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# Validation of mercury free methods for analysis of Chemical Oxygen Demand in municipal wastewater

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# Abstract

## Validation of mercury free methods for analysis of Chemical Oxygen Demand in municipal wastewater

*Sandra Jonsson*

Water is used every day in society and to be able to recycle this water we depend upon efficient wastewater treatment. It is vital to test the wastewater based on different parameters. One parameter is the Chemical Oxygen Demand (COD), which defines the amount of organic substances that can be chemically oxidized within the water. The Swedish standardized analytical method for COD (SS-028142), COD(Cr) is dependent on mercury, a substance which was banned according to Swedish regulations in year 2009 but is still used due to time limited dispensations.

This report is a part of a pre-procurement innovative project initiated by the Swedish Water and Wastewater Association (SWWA) in order to bring forward and evaluate mercury free analytical methods for COD for municipal wastewater. The aim was to validate three analytical methods for COD: Chloride Determination, Chloride Elimination and PeCOD and compare the analytical results to the standardized COD(Cr). Three laboratories, Käppala (Stockholm), Gryaab (Gothenburg) and Komlab (Örnsköldsvik) were included in the validation process by providing analytical data. The validation was conducted using the data as input for the statistical methods regression, correlation and analysis of variance to investigate the performance of the individual methods. As a complement to the statistical results, comments regarding the methods brought up by the laboratory staff were compiled in order to reflect on the usability and robustness of the methods.

The results indicated that the method Chloride Determination was the method most similar to the COD(Cr) method, when investigating obtained COD concentrations, required analytical time and implementation steps needed to obtain a final COD value. This result was evident by high coefficient of determination values for influent wastewater samples. The PeCOD method, which was submitted in two versions, one manual and one automatic was only able to analyze soluble COD. It was found that the PeCOD methods obtained lower COD concentrations compared to the standardized method when analyzing filtered samples. Due to highly variable correlation coefficients between the PeCOD and COD(Cr) for various types of samples indicated that no uniform linear relation between the methods was present. Analysis with the Chloride Elimination method was halted early in the validation process, but was found to receive approximately 50 percent lower COD values than the reference method COD(Cr). Finally it can be said that the input data for conducting the statistical test were limited and further analysis should be recommended in order to validate the results with a higher certainty.

**Keywords**: Chemical oxygen demand, COD, Mercury free COD, Pre-commercial innovation procurement, dichromate, wastewater, comparison validation

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## Referat

### Validering av kvicksilverfria analysmetoder för bestämning av kemiskt syreförbrukande ämnen (COD) i kommunalt avloppsvatten

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Varje dag produceras avloppsvatten i samhället och för att kunna återanvända detta vatten krävs en tillförlitlig reningsprocess. För att rena avloppsvatten effektivt är det betydelsefullt att kontinuerligt testa avloppsvattnet utifrån ett antal viktiga parametrar. En av dessa är kemisk syreförbrukning, COD, som definieras av den mängd syre som förbrukas genom fullständig kemisk oxidation av organiskt material. Den svenska standardiserade analysmetoden för COD (SS-028142) , COD(Cr) är beroende av kvicksilver för att erhålla ett korrekt analysresultat utan påverkan av kloridjoner. Kvicksilver är enligt Svensk lag förbjudet sedan år 2009, men analysmetoden är dock vanligt använd på svenska avloppsreningsverk tack vare årliga dispenser.

Detta examensarbete är en del av en förkommersiell innovationsupphandling som initierats av Svenskt Vatten med mål att undersöka och validera kvicksilverfria analysmetoder för COD tillgängliga på den internationella marknaden. Projektets syfte var att utföra en validering av tre analysmetoder: Klorid Determination, Klorid Elimination och PeCOD och jämföra dess resultat med referens metoden COD(Cr). Tre olika laboratorier, Käppala (Stockholm), Gryaab (Göteborg) och Komlab (Örnsköldsvik) medverkade i projektet. Valideringen genomfördes med de statistiska metoderna regression, korrelation och variansanalys, utifrån insamlade mätdata i syfte att undersöka de givna metodernas prestanda. Som ett komplement till det statistiska testerna sammanställdes synpunkter som framkommit under analysarbetet av laboratoriepersonal, för bedömning av metodernas användarvänlighet och robusthet.

Utifrån valideringen var det tydligt att metoden Klorid Determination hade störst likhet med COD(Cr) metoden utifrån givna analysresultat, analystid samt utförda analyssteg. Detta resultat styrktes av höga värden för determinationskoefficients för inkommande avloppsvatten mellan innovatios metoden och referense metoden COD(Cr). Analysmetoden PeCOD bestod av två olika versioner, skildrade den lösliga COD innehållet i provet istället för den total COD koncentrationen som hos COD(Cr). Oavsett vilken version av PeCOD som används erhålls ett lägre COD resultat jämfört med referens metoden COD(Cr) då filtrerade prover analyserades. De framtagna varierande korrelations koefficienter mellan PeCOD och COD(Cr) indikerade att ingen enhetlig korrelation gick att finna mellan metoderna hos de olika laboratorierna. Analysmetoden COD Elimination pausades tidigt i processen men de tidiga testerna visade på halverade COD koncentrationer jämfört med referens metoden.

Slutligen kan det nämnas att mätdata som användes som indata till de beskrivna statistiska testerna var begränsade och att vidare analyser rekommenderas för att kunna bevisa givna resultat med ökad sannolikhet.

**Nyckelord**: Kemisk syreförbrukning, COD, Kvicksilverfri COD, för-kommersiell innovations upphandling, dikromat, avloppsvatten, jämförelse validering

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# Preface

This report is a master thesis of 30 ECST conducted as a final part of the Master Program in Environmental and Water Engineering at Uppsala University. The overall supervisor for this project has been Emma Lundin at SP Urban Water Management, helping with questions regarding the project and report compilation. Dervisa Karat, Laboratory Manager at Käppala Association has been supervising and supporting the analytical work conducted within the project. Another important mentor for the validation setup has been Manuela Lopez, Laboratory Manager at Komlab and laboratory coordinator within the project, providing valuable recommendations throughout the process. Subject reviewer was Jean Pettersson, Senior Lecture at the Department of Analytical Chemistry at Uppsala University. Examiner was Anna Sjöblom, Senior Lecture at the Department of Earth Sciences at Uppsala University.

The project was conducted in collaboration with SP Urban Water Management and Käppala Association. The thesis was performed within the a pre-commercial procurement project of finding a mercury free analytical method for analyzing COD in wastewater and wastewater products initiated by the Swedish Water and Wastewater Association.

Figure 1 has been downloaded and rework from the open software provided by Lantmäteriet and are published with permission from Lantmäteriet.

To begin with I would like to thank you **Emma Lundin**, for being a steady support during this project. You have with great encouragement guided me thru decisions and problems, big and small, always prepared to answer my never ending questions and for that I am really grateful. My gratitude is also directed to **Dervisa Karat** for your patience and your positive attitude. I will always remember your words “It will be alright” with warmth inside of me. I also like to thank **Manuela Lopez** for letting me visit you and your colleagues at Komlab to learn more about laboratory work as well as discussing the project.

Furthermore I would like to thank my subject reviewer **Jean Pettersson** for your valuable guidance and recommendation throughout the project. I would also like to show my gratitude for the wonderful people that I have had the honor of working with during this project at **Käppala Association**, **SP Urban Water Management** and in other ways been involved in the pre-commercial procurement. I have not felt anything else but welcomed from day one and I wish you all the best.

Lastly I like to thank my wonderful family for always cheering me on and support me as well as **Oskar Nydahl** for being one of the most patient and loving people I know.

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# Populärvetenskaplig sammanfattning

## Validering av kvicksilverfria analysmetoder för bestämning av kemiskt syreförbrukande ämnen (COD) i kommunalt avloppsvatten

*Sandra Jonson*

När vatten används i samhället produceras avloppsvatten med olika sammansättning beroende på hur vattnet smutsats ned. Detta avloppsvatten förs sedan vidare till avloppsreningsverk, där det renas från bland annat näringsämnen och tungmetaller. En av de parametrar som är viktig för såväl reningsprocessen inom ett reningsverk och för de vattendrag som tar emot de renade avloppsvattnet att mäta är de organiska substanserna som finns i avloppsvattnet. Då organiska ämnena bryts ned förbrukas syre och om detta sker genom fullständig kemisk oxidation i vatten kallas den behövda syremängden för kemisk syreförbrukning och anges då i måttet COD (efter engelska benämningen Chemical Oxygen Demand).

Parametern COD är värdefull vid energieffektivisering och processreglering inom avloppsreningsverk. Den svenska analysmetoden som används för att bestämma COD värdet i avloppsvatten heter COD(Cr) och är beroende av miljöfarliga kemikalier så som kvicksilversulfat och kaliumdikromat. Kvicksilversulfat används för att reagera med klorid joner som är vanligt förekommande i avloppsvatten och motverka inverkan av dessa då det bidrar till ett förhöjt, falskt COD värde. Sedan 2009 är användandet, export och import av kvicksilver förbjudet i Sverige men det är än så länge tillåtet att årligen ansöka dispens från detta förbud, vilket avloppsverken hitintills gjort. Dessa dispenser har gjort det möjligt att utföra COD analyser på de svenska avloppsreningsverken, men en ny kvicksilverfri analysmetod är önskvärd från branschen. Utifrån denna önskan startade branschorganisationen Svenskt Vatten en för-kommersiell innovationsupphandling med målet att hitta kvicksilverfria COD analysmetoder som skulle kunna ersätta dagens COD(Cr) metod.

Denna studie är en del av denna upphandling och är en utvärdering mellan tre olika kvicksilverfria COD analysmetoder som inkommit i upphandlingen. Utgångspunkten i utvärderingen var att metoderna skulle kunna användas på kommunalt avloppsvatten och vara möjliga att brukas på alla avloppsverk i Sverige. Metoden skulle vara kvicksilverfri och helst även fri från andra kemikalier listade inom den Europeiska kandidatlistan för miljöfarliga kemikalier. Examensarbetet kom med bakgrund av de ställda kraven att beröra de för- och nackdelarna som metoderna hade för användarvänligheten, påverkan på miljön och hur korrekta COD värden de redovisade. Stor vikt lades även vid att utvärdera hur de olika metoderna kunde likställas med dagens analysmetod, COD(Cr) som var referensmetod i studien.

Analysmetoderna som jämfördes var Klorid Determination, Klorid Elimination och PeCOD. Metoderna Klorid Determination och Klorid Elimination, som kom från det tyska företaget Macherey-Nagel, byggde på att prover värmdes upp i små provrör där kaliumdikromat var tillsatt. Dessa provrör fick därefter svalna och genom att bestråla dem med ljus kunde man utifrån dess intensitet bestämma COD koncentrationen. PeCOD metoden var utvecklad av det kanadensiska företaget ManTech och var en elektrokemisk metod. Genom att mäta den elektriska spänningen som produceras då organiskt ämnen i ett prov bryts ned under bestrålning med UV-ljus kunde ett COD värde fastställas. PeCOD metod innehöll inga miljöfarliga kemikalier och producerade inte heller något farligt avfall. De tre metoderna testades genom att utföra analyser på så kallade standardlösningar och olika avloppsvatten. Standardlösningar kännetecknas av en lösningens som består av enbart ett ämne, exempelvis sockerämnet sorbitol, där koncentration av ämnet är känt. Genom den enklare sammansättningen i lösningen och dess kända koncentration är det lättare att utvärdera hur väl en metod kan återge rätt värde på det studerade ämnet. Provtagning och analys genomfördes på såväl standardlösningar som på inkommande och utgående avloppsvatten för att se hur olika COD koncentrationer påverkade analysresultaten hos de olika metoderna.

För att undersöka hur den geografiska spridningen i Sverige och även varierande sammansättningen på avloppsvatten skulle kunna inverka på COD resultaten, utfördes analyser vid tre olika laboratorier: Käppala Förbundet (Stockholm), Gryaab (Göteborg) och Komlab (Örnsköldsvik).

Resultaten från analyserna utvärderades med tre olika statistiska metoder som valts ut för att bundersöka olika egenskaper hos metoderna. Utifrån detta var det tydligt att Klorid Determinationsmetoden var den metod som överensstämde bäst med referensmetoden, COD(Cr). Detta gällde såväl de givna analysresultat men även tiden för analys och liknande analysmetodik. För metoden Klorid Elimination var mängden resultat till stor grad begränsad. Det var dock tydligt att denna metod gav mycket lägre COD värden än referensmetoden och inget samband mellan dessa två kunde därför fastställas. PeCOD metoden krävde filtrering ac avloppsproverna innan analys genomfördes då den inte tolererade partiklar i provet och gav därför enbart den lösliga COD koncentrationen i avloppsprovet. I studien jämfördes även två olika versioner av PeCOD-metoden, en som hanterades manuellt av laboratoriepersonal och en som var automatisk. Resultaten visade att båda versionerna av metoden producerade lägre COD värden än referensmetoden, men den manuella enheten gav dock högre värden än den automatiska metoden och hade därmed högre korrelation med COD(Cr).

Utifrån utvärderingen mellan metoderna kunde vissa rekommendationer ges för det fortsatta arbetet inom upphandlingsprojektet. En av dessa var att fortsätta utföra analyser på avloppsvatten för metoden Klorid Determination samt den manuella versionen av PeCOD metoden. Då kloridkoncentrationen i avloppsproverna som studerats varit låga, skulle det med fördel kunna tillsättas en känd mängd av klorid i proverna. Genom denna provberedning skulle det vara möjligt att undersöka hur metoderna reagerar på olika kloridkoncentrationer och hur de inverkar på COD resultatet.

Slutligen så har denna studie visat att vidare arbetet krävs för att finna en ny COD analysmetod som kan ersätta dagens alternativ, vilket anses möjligt då många forskningsprojekt genomförs inom området och då ett ökat behov finns inom branschen.

## Abbreviations

| | |
|---|---|
| **ANOVA** | Analysis of variance, statistical methods for hypothesis testing |
| **BOD** | Biological oxygen demand |
| **CEN** | European Committee for Standardization |
| **COD** | Chemical oxygen demand |
| **COD(Cr)** | The analysis method currently used for determined the COD concentration |
| **df** | Degree of freedom, a statistical notion |
| **F** | F-value, statistical notation that describes the independent set of variable in a obtained data set |
| **F crit** | The critical F-value tabulated assigned to the given number of degree of freedom for involved datasets which if exceeded means that the set null hypothesis would be rejected |
| **ISO** | International Organization for Standardization |
| **M-N CL EL** | Macherey-Nagel Chloride Elimination |
| **M-N CL DET** | Macherey-Nagel Chloride Determination |
| **MS** | Mean squares, statistical notion, the mean deviation assigned to the number of degree of freedom |
| **PCP** | Pre-Commercial Procurement |
| **TOC** | Total organic carbon |
| **SWWA** | Swedish Water and Wastewater Association |
| **SS** | Sum of squares, a statistical notion |
| **VINNOVA** | Swedish innovation Agency |

## Statistical Designations

| | |
|---|---|
| $r$ | Correlation coefficient, used in correlation analysis that represent how close invested variables are to be linear conjunctional |
| $R^2$ | Coefficient of Determination, used in regression analysis, refers to how variation in to individual data sets can be explained in each other under the assumption that a linear conjunction is present between the data sets |
| $\bar{x}$ | Mean value of the parameter x |
| $\alpha$ | Intercept of regression line |
| $\beta$ | Slope of regression line |
| $\sigma^2$ | Variance |

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# 1 INTRODUCTION

Measurements and tests are carried out every day for estimating parameters and to produce values that should be related to in everyday life. It can be the measurement of the temperature outside to get an idea of how one should dress in the morning, but it can also be testing of our drinking water to ensure that it is safe enough to drink. Our society depends on execution of daily analytical work to guarantee peoples safety and for different processes to function correctly. Chemical Oxygen Demand (COD) is a central parameter in process operation and control as well as modelling of wastewater treatment plants (WWTP). The ability to measure COD plays a crucial role in:

- Optimization in the WWTP operations regarding overall operating and detailed control strategies
- Simulation of process start-ups and commissioning
- Evaluating proposed plans for renovation and expansions
- Performing scenario analyzes with different organic load to the WWTP
- Achieving a sufficient treatment process

The standardized method for measuring COD in wastewater involves a mercury compound in order to limit the interference by chloride ions, which are often present in high concentrations in municipal wastewater. The use of mercury is regulated by Swedish law and should be completely avoided due to its extremely toxic nature (Benz et al., 2008). Swedish WWTPs are able to on a yearly basis apply for an exemption from this regulation in order to continue analyzing COD using the today standardized analytical method for COD, COD(Cr). Because of the uncertainty regarding the future allowed usage of the COD(Cr) method, the need for an analytical method for COD without the toxic and hazardous content of mercury is considered high. The COD(Cr) is currently (2015) allowed to be used according to Swedish law until 2017 (Olsson, 2014).

In order to meet the need for a new analytical method to measure the parameter COD the Swedish Water and Wastewater Association (SWWA) initiated a project called "Pre-Commercial procurement of a Mercury free COD analysis method for Wastewater and Wastewater products". This project, which is financed by the Swedish Innovation Agency, VINNOVA, has the overall goal to find a mercury free and environmental friendly analytical method for measuring COD in municipal wastewater, giving results comparable to the standardized method COD(Cr).

## 1.1 AIM

The aim was to perform a method comparison validation in order to find a suitable mercury free analytical method to analyze chemical oxygen demand (COD) in municipal wastewater. The investigation was done as a validation of three analytical methods, which were included in the innovation procurement project described above, were Käppala Association was the contracting authority.

The goal of the validation was to identify the most suitable analysis method and define its ability to represent reliable results based on predetermined specifications listed in Section 1.2. The samples used were wastewater collected from geographically varying WWTP in Sweden at Stockholm, Gothenburg and Örnsköldsvik. The three participating laboratories within the validation work were Käppala Association (Stockholm), Gryaab (Gothenburg) and KOMLAB

(Örnsköldsvik). Analytical results from wastewater samples were collected between the April and July 2015.

## 1.2 GOAL/RESEARCH QUESTIONS

The goal was to compile a first evaluation in the pre-commercial procurement (PCP) of the selected analytical methods. The requirements stated within the PCP were acting as guidelines when conducting the comparison validation and were summarized for the methods as below:

- free from mercury,
- desirably free from other chemicals listed in REACH by the European Chemical Agency
- able to correlate to the presently used COD(Cr) analysis to enable comparison to historical COD values, international benchmarking and to be used in process models which have been developed for wastewater treatment plants, independent of municipality
- able to, if possible to, generating analytical result faster than the present method, COD(Cr), which require approximately three hours,
- user-friendly and appropriate to use and handle, regarding environmental aspects
- able to execute on-line measurements in the WWTP which would be controlled regularly using analytical test performed in a laboratory environment.

Four research question where designed and these problem definitions are the cornerstones in the project.

- *What are the major differences in design between the three COD analytical methods and what are the advantages and disadvantages of the methods based on the overall established requirements?*
- *Which analytical method is considered to be the most suitable in terms of accuracy, robustness and user friendliness?*
- *Can the methods be correlated to the COD(Cr) analytical method and its historical measurement data as well as used for modelling purposes?*
- *Can one/several analytical methods in the validation replace the utilization of the today used COD(Cr) method?*

This assessment will be used as a basis for further comparison validation and also for an external midterm report within the PCP. The report is part of the dissemination of the project outcome and will be shared with stakeholders in the wastewater sector.

## 1.3 DELIMITATIONS

In order to clarify the scope of the project, this master thesis is limited by three general restrictions were made. (1)The project solely regards wastewater samples collected and analyzed during the spring and summer of year 2015. The sampling was executed by the internal staff at each of the participating WWTPs. (2) The project does not investigate the correlation between the parameters COD, Total Organic Carbon (TOC) or Biological Oxygen Demand (BOD). (3) Subsequently, discussion and further research regarding the ability to replace analysis of COD with TOC analysis in the future is excluded.

## 1.4 OUTLINE OF THE REPORT

The structure of this report is given in the following way:

Chapter 2 describes the background for the project and this section aims to describe the definition of the parameter COD and its field of application for the WWTP.

Chapter 3 is concerning the theoretical framework of the report, were the standardized analytical method used today at the laboratories, COD(Cr) and the chemicals active in this method are described in detail. The chapter also gives an introduction to the method validation and the selected statistical methods used in for providing results.

Chapter 4 is divided into three sections with the aim declare the methods that has been used throughout the study.

Chapter 5 illustrates the results given in the study and are divided based on the types of wastewater samples that have been analyzed. Additional result such as regression curves for standard solutions and laboratory observation are also a vital part of the results in the same chapter.

Chapter 6 and 7 summarized the report with a discussion and conclusion. In addition to the report, three appendixes were conducted, containing analytical data and additional representation of the results.

# 2 BACKGROUND

An important aspect in the PCP was to bring forward a sustainable COD analysis method with high reliability and robustness, with the intension of being used all over Sweden. Based on this demand, it was important to analyze varied types of municipal wastewater with different compositions taken from geological spread WWTPs. The location of the involved laboratories Käppala, Gryaab and Komlab can be seen in Figure 1. The laboratories Käppala and Gryaab are both analyzing samples from one connected WWTP, Käppala and Rya respectively. The WWTPs are equipped with mechanical, chemical and biological treatment steps ("Clean facts about Gryaab," n.d.). The Komlab laboratory however, receives wastewater samples from 28 municipal WWTPs, with various purifications techniques ("Avloppsreningsverk - Miva," 2014).

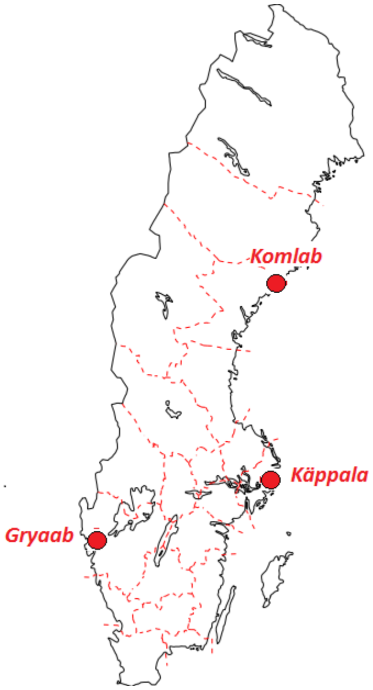

*Figure 1. Sweden, are marked for the laboratories Gryyab, Käppala and Komlab ©Lantmäteriet 2015*

The most southern participating WWTP is Rya, located in Gothenburg. Seven communities are connected to Rya which equals over seven hundred thousand people. This gives a mean inflow of approximately 4,380 liter per second ("Om Gryaab - Gryaab - för ett renare hav," 2014). The composition of the influent wastewater is mainly consisting of storm water, 61% and of wastewater from households, 35% and to a small extent generated from the industry sector, 4%. Because of the large part of storm water, the wastewater is to a great extent diluted. This can complicate the purification processes due to high flow variations depending seasons and the weather (Enache, 2015, personal communication).

The WWTP of Käppala, is as the WWTP of Rya, one of the largest in Sweden and located east of Stockholm. The WWTP receives wastewater from eleven member municipalities with a total of over half million citizens and has a mean inflow of 1,800 liter per second ("Käppala Association and the Käppala Wastewater Treatment Plant," 2011). The content of the incoming wastewater is consisting of storm water estimated as 40% and to 45% of households. The reminding part of the incoming wastewater is to 15% produced by various industries (Frenzel, 2015, personal communication).

At the Komlab laboratory, wastewater samples are received from the different municipal WWTPs, to which about 36,500 people are connected (“Avloppsreningsverk - Miva,” 2014). By using wastewater samples originated from various locations and varied composition of organic substances, the expectation was that it would compose a good basis for the validation process.

## 2.1 CHEMICAL OXYGEN DEMAND AND ORGANIC SUBSTANCES

Organic compounds in wastewater are mainly made up of the elements carbon, hydrogen and oxygen. These elements can together form several different molecule structures that can be variously difficult to oxidize or decompose. Different analytical methods can be utilized in order to determine the content of organic matter in a water sample. These methods are often divided into two groups depending on their detection limit: analytical methods that are able to measure gross concentrations of organic compounds larger than 1.0 mg/l, are often gathered as one group of methods. Other methods are aligned for identifying trace concentrations less than 1.0 mg/l. For process management and measuring organic compounds at WWTPs the gross concentrations of organic compounds are often determined in parameters such as COD, biochemical oxygen demand (BOD) and TOC (Metcalf & Eddy, 2014).

By definition, COD is a parameter that estimates the total quantity of oxygen-consuming substances during a complete chemical breakdown of organic matter in a sample using dichromate in an acid solution (Metcalf & Eddy, 2014). The COD parameter, unlike the BOD is able to represent a larger fraction of the organic compounds that is oxidized, due to a more intensive chemical oxidation. Both organic and inorganic compounds in a wastewater sample are oxidized in a COD analysis in comparison to the BOD analysis which is only able to oxidize the organic substances. Also the organic fraction in the sample may be more oxidized in a COD analysis then in a analysis for BOD, due to stronger oxidants (Miller et al., 2001). Metcalf and Eddy (2014) continue to describe that the analytical method BOD can be effected by the internal variation to a larger extent, because of its dependence on biological processes by microorganisms in the analytical method. An example of this is that some types of organic compounds may have a toxic effect on the microorganism used in the analysis. These toxic substances can inhibit a fully biological oxidation process or even kill the needed microorganisms. Another advantage that the analysis of COD possess compared to the BOD process is that it can be done in a shorter time. The Swedish standard method for analyzing COD is performed in approximately 3 hours compared to the BOD analytical methods that require seven days.

The TOC is in comparison to the other mentioned analytical methods a method that takes all the oxygen demanding components into account. The method to determining the TOC value for a sample is similar to the one for COD using a wet chemical oxidation. TOC is, however, a method that also can be used for online measurement, which for the moment is difficult to implement for the COD parameter (Metcalf & Eddy, 2014).

## 2.2 THE ROLE OF THE COD PARAMETERS IN OPERATION AND CONTROL OF WASTEWATER TREATMENT

Organic substances in wastewater can be analyzed by various methods and the most common parameters to indicate the organic concentration are, as mentioned, BOD, TOC and COD. By estimating the oxygen demand through COD and BOD in the influent and effluent wastewater, these gives an insight of the efficiency for the treatment processes within a plant. By examine the COD value for effluent wastewater, it will also give an estimated value of the content of the oxidizable substance that is released to the surrounding environment (Miller et al., 2001).

