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Process Evaluation and Microbial Data Analysis of Randvik Wastewater Treatment Plant, Risør Norway

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Summary:

The Randvik wastewater treatment plant at Risør, Norway is a biological wastewater treatment plant consisting of nitrification and denitrification processes both for organic and nutrient removal. The treatment plant is located at Risør municipality in Agder County Norway. The sewage treatment plant is the smallest of its kind, with an ultimate design capacity of 7000 PE (population equivalent). This study has investigated the process evaluation and microbial data analysis of the wastewater treatment plant. The physiochemical data collected over the last five years (2018-2022) was studied, and the correlation between microbial data (genomic sequencing data) obtained from polymerase chain reaction (PCR) techniques was attempted along with reviewing the literature to understand the results of the data analysis.

The physiochemical data analysis was based on secondary data which includes biochemical oxygen demand (BOD), total phosphorus (TP), total nitrogen (TN), and influent flow rate provided by the Risør municipality. The data analysis was performed by plotting and comparison on Microsoft Excel. A single factor ANOVA test was performed in Excel to find out the significance of the data. The most prevalent microbial species in the Randvik wastewater treatment plant were identified using samples from the micro-biome sequencing results. The main results of the project were that the removal efficiency of the biological oxygen demand (BOD) and the total nitrogen (TN) was satisfied with the emission standards of the Norwegian legislative whereas the requirement for purification effect for total phosphorus (TP) concentration was nearly acceptable. The average treatment efficiency of organic pollutants for five years was found to be 98% while the total phosphorus removal efficiency was calculated as 88.42%. Likewise, the trend analysis between BOD and TP shows that it fluctuates at different times of the year and doesn't remain constant in both the influent and effluent wastewater.

The microbial profile of all the samples collected from the plant showed that the *Actinomycetales, Saprospirales, Caldilineales, Acidimicrobiales, Rhizobiales, etc* are the major microbial orders that dominate the wastewater. Further studies should focus on improving the conditions for phosphorus removal, which mean optimization of the anoxic/anaerobic tanks and reducing the sludge level in the settling tanks. Likewise, initiatives should be taken to reduce the sludge volume index of the treatment plant as it is quite higher (500-700 ml/g) out of the aerobic basin based on the treatment plant data. Based on recent investigations, this thesis also suggests a promising treatment method known as simultaneous nitrification-denitrification and phosphorus removal (SNDPR). Hence, the Randvik wastewater treatment plant can achieve an efficient SNDPR process with the current treatment plant setup.

Preface

This master's thesis study has been performed as part of a master's degree program in Energy and Environmental Technology at the University of South-Eastern Norway. The research was carried out to analyze the physiochemical data collected over the last five years as well as the microbiome data and correlate it with the wastewater characteristics and treatment process in the Randvik WWTP. The research was carried out based on the secondary data provided by the treatment plant and microbiome data collected from a project. This study was organized by USN in collaboration with Randvik WWTP, Risor municipality.

I would like to express my sincere gratitude to researcher Dr. Eshetu Janka Wakjera, my esteemed advisor, for his continuous support during my master's thesis project and for his technical guidance, feedback, and beneficial suggestion throughout the project.

I would also like to thank Nina Lieng Christiansen, department engineer at Randvik WWTP, Risør Municipality for providing me access to the treatment plant data. Special thanks go also to SINTEF Industry for genomic analysis services obtained in a regional fund collaborative project with USN.

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Porsgrunn, 15.05.2023

Sandesh Kuikel

Contents

Nomenclature

ANAMMOX	Anaerobic Ammonium Oxidation
AOB	Ammonia Oxidizing Bacteria
ASP	Activated Sludge Process
BNR	Biological Nutrient Removal
BOD	Biological Oxygen Demand
C/N	Carbon to Nitrogen ratio
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphorus Removal
EPS	Extracellular Polymeric Substances
GAOs	Glycogen Accumulating Organisms
HDB	Heterotrophic Denitrifying Bacteria
HRT	Hydraulic Retention Time
MBBR	Moving Bed Biofilm Reactor
MLSS	Mixed Liquor Suspended Solids
N	Nitrogen
NOB	Nitrite Oxidizing Bacteria
P	Phosphorus
PAOs	Polyphosphate Accumulating Organisms
PHAs	Poly-hydroxy-alkanoates
PCR	Polymerase Chain Reaction
RAS	Return Activated Sludge
SHARON	Single reactor High Activity Ammonia Removal Over Nitrite

SRT	Sludge Retention Time
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solid
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solid
WAS	Waste Activated Sludge

1 Introduction

One of the biggest environmental problems facing today's society is the steadily rising generation of municipal wastewater due to growing populations and urban expansion. Municipal wastewater is characterized as a mixture of liquid wastes eliminated from homes, institutions, commercial enterprises, and industries, as well as groundwater, surface water, and stormwater [1]. Wastewater contamination is still a significant issue in modern civilization because of industrialization and the ongoing growth of the global population. Global eutrophication (algal blooming in an aquatic ecosystem) is a major problem caused due to the emission of wastewater containing a higher concentration of phosphorus (P) and nitrogen (N) into the water bodies. Hence, the major goal of wastewater treatment is to eliminate organics, suspended solids, nutrients (P &N), and other contaminants that, if released into aquatic habitats, could harm the ecosystem.

Wastewater can be treated using physical, chemical, and biological unit processes. Furthermore, wastewater containing biodegradable constituents can be treated biologically. The biological wastewater treatment process uses a variety of microorganisms, primarily bacteria, are used to biologically remove dissolved and particulate carbonaceous BOD and stabilize organic materials found in wastewater [2]. Therefore, the biological treatment process has an economic advantage over other treatment processes such as chemical and physical in terms of capital investment and operation-maintenance costs. The biological process used for wastewater treatment can be divided into two main categories: i) suspended growth (activated sludge), and ii) attached growth (biofilm).

The Randvik (Risør municipality in Agder County, Norway) wastewater treatment plant has implemented biological treatment known as enhanced biological phosphorus removal (EBPR) instead of chemical treatment. EBPR for the removal of phosphorus is a practical, affordable, and sustainable approach. The basic EBPR process consists of an anaerobic zone followed by an aerobic zone. The EBPR process relies on microorganisms that can collect phosphorus (P) from wastewater for cellular growth, thereby eliminating P from the liquid phase. The key organisms in the EBPR process are polyphosphate accumulating organisms (PAOs) and they remove most of the P from the wastewater [3]. They are capable of accumulating phosphorus over and beyond what is necessary for growth. Conventional activated sludge organisms normally accumulate 2% of dry biomass as phosphorus whereas PAOs often accumulate 4 to 8% in full-scale treatment plants [4]. Phosphorus and nitrogen removal in municipal wastewater is done to prevent or reduce eutrophication in the receiving water bodies. Environmental aspects, process operational factors, and the composition of wastewater play a vital role in the efficient operation of the EBPR processes.

1.1 Scope of Work

This study is a project with the Randvik Wastewater treatment plant at Risør municipality in Agder County Norway. It is a biological wastewater treatment system combining oxidation of organics, nitrification-denitrification, and phosphorus removal process. The Activated Sludge (AS) process (because of the presence of active microorganisms) is widely adopted for the treatment of municipal and industrial wastewater on a small scale. In this study, the physiochemical data collected over the last five years (from 2018 –2022) was analyzed and tried to find the correlation between microbial data (genomic sequencing data) obtained from the polymerase chain reaction (PCR) techniques. Process evaluation and microbial data analysis based on the previous five years' data are performed to provide a good prediction of process dynamics and to study possible improvements in process operation and performance. The wastewater treatment process at Risør is presented in the figure below.

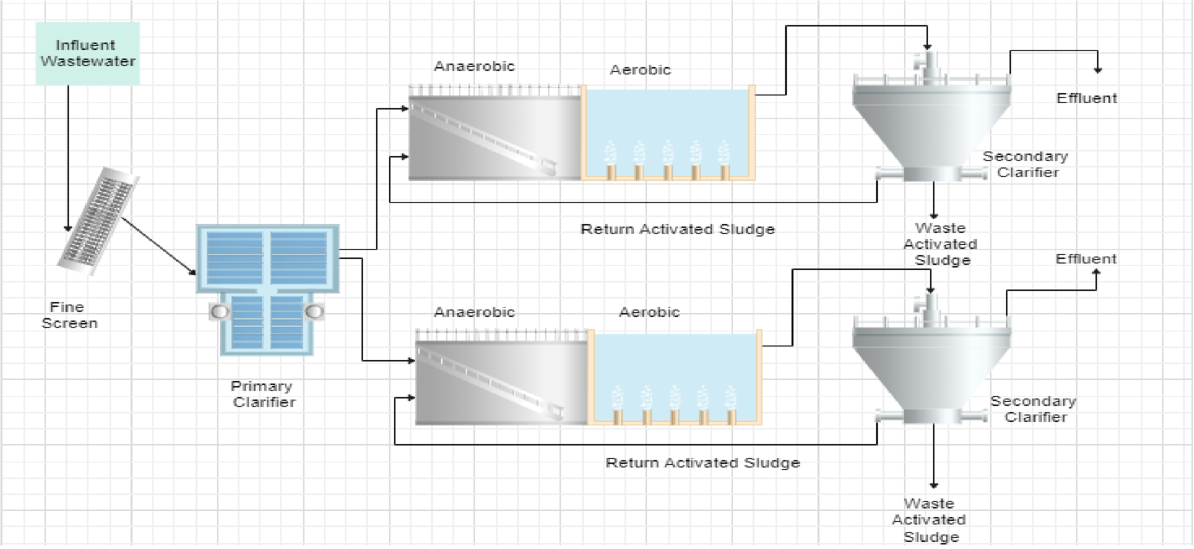


Figure 1.1: Wastewater Treatment Process at Randvik WWTP, Risør- (adopted and modified from the Risør treatment plant flow diagram without including reed beds)

1.2 Objectives

The project is solely focused on the biological treatment of municipal wastewater. The main objectives of this thesis are to investigate the process performance and microbial data analysis of the Randvik WWTP. The specific objectives of the project are listed below:

1. To analyze the physiochemical data collected over 5 years and undertake the treatment plant mass balance analysis.
2. To analyze the PCR microbiome data and correlate it with the wastewater's physical and biochemical characteristics.
3. To correlate the physiochemical data with the microbial data analysis and evaluate the process.
4. To review the literature to interpret the results of the data analysis.

1.3 Thesis Outline

The thesis report is divided into seven chapters. Chapter 1 provides a brief introduction to the biological treatment process and the objectives of the project. The theory and the literature study regarding the biological treatment process highlighting activated sludge systems with both denitrification and nitrification zone are explained the Chapter 2. Chapter 3 deals with the materials and methods adopted to achieve the thesis objectives. The fourth chapter presents the results obtained with appropriate graphical representation and explains the discussion of the obtained results comparing literary works. Chapters 5, 6, and 7 describe the conclusion based on discussion, the recommendation for future research, and references respectively.

2 Theory and Literature Study

This chapter deals with the different steps incorporated with the biological wastewater treatment process, the biological oxygen demand (BOD) removal process, nitrification, and denitrification for nitrogen removal, and enhanced biological phosphorus removal (EBPR) techniques. Furthermore, this topic covers the overview of Randvik WWTP, and the biological process associated with it.

2.1 Biological Wastewater Treatment Process

The biological treatment process is a conventional method of wastewater treatment where the naturally occurring bacteria, protozoa, and other microbes are primarily responsible for the oxidation of the organic materials, and cleaning the contaminated water [5]. A biological process is efficient and economical in comparison to physical and chemical processes. The two major biological processes used for the treatment of wastewater are suspended growth(activated sludge) and attached growth (or biofilm) processes [1].

2.1.1 Suspended growth process

The microorganisms suspended or floating in the wastewater are utilized to treat the wastewater in the suspended growth of the biological treatment process. With the help of pneumatic aeration or mechanical agitation, microorganisms and bacteria that treat wastes are suspended inside the liquid [6]. Microorganisms ingest organic material to develop and generate biomass flocs. The wastewater's organic content and other components are transformed into gases and cell tissues [7]. This approach uses an enrichment culture of microbial association to remove contaminants and make wastewater of quality acceptable to the environment [8]. The following equation shows the aerobic biological oxidation of organic matter.

$$v_1(organic\ material) + v_2O_2 + v_3NH_3 + v_4PO_4^{3-} \rightarrow v_5(new\ cells) + v_6CO_2 + v_7H_2O \tag{2.1}$$

Where, v_i is the stoichiometric coefficient.

The suspended growth process can be classified into two types based on the requirements of oxygen: i) aerobic suspended growth process, and ii) anaerobic suspended growth process. Aerobic processes are used in municipal and industrial wastewater treatment while anaerobic processes are used for the treatment of organic sludge and high-strength industrial wastewater [9]. The advantages of the suspended growth processes are to increase active microbial mass per unit volume, reduce suspended solids loading to the clarifier, enhance nitrification, improve sludge settling characteristics, and flexibility to diverse influent circumstances (shock load) [10]. Activated sludge process, aerated lagoons, and aerobic digestion are examples of aerobic suspended growth processes [11]. On the other hand, anaerobic digestion and anaerobic contact fall under an anaerobic suspended growth process [9]. The classification of the suspended growth process is shown in Fig (2.1).

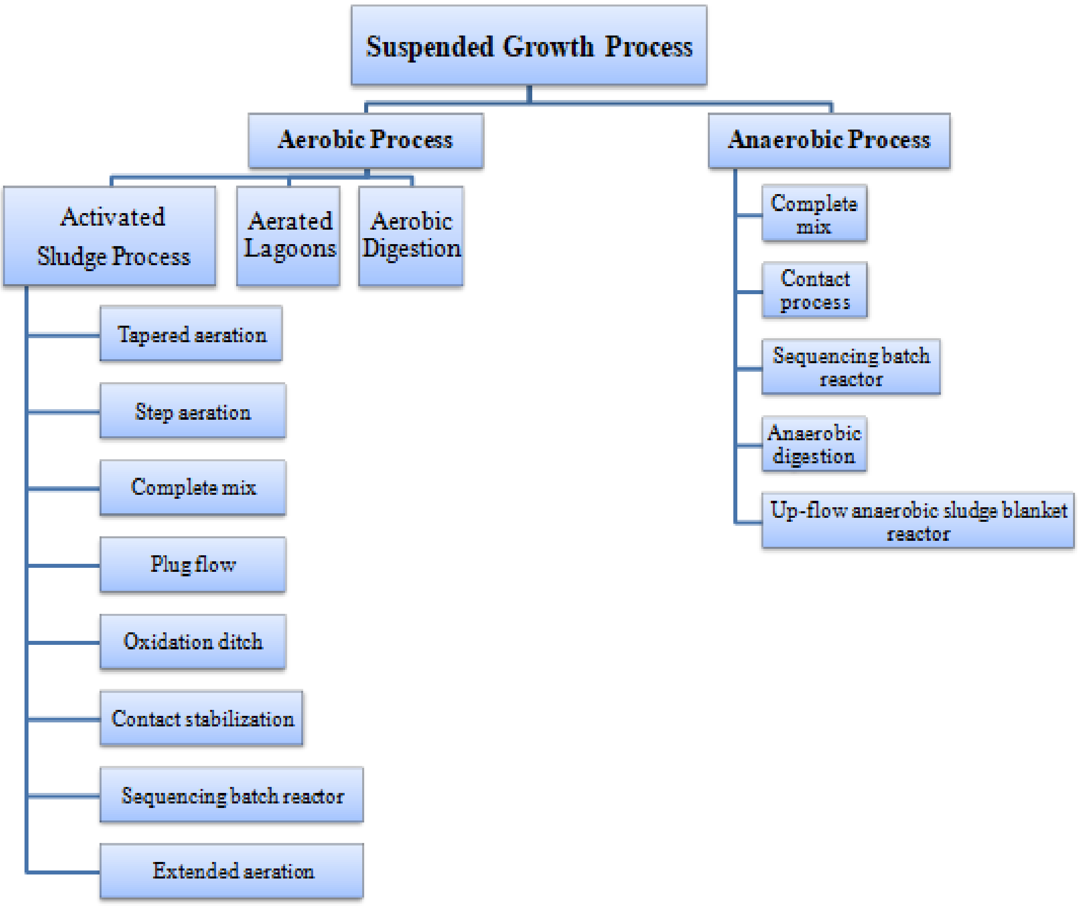


Fig 2.1: Classification of Suspended Growth Process [9]

The activated sludge process (ASP) is a widely adopted method of suspended growth system for the biological treatment of municipal and low-strength industrial wastewater [1]. The three major components of the ASP are i) the aeration tank ii) the settling tank (secondary clarifier), and iii) the recycling system. The aeration tank serves as a bioreactor, a secondary clarifier for the separation of sludge and treated wastewater, and a recycling system for the transfer of the return activated sludge (RAS) from the clarifier to the aeration tank [12]. The activated sludge is a biological floc having a mixture of microorganisms, non-living organic matter, and inorganic materials. The floc mixes with the stream of wastewater and oxidizes organic substances for bio-oxidation and nitrification reaction in the presence of oxygen or de-nitrification in the absence of oxygen [13]. Clark and Gage (1913) first experimented on the activated sludge process in 1913 at the Lawrence experiment station in the USA. The activated sludge procedure was developed by Ardern and Lockett, who also performed research and published findings on the advantages of reusing "*activated sludge*" for aerobic wastewater treatment in 1913-1914 [14]. Mixed liquor is a mixture of raw wastewater and activated sludge in an aeration tank. Mixes liquor suspended solid (MLSS) consists mainly of microorganisms and non-biodegradable suspended solids [15].

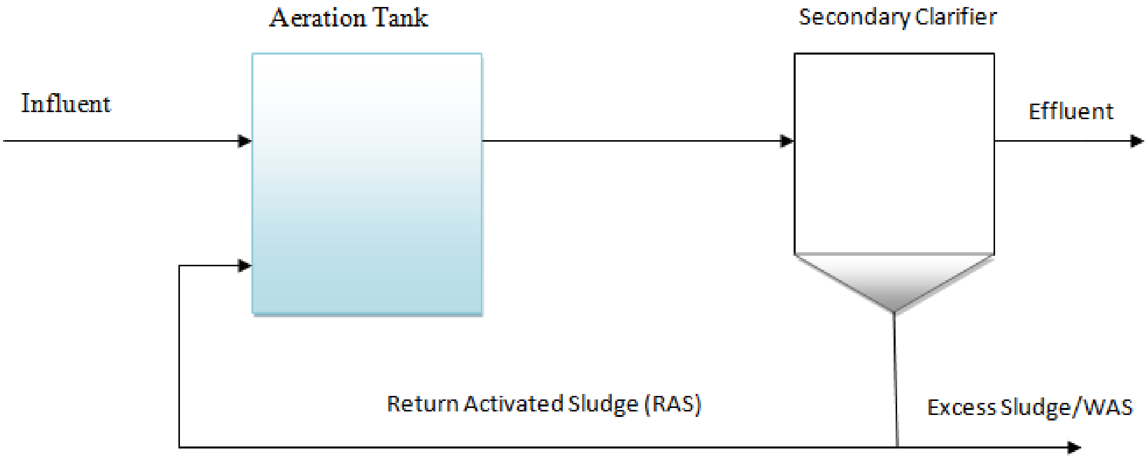


Fig 2.2: Layout of conventional activated sludge process [11]

Fig (2.2) shows the conventional activated sludge process consisting of an aeration tank, a settling tank (secondary clarifier), and return activated sludge. The waste-activated sludge goes to the disposal unit or is further treated as sludge stabilization. e.g., biomass

2.1.2 Attached Growth Process

Attached growth processes are biological wastewater treatment processes where biomass is attached with some type of media such as rock, ceramic, gravel, sand, plastic material, and slag [16]. The packing material in the attached growth systems might be fixed or suspended in the reactor. Polymeric materials (plastics) are widely used as packing materials for the attached growth process because they are inexpensive, lightweight, adaptable to various sizes and forms, and have a relatively high surface area [17]. Very short hydraulic retention time, high removal efficiency, low chemical addition rates, and relatively steady effluent independent of hydraulic and organic shock loads are the major characteristics of the attached growth process. A biofilm made up of bacteria, particulate matter, and extracellular polymers is attached and covers the support packing material [18]. The group of microorganisms which can be attached to the surface is known as a biofilm. They are complex structures consisting of microorganisms held together by extracellular polymeric substances (EPS) [19]. The choice of packing material is crucial for sustaining high levels of active biomass and a range of microbial populations because it provides a large surface area per unit volume for the growth of biofilms [20]. The packing material must have a large contact surface area, a high void space, and sufficient mechanical strength [16].

