of specific COD fractions, they can be utilized to calibrate the process model to the facility's specific characteristics.

A limiting factor for this study was time, as there were only 3 months to perform data collection prior to analysis. This study period did allow for the capture of trends through the days of that the week. However, an additional field observation is the effect ambient that has on the process efficiency. A second limiting factor of this study was available funding, as a long collection period does incur greater cost. Due to these limitations, an estimated nbsCOD value was utilized based on literature values and known nbsCOD vs. tCOD values of the treatment plants' effluent. This limitation may impact the accuracy; however, it could be easily corrected in future studies.

Due to the limited budget, only two sample locations were included. With more resources, expanding testing to the anaerobic zones of the treatment plant would provide a better understanding of the available rbCOD values available to the PAO population within the specific selector zones. Further sampling and sample preparation procedures would need to be developed, as the sample is biologically active and quickly changing due to the present biomass. Fast evaluation would be essential to capture the current bCOD portions.

4.3 Future Considerations

4.3a Faster Analysis

With cost and time being concerns for determining the rbCOD values consistently to allow for the formation of trends, alternative technologies could be utilized to reduce the time spent analyzing the COD concentrations of the plant's influent. peCOD is a nanotechnology-based approach that lacks dichromate and mercury as compared to the HACH 8000 procedure and is faster than traditional analysis. A peCOD system, although shown to produce similar results as the colorimetric method, does have the advantage of more rapidly analyzing samples, providing results in only 10-15 minutes, as compared to 2-3 hours, allowing for real-time process adjustments.

4.3b Expand Sampling Preparation Methods

Expanding laboratory sample handling practices, including written plans and staff training, is also suggested to ensure proper sample storage and handling. Despite its expansive capabilities, the onsite process control laboratory is uncertified. It lacks written quality control plans to properly and systematically handle samples and their storage. Developing a standardized sample handling, storage, and analysis plan for COD fractionation can reduce the sample processing time. An aspect of the sample preparation method that varies among studies is the reagent used for the flocculation of particulate material in the sample preparation method three. This study utilized Zinc Sulfate; however, it is not the only metal salt that can be utilized for the precipitation of suspended solids. Further exploration of available flocculants is an area identified for future study.

4.3c Target Specific Selector Zones

If future studies take place, it is suggested that sampling be expanded to targeted selector zones within the EBNR process to address the identified limitation. Specific zones, such as the anaerobic and anoxic zones could be sampled to better understand the exact amount of readily available carbon for the PAOs to utilize. The influent analysis shows the plant loadings; however, it does not account for the dilution factor associated with RAS return into the basins, NRCY return, and its effects on rbCOD consumption. With a small sample size due to the constraints of the study, it would be beneficial to repeat or continue the sample collection and analysis to increase the sample size, as well as expand to different selector zones throughout the plant. Although not a zone within the process, this method could also be utilized in bench-top phosphorus uptake testing to fractionate the portions available to the PAOs when performing experimental process testing in a laboratory setting.

4.3d Include Seasonal Analysis

To address another limitation, expansion of seasonal analysis should be considered. Due to the plant's specific process and size, the seasons significantly contribute to the plant's treatment efficiency due to changes in rainfall and ambient temperature due to the plant's specific process and size. It is hypothesized that the increase in temperature and flow variation creates conditions within the collection system that lead to fermentation of the wastewater and an increase in VFAs in the influent. It is suggested that this field observation be validated by performing physicochemical fractionation methods at varying temperatures and weather patterns and comparing the flow, ambient temperature, rbCOD, and VFA speciation data with the plant's phosphorous removal efficiency to determine if there is a statistical significance between ambient temperature and rbCOD values.

Expanding sampling through the seasons would allow for the formation of trends related to seasonality. Through proper budgeting and staffing, this is feasible, and as shown, a practical flocculation and filtration method, such as the one utilized in this study, could yield useful data.

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APPENDICES

Appendix A*Analytical Equipment List*

Item Description	Manufacture	Serial Number	Service/ Calibration Frequency
Hach DR6000 S/N: 2180246 Lamp Hours: 64.8 Cycles: 18589 Version: 1.2	HACH	2180246	Yearly
Hach HQ411d pH/mV	HACH	Meter S/N: 190200013189 Probe S/N: 190662568978	Daily