The COD parameter also plays an important role in operating a WWTP and its internal processes. COD is a stoichiometric parameter that takes into consideration the ration of which chemical substances reacts with each other. This can be used to calculate the theoretical load of oxygen demand in different purifications steps at the WWTP. Through these characteristics the extraction of methane gas produced by the anaerobic digester can be predicted. The COD is also normally used in mass balance calculations for processes within the treatment plant and is a basic parameter for the optimization of the biogas production (Thunberg, 2015, personal communication).

Another factor that makes the estimation of COD valuable, is the fact that it is the parameter most used in literature regarding waste water treatment and process management. Another benefit of COD as an indicator of oxygen demand compared to BOD, is the globally recognized definition. “The measurement of the oxygen equivalent of the organic material in wastewater that can be oxidized chemically using dichromate in an acid solution” (Metcalf & Eddy, 2014). In addition, the analysis procedure for determination of the BOD may vary between different countries and laboratories, due to various duration time for the analysis (Thunberg, 2015, personal communication). The international standard to determine BOD in a wastewater sample is $BOD_5$, which measure the consumed oxygen in a wastewater sample after a five day period in the presence of oxygen consuming microorganisms, while in Sweden the standard is to do the process over seven days (Boyles, 1997). The varying conditions in the analysis process of BOD makes the definition uncertain and dependent on where the analysis has been performed.

If the treatment of the organic substances within the wastewater is insufficient, it could have a major effect on the adjacent recipient. Oxygen demanding substances are naturally present in the aquatic environment as humus and also added naturally through the metabolism of water living plants and organisms. Oxygen demanding substances could also be added to the recipient through human disposal (“Utsläpp av syreförbrukande ämnen - Länsstyrelsen i Dalarna,” n.d.). Regardless of the origin of the organic substances, they are decomposed by microorganisms in the water under the consumption of oxygen. If the concentration of organic substances is too high in the recipient, it could lead to oxygen depletion which would hamper the survival of aquatic organisms. Oxygen depletion can occur naturally in deep lakes where the water can be divided in layer due to a temperature gradient within the water volume. However, if the emissions of oxygen consuming substances are not regulated, it will enhance the risk of oxygen depletion due to unnatural circumstances (“Utsläpp i siffror - Kemisk syreförbrukning, COD-Cr,” 2010).

# 3 THEORETICAL FRAMEWORK

This chapter aims to describe the theoretical framework for the study and are divided in four sections.

## 3.1 STANDARDS FOR COD ANALYSIS

According to the National encyclopia (2015) a standard is defined as *"a way of creating systematic order and rule-making activity in order to achieve optimal technical and economical solution to recurring problems"*. The purpose of a standard may widely vary, but is often used to create guidelines to ensure function and quality in a product or process. This is also the case with the analysis of COD in wastewater. The internationally standard used for analysis of COD is ISO-6060. It is constructed on the historically approach of analyzing COD which is an open reflux methodology, where the organic substances in the sample are oxidized while boiling in dichromate and sulphuric acid for two hours. The organic substances will then oxidized with the dichromate and the sample is then analyzed through a titrimetric detection to evaluate the remaining content of substance. The common analytical method used today is, however, the closed tube method illustrated in Figure 2 and described in the ISO standard 15705 (Lopez, 2015, personal communication). Instead of performing the digestion of the samples in a volumetric flask, the samples are pipetted in two milliliter amounts to cuvettes, which can be sealed and thereafter heated for digestion. A benefit with the closed tube method is the minimization of the waste amount of dichromate, due to prepared regents in the cuvettes (Axén and Morrison, 1994).

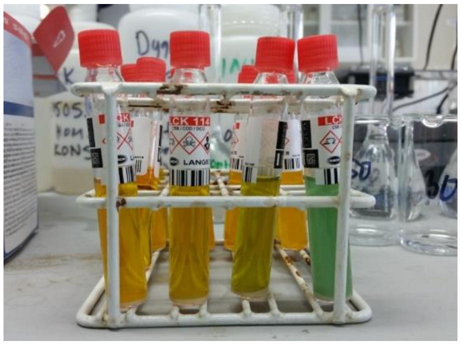

*Figure 2. The small cuvettes of COD(Cr) used for analysis of COD manufactures of the company Hach Lange. Photo: Sandra Jonsson*

The analysis to determine the parameter COD for waste water in Sweden is regulated under the national standard SS-028142 that is customized to the international standard ISO 6060 2^nd^ edition ("CODcr Lange", 2015). This analysis regarding the total COD value, includes both the soluble and the particular fraction of the organic compounds in the investigated sample. The reliability of the COD(Cr) method is determined by the composition of the waste water (SS028142).

The Swedish standard covers analysis of water containing concentrations of organic substances, resulting in a COD-value between 30-1000 mg/l. If the sample would contain a higher amount of chemically oxidizable substances it needs to be diluted. To enable a high precision of determination of the COD(Cr) value, the level of COD should be within the range of 300-600 milligrams per liter. Several chemicals are active in the COD analysis and are involved in different parts of the analytical process (SS028142). One of these chemicals is mercury sulphate that is added to reduce the interference from free chloride ions to the COD value. Chloride ions are commonly present in municipal wastewater, due to winter road deicing, wastewater from households and saltwater infusion. It is also transported to the wastewater through human urine. This type of ions represent the most common interference in the analysis of COD if not counteracted (Axén and Morrison, 1994).

In both the Swedish and international standard for measuring COD it is stated that the concentration of chloride ions should not exceed 1,000 mg/l. If a higher chloride concentration is present, it would have a great effect on the analysis result and thereby make the response of the analysis untrustable (SS028142). Chloride is oxidized in an acid solution with dichromate in the COD(Cr) analysis, but does not affect the BOD analysis method or natural oxidation processes (Axén and Morrison, 1994). Together, chloride and mercury form a soluble mercuric chlorine complex. Through this reaction, the chloride ions interference is not totally eliminated, but highly reduced. Another chemical compound used in the analysis method is potassium dichromate, which is added in a predetermined amount and are then reduced by the oxidized material within the sample (SS-028142). The residual quantity of dichromate is then measured to obtain the consumed amount of oxidants to generate the concentration of COD. By using the COD(Cr) method to determine the COD value generates remaining amounts of dichromate and mercury as analytical waste which constituting to a potential environmental hazard (Axén and Morrison, 1994).

## 3.2 CHEMICALS ACTIVE IN THE COD(CR) STANDARD

The standard analytical method, COD(Cr) is dependent on the chemicals mercury sulfate, potassium dichromate, silver sulfate and sulfuric acid in order to perform an COD analysis. Sulfuric acid is added to shorten the reaction time and silver sulfate is present as a catalyst. For this study, the selected chemicals of importance were mercury sulfate and potassium dichromate, due to their prominent role within the scope of the PCP. With this said, it is not an indication that the other chemicals included in the COD(Cr) method have an irrelevant impact to the nature or working environment for the chemist conducting the analysis.

### 3.2.1 Mercury

Mercury sulfate has a great toxic effect on both the environment and human health. Mercury is a metallic element that is liquid in room temperature. It is extracted by mining and heating of the mineral cinnabar (HgS). Mercury is used in various applications such as dental filling with amalgam, energy saving light bulbs, battery and thermometers. For humans, exposure to the substance can cause extensive damage on the nervous system, kidneys and the cardiovascular system (“Kvicksilver i sill/strömming,” 2014).

Mercury is mainly released into the environment due to the combustion of fossil fuel, but also through natural processes such as volcanic eruptions and can in the natural environment be present in different form and compounds (Mellin, 2010). The most common form in the atmosphere is mercury vapor, while in the ground or water, it is usually bound in compounds with organic matter or inorganic salt. The biggest threat to humans and ecosystems is inorganic mercury that is converted to methylmercury by microorganisms in the water, ground and bottom sediment (Sundblad et al., 2012). The fact that methylmercury is lipophilic makes it easily absorbed and bioaccumulative in living organisms (“Kvicksilver,” 2014). It is estimated that approximately 15% of the mercury that is deposit in Sweden actually originates from Sweden. The major part is therefore transported by atmospheric deposition to Sweden from other parts of the world (Sundblad et al., 2012).

The regulation of products and processes containing mercury has in recent years been restricted drastically both in Sweden and worldwide. Since the first of June 2009, a ban of usage, export and import is regulated by law in Sweden. Only a few exceptions is allowed due to the European common acts. An example is batteries and different kinds of electronic instruments (Swedish Chemical Agency, 2010). Globally, the major limiting treaty is the Mimata Convention, which was ratified the 19:th of January 2013. The convention is today signed by 140 delegates and has the overall aim to limit new establishment of and to phase out existing mercury mines (“Minamata Convention on Mercury,” 2015).

### 3.2.2 Dichromate

Potassium dichromate, $K_2Cr_2O_7$, is a salt of chromium formed by a reaction between chromium trioxide and potassium hydroxide (Castanedo-Tardan and Jacob, 2008). In the COD(Cr) method, the substance acts as an important oxidant to reduce organic matter. In this process, chromium reduces electrons, from a hexavalent valence state chromium to transform trivalent chromium ions (“COD(Cr) Lange”, 2015). Both of these states are toxic and carcinogenic and can in various extent pass through cell membranes. Inside the cells, these types of chromium form reactive intermediates that produces reactive oxygen radicals. The radicals can cause damage to the DNA, cellular proteins and lipids (Patlolla et al., 2009). Exposure to potassium dichromate is also known to generate chromium-related dermatitis, which is a type of skin inflammation (Castanedo-Tardan and Jacob, 2008).

Despite both environmental and health risks, the substance is widely used in a range of applications. It is commonly used in the production of pyrotechnics, cement, the tanning process of leather and production of matches (Castanedo-Tardan and Jacob, 2008). It has been listed on the European Chemical Agency´s candidate list since 2008 and is scheduled to be totally banned in September year 2017 (“Översyn av Utgående undantag från kvicksilverförbudet, år 2014,” n.d.). The reason is the substance mutagenic, reproductive toxicity and cancerogenic properties (Patlolla et al., 2009).

## 3.3 PREVIOUS STUDIES OF MERCURY FREE METHODS FOR ANALYSING COD

Several studies have identified and developed new analytical methods for COD in hope to replace the currently used method, COD(Cr) and thereby minimizing the use of mercury. The following section aims to summarize this studies. Even though these methods are based on various chemicals principals and design, an assumption can be made that these methods have not been able to assimilate the COD(Cr) in such a way that it could be substituted. This is based on the fact that the standardized method COD(Cr) is still used today and the reason why the PCP was initiated by the SWWA .

### 3.3.1 Ag-COD analysis method

The first method investigated was an Ag-COD analysis method similar to the Swedish standard SS028142 used today. Benito and Morrison (2003) propose an analytical approach using silver nitrate ($AgNO_3$) as the reagent substance instead of mercury sulfate, to minimize the interference of chloride ions in the wastewater sample. The method was dependent upon both potassium dichromate and sulfuric acid, two chemicals used in the standardized COD(Cr) method. It was developed to work in two different COD concentrations intervals, one for 0 to 200 mg/l and the other for 200 to 1500 mg/l. The difference was the varying content of silver nitrate in the reagent solution. The solution added for the analysis of the lower COD content contained 20% silver nitrate, while 50% were needed to determine the higher COD values. From the study, it was shown that the Ag-COD method was applicable for influent, process and effluent wastewater samples (Benito and Morrison, 2003).

The Ag-COD method was similar with the COD(Cr) method in both analytical approach and time requirements. The method was undertaken in closed tubes which was heated for two hours using a heating block. After the samples been cooled to room temperature, they were scanned using a spectrophotometer. In a comparative analysis between the COD(Cr) and Ag-COD analysis method, it was showed that the Ag-COD analysis method resulted in higher COD value then the COD(Cr) method used today (Benito and Morrison, 2003).

### 3.3.2 COD Microwave analysis method

The COD microwave method was based on a potassium dichromate oxidation in a digestion bomb which was heated in a microwave oven and developed for all types of waste water samples. The microwave method begins with carefully adding sulfuric acid into ultrapure water and thereafter adding potassium dichromate solution to the mixture. A limited volume of the wastewater sample is then put into a teflon cup together with the produced regent solution. The cup is then set into a digestion bomb, which was placed inside a household microwave with the effect of 550 Watt for two minutes. After two minutes the organic substances in the sample was completely oxidized and the sample then needs to be cooled and diluted before conducting analysis in a spectrophotometer (Axén and Morrison, 1994).

The microwave method was timesaving compared to the today used closed tube method, due to the reduced digestion time. This reduction in time was achieved due to increased pressure developed under the digestion process in the microwave. The increased pressure enables a usage of a lower sulfuric acid concentration, due to the increased boiling temperature provided by the acids which also reduced interference of chloride oxidation (Axén and Morrison, 1994).

Another factor of the reduced digestion time was that the microwave method heats up the sample evenly, unlike the COD(Cr) analysis method that only heats up the sides of the sample tube. An advantaged of the COD microwave analysis was its ability to work satisfactory and reducing the chloride interference without adding mercury sulphate. A downside was the uncertainty regarding how complete the oxidation would be if a high concentration of chloride ions were present. This made the microwave analysis method suitable for wastewater analysis with fairly low chloride concentration (Axén and Morrison, 1994). In the study, Axén and M Morrison (1994) found that chloride concentrations under 250 mg/l made chloride interference negligible.

### 3.3.3 Trivalent Manganese oxidant analysis method with chloride removal by sodium Bismuthate pretreatment

The fundamental idea behind the method was that the analysis was initiated with a homogenization of the sample that thereafter was acidified with sulfuric acid. A manganese(III) COD reagent was added to the fluid which underwent a chloride removal process. The removal system was conducted of a separation between the solid and liquid organic components in the wastewater sample instead of using mercury sulphate to reduce the impact of interference. This separation was performed to promote oxidation of chloride to chlorine using solid sodium bismuthate in the liquid phase, were the chlorine thereafter was able to evaporate. This process was executed through a vacuum added column packed with both solid sodium bismuthate and a free-flowing agent. A glass filter was placed on the top of the column to distinguish solid organic compounds in the sample from the rest of the sewage water. The sample was then forced through the column by increased vacuum pressure of -5 kilopascal relative to atmospheric pressure. Because of both the acidification of the vial and the contact area of the solid sodium bismuthate, chloride was then able to oxidize to vapor form as chlorine gas. This oxidation did not according to Miller et al (2001) effect the organic compounds in the wastewater sample. For this process to occur it was however essential that the conditions such as temperature, contact time and flowrate was checked and optimized (Miller et al., 2001).

A byproduct produced in the column was trivalent bismuthate, which gathered in the liquid phase and therefore pass though the column with the water sample. This substance was said to not affect the organic compounds in the sample and therefore had no impact on the given COD value. To clean the column the vacuum can be increased and both the liquid and the chlorine gas is then released. When the samples have undergone the above described procedure the solid organic compounds trapped on the glass fiber was finally added to the liquid phase. The mixture was then digested for one hour at the temperature of 150 degree Celsius, cooled and could thereafter be analyzed with a spectrophotometer or by titration. The manganese method had according to the authors the advantages of being quick, rather simple and economically justifiable compared to the standardized COD(Cr) method (Miller et al., 2001).

### 3.3.4 Ultrasound digestion and oxidation-reduction potential based titration

This method was based on the phenomena cavitation, which occurs when high-frequency ultrasound is subjected to a water sample and produce vacuum and compression waves (Kim et al., 2007). Cavitation occurs in a liquid when the pressure falls below the vapor pressure and the liquid will locally transform to vapor. When the pressure increase again over the vapor pressure point, a condensation of the vapor will take form (Dyne, 2015).

Under the influence of ultrasound, low-pressure bubbles are formed, implodes and release excessive energy released in the cavitation process. If the realized energy is sufficient, it has the ability to initiate various chemical reactions. In the presence of concentrated sulfuric acid and dichromate, the additional energy makes the organic substances in the sample to oxidize. The oxidation was proven to be fully developed and completed within 2 minutes by using a sonication effect of 450 Watt. After the oxidation was completed, determination of the remaining dichromate was conducted through a ferrous ammonium sulfate titration. Meanwhile, the oxidation reaction potential was measured with a silver chloride electrode in order to compute the final COD value in the sample (Kim et al., 2007)

That the ultrasound digestion method produced lower COD values than the comparing standard method. The conclusion was that the energy from the process might be too small for digesting the total amount of the organic substances in the wastewater samples which made the COD value lower. When analyzing the same sample multiple times, the error from the estimated COD value also become greater for each analysis. The source of error was characterized due to decreasing ultrasonic, but the reason for weakened ultrasonic power was not identified (Kim et al., 2007).

## 3.4 METHOD VALIDATION

To ensure that analysis and measurements provides accurate and reliable results, verification is needed and this can be done by validating an analytical method (Magnusson and Örnemark, 2014).

Two terms often used in the process of developing and evaluating new analytical methods are verification and validation, which concerns different stages within the process. Usually a validation of an analytical method is linked to the development phase of the method. It should result in a confirmation that specified demands for the tested method are fulfilled, based on the intended usage (Nilsson et al., 2000). A verification, on the other hand, is a production of evidence that shows that the method is able to meet established requirement, regardless of the intended usage (Magnusson and Örnemark, 2012).

It can be essential to perform a validation due to development of a new analytical method, or a change in an existent method. Validation is also suitable if the analytical method will to be launched on a new market (Magnusson and Örnemark, 2009).

The extent of the validation may vary depending on sector specific requirements, if the laboratory is accredited, or the purpose of the validation (Nilsson et al., 2000). An investigation of the parameters such as accuracy, measurement uncertainty and precision are common. In the ISO standard 17025, clause 5.4.5.3 the citation *"Validation is always a balance between cost, risk and technical possibilities"* summarizes the challenges and constraints valid when performing a method validation (Magnusson and Örnemark, 2014). Even if the scope may vary depending on the type of validation, it is often structured using similar specifications. The first step is to define the current requirements that should be stated for the analytical method. Requirements are then compiled to a list of demands describing what needs to be fulfilled in order to be accepted and act as a foundation for further validation (Magnusson and Örnemark, 2009).

It is important to clarify which steps that should be included in the measurement process in the validation. It is vital to map how and to what extent various steps in the execution of an analytical method counteracts to the overall accuracy and precision. In general, the validation usually covers the analysis in the laboratory, but excludes sampling and transport between the sampling site and the laboratory. The condition that the sample was in before it entered the laboratory is therefore excluded in the validation process (Nilsson et al., 2000).

After the preparatory plan of the validation is completed, the practical analytical work can begin. It is important that the personnel executing the analysis are qualified and well educated in order to minimize the human impact on the result. To examine a method precision limit, it is common to perform tests to investigate the reproducibility and repeatability. One way is, according to Magnusson and Örnemark (2009), to conduct duplicates of each analyzed sample. If the duplicates generate equivalent analytical result the repeatability is proven to be high and reliable. The reproducibility is another way of testing the precision of a method and by analyzing the same sample over several consecutive days and investigate if the analytical result may vary, depending on time of analysis. If the result is similar, then the method is said to have a high reproducibility accuracy.

Another factor is the trueness of the results. It is a parameter that indicates how close the analytical result is to the "true value" of the analyte in the sample. This can be tested by comparing the analytical result from a new method to a known method by conducting analysis using reference solutions with a known concentration (Magnusson and Örnemark, 2014). The measurement uncertainty is often unique for each analytical method, but is normally between 10-30% of the received value (Karat, 2015, personal communication).

As a final stage documenting the obtained results and conclusions in a report is needed. This should account the requirements of the analytical method, measurement data, critical factors in the validation and a summation describing the findings (Magnusson and Örnemark, 2014).

The goal is to identify the benefits and drawbacks of the analytical methods and to outline the accuracy and delimitations. A decision can hence be made regarding the suitability of the method within the intended usage area (Nilsson et al., 2000).

## 3.5 STATISTICAL ANALYSIS

One approach to compare the different analytical methods is to use several statistical methods. These methods are chosen to best compare the methods and are selected in collaboration between the author and the subject reviewers. James N Miller and Jane C Miller (2010) has been the main scientific reference for the theoretical definition of the statistical methods.

### 3.5.1 Regression

A way of comparing analytical methods, often referring to a new method and its comparability with analytical result for an established and known method, is the usage of regression lines. The reference method, should be reliable and used to detect systematic errors in the data produced by the new method. Analysis most be conducted with both methods of interest using the same samples. When plotting the line of regression, the assumption is made that errors will only be present on the y-axis. Measurements from an innovative method will therefore be on the y-axis and the analytical result provided by the COD(Cr) method on the x-axis. The assumption is that the validated methods have a higher tendency of errors in the data than the reference method. In a comparison analysis, which a line of regression follows, random errors may of course arise for both methods. Even if the assumption may not be met, regression plots is still a common used approach when comparing analytical methods according to Miller and Miller (2010).

The parameters of interest is the intercept, slope of the line and the coefficient of determination $R^2$. $R^2$ explains how well the investigated variables on the x- and y-axis correlates to each other under the presumption that a linear relation between the two is present. The regression plot is based on the linear equation, where the slope is denoted as $\beta$ and the intercept of the line as $\alpha$ below in equation (1).

$$y = \beta x + \alpha \tag{1}$$

When comparing two different analytical methods, the optimal outcome is to achieve an intercept value close to zero, and a slope and coefficient of determination value close to one. These resulting parametric values would then indicate that the two different methods are able to produce the same result, when analyzing the same sample. However, this is an unlikely event and by investigating the parameters individually for the methods, an overall insight in the performance of the methods can be generated.

### 3.5.2 ANOVA

A statistical technique often used to estimate and determine where and to what extent different causes of variation occur among datasets, is analysis of variance, abbreviated as ANOVA. The basic principle is that it can be used as a way to determine if a multiple of data groups differ significantly from one another by comparing and testing hypothesis regarding their mean value (Grandin, 2003). The datasets in this case can refer to known changes in a controlled parameter such as the temperature in a room measure by various kinds of thermometers. In a specific dataset, it is common to have two types of variation.

Random errors which cannot be predetermined or calculated and a type of variation, which goes under the name controlled variation. A controlled variation is a known parameter, which will be altered to determine to what extent the parameter will affect the end result. Here the main controlling parameter will be the various analytical methods used to analyze the COD content in different samples. A presumption for using the ANOVA statistical test is that the random errors must be truly random. If the errors are caused by an underlying unknown factor or trend, the result may not be random and the data cannot be used in ANOVA. Another requirement is that the data used is normal distributed.

A test that is often used in comparison studies is the statistical test, t-test, which like the ANOVA also uses the mean value of different datasets to determine whatever they are significantly separated from each other. The difference in these two test and the reason why ANOVA is favorably in this study is the accumulation of errors due to repeated calculations of the t-test. The type of error handed can lead to that the null hypothesis can be rejected even though its true (Miller and Miller, 2010). According to Grandin (2003), if one would perform repeated t-test on the same dataset, this error would be five percent in the first run and thereby increase in the following tests, based on a 95% confidence interval. By ANOVA, the accumulation of error is then avoided and the accuracy for the resulting parameters will be higher. The ANOVA test is less sensitive to irregularities in the data if it is not completely normal distributed than the t-test, which is a benefit if the data are limited.

An ANOVA is performed by setting up a null hypothesis which says that the involving sets of data do not significantly differ from each other. The principal of the test is to examine variation both between and within each data group (Grandin, 2003). From these parameters it can be establish if the variance within the individual datasets is smaller than the variance between the separate data groups. If this is the case and if the between-samples-variance are greater, then the null hypothesis can be rejected. To be able to examine which type of variance is greater then the other Miller and Miller (2010) recommend to combine the ANOVA with a F-test. An F-test measures the ratio between the variances and the result is then compared to a critical tabulated F-value, to verify if the null hypothesis is true or could be rejected. For further description regarding the F-test, see Section 3.5.3.

For further description and review regarding calculations used i ANOVA, see Appendix D.

In a report commissioned by the Swedish Environmental Protection Agency regarding guidelines of data analysis for statistical users, an important rule of thumb is described which can be used if the distribution of the dataset is unknown. If a dataset consist of twenty observations or more, then it can be assumed to the approximately normally distributed. This assumption is based on the mathematical regulations on the limit theorem, which plays a central role in general statistics. This enable statistical test like analysis of variance to be used even if the data population may not be perfectly normal distributed (Grandin, 2003).

### 3.5.3 F-test

To determine whether several datasets differ in precision or if one data group is more precise than another reference group, an F-test is normally used. The F-test is calculated based on the ration of variance, $\sigma^2$, of the two samples to investigate if the null hypothesis, $H_0$ is true. The hypothesis in this type of test is that the two variance values adopts the same value. The data groups in this case are results collected by different analytical COD methods. To be able to implement the F-test on the data, one need to choose between two possible types of F-test.

If the goal is to investigate if one method is more precise than another, it is suitable to use a one-sided F-test. Here the main target is to decide if the different method differ significantly in precision from one another. Then it is better to carry out a two-sided version of F-test. The difference between the two types of F-test and when to use them are based on if the outcome of the difference of variance are known in advance or not.

If for example previously studies have indicated that an analytical method A performs lower results then method B, then an assumption of the outcome can be made and therefore the one-sided F-test are the right version to use. This should be done with caution because of the assumption of high suspicion of a positive bias need to be true for receiving proper results. For the majority of cases the two-sided F-test are the most adequate and is also the one performed in this report. Both version of the F-test is performed by the same equation, which is based on the null hypothesis H0: $\sigma^2_{dataset\ 1} = \sigma^2_{dataset\ 2}$. When the F-test are integrated as a part of an ANOVA described above it will be calculated using the ratio of variance between data sets and the variance within each of the individual sets, see equation 2.

$$F = \frac{\sigma^2_{dataset\ 1}}{\sigma^2_{dataset\ 2}} = \frac{\sigma^2_{Between\ groups}}{\sigma^2_{Within\ groups}} \tag{2}$$

If a value is obtained close to one it is said to prove that the null hypothesis is true. The calculated F-value is then compared to a critical F-value, which value is depending on the version of F-test used. The critical values are obtained from tables, indicating probability and type of F-test. If the calculated F-ratio however would exceed the critical F-value, determined by the degree of freedom, than the null hypothesis is proven to be false and can therefore be rejected.

### 3.5.4 Correlation

A correlation is according to Grandin (2003) an approach to investigate if two variables are connected to each other and how strong this joint variation may be. This is practically done by producing a correlation coefficient,  . This parameter represent how close the investigated variables are to a linear relationship between one another. The correlation is calculated using the covariance divided by the product of all standard deviations for variables included in the correlation study. This calculation summarized in equation (3), taken from Miller and Miller (2010). The parameters $x$ and $y$ designate observed values from two different datasets and $\bar{x}$ and $\bar{y}$ are designations for the mean value of each set of data.  As can be seen in equation (3) the correlation coefficient is based on that all observations in a dataset are compared to the sets mean value.

$$r = \frac{\sum_i[(x_i - \bar{x})(y_i - \bar{y})]}{\{[\sum_i(x_i - \bar{x})^2][\sum_i(y_i - \bar{y})^2]\}^{1/2}} \tag{3}$$

The assumptions that are made when performing a correlation analysis are that the relation between the variables is linear and independent of each other. The data also need to be normal distributed, when performing the test using Microsoft Excel which uses the Pearson correlation test, that's only valid for normal distributed data. In this report assumptions are made that the data collected will be possible to approximate as normal distributed according explanation in section 3.5.1.