The attached growth system can be classified into two different categories based on the carrier movements i.e., fixed biofilm and moving biofilm system. It can be used as an aerobic or anaerobic process. Trickling filters, biological disks, and anaerobic up-flow filters are regarded as the most common example of fixed biofilm processes. Similarly, aerated biofilters, biological fluidized beds, biofilm reactors, etc. are some examples of moving biofilm systems. The major drawback of the attached growth process is media clogging. There have been reports of bio-media clogging due to biofilm thickness, poor water flow, media selection, a lack of aeration, a lack of backwashing, and mechanical failure [21]. There are several advantages of using biofilm in wastewater treatment systems over suspended growth systems, including more flexible procedures, less space requirement, low operation costs, shorter hydraulic retention time, increased resiliency, longer biomass retention period, and increased active biomass clusters [22]. The attached growth process is more stable when there are significant changes in the flow rate and concentration of the wastewater [23].

Moving bed biofilm reactor (MBBR) is an advanced wastewater treatment technology where freely moving plastic carriers with attached biofilm remove organic matter along with an innovative method for nitrification and denitrification. It is a novel aerobic wastewater treatment technology having higher efficiency and low maintenance costs which can handle wastewater flows ranging from 100,00 to 150,000 m^3 in different situations [24]. MBBR concepts based on biofilm are widely used for both organic and inorganic removal in industrial and municipal sewage treatment processes [25].

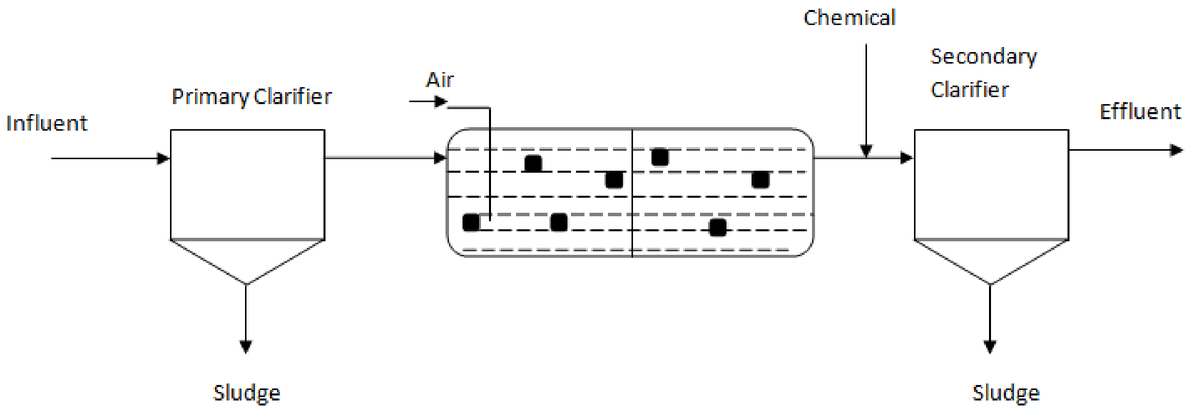


Fig 2.3: MBBR system for BOD and Phosphorus removal by chemical precipitation [1]

2.2 Phosphorus Removal

The characteristics of municipal wastewater depend upon various factors such as inflow and infiltration in a sewer line, discharges from industries, sewer system types, presence of phosphate detergents, and it varies from place to place as it is very site-specific [26]. Norwegian wastewater is typically cold, diluted, and has low nutrients due to the high amount of rainfall during wastewater transportation [27]. Phosphorus is a necessary ingredient for the growth of algae, crops, and other biological creatures. Municipal and industrial wastewaters along with agricultural activities are the major sources of phosphorus. The presence of large amounts of phosphate in wastewater is one of the primary causes of eutrophication, which has a severe impact on many natural water bodies [28].

There are two main phosphorus removal technologies: i) enhanced biological phosphorus removal, and ii) chemical phosphorus removal. Chemical phosphorus removal has been the most accepted technique but, these days EBPR has gained wider interest due to its low cost, less sludge production, and higher probability of phosphorus recovery [29]. Chemical precipitation removes phosphorus in three steps: coagulation, flocculation, and separation. In the chemical precipitation process, the chemicals mostly used for the removal of phosphorus are iron (Fe), calcium(Ca), and Aluminum(Al) cat-ions used for the precipitation of phosphorus [30].

Examples of phosphate removal by the addition of aluminum and iron are shown below.

1. Phosphate precipitation with aluminum

$$Al^{3+} + H_nPO_4^{3-n} \rightleftarrows AlPO_4 \downarrow + nH^+ \tag{2.2}$$

2. Phosphate precipitation with iron

$$Fe^{3+} + H_nPO_4^{3-n} \rightleftarrows FePO_4 \downarrow + nH^+ \tag{2.3}$$

2.2.1 Phosphorus removal by biological methods

Biological wastewater treatment is used to remove organic matter along with the removal of nutrients such as nitrogen and phosphorus. Biological phosphorus removal was based on research in the 1950s which states that activated sludge could take up excess phosphorus required for general biomass growth. This process is termed luxury uptake, and various processes and applications have been developed on this principle [29].

The polyphosphate accumulating organisms (PAO) is responsible for the EBPR process to remove phosphorus in the activated sludge system. The major advantage of the EBPR process is less sludge production and ease of phosphorus recovery [31]. Polyphosphate-accumulating organisms (PAO) are crucial for removing higher amounts of phosphorus since they can store roughly 0.38 g P/g VSS [32]. The EBPR approach can remove an adequate quantity of phosphorus from the system up to 90% in contrast to standard biological treatment, which can only remove 15 to 25% of phosphorus [33]. PAOs as well as Volatile fatty acids (VFA) are the key factors to enhance the efficiency of the process [34]. EBPR process removes phosphorus if the wastewater contains organic materials in the form of volatile fatty acids [35]. Figure (2.4) shows the EBPR process having four different units' anaerobic zone, aerobic zone, secondary clarifier, and recirculating sludge system.

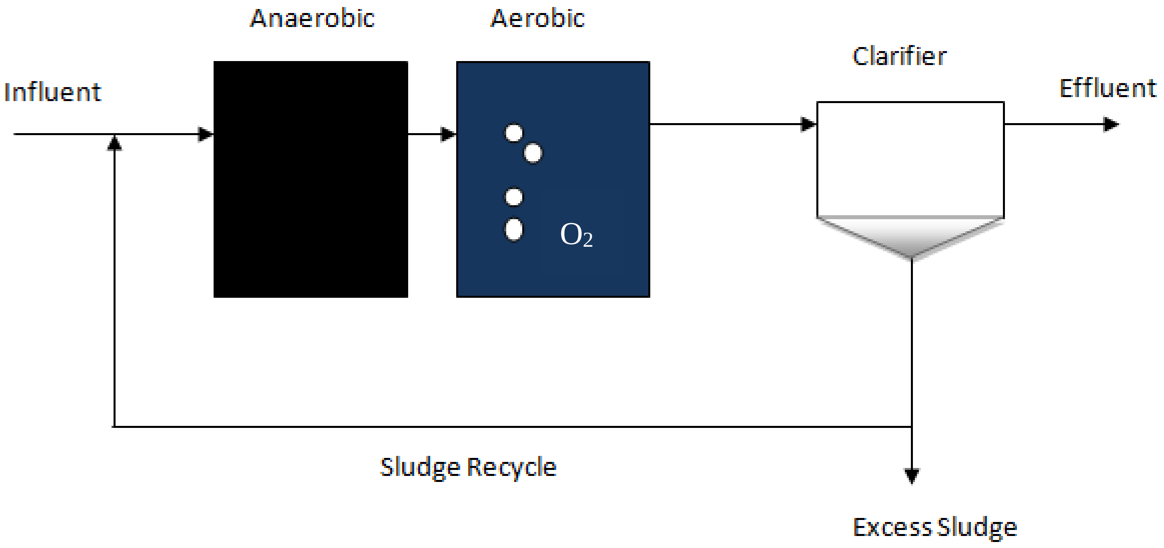


Fig 2.4: EBPR process configuration [36]

2.2.1.1 Anaerobic Zone

The anaerobic zone of EBPR is placed first in the bioreactor where the wastewater and the return-activated sludge (RAS) are mixed. The PAOs convert organic materials into energy-rich carbon compounds known as polyhydroxyalkanoates (PHAs). The required energy for the process is achieved through the breakdown of polyphosphate molecules thereby increasing phosphate concentration. The polyphosphate breaks down releasing phosphate in the mixed liquor where depletion of polyphosphate occurs. Volatile fatty acids are present in the influent or are created by fermentative bacteria in the anaerobic tank [37]. Hydraulic retention time is important in the anaerobic zone to give enough time for carbon and polyphosphate metabolism, normally one to two hours is suitable [38]. However, glycogen-accumulating organisms (GAO) co-exist with PAOs and tend to take up VFAs in the anaerobic zone. Due to the competition between two sets of organisms (PAOs and GAOs), there might be the possibility of degradation of P removal. GAOs can proliferate in anaerobic and aerobic environments without performing anaerobic P release or aerobic P absorption [38,39].

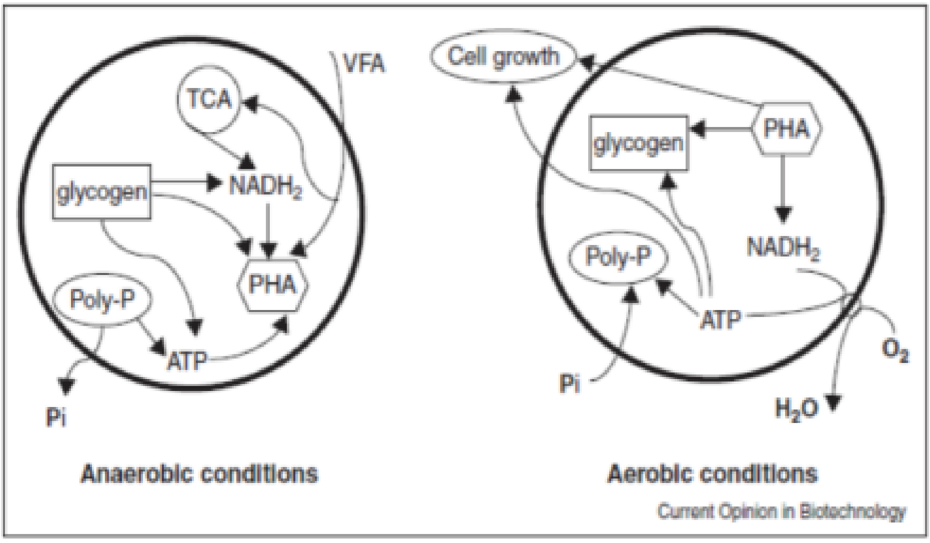


Fig 2.5: Schematic diagrams of the anaerobic (phosphorus release) and aerobic (phosphorus uptake) PAO metabolism in the EBPR process [41]

2.2.1.2 Aerobic Zone

The PAOs take up orthophosphate using the energy from the oxidation of organic matter in the presence of oxygen and convert it into polyphosphate. The PAOs take up more orthophosphate than they released in anaerobic conditions, this phenomenon is known as luxury uptake. Under aerobic conditions, the PAOs receive the ability to utilize the intercellular stored PHA as a source of energy for the growth of new cells, giving them the ability to take up more phosphate than what was released during an anaerobic phase [32].

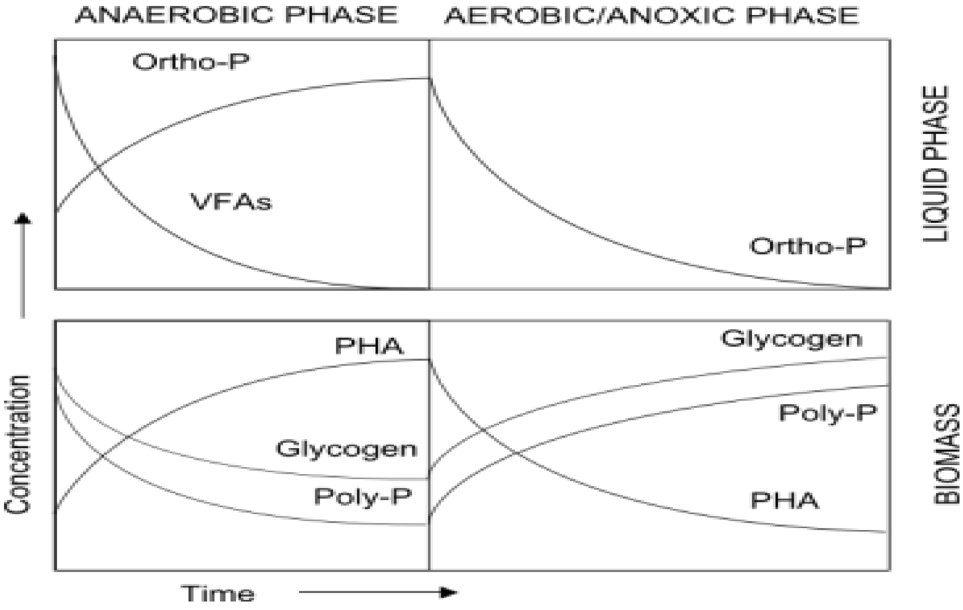


Fig 2.6: Concentration of various compounds in an anaerobic and aerobic reactor in the Biological phosphorus removal process [36]

2.2.2 Factor affecting the EBPR Process

The effectiveness and efficiency of the EBPR process are influenced by different parameters, among them, environmental and operational parameters play a vital role. The process becomes unstable due to a lack of understanding of the microbiology associated with the process. Moreover, pH, temperature, oxygen level in the aeration basin, wastewater source, and ratio of P to acetate acid have been regarded as key factors that affect the EBPR process [42]. For instance, a small pH drop from 7.0 to 6.5 resulted in a total loss of phosphate-removing capacity and a significant shift in microbial populations [43]. The rate of phosphate release in the anaerobic period will increase as the pH rises, and the aerobic period will see higher phosphorus removal. The optimal pH for the performance of PAOs in the EBPR is about 7.5 [44]. Generally, pH lower than 7 and temperature as high as 30°C hinder phosphorus removal due to the increase of GAOs. A higher COD/P ratio favors GAOs while a lower ratio such as 10-20 mgCOD/mgP is beneficial for the PAO's growth [39]. EBPR operation has been observed successful at a temperature as low as 5°C [45]. It has been discovered that PAOs develop more favorably at low temperatures (10–20°C), which enhances EBPR performance [46].

2.3 Nitrogen removal biological process

The wastewater generated from houses, agricultural activities, and industrial sectors has a high amount of nitrogen derivatives. The partially treated or untreated wastewater discharged to the environment causes damages to the environment such as the eutrophication of lakes and acidification of rivers [47]. The most common biological method for eliminating nitrogen species from wastewaters has been nitrification/denitrification which involves converting ammonia to nitrate and then organically reducing the nitrate to diatomic nitrogen [48]. The growth and activity of the microbial communities in the bioreactor, which comprises ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), and heterotrophic denitrifying bacteria (HDB), is crucial to the success of the biological nitrogen removal (BNR) process [49]. This chapter deals with different types of biological nitrogen removal processes along with their merits and demerits.

2.3.1 Conventional nitrification and denitrification

It is the most common method of biological nitrogen removal where microbial elimination of ammonium occurs. Ammonium is oxidized into nitrate in the presence of oxygen during nitrification while in denitrification steps, nitrate is reduced into molecular nitrogen in anoxic conditions [50].

2.3.1.1 Nitrification

It refers to the biological process in which ammonia (NH_3) or ammonium (NH_4^+) primarily converts into nitrites (NO_2^-) and then into nitrates (NO_3^-) in the presence of oxygen. The most common bacteria responsible for aerobic nitrification are autotrophic organisms, such as *Nitrosomonas* and *Nitrobacter*. The ammonia is converted into nitrite by ammonia-oxidizing bacteria (AOB) known as nitritation and nitrite-oxidizing bacteria (NOB) oxidize nitrite to nitrate called nitration [51]. Ammonia or nitrite is used as an energy source by the bacteria and molecular oxygen as an electron acceptor, while carbon dioxide is used as a carbon source. Currently, five AOB genera have been identified and classified as Proteobacteria where four comes under β-Proteobacteria such as *Nitrosomonas, Nitrosospira, Nitrosovibrio, and Nitrosolobus* while *Nitrosococcus* fall under γ-Proteobacteria subclass [52]. The NOB phylogeny contains four genera; *Nitrobacter* lies in α-Proteobacteria, *Nitrococcus* within γ-Proteobacteria, and *Nitrospina* and *Nitrospira* within the δ-Proteobacteria [53].

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \tag{2.4}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \tag{2.5}$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \tag{2.6}$$

The number of AOB should be larger than the NOB in a balanced nitrifying system, theoretically, the numerical ratio between AOB to NOB should be 2:1. An important factor in nitrifying community optimization is the growth balance between AOB and NOB. If AOB increases faster than NOB and the ammonium oxidizing rate is greater than the nitrite-oxidizing rate, nitrite will easily accumulate as an intermediate [54]. Nitrite is a great threat to the aquatic ecosystem and human health. Nitrite will be transformed into nitrous oxide in the anoxic environment, which is a major greenhouse gas for ozone layer depletion [55].

Oxygen is a necessary parameter as nitrification occurs in the presence of oxygen. Nitrifying microorganisms consume dissolved oxygen for the oxidation of ammonia to nitrite and then to nitrate. The concentration of dissolved oxygen for the complete nitrification is 4 mg/l and when it is below 0.2 mg/l, the nitrification process stops [56]. Likewise, pH is also the sensitive operating parameter for nitrification. For *Nitrosomonas*, the ideal pH range is roughly between 7.0 and 8.0, whereas *Nitrobacter* prefers a pH range of approximately 7.5 to 8.0 [57]. Nitrification stops at a pH below 6, the acid formation in the nitrification lowers the pH and hinders the growth of nitrifying bacteria [58].

The nitrification activity increases with an increase in temperature but under a certain limit because the level of free ammonia increases with temperature. Nitrification reaches a maximum rate at a temperature between 30°C and 35°C, and above 40°C, the nitrification rate decreases to approximately none. At temperatures below 10°C, nitrification is limited. Moreover, suspended growth cultures are more sensitive to temperature changes than biofilms [59].

The hydraulic retention time (HRT) variations have a significant effect on the microbial community structures. Less contact time between microorganisms and wastewater leads to decreased nitrification efficiency in shorter HRT. Hence, longer HRT is preferred at the startup of the process to grow more nitrifying bacteria [60].

Alkalinity is defined as the ability of water to neutralize hydrogen ions produced during the oxidation of ammonium ions into nitrate ions. It is also known as the buffering capacity of water. During the nitrification process, alkalinity is lost in an activated sludge system. For every milligram of ammonium ions oxidized, 7.14 mg of alkalinity as CaCO3 is lost. Nitrification is a pH-sensitive process and the rate of nitrification will decline abruptly at pH values less than 6.8 [61]. Equation (2.7) shows the stoichiometry of the alkalinity requirement in the nitrification process.

$$NH_4^+ + 2HCO_3^- + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_2O \tag{2.7}$$

Similarly, a larger amount of alkalinity also affects the nitrification process. The higher the alkalinity, the higher will be the pH. Hence, if more than the necessary amount of alkalinity is added, the pH will rise, and the ammonium nitrogen converted to free ammonia which inhibits the performance of AOB and NOB bacteria [62].