Appendix B*Reagents List*

Item Description	Volume	Concentration	Manufacture	Lot Number
Sulfuric Acid	500ml	98%	AquaPhoenix	2GL22J076201
Sodium Hydroxide	500ml	99%		
Zinc Sulfate	500ml	99%		
TNT [®] 820 (1-60 mg/l)	N/A	N/A	Hach	
TNT [®] 821 (3-150 mg/l)	N/A	N/A	Hach	
TNT [®] 822 (20-1500 mg/l)	N/A	N/A	Hach	
TNT [®] 879 (1-1000 mg/l)	N/A	N/A	Hach	
COD Standard Solution	250	1000 m/l	Hach	A4071

Appendix C

Materials List

Item Description	Manufacture & Model	S/N	Service/ Calibration Check Frequency
Sample Bottles	Environmental Express	4108244000	N/A
1000ml Flask	-	-	N/A
500ml Flask	-	-	
200ml Beakers	-	-	N/A
10ml Beakers	-	-	
Pipettes	-	-	Daily
Pipette Tips	-	-	
KimTech Wipes	-	-	
Magnetic Stir Plate	-	-	As Needed
Magnetic Stir Bar	-	-	
Ultra-Pure Water	Direct-Q 3 - Miliport Type 1 Machine	F4DB43127C	Yearly
Analytical Balance	Scientech ZSA 210 Analytical Balance	35743	Weekly
Vortexer	OniLab MX-F	VA233AM0000403	As Needed
Thermoreactor	YSI CR2200	23060067	As Needed
Thermoreactor	YSI CR2200	24200342	As Needed
Sample Refrigerator	-	-	Daily
10 ml Luer Lock Syringes	-	-	
0.45 um Syring Filters	-	-	

Appendix D

Stock Solution Preparation

Zinc Sulfate (ZnSO₄)

Zinc Sulfate Standard – 0.620 M

- Certificate of Analysis (COA) present
 - Lot No.: 2MJ0126
 - Expiration Date: 05-30-2026
- Preparation: $0.620 \text{ mol ZnSO}_4 \times 161.44 \text{ g/mol} \times 1.0 \text{ L} = 100 \text{ g}$ of ZnSO₄ to be dissolved in 1000 ml of H₂O
 - Water temperature: 21.4 °C
 - Conductivity: 18.2 μS/cm (measured at 25 °C)

Sodium Hydroxide (NaOH)

Prepared Sodium Hydroxide Solution – 2 M

- Certificate of Analysis (COA) present
 - Lot of No.: AD-24169
- Preparation: $2.0 \text{ mol NaOH} \times 40.0 \text{ g/mol} \times 0.50 \text{ L} = 40.0 \text{ g}$ NaOH to be dissolved in 500 ml of H₂O
 - Water temperature: 21.4 °C
 - Conductivity: 18.2 μS/cm (measured at 25 °C)

Appendix E

USEPA¹ Reactor Digestion Method²

Method 8000

0.7 to 40.0³ mg/L COD (ULR); 3 to 150 mg/L COD (LR); 20 to 1500 mg/L COD (HR); 200 to 15,000 mg/L COD (HR Plus)

Scope and application: For water and wastewater. Digestion is required.

¹ Ranges 3 to 150 mg/L COD and 20 to 1500 mg/L COD are USEPA approved for wastewater analyses (Standard Method 5220 D), Federal Register, April 21, 1980, 45(78), 26811-26812.

² Jirka, A.M.; Carter, M.J., Analytical Chemistry, 1975, 47(8), 1397.

³ The ULR is only available with spectrophotometers that can measure at a wavelength of 350 nm.



Test preparation

Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter and light shield requirements for the instruments that use them.

To use the table, select an instrument, then read across to find the applicable information for this test.

Table 1 Instrument-specific information for test tubes

Instrument	Adapters	Light shield
DR6000, DR5000	—	—
DR3900	—	LZV849
DR3800, DR2800, DR2700	—	LZV646
DR1900	9609900 (D ¹)	—
DR900	4846400	Cover supplied with the instrument

Before starting

Install the instrument cap on the DR900 cell holder before ZERO or READ is pushed.

DR3900, DR3800, DR2800 and DR2700: Install the light shield in Cell Compartment #2 before this test is started.

The reagent that is used in this test is corrosive and toxic. Use protection for eyes and skin and be prepared to flush any spills with running water.

The reagents that are used in this test contain mercury. Collect the reacted samples for safe disposal.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Run one blank with each set of samples. Run all tests (the samples and the blank) with the same lot of vials. The lot number is on the container label. Refer to [Blanks for colorimetric determination](#) on page 4.

Store unused (light sensitive) vials in a closed box.

If the samples contain high concentrations of chloride, refer to the Alternate reagents section.

Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

¹ The D adapter is not available with all instrument versions.