When calculating a correlation coefficient it´s value may vary between $-1 \leq r \leq +1$. If a calculated correlation coefficient is close to positive one, then the variables or datasets are said to have a strong positive correlation. This would be the case if two different analytical methods produced very similar COD values for the same samples. On the contrary if the coefficient of correlation would adopt the negative value, it would suggest that the methods are conduction opposite results. A correlation coefficient close to zero indicates that no linear trend between the variables are present.

# 4 METHOD

This chapter is divided into three parts describing the methods description for the reference method and the validated analytical methods and also a review regarding the plan of validation.

## 4.1 REFERENCE METHOD COD(CR)

The method used as a reference was the COD(Cr) method through the brand Hach Lange named Lange LCK 114/814 by the providing retailer. This following description is based on the method description used at Käppala Laboratory for the Hach Lange method ("CODcr Lange," 2015). Hach Lange is a COD cuvette method that is delivered with all the digestion chemical placed inside the cuvettes and locked with a screw cap. The chemicals used within the method are described in Section 3.1; concentrated sulfuric acid, mercury sulfate, silver sulfate and potassium dichromate. The sulfuric acid and the potassium dichromate are active in the digestion process of the organic substances when the sample is heated to 148 degrees Celsius for two hours. The silver sulfate acts as a catalysis for the reaction and the dichromate is reduced from six to three worthy chromium ions. By measuring the amount of reduced dichromate in the cuvette using a spectrometer the total COD value can be determined.

In order to counteract the interference of chloride ions in the wastewater, mercury sulfate is added in the cuvettes which are able to minimize the impact of chloride ions if the concentration do not exceeds 1500 mg/l. The wastewater samples need to be homogenized before performing analysis and if the samples are not analyzed directly they should be preserved with 4 molar sulfuric acid to a pH-value of 1-2. If no preservation is performed then the organic compounds start to decompose which would generate an incorrect value of the COD content when performing analysis.

The samples are analyzed by initially shaking the cuvettes and distributing the active chemicals within the total cuvette volume. The lid is removed and 2 milliliter of the homogenized sample is pipetted into the cuvette. The lid is screwed back on and the test tube is shaken to generate a homogeneous sample solution. Before digesting the sample in a heating block the cuvettes need to be cleaned using a napkin. After the digestion is complete the cuvettes are moved to a sample rack to cool before measuring the COD value with a spectrometer at the wavelength of 605 nm.

## 4.2 INNOVATIVE ANALYTICAL METHODS OF COD

Three innovative analytical methods were selected to take part in the validation process and are all mercury free, which were a basic requirement to participate in the procurement.

### 4.2.1 COD Chloride Detection

The analysis method COD Chloride Determination was developed by the German company MACHEREY-NAGEL and was a small cuvette method similar to the reference method (Figure 3). Overall, the method was based on a determination of the chloride concentration in the sample before analyzing the COD concentration in the sewage sample by a traditional dichromate and sulfuric acid treatment. After the analysis was completed, the estimated COD value was adjusted by subtracting the chloride concentration multiplied by a correction factor. This calculation was performed to remove the chloride interference that otherwise would result in a higher incorrect COD value.

*Figure 3. An illustration of the appearances of the small cuvettes used in the method Chloride determination and Chloride Elimination. Photo: Sandra Jonsson*

In order to generate the correct correction factor the company used standard solutions in various concentrations based on the chemical potassium hydrogen phthalate (PHP). Different known chloride concentrations were added to the PHP standard solutions. By examine the resulting COD value for the different type of solutions a correction factor could be calculated (Prokish, 2015 (personal contact)). The method reproduce the COD content in the unit mg/l and enables analysis of sample containing a COD value between 15-1500 mg/l.

The method was similar to the COD(Cr) method and utilizes the transformation of chromium which occurs when organic and inorganic matter in the sample is digested. Chromium that reacts with the matter and changes configuration from a hexavalent state to a trivalent one. These two types of chromium have different colors and their amount can therefore be determine using a spectrophotometer. The method consisted of two measurement ranges based on the expected COD concentration in the wastewater sample. For incoming wastewater with a high COD concentration the higher range was used, for COD concentrations between 150-1500 mg/l. The lower range was used for outgoing wastewater and could predict COD values from 15-160 mg/l.

Sample needed to be homogenized and preserved to a pH ≤ 2 for executing analysis with the Chloride Determination method. The samples needed to be cooled during the time between sampling and performing of analysis to prevent degradation of the organic matter. If the sample preparation was carried out efficiently the samples could be stored for up to 28 days and still provide accurate results.

The analysis was performed by measuring two ml of homogenized sample using a pipette into a cuvette tube of proper range, depending on the expected COD concentration. The tube was thereafter sealed with a lid and shaken, before placed into a heating block (Figure 4). In the heating block digestion of the sample was performed for 2 hours at temperature 148° C. The cuvette were then shaken once again and placed into a rack to cool. This was done to obtain sedimentations of particles in the sample and to minimize interference of turbidity otherwise causing a higher false COD concentration. The method was able to analyze samples containing a chloride concentration of 1000 mg/l or less, which if higher would provide a high interference.

*Figure 4. Instruments corresponding to both of the Macherey-Nagel methods, COD Chloride Determination and COD Chloride Elimination. The heating block used for digesting of the samples cuvettes on the left and the spectrophotometer used for measuring the final COD concentration in the sample to the right. Photo: Sandra Jonsson.*

When the cuvette had been cooled it was cleaned and scanned by a spectrophotometer shown in Figure 4 at the wavelength of 436 nm for samples using low range cuvettes and 620 nm for the higher COD range. The total time for analyzing COD using the Chloride Determination method was approximately 3 hours.

### 4.2.2 COD Chloride Elimination

This method was developed by the German company Macherey-Nagel. In order to perform the analysis without using mercury sulfate to counteract the interference of chloride ions the method started with a removal step of chloride ions. This removal was done by inserting the sample in a chloride elimination cartridge shown in Figure 5. The total volume of the cartridge was five milliliter and silver nitrate was present in the cartridge to react with the free chloride ions present in the wastewater.

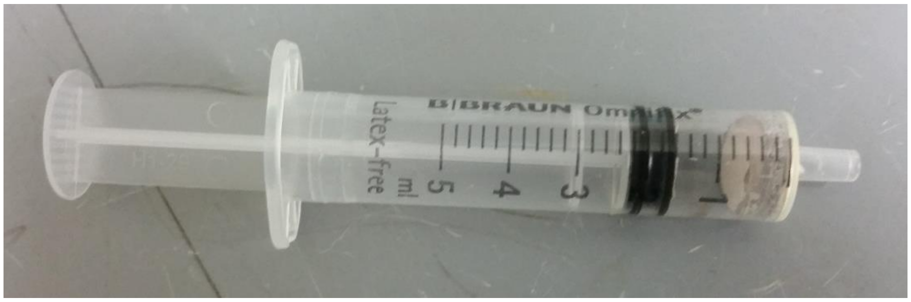

*Figure 5. Chloride Elimination cartridge used to pretreat the samples to remove free chloride ions and counteract their interference which would otherwise contribute to the final COD concentration value. Photo: Sandra Jonsson.*

The sample was firmly injected in the cartridge and tilted from side to side in 30 seconds to favor mixing between the sample and the active chemical. The sample was then pushed back through the membrane at the bottom side of the cartridge into a suitable container. Two ml of treated sample was pipetted into a small cuvette containing both potassium dichromate and sulfuric acid. The cuvette were digested in a heating block for two hours at 148 degrees Celsius, before cooled and measured by a spectrophotometer. The method was sensitive to interference of turbidity and it was therefore important that the sample had time to settle before the reading was performed.

The Chloride Elimination method was divided in two ranges. The lower range was for effluent wastewater samples containing a COD concentration from 15-160 mg/l. The higher range was limited to COD concentrations of 100-1500 mg/l. In both of these ranges, silver sulfate was used in the sample cuvettes as a catalysis to ensure an equivalent result as to the today used COD(Cr) method. The photometer as well had two different settings of wavelengths depending on the use COD range, which were 436 nm for the lower range and 620 for the higher range. This was due to the transformation of chromium and its different compositions described earlier in Section 4.2.

The difference between the two methods supplied by the company Macherey-Nagel were the way the chloride interference were treated. In the Chloride Elimination method the chloride ions were removed before analysis of COD was initiated, while the Chloride Determination uses the chloride concentration in retrospect to correct the obtained COD value.

### 4.2.3 PeCOD

The third innovation method was PeCOD L100, a photo electrochemical method developed by the Canadian company MANTECH. The method is designed to measure the soluble COD fraction in the samples by measuring and recalculate a photocurrent generated from oxidation of organic compounds in the injected sample. The method required sample to be filtrated in order to remove particular larger than fifty μm to perform analysis.

The method was based on a photo electrochemical technique that included a sensor consisting of a UV activated nano-particulated titanium dioxide ($TiO_2$) photo catalysis (Figure 6). In the beginning of an analysis the sample was inserted into a microcell, containing the sensor. UV light was irradiated on the sensor containing titanium dioxide and a potential bias was applied. This treatment of the sensor gave rise to a strong oxidizing agent (+3.1 V) that started a transfer of electrons from organic species in the sample introduced in the cell. Because of the introduced potential bias the liberated electrons were then forced into an external circuit were reduction of oxygen was executed creating a charge. By monitoring this charge it gave an indirect value of the oxidation grade of the organic compounds in the sample.

To conduct analysis, the sample needed to be mixed with an electrolyte solution made of lithium nitrate ($LiNO_3$). The proportion between sample and electrolyte solution varied depending on the COD concentration in the sample. For lower COD concentrations, as expected in effluent wastewater samples, the ratio was set to be 1:1 of sample and electrolyte solutions. For high COD content the ratio was changed to 1:9, with the mayor part being electrolyte solution. PeCOD were divided into different ranges, where the lower range was suitable for samples with COD concentrations of 15-150 mg/l. The higher range was used for samples with a greater COD content then 150 mg/l up to of 1500 mg/l (Peddle, 2015, personal contact).

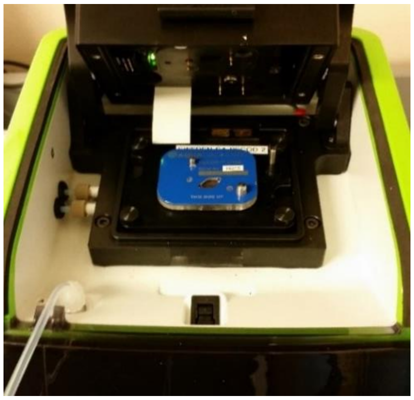

*Figure 6. The PCOD unit with open protection cover, revealing the elector block (black plate) and electro sensor (blue sensor) Photo: Sandra Jonsson.*

The company ManTech, provided two different versions of the method, one manual and on automatic system (Figure 7), where the PeCOD unit was identical for both versions. The only differences between the versions was the performance of sample preparation and how the samples were injected into the unit. For the manual PeCOD the samples needed to be mixed by a chemist with t electrolyte solution according to described quantities. When running an analysis the chemist needed to manually place the inlet hose into various beakers according to the computer software. If analyzing with the automatic PeCOD system the sample preparation would automatically be carried out in a connected vessel. The sample was then inserted in the right amount into the PeCOD unit and sensor according to the computer software, without any delay caused by human action.

*Figure 7. The manual PeCOD unit shown to the left and the automatic PeCOD system to the right, with attached pumps above and to the right of the PeCOD unit. To the right of the automatic PeCOD unit a sample holder is attached along with a sample arm used to transport sample solution from the racket into the vessel. Photo: Sandra Jonsson*

The time required to execute an analysis was approximately 15 minutes per sample, regardless version being used. The PeCOD method was free from both the substances mercury and dichromate and in the writing moment all other chemicals listed in REACH ("Kandidatförteckningen i Reach - Kemikalieinspektionen," 2015). Sample matrices such as natural water and wastewater were according to the company proven to have a good accuracy compared to the traditional COD(Cr) method. The method was also available for online measurements, which enable analysis without supervision direct in the treatment process (Peddle, 2015, personal contact).

### 4.3 PLAN OF COMPARISON VALIDATION

This section aims to describe the structure used when conducting the validation between the three innovative methods participating in the PCP. The validation is formed as a comparison validation, where the methods were compared against the reference method COD(Cr). The purpose of the validation plan was to produce measurement data using equivalent samples for each of the involved method and in this way enable statistical methods confirming the most comparable method to the COD(Cr).

The sample types that were analyzed in the validation were both influent and effluent municipal wastewater in combination with standard and control solutions. The comparison validation was initiated by performing analysis on two types of standard solutions based on the substances potassium hydrogen phthalate (PHP) and sorbitol with predetermined concentrations. By executing analyzes on standard solution it was possible to get a first insight in the performance regarding the methods due to the simplified sample matrix. This favored detection of systematic errors or bias within the analytical methods. The used standard solutions was produced from PHP or Sorbitol into five different concentrations. The concentration values were selected to cover the working range of COD values for the used methods. The details regarding the development process of the preparation of standard and control solutions which can be found in Appendix B and the regression curves are described further in Section 4.3.2

The municipal wastewater samples, standard and control solutions were used to analyze COD for all innovative methods and for the today standardized COD(Cr) through the brand Hack Lange.

In addition to the COD analysis a variety of additional analytical test were also performed according the validation plan. These test were determination of the chloride concentration, determination of suspended substances (SS) and biological oxygen demand (BOD) for each wastewater sample. The parameters BOD and the chloride concentration were analyzed at external laboratories, while SS analysis were done on the individual laboratories. To illustrate the required analysis and sample preparation within the validation process, a flowchart (Figure 8) of the plan is illustrated. As both the flowchart and the Section 4.2 indicates, different types of sample preparation were needed to enable analysis. Further description regarding the sample preparation and how the samples were collected for the three laboratories is available in Subchapter 4.3.1.

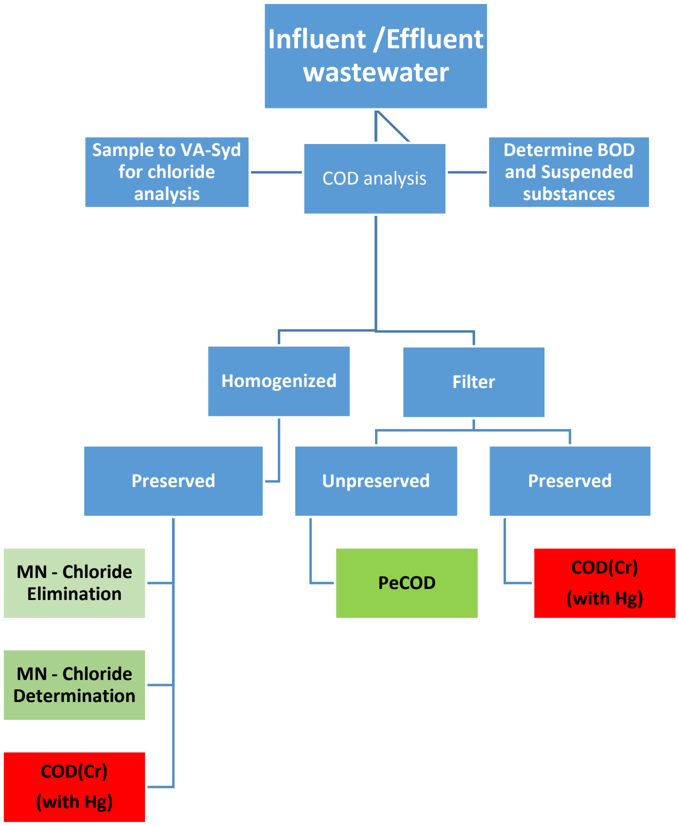

*Figure 8.Test plan for influent and effluent wastewater including the variation of sample treatment for the innovative methods.*

An analysis of SS gives the dried weight of a residue conducted on a wastewater sample. The SS includes both the inorganic and organic substances in the sample and are often combined by a residue of ignition test, which accounts the proportions between organic and inorganic matter. The chloride concentration is used to compute the final COD value for the Chloride Determination method describe in Section 4.2.1.

The additional two analysis, BOD and SS were executed in order to acquire better data to validate the PeCOD method. Because PeCOD is unable to analyze the suspended matters in the wastewater samples this parameter was intended to be used to find a correlation between filtered and unfiltered samples. The BOD analysis had according to the company ManTech historically shown a better accuracy and correlation to the PECOD than comparing the results to the COD(Cr) method. Based on wishes from the company, BOD results were therefore also presented to enable comparison to both the BOD and COD(Cr) method.

The reference method, COD(Cr) was selected for all innovative method based on the main goal of the PCP to find and evaluate a COD method which would correlate to COD(Cr). The data for the requested parameters BOD and SS were however compiled in this report as a basis for further studies within the PCP in the future.

The PCP did unfortunately encounter some delivery delays regarding the participating innovative methods. For the Macherey-Nagel methods this resulted in only receiving one of the two ranges for each method at the first delivery. The lower rage for the method Chloride Elimination and the higher range for Chloride Determination were provided within the first shipment. The two missing ranges for the Macherey-Nagel methods and the both versions of PeCOD were supplied to the project approximately one month after initial analytical work had begun. After performing analysis with the methods for a couple of weeks the decision was made to finish the validation of the Chloride Elimination method, because to divergent COD values compared to the reference method. This resulted in a more limited amount of analytical measurement for this specific method and a higher uncertainty were therefore obtained for the final statistical results.

### 4.3.1 Sampling and sample preparation

Samples of influent and effluent wastewater were analyzed weekly and consist of daily samples collected over 24 hours. The samples were then analyzed with control solutions using the three innovative methods and compared to the reference method COD(Cr). The influent wastewater was collected after the grid in the WWTP and the effluent wastewater at the outlet of the WWTP, after all treatment steps. For Käppala and Gryaab the samples were collected proportional to the flow under 24 hours. For Käppala WWTP the sampling began at midnight and continue to the following midnight, while Gryaab was starting the collection at seven in the morning and continued to the same time the following day. The collection of the samples were carried out various days of the week to include daily shifts in composition for the wastewater. In the moment of writing it has not been possible to identify how sampling has been performed on the different WWTPs from where Komlab gets their samples.

As illustrated in Figure 8 it is necessary to pretreat the sample differently depending on type of analytical method. For the methods Chloride Elimination, Chloride Determination and the reference method COD(Cr) the wastewater samples needed to be homogenized and preserved. This process was performed to receive a homogenous sample and to prevent degradation of the organic matter in the sample. This was achieved by lowering the pH value from approximately 7 to around 2, according to the standard SS-EN ISO 5667-3:2012. Samples analyzed with the PECOD method were obligated to obtain a pH value of 4-10 and could therefore not be stored for more than 24 hours and analysis were therefor needed to be perform the same day as sampling.

The sample needed to be filtrated through a glass fiber filter when analyzing with the PeCOD method. In an initial stage of the project attempts were made to order 50 micrometer glass fiber filter by ordinary laboratories supplier. Unfortunately this specific filter size was not found, so an alternative solution had to be used. Käppala and Gryaab used a glass fiber filter with a tread count of 1,6 μm. This filter is normally used separating solid matter from wastewater samples when analyzing suspended matter in a sample. Because a higher thread density were used than recommended, a smaller portion of the particulate COD was included in the sample analyzed with the PeCOD method. To examine the impact of varying filter sizes, Komlab chosen to filtered the samples with a 20 micrometer glass fiber filter instead.

### 4.3.2 Regression lines for measurement standard solutions with known concentrations

To be able to assess how much of a measured signal that truly originates from the analyte for a specific analytical method, measurement of standard solutions is often used. Standards are normally solutions made out of one single substance (Magnusson and Örnemark, 2014).

The standard solutions were prepared to obtain predetermined concentrations evenly distributed over the working range for the analytical methods. It was done to determine the methods capacity to give accurate results independent on the evaluated concentration. Two different standard solutions was produced. The first solution was made of PHP and used for the Chloride Determination, Chloride Elimination and the reference method, COD(Cr).The second were a sorbitol solution applied to the two PECOD systems, as illustrated in the flowchart in Figure 9. The reason for using two types of solutions were because the PeCOD method historically proven to enable better calibration properties using sorbitol solutions than PHP solutions.

The PHP and the sorbitol standard solutions were made into two different COD concentration ranges. The first, low range, were compiled of solutions with 15, 25, 50, 100 and 160 mg/l and tested on the lower ranges of the various methods. For the higher range, solutions with concentrations of 100, 200, 500, 1000 and 1500 mg/l were made.

The standard solutions were analyzed as duplicates and a mean value was then calculated from the two obtained values and used as an input for the regression lines. These graphs were performed by plotting the analytical data for the various methods on the y-axis against the theoretical calculated concentrations on the x-axis.

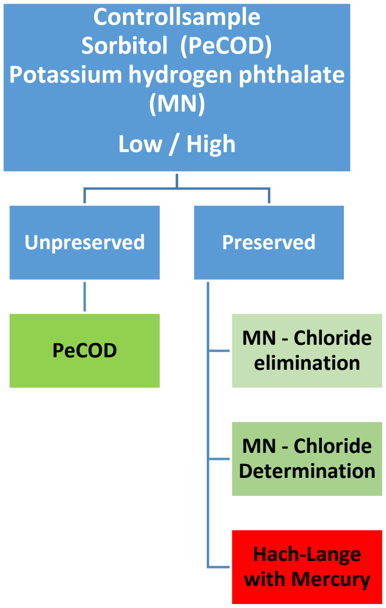

*Figure 9. Test schedule for sample preparation of standard solutions used for generating a five point regression line for the various methods using standard solutions.*

The two substances PHP and sorbitol were also used to create control samples in the concentrations 50 and 500 mg/l. The control solutions were analyzed with the COD(Cr) method for verification of the concentrations and used as a single sample every time analysis of wastewater samples were performed. The purpose of the control sample was to be a references for real samples and to easier detect systematical errors in the method (Hovind et al., 2006). The control solution were treated in the same way as the influent and effluent wastewater samples when performing analysis according to the various methods.

### 4.3.3 Analysis of influent and effluent wastewater samples

The wastewater samples and standard solutions were analyzed as duplicates for all the innovative COD method as well as for the COD(Cr) method, but reported as individual test results. The decision was made in order to provide an increased amount of data, but because the duplicates were taken from the same sample they are not consider independent from each other.

The data obtained from analysis of wastewater samples have been used for three different statistical methods. The first statistical illustration of the data were was regression curves, where data for the innovative methods were set against the results obtained by the reference method, COD(Cr). For the regression study the assumption was made that the error produced by the COD(Cr) method was smaller than for the innovative COD methods. Data from the reference method, COD(Cr) were therefore plotted on the x-axis and results from the innovated methods on the y-axis. An additional assumption was that the errors obtain in the y-axis were homoscedastic and constant. At the contrary if the data is heteroscedastic the error margin increases with increasing concentration values, for the values on both the x- and y-axis. When the error is constant it is of the same magnitude for each investigated concentration. Normally this is not the case, due to the fact that random errors often increase with higher analytic concentrations and a weighted regression should therefore be a more appropriate selection. The decision was made to overlook this matter because the regression analysis was one of several statistical methods used on the data and therefor one part of the validation of the various methods.

The second statistical method was ANOVA which was analyzed in two groups based on result of influent and effluent wastewater samples. The first group was based on result from the methods; COD(Cr), COD Chloride Elimination and COD Chloride Detection. Because filtration was needed for the samples analyzed but the PeCOD method, the two versions of the method were set in a group with filtered sample analyzed on the COD(Cr) method for performing a ANOVA. By dividing the groups in this order, the individual mean values were estimated to adopt similar values and would therefore generate reliable result from the ANOVA.

For the correlation analysis the correlation of interested between the methods was to be linear and positive, which is the normal approach when validating methods (Karat, 2015, personal contact). These three statistical methods did not describe all the detailed trends of the participating methods but were chosen to give a general description of their individual characteristics. All the statistical tests were performed using a confidence interval of 95 percent, indicating that the results are proven to be valid within a 95 percent certainty.

# 5 RESULTS

Results from the selected statistical tests are in this chapter presented for the various methods for both influent and effluent wastewater sample. The two types of wastewater are presented separately to investigate if the different amount of COD would affect the statistical outcome. Laboratory observations, which aims to describe the usability of the methods and their practical benefits and drawbacks are presented in the end of this chapter.

## 5.1 RESULTANT REGRESSION CURVES USING STANDARD SOLUTIONS

In order to evaluate the performance of the analytical methods, regression lines were plotted with result from analyzing standard solutions with known concentrations, using CD(Cr) as the reference method. More detailed information regarding the preparation of standard solutions, used substances and concentrations are available in Section 4.3.2.

All the regression results and plots described in the following section are based on the measurement data for Käppala laboratory. Regression curves of identical standard solution obtained by the laboratories Gryaab and Komlab were similar and therefore excluded from this report.

### 5.1.1 Hach Lange, COD(Cr)

The reference method, COD(Cr) had a working range of 25-1000 mg/l, compared to the innovative methods which were able to analyze sample with COD concentrations up to 1500 mg/l. The standard solution with the highest concentration of 1500 mg/l was therefore not analyzed using this method. The regression curve shown in Figure 10 represent the COD(Cr) results from analyzing standard solution with concentrations from 15-1000 mg/l. The value of the coefficient of determination, $R^2$ is close to one, as desired, which indicates that the relations between the data are linear. This, in combination with an intercept of nearly zero and a slope of one, leads to the conclusion that the regressions curve is as desired and expected for the investigated method.