2.3.1.2 Denitrification

Denitrification is an important process in the global nitrogen cycle. It is the process by which nitrates are reduced to gaseous nitrogen by facultative anaerobes having different intermediate products. *Acinetobacter, Agrobacterium, Achromobacter, Arthrobacter, Alcaligenes, Bacillus, Corynebacterium, Chromobacterium, Flavobacterium, Halobacterium, Hypomicrobium, Moraxella, Methanomonas, Neisseria, Paracoccus, Pseudomonas, Propionibacterium, Rhodopseudomonas, Rhizobium, Spirillum, and Vibrio* are some heterotrophic microorganisms which can do denitrification [2]. Unlike autotrophic bacteria, heterotrophic bacteria consume organic carbon sources. For denitrification, it is necessary to maintain an anaerobic or anoxic environment because if oxygen is available, bacteria use it for metabolism before they use nitrate. The energy reactions (oxidation/reduction) in the denitrification process can be depicted below by equation (2.10) [63]. The organic substrate acts as an electron donor while the nitrite/nitrate is an electron acceptor.

$$6NO_3^- + 2CH_3OH \rightarrow 6NO_2^- + 2CO_2 + 4H_2O \tag{2.8}$$

$$6NO_2^- + 3CH_3OH \rightarrow 3N_2 + 3CO_2 + 3H_2O + 6OH^- \tag{2.9}$$

Overall reaction,

$$6NO_3^- + 5CH_3OH \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^- \tag{2.10}$$

Reduced biological activity related to temperature drops is an especially important feature of biological denitrification when wastewater temperatures might drop to around 5°C during the winter. The key regulating elements for denitrification are the availability of a substrate, the absence of oxygen, and the presence of active denitrifiers. The optimum pH for denitrification based on denitrification activity is found to be 7 to 7.5 [64]. The wastewater has a high concentration of ammonia and low COD doesn't favor denitrification as there will be an additional cost of supplying organic carbon [58].

Nitrate reduction goes through various intermediate products which are shown in the equation (2.11).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{2.11}$$

2.3.2 ANAMMOX Process

The anaerobic ammonium oxidation (ANAMMOX) is a new and unique wastewater treatment process that involves the conversion of $NH_4^+ - N$ and $NO_2^- - N$ into N_2 by ANAMMOX bacteria under anaerobic or anoxic conditions [65]. The process was discovered in the Kluyver Laboratory of Biotechnology, the Delft University of Technology, the Netherlands in 1995 [66]. Conventional nitrogen removal, which consists of the aerobic conversion of ammonium to nitrate (autotrophic nitrification) combined with the anaerobic conversion of nitrate to nitrogen gas in the presence of organic carbon (heterotrophic denitrification), is energy-intensive, owing primarily to too much aeration costs. The main advantages of the ANAMMOX process are high nitrogen removal efficiency and requiring no additional carbon source, high nitrogen load, low operating costs, and less sludge production [67]. It has been applied to treat high-strength ammonia wastewater such as pig breeding wastewater, landfill leachate, etc. At present, more than 100 Anammox wastewater processes are in operation worldwide [68]. Strous et al.(1998) proposed the overall reaction for cell synthesis presented in equation (2.12) [69].

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} \tag{2.12}$$

A crucial parameter for the anammox process is the nitrite concentration. Nitrite is an essential substrate but also inhibitory to the reaction. The need for organic carbon decreases by 100%, aeration requirements by about 60%, and sludge production by about 90% in the anammox process compared to the conventional nitrification-denitrification process [67]. There are only five genera of anammox bacteria have been identified such as *Brocadia, Kuenenia, Scalindua, Jettenia, and Anammoxoglobus* [70].

2.3.3 Nitritation-Denitritation

A new biological system for the removal of nitrogen from wastewater has been developed as an alternative to the conventional nitrification-denitrification process. The partial nitrification of ammonia to nitrite has been named nitritation, and the direct reduction of nitrite to N_2 gas is termed denitritation [71]. The application of this process could lead to a reduction in the aeration costs and external carbon sources as compared to the previous methods. In the nitritation process, AOB converts ammonia to nitrite under aerobic conditions, while heterotrophic bacteria reduce nitrite under anaerobic conditions to produce nitrogen gas known as denitritation [72]. The two-step reactions of the process are shown in equations (2.13) and (2.14) [73].

Nitritation: $NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H_2O + 2H^+$ (2.13)

Denitritation: $NO_2^- + 0.5CH_3OH \rightarrow 0.5N_2 + 0.5CO_2 + 0.5H_2O + OH^-$ (2.14)

The process can be run in a single reactor and requires less oxygen and less organic carbon [74]. The main goal of this process is to stop the oxidation of ammonia to nitrite creating an unsuitable environment for immediate oxidation to nitrate as well as to save cost from aeration and carbon source. The process is also known as SHARON (Single reactor system for High activity Ammonium Removal Over Nitrite) [75].

2.4 Biological Oxygen Demand (BOD)

The amount of oxygen utilized by bacteria and other microorganisms while decomposing organic materials under aerobic circumstances is referred to as BOD. Dissolved oxygen is an essential component of natural water bodies, sustaining aquatic life and the aesthetic qualities of streams and lakes. The amount of oxygen needed to eliminate waste organic matter from water during the aerobic bacteria's breakdown process is known as the "*biological oxygen demand*." It is regarded as an index of organic pollution in wastewater treatment plants [48,76]. The BOD_5 value is usually expressed in milligrams of oxygen consumed per liter of the sample during five days of incubation at 20°C [33].

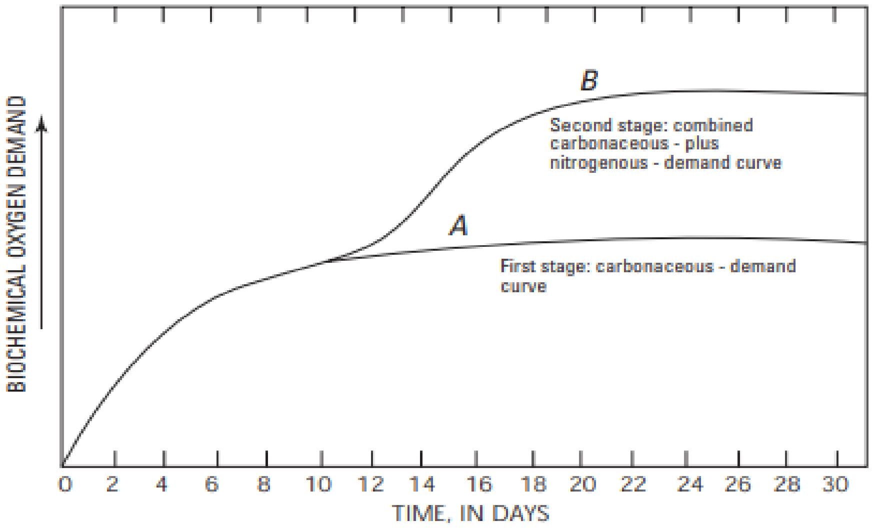


Fig 2.7: Biochemical Oxygen Demand with respect to time [77]

There are two stages of decomposition in the BOD test: i) the carbonaceous stage, and ii) the nitrogenous stage. The carbonaceous stage represents that portion of oxygen demand taking part in the conversion of organic carbon to carbon dioxide. The nitrogenous stage represents the oxygen demand involved in the conversion of organic nitrogen, ammonia, and nitrite into nitrate [77].

The biological measurement "*Biochemical Oxygen Demand*" (BOD) was selected in 1908 as an indicator of the organic pollution of rivers by the U.K. Royal Commission on River Pollution [78]. The standard method to find out the BOD of a sample in the laboratory is the dilution method. The water sample is diluted with aerated water and the initial DO is found, then it is incubated for 5 days at 20°C. After these 5 days, we measure the DO which is now known as the final DO [79].

$$BOD_5 = (Initial\ DO - Final\ DO) * DilutionFactor \qquad (2.15)$$

$$Dilution\ Factor, DF = \frac{Diluted\ Sample\ Volume}{Undiluted\ Sample\ Volume} \qquad (2.16)$$

Clean rivers will have a 5-day carbonaceous BOD below 1 mg/l whereas, partially polluted rivers may have a BOD value in the range of 2 to 8 mg/l [79].

2.5 Overview of Randvik WWTP

Randvik WWTP is a small biological wastewater treatment system consisting of nitrification and denitrification processes, located at Risør municipality in Agder County Norway. The plant was put into operation in 2002 as a biological wastewater treatment plant and designed for 7000 person equivalents (pe). The UTM coordinate based on zone 33 of the plant site is (165219°E,6522120°N) while the coordinate of the discharge point is (165963°E,6522270°N).

The plant is divided into two process lines, each consisting of a bioreactor and a secondary clarifier. The bioreactor contains an anaerobic and an aerobic tank. The sewage goes through a primary screen and cyclone (remove sand and fat), denitrification and nitrification chambers, and a sludge-settling secondary clarifier. The aeration in the nitrification tank is done by four air pumps. The waste-activated sludge pumped to the reed beds has an overall area of 3200 m^2,8 cells of 400 m^2 each.

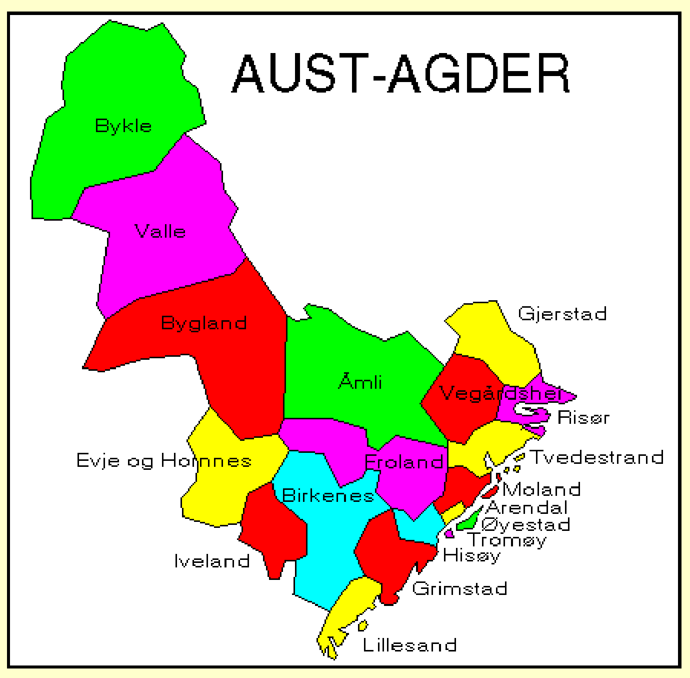


Fig 2.8: Geographical map of Aust Agder County, Norway showing Risør Municipality [80]

3 Materials and Methods

This section describes the materials and methods adopted for the process evaluation and physiochemical data analysis based on the secondary data provided by the treatment plant. An in-depth literature review covering the principles of wastewater treatment (particularly biological treatment) and performance indicators was first carried out to establish a solid foundation for scientific knowledge. The secondary data obtained from the Randvik wastewater treatment plant includes BOD, total phosphorus, total nitrogen, and the influent flow rate. These data were collected after a 24-hour mixture test as a sampling period. The data was provided by Risør municipality.

3.1 Plant description

The Randvik wastewater treatment plant is located at Risør municipality in Agder County Norway. The sewage treatment plant is the smallest of its kind, with an ultimate design capacity of 7000 PE (population equivalent). The plant has been functional since 2002. The treatment plant with activated sludge is a biological treatment system consisting of primary and secondary treatment systems. The sewage passes through the screening and grit removal chamber to prevent damage to the mechanical equipment, and cyclone to remove sand and fat. These steps are designed for the preliminary treatment, and the biological nutrient removal system has de-nitrification and nitrification reactors for the secondary treatment as shown in Fig (1.1).

The plant has different components such as a grate, cyclone, denitrification and nitrification reactors, and secondary clarifiers for sludge settling. The wastewater from the primary clarifier bifurcates into two parallel sets of denitrification /nitrification reactor and secondary clarifier. The maintenance and supervision of the combined sewerage system as well as wastewater treatment device are the responsibility of Risør Municipality. The inflow of wastewater is 30 L/s whereas, the sludge return rate from the clarifier is 14 L/s. The aeration of the nitrification chamber is done by the four air pumps, each having a power of 11KW. 30 m^3/day sludge is taken out and stored in the intermittent storage tank which has a provision of aeration to control smell. The stored sludge is pumped out to the reed bed at a 25.7 m^3/hr flow rate for even distribution. The reed beds have a rubber lining, sand, gravel, and reeds (*Phragmites Australis*).

For natural aeration, the beds are attached with PVC tubing. The soil level increases by 10 cm/year. The reed beds cover 3200 m^2 of area, which is divided into 8 cells of 400 m^2 each. The plant produces approximately 110 tone sludge per year and the sludge volume index is between 500-700 ml/g.

3.2 Data Collection

Overall historical statistical data of the Randvik wastewater treatment plant from 2018 to 2022 included in this study, which receives urban domestic and industrial wastewater discharge. The raw data were collected by the Randvik treatment plant. The treatment facility monitors the physical and chemical properties of influent and effluent wastewater regularly for treatment process management and compliance with discharge regulations. The plant is designed for the population equivalent (PE) of 7000. The BOD, total phosphorus, total nitrogen, and the influent flowrate data are provided by the treatment plant. BOD and total phosphorus were measured at both the influent and the effluent wastewater while the total nitrogen was measured with the effluent only. There were 12 influent and effluent samples for each year of data collection for total phosphorus and BOD measurement while only 6 samples were for the effluent total nitrogen measurement. The influent flow rate was measured every month of the year having 12 sampling points.

The average monthly rainfall and temperature data between 2018 to 2022 of the meteorological station, Adger, Norway (elevation 36m) are collected from the Norwegian Meteorological Institute and Norwegian Broadcasting Organization. The water consumption data of the Risør Municipality has been collected from the statistics office website in Norway.

3.3 Microbiome sampling and DNA analysis

In the Regional research fund (RFF) project ‘Biofilm characterization for wastewater treatment’, biological samples were collected from the Risør wastewater treatment plant.to investigate microbiome diversity. Three samples from i) aerobic reactor, ii) anaerobic reactor, and iii) return activated sludge were harvested on 26 September 2022. The extracted DNA was used for barcode sequencing and taxonomic classification. The DNA analysis was done by SINTEF, the project partner.

3.4 Data Analysis

Data analysis is a statistical method for describing data. Mean, median, mode, percentiles, range, variance, and standard deviation are the common statistical measurements of data that can be used to give detailed information about a data set and comparison between different data sets. Likewise, regression and correlation analyses can be used to compare the different data sets. Linear regression analysis is used to predict the estimated data set based on the measured set of data. The concentration of and the ratios between the different water quality parameters in the influent wastewater play a major role in the selection and functioning of the treatment plant. Similarly, the concentration and ratios in the effluent wastewater have the foremost importance in evaluating the treatment plant performance and its impact on human health, the environment, and the design of advanced novel wastewater treatment processes. Therefore, it is of utmost importance to conduct data analysis on the water quality parameters of municipal wastewater treatment plants. Hence, data analysis on influent and effluent wastewater quality at Randvik WWTP was performed by data plotting and comparison on Microsoft Excel. A single factor ANOVA test was performed in excel to find out the significance of the different parameters of the data. The influent discharge, biological oxygen demand (BOD), total phosphorus (TP), and total nitrogen (TN) data collected over the last five years (2018-2022) were analyzed and expressed in the graph. Samples of the microbiome sequencing result were used to identify the most common microbial species in the Randvik wastewater treatment plant.

4 Results and Discussion

This section deals with the results and discussion based on the project work determined through the analysis of the secondary data obtained from the treatment plant. The results are presented in graphical form. The graphs were plotted based on the data provided by the treatment plant. MS Excel was used for the data analysis, comparison, and plotting of graphs.

4.1 Quantity of influent wastewater

Knowledge of wastewater flow rate is very important to the design and operation of the treatment plants. Randvik WWTP has combined sewer systems that are designed to collect both sanitary sewage and storm-water runoff. The rate of water supply, population density, type of area, type of sewer system, and infiltration/ex-filtration are the major factors responsible for the variation of influent wastewater flow rate. The hydraulic design of collection and treatment facilities are directly affected by the variations in the flow rates [81].

The variations of influent flow rate to the treatment plant at the different times of the year starting from 2018 to 2022 are plotted in Fig (4.2). The flow during January, February, July, and December are relatively high in 2018 but, the maximum flow occurs during December. The flow rates are somewhat similar in all months except February, March, and November in 2019. Higher influent flow caused by rainwater makes wastewater dilute and the concentration of organic loads and contaminants in the water decreases. The inflow rates in 2020 are high during March, October, and December and lowest in May and June. Likewise, May, June, July, and November 2021 had higher inflow rates. In 2022, the treatment plant received more influent in January, September, and November. The inflow rate is low during December as the precipitation was quite low at that time but the average five-year rainfall in December was relatively high. For instance, Fig (4.5) shows the average monthly rainfall variations since 2018 in Risør municipality. The highest inflow rate recorded in September 2022 during the last five years was 3326 m^3/day and the lowest was 794 m^3/day in June 2020.

Domestic, industrial, infiltration, and stormwater are the major components of wastewater flow rates. The ratio of wastewater components varies with the local conditions and the time of the year [82]. Municipal water use is comprised of domestic, industrial, and public service, and a less significant amount of losses and leakage. The water provided to residential and commercial areas, recreational facilities, and institutions has come under domestic water use. The wastewater flow rate increases with the number of people residing in a particular area and vice versa. On the contrary, the average wastewater flow rate per capita decreases with the increase in the number of persons per residence [83].

Table 4.1: Estimated average household water consumption per inhabitant in Risør municipality (collected from the Central Bureau of Statistics of Norway) [84]

	2020	**2021**	**2022**
Estimated average household water consumption per associated inhabitant per day (L/person/day)	240	213	200

It is evident from this data that the household water consumption per capita per day in Risor municipality is highest during the breakdown of COVID-19. It is found to be 240 l/person/day in 2020 and decreases proportionately to 213 L/person/day and 200 L/person/day in subsequent years respectively. The increase in tap water consumption in 2020 coincided with the first Covid-19 year when people stayed home more as they flushed the toilet more often at home than at work or school. Springs and summers were also relatively warm and dry, prompting households to use more potable water for watering their gardens and pools [79].

If the community has well-constructed sewers and stormwater drainage is excluded, and constant use of water by the industries, then there will be less variation between water supply and wastewater quantity (flowrates) [86]. The minimum flows occur during the early morning when the consumption of water is lowest. The peak flow occurs during the late morning as the water takes some time to reach the treatment plant after using it during peak morning. The second peak flow generally occurs during the early evening [2]. The data obtained from the Randvik wastewater treatment plant was an average daily flow which is used in determining the plant capacity, flowrates, and constituent loadings. The wastewater treatment plants used for the small communities having less number of person equivalents have higher peak flow in comparison to average flow values [87]. Therefore, there are not many variations of the flow rate in the Randvik WWTP as shown in Fig (4.4) except for some exceptional conditions.

Table (4.2) shows the average influent flow (m^3/day) of wastewater along with the standard deviation (SD) in the respective year to the treatment plant.