Items to collect

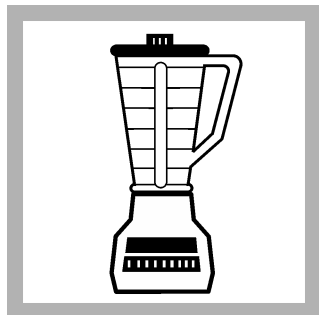
Description	Quantity
Beaker, 250-mL	1
Blender	1
COD Digestion Reagent vials	varies
DRB200 Reactor	1
Light shield or adapter (For information about sample cells, adapters or light shields, refer to Instrument-specific information on page 1.)	1
Magnetic stirrer and stir bar	1
Opaque shipping container for storage of unused, light-sensitive reagent vials	varies
Pipet, TenSette, 0.1- to 1.0-mL, with pipet tips (for use with the 200–15,000 mg/L range)	1
Pipet, volumetric, 2.00-mL	2
Pipet filler safety bulb	1
Test tube rack	2

Refer to [Consumables and replacement items](#) on page 7 for order information.

Sample collection and storage

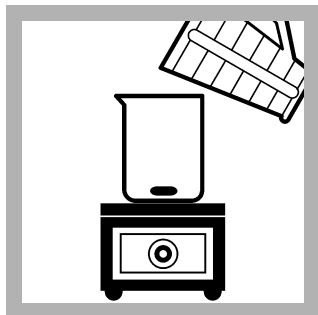
- Collect samples in clean glass bottles. Use plastic bottles only if they are known to be free of organic contamination.
- Test biologically active samples as soon as possible.
- Homogenize samples that contain solids to get a representative sample.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated sulfuric acid (approximately 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- Keep the preserved samples at 2–6 °C (36–43 °F) for a maximum of 28 days.
- Correct the test result for the dilution caused by the volume additions.

Reactor digestion procedure

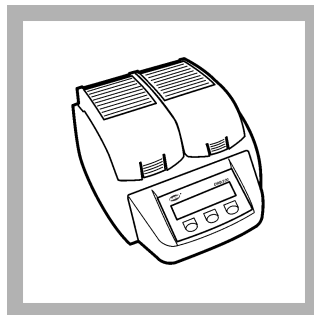


1. Put 100 mL of sample in a blender. Blend for 30 seconds or until homogenized.

For samples with large amounts of solids, increase the homogenization time. If the sample does not contain suspended solids, go to step 3.

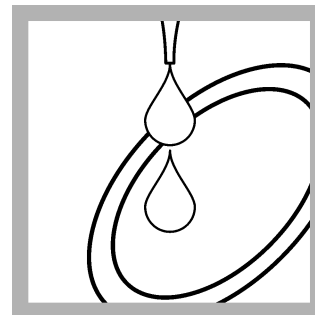


2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.



3. Set the DRB200 Reactor power to on. Preheat to 150 °C.

Refer to the DRB200 User Manual for selecting pre-programmed temperature applications.



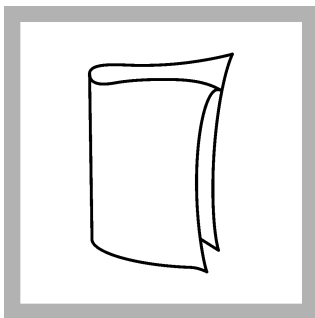
4. Prepare the sample: Remove the cap from a vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of sample to the vial.

For 250–15,000 mg/L vials: Use a TenSette Pipet to add 0.20 mL of sample to the vial.

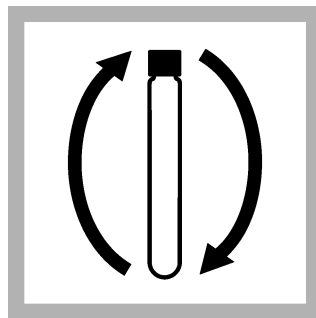


5. Prepare the blank:
Remove the cap from a second vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of deionized water to the vial.

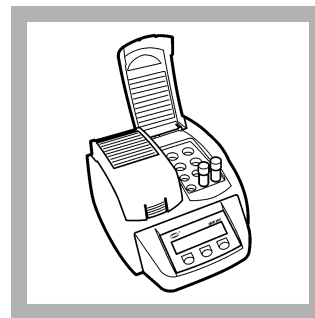
For 250–15,000 mg/L vials:
Use a TenSette Pipet to add 0.20 mL of deionized water to the vial.



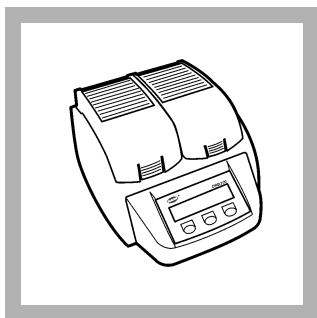
6. Close the vials tightly. Rinse the vials with water and wipe with a clean paper towel.