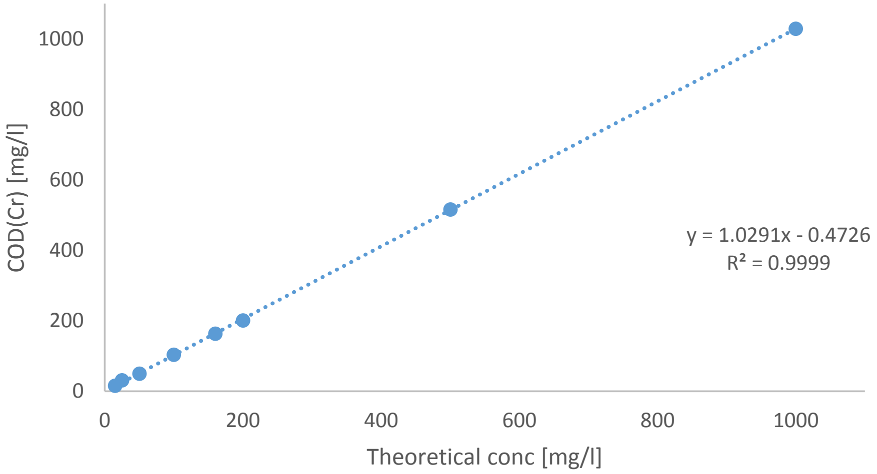

*Figure 10. Regression plot for the total measurement range of 15-1000 mg/l COD for the COD(Cr) method. The calculated concentrations for the standard solutions are plotted on the x-axis and the obtained measurement results on the y-axis.*

The innovative analytical methods Chloride Elimination, Chloride Detection and PeCOD did not consist of one contiguous range, but of two ranges as described. To be able to compare the result for standard solutions between the COD(Cr) method and the innovative methods the regression line for the former method where split into two ranges as illustrated in Figure 11. The lower range consisted of concentrations from 15-160 mg/l and the higher range obtained values from 100-1000 mg/l COD. This overlap in concentration between the two ranges are chosen to avoid results on the border of the ranges which can otherwise occur.

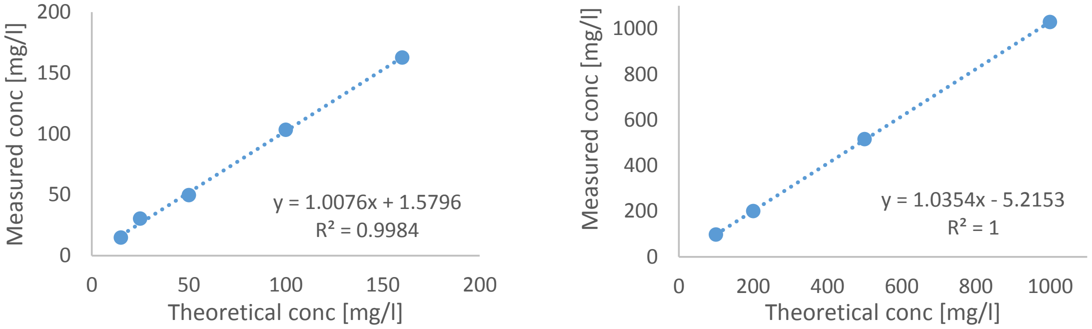

*Figure 11. Regression plots from standard solutions analyzed with the COD(Cr) method. The lower range with standard solutions from 15-160 mg/l are presented to the left while standard solution with higher concentrations, 100- 1000 mg/l are featured to the right.*

From Figure 11 it can be seen that the two plots have divergent intercepts from the regression line shown in Figure 10. This is a result of dividing the normal total measurement range for the COD(Cr) method into two parts, with less data points to each plot.

### 5.1.2 COD Chloride Determination

Regression curves were conducted for the low and high COD range for the Chloride Determination method, each containing standard solutions with COD concentrations of 15-160 and 100-1500 mg/l. The regression line and the coefficient of variation, $R^2$ were estimated and in Figure 12 it is shown that the $R^2$ value for both ranges was high and the intercept for the lower range was close to the desired zero-value for the measured concentrations.

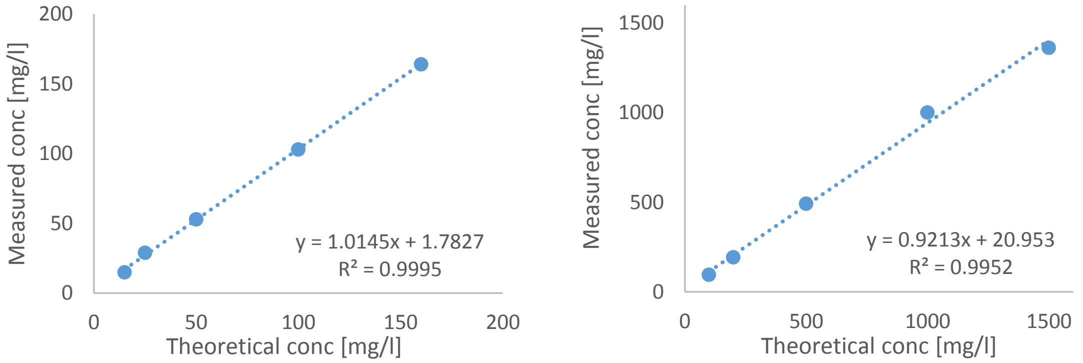

*Figure 12. Regression plots for the Chloride Determination method analyzing standard solutions for the low COD range with concentrations of, 15-160 mg/l, presented to the left and for the high range concentrations, 100 to 1000 mg/l in the right plot.*

The $R^2$-value was high for both ranges of the method, but the intercept for the plots in Figure 12 obtain divergent values when comparing them. The lower range generated an intercept close to one while the higher range received a higher intercept. A high positive intercept indicates that the analytical method will report a COD value higher than the actual value for a samples then the reference method COD(Cr).

### 5.1.3 COD Chloride Elimination

For the Chloride Elimination method it was only possible to produce a regression line for standard solutions using the lower COD range (Figure 13) due to circumstances explained further in Section 4.3.

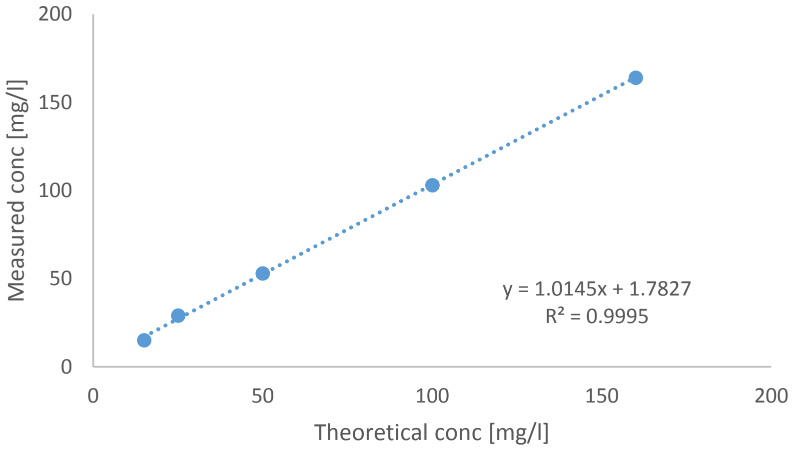

*Figure 13. Regression plot for the lower range of the Chloride Elimination method using standard solutions with concentrations ranging between 15-160 mg/l. The theoretical calculated concentration values corresponds to the x-axis, while the measured COD values are listed on the y-axis.*

The intercept, the value regarding the slope of the line and $R^2$ -value had satisfactory values, based on the criteria listed earlier in the chapter.

### 5.1.4 PeCOD automatic and manual

When investigating the regressions line produced from standard solutions for the automatic PeCOD system (Figure 14), the intercept are seen to be more separate between the two ranges then for the regression plots from data generated by the manual PeCOD unit (Figure 15).

The intercept are more inaccurate for the higher range with higher COD concentrations, which is expected due to higher measurement uncertainty for low COD values under the investigated range. A higher intercept can therefor be accepted for the higher range, even though a value close to the origin is favored. The $R^2$-value for the two ranges are both high for the automatic PeCOD (Figure 14). The intercept of exactly one is likely to depend on random errors such as measurement uncertainty.

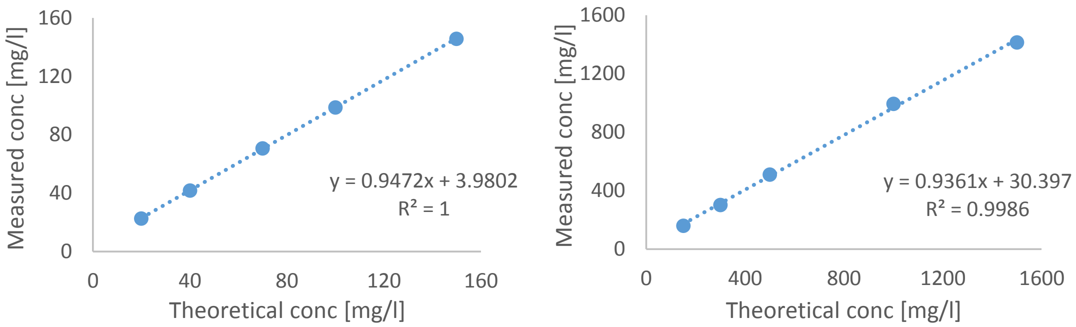

*Figure 14. The automatic PeCOD method for lower range (15-160 mg/l) of COD concentrations for standard solutions to the left and data analyzed with the higher range (160-1500 mg/l) to the right figure.*

The manual version of the method (Figure 15) had a better slope value for the higher COD range than the lower, with a value lose to positive one. On the contrary the lower range received a better intercept, as expected and the $R^2$- values were high independent on investigated COD range.

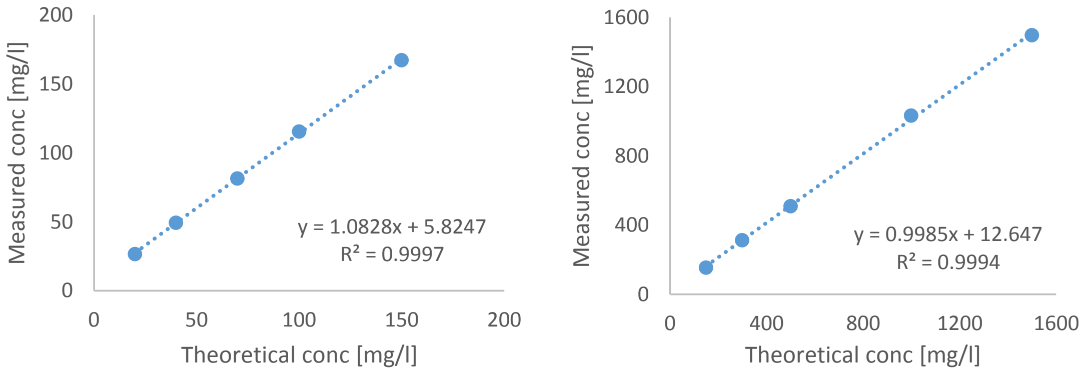

*Figure 15. Regression plots for the Manual PeCOD method for the lower COD range (15-160 mg/L) to the left and the higher range (160-1500 mg/l) using sorbitol standard solutions.*

By comparing the regression results between the manual (Figure 15) and the automatic PeCOD (Figure 14) it were clear that the variation between the ranges are smaller for the manual unit then for the automatic version regarding intercept and the $R^2$- value.

## 5.2 ANALYTICAL RESULT FOR INFLUENT WASTEWATER SAMPLES

Following section presents the results obtained from statistical test performed on incoming wastewater samples sampled and analyzed at the laboratories Käppala, Gryaab and Komlab. The data used within for the results generated of analyzing influent and effluent wastewater are listed in Appendix A for the three laboratories. Both the influent and effluent wastewater for the laboratories had a chloride concentration between 10-140 mg/l. The COD concentrations for influent wastewater samples were between 200-900 mg/l including all the participating laboratories.

### 5.2.1 Regression analysis

This section present the regression analysis for the influent wastewater samples analyzed with the three innovative methods by the three participating laboratories. The result were thereafter compared to the data obtained by the reference method COD(Cr). In the figures listed below does the red solid line corresponds to the line of theoretical values, which illustrate the optimal outcome of data between the compared methods. The results produced from analysis are marked by blue points and their trend lines are illustrated as blue, dashed lines. This presentation of data are implemented for both influent and effluent wastewater and are inserted as a reference to indicate were the desired values would be if the methods would generate similar COD concentrations.

#### 5.2.1.1 M-N Chloride Determination

As can be seen in the regression plots in Figure 16, the incoming wastewater samples analyzed by the Chloride Determination method gives a wider spread in the regression parameters than when analyzing standard solutions (Figure 12). The $R^2$-value obtained for the different laboratories are rather close to one, which indicate that a linear relation between the methods exist. When investigating the intercept, Gryaab received the lowest value of approximately 29.9 which is similar to Komlab. Käppala receives an intercept that is more than double in size and can be seen as divergent compared to the other laboratories.

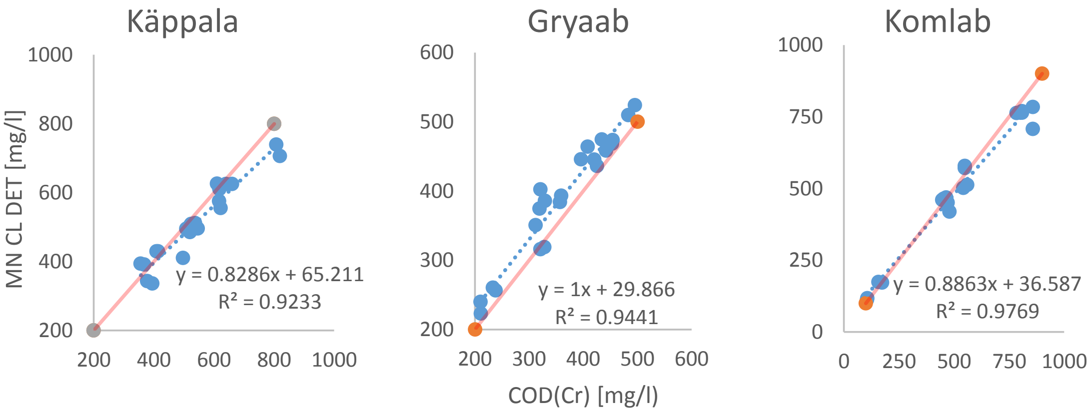

*Figure 16. Regression plots for the Chloride Determination method by the laboratories; Käppala, Gryaab and Komlab. The data produced by the method was plotted on the y-axis against the result for the COD(Cr) method on the x-axis. The blue trend line correspond to the analytical data, while the red line illustrates the theoretical desired values.*

Based on Figure 16, the regression line for Gryaab was closes to a theoretical line due to a low intercept value, a slope of one and a $R^2$-value close to one. If the spread of data where to be investigated, the result for Gryaab have the most even distribution between the lowest and highest COD value. An even spread of data was provided for Käppala as well, but with higher COD concentrations does the innovative method generate lower values compared to the reference method. Analytical results produced by Komlab however appears to be gathered in three groups, one for low COD concentration, one in the middle and the third containing the highest COD results. A possible reason for this could be that the wastewater samples for Komlab are collected at different WWTP which could generate different levels of COD concentrations within a narrow interval.

#### 5.2.1.2 M-N Chloride Elimination

Because the late delivery of the high range of the Chloride Elimination method there were only a few wastewater samples analyzed before the method was removed from the PCP. The results for the influent wastewater samples are therefore limited but summarized in Figure 17. A trend in the data indicates that the Chloride Elimination method generates almost 5-10 times lower COD concentration values than the reference method, COD(Cr). The data was widely spread from the theoretical line for all laboratories which is seen as an undesired trend in the data.

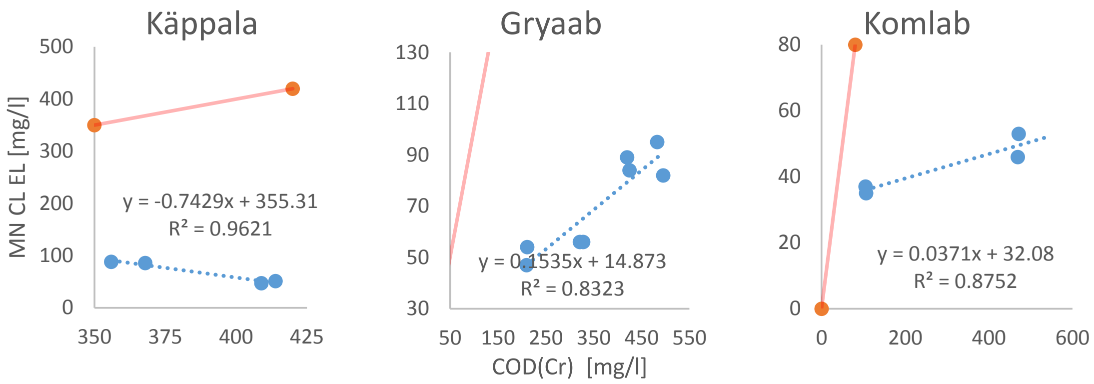

*Figure 17. Regression plots for the Chloride Elimination method analyzing influent wastewater samples for the laboratories; Käppala, Gryaab and Komlab. The obtained values by the reference method, COD(Cr) are illustrated on the x-axis while the y-axis present the data from the Chloride Elimination method.*

The plots shown in Figure 17 indicates that the correlation are both negative and positive comparing to the reference method, but is a result without reliable basis. Overall it can be stated that the regression results for this method and range are highly uncertain do to the limited amount of data.

### *5.2.1.3 PeCOD automatic and manual*

Analysis of incoming wastewater was executed simultaneously on the manual PeCOD unit as well as the automatic PeCOD system by all the laboratories. The result from the COD(Cr) method that is plotted on the x-axis in Figure 18 and Figure 19 were generated analyzing the same filtered samples used for the PeCOD units. The results from the automatic PeCOD are shown in Figure 18 while Figure 19 describes the results obtained by analyzing the samples on the manual unit. Both versions of PeCOD had a weak linear relation against the reference method compared to the theoretical lines. The two versions of the method did both generate lower COD values relatively the COD(Cr) method which is seen in both Figure 18 and Figure 19.

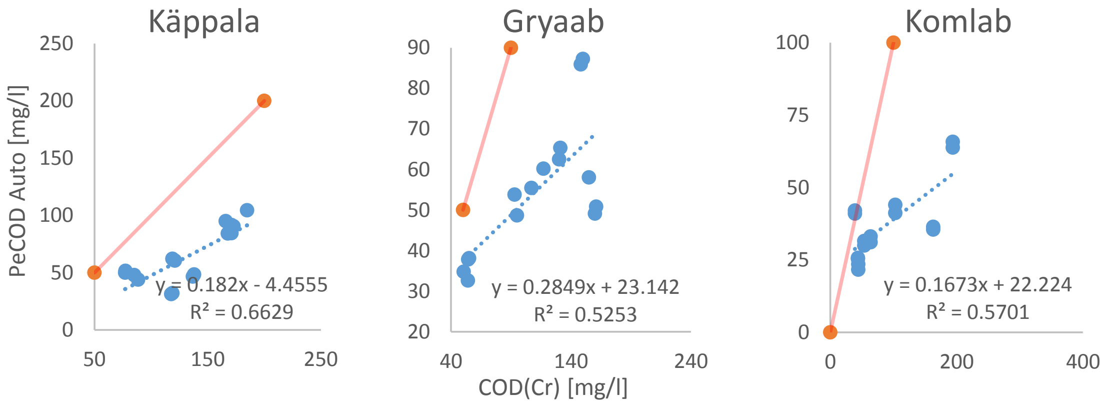

*Figure 18. Regression plots for the automatic PeCOD unit when analyzing influent wastewater samples for the laboratories; Käppala, Gryaab and Komlab. The data produced by the PeCOD is illustrated on the y-axis and compared to the result for the same filtered*

*samples with the COD(Cr) method. The red solid line represent the theoretical values of COD concentrations and the blue trend line is predicted from the analytical data.*

The two versions of PeCOD were compared in order to identify various trends between the different types of the method, as well as between the laboratories. Overall, the two units of PeCOD gave a similar spread of data, but the appearance of the regression plots varied. Komlab had the most similar result when comparing the regression plots produced by the two versions of PeCOD against one another.

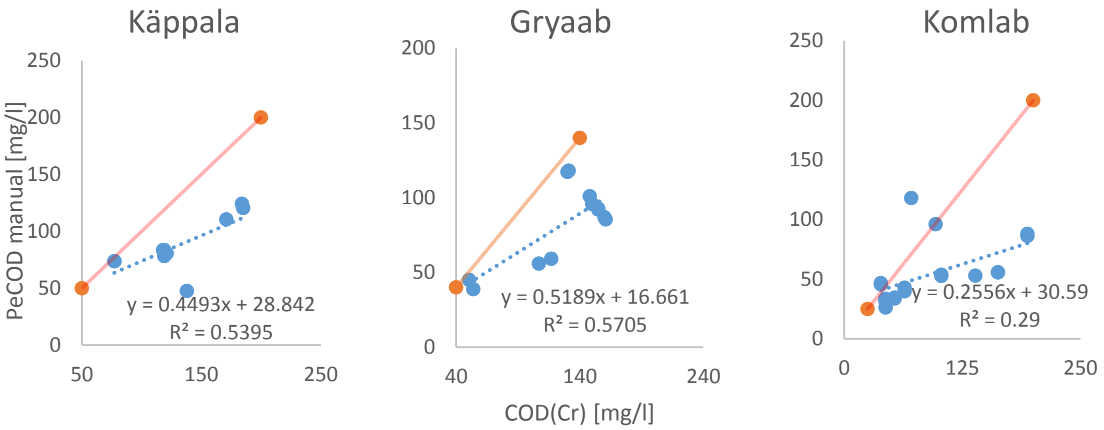

*Figure 19. Regression plots for the manual PeCOD unit when analyzing influent wastewater samples for the laboratories; Käppala, Gryaab and Komlab. The data produced by the PeCOD is illustrated on the y-axis and compared to the result for the same filtered samples with the COD(Cr) method. The red solid line matches the theoretical values assuming that the methods produce equal COD concentrations. The blue trend line was predicted from the obtained analytical data.*

The regression plots generated from data produced by the manual and automatic PeCOD at Komlab indicated that two data points differ from the rest of the data. These two values generated high COD concentrations for the PeCOD method while low COD values for the reference, COD(Cr) method. If these data would be removed it would affect the regression line and the $R^2$-value as illustrated in Table 1. The change in input data affects the $R^2$-value, which more than tripled, due to the elimination of the identified data. The slope value of the line and the intercept were in comparison to the $R^2$-value not influenced to the same degree.

*Table 1. Result from regression analysis for influent wastewater at Komlab laboratory with and without the removal of two suspected outlier values*

| | Automatic PeCOD | Manual PeCOD |
|---|---|---|
| With all data points | $y = 0.174x + 30.9$<br>$R^2 = 0.1374$ | $y = 0.2556x + 30.59$<br>$R^2 = 0.295$ |
| Without 2 data points | $y = 0.1673x + 22.224$<br>$R^2 = 0.5701$ | $y = 0.2852x + 21.927$<br>$R^2 = 0.7979$ |

As complimentary results to the regression plots for the two versions of PeCOD has regression plots been performed (Figure 20, Figure 21) comparing COD values obtained by the PeCOD and values from the COD(Cr) method when analyzing unfiltered samples. This would give an indication on how the PECOD method would correspond to historical COD data, which is produced by the COD(Cr) method from unfiltered samples.

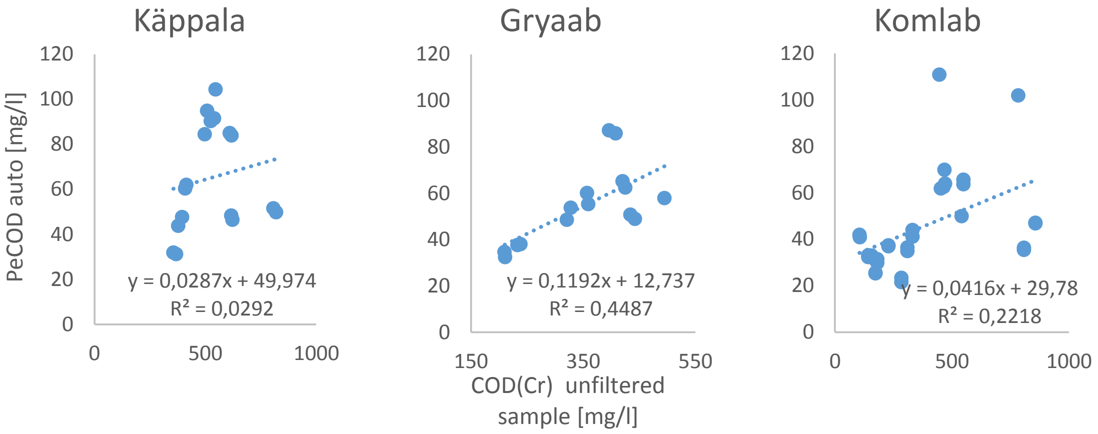

*Figure 20. Regression plots for the automatic PeCOD for influent wastewater samples at the laboratories; Käppala, Gryaab and Komlab. The samples were filtrated before analyzed with the PeCOD method but kept unfiltered when analyzed by the COD(Cr) method.*

The $R^2$-value and slopes values for the regression lines for the different laboratories in Figure 20 and Figure 21 indicate that no similar value for the correlation factor could be found between the PeCOD versions and the reference method, COD(Cr).

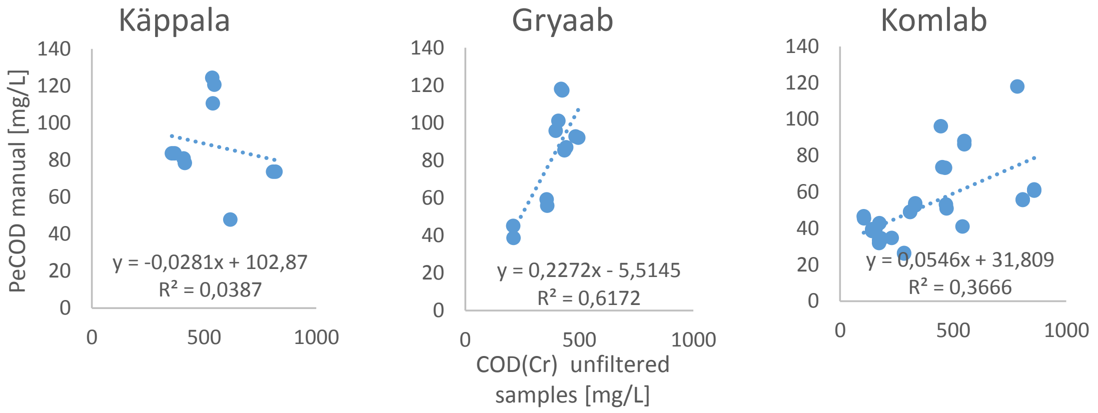

*Figure 21. Regression plots for the manual PeCOD for influent wastewater samples at the laboratories; Käppala, Gryaab and Komlab. The samples were filtrated before analyzed with the PeCOD method but kept unfiltered when analyzed by the COD(Cr) method*

The data obtained for the two versions of PeCOD are additionally compared to the BOD concentrations in Appendix C due to recommendations from the company ManTech. They had historically received better correlations between PeCOD data and BOD values, than compared to data obtained by the COD(Cr) method. The comparison were performed for both influent and effluent wastewater samples and the result did not indicate that the BOD parameter would have a better correlation to the PeCOD data than the COD(Cr) method.