Table 4.2: Average influent flow of wastewater in Randvik WWTP

	Average flow, m^3/day			
2018	**2019**	**2020**	**2021**	**2022**
1176±344.54	1555±705.27	1476±738.98	1298±309.48	1703±894.30

The error bars in the year 2019, 2020, and 2022 have higher lengths which show the larger spread of data from the respective mean value in Fig (4.1). In contrast to this, the years 2018 and 2021 have small error bars of standard deviation which demonstrates the smaller spread of data from the mean value.

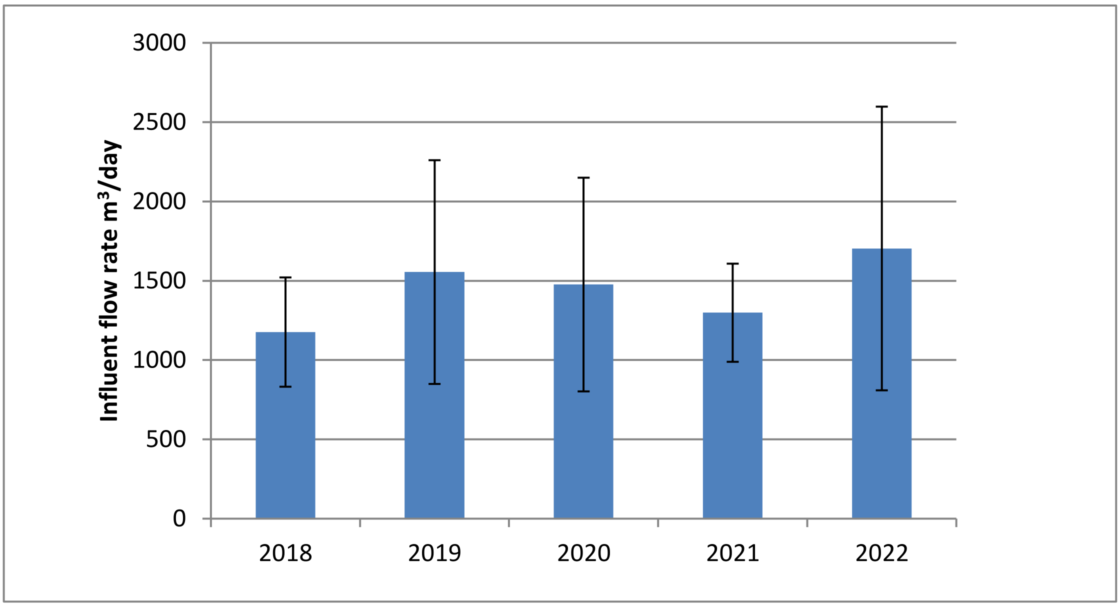
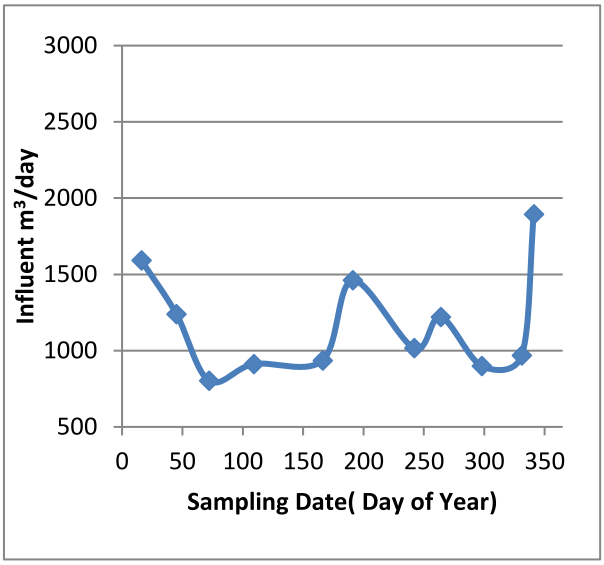
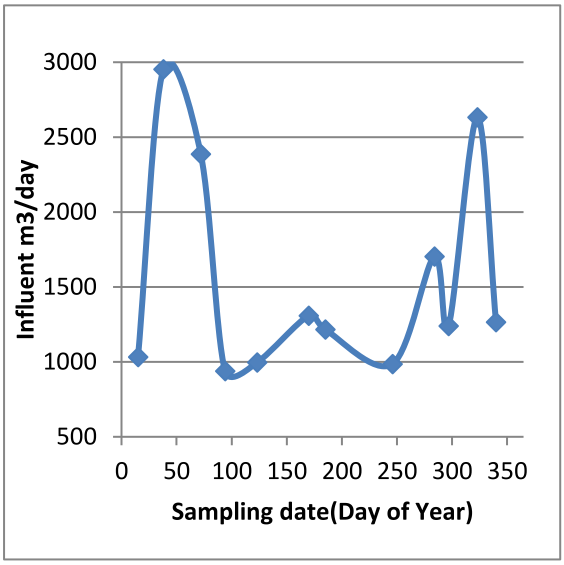


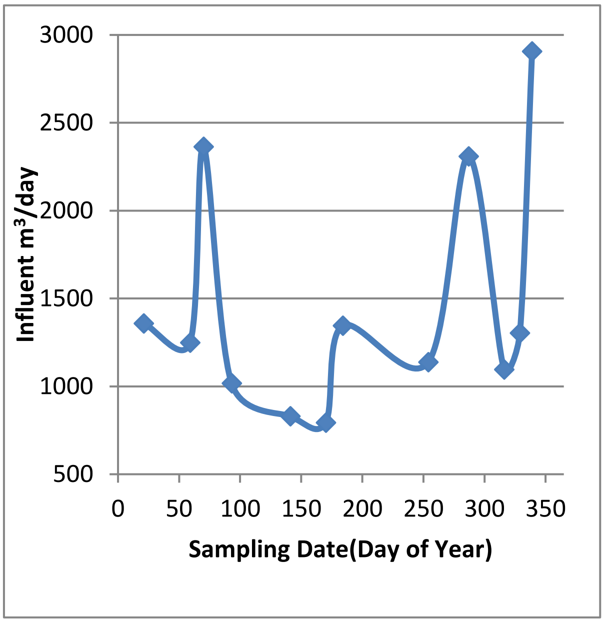
Fig 4.1: Average influent flow rate (m^3/day) over five years from 2018-2022



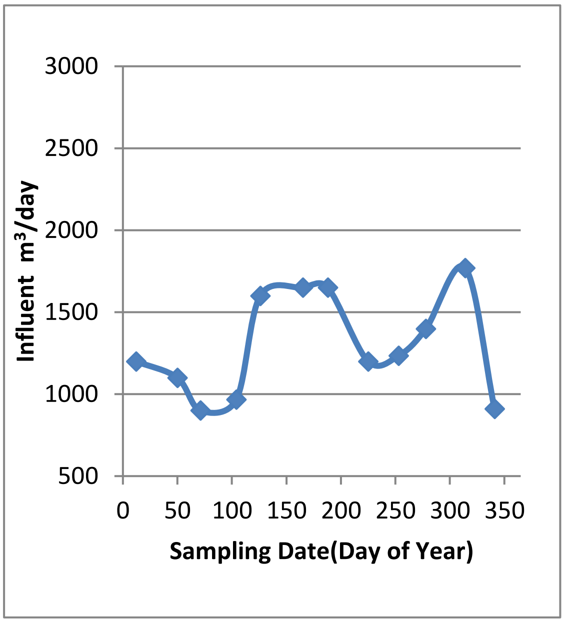
(a) 2018



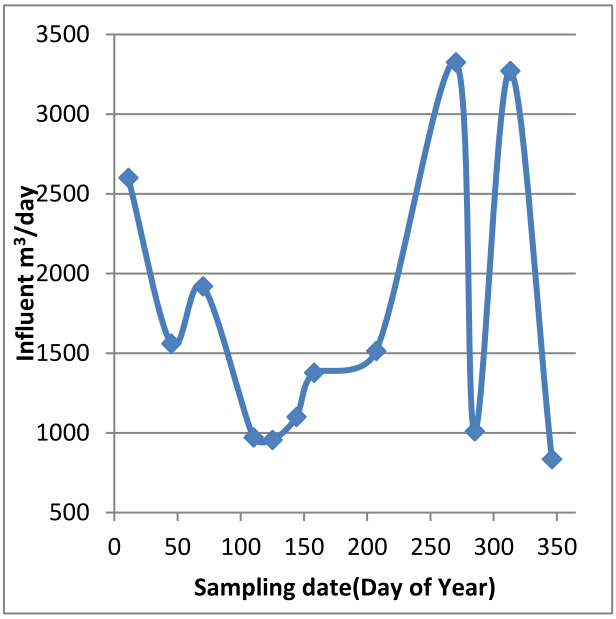
(b) 2019



(c) 2020



(d) 2021



(e) 2022

Fig 4.2: Influent flow rate (m3/day) vs day of the year to the Randvik wastewater treatment plant (a) 2018 (b) 2019 (c) 2020 (d) 2021, and (e) 2022 respectively

The wastewater flow rate and constituent loading differ during the time of day, day of the week, season of the year, and year to year. The amplitude of the flow rate normally goes to peak during the late morning as it is dependent on the length of the collection systems and the size of the residence area [1].

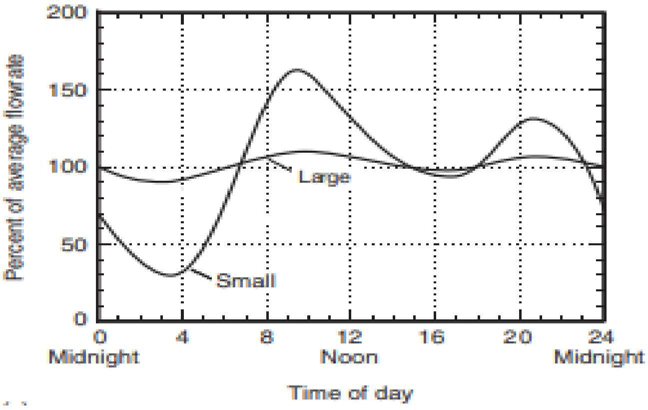


Fig 4.3: Flow rate variation for small and large communities [1]

The variations of wastewater from industrial areas are quite difficult to predict. The seasonal variation depends upon the location and nature of the community. The flow rate is considerably high during the wet season in comparison to the dry one. The rate of infiltration increases due to snowmelt in the spring season [88]. As Randvik WWTP has a combined sewer system, flow in the collection system is composed of a sanitary sewer system along with storm water runoff and snowmelt. Therefore, the variation in the flow rate could be associated with water use, collection system, and environmental conditions.

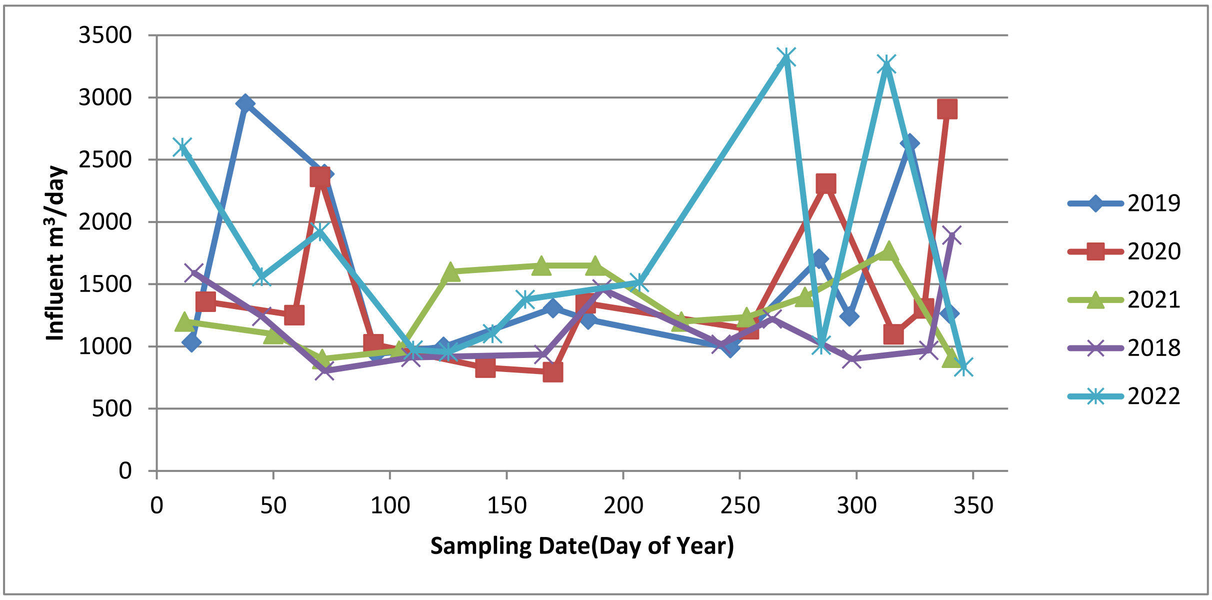


Fig 4.4: A comparison between influent flow rates among five different years, from 2018-2022

Due to global climate change, it is observed that occasionally the flow rate becomes too high because of sudden heavy rain and early snow melt i.e. temperature increase [89]. Fig (4.4) depicts the comparison between the influent flow patterns of wastewater for five consecutive years. In the Randvik wastewater treatment plant, the flow pattern is to some extent equivalent to each other except for some months. The flow during December and January is relatively high each year as it may be festival time and people stay at home and use comparatively more amount of water. Another reason might be the intensity of rainfall occurring at that time as precipitation is quite high which is shown in Fig (4.5).

The flow rates during April, May, June, July, and August didn't show significant differences and were almost similar to each other. The inhabitants preferred to travel outside of the home during the vacation period and they spent the majority of time outside the home. So, municipal water use becomes very less which directly reduces the wastewater flow into the collection system. The rainfall has less effect on flow rates during the dry weather season because of less runoff. Fig (4.5) shows the average monthly variation in the rainfall in Risør Municipality. The rainfall pattern increases from July to December while it is less and follows the constant pattern from March to July. Although there is less amount of rainfall, the flow rate was not much low because of the surface runoff due to the melting of snow, which fell in the winter season as the temperature gradually rose shown in Fig (4.6).

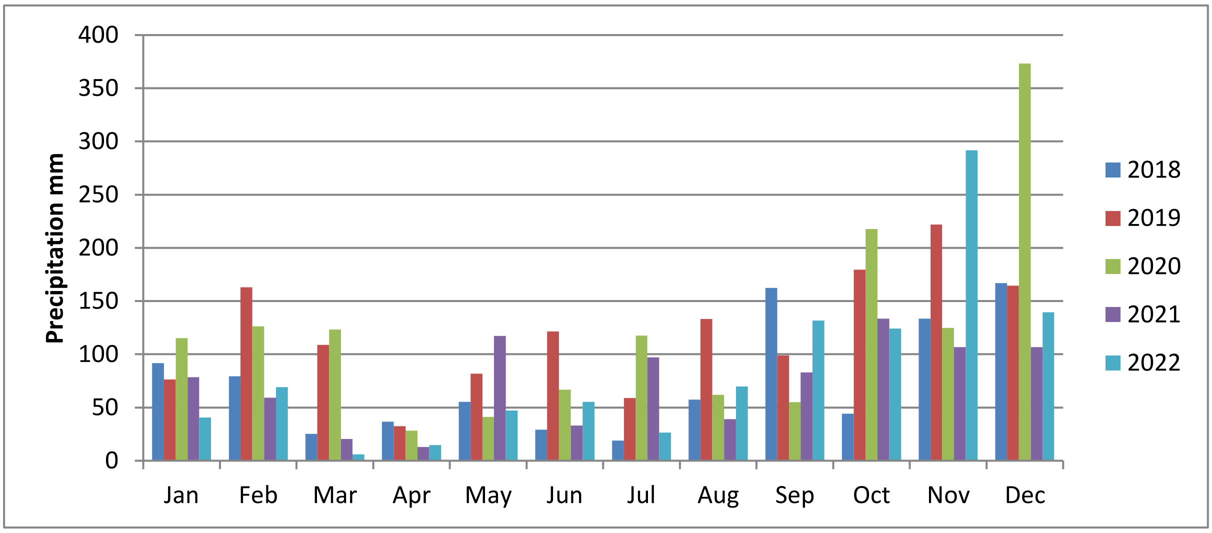


Fig 4.5: Average monthly precipitation, served by Norwegian Meteorological Institute and NRK (meteorological station, Adger, 36m elevation) [90]

Though the temperature is high, and people need a maximum amount of water for daily use, because of a smaller number of inhabitants during that time to use municipal water, there are not many variations in the quantity of wastewater. On the other hand, the influent flow rate after the COVID-19 breakdown in February 2021 increased as people had to stay within their houses because of travel restrictions. They were taught to wash their hands frequently and use more amount of water for household activities. The lowest average monthly rainfall recorded in five years is in April while the highest amount was found to be in December.

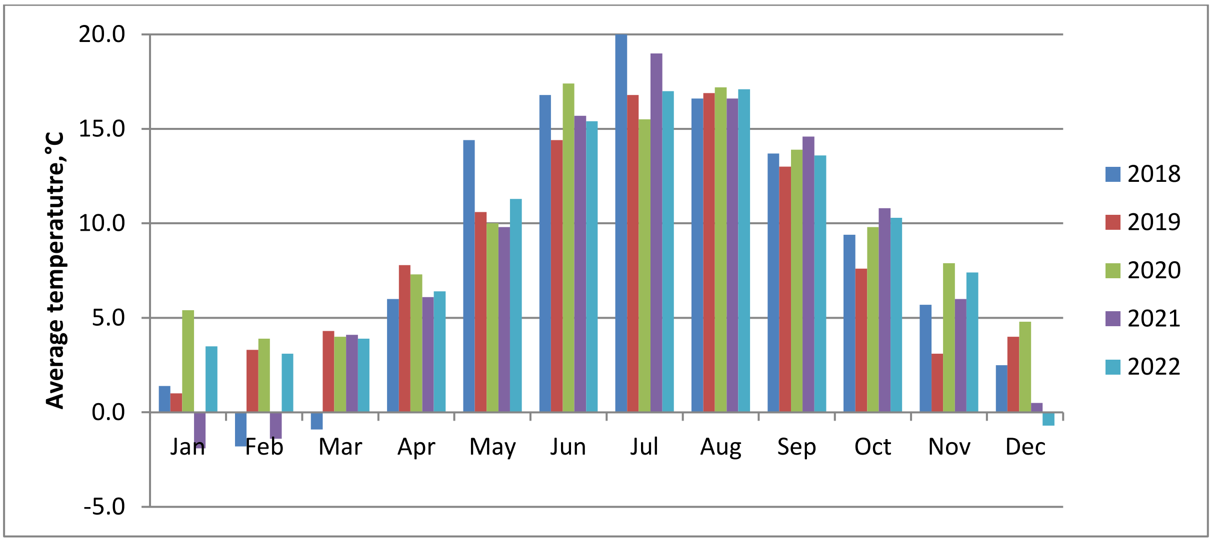
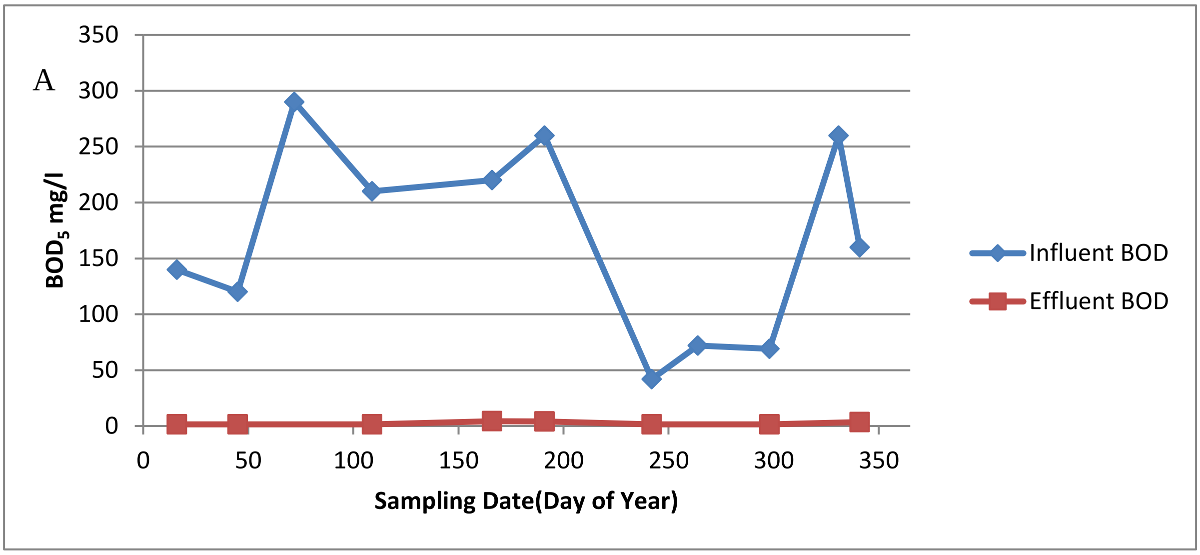