7. Hold the vials by the cap, over a sink. Invert gently several times to mix. **The vials get very hot during mixing.**



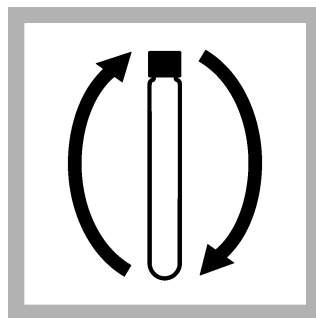
8. Put the vials in the preheated DRB200 reactor. Close the lid.



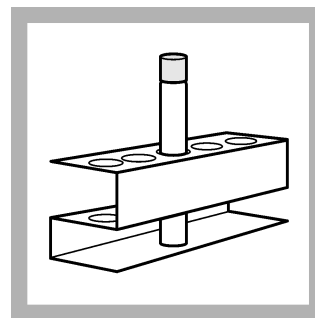
9. Heat the vials for 2 hours.



10. Set the reactor power to off. Let the vials cool in the reactor for approximately 20 minutes to 120 °C or less.

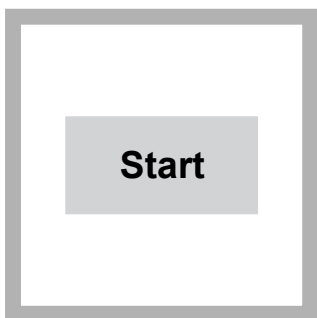


11. Invert each vial several times while it is still warm.

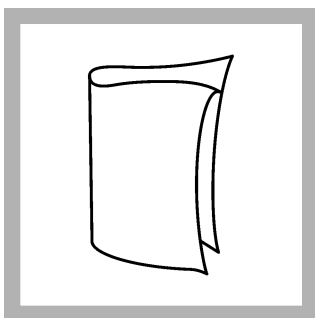


12. Put the vials in a tube rack to cool to room temperature.

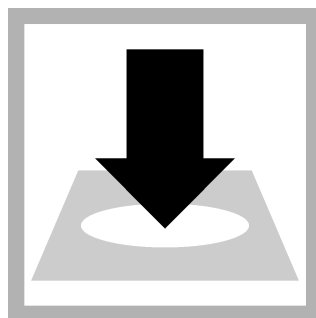
Colorimetric procedure



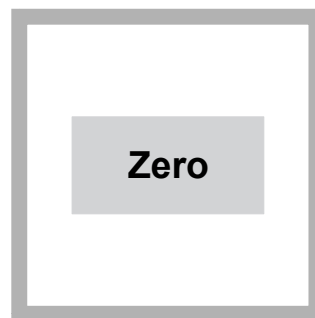
1. Start program **431 COD ULR**, **430 COD LR** or **435 COD HR**. For information about sample cells, adapters or light shields, refer to [Instrument-specific information](#) on page 1.



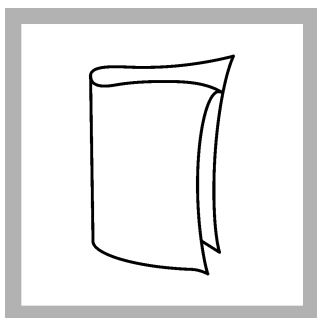
2. Clean the blank sample cell.



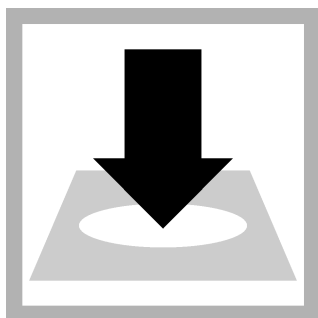
3. Insert the blank into the cell holder.



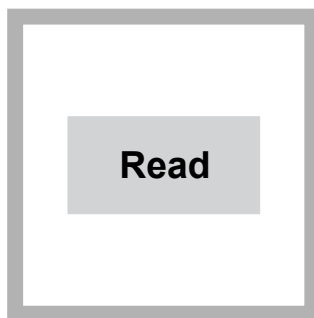
4. Push **ZERO**. The display shows 0 or 0.0 mg/L COD.



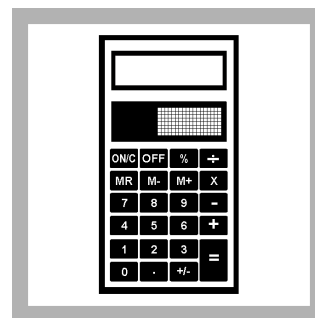
5. Clean the prepared sample cell.