### 5.2.2 Analysis of Variance

As described in section 3.5.1 an ANOVA is a statistic test that can be conducted on several datasets to investigate if various datasets mean value differ significantly from each other. The first datasets that were used to perform the ANOVA is compiled in Table 2 and consist of data produced by the methods Chloride Elimination, Chloride Determination and COD(Cr). The null hypothesis for this ANOVA was stated that the mean values for the different datasets was not significantly divergent from one another. By investigating Table 2 it is clear that the null hypothesis can be rejected for all the laboratories, due to a considerably higher calculated F-value than the critical F-value.

*Table 2. Compilation of result from ANOVA for the methods COD(Cr), Chloride Detection and Chloride Elimination conducted on influent wastewater samples presented individually for the three laboratories; Käppala, Gryaab and Komlab.*

| | F-value | P-value | F crit |
|---|---|---|---|
| **Käppala** | 29.2 | $7.5 \cdot 10^{-9}$ | 3.93-4.05 |
| **Gryyab** | 47.7 | $3.2 \cdot 10^{-12}$ | 3.93-4.05 |
| **Komlab** | 7.8 | $1.0 \cdot 10^{-3}$ | 3.93-4.05 |

A second group of methods, the two versions of the PeCOD and COD(Cr9) are compiled generating the ANOVA result listed in Table 3. This ANOVA resulted in the conclusion that the methods are not able to report similar COD concentrations which corresponds to similar mean values.

*Table 3. Compilation of result from ANOVA for the COD(Cr) method and the automatic and manual PeCOD method conducted on influent wastewater and individually represented for the laboratories; Käppala, Gryaab and Komlab*

| | F-value | P-value | F crit |
|---|---|---|---|
| **Käppala** | 25.5 | $5.5 \cdot 10^{-8}$ | 3.93-4.05 |
| **Gryyab** | 14.2 | $2.2 \cdot 10^{-5}$ | 4.05 |
| **Komlab** | 12.6 | $1.79 \cdot 10^{-5}$ | 3.80-3.93 |

An observation that could be made from the ANOVA was that Komlab laboratory received the lowest calculated F-values, which, however, are considerably higher than the critical F-values which still proves that a significant difference in mean value exist.

### 5.2.3 Correlation analysis

A way to understand how the various methods correlated to each other were to conduct correlation analysis based on the results for influent wastewater samples. The correlation coefficients, $r$, are stated in Table 4 and the decision was made to only represent the correlation coefficient between the COD(Cr) method and the innovative methods. It was also relevant to display the valid correlation between the two versions of the PeCOD method and the COD(Cr) method analyzing filtered samples. The result indicated that the Chloride Determination method had a high, positive correlation to the COD(Cr) method for all the laboratories. The correlation coefficient generated for the Chloride Elimination method are represented in parenthesis due to the limited set of data and are therefore presented with a large uncertainty. The values are, however, included in this summary to give statistical support for the decision to interrupt analysis with the given method.

The correlations factor for Chloride Elimination was high and positive for both the laboratories Gryaab and Komlab, but had a high negative correlation to the COD(Cr) method for Käppala. When investigating the correlation between the PeCOD units and the COD(Cr) method, it was as expected higher for filtered samples than unfiltered ones for both Käppala and Gryaab. Unexpectedly this was not the case for Komlab, which received lower correlation to the results obtained on filtered samples for the COD(Cr) method. It should be noted that all correlation factors for Komlab was low in this context, compared to the desired correlation value of one.

Another abnormal result provided from Table 4 was the calculated coefficient of correlation values between the filtered and unfiltered samples analyzed on the COD(Cr) method at Käppala laboratory. Instead of generating a high positive correlations a small and negative correlations factor, close to zero were produced. A value this small shows that no correlation exist between the investigated methods.

*Table 4. Correlation factors calculated for Käppala, Gryaab and Komlab, based on the innovative methods relationship to the reference method COD(Cr) for filtered and unfiltered samples of influent wastewater.*

| | Käppala | | Gryaab | | Komlab | |
|---|---|---|---|---|---|---|
| | *COD(Cr)* | *COD(Cr) (filtrered samples)* | *COD(Cr)* | *COD(Cr) (filtrered samples)* | *COD(Cr)* | *COD(Cr) (filtrered samples)* |
| *COD(Cr)* | 1 | | 1 | | 1 | |
| *CL DET* | 0.96 | | 0.97 | | 0.99 | |
| *CL EL* | (-0.98) | | (0.91) | | (0.94) | |
| *COD(Cr) (filt samples)* | -0.05 | 1 | 0.97 | 1 | 0.75 | 1 |
| *PeCOD auto* | 0.17 | 0.81 | 0.67 | 0.72 | 0.47 | 0.37 |
| *PeCOD manual* | -0.20 | 0.73 | 0.79 | 0.76 | 0.61 | 0.54 |

## 5.3 ANALYTICAL RESULTS FOR EFFLUENT WASTEWATER SAMPLES

The following section describes the results obtained by statistical analysis for all the innovative methods for COD included in the PCP, based on measurement data for effluent wastewater. The COD concentrations for effluent wastewater included within the study were ranging from 20 to 60 mg/l.

### 5.3.1 Regression analysis

As for subchapter 5.2.1 the innovative methods are listed as followed; Chloride Determination, Chloride Elimination and automatic and manual PeCOD. As for influent wastewater, a red, solid trend line is inserted in the regression plots to illustrate the theoretical values generated if the methods were to produce equal COD concentrations. The measurement data are marked as blue dots and the trend line as a blue, dashed line.

#### *5.3.1.1 M-N Chloride Determination*

When analyzing the results obtained by the Chloride Determination method for effluent wastewater samples, it was clear that the results vary among the laboratories.

In the Figure 22 it is shown that Käppala did not have a linear relation between the innovative method and the reference method, COD(Cr). This was evident by examine the spread of data and the $R^2$-value which was close to zero. The data was located far from the theoretical line indicating that the method were unable to generate similar COD values as the reference method. This was an unexpected result compared to the regression plots performed for influent wastewater at Käppala laboratory presented in Figure 16. More consistent are the regression lines for Gryaab and Komlab for influent and effluent wastewater, which received a higher linear relationship between the methods. The $R^2$-value was reduced for Gryaab for effluent wastewater compared to the influent wastewater samples, but got an intercept value close to zero.

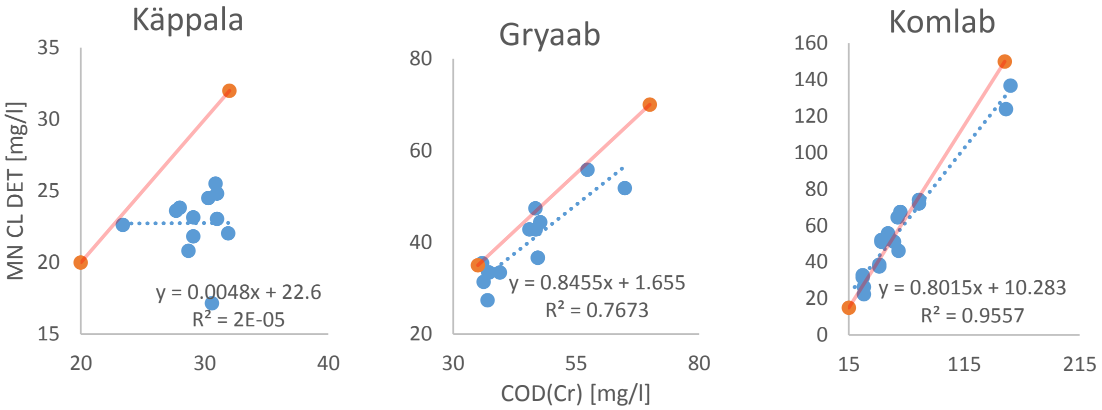

*Figure 22. Regression plots for the Chloride Determination method analyzing effluent wastewater at the laboratories; Käppala, Gryaab and Komlab. The data produced by the method was plotted on the y-axis against the result for the COD(Cr) method on the x-axis. The blue trend line correspond to the analytical data, while the red line illustrates the theoretical desired values.*

Komlab was the laboratory that had the highest similarity when comparing regression plots for influent and effluent wastewater samples. One samples that was analyzed as duplicates received a much higher COD value than the rest of the collected data from Komlab. These data seems to be valid though both the reference method and the Chloride Detection method received high COD concentrations for the specific samples. An additional regression plots was conducted (Figure 23) where the two points were removed in order evaluate what impact they may have on the overall regression result.

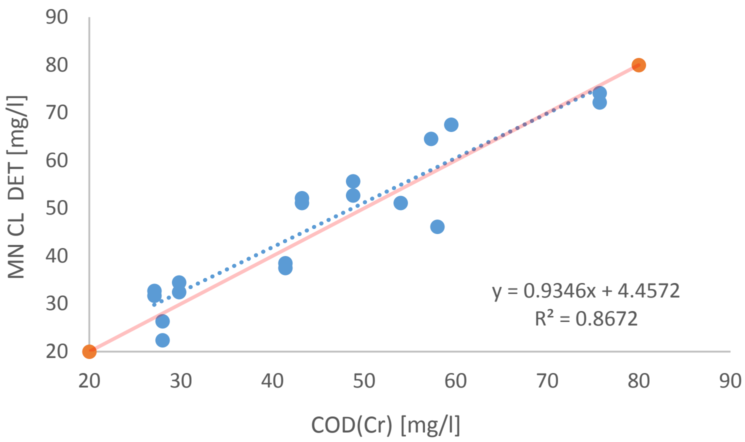

*Figure 23. Regression plot based on measurement data from the method Chloride Determination at the laboratory Komlab on effluent wastewater samples. Two values have been removed due to high divergent COD concentrations.*

When comparing the two regression plots for Komlab a lower coefficient of determination value was obtained when modifying the data set. A higher slope value closer to one and a lower intercept value were however achieved for the second regression line (Figure 23), which indicates that a better agreement between the two analytical methods COD(Cr) and Chloride Determination were obtained with the modified data.

#### *5.3.1.2 M-N Chloride Elimination*

From the regression plots illustrated in Figure 24 it could be seen that the coefficient of determination was low regardless of laboratory. This indicates that the COD value obtained by the Chloride Elimination method was not equivalent to reference method COD(Cr) for measuring COD. It could also be noted that the intercept for the involved laboratories were varying. For all the compilations of analytic results collected from outgoing wastewater samples it were consistent that the intercept obtains a value higher than zero. This indicates that a content of COD would be recognized with the Chloride Elimination method while the COD(Cr) would detect a zero level of COD. A large distribution of the measurement result along the trend line also displays that the two COD methods produced unequal COD values while analyzing the same sample.

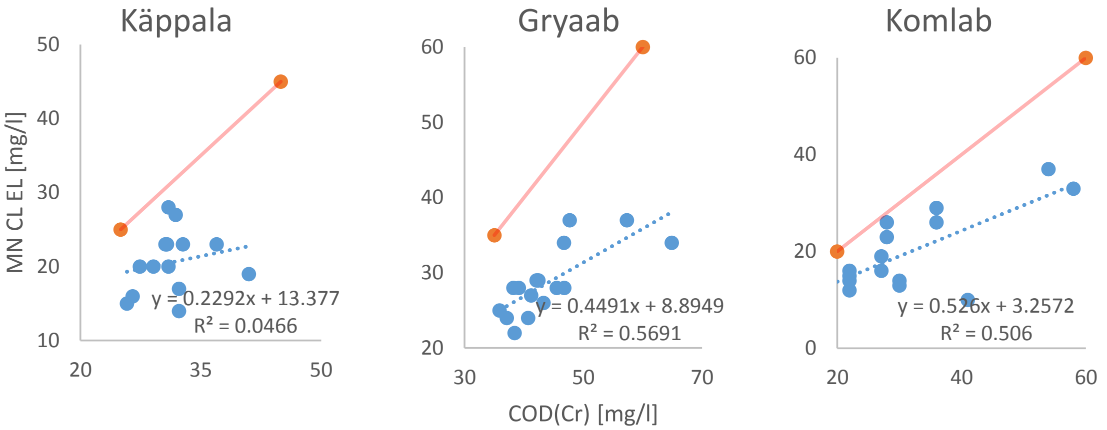

*Figure 24. Regression plots produced by the Chloride Elimination method for effluent wastewater compared to the method COD(Cr)where the data are obtained at the three laboratories; Käppala, Gryaab and Komlab.*

When regression plots for effluent wastewater (Figure 24) was compared to influent wastewater (Figure 17) for the Chloride Elimination method it was seen that the method was unable to produce equivalent COD values comparable to the reference method. This result was valid for all the laboratories, but to various extent. When examine data for the Komlab laboratory it was clear that some measurements had a lower COD value then the measurement uncertainty limit set for the involved methods. The limit were set to 25 mg/l for the reference method, COD(Cr) and 15 mg/l for the Chloride Elimination method. Additional regression plots were therefore conducted for this laboratory removing five data points with COD concentrations smaller than the set uncertainty limit (Figure 25).

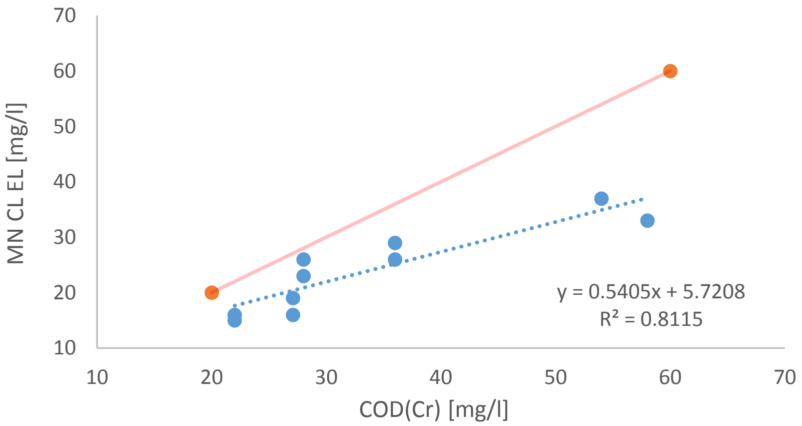

*Figure 25. Regression plot for the Chloride Elimination method compared to the COD(Cr) method at Komlab when analyzing effluent wastewater samples. Five measurement data values were removed due to low COD concentrations.*

This change in input data impacted the R$^2$-value to rise from 0.51 to 0.81, without the slope and intercept values changing considerably.

#### *5.3.1.3 PeCOD automatic and manual*

The samples analyzed at Komlab were filtered with a filter of 20 µm, compared to the 1.6 µm filters used at Käppala and Gryaab. This lead to an increase of particulate organic matter in the sample and thereby to a higher COD content in the analyzed wastewater samples sampled and analyzed at Komlab. The biggest spread in results between the innovative and COD(Cr) method for the automatic PeCOD unit were obtained for Käppala laboratory, which was evident in the low $R^2$-value (Figure 26).

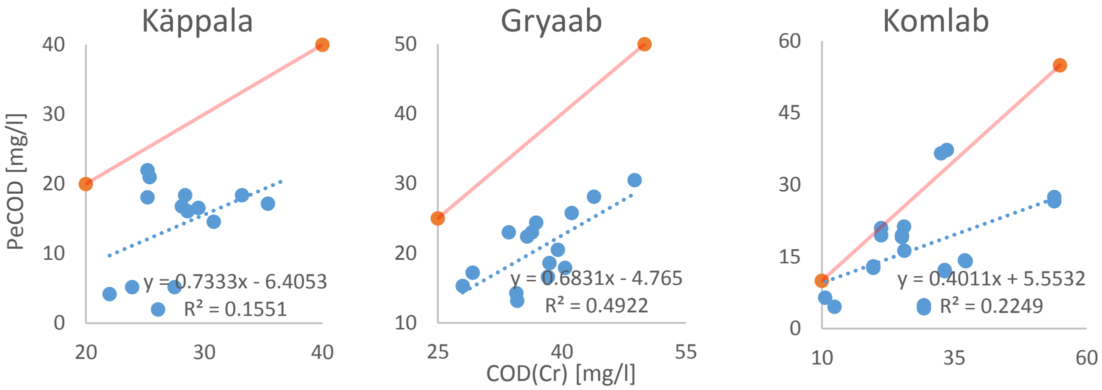

*Figure 26. Regression plots produced by the automatic PeCOD compared to the COD(Cr) method for effluent wastewater with the analytical data obtained at the three laboratories; Käppala, Gryaab and Komlab*

The regression plots in Figure 26 were performed by using all data from analysis conducted at the individually laboratories. For Käppala and Komlab, some of the data points had COD concentrations below the measurement uncertainty limit for the two methods. These data was therefore removed from the data set for the two laboratories, to verify that the studied data were in the acceptable uncertainty range. For Gryaab it was only one value, of 15 mg/l, that was lower than the set uncertainty limit for the PeCOD method. The decision was made that this data point could be included into the data set, though the value was close to the limit of uncertainty and was believed not to effect the final parameters significantly.

It could also be suspected that the two highest COD values from Komlab affected the regression analysis, due to their deviation in concentration compared to the rest of the data in. The regression plots for Käppala and Komlab were therefore recalculated for the automatic PeCOD unit (Figure 27) were data was removed from the set. This change in in input data had a minor effect on the regression result for Käppala laboratory, but led to a small increase in the $R^2$ value and intercept and a decrease for the slope value. This plot in connection to the result in earlier regression analysis for Käppala studied effluent wastewater indicates that no linear relation between the automatic PeCOD and the COD(Cr) method for filtered samples exist.

For the data obtained by Komlab a comparison between the plots in Figure 26 and Figure 27 generated a small decrease in the slope and $R^2$ value, while the intercept was increased. The most consistent correlation between the COD(Cr) and the automatically PeCOD system was stated for Komlab.

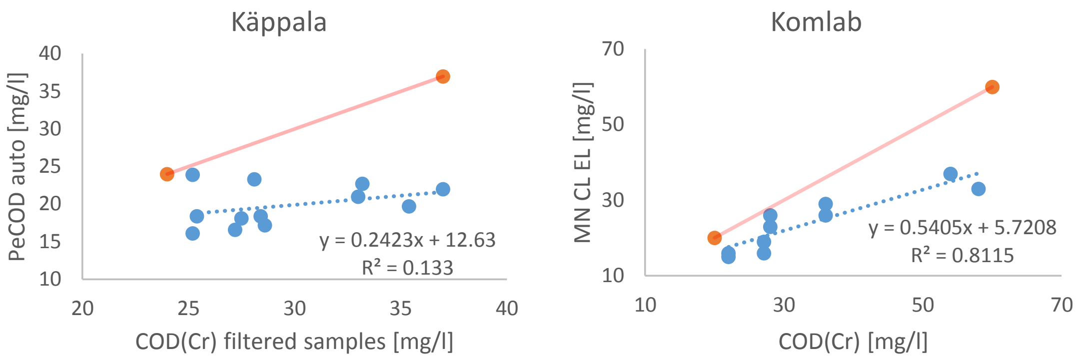

*Figure 27 Regression plots for effluent wastewater samples analyzed on the COD(Cr) method and the automatic PeCOD system for Käppala (to the left) and Komlab (to the right). From the input data were 6 measurement points removed from Käppala and Komlab due to too low COD concentration.*

When comparing the Manual PeCOD unit to the COD(Cr) (Figure 28) for filtered samples, the results continue to vary depending on the studied laboratory. For Käppala it was found that no linear relation between the methods were present, which was proven with a low $R^2$ value close to zero. The results indicated that Gryaab had a higher correlation between the manual PeCOD and the reference method (Figure 28), compared to the automatic PeCOD system (Figure 26, Figure 25), due to a higher slope value of the regression line. The highest $R^2$ value for the three laboratories was for Komlab, with a value of 0.9515.

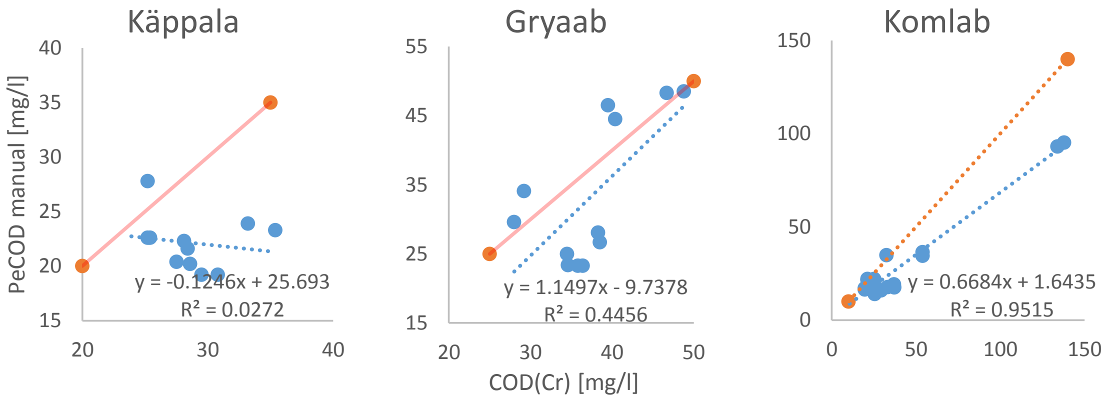

*Figure 28. Regression plots for effluent wastewater samples from the manual PeCOD method compared to the COD(Cr) method on filtered samples for the three laboratories; Käppala, Gryaab and Komlab.*

The Komlab laboratory was seen to have a high correspondence between the PeCOD method and the reference method, COD (Cr). There were however two data points diverging from the rest of the dataset for Komlab in Figure 26 and Figure 28, which impacted the regression line to a great extent. It was determined that the values should be included in the datasets thought the samples had similar COD concentrations for all COD methods and could therefore not be seen as outliers.

As additional results to the above compiled regression plots for the two versions of the PeCOD was additional regression plots performed on the effluent wastewater samples, were the samples had not been filtered before analyzed with the reference method.  These plots are listed below in Figure 29 and Figure 30 and includes all the measurement data, regardless of COD content. These figures are incorporated to understand to what extent the results produced by the two PeCOD units complied to results for the COD(Cr) method for unfiltered samples and therefor how they would correlate to historical COD values.

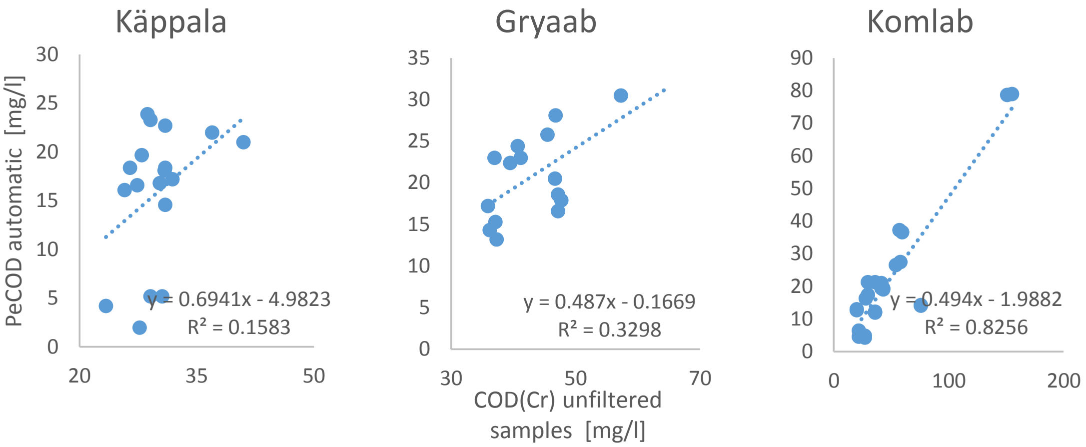

*Figure 29. Regression plots for the automatic PeCOD analyzing filtered samples compared with unfiltered samples analyzed by the COD(Cr) at the three laboratories: Käppala, Gryaab and Komlab using effluent wastewater samples.*

Both Figure 26 and Figure 29 have been produced of results from the automatic PeCOD and COD(Cr) method. The difference between the compilations was that Figure 29 COD(Cr) method in the later mentioned figure analyzed unfiltered samples. Even though the samples were pretreated differently for the figures did the $R^2$ values have similar values for the individual laboratories. The lowest $R^2$ value was obtained for Käppala and the highest for the Komlab laboratory, regardless if the samples had been filtrated or not before analyzed with the COD(Cr) method.

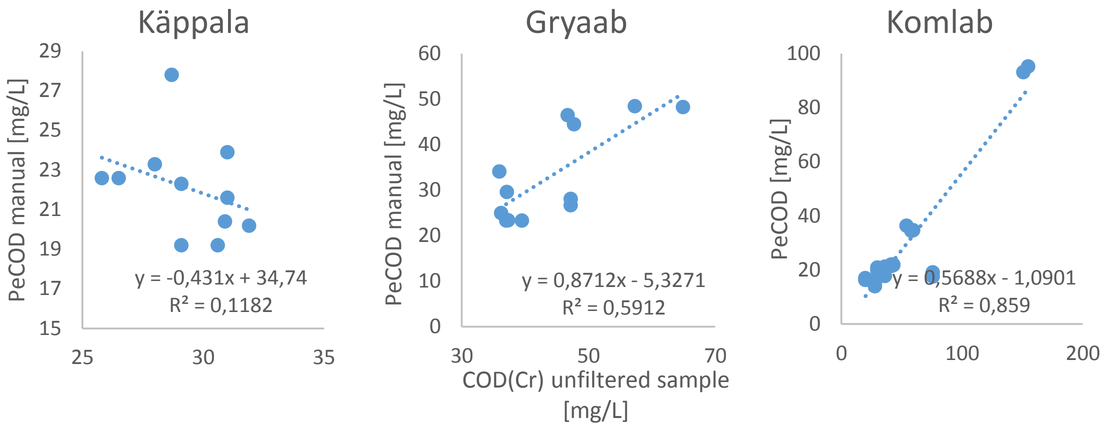

*Figure 30. Regression plots for the manual PeCOD analyzing filtered samples compared with unfiltered samples analyzed by the COD(Cr) at the three laboratories: Käppala, Gryaab and Komlab using effluent wastewater samples*

### 5.3.2 Analysis of Variance

Two groups of ANOVA was produced for effluent wastewater to investigate if there was any significantly difference in the mean values produced for each of the involving methods, which was performed in the same manner as for the influent wastewater samples. When comparing effluent wastewater samples for the methods; COD(Cr), Chloride Elimination and Chloride Determination it was shown that a significantly difference between the methods mean values were present for all the laboratories (Table 5). The highest F-value of 37.7 was calculated for Käppala, which means that the values the greatest dispersion between them for this laboratory. A small p-value indicated that the probability that a true null hypothesis could be falsely rejected was close to none existing.