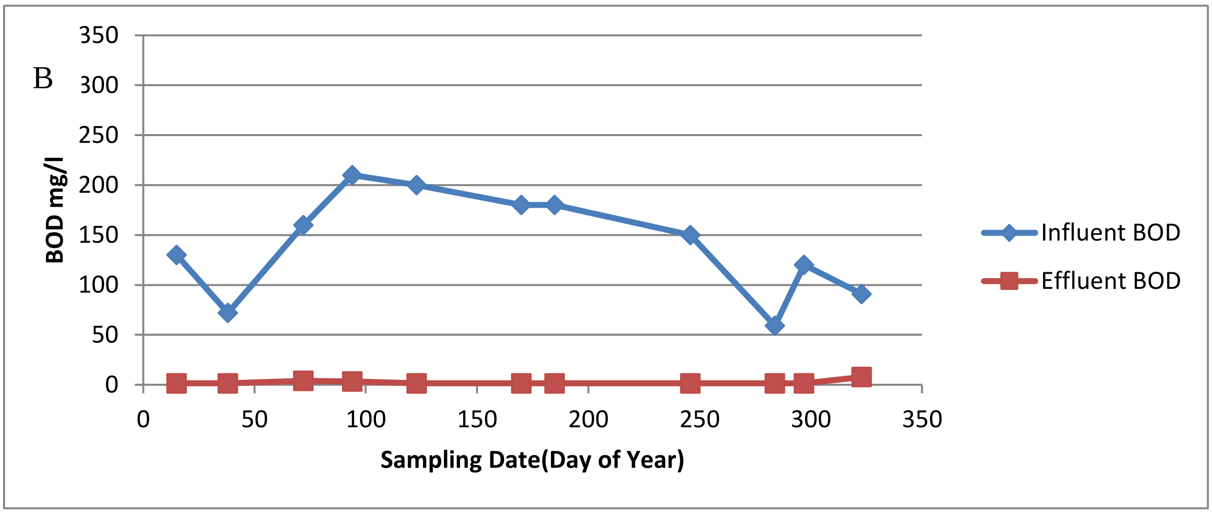
Fig 4.6: Average monthly temperature measured by Norwegian Meteorological Institute and NRK (Lyngør lighthouse measuring station, Risør) [90]

4.2 Characteristics of wastewater quality

4.2.1 Variation of BOD_5 and its Effect on the treatment plant

The variation of influent and effluent BOD with a different sampling time of five different years starting from 2018 to 2022 is shown in Fig (4.7). It can be depicted from the graph that the amount of BOD was significantly reduced after the wastewater treatment process. The maximum influent BOD value recorded in the last five years was in May 2020 and it was found to be 600 mg/l. The higher BOD value indicates a high level of pollution in the water and there is a greater concentration of organic matter. BOD is referred to as the amount of oxygen present in the wastewater which is required by the microorganisms to decompose the organic matter in an aerobic environment [91].


A
BOD$_5$ mg/l
350
300
250
200
150
100
50
0
0
50
100
150
200
250
300
350
Sampling Date(Day of Year)
Influent BOD
Effluent BOD


B
BOD mg/l
350
300
250
200
150
100
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0
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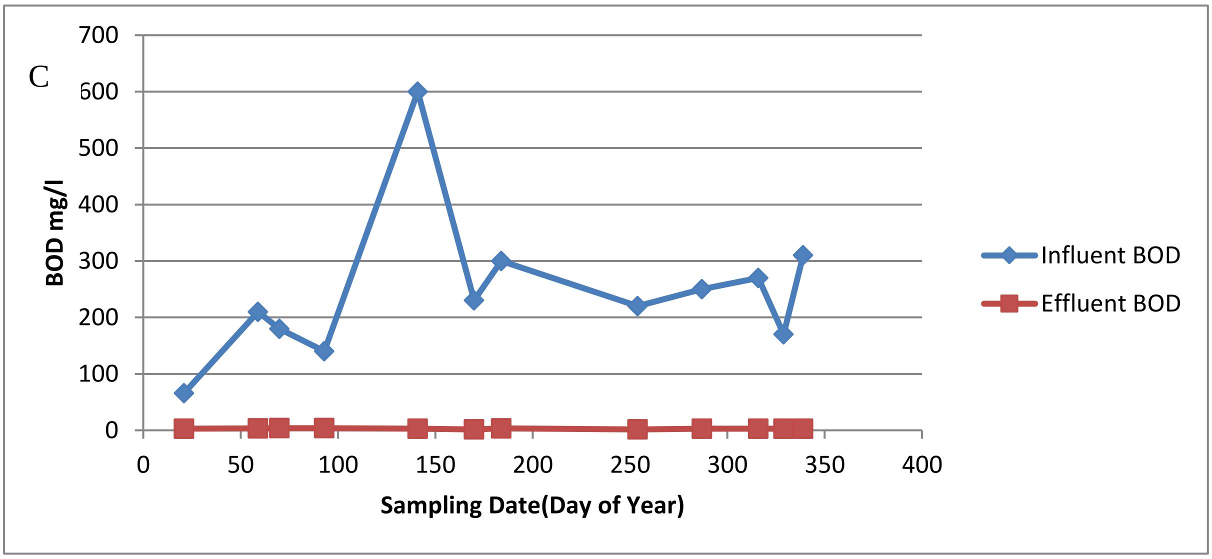
Sampling Date(Day of Year)
Influent BOD
Effluent BOD


C
BOD mg/l
700
600
500
400
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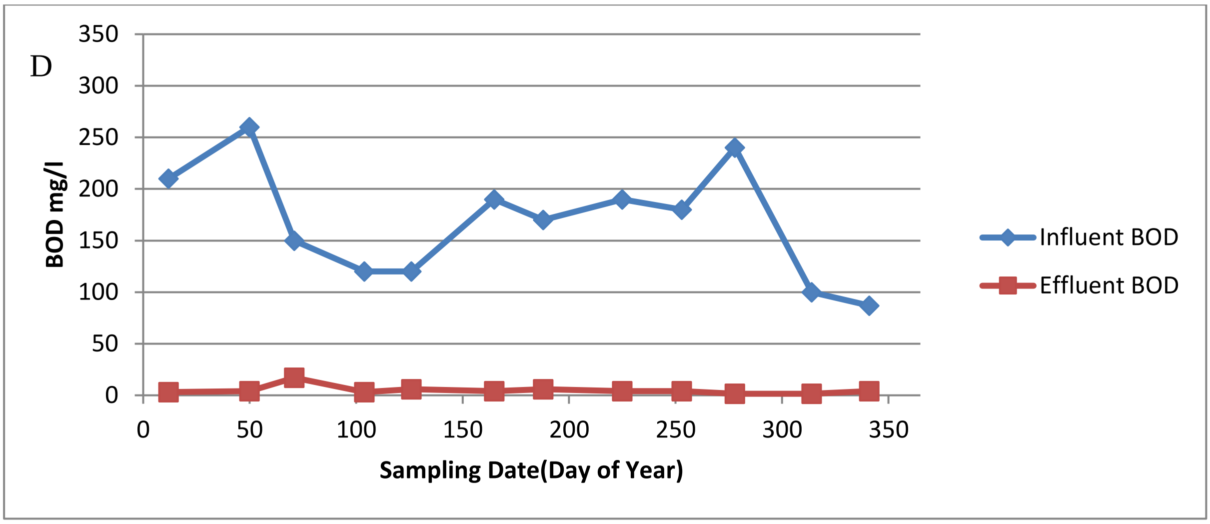
Sampling Date(Day of Year)
Influent BOD
Effluent BOD



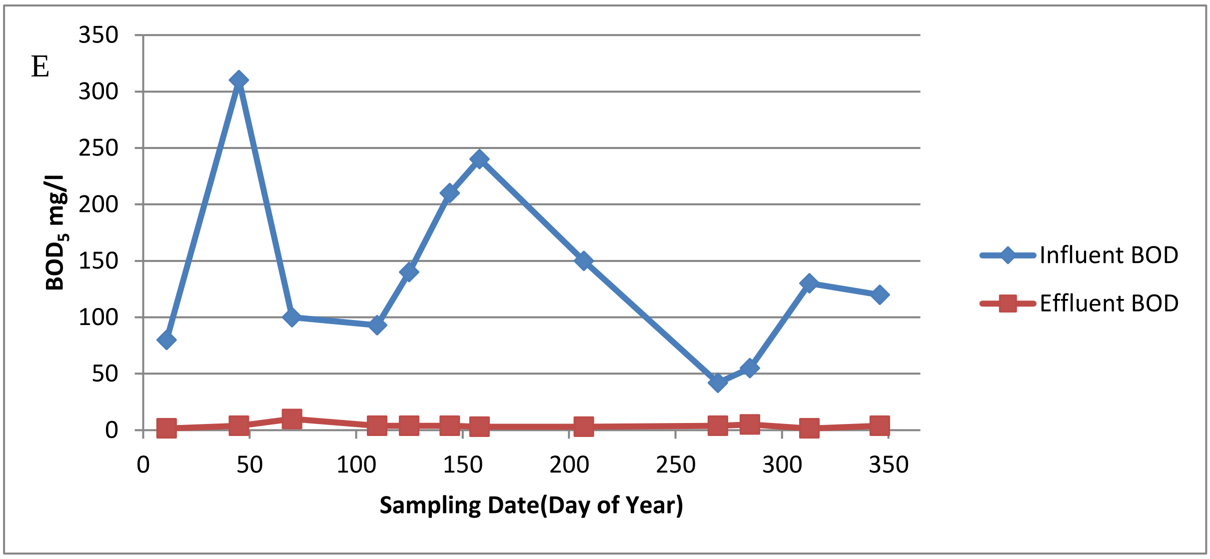


Fig 4.7: Variation of influent and effluent BOD (mg/l) with a day of year A) 2018 B) 2019 C) 2020 D) 2021 and, E) 2022 respectively

Fig (4.8) shows the changes in the BOD concentration of influent wastewater in the treatment plant while Fig (4.9) displays the variation of BOD concentration after the treatment process. The influent BOD concentration follows a similar pattern in all years except 2020. There were huge changes in the graph that occurs after April and reaches a maximum of 600 mg/l. These distinct variations of BOD in the wastewater might be due to the breakdown of COVID-19 and people staying at home for a longer period, which causes more organic matter loadings in the wastewater [92]. Temporary lockdowns, stay-at-home regulations, movement limitations, and personal health care activities have significantly altered the everyday life routine in towns and cities, which may have an impact on the characteristics of municipal wastewater [85].

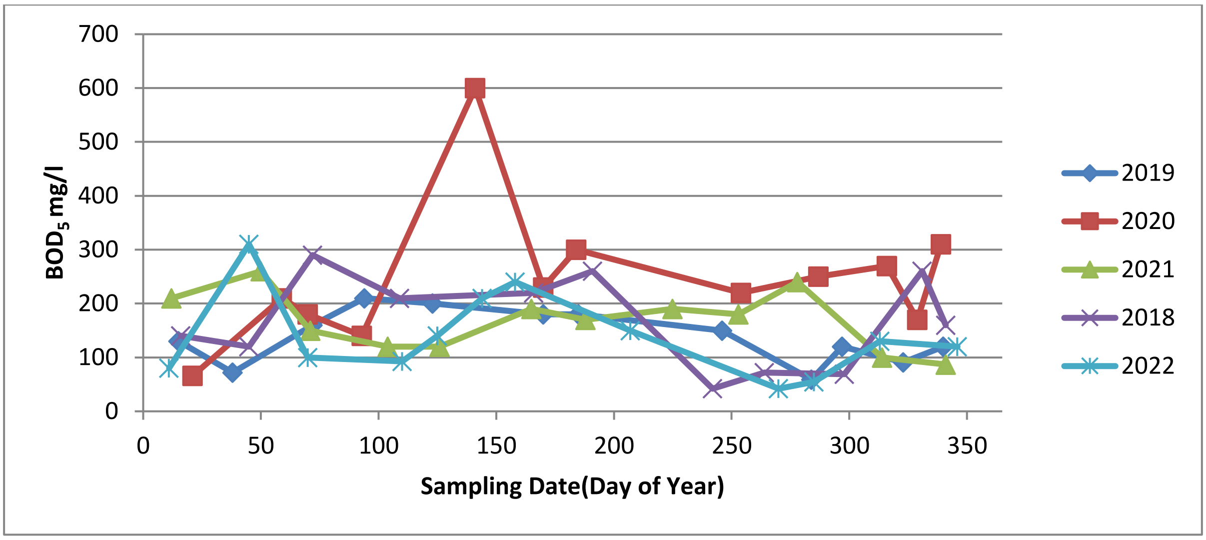


Fig 4.8: Influent BOD concentration (mg/l) variation over the years

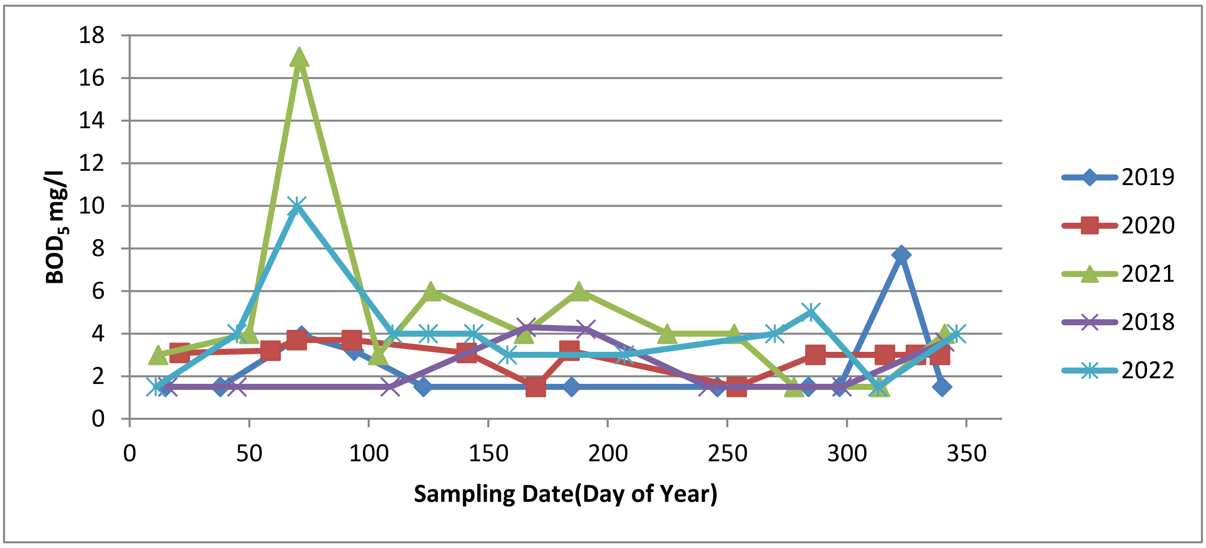


Fig 4.9: Effluent BOD concentration (mg/l) variation over the years

The influent BOD is found to be lower during August and September of 2018 and 2022 respectively. Although the influent BOD is low at the start of the year in 2020, it rises progressively up to May and becomes stable afterward. Even though it has shown stability, it was quite higher than the BOD concentration rest of the years (Fig 4.8). It was indicated from Fig (4.8) that the organic loading pattern is almost similar except for a few months in 2020. Moreover, the flow pattern of the effluent BOD concentration in all five years seems agreeably similar for the first three years. The effluent BOD concentration in March 2021 and March 2022 jump up to a value of 17 mg/l and 10 mg/l respectively. Besides these, the effluent BOD is a bit high in November 2019 and was found to be 7.7 mg/l. Hence, it could be due to the high BOD overload which occurred during that time of the year. If there is a presence of food and beverage industries experiencing seasonal production variability, it is better to consult with the treatment plant to enhance its capacity. Excess odors and sludge are some symptoms of BOD overload and if BOD increases regularly, it can damage the biomass [93].

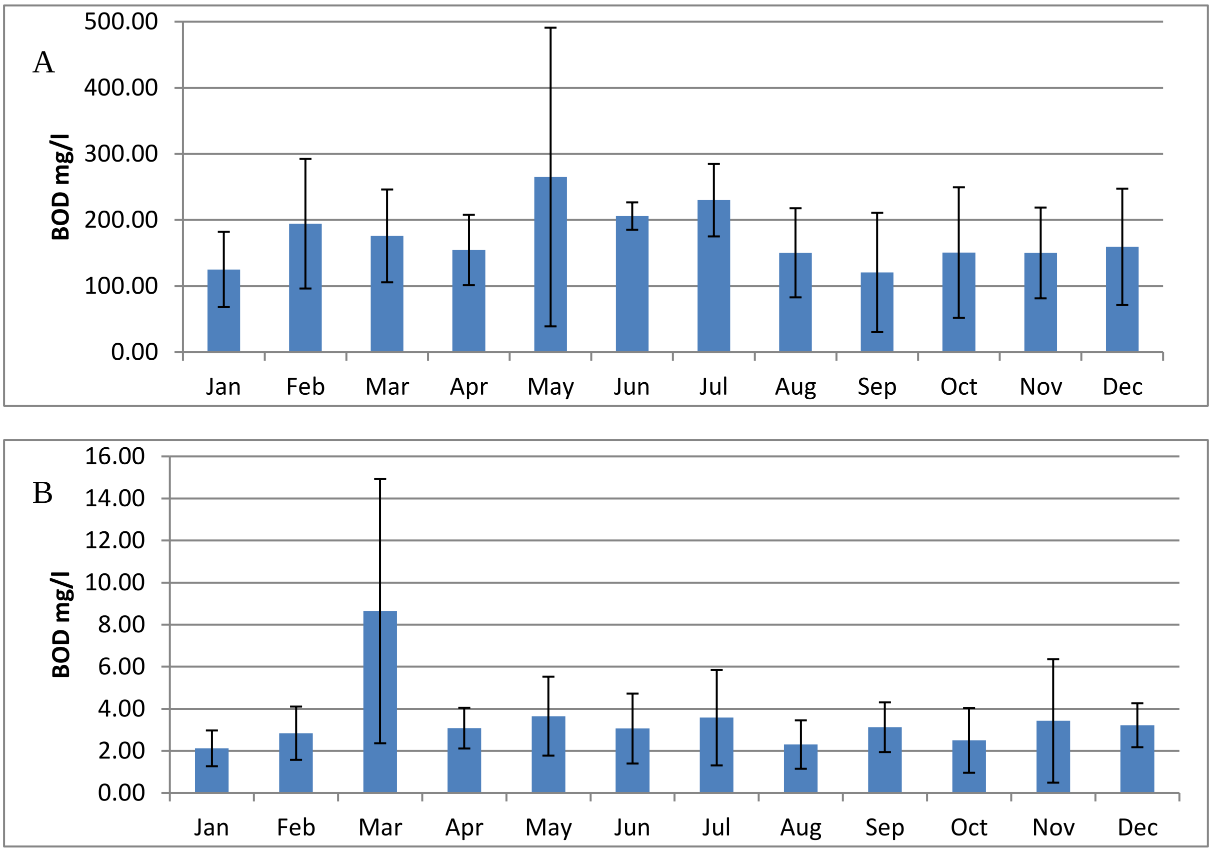


Fig 4.10: Average daily BOD variation over different months of five years data A) Influent B) Effluent

Fig (4.10) shows the graphical representations of the variation of BOD in the average monthly daily flow of influent and effluent wastewater over five years. The error bar shows the spread of data around the mean value. The small error bar represents a low spread of data while the larger error bar shows the larger spread of data indicating more variables from the mean. The length of the error bar in the influent BOD of May shows a higher value which indicates that the data collected over that time was more variable and less reliable. Similarly, the error bar generated in the effluent BOD in March shows higher uncertainty of the data. The graph shows error bars that overlap in all the months of the mean monthly BOD variation in the influent and effluent wastewater respectively. This could provide a hint that the difference was not statistically significant but needs to perform a statistical test to conclude.