6. Insert the prepared sample into the cell holder.



7. Push **READ**. Results show in mg/L COD.



8. If using High Range Plus COD digestion reagent vials, multiply the result by 10. For the most accurate results with samples near 1500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.

Blanks for colorimetric determination

The blank vial can be used again and again for measurements that use the same lot of reagent vials. Measure the absorbance of the blank vial over time and prepare a new blank vial when the absorbance changes.

1. Put the instrument in the absorbance mode at the applicable wavelength. Refer to [Table 3](#) on page 6.
2. Add 5 mL of deionized water into an empty vial.
3. Put the vial in the instrument and zero the instrument.
4. Put the blank vial that is used in the test procedure into the instrument and record the absorbance value.
5. Keep the blank vial in the dark.
6. Prepare a new blank when the absorbance has changed by approximately 0.01 absorbance units.

Interferences

Chloride is the primary interference in this test procedure. Each COD vial contains mercuric sulfate that removes chloride interference to the level specified in Column 1 of [Table 2](#). Dilute samples that have higher chloride concentrations to the level given in Column 2.

Note: For best results, use the low range and ultra-low range vials for samples that have high chloride concentrations (near maximum concentration) and low COD concentrations.

If sample dilution causes the COD concentration to be too low for accurate measurements, add 0.50 g of mercuric sulfate (HgSO_4) to each COD vial before the sample is added. The additional mercuric sulfate will increase the maximum chloride concentration to the level given in Column 3.

Note: Bromide interference is not removed with mercuric sulfate.

Table 2 Chloride concentration limits in the sample

Vial range	Column 1 (maximum mg/L Cl^-)	Column 2 (mg/L Cl^- for diluted samples)	Column 3 (maximum mg/L Cl^- with mercuric sulfate)
ULR ² (0.7–40.0 mg/L)	2000	1000	N/A
LR (3–150 mg/L)	2000	1000	8000

² The ULR is only available for spectrophotometers that can measure at a wavelength of 350 nm.

Table 2 Chloride concentration limits in the sample (continued)

Vial range	Column 1 (maximum mg/L Cl ⁻)	Column 2 (mg/L Cl ⁻ for diluted samples)	Column 3 (maximum mg/L Cl ⁻ with mercuric sulfate)
HR (20–1500 mg/L)	2000	1000	4000
HR Plus (200–15,000 mg/L)	20,000	10,000	40,000

Accuracy check

Standard solution method

Items to collect:

- 1000 mg/L COD standard solution
- 100-mL volumetric flask, Class A
- Volumetric pipets, Class A and pipet filler
- Deionized water
- Potassium acid phthalate (KHP), dried overnight at 120 °C (HR Plus only)

0.7 to 40.0 mg/L ULR

1. Prepare a 30-mg/L COD standard solution as follows:
 - a. Use a pipet to add 3.00 mL of the 1000 mg/L standard solution into a 100-mL volumetric flask.
 - b. Dilute to the mark with deionized water. Mix well.
2. Use the test procedure to measure the concentration of the standard solution.
3. Compare the expected result to the actual result.

Note: The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.

3 to 150 mg/L LR

1. Prepare a 100-mg/L COD standard solution as follows:
 - a. Use a pipet to add 10 mL of the 1000 mg/L standard solution into a 100-mL volumetric flask.
 - b. Dilute to the mark with deionized water. Mix well.
2. Use the test procedure to measure the concentration of the standard solution.
3. Compare the expected result to the actual result.

Note: The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.

20 to 1500 mg/L HR

1. Use the test procedure with a 300-mg/L, 800 mg/L or 1000 mg/L COD standard solution to measure the concentration of the standard solution.
2. Compare the expected result to the actual result.

Note: The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.

200 to 15,000 mg/L HR Plus

1. Prepare a 10,000 mg/L COD standard solution as follows:
 - a. Dissolve 8.500 g of dried KHP in 1000-mL of organic-free deionized water.
2. Use the test procedure to measure the concentration of the standard solution.
3. Compare the expected result to the actual result.

Note: The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are small variations in the reagents or instruments.

Alternate reagents

Mercury-free COD2 Reagents are available as a mercury-free alternative. These reagents are fully compatible with test procedures and stored programs in the instruments. Chloride and ammonia determinations are recommended for accurate results.

NOTICE

COD2 reagents are not approved for USEPA reporting purposes. Because COD2 reagents do not contain mercury as a masking agent, they exhibit a positive interference from chloride. More information is available for use with specific applications.