*Table 5. The results obtained by performing ANOVA calculations for the methods; COD(Cr), Chloride Determination and  Chloride Elimination based on their result analyzing effluent wastewater samples*

| | F-value | P-value | F crit |
|---|---|---|---|
| Käppala | 37.7 | $2.43 \cdot 10^{-10}$ | 3.93-4.05 |
| Gryaab | 21.9 | $1.62 \cdot 10^{-7}$ | 3.93-4.05 |
| Komlab | 7.7 | $9.98 \cdot 10^{-4}$ | 3.80-3.93 |

In Table 6 it was showed that the mean values calculated by the data obtained by the methods, COD(Cr) and the two versions of PeCOD did not differ significantly when analyzing the results at Komlab. This indicates that the results were more similar between the participating methods when conducting analysis at Komlab than the other two laboratories, which was a deviant result compared to the rest of the performed ANOVA calculations. When examine the calculations obtained for Komlab in Table 6 it should be mentioned that this result was produced with a high p-value. The p-value of 0.1077 indicates that in approximately one of ten cases the null hypnosis will be falsely rejected and the result can therefore not be represented with a high probability.

*Table 6. The results obtained by performing ANOVA calculations for the methods; COD(Cr), automatic PeCOD and manual PeCOD based on their result analyzing effluent wastewater samples*

| | F-value | P-value | F crit |
|---|---|---|---|
| Käppala | 27.2 | $2.07 \cdot 10^{-8}$ | 3.93-4.05 |
| Gryyab | 23.5 | $1.79 \cdot 10^{-7}$ | 4.05 |
| Komlab | 2.3 | 0.1077 | 3.80-3.93 |

When performing an ANOVA for Komlab excluding the two higher COD concentration values described previously in section 5.3.1, the result for the calculated F-value become 8.03, which is an indication that the results is to a large extent affected by these higher COD values.

### 5.3.3 Correlation analysis

A correlation analysis with the aim of developing correlation coefficients between the COD(Cr) method and the various innovative methods were performed and the result compiled in Table 7. As for the influent wastewater samples the Chloride Determination was found to have the highest correlation to the COD(Cr) method for both Gryaab and Komlab. When investigating the correlation factor achieved for Käppala, the factor was close to zero, which indicate that no linear correlation between the two methods exist. This was equivalent to the results that could be seen in Figure 22, where the $R^2$ value as well was close to zero.

The Chloride Elimination method are shown to have positive correlation to the COD(Cr) method for all laboratories. The factor of 0.22 for Käppala were in this context seen as a low correlation rate. The laboratories, Gryaab and Komlab had higher and similar coefficient factors compared to each other, which more strongly indicated on a present covariation.

*Table 7. Correlation coefficients calculated for Käppala, Gryaab and Komlab, based on the innovative methods relationship to the COD data conducted by the COD(Cr) method for both filtered and unfiltered samples on effluent wastewater samples*

| | Käppala | | Gryaab | | Komlab | |
|---|---|---|---|---|---|---|
| | *COD(Cr)* | *COD(Cr) filtrered samples* | *COD(Cr)* | *COD(Cr) filtrered samples* | *COD(Cr)* | *COD(Cr) filtrered samples* |
| *COD(Cr)* | 1 | | 1 | | 1 | |
| *CL DET* | 0.01 | | 0.88 | | 0.98 | |
| *CL EL* | 0.22 | | 0.75 | | 0.71 | |
| *COD(Cr) filt* | 0.64 | 1 | 0.87 | 1 | 0.94 | 1 |
| *PeCOD auto* | 0.40 | 0.35 | 0.57 | 0.70 | 0.91 | 0.92 |
| *PeCODmanual* | -0.34 | -0.16 | 0.77 | 0.67 | 0.93 | 0.98 |

When investigating the PeCOD method for Käppala it was found that the correlation factors were positive for the automated device, but negatively correlated for the manual PeCOD. This result was evident regardless of the samples being filtered or not. If comparing to the correlation factors obtained for Käppala in section 5.2.3 for incoming wastewater, the correlation between the COD(Cr) and manual PeCOD was for effluent wastewater much lower and even negative, which was contrary to the earlier findings. This indicated that the PECOD method had a better correlation with the COD(Cr) method for higher COD concentrations at the Käppala laboratory.

Gryaab had higher correlations between the PeCOD and the COD(Cr) method than Käppala which were also positive for all method combinations. A result was that the correlation coefficients were comparable in size regardless of the samples being filtered or not for the COD(Cr) method. The conclusion could therefore be made that the treatment of samples did not seemed to affect the correlation between the reference and the PeCOD method when examine data from Gryaab.

For Komlab, it could be seen that the correlation was high for all method combinations between COD(Cr) and the PeCOD. This was a contradictory result compared to the correlation analysis for influent wastewater, which indicated on low correlation for the investigated methods. Summarized, the PeCOD method seemed to have alternative high and low correlation with COD(Cr) for effluent wastewater depending on the laboratory conducting the analysis, which could be a result of various composition in the analyzed wastewater.

## 5.4 LABORATORY OBSERVATION

Through the analytical work of analysis and data collections, remarks regarding the workability of the innovative methods and their usability were noted, which were intended to serve as a compliment to the statistical methods.

### 5.4.1 COD Chloride Detection

The Chloride Determination method was observed to be the method most in common with the reference method COD(Cr), based on the required sample treatment and execution steps of analysis in combination with equal operational time. The similarity in performing the analysis between the methods could be a benefit due to a simplified learning process. This would generate a minimized work effort before the method could be used in the laboratory routine work. The mayor differences between the COD(Cr) and the Chloride Determination method was the required processing of data in order to achieve a final COD value. The innovative method was however also reliant on the result for the chloride concentration in the sample in order to calculate the final COD. This leads to an increase in measurement uncertainty for the definitive COD value due to the need of multiple analytical test which individually contributes to a uncertainty of measurement. Because the analytical test of chloride concentration were performed on an external laboratory, it resulted in longer time before a final COD value could be determined. If this method were to be selected as a subsequent method to the COD(Cr), this problem would need to be addressed in order to generate faster analytical result for COD. An additional economical cost could therefore be added if the method would be found suitable in order to buy the required analytical equipment for performing analysis of chloride concentration.

### 5.4.2 COD Chloride Elimination

As mentioned in section 4.2.2 was a cartridge used for pretreatment of the samples in order to remove the chloride ions before performing analyzes of COD and thereby enhance interference of the ions. A problem which were detected with the cartridge was that the membrane designed to separate the active solid chemical from being pushing out of the cartridge together with the treated sample was often released from its anchor points. The samples was then contaminated and could not be used for further analysis. The larger consumption of cartridges for producing a useable sample, compared to the available cuvettes, did generate an unequal distribution between the two analysis parts. This led to a growing amount of unusable cuvettes. When the membrane loosen within the cartridge it could generate brown colored spots on the skin if the active chemicals within the cartridge were to be in contact with it. This occurred in one occasion and was due to a missed drop on the working bench.

As for the Chloride Determination, the Chloride Elimination method was dependent on potassium dichromate, which could be undesirable due to the limited usage of the substance within the near future and also unsafe for the working environment for the chemist.

### 5.4.3 PeCOD

To be able to analyze with the PeCOD units a calibration of the sensor and system were needed. The calibration process was required to be perform daily or if more than 4-6 hours had passed since the last calibration was performed. When the daily calibration was conducted for the PeCOD unit it was often needed to perform a recalculation due to unsatisfying result for the calibration parameters. It could also be that the first calibration would indicate on accepted calibration results, but when analyzing calibration solutions with known COD concentration as a quality check of the instrument, it would generate incorrect values. In order to achieve correct analytical results with the method a new calibration were therefor needed.

In the beginning of the analytical work, attempts were made to analyze effluent wastewater samples without filtering the samples as a pretreatment. The analysis were then performed as duplicates by taking the sample twice from the same sample container. The result indicated that the first sample had an almost twice as high COD concentration as measured for the second sample. This was thought to be due to the suspended matter (SS) in the sample that settled to the bottom of the beaker, which could have been absorbed by the sample probe in the first sample. When the sample are injected in the automatic PeCOD system, the probe is put down to the bottom of the sample container. The laboratory staff expressed the need to provide the probe with a stirrer, in order to ensure that the sample would be homogeneous and thereby representable.

The fact that the PeCOD method was unable to analyze the partical COD content in the samples was stated as a drawback for the method by the laboratory staff. This was mainly due to that the COD plays a vital role in the operation process and modelling of a WWTP, but also the ability to see how the COD load have changed historically (Lundin, 2015).

# 6 DISCUSSION

A discussion regarding the statistical results and the laboratory comments for the innovative method participating in the study will be given in this chapter, divided into 6 sections. The first Section 6.1 discussed the choice of method used in the study. Thereafter was the result from the various method taken up for discussion individually in Section 6.2, 6.3 and 6.4 and finally compared in Section 6.5. The discussion was finalized by highlighting eventual sources of errors within the study in Section 6.6.

## 6.1 METHOD

The statistical methods chosen for this thesis were selected from a number of suitable tests that could be performed on this type of data. The methods that were selected were regression analysis, ANOVA and calculation of the correlation coefficients and were chosen to evaluate various properties for the innovative methods.

Regression analysis was selected as a statistical method to illustrate how the validated methods would correlate to the reference method, COD(Cr) and was a good approach to represent individual data in a figurative form. This illustration of data simplifies outlier detection but also describes illustrative properties of the data, which otherwise could be hard to detect. Additional regression plots where conducted for some datasets using processed data when strong evidence existed for treating part of the data as deviant. An example of this was when a few COD data points deviated from the rest of the data for Komlab, even when these results could not be proven as outliers (Figure 26). The ANOVA examines the overall ability of the methods to present similar results but were not dependent on specific deviating values in the same way as the regression analysis. It could be seen that this statistical method did not provide crucial result for the individual methods, due to the fact that it was not able to prove which of the methods that had a divergent mean value of the involved three methods. The ANOVA can for this purpose be followed up by further statistical tests that states which set of measurement data that may be divergent. This was not made as a part of this study because the main focus was to evaluate if any inequality was present or not. As a complement to the regression analysis was correlations factors calculated, which indicated how well the innovative methods covariated to the reference method. The underlying aim of all the statistical methods and processing of the data sets, was to investigate the overall tendencies within the different innovative methods compared to the COD(Cr) method.

An initial approach when performing the statistical methods was to include all the analytical data provided within the validation, in order to investigate the overall performance of the various methods. Because the knowledge of the validated methods and their ability to provide accurate results were limited it was difficult with high certainty to point out data as eventual outliers and duplicates were therefor made for all samples. The result was then included in the data set as two separate values. If the duplicates generated deviating values, they were further compared to the COD concentration provided for the COD(Cr) method. The drawback of this treatment of data was that the two values were dependent of each other though the samples were collected from the same beaker.

The data from the influent and effluent wastewater samples were examined separately to enable characterization of method performance with varying COD concentrations. It was seen that the COD content had a varying impact for the methods compared to the reference method, COD(Cr). The influent wastewater had a much higher concentration of COD, compare to the effluent wastewater, due to a higher particular COD content. By comparing the different types of wastewater to each other and to involve multiple laboratories and WWTP it was found that the innovative methods were applicable to different degree depending on type of wastewater analyzed. Both Gryaab and Käppala received samples from large WWTP with many person equivalents, which resulted in wastewater with a large diversity of inorganic and organic compounds. For these types of WWTP it was said that the total organic load in incoming wastewater are relatively constant. For Komlab laboratory, which received wastewater samples from a variety of WWTP, it was believed that the COD concentration for influent wastewater would be shifting to a larger extent than for the other two laboratories. The measurement data could however reveal that the incoming COD concentrations for all laboratories did vary in the same extent. The chloride concentration was however more consistent for influent and effluent wastewater and around 55-130 mg/l for Gryaab and Käppala and a bit lower for Komlab, with concentrations of 15-85 mg/l.

Some factors regarding the methods could not be validated within the statistical test described above. The usability and the environmental impact of the various methods were two aspects that should be taking in consideration when validating the methods. Different viewpoints brought up by chemists and other involved parties in the PCP was therefore summarized for each analytical method. This sections aims to describe the benefits and drawbacks that were experience of the laboratory personnel. The laboratory observations has not been weighted against the statistical results but were treated as additional input regarding the overall performance of the participating analytical methods.

## 6.2 COD CHLORIDE DETERMINATION

When analyzing PHP standard solutions for the COD Chloride Determination method (Figure 12) the results were as expected close to the calculated theoretical COD values. When analyzing wastewater samples and conducting regression plots, the result indicated on a high linear correlation to the COD(Cr) method, for both influent (Figure 16) and effluent (Figure 22) wastewater samples. The correlation coefficients for influent wastewater were for all laboratories higher than 0.95 (Table 4) which indicates that the investigated method had a good correlation regardless of variation in the composition of the municipal wastewater. The only discrepant result for the regression analysis was obtained for effluent wastewater samples analyzed at the Käppala laboratory, which indicated on no correlation with a $R^2$ value of close to zero. This outcome could be due to that Käppala received the lowest COD concentration values compared to the other laboratories and thereby had a greater measurement uncertainty connected to the data for both methods. This may have generated in a wider spread of the COD concentrations for the laboratory which gave a less linear fit between the methods.

The Chloride Determination method is the method most similar to the standardized COD(Cr) method, when investigating the results from performed statistical methods and required analytical steps, described in detail in Section 4.2.1. This indicates that the method could be used as a replacement for the COD(Cr) method without mayor changes in analytical procedure performed by the staff on the laboratory. The drawback of the method is that it is not able to be used for online measuring and required equivalent analytical time as the reference method.

The Chloride Determination method is mercury free and counteract the interference from chloride ions by a calculation subtracting the chloride concentration multiplied by a factor from the photometer measured COD value. The method does however depend on potassium dichromate, which is listed on the European Chemical Agency's candidate list and has a sunset data in September 2017. For the method to be a sustainably selection for replacing the COD(Cr) in the long term, it would be necessary to further develop the method to replace the need for potassium dichromate to a more environmental alternative. If not developed, this method would be seen as a short term solution for the environmental and hazard problems that the standardized COD method generates today.

## 6.3 COD CHLORIDE ELIMINATION

The second analytical method submitted from the company Macherey-Nagel was the COD Chloride Elimination method. The measurement results for this method was limited due to a decision taken early in the validation process to place the method on hold. The underlying reason for this decision was that the analytical result obtained, generated consistently lower COD values compared to the COD(Cr).

The low COD values were probably caused by the cartridge which was used to pretreat the samples before entering the cuvettes for analysis. It was thought that the chemicals in the cartridge partially reacted with the organic substance in the wastewater samples in addition to the intended chloride ions, resulting in a lower analyzed COD concentration. An additional cause for the low COD concentration may be a result of organic particles from the sample could have been trapped in the membrane when injecting the sample into the cartridge. The membrane in the cartridge was design to separate any solid COD from the sample when passing through the membrane into the cartridge. This fraction of COD should then be reunite to the sample as the sample were ejected from the cartridge. It is not unlikely that particles were caught up in the membrane, resulting in a smaller solid fraction of solid COD than was originally in the sample, causing the lower obtained COD concentrations.

When an analytical method obtains only lower or higher analyte values compared to a reference method, the result could theoretically be multiplied by a scalar to achieve equivalent values. The downside of this process of data is that a measurement uncertainty is present for all analyzed COD result. The uncertainty was as previous varying depending on used analytical method, but are normally around 10-30 % of the receive concentration. It is therefore important to consider the measurement uncertainty when comparing measurement values for a parameter produced by a diversity of analytical methods. Because the analytical result obtained by the chloride elimination method were almost half the COD value obtained by the COD(Cr) method (Appendix A), the correlation factor would have been relatively high, close to two. While scaling up the received analytical value for the innovative method to correspond to the COD(Cr) results it would simultaneously increase the absolute uncertainty for the final calculated COD concentrations.

The decision to interrupt further analysis with the Chloride Elimination method in combination with the late delivery of the higher range of the method resulted in that no standard solutions were possible to be analyzed at that specific range. The lower COD range was however investigated using PHP standard solutions ranging between 15 to 160 mg/l and it was found that the method received a good agreement between the theoretical values and measured COD values. When performing analysis for wastewater samples, it yield more discrepant results compared to the reference method, COD(Cr). The statistical result conducted on the influent wastewater samples should be analyzed with caution due to the limited amount of samples analyzed at the various laboratories which result in a high uncertainty in the available input data.

The regression plots for influent wastewater samples (Figure 17) resulted in varying conformity to the COD(Cr) method depending on laboratory. The most notable result was for Käppala which against expected results generates a negative trend line and an intercept of +355. Gryaab and Komlab received, compared to each other, equivalent results with a low slope values for the trend lines and a $R^2$ value of approximately 0.85 for both laboratories. For the effluent samples (Figure 24) there were no clear agreement between the innovative and reference method, COD(Cr) when comparing the laboratory. As for influent samples, the laboratory Käppala had the lowest correlation between the methods, which for effluent wastewater samples were close to zero. The other two laboratories, Gryaab and Komlab did obtain higher correlation between the methods, but the with lower slope values compared to the value calculated for influent wastewater.

The correlation coefficients were higher for both Gryaab and Komlab for the influent samples, but did not receive factors lower than 0.7 for both laboratories evaluating the effluent samples (Table 7). Due to the limited amount of influent wastewater samples was the statistical results generated with a large uncertainty. When examining the overall statistical results produced by Käppala, the conclusion could was made that the Chloride Elimination was unable to produce COD results comparable to the COD(Cr) method. This is obvious in the correlations coefficient that adopted a negative value of -0.98 for influent samples (Table 4) and 0.22 for effluent sewage samples (Table 7).

Several remarks were stated from the staff of the involved laboratories regarding the execution of the analysis using the Chloride Elimination method. One of these was concerning the membrane in cartridge which had the tendency to loosen while injection of the sample was conducted, which contaminated the sample and made it unusable. It was important that protective clothing was used at all time when analyzing or cleaning the used laboratory equipment from the method, to prevent staining on the skin from the leaking chemicals from the cartridge. For Käppala the contamination of samples could also cause further problems because the collected sample volume was often limited. As for the previous described method, Chloride Determination, the Elimination method is also base on the usage of potassium dichromate as an essential oxidant. Due to the environmental and safety hazard that this substance represent it would be vital to renew the content of active chemicals within the method in order for the method to be a suitable candidate for replacing the COD(Cr) method.

If the COD Chloride Elimination method should be compared to earlier studies of mercury free COD method it had similarities to the Ag-COD analytical method described in section 3.3.1.Silver nitrate is used in both of these methods to counteract the interference of chloride ions as well as potassium dichromate to enhance oxidation of organic matter within the sample. The differences between the two analytical methods is when the free chloride ions are processes, as a pretreatment is a separated cartridge or within the analysis in the analysis cuvette, which was used for the Ag-COD method.

## 6.4 PECOD

The third analytical method included in the validation process was the PeCOD L100 which were represented with two version of the method, a manual and an automatic system. The method was supplied by the company ManTech and based on different ranges, which used various electrolyte solutions and programs in the software.

Standard solutions based on the substance sorbitol were initially analyzed and resulted in a good correlation between the theoretical COD concentrations and the PeCOD units for both the manual and automatic version of the method (Figure 15 and Figure 14). Both versions, regardless of range, generated $R^2$ values above 0.99, which were an indication of good agreement between the plotted data.

When comparing all the analytical results for wastewater for two versions of PeCOD it was evident that the manual unit generated higher COD concentrations than the automatic PeCOD (Appendix A). Both PeCOD units generally generated lower COD concentrations than the COD(Cr) method when analyzing the same samples. This result can be seen for both influent wastewater samples (Figure 18, Figure 19) as well as effluent wastewater samples (Figure 26, Figure 28) for the PeCOD method.

When the company ManTech received the data from the validation process they listed more than one quarter of the analytical results as uncertain. The reason for this was that the it differed more than 30% between the automatic unit manual. This outcome was present for both the influent and effluent wastewater samples, but when analyzing effluent wastewater it was only analytical data obtained at Gryaab, which contributed to this deviating data. ManTech believes this was due to deformity in the pumps that deliver solutions in proper amount to the vessel connected to the automatic PeCOD unit, but it needs further investigation to establish this statement (Peddle, 2015, personal communication).

Despite the difference in COD concentration between the manual and automatic PeCOD, both unit did generate the same trends in the data compared to the standardized COD(Cr) method. An example of this is illustrated by comparing the regression plot generated for the automatic PeCOD (Figure 18) and the manual PeCOD (Figure 19) for influent wastewater samples. It is there evident that the samples cause the similar spread between the PeCOD methods and the COD(Cr). The results for Komlab indicated that two data points was deviated from the rest of the data when examining the regression plots for influent wastewater and were therefor suspected to be outliers. The result originated from the first week of analyzing when the laboratories were still in training and some minor difficulties encountered considering the execution of analysis. If these data points was to be removed the $R^2$ value would increase from 0.1374 to 0.5701 for the automatic PeCOD (Table 1). For the manual unit the difference was even greater, where the R2 value was improved from 0.295 to 0.7979 (Table 1). These were assumed to effect the regression line by influence the weighting of the data in the set and generate a better correlation between the PeCOD and the COD(Cr) than was actually present.

When investigating the regression analysis conducted for filtrated influent wastewater samples, both Gryaab and Komlab received similar spread of data when comparing the two PeCOD versions to the reference method. This result was not accurate for Käppala laboratory, where the data was scattered for both the PeCOD unit. The data in Figure 26 and Figure 28 present a nonlinear correlations between the PeCOD and COD(Cr) method for the Käppala laboratory. Some of the COD values obtained when analyzing effluent wastewater samples at both Käppala and Komlab were generally too low and under the measurement range for the PeCOD and COD(Cr) methods. Additional regression plots were therefore created to investigate if these data points had a major impact on the obtained results. For Käppala it can be seen in Figure 27, that the elimination of data do not have a large influence. Komlab generated a lower correlation between the investigated methods with a lower $R^2$ value and a more deviating intercept than in the original regression analysis (Figure 26). It was of course vital to perform statistical tests where all the measurement data for an analytical method had the same measurement uncertainty connected to it. As the data showed, both Käppala and Komlab received lower COD values for effluent wastewater than the innovative methods were able to analyze with a high certainty. Because these low values did appear it is likely to reoccur low COD values again and it is therefore desirable that the future method are able to establish COD values with high certainty regardless of amount of analyte in the sample.

One important aspect of consideration when evaluating the PeCOD method is to examine to what extent the method could correlate to historical COD data. Normally for the operational and modelling aspect for a WWTP the COD value that is requested is the total COD, included both the soluble and particular fraction of COD. The PeCOD which represent the soluble COD content where therefore compared to unfiltered samples analyzed with the reference method, COD(Cr). When evaluating this analysis for both influent and effluent wastewater it could be established that the same tendency in obtained data were present, regardless of the samples being filtrated or not for the COD(Cr) method. Interestingly the $R^2$ values were higher for Gryaab and Komlab when analyzing the results from the manual PeCOD. By gathering additional parameters of the wastewater samples, such as suspended matter, the various COD types could be used to investigate a conversion factor between the PeCOD and total COD value. This factor would probably vary locally on the different laboratories and would generate a high workload for the specific laboratories to produce. The fact that the PeCOD method is unable to analyze the particulate content of COD in the samples was stated as a drawback, due to the vital role which the total COD values has in operation process and modelling of a WWTP (Lundin, 2015). Even if a conversion factor could be found between PeCOD and COD(Cr) for unfiltered samples it would add a uncertainty to the final COD value when depending on a multiplier to receive the total COD value.

The correlation analysis resulted in varying results for the laboratories depending on the type of wastewater samples that were analyzed. For influent samples Käppala and Gryaab had similar correlation of 0.72 to 0.81 (Table 4) for the two versions of PeCOD compared to COD(Cr) for filtered samples. Gryaab did obtained similar correlation coefficients for effluent wastewater, where Käppala was found to have almost no correlation for the PeCOD methods to the COD(Cr) irrespective to the pretreatment of the samples (Table 7). Komlab received higher correlation for the manual PeCOD than for the automatic system and the highest values were evident for effluent wastewater samples (Table 7). The correlation coefficient for Komlab for effluent samples were 0.9086 at minimum and 0.9754 as highest, depending on whatever the samples were filtrated or not before conducting analysis.

The laboratory which achieved the most uniform coefficient of correlation were Gryaab with ranging values between 0.5743 up to 0.7856 for the COD(Cr) method (unfiltered and filtered samples) or version of PeCOD method. The highest values were set for effluent wastewater samples at Komlab laboratory and Käppala did receive the correlation values that were most contradictionary when comparing the manual and automatic PeCOD.

According to the company responsible for the PeCOD method, the method was proven to have a lower correlation if measurement data from different WWTP were mixed together (Peddle, 2015). The reason for this was that the samples contained different composition regarding organic substances which could react and decompose in various degree. Based on this argument it would be possible to compare analytical data within a WWTP, were the composition may be less altering then to compare measurement data obtained for several different WWTPs. Due to the fact that Komlab receives wastewater samples from different WWTPs it was therefore unexpected that this laboratory had the best correlations between the PeCOD and the COD(Cr) method on 0.9086 and higher.

The PeCOD method was the most environmental friendly method in the validation, without the usage of mercury as well as potassium dichromate. The technique used to conduct analysis of COD is innovative and not found in prior studies. If comparing the submitted versions of the method, the manual method could be seen to generate higher COD values than the automatic unit. This result was not expected because the PeCOD units used for both versions should be equivalent according to the company and therefore receive equal results. Both the PeCOD versions needed to be calibrated before analyzing samples. This process did often require multiple calibrations to be performed in order to receive proper calibration results, which leads to additional work for the laboratory staff. After the PeCOD had been calibrated the automatic system was completely self-propelled as long as the sample were correct placed in the related rack and the software was programmed for the given run. The manual version of the method needed supervision every tenth minute while the software was running in order to change place on the injection cable which provided the unit with relevant solutions. This gives that the manual PeCOD needed more oversight and was harder to combine with other laboratory work.

The need for multiple calibration of the PeCOD was a problem that all of the laboratories encountered to various degrees. If the sensors within the PeCOD unit had been extensively used and needed to be replaced it was thought to generate insufficient calibration results, but because the need of repeated calibration was constant it was however thought to depend on another underlying cause. Another explanation could have been that the analysis conducted on the unit were too far apart in time, due to only weekly sampling of wastewater samples. Even though standby runs with blank solutions were executed in order to keep the sensors hydrated it could have in some point gone dry and therefore not operated as preferred according to the suppling company ManTech (Lindsay, 2015, personal communication).