ANOVA single factor test was performed in excel to find out the significance of the data and the results are shown in the tables below.

Table 4.3: ANOVA Single Factor test of influent BOD concentration in different months (January to December) from 2018 to 2022

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	93209.6	11	8473.60	1.024	0.441	1.999
Within Groups	388952.4	47	8275.583			
Total	482162	58				

The p-value as calculated by the ANOVA test in Table (4.3) is greater than 0.05 (significance level). The null hypothesis remains valid if the p-value is greater than the significance level, yet the results are not statistically significant for the influent BOD concentrations in different months.

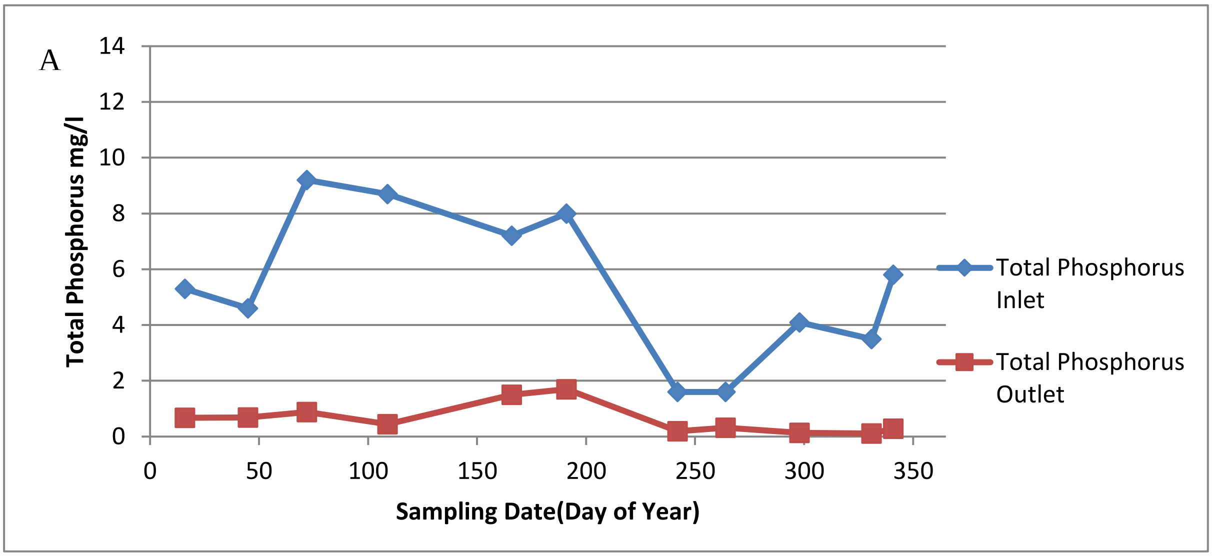
Table 4.4: ANOVA Single Factor test of effluent BOD concentration in different months (January to December) from 2018 to 2022

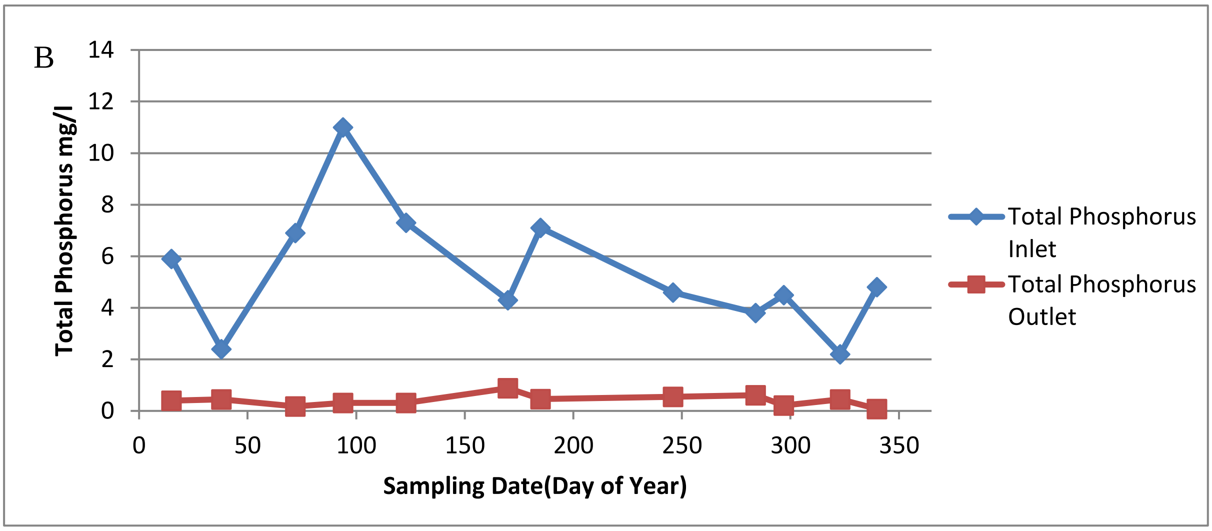
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	132.039	11	12.004	2.507	0.015	2.014
Within Groups	210.671	44	4.788			
Total	342.710	55				

Likewise, the p-value obtained from the ANOVA test in Table (4.4) was 0.0152 which is smaller than the significance level for effluent BOD concentrations in different months. Hence, the null hypothesis was rejected, and the variables are reported as statistically significant.

4.2.2 Total Phosphorus variation in influent and effluent wastewater

Phosphorus reduction can provide greater environmental protection than organic matter removal. The most effective method to protect most oxygen-rich water bodies which are neither susceptible to living matter nor nitrogen is to reduce phosphorus [94].





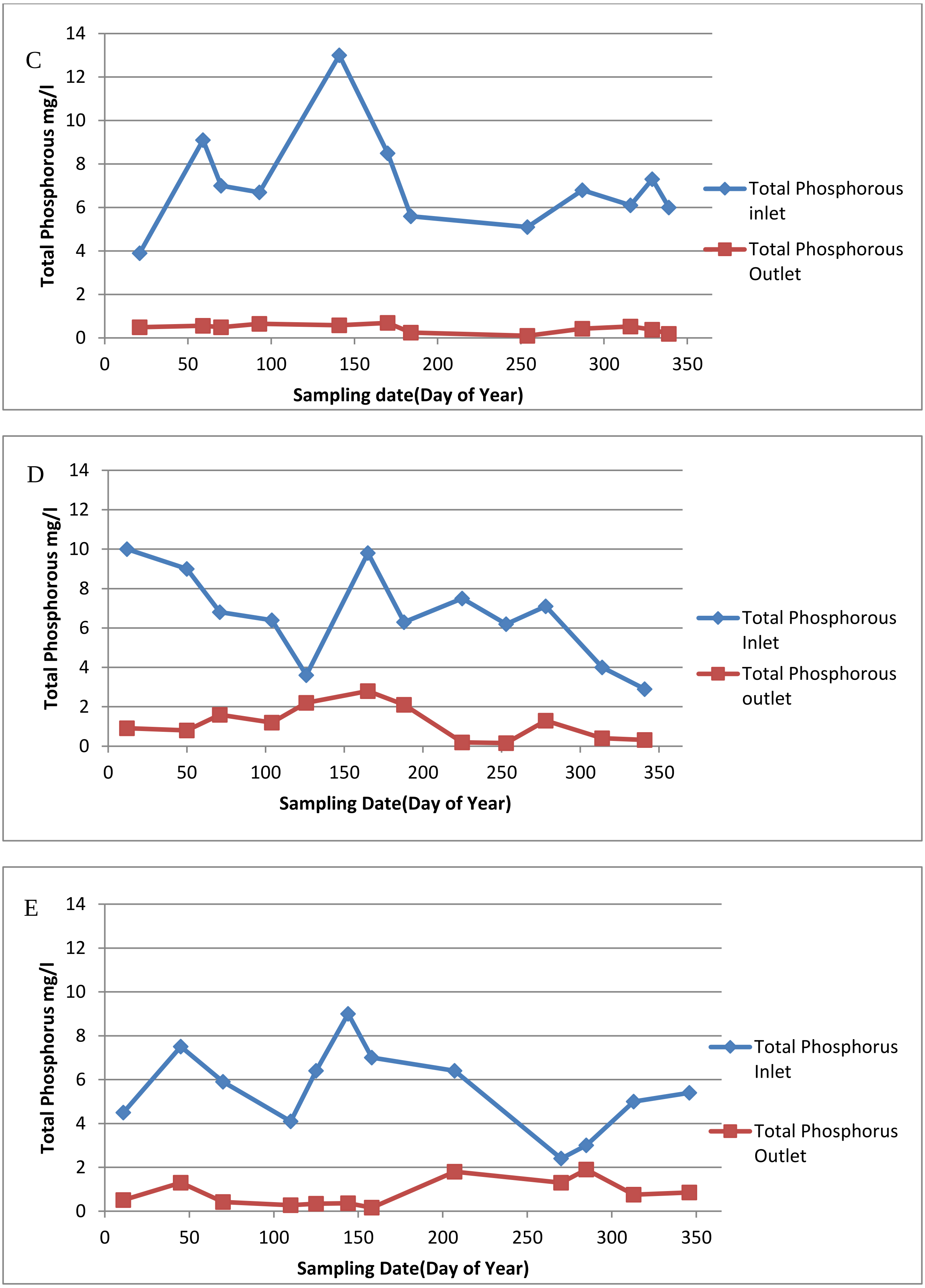


Fig 4.11: Variation of Total Phosphorus at influent and effluent wastewater A) 2018 B) 2019 C) 2020 D) 2021 and, E) 2022 respectively

Fig (4.11) shows the variation of phosphorus levels in the wastewater influent and effluent for five different years starting from 2018 to 2022 respectively. The pattern of total phosphorus concentration in the influent wastewater looks a bit similar between the different years. During the early days of the year, the total phosphorus level in 2020 and 2022 rise while decreasing in 2018, 2019, and 2021 respectively. The highest phosphorus level recorded in January was 10 mg/l in 2021. The total phosphorus in the influent wastewater decreases with the days of the year. The graph follows a similar pattern in the summer months and the maximum influent phosphorus was found to be 13 mg/l in May 2020. The phosphorus level in wastewater was higher in 2020 in comparison to the other four years. It can be concluded from the graph that the phosphorus in the wastewater rises during the summer months while falls in winter and autumn. Human and animal waste, detergents, agriculture runoff, and industrial discharges are the major sources of phosphorus in wastewater [95]. During the rainy season, the precipitation runoff contributes a little phosphorus load due to the dilution of the phosphorus in the wastewater if the sewerage system is combined [96]. The lowest influent total phosphorus (TP) measured was in August and September of 2018 and was found to be 1.6 mg/l as shown below in Fig (4.12).

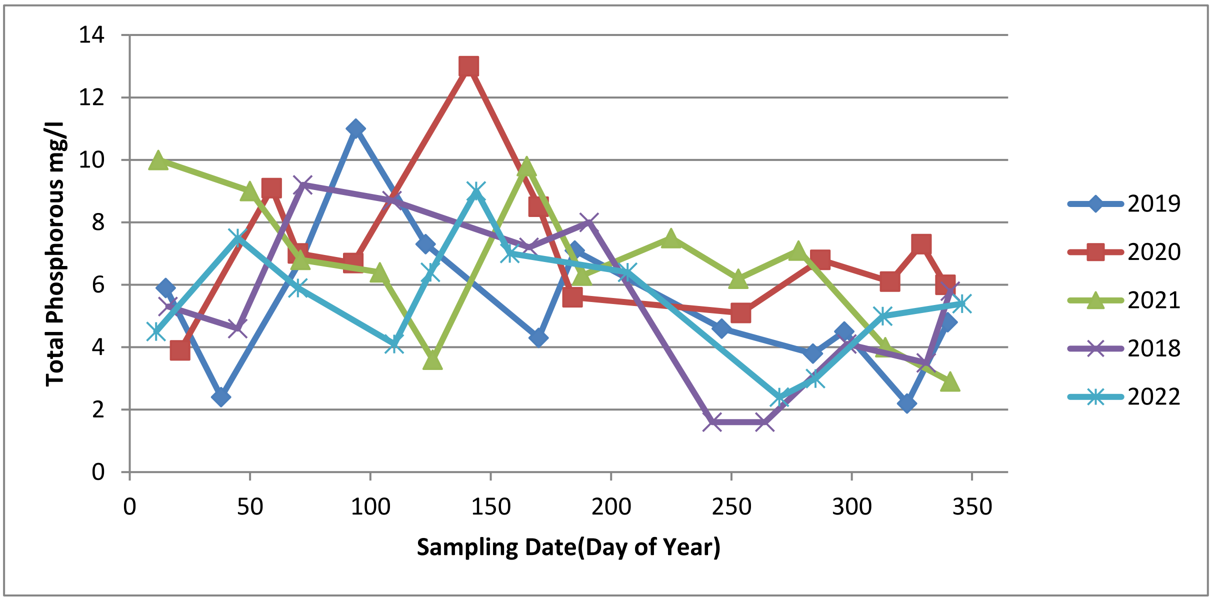


Fig 4.12: Influent phosphorus variation over the years

Fig (4.13) shows the TP variation with the days of the year in the wastewater after the treatment process. The phosphorus removal in the years 2019 and 2020 seems to be satisfactory and almost all the data was found to be around 0.5 m g/l. Though the influent TP was high in 2020, the treatment plants works properly, and relative treatment efficiency was found to be good.

The maximum value of TP in the effluent was 2.8 mg/l in June 2021 whereas the minimum was recorded as 0.077 mg/l in December 2019.

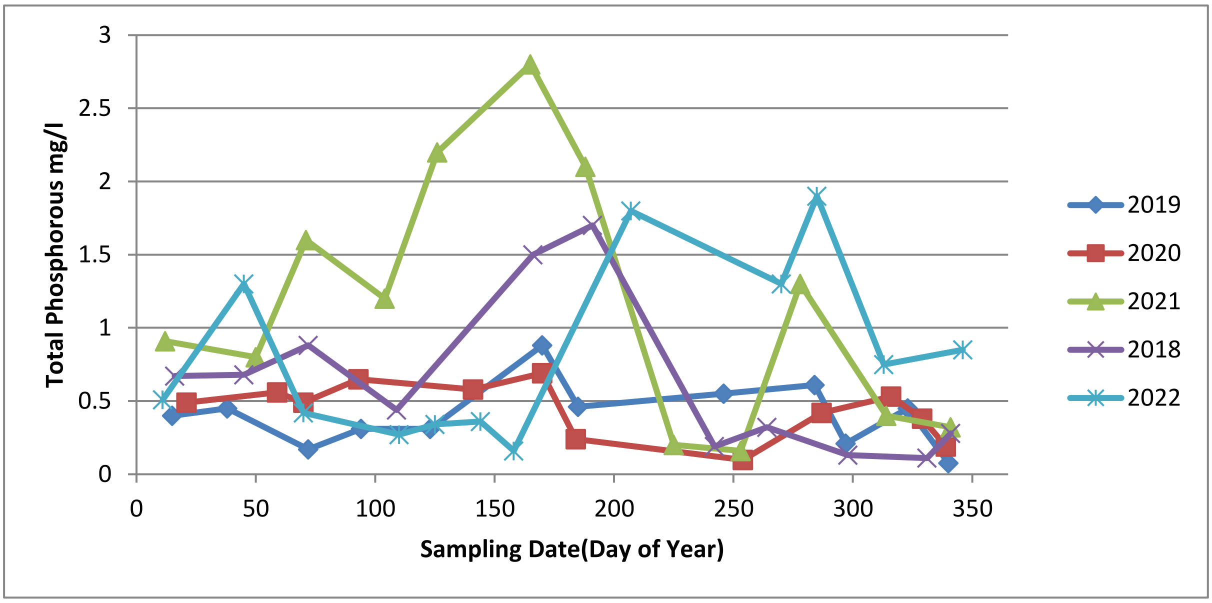


Fig 4.13: Effluent phosphorus variation over the years

The trend of phosphorus removal in the effluent shows that it was similar in the year 2019 and 2020 respectively while in the other three years, the trend differs from each other. Fig (4.13) shows that the TP in the effluent wastewater during 2018, 2020, and 2021 is quite high in the summer months. Hence, it can be interpreted that the trend of phosphorus removal seems to be irregular based on these five years' data. The biological removal of phosphorus necessitates an anaerobic environment and consumes carbon and alkalinity. With influent flows that already have decreased DO content before aeration, the first basin's anaerobic requirement is more readily met [97]. In conventional biological nutrient removal, the limited carbon sources are prioritized by denitrifying bacteria, and PAOs are frequently surpassed in the competition for carbon sources, leading to poor P removal efficiency [98]. Due to its great efficiency and low cost, the simultaneous nitrification, denitrification, and phosphorus removal method shave attracted a lot of interest which depends on autotrophic nitrifying bacteria, heterotrophic denitrifying PAOs, and denitrifying GAOs in addition to ordinary PAOs and denitrifying microbes [99].

Fig (4.14) represents the average daily total phosphorus variation in the different months of the year starting from 2018 to 2022. The error bars in the average influent phosphorus have overlapped with each other, which illustrates the data are statistically significant. The error bar in May for influent phosphorus shows that the data are less reliable and vary significantly from the mean. On the other hand, the error bar in July shows more reliable data with less variation from the mean. Likewise, the error bars in the effluent phosphorus also overlap with each other which validates that the data has a statistically significant difference between the years. The error bar is longest in June and shortest in November, showing that the data collected over June varies from mean and less reliable while more reliable and low variation from mean in November. From Fig (4.14) for effluent TP, the error bars of March, May, and July show unusual length, more than the average value. Therefore, statistical tests should be performed to conclude whether these data are statistically significant or not.