Method performance

The method performance data that follows was derived from laboratory tests that were measured on a spectrophotometer during ideal test conditions. Users can get different results under different test conditions.

Program	Standard	Precision (95% Confidence Interval)	Sensitivity Concentration change per 0.010 Abs change
431 (ULR)	30 mg/L COD	28.8–31.2 mg/L COD	0.5 mg/L COD
430 (LR)	80 mg/L COD	77–83 mg/L COD	3 mg/L COD
435 (HR)	800 mg/L COD	785–815 mg/L COD	23 mg/L COD
435 (HR Plus)	8000 mg/L COD	7850–8150 mg/L COD	230 mg/L COD

Summary of method

The results in mg/L COD are defined as the milligrams of O₂ consumed per liter of sample under the conditions of this procedure. The sample is heated for 2 hours with sulfuric acid and a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr₂O₇²⁻) to green chromic ion (Cr³⁺). When the 0.7–40.0 or the 3–150 mg/L colorimetric method is used, the amount of Cr⁶⁺ that remains is measured. When the 20–1500 mg/L or 200–15,000 mg/L colorimetric method is used, the amount of Cr³⁺ that is produced is measured. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences.

Test results are measured at the wavelengths that are specified in [Table 3](#).

Table 3 Range-specific test wavelengths

Range in mg/L COD	Wavelength
0.7–40.0 mg/L	350 nm (for applicable instruments)
3–150 mg/L	420 nm
20–1500	620 nm (610 nm for colorimeters)
200–15,000 mg/L	620 nm (610 nm for colorimeters)

Pollution prevention and waste management

Reacted samples contain mercury, silver and chromium and must be disposed of as a hazardous waste. Dispose of reacted solutions according to local, state and federal regulations. Users in the United States can use the ez COD Recycling Service for disposal of COD vials. Refer to [Consumables and replacement items](#) on page 7.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
COD, Ultra Low Range, 0.7–40 mg/L	1–2 vials	25/pkg	2415825
COD, Low Range, 3–150 mg/L	1–2 vials	25/pkg	2125825
COD, High Range, 20–1500 mg/L	1–2 vials	25/pkg	2125925
COD, High Range Plus, 200–15,000 mg/L	1–2 vials	25/pkg	2415925
Water, deionized	varies	4 L	27256

Alternate reagents and package sizes

Description	Quantity/test	Unit	Item no.
COD2, Low Range, 0–150 mg/L COD	1–2 vials	25/pkg	2565025
COD2, High Range, 0–1500 mg/L COD	1–2 vials	25/pkg	2565125
COD2, High Range, 0–1500 mg/L COD	1–2 vials	150/pkg	2565115
COD2, High Range Plus, 0–15,000 mg/L COD	1–2 vials	25/pkg	2834325
COD Digestion Reagent Vials, 3–150 mg/L COD	1–2 vials	150/pkg	2125815
COD Digestion Reagent Vials, 200–1500 mg/L COD	1–2 vials	150/pkg	2125915
COD Digestion Reagent Vials, ULR 0.7–40.0 mg/L	1–2 vials	150/pkg	2415815
COD Digestion Reagent Vials, HR plus, 200–15,000 mg/L	1–2 vials	150/pkg	2415915

Required apparatus

Description	Quantity/test	Unit	Item no.
Blender, 2-speed, 120 VAC option	1	each	2616100
OR			
Blender, 2-speed, 240 VAC option	1	each	2616102
DRB200 Reactor, 110 VAC option, 15 x 16-mm wells	1	each	LTV082.53.40001
OR			
DRB200 Reactor, 220 VAC option, 15 x 16-mm wells	1	each	LTV082.52.40001
Pipet filler, safety bulb	1	each	1465100
Pipet, volumetric, Class A, 2.00 mL	1	each	1451536

Recommended standards and apparatus

Description	Unit	Item no.
Beaker, 250 mL	each	50046H
COD Standard Solution, 300 mg/L	200 mL	1218629
COD Standard Solution, 300 mg/L	500mL	1218649

Recommended standards and apparatus (continued)

Description	Unit	Item no.
COD Standard Solution, 800 mg/L	200 mL	2672629
COD Standard Solution, 1000 mg/L	200 mL	2253929
Oxygen Demand Standard (BOD, COD, TOC), 10-mL ampules	16/pkg	2833510
Pipet, TenSette [®] , 0.1–1.0 mL	each	1970001
Pipet tips for TenSette [®] Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette [®] Pipet, 0.1–1.0 mL	1000/pkg	2185628
Potassium Acid Phthalate (KHP), ACS	500 g	31534
Stir bar, octagonal	each	2095352
Stirrer, electromagnetic, 120 VAC, with electrode stand	each	4530001
Stirrer, electromagnetic, 230 VAC, with electrode stand	each	4530002
Test tube rack, stainless steel	each	1864100
Wipes, disposable	70/pkg	2096900