In order to analyze with the PeCOD method, it is vital that the sample obtain a pH value of 4-10 in order to not harm the connected sensor in the unit. The samples were therefor not able to be preserved and it was desired to conduct analysis the same day as sampling in order to counteract degradation of organic substances within the sample. Due to this timeframe and calibration process needed, it was difficult to conduct reruns of the sample within the same work day.

## 6.5 COMPARISON OF THE METHODS

All the methods were mercury free, which were a basic requirement stated in the PCP. A desired requirement was that the methods also should be free from potassium dichromate, which was fulfilled for the PeCOD method but not for the Chloride Determination and Elimination methods. Both the Chloride Determination and Elimination methods were, due to their content of dichromate, more similar to the COD(Cr) method in the sense of having the same basic chemical composition. This makes the methods more likely to generate comparable COD values against the COD(Cr) method than for the PECOD, which was based upon a different chemical principle.

In addition to the result provided by regression and correlation analysis was ANOVA studies executed. This statistical test was performed in two groups, where the first group were using data for the methods Chloride Determination and Chloride Elimination in comparison to the COD(Cr) method (Table 2, Table 5). The second group was conducted f data from the manual and automatic PeCOD and the COD(Cr) for filtrated samples (Table 3, Table 6). The outcome from the ANOVA was that the methods mean values were significantly different regardless of group of method, laboratory or wastewater that was studied. It was only one ANOVA calculation, investigating Influent wastewater at Komlab for the PeCOD and COD(Cr) group (Table 6), that generated a larger calculated F-values than a critical F-value. Komlab received calculated F-values nearest the critical F value for all type of ANOVA, regardless of the methods or wastewater studied. However the calculated values were significantly higher than the critical F-value, which indicates that the mean values were deviating from each other. It is important to remember that even if a rejection of the null hypothesis is stated it does not imply that all the data sets differ from each other, just that at least one of the three sets are deviating. Based on these ANOVA results it is difficult to draw any conclusions to which analytical method that is to prefer for a further validation process.

There are both differences and similarities between the methods and laboratories present in the validation process. The COD concentrations for effluent wastewater was regardless of laboratory often low and on the boarder of the detection limit for the various methods. This generates an increased uncertainty to the statistical result performed on the effluent wastewater samples. In some cases data were removed due to too low COD concentrations which generated a smaller dataset to perform the statistical test with. It is therefore more uncertain to draw overall conclusions if using only the effluent wastewater samples as data.

When investigating the aspect of learning to work and conduct analysis with the various methods was the Chloride Determination method found to be most similar to the COD(Cr) method. This was becuase the similar time for analysis and working step needed to generate COD results. The same procedure was needed for the Chloride Elimination method, excluding the pretreatment of the sample by the cartridge. The training required for the laboratory staff which are already familiar to the COD(Cr) method are there for limited for both the Chloride Determination and Elimination methods. The PeCOD however was more divergent in underlying analysis technique compared to the COD(Cr) method, but has the advantage of generating COD results in less time (15 minutes), based on the PeCOD being calibrated before analysis. The PeCOD method was also the most environmental friendly method with no hazardous waste, which could be seen as a advantages, due to the minimized cost of waste disposal for the laboratories.

When examining the regression curves for standard solutions for the three methods, no mayor differences or indications that a method would be unsuited for upcoming validation were found. Due to the regression results for standard solutions it were clear that all the methods were capable to analyze COD concentrations for simplified sample matrixes as standard solutions made of PHP or sorbitol. Unfortunately, the higher range for the Chloride Elimination method could not be tested this way and a lack of data was therefore provided for this specific method.

By comparing the regression plots methods it was clear that Chloride Determination had a stronger linear relation to the COD(Cr) methods than the other two innovative methods. This was especially evident when evaluating the regression plots obtained for influent wastewater (Figure 16) where it could be seen that the method had a high conjunction for the two methods for all the laboratories with a $R^2$ value of 0.92 and higher. Based on the statistical results and the discussed laboratory observations the Chloride Determination could be recommended for further validation. It would however be favorable to continue developing the method to obtaining an even better correlation to the COD(Cr) method without the need of potassium dichromate. The Chloride Elimination method was unable to generate equal COD values compared to the COD (Cr) method, which was the primary requirement stated in the validation and are therefore not recommended for further testing.

The PeCOD had a steady performance level, which had a poor correlation to the COD(Cr) method. Because the PeCOD method analyzed the soluble COD content, it would require an equation or conversion factor in order to generate a total COD concentration similar to the COD(Cr) method. This transformation of analytical would be needed in order to relate the PeCOD results to historical obtain COD measurement data. The possibility of using the value of suspended solids within the wastewater in combination with the PeCOD result could be one approach to obtain a total COD value, which however would result in a absolute higher measurement uncertainty, due to the individual measurement uncertainty for each of the analysis. In order to solve this problem for the PeCOD method, the submitted company has indicated that a development of the method are in process which would enable analysis of the particulate COD content in the samples. If this method development was to function as intended it would be a natural step to include this upgraded version of the method into the upcoming phase in the validation. Not only would it add an additional method measuring the total COD concentration to the validation, but also enhance the probability to find a more greener method than the COD(Cr) method which was desirable.

## 6.6 SOURCES OF ERROR

Some aspects were consider to contribute to uncertainty for the produced results in the validation study. The mayor uncertainty factor when performing the statistical analysis for wastewater samples were the limited amount of analytical data. Due to the delayed arrival of the analytical methods, was the analytical work postpone, which resulted in a reduced period of time for intended analytical work. One of the basic requirements for the data used in the statistical test was that it should be normal distributed. No trend analysis were executed in order to prove relevant distribution, but the data was assumed to be approximately normal distributed according to earlier explanation in the middle of section 3.5.2, if 20 or more data points were available. In those cases were the data sets were conducted of less than the required amount of data, the test were executed as normally but marked as having a higher uncertainty.

When conducting the analysis with the various innovative methods, the analysis were at the greatest extent performed for all the methods at the same time on the day of sampling. In a few occasions this approach could not be fulfilled due to required reanalysis in order to receive valid results. Analysis with the PeCOD was as discussed needed to be performed as quickly as possible to avoid degradation of the organic substances within the sample. Because preservation was allowed for the other innovative method as well as for the COD(Cr), these analysis were able to postponed without changing the COD content in the sample.

Both the methods submitted by Macherey-Nagel were based on cuvettes being scanned in the spectrometer to receive a final COD value. The spectrometer used, regardless of method the same wavelength for a specific COD range even when the solution in the unused cuvettes had different color. This could be seen to contribute with an uncertainty to the COD concentrations performed with the Chloride Elimination and Determination methods.

# 7 CONCLUSIONS

Based on the validation of the three innovative methods, COD Chloride Determination, COD Chloride Elimination and PeCOD the following conclusions could be drawn.

The Chloride Determination method was shown to be the method most comparable to the COD(Cr) method based on analysis performance, time of analysis and analytical result. The Chloride Elimination method was seen to produced much lower result than the reference method, COD(Cr).This was thought to be a result of the pretreatment of the sample when using the cartridge device and the method could therefore not be comparable to the COD(Cr) method.

When comparing the two versions of the PeCOD with the COD(Cr) method they were both seen to produce lower COD concentrations than the reference method. The manual PeCOD unit did generally give higher COD concentrations than the automatic PeCOD when analyzing the same samples. This gave that the manual PeCOD was the version most comparable to the COD(Cr) method. The comparability between the PeCOD versions and the reference method was also found to differ between the laboratories and no uniform correlation factor could be established.

For the extended validation process is it recommended to continue the testing of Chloride Determination. It is also recommended to continue with the manual PeCOD version if the company is able to develop the method for analysis of the particular COD. The Chloride Elimination method has not shown sufficient correlation compared to the reference method and should therefore not be further developed or tested.

Lastly it could be stated that due to low COD concentrations for effluent wastewater samples the statistical results for these samples was uncertain, because the values were close to the detection and quantification limit for the methods. Overall, the data obtained within the validation were limited but general characteristics for the participating methods have been predicted and could act as a foundation for future validating work.

# 8 RECOMMENDATIONS FOR FURTHER STUDIES

To generate better conditions for comparing the validate methods additional aspects could be entered within the validation work. The first recommendation is to continue analyzing COD for influent and effluent wastewater and favorably increase the sampling to twice a week in order to generate more data. It would also be positive if the analysis could be executed on a variety of yearly seasons including autumn and winter conditions for the influent wastewater. This is mainly due to yearly varying chloride concentrations which within this study has been covering a small concentration span.

It would also be preferred to perform analysis on a wider range of COD concentration and especially for higher COD concentrations for effluent wastewater. This could be done by spiking wastewater samples with different COD concentration. This would enable analysis of COD concentrations for the whole range between influent and effluent wastewater and provides a wider spread of data. The same sample treatment could also be done for the chloride concentrations to achieve higher variation of chloride content within the samples. This would be highly recommended if the future testing time would not include the seasonal variation of chloride concentration.

When working within this project it was evident that chloride concentration was a parameter that was not analyzed on a regular basis for wastewater at the Käppala Association or the other laboratories. If the concentration of chloride was to be analyzed for a whole year, it could form the basis for developing seasonal concentration values. Depending on the yearly variance of the obtained chloride concentrations a fixed summer and winter value could thereafter be set.

If several analysis were to be performed and the data for the various method would be enhanced it would be recommended to implement the statistical method Principal Component Analysis (PCA). The test are able to investigate how the wastewater composition are influencing the analytical measurements for the innovative methods obtained in the study based on all the data for a specific sample. A requirement for the PCA test is that the data are of sufficient quantity in order to be performed and the limitation of data has made it difficult to conduct the test within this study.

Finally it would be recommended to not limit the search for a mercury free COD method to the methods provided within the procurement. The method participating in the validation was a result of the development that had been done at the time for tendering and new methods are constantly developed on the market. An evident of this was that several companies have been in contact with the project management during the project asking to attend the procurement.

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## 9.2 PERSONAL COMMUNICATIONS

Enache, L.; Laboratory Engineer at Gryaab, 2015, 20:th of July

Frenzel, M., Environmental Engineer at Käppala Association, 2015-07-22

Karat, D., Laboratory manager at Käppala Association, 2015, 24:th of August

Lopez, M., Laboratory Manager at Komlab Laboratory, 2015, 7:th of July

Lundin, E., Project Manager for the pre-procurment project, 2015, 25:th of August

Peddle, L., Quality Control and Research Application Supervisor at ManTech inc, 2015

Prokish, C.,Development Engineer at Machery-Nagel, 2015. 25:th of June
Thunberg, A., Production Manager at Käppala Association, 2015, 28: of July

# APPENDIX A MESURMENTDATA FROM THE THREE LABORATORIES: KÄPPALA, GRYAAB AND KOMLAB

The analytical data received within the project are presented separated for influent and effluent wastewater samples for the individual laboratories in the following way: Käppala, Gryaab and Komlab. The grey shading indicated that analysis had not started or had ended at the time for the specific wastewater sample. The dashed line indicated that analytical data were lacking and the yellow shade represented that the values were lower than the set detection limit for the method.

*Table 8. Käppala laboratory, Influent wastewater samples*

| Date | Sample identity | SS mg/l | BOD-7 mg/l | Chloride mg/l | COD (Cr) mg/l | COD (CR) filtered mg/l | MN - Chloride Elim. mg/l | MN – Chloride Detek. scanned mg/l | MN - Chloride Detek. Recalc. mg/l | Auto PeCOD mg/l | Manual PeCOD mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20150423 | ink 150420 | - | 180 | 75 | 520 | | | 520 | 503,5 | | |
| 20150423 | ink150420 | - | 180 | 75 | 520 | | | 502 | 485,5 | | |
| 20150430 | ikn150428 | - | 270 | 85 | 660 | | | 644 | 625,3 | | |
| 20150430 | ink150428 | - | 270 | 85 | 640 | | | 644 | 625,3 | | |
| 150508 | ink 150506 | 330 | 240 | 68 | 618 | 168 | | 625 | 610,04 | 84 | |
| 150508 | ink 150506 | 330 | 240 | 68 | 610 | 171 | | 641 | 626,04 | 85 | |
| 150519 | ink 150517 | 287 | 110 | 51 | 395 | 85,2 | - | 348 | 336,78 | 47,7 | |
| 150519 | ink 150517 | 287 | 110 | 51 | 378 | 88,7 | - | 355 | 343,78 | 43,9 | |
| 150520 | ink 150519 | 383 | 210 | 52 | 819 | 77,1 | - | 718 | 706,56 | 49,9 | 73,7 |
| 150520 | ink 150519 | 383 | 210 | 52 | 807 | 77,5 | - | 751 | 739,56 | 51,6 | 73,7 |
| 20150525 | ink 150525 | 217 | 170 | 65 | 368 | 118 | 86 | 406 | 391,7 | 31,3 | 83,5 |
| 20150525 | ink 150525 | 217 | 170 | 65 | 356 | 119 | 88 | 408 | 393,7 | 31,9 | 83,5 |
| 20150608 | INK 150607 | 197 | 160 | 66 | 414 | 119 | 51 | 445 | 430 | 62 | 78,3 |
| 20150608 | INK 150607 | 197 | 160 | 66 | 409 | 121 | 47 | 445 | 430 | 60,4 | 80,7 |
| 150611 | INK 150610 | 240 | 220 | 72 | 539 | 171 | | 513 | 497 | 91,6 | 110,6 |
| 150611 | INK 150610 | 240 | 220 | 72 | 524 | 173 | | 525 | 509 | 90,4 | - |
| 150616 | INK 150615 | 227 | 230 | 69 | 546 | 185 | | 511 | 496 | 104,5 | 120,7 |
| 150616 | INK 150615 | 227 | 230 | 69 | 537 | 184 | | 527 | 512 | - | 124,4 |
| 150625 | Ink 150623 | 253 | 100 | 75 | 622 | 137 | | 572 | 555,5 | 46,5 | - |
| 150625 | Ink 150623 | 253 | 100 | 75 | 617 | 138 | | 592 | 575,5 | 48,4 | 47,7 |
| 150625 | Ink 150623 | 253 | 100 | 75 | - | - | | 569 | 552,5 | 50,5 | 48,7 |
| 150625 | Ink 150623 | 253 | 100 | 75 | - | - | | 572 | 555,5 | 52,1 | 46,5 |
| 150625 | Ink 150623 | 253 | 100 | 75 | - | - | | 585 | 568,5 | 53,7 | 50,4 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150626 | Ink 150623 | 253 | 100 | 75 | - | - | | 576 | 559,5 | - | - |
| 150630 | Ink 150623 | 253 | 100 | 75 | - | - | | 569 | 552,5 | - | - |
| 150701 | Ink 150623 | 253 | 100 | 75 | - | - | | 576 | 559,5 | - | - |
| 20150708 | INK 150702 | 313 | - | 82 | 508 | 166 | | 513 | 494,96 | 94,9 | - |
| 20150708 | INK 150702 | 313 | - | 82 | 497 | 171 | | 429 | 410,96 | 84,5 | - |

*Table 9.Gryaab laboratory, influent wastewater samples*

| Date | Sample identity | SS mg/l | BOD-7 mg/l | Chloride mg/l | COD (Cr) mg/l | COD (CR) filtered mg/l | MN - Chloride Elim. mg/l | MN – Chloride Detek. scanned mg/l | MN - Chloride Detek. Recalc. mg/l | Auto PeCOD mg/l | Manual PeCOD mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150421-22 | 2015-1042-01 | 224 | 240 | 130 | 453 | | | 497 | 468,4 | | |
| 150421-22 | 2015-1042-01 | 224 | 240 | 130 | 454 | | | 502 | 473,4 | | |
| 150426-27 | 2015-1084-01 | 188 | 180 | 110 | 312 | | | 375 | 350,8 | | |
| 150426-27 | 2015-1084-01 | 188 | 180 | 110 | 329 | | | 410 | 385,8 | | |
| 150503-04 | 2015-1140-01 | 184 | 160 | 130 | 319 | | | 403 | 374,4 | | |
| 150503-04 | 2015-1140-01 | 184 | 160 | 130 | 321 | | | 431 | 402,4 | | |
| 150512-13 | 2015-1236-01 | 162 | - | 66 | 238 | 54,9 | | 271 | 256,48 | 38,1 | - |
| 150512-13 | 2015-1236-01 | 162 | - | 66 | 233 | 54,3 | | 275 | 260,48 | 37,8 | - |
| 150520-21 | 2015-1306-01 | 148 | 110 | 110 | 328 | 92,8 | 56 | 343 | 318,8 | 53,8 | - |
| 150520-21 | 2015-1306-01 | 148 | 110 | 110 | 321 | 94,8 | 56 | 340 | 315,8 | 48,7 | - |
| 150528-29 | 2015-1379-01 | 308 | 210 | 110 | 495 | 155 | 82 | 548 | 523,8 | 58 | 92 |
| 150528-29 | 2015-1379-01 | 308 | 210 | 110 | 483 | 155 | 95 | 534 | 509,8 | - | 92,7 |
| 150602-03 | 2015-1418-01 | 128 | 84 | 55 | 210 | 50,5 | 47 | 252 | 239,9 | 34,8 | 45,1 |
| 150602-03 | 2015-1418-01 | 128 | 84 | 55 | 211 | 53,9 | 54 | 235 | 222,9 | 32,6 | 38,7 |
| 150610-11 | 2015-1490-01 | 220 | 200 | 140 | 420 | 131 | 89 | 476 | 445,2 | 65,3 | 118,1 |
| 150610-11 | 2015-1490-01 | 220 | 200 | 140 | 425 | 130 | 84 | 467 | 436,2 | 62,5 | 117,3 |
| 150616-17 | 2015-1548-01 | 204 | 190 | 96 | 396 | 150 | | 467 | 445,88 | 87,2 | 95,7 |
| 150616-17 | 2015-1548-01 | 204 | 190 | 96 | 408 | 148 | | 485 | 463,88 | 85,9 | 101 |
| 150622-23 | 2015-1587-01 | 268 | 220 | 120 | 442 | 160 | | 485 | 458,6 | 49,1 | 86,9 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150622-23 | 2015-1587-01 | 268 | 220 | 120 | 434 | 161 | | 501 | 474,6 | 50,8 | 85,4 |
| 150628-29 | 2015-1636-01 | 188 | 180 | 140 | 357 | 117 | | 415 | 384,2 | 60,2 | 59,2 |
| 150628-29 | 2015-1636-01 | 188 | 180 | 140 | 359 | 107 | | 424 | 393,2 | 55,4 | 55,8 |

*Table 10. Komlab Laboratory, Analytical measurement from influent wastewater samples*

| Date | Sample identity | SS mg/l | BOD-7 mg/l | Chloride mg/l | COD (Cr) mg/l | COD (CR) filtered mg/l | MN - Chloride Elim. mg/l | MN – Chloride Detek. scanned mg/l | MN - Chloride Detek. Recalc. mg/l | Auto PeCOD mg/l | Manual PeCOD mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150423-24 | 339 | - | 120 | 26 | 560 | | | 518 | 512,28 | | |
| 150423-24 | 339 | - | 120 | 26 | - | | | 471 | 465,28 | | |
| 150423-24 | 343 | - | 200 | 51 | 479 | | | 431 | 419,78 | | |
| 150423-24 | 343 | - | 200 | 51 | - | | | 429 | 417,78 | | |
| 150506 | 391 | 840 | 210 | 49 | 784 | 71 | | 774 | 763,22 | 102 | 118 |
| 150506 | 391 | 840 | 210 | 49 | - | - | | 766 | 755,22 | - | - |
| 150506 | 401 | 340 | 320 | 49 | 446 | 97 | | 471 | 460,22 | 111 | 96 |
| 150506 | 401 | 340 | 320 | 49 | - | - | | 479 | 468,22 | - | - |
| 20150521 | 442 | - | 200 | 51 | 469 | 139 | 46 | 459 | 447,78 | 70 | 53 |
| 20150521 | 442 | - | 200 | 51 | 471 | - | 53 | 462 | 450,78 | 64 | 51 |
| 20150521 | 444 | - | 150 | 33 | 542 | - | - | 509 | 501,74 | 50 | 41 |
| 20150521 | 444 | - | 150 | 33 | - | - | - | 522 | 514,74 | 45 | 36 |
| 20150602 | 480 | 33 | 4 | 16 | 105 | 39,1 | 37 | 116 | 112,48 | 42 | 46,6 |
| 20150602 | 480 | 33 | 4 | 16 | 106 | 39,1 | 35 | 121 | 117,48 | 41 | 45,5 |
| 20150602 | 482 | 120 | 52 | 21 | 173 | 43,8 | - | - | - | 25,7 | 33,6 |
| 20150602 | 482 | 120 | 52 | 21 | 173 | 43,8 | - | - | - | 25,4 | 31,9 |
| 20150602 | 484 | 180 | 140 | 39 | 332 | 103 | - | - | - | 41,2 | 53,8 |
| 20150602 | 484 | 180 | 140 | 39 | 332 | 103 | - | - | - | 44 | 52,6 |
| 20150602 | 486 | 300 | 65 | 21 | 284 | 44,2 | - | - | - | 23,5 | 26,1 |
| 20150602 | 486 | 300 | 65 | | 284 | 44,2 | - | - | - | 21,6 | 26,4 |
| 20150602 | 489 | - | 48 | 16 | 181 | 53,7 | - | - | - | 31,5 | 34,5 |
| 20150602 | 489 | - | 48 | 16 | 181 | 53,7 | - | - | - | 29,9 | 33,8 |
| 20150616 | 557 | 221 | 210 | 44 | 465 | - | | 478 | 468,32 | 62,6 | 73,3 |
| 20150616 | 557 | 221 | 210 | 44 | 453 | - | | 474 | 464,32 | 61,9 | 73,6 |
| 20150616 | 565 | 96,8 | 45 | - | 142 | - | | - | - | 33,2 | 38,5 |
| 20150616 | 565 | 96,8 | 45 | - | 142 | - | | - | - | 32,4 | 39,5 |
| 20150616 | 567 | 713 | 200 | 83 | 858 | - | | 802 | 783,74 | 47,2 | 61,4 |
| 20150616 | 567 | 713 | 200 | 83 | 858 | - | | 726 | 707,74 | 46,9 | 60,6 |
| 20150616 | 577 | 261 | 140 | - | 310 | - | | - | - | 36,5 | 49,1 |
| 20150616 | 577 | 261 | 140 | - | 310 | - | | - | - | 35 | 48,9 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20150616 | 582 | 177 | 73 | - | 229 | - | | - | - | 37,1 | - |
| 20150616 | 582 | 177 | 73 | - | 229 | - | | - | - | 37,4 | 34,7 |
| 20150630 | 618 | 88 | 58 | 34 | 157 | 64 | | 182 | 174,52 | 33,1 | 39,7 |
| 20150630 | 618 | 88 | 58 | 34 | 174 | 64 | | 180 | 172,52 | 31,1 | 42,8 |
| 20150630 | 620 | 267 | 210 | 64 | 549 | 194 | | 585 | 570,92 | 65,7 | 86,2 |
| 20150630 | 620 | 267 | 210 | 64 | 549 | 194 | | 593 | 578,92 | 63,8 | 88 |
| 20150630 | 622 | 520 | 220 | 45 | 808 | 163 | | 774 | 764,1 | 36,4 | 55,6 |
| 20150630 | 622 | 520 | 220 | 45 | 808 | 163 | | 779 | 769,1 | 35,5 | 55,8 |

*Table 11. Käppala Laboratory, Analytical result for effluent wastewater samples*

| Date | Sample identity | SS mg/l | BOD-7 mg/l | Chloride mg/l | COD (Cr) mg/l | COD (CR) filtered mg/l | MN - Chloride Elim. mg/l | MN – Chloride Detek. scanned mg/l | MN - Chloride Detek. Recalc. mg/l | Auto PeCOD mg/l | Manual PeCOD mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20150423 | Utg 150420 | | 2 | 75 | 32,3 | | 14 | | | | |
| 20150423 | Utg 150420 | - | 2 | 75 | 32,3 | | 17 | | | | |
| 20150430 | Utg 150428 | - | 2 | 74 | 30,8 | | 23 | | | | |
| 20150430 | Utg 150428 | - | 2 | 74 | 32,8 | | 23 | | | | |
| 150508 | Utg 150506 | 1,2 | 2 | 80 | 41 | 33 | 19 | | | 21 | - |
| 150508 | Utg 150506 | 1,2 | 2 | 80 | 37 | 37 | 23 | | | 22 | - |
| 150518-19 | utg 150517 | 1 | 2 | 65 | 27,4 | 27,2 | 20 | | | 16,6 | - |
| 150518-19 | utg 150517 | 1 | 2 | 65 | 31 | 26,2 | 20 | | | 14,6 | - |
| 150520 | utg 150519 | 2 | 2 | 48 | 26,5 | 25,4 | 16 | | | 18,4 | 22,6 |
| 150520 | utg 150519 | 2 | 2 | 48 | 25,8 | 25,2 | 15 | | | 16,1 | 22,6 |
| 20150525 | utg 150525 | 2 | 2 | 63 | 30,6 | 29,5 | 23 | 31 | 17,14 | 5,2 | 19,2 |
| 20150525 | utg 150525 | 2 | 2 | 63 | 29,1 | 30,8 | 20 | 37 | 23,14 | 5,2 | 19,2 |
| 20150608 | UTG 150607 | 1,2 | 2 | 68 | 31 | 28,4 | 28 | 38 | 23,04 | 18,4 | 21,6 |
| 20150608 | UTG 150607 | 1,2 | 2 | 68 | 31,9 | 28,6 | 27 | 37 | 22,04 | 17,2 | 20,2 |
| 150611 | UTG 150610 | 1,2 | 2 | 75 | 30,9 | 27,5 | | 42 | 25,5 | 18,1 | 20,4 |
| 150611 | UTG 150610 | 1,2 | 2 | 75 | 30,3 | 23,9 | | 41 | 24,5 | 16,8 | - |
| 150616 | UTG 150615 | 0,2 | 2 | 69 | 31 | 33,2 | | 40 | 24,82 | 22,7 | 23,9 |
| 150616 | UTG 150615 | 0,2 | 2 | 69 | 28 | 35,4 | | 39 | 23,82 | 19,7 | 23,3 |
| 150625 | Utg 150623 | 1 | 2 | 69 | 28,7 | 25,2 | | 36 | 20,82 | 23,9 | 27,8 |
| 150625 | Utg 150623 | 1 | 2 | 69 | 29,1 | 28,1 | | 37 | 21,82 | 23,3 | 22,3 |
| 150625 | Utg 150623 | 1 | 2 | 69 | - | - | | 38 | 22,82 | 21,3 | 20,5 |
| 150625 | Utg 150623 | 1 | 2 | 69 | - | - | | 41 | 25,82 | 20,6 | 16,3 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150625 | Utg 150623 | 1 | 2 | 69 | - | - | | 38 | 22,82 | 19,9 | 13,8 |
| 150626 | Utg 150623 | 1 | 2 | 69 | - | - | | 37 | 21,82 | - | - |
| 150630 | Utg 150623 | 1 | 2 | 69 | - | - | | 39 | 23,82 | - | - |
| 150701 | Utg 150623 | 1 | 2 | 69 | - | - | | 42 | 26,82 | - | - |
| 20150708 | UTG 150702 | 2,6 | - | 79 | 23,4 | 22 | | 40 | 22,62 | 4,2 | - |
| 20150708 | UTG 150702 | 2,6 | - | 79 | 27,7 | 26,1 | | 41 | 23,62 | 2 | - |