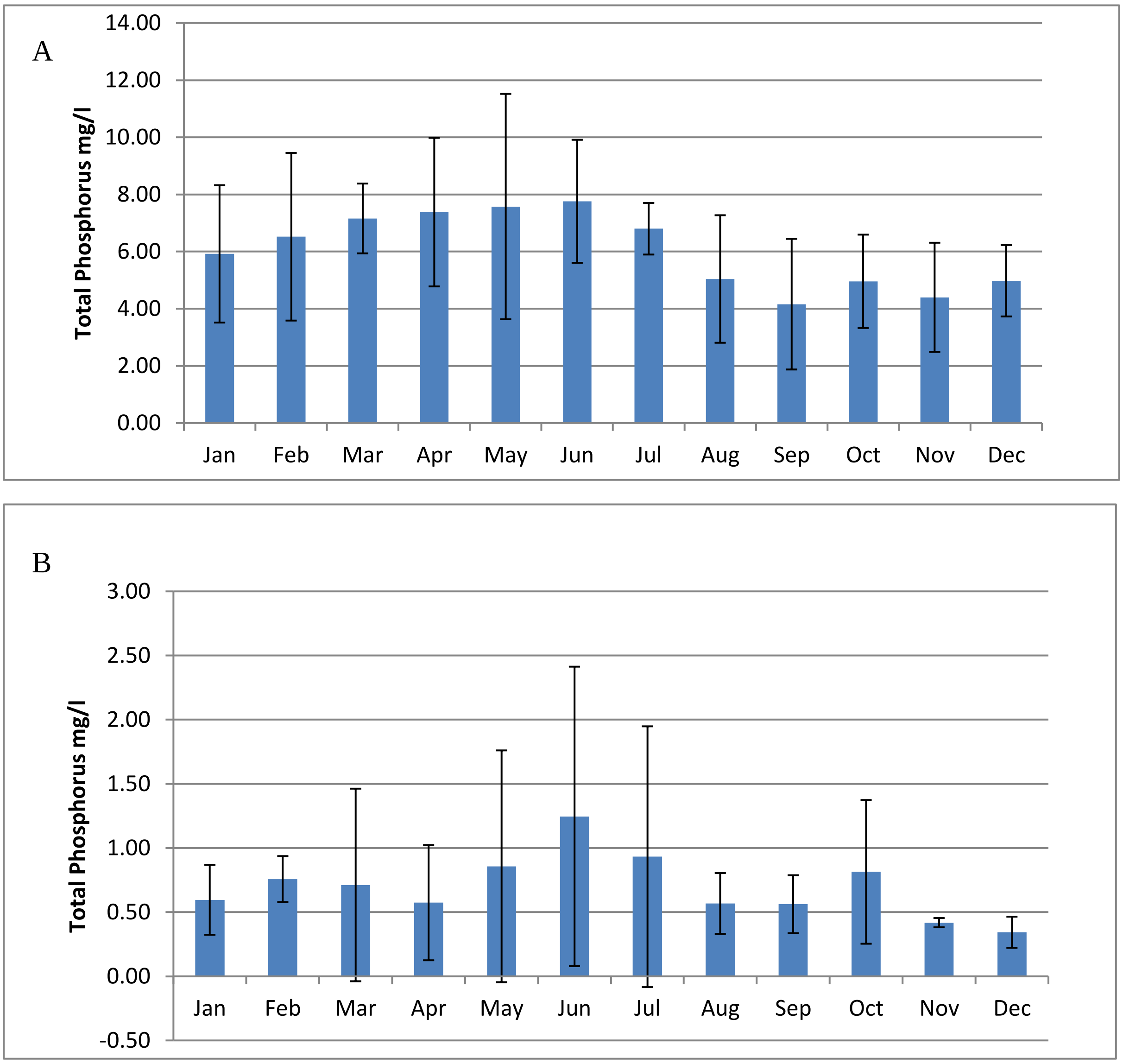


Fig 4.14: Average daily TP variation over different months of five years' time A) Influent B) Effluent

ANOVA test was carried out for both the influent and effluent TP concentration of the different months of the year and test results were presented in Table (4.5).

Table 4.5: ANOVA Single Factor test of influent TP concentration in different months (January to December) from 2018 to 2022

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	91.142	11	8.286	1.683	0.107	1.999
Within Groups	231.380	47	4.923			
Total	322.521	58				

The p-value as calculated by the ANOVA test in Table (4.5) is 0.107 which is greater than 0.05 (significance level). The null hypothesis remains valid as the p-value is greater than the significance level, yet the results are not statistically significant for the influent TP concentrations in different months of the year.

Table 4.6: ANOVA Single Factor test of effluent TP concentration in different months (January to December) from 2018 to 2022

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.290	11	0.299	0.802	0.637	1.999
Within Groups	17.523	47	0.373			
Total	20.813	58				

Similarly, the p-value obtained from the ANOVA test in Table (4.6) was 0.637 which is greater than the significance level for effluent TP concentrations in different months of the year. Hence, the null hypothesis was accepted, and the variables are reported as statistically not significant.

4.2.3 Removal Efficiency of BOD_5 and TP

The removal efficiency is calculated as,

$$Removal\ efficiency = \frac{(Average\ Influent - Average\ Effluent)}{Average\ Influent} \times 100\% \qquad (4.1)$$

The average removal efficiency of TP is calculated by considering equation (4.1),

$$= ((6.05 - 0.7))/6.05 \times 100$$

$$= 88.42\%$$

Table 4.7: Influent and Effluent TP (mg/l) variation in different months over five-year period

	Month	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
2018	Influent TP	5.3	4.6	9.2	8.7		7.2	8	1.6	1.6	4.1	3.5	5.8
	Effluent TP	0.67	0.68	0.88	0.44		1.5	1.7	0.19	0.32	0.13	0.11	0.28
2019	Influent TP	5.9	2.4	6.9	11	7.3	4.3	7.1	4.6	3.8	4.5	2.2	4.8
	Effluent TP	0.4	0.45	0.17	0.31	0.31	0.88	0.46	0.55	0.61	0.21	0.45	0.08
2020	Influent TP	3.9	9.1	7	6.7	13	8.5	5.6	5.1	6.8	6.1	7.3	6
	Effluent TP	0.49	0.56	0.49	0.65	0.58	0.69	0.24	0.1	0.42	0.53	0.38	0.19
2021	Influent TP	10	9	6.8	6.4	3.6	9.8	6.3	7.5	6.2	7.1	4	2.9
	Effluent TP	0.91	0.8	1.6	1.2	2.2	2.8	2.1	0.2	0.16	1.3	0.4	0.32
2022	Influent TP	4.5	7.5	5.9	4.1	6.4	9	7	6.4	2.4	3	5	5.4
	Effluent TP	0.51	1.3	0.42	0.27	0.34	0.36	0.16	1.8	1.3	1.9	0.75	0.85
	Influent Average	5.92	6.52	7.16	7.38	7.58	7.76	6.80	5.04	4.16	4.96	4.40	4.98
	SD	2.40	2.93	1.22	2.60	3.94	2.15	0.90	2.23	2.29	1.63	1.91	1.25
	Effluent Average	0.60	0.76	0.71	0.57	0.86	1.25	0.93	0.57	0.56	0.81	0.42	0.34
	SD	0.27	0.18	0.75	0.45	0.90	1.17	1.02	0.24	0.23	0.56	0.04	0.12

The removal efficiencies of total phosphorus each year from 2018 to 2022 are found to be 88.37%, 92.41%, 93.80%, 82.36%, and 85.05% respectively.

The average removal efficiency of BOD_5 is calculated by applying equation (4.1),

$$= \frac{(173.55 - 3.46)}{173.55} \times 100\%$$

$$= 98\%$$

Table 4.8: Influent and Effluent BOD_5 (mg/l) variation in different months over five year period

	Month	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
2018	Influent BOD_5	140	120	290	210		220	260	42	72	69	260	160
	Effluent BOD_5	1.5	1.5		1.5		4.3	4.2	1.5		1.5		3.6
2019	Influent BOD_5	130	72	160	210	200	180	180	150	59	120	91	120
	Effluent BOD_5	1.5	1.5	3.9	3.2	1.5	1.5	1.5	1.5	1.5	1.5	7.7	1.5
2020	Influent BOD_5	66	210	180	140	600	230	300	220	250	270	170	310
	Effluent BOD_5	3.1	3.2	3.7	3.7	3.1	1.5	3.2	1.5	3	3	3	3
2021	Influent BOD_5	210	260	150	120	120	190	170	190	180	240	100	87
	Effluent BOD_5	3	4	17	3	6	4	6	4	4	1.5	1.5	4
2022	Influent BOD_5	80	310	100	93	140	210	240	150	42	55	130	120
	Effluent BOD_5	1.5	4	10	4	4	4	3	3	4	5	1.5	4
	Average Influent	125.20	194.40	176.00	154.60	265.00	206.00	230.00	150.40	120.60	150.80	150.20	159.40
	SD	56.97	98.01	70.21	53.25	225.91	20.74	54.77	67.39	90.29	98.72	68.70	88.07
	Average Effluent	2.12	2.84	8.65	3.08	3.65	3.06	3.58	2.30	3.13	2.50	3.43	3.22
	SD	0.85	1.27	6.29	0.97	1.88	1.66	2.27	1.15	1.18	1.54	2.94	1.04

Hence, the removal efficiency of biochemical oxygen demand is found to be 98% while the total phosphorus removal efficiency is determined as only 88.42%. The Norwegian legislation already makes the reduction of phosphorus by 90% a basic requirement for wastewater treatment facilities in a typical and sensitive location [100]. The removal efficiencies of the BOD each year from 2018 to 2022 are 98.54%, 98.31%, 98.81%, 97.13%, and 97.13% respectively.

4.2.4 Trend analysis between BOD and TP in influent and effluent wastewater

Fig (4.15) shows the trend of BOD and TP concentration in wastewater influent and effluent respectively in 2018 at Randvik WWTP. The trend line of influent BOD having a negative slope decreases with the days of the year. However, the trend of the influent TP remains almost constant. On the contrary, the trend line demonstrates that the effluent BOD concentration increases, and effluent TP decreases with the start to the end of the year.

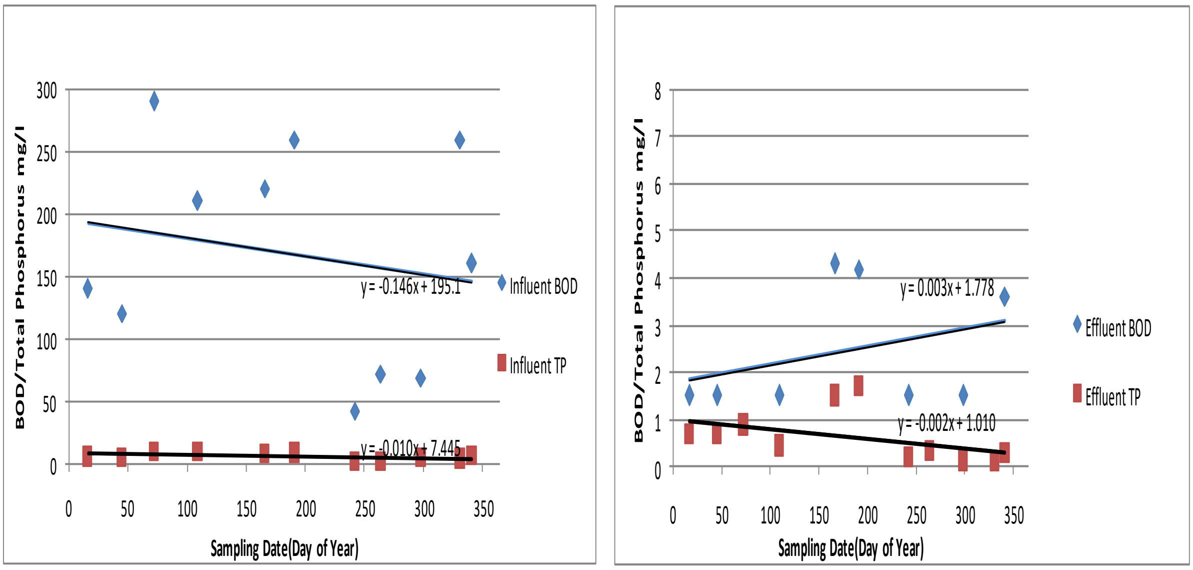


Fig 4.15: Trend analysis of influent and effluent BOD and TP at different times of year in 2018

Similarly, Fig (4.16) validates that the trend of total phosphorus is somewhat constant in both influent and effluent wastewater throughout the year 2019. The trend line of influent BOD shows it decreases with the days of the year whereas the effluent BOD concentration increases over time. The BOD concentration increases from January to December while the total phosphorus remains constant in the influent wastewater in 2020. Likewise, Fig (4.17) signifies that the trend of both effluent BOD and total phosphorus has a negative slope. Fig (4.18) reveals that the trend lines of all the parameters have a negative slope which means the concentration decreases with the days of the year in 2021. The trend of influent BOD concentration seems to be decreased from January to December while the total phosphorus remains firm throughout the year in 2022. However, the BOD concentration in the effluent wastewater having a negative slope, suggests that BOD level in water decreases with time. The effluent total phosphorus in the trend line has a positive slope, concentration increases from January to December in 2022 as shown in Fig (4.19).

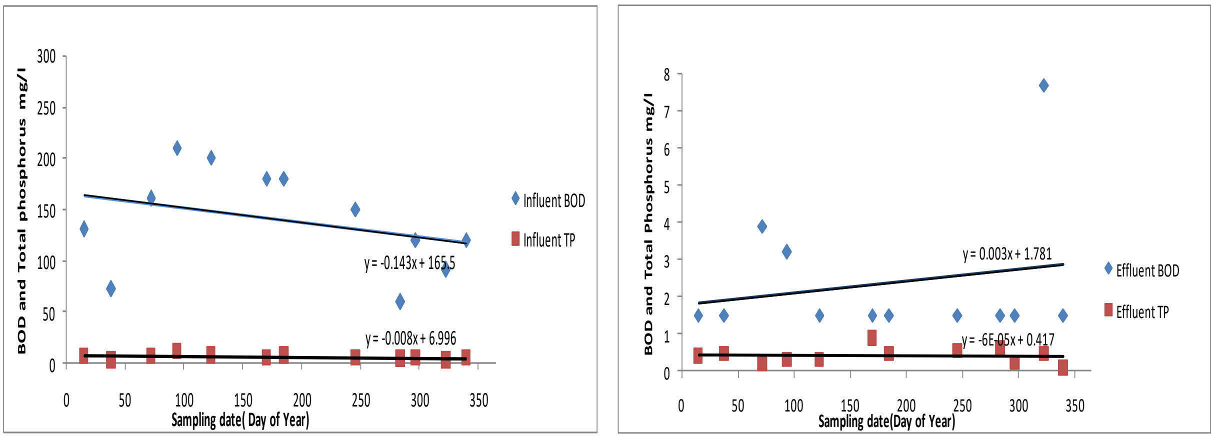


Fig 4.16: Trend analysis of influent and effluent BOD and TP at different times of year in 2019

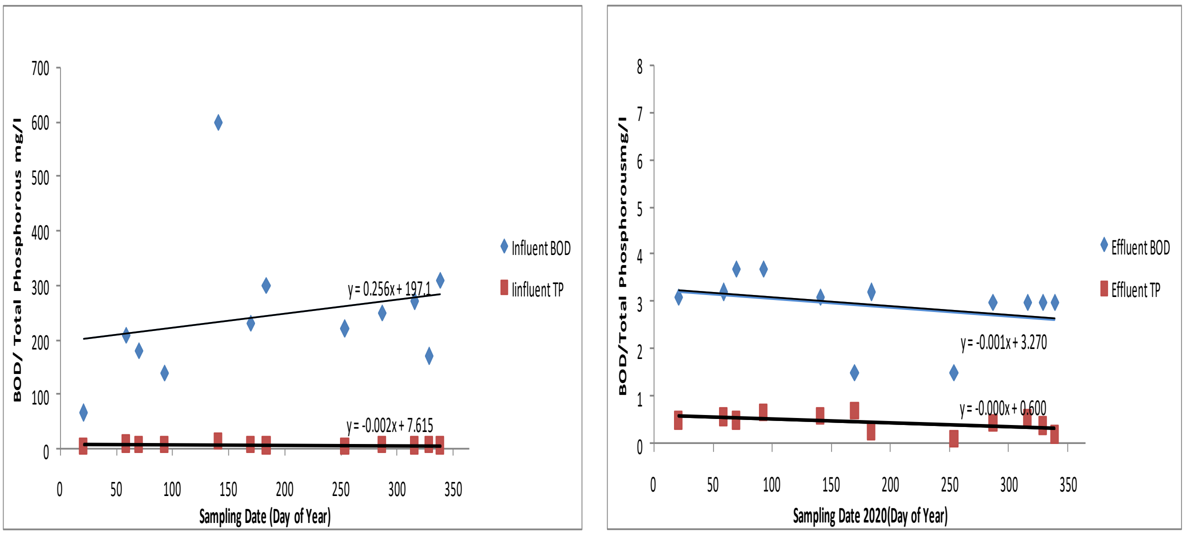


Fig 4.17: Trend analysis of influent and effluent BOD and TP at different times of year in 2020

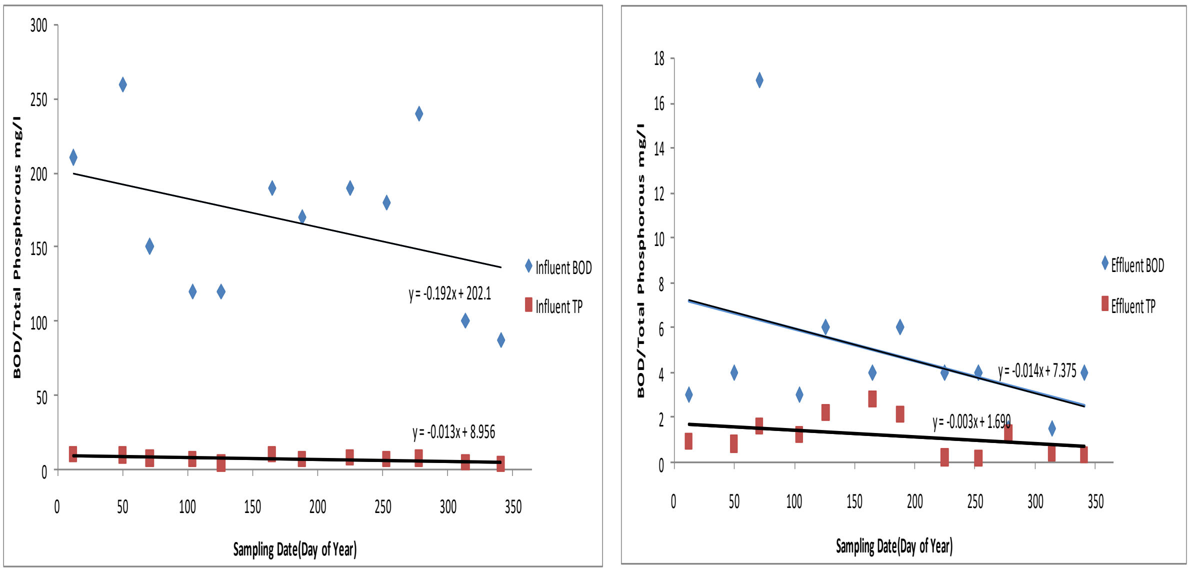


Fig 4.18: Trend analysis of influent and effluent BOD and TP at different times of year in 2021