Optional reagents and apparatus

Description	Unit	Item no.
Balance, analytical, 80 g x 0.1 mg 100–240 VAC	each	2936701
Flask, volumetric, Class A, 1000 mL glass	each	1457453
Flask, volumetric, Class A, 100 mL, glass	each	1457442
Pipet, volumetric, Class A, 3 mL	each	1451503
Pipet, volumetric, Class A, 10 mL	each	1451538
Sulfuric Acid, ACS	500 mL	97949
Wastewater Influent Standard Solution, Mixed Parameter, for NH ₃ -N, NO ₃ -N, PO ₄ ³⁻ , COD, SO ₄ ²⁻ , TOC	500 mL	2833149
EZ COD™ Recycling Service with 5-gal bucket-mail back option (For US customers only. 20 and 55 gallon sizes are also available.)	each	2895405
EZ COD™ Recycling Service with 5-gal bucket- pick up option. (For US customers only. 20 and 55 gallon sizes are also available.)	each	2895405P
Finger cots	2/pkg	1464702
Gloves, chemical resistant, size 9–9.5	pair	2410104 ³
Paper, for weighing, 100 x 100 mm	500/pkg	1473885
Safety goggles, vented	each	2550700
Wastewater Effluent Standard Solution, Mixed Parameter, for NH ₃ -N, NO ₃ -N, PO ₄ ³⁻ , COD, SO ₄ ²⁻ , TOC	500 mL	2833249

³ Other sizes available

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
 In the U.S.A. – Call toll-free 800-227-4224
 Outside the U.S.A. – Contact the HACH office or distributor serving you.
 On the Worldwide Web – www.hach.com; E-mail – techhelp@hach.com

HACH COMPANY
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 Telephone: (970) 669-3050
 FAX: (970) 669-2932

Appendix F

Measuring range I: 1.0–70 mg/L Cl⁻,
measuring range II: 70–1000 mg/L Cl⁻

TNTplus[®] 879—Method 10291

Scope and application: For water and wastewater.



Test preparation

Reagent storage

Storage temperature: 2–8 °C (35–46 °F)

pH/Temperature

The pH of the water sample must be between pH 3–10.

The temperature of the water sample and reagents must be between 15–25 °C (59–77 °F).

Before starting

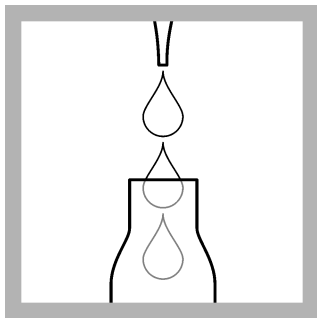
This method is applicable on DR1900, DR2800, DR3800, DR3900, DR5000 and DR6000 only.

Review safety information and expiration date on the package.

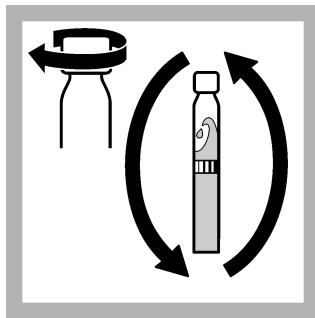
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

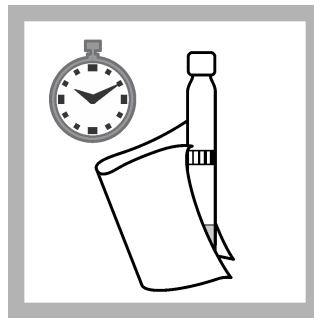
Procedure Measuring range I



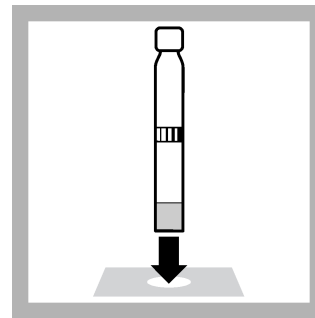
1. Carefully pipet 1.0 mL of **sample** into the **sample vial**.



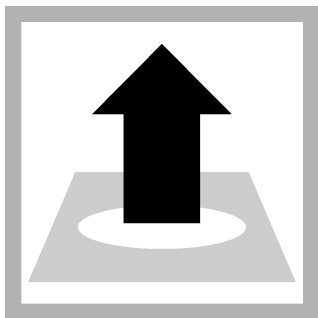
2. Close the vial and invert a few times.