*Table 12. Gryaab Laboratory, analytical result for effluent wastewater samples*

| Date | Sample identity | SS mg/l | BOD-7 mg/l | Chloride mg/l | COD (Cr) mg/l | COD (CR) filtered mg/l | MN - Chloride Elim. mg/l | MN – Chloride Detek. scanned mg/l | MN - Chloride Detek. Recalc. mg/l | Auto PeCOD mg/l | Manual PeCOD mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150421-22 | 2015-1049-01 | 4,4 | 10 | 130 | 43,3 | | 26 | | | | |
| 150421-22 | 2015-1049-01 | 4,4 | 10 | 130 | 42,4 | | 29 | | | | |
| 150426-27 | 2015-1089-01 | 3,6 | 7 | 98 | 38,2 | | 28 | | | | |
| 150426-27 | 2015-1089-01 | 3,6 | 7 | 98 | 38,4 | | 22 | | | | |
| 150503-04 | 2015-1150-01 | 6,4 | 8 | 130 | 42,1 | | 29 | | | | |
| 150503-04 | 2015-1150-01 | 6,4 | 8 | 130 | 39,1 | | 28 | | | | |
| 150512-13 | 2015-1239-01 | 7,6 | 10 | 88 | 40,7 | 36,9 | 24 | | | 24,4 | - |
| 150512-13 | 2015-1239-01 | 7,6 | 10 | 88 | 41,2 | 33,6 | 27 | | | 23 | - |
| 150520-21 | 2015-1311-01 | 8 | 11 | 110 | 46,8 | 43,9 | 28 | 67 | 42,8 | 28,1 | - |
| 150520-21 | 2015-1311-01 | 8 | 11 | 110 | 45,5 | 41,2 | 28 | 67 | 42,8 | 25,8 | - |
| 150528-29 | 2015-1367-01 | 9,2 | 15 | 110 | 57,3 | 48,8 | 37 | 80 | 55,8 | 30,5 | 48,5 |
| 150528-29 | 2015-1367-01 | 9,2 | 15 | 110 | 64,9 | 46,7 | 34 | 76 | 51,8 | - | 48,3 |
| 150602-03 | 2015-1412-01 | 6 | 10 | 57 | 35,9 | 29,2 | 25 | 48 | 35,46 | 17,2 | 34,1 |
| 150602-03 | 2015-1412-01 | 6 | 10 | 57 | 37,1 | 28 | 24 | 46 | 33,46 | 15,3 | 29,6 |
| 150610-11 | 2015-1495-01 | 5 | 10 | 130 | 46,7 | 39,5 | 34 | 76 | 47,4 | 20,5 | 46,5 |
| 150610-11 | 2015-1495-01 | 5 | 10 | 130 | 47,7 | 40,4 | 37 | 73 | 44,4 | 17,9 | 44,5 |
| 150616-17 | 2015-1547-01 | 4,2 | 6 | 120 | 47,2 | 38,3 | | 63 | 36,6 | 16,6 | 28,1 |
| 150616-17 | 2015-1547-01 | 4,2 | 6 | 120 | 47,2 | 38,5 | | 63 | 36,6 | 18,6 | 26,7 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150622-23 | 2015-1593-01 | 2,6 | 6 | 130 | 36,2 | 34,5 | | 60 | 31,4 | 14,3 | 25 |
| 150622-23 | 2015-1593-01 | 2,6 | 6 | 130 | 37,3 | 34,6 | | 62 | 33,4 | 13,2 | 23,4 |
| 150628-29 | 2015-1639-01 | 3,6 | 6 | 130 | 39,5 | 35,8 | | 62 | 33,4 | 22,4 | 23,3 |
| 150628-29 | 2015-1639-01 | 3,6 | 6 | 130 | 37 | 36,4 | | 56 | 27,4 | 23 | 23,3 |

*Table 13. Komlab laboratory, analytical measurement results for effluent wastewater samples*

| Date | Sample identity | SS mg/l | BOD-7 mg/l | Chloride mg/l | COD (Cr) mg/l | COD (CR) filtered mg/l | MN - Chloride Elim. mg/l | MN – Chloride Detek. scanned mg/l | MN - Chloride Detek. Recalc. mg/l | Auto PeCOD mg/l | Manual PeCOD mg/l |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150423-24 | 335 | 22 | 4 | 28 | 30 | | 13 | | | | |
| 150423-24 | 335 | 22 | 4 | 28 | 30 | | 14 | | | | |
| 150506 | 397 | 13 | 11 | 12 | 22 | 10,6 | 14 | | | 6,5 | - |
| 150506 | 397 | 13 | 11 | 12 | 22 | - | 12 | | | - | - |
| 150506 | 404 | 13 | 4 | 28 | 22 | 12,4 | 15 | | | 4,6 | - |
| 150506 | 404 | 13 | 4 | 28 | 22 | - | 16 | | | - | - |
| 150506 | 405 | 8,1 | 15 | 5 | 36 | 25,5 | 29 | | | 21,3 | 21,3 |
| 150506 | 405 | 8,1 | 15 | 5 | 36 | - | 26 | | | - | - |
| 20150521 | 439 | - | 4 | 27 | - | - | - | - | - | - | 10,3 |
| 20150521 | 439 | - | 4 | 27 | - | - | - | - | - | - | 10 |
| 20150521 | 445 | 5,7 | 4,1 | 74 | 27,1 | 29,3 | 19 | 48 | 31,7 | 4,3 | 16 |
| 20150521 | 445 | 5,7 | 4,1 | 74 | 27,1 | 29,3 | 16 | 49 | 32,7 | 4,9 | - |
| 20150527 | 464 | 6,5 | 4 | 8 | 20 | 19,7 | - | - | - | 13 | 17,1 |
| 20150527 | 464 | 6,5 | 4 | 8 | 20 | 19,7 | - | - | - | 12,7 | 16,4 |
| 20150527 | 468 | 11 | 13 | 64 | 36 | 33,2 | - | - | - | 12 | 17,8 |
| 20150527 | 468 | 11 | 13 | 64 | 36 | 33,2 | - | - | - | 12,3 | - |
| 20150527 | 470 | 18 | 23 | 22 | 54 | 53,9 | 37 | 56 | 51,2 | 26,6 | 36,4 |
| 20150527 | 470 | 18 | 23 | 22 | 58 | 53,9 | 33 | 51 | 46,2 | 27,5 | 34,5 |
| 20150527 | 471 | 6,6 | 9,7 | 21 | 28 | 25,6 | 23 | 27 | 22,4 | 16,3 | 14,9 |
| 20150527 | 471 | 6,6 | 9,7 | 21 | 28 | 25,6 | 26 | 31 | 26,4 | - | 14 |
| 20150602 | 487 | 12 | 4 | - | 41 | - | 10 | 35 | - | - | - |
| 20150602 | 487 | 12 | 4 | - | - | - | 18 | 35 | - | - | - |
| 20150610 | 551 | 14 | 28 | 43 | 57,3 | 33,6 | | 74 | 64,5 | 37,3 | - |
| 20150610 | 551 | 14 | 28 | 43 | 59,5 | 32,5 | | 77 | 67,5 | 36,6 | 34,8 |
| 20150610 | 552 | 7,9 | 18 | 43 | 41,4 | 21,2 | | 48 | 38,5 | 21 | 22,1 |
| 20150610 | 552 | 7,9 | 18 | 43 | 41,4 | 21,2 | | 47 | 37,5 | 19,5 | 21,5 |
| 20150610 | 553 | 14 | 18 | 54 | 43,2 | 25,1 | | 63 | 51,1 | 19,5 | 21,8 |
| 20150610 | 553 | 14 | 18 | 54 | 43,2 | 25,1 | | 64 | 52,1 | 19,1 | 22,1 |
| 20150616 | 558 | 7,3 | 10 | 65 | 48,8 | - | | 67 | 52,7 | - | - |
| 20150616 | 558 | 7,3 | 10 | 65 | 48,8 | - | | 70 | 55,7 | - | - |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20150623 | 597 | 40 | 32 | 22 | 75,7 | 37 | | 77 | 72,2 | 14,3 | 19,2 |
| 20150623 | 597 | 40 | 32 | 22 | 75,7 | 37,2 | | 79 | 74,2 | 14,1 | 17,6 |
| 20150623 | 598 | 8,3 | 97 | 160 | 155 | 138 | | 172 | 136,8 | 79 | 95,3 |
| 20150623 | 598 | 8,3 | 97 | 160 | 151 | 134 | | 159 | 123,8 | 78,7 | 93,2 |
| 20150623 | 603 | 11 | 6,2 | 16 | 29,8 | - | | 36 | 32,5 | 21,3 | 21 |
| 20150623 | 603 | 11 | 6,2 | 16 | 29,8 | - | | 38 | 34,5 | 17,4 | 20 |

# APPENDIX B PREPERATIONDESCRIPTION OF STANDARD AND CONTROLL SOLUTIONS

The PHP solution was prepared of solid potassium hydrogen phthalate (PHP) which dried in an oven at 120°C for two hours. The substance was then weight and dissolved with deionized water and four molar sulfuric acid ($H_2SO_4$) to achieve two stock solution. These solutions were produces according to the quantities in Table 14 with concentration of 1000 mg/l for the low stock solution and 2000 mg/l for the higher range.

*Table 14. Input parameters for PHP stock solutions used as foundation for further dilutions to conduct standard solution of various COD concentration.*

| Solution | PHP [g] | $H_2SO_4$ 4 M [ml] | Diluted with deionized $H_2O$ to [ml] | Concentration [mg/l] |
|---|---|---|---|---|
| Stock solution (PHP 1)<br>(high COD range ) | 0.425 | 5 | 500 | 1000 |
| Stock solution (PHP 2)<br>(low COD range ) | 0.4251 | 2.5 | 250 | 2000 |

Based upon the two stock solution, standard solutions where prepared in varying concentrations with the aim of covering the whole working range for both the lower and the higher COD range according to Table 15 and Table 16. The measured amounts of stock solution were thereafter diluted with acidulated deionized water containing1 ml 4 M sulfuric acid for each 100 ml of water. The acidification of the water was made to achieve an equal pH-value as for the preserved wastewater samples which were to be analyzed later on.

*Table 15. Desired concentrations and the volumes needed from the stock solution (2) for producing standard solution for the lower COD range for the analytical methods: Hach Lange, Macherey-Nagel Chloride Detection and Chloride Elimination*

| Concentration [mg/l] | Stock solution (PHP 2) [ml] (100 ml volumetric flask) |
|---|---|
| 15 | 1,5 |
| 25 | 2,5 |
| 50 | 5 |
| 100 | 10 |
| 160 | 16 |

*Table 16. Desired concentration and the volumes needed from the stock solution (1) for producing standard solution for the higher COD range for the analytical methods: Hach Lange, Macherey-Nagel Chloride Detection and Chloride Elimination*

| Concentration [mg/l] | Stock solution (PHP 1) [ml] (100 ml volumetric flask) |
|---|---|
| 100 | 5 |
| 200 | 10 |
| 500 | 25 |
| 1000 | 50 |
| 1500 | 75 |

The sorbitol standard solutions were produced from solid sorbitol and dried according to the same embodiment as PHP substrate. It was then weighted in the right amount described in Table 17 to produce two stock solutions with the concentration of 1000 and 2000 mg/l in separate volumetric flasks of thousand ml.

*Table 17. Input parameters for the two sorbitol stock solutions used as foundation for further dilutions to conduct standard solutions of variated COD concentrations.*

| Solution | Sorbitol [g] | Diluted with deionized $H_2O$ to [ml] | Concentration [mg/l] |
|---|---|---|---|
| Stock solution (S1) (high COD range ) | 1.7519 | 1000 | 2000 |
| Stock solution (S2) (low COD range ) | 0.8756 | 1000 | 1000 |

The stock solutions for sorbitol were not preserved, because the PeCOD instrument was not able to run sample solutions lower than pH 2. From the stock solutions, a predetermine amount were placed into 100 ml volumetric flasks described in Table 18 and Table 19 and then diluted with deionized water to achieved an accurate volume.

*Table 18. Desired concentration and the volume needed from the stock solution (S2) to produce standard solution for the lower COD range for the analytical method PeCOD*

| Concentration [mg/l] | Stock solution (S2) [ml] (100 ml volumetric flask) |
|---|---|
| 20 | 2 |
| 40 | 4 |
| 70 | 7 |
| 100 | 10 |
| 150 | 15 |

*Table 19 Desired concentrations and the volumes needed from the stock solution (S1) for producing standard solution for the higher COD range for the analytical method PeCOD*

| Concentration [mg/l] | Stock solution (S1) [ml] (100 ml volumetric flask) |
|---|---|
| 150 | 7.5 |
| 300 | 15 |
| 500 | 25 |
| 1000 | 50 |
| 1500 | 75 |

## APPENDIX C REGRESSION PLOTS OF PECOD COMPARED TO BOD$_7$

As supplement to the regression plots performed for the two versions of the PeCOD method against the reference method COD(Cr), were additional regression plots made comparing the PeCOD results to the measured BOD concentration. This comparison was performed due to a recommendation from the submitted company ManTech which earlier had found better correlation between the PeCOD method and BOD, than comparing to the COD(Cr) method. The results were as previous in the report divided for influent and effluent wastewater samples. The influent wastewater did for both the automatic (Figure 31) and manual (Figure 32) PeCOD show a weak linear correlation and the wide spread of data.

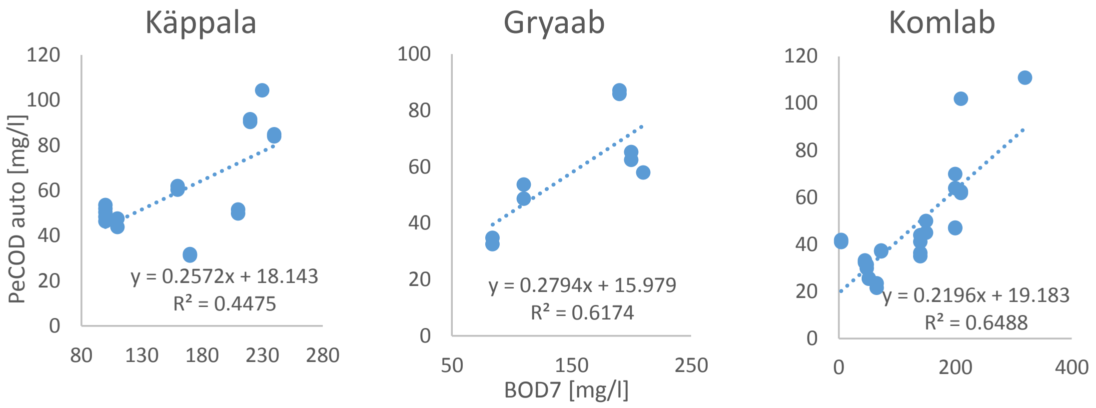

*Figure 31. Regression plot performed on influent wastewater for the automatic PeCOD compared to the BOD concentrations for the samples.*

When comparing the intercept for the two versions of PeCOD for influent wastewater it was clear that similar values were obtained for the automatic PeCOD. The manual PeCOD did however give different intercept depending on laboratory. This dissimilarity could also be found for the R$^2$ value for the manual PeCOD but tend to be more uniform for the automatic PeCOD comparing Figure 31 and Figure 32.

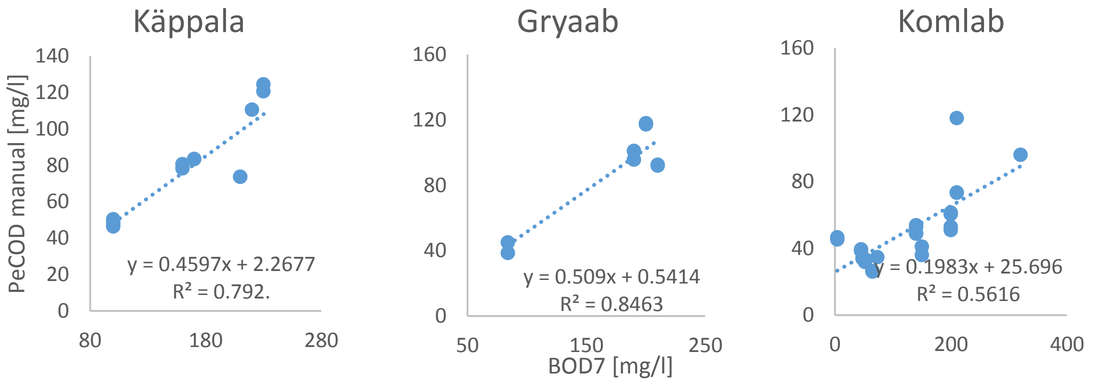

*Figure 32. Regression plot conducted on influent wastewater samples for the manual PeCOD compared to the obtained BOD value for the samples.*

A plot could not be conducted for the Käppala laboratory on effluent wastewater samples, because all samples collected and analyzed had received a BOD value of <2, which was rounded off to the value 2. Regression plots were however conducted for the other laboratories Gryaab and Komlab. Two analytical results for Komlab analyzed as duplicates were excluded from the dataset, because the values were more than 3 times higher than the other data collected for both methods. These values were not excluded due to incorrect measurements or inaccurate content of COD or BOD, but because these values had a high overall impact on the regression parameter, which could be questioned. The removal of data was done for the same sample for both the automatic and manual PeCOD systems using analytical data from Komlab (Figure 33, Figure 34).

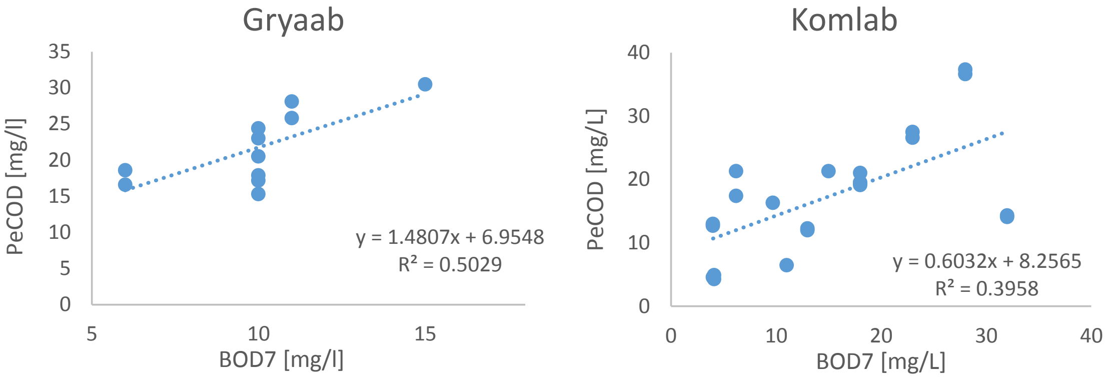

*Figure 33. Regression plot conducted on effluent wastewater samples for the automatic PeCOD compared to the obtained BOD value for the samples.*

An interesting result for Gryaab was that the data was grouped to different BOD values with varying COD values according to the PeCOD methods. This result was evident for both the automatic (Figure 33) and manual (Figure 34) PeCOD. The data was however too limited in order to draw any conclusions regarding the cause of this effect.

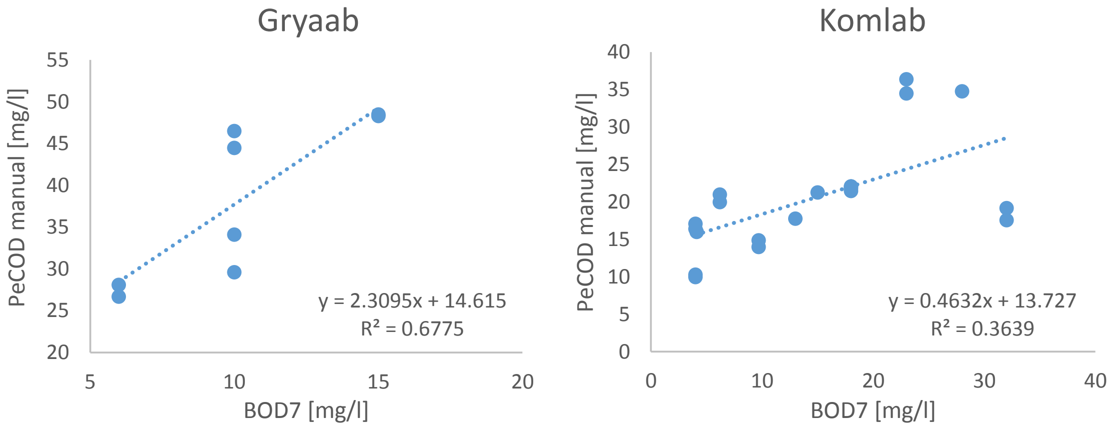

*Figure 34. Regression plots conducted on effluent wastewater samples for the manual PeCOD compared to the obtained BOD value for the samples.*

Based on this regression plots it could not be proven that the PeCOD method would have had a better correlation to the BOD concentrations than the COD(Cr) values for the investigated wastewater samples.

# APPENDIX D FURTHER EXPLANATION REGARDING ANOVA CALCULATIONS

A practical example is of the ANOVA in Section 3.5.2 based on fiction values in Table 20. The calculation is based on three different data sets named 1, 2 and 3.

*Table 20. Input values for three different data sets used to conduct the ANOVA represented in Table 21*

| Groups | Data |
|---|---|
| 1 | 5, 8, 9.5, 6, 7, 7.2 |
| 2 | 6.7, 8.2, 9, 4, 5.3, 5.5 |
| 3 | 7, 9, 10.4, 6.3 |

*Table 21. One- way ANOVA performed using values listed in Table 20 combined with an F-test*

| Groups | Count, $n_1$ | Sum | Mean value | Variance | | |
|---|---|---|---|---|---|---|
| 1 | 6 | 42.7 | 7.1 | 2.44 | | |
| 2 | 6 | 38.7 | 6.5 | 3.57 | | |
| 3 | 4 | 32.7 | 8.2 | 3.51 | | |
| Source of variation | SS | df | MS | F | P-value | F crit |
| *Between groups* | 7.14 | 2 | 3.57 | 1.14 | 0.35 | 3.81 |
| *Within groups* | 40.59 | 13 | 3.12 | | | |
| Total | 47.73 | 15 | | | | |

The first calculation is to estimate, for each dataset, how many data points are obtained (noted as $n_1$) and what their total sum are. These values are summarized in the upper part of Table 21 in column two and three. The mean value, $\bar{x}$, is then calculated (third column). The variance, denoted as $\sigma^2$, is thereafter calculated according to equation (4) for all the individual sets of data. The designation $x_i$ indicates each of the values listed in Table 20. The computed values of variance are compiled in the upper ending column in Table 21.

$$\sigma^2 = \sum \frac{(x_i - \bar{x})^2}{(n_1 - 1)} \tag{4}$$

These calculated variables are the basis for further calculations in ANOVA, established for *between* or *within* the provided data sets.

The sum of squares (SS) is calculated where all the input data values in Table 20 are subtracted by their datasets mean value (equation (5)). The resulting value is found in lower part of Table 21 in the second column.

$$SS_{Within\ datasets} = \sum_i (x_i - \bar{x})^2 \tag{5}$$

The mean square (MS) which describes the mean values of the squared differences between the measured value and the data sets mean value, are obtained by dividing the SS value with the degree of freedom. The degree of freedom used for within data sets calculations, is the amount of datasets subtracted by one as equation (6) illustrates. The ratio between the SS and degree of freedom is described in equation (7) and the value of MS is summarized in the lower part of Table 21 in the fourth column. The index number two indicates the number of datasets compared to index one that describes the amount of measurement data for each set of data.

$$df_{Within\ datasets} = n_1 - 1 \tag{6}$$

$$MS_{Within\ datasets} = \frac{SS}{df} = \frac{\sum_i (x_i - \bar{x})^2}{n_2 - 1} \tag{7}$$

For the calculations *between the data groups*, the calculations will follows the same procedure but with different input data. To start, the SS are calculated by using a mean value predicted for all the measurement values within the data groups subtracted by the mean value of the total measurements, summarized in equation (8).

$$SS_{Between\ datasets} = \sum_j \left(x_j - \bar{x}_j\right)^2 \tag{8}$$

To be able to calculate a MS value between the data sets, is the degree of freedom need to be determined. This is done by summarize the number of measurement values subtracted by the amount of data groups as the data is gathered from. The calculation for the degree of freedom is illustrated in equation (9) where $n_j$ is the notation for total number of data and $h$ the amount of data groups.

$$df_{Between\ datasets} = n_j - h \tag{9}$$

The MS are then calculated using the SS value divided by the degree of freedom obtained between the samples and the resulting value listed in Table 21, column four according to equation (10).

$$MS_{Between\ datasets} = \frac{SS_{Between\ datasets}}{df_{Between\ datasets}} = \frac{\sum_j \left(x_j - \bar{x}_j\right)^2}{n_j - h} \tag{10}$$

When the MS values have been produced for both the *within dataset* group and the one for *between datasets*, the ANOVA is completed by calculating an F-value using an F-test in agreement of equation (11). This value is obtained by dividing the mean square value between the data groups with the within data set calculated mean square value, compiled in Table 21, column four.

$$F = \frac{MS_{Between\ datasets}}{MS_{Within\ datasets}} \tag{11}$$

As further explained in the Section 3.5.3 about F-test, if a calculated F-value obtains a higher value than the critical tabulated F-value for a specific degree of freedom, the null hypothesis are able to be rejected. For the example given above, the calculated F-value is 1.14 and the critical value for F is predicted to 3.81. This indicates that null hypothesis are in fact valid for thought the mean values for the three sets of data did not significantly diverse from each other.