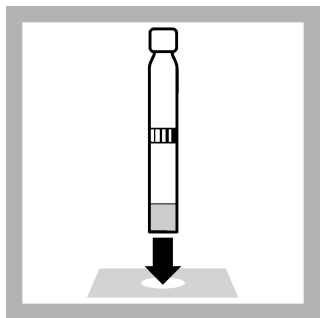
3. After **3 minutes**, thoroughly clean the outside of the **sample vial**.



4. Insert the **zero vial** into the cell holder.
DR1900: Go to LCK/TNTplus methods.
Select the test: push **ZERO**.

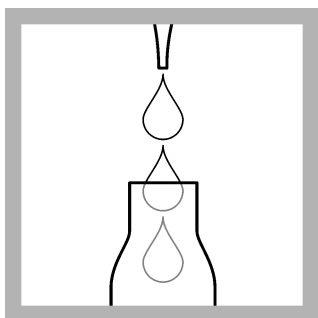


5. Remove the zero vial.

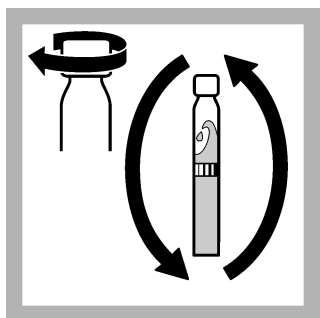


6. Insert the **sample vial** into the cell holder.
DR1900: Push **READ**.

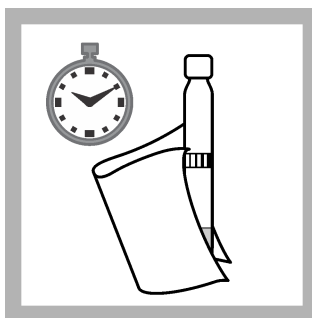
Procedure Measuring range II



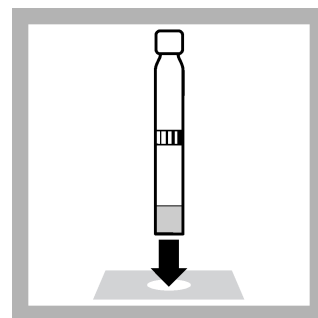
1. Carefully pipet **0.1 mL of sample** into the **sample vial**.



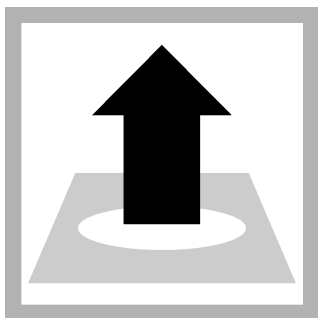
2. Close the vial and invert a few times.



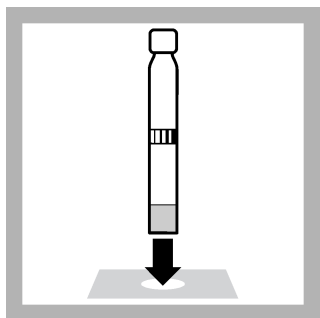
3. After **3 minutes**, thoroughly clean the outside of the **sample vial**.



4. Insert the **zero vial** into the cell holder.
DR1900: Go to LCK/TNTplus methods.
Select the test: push **ZERO**.



5. Remove the zero vial.



6. Insert the **sample vial** into the cell holder.
DR1900: Push **READ**.

Interferences

The ions listed in the table have been individually checked against the given concentrations and do not cause interference. The cumulative effects and the influence of other ions have not been determined.

Silver interferes due to the precipitation of silver chloride (low-bias results). Mercury hinders the reaction (low-bias results). Bromides and iodides, which are found in particular in many mineral waters, undergo the same reaction (high-bias results). Substances which form colored complexes with iron(III) salts interfere with the determination.

The measurement results must be subjected to plausibility checks (dilute and/or spike the sample).

Interference level	Interfering substance
1000 mg/L	SO ₄ ²⁻ , NO ₃ ⁻
50 mg/L	Pb ²⁺ , Zn ²⁺ , Ni ²⁺ , Cu ²⁺ , Cr ³⁺ , Cr ⁶⁺
10 mg/L	Cd ²⁺
0.4 mg/L	CN ⁻ , S ²⁻

Summary of method

During the reaction of chloride ions with mercury thiocyanate the slightly dissociated mercury(II) chloride is formed. Simultaneously an equivalent amount of thiocyanate ions are set free, which react with iron(III) salts to form iron(III) thiocyanate.

TNT^{III}plus[®]



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