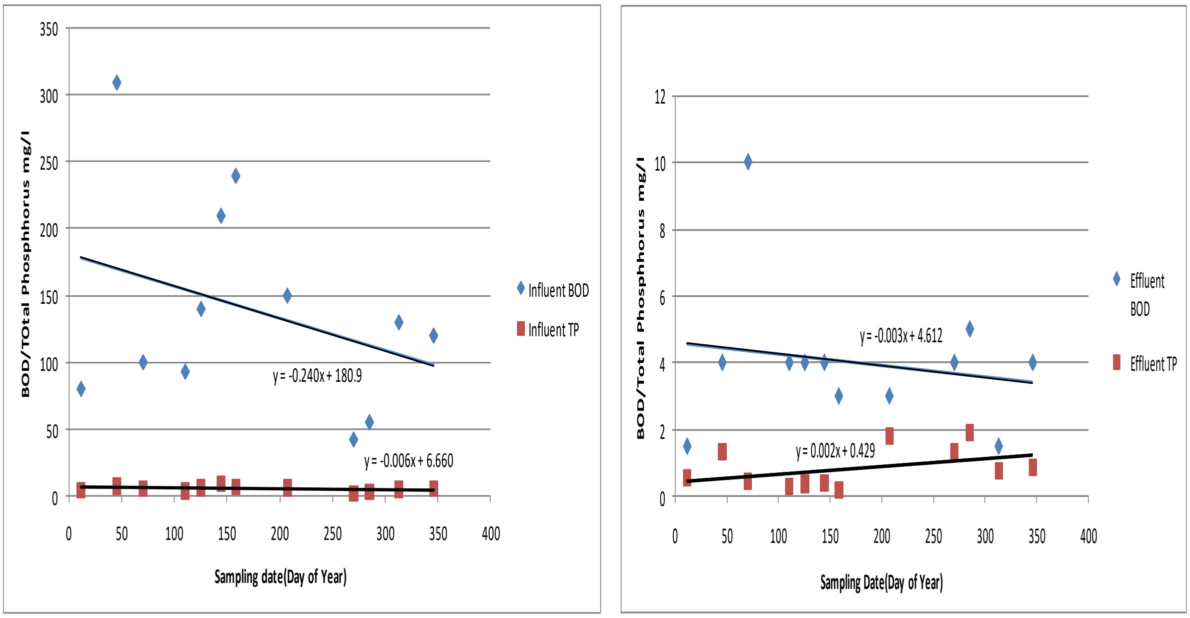
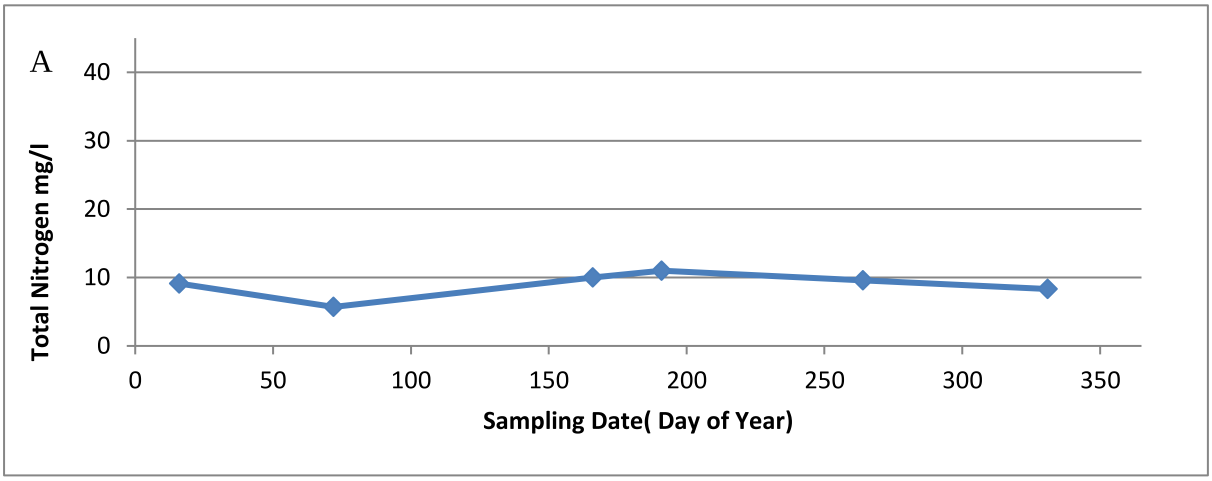


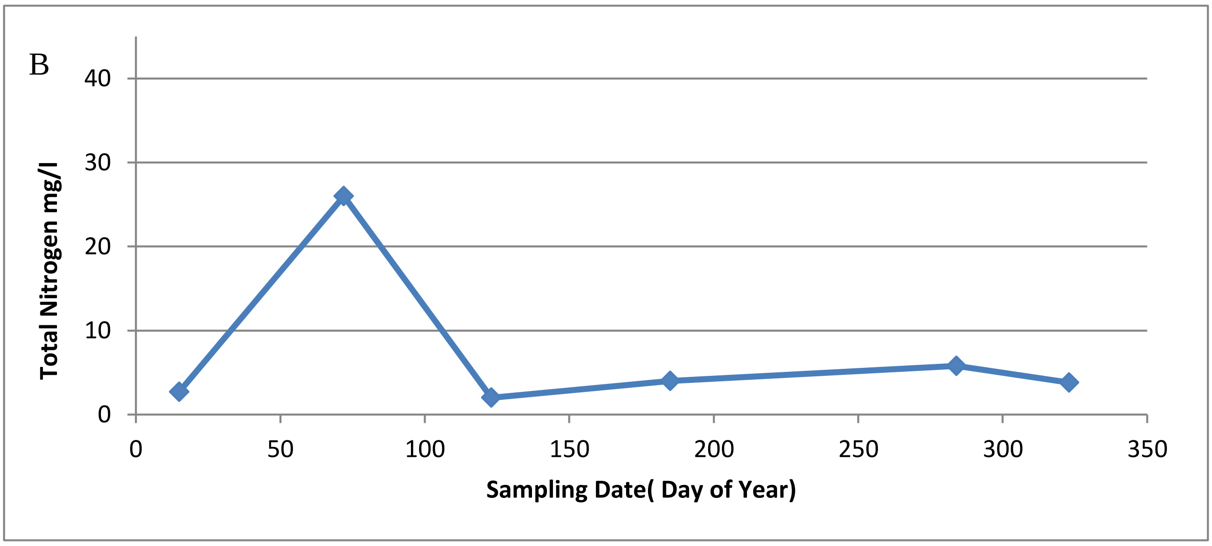
Fig 4.19: Trend analysis of influent and effluent BOD and TP at different times of year in 2022

It is shown from the graphs that the concentration of BOD and total phosphorus in the influent wastewater over five years starting from January to December follow the same trend except in the case of influent BOD in 2020. The higher BOD concentration shows a higher level of organic pollution in the water. The BOD value in wastewater is significantly lower during the spring and the summer months because of the spring thaw and summer rainfall [1].

4.2.5 Concentration of Nitrogen in the effluent wastewater

Nitrogen removal requires a biological process i.e., nitrification and/or denitrification which is dependent on many factors including temperature and takes place at a slower rate in cold influent water. Nitrogen removal under a cold climate requires a higher hydraulic retention time, more energy (i.e., aeration), and an extra carbon source for denitrification processes. The removal efficiency of Nitrogen should be 85% or 6 mg/l concentration in the treated wastewater, which is quite difficult to achieve in cold climates [100]. Fig (4.20) shows the concentration of nitrogen in the effluent wastewater after the treatment process at different sampling points of the year starting from 2018 to 2022.





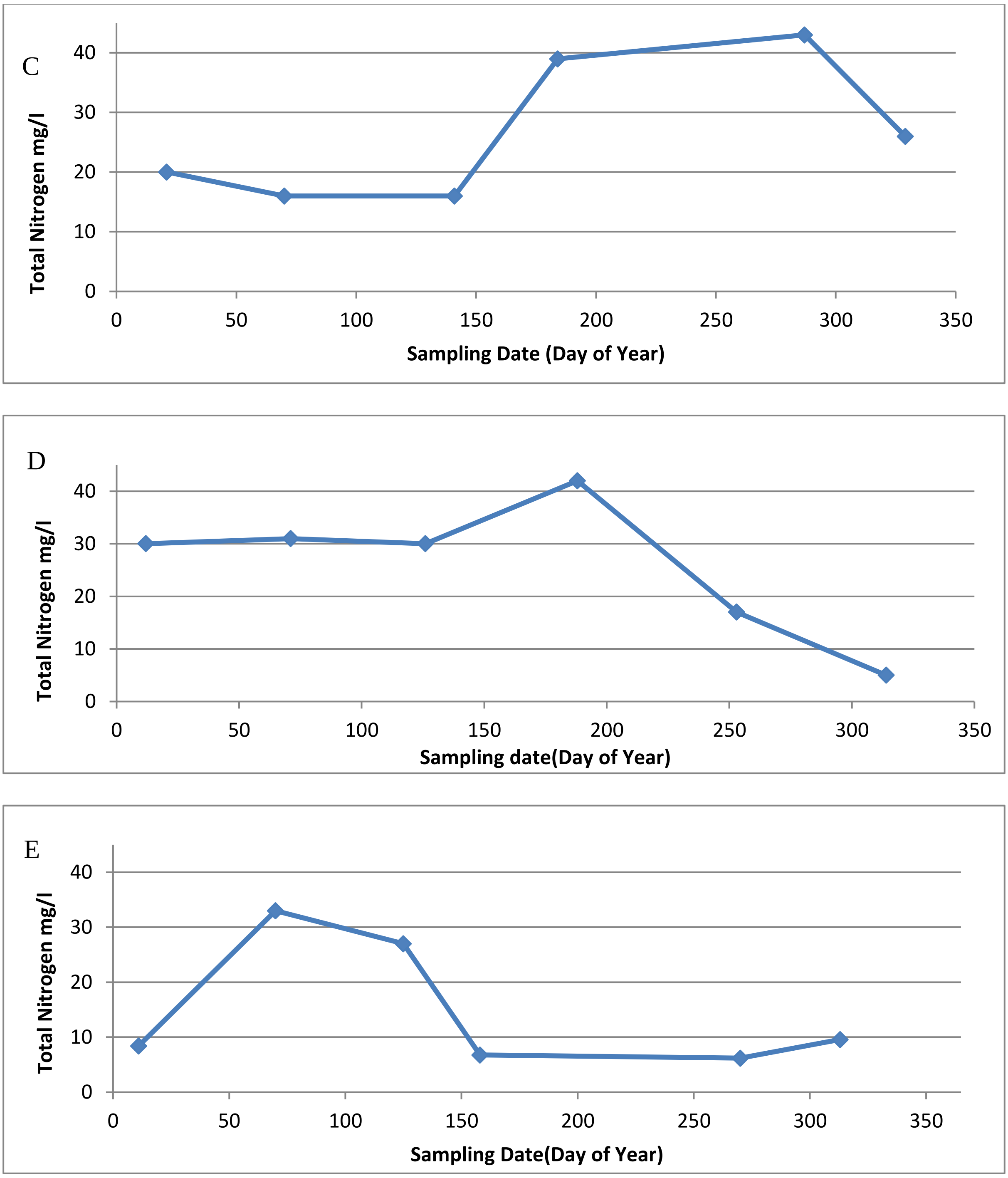


Fig 4.20: Variation of total nitrogen concentration in various sampling points of a year A) 2018 B) 2019 C) 2020 D) 2021 and, E) 2022 respectively

For medium WWTPs (PE 10,000-100,000) and large WWTPs (PE ≥100,000), the effluent standard for total nitrogen is 15 mg/l and 10 mg/l, respectively, as a result of suggestions made in the EU Directive regarding the quality of treated wastewater [101]. The Randvik WWTP is designed for the 7000 PE; it comes under the category of small WWTP. The concentration of

TN in the effluent wastewater is more or less 10 mg/l in 2018 throughout the year. The nitrogen removal efficiency is quite good in 2019 as five out of six sampling data show TN below 5 mg/l. The nitrogen concentration in the water after the treatment process seems a bit higher in 2020 and 2021. The largest value of TN in effluent water was found to be 43 mg/l in October 2020 whereas the lowest value was 2 mg/l in May 2019 respectively. From the graph, it can be seen that the concentration of TN is getting lower at the end of the year.

4.3 Microbial Diversity in the treatment plant

The microbial profile of all three sample points (Risør R1, Risør R2, and Risør R1 and R2 mix) is quite similar where *Actinomycetales* dominates. Figure (4.21) shows the operational taxonomic unit (OTU) plot. Table (4.9) shows the top microbial assemblage at the order level extracted from the OTU plot.

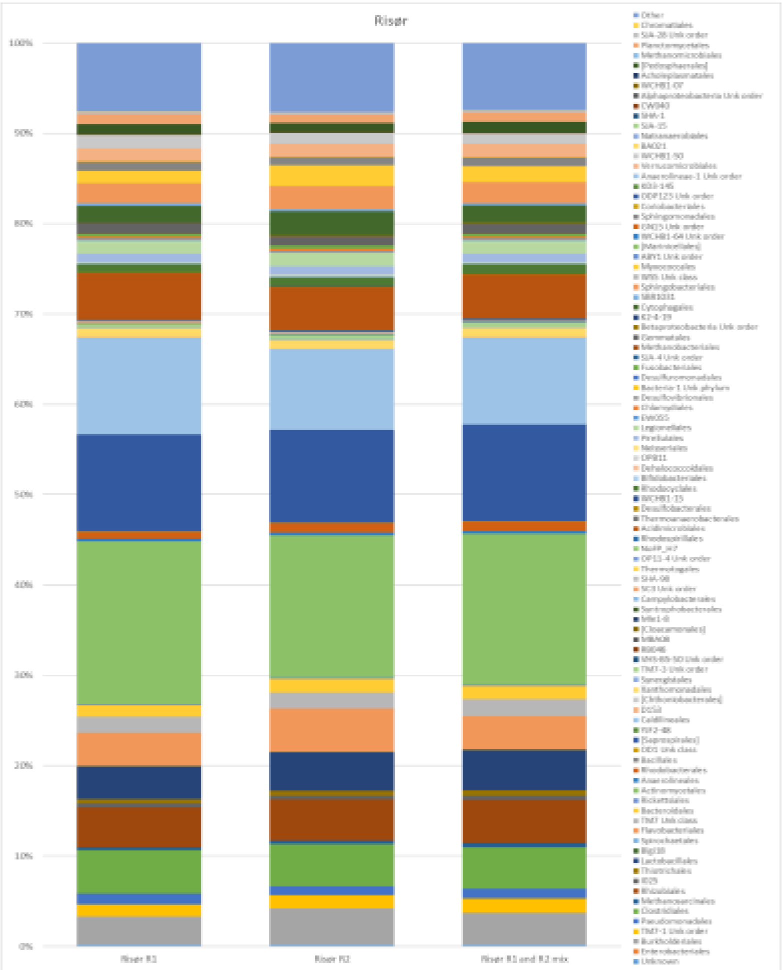


Fig 4.21: Microbial assemblage at the Order level at Randvik Wastewater Treatment Plant

Table 4.9: Top Microbial assemblage at the Order level

	Microorganisms		
S.N	**R1**	**R2**	**R1 & R2 Mix**
1	Actinomycetales	Actinomycetales	Actinomycetales
2	Saprospirales	Saprospirales	Saprospirales
3	Caldilineales	Caldilineales	Caldilineales
4	Acidimicrobiales	Acidimicrobiales	Rhizobiales
5	Clostridiales	Flavobacteriales	Acidimicrobiales
6	Rhizobiales	Clostridiales	Clostridiales
7	Flavobacteriales	Rhizobbiales	Lactobacillales
8	Lactobacillales	Lactobacillales	Flavobacteriales
9	Burkholderiales	Burkholderiales	Burkholderiales
10	Sphingobaccteriales	Cytophagales	Sphingobacteriales
11	Cytophagales	Sphingobaccteriales	Cytophagales
12	TM7 Unk class	Myxococcales	TM7 Unk Class
13	Legionellales	TM7 Unk Class	Myxococcales
14	Verrrucomicrobiales	Legionellales	TM7-1 Unk Order
15	WCHB1-50	Bacteroidales	Legionellales

Actinomycetales, Saprospirales, Caldilineals, Acidimicrobiales, Rhizobiales, Clostridiales, etc are the dominant microbial order found in the wastewater. The investigation of microorganisms in a WWTP offers information on the community's species structure, revealing the dominant microbial groups responsible for effective wastewater treatment. *Actinomycetales* are gram-positive and anaerobic bacteria having mycelia in a filamentous and branching growth pattern. The optimum environment required for the growth of *Actinomycetales* has a pH between 7 and 8 and a temperature between 15 and 20 °C [102]. Some *Actinomycetales* are nuisance microorganisms in the wastewater as they emit an earthy odor that degrades the quality of municipal water. It has been recognized as the cause of disruptions in wastewater treatment as the growth of microorganisms can produce thick foam in the activated sludge process. Because of the hydrophobicity of their cell wall, *Actinomycetales* have an advantage over other microbes in adhering to water-immiscible interfaces and degrading chlorinated hydrocarbons and complex organic molecules [103]. *Actinomycete* helps in the removal of heavy metals and degrades aromatic compounds from wastewater. The phylum Proteobacteria (21-65%) predominates in municipal WWTPs, with β-Proteobacteria being the most numerous classes and being substantially responsible for organic and nutrient removal [104]. The *Acitomycetales* and *Acidimicrobiales* come under the phylum Actinobacteria, *Saprospirales* under

Bacteriodetes, *Clostridiales*, *Caldilineales* and *Rhizobiales* under Firmicutes, Chlorofexota and Proteobacteria phyla respectively[103]. Specific environmental conditions such as temperature, pH, DO, HRT, SRT, and inter-species interactions may influence bacterial selection from the influent wastewater [105].

5 Conclusion

This master thesis project aimed to review and investigate the nitrification/denitrification process for biological nutrient removal of the Randvik wastewater treatment plant. The treatment efficiency of organic pollutants was found to be 98% reducing average BOD concentration in influent 175.33 mg/l to effluent 3.46 mg/l. Likewise, the removal efficiency of the total phosphorus was determined as 88.42% where the TP concentration in influent 6.05 mg/l reduces to 0.7 mg/l in the effluent. The plant has excellent results in the treatment of organic pollutants (BOD removal), and nitrogen removal whereas the phosphorus removal efficiency hasn't met the emission standard set by the Norwegian legislation. Aquatic ecosystems get more eutrophicated as a result of anthropogenic phosphorus (P) discharges.

The data analysis on wastewater influent and effluent quality was performed by data plotting, ANOVA test, and mean comparison in Microsoft Excel. The maximum average daily flow into the treatment plant was found to be 1703±894.30 m^3/day in 2022 whereas the minimum flow occurred as 1176±344.54 m^3/day in 2018. The influent flow fluctuates because of the variation of water consumption by the users in different seasons, and global warming which affects temperature and precipitation variation. The total nitrogen concentration in the effluent was found to be lower (<15 mg/l) in the first two years i.e., 2018 and 2019 respectively while it increased significantly onwards reaching a maximum value of 43 mg/l in 2020. Excessive ammonia is toxic to fish and other aquatic life and leads to eutrophication. The trend analysis between BOD and TP shows that it fluctuates at different times of the year and doesn't remain constant in both the influent and effluent wastewater. The SVI of the sludge was calculated by the treatment plant as 500-700 ml/g, which is high for the normal range of 80-1500 ml/g in a conventional activated sludge process. However, it appears that the sludge settles very quickly, and the effluent water is very clear. Sludge-drying reed beds are a technically simple method for dewatering and sludge stabilization compared with other sludge handling techniques.

The biological treatment of wastewater depends on bacteria, nematodes, or other small micro-organisms to break down organic waste. Wastewater may contain organic matter, partially digested food, pathogenic organisms, heavy metals, or toxins. The influent wastewater to the Randvik treatment plant contains different microorganisms, among them *Actinomycetales* order dominates. *Saprospirales, Caldilineales, Acidimicrobiales, Clostridiales, Rhizobiales,* *Flavobacteriales*, etc. are the other major microbial assemblage found in the wastewater as shown in the operational taxonomic unit (OUT) plot.

6 Recommendation

The Randvik WWTP is a biological treatment system that consists of both nitrification and de-nitrification processes for wastewater treatment. The plant has achieved tremendous success in the removal of the BOD concentration along with total nitrogen while the removal efficiency of the total phosphorus is found to be below the emission standards set by the Norwegian legislation. Based on the results, there is still a need for the optimization of the biological nutrient removal process. Biological phosphorus removal depends upon the phosphorus uptake by the aerobic heterotrophs having the ability to store orthophosphate over their requirements for biological growth. Phosphorus can also be removed from the wastewater through chemical precipitation which mainly uses aluminum, and iron or lime to form chemical flocs. Though a higher amount of phosphorus can be removed through this system, it produces more sludge and has higher operating costs. Therefore, a combination of biological and chemical processes could be a better option when the TP concentration in the effluent needed is very low. Recent studies showed that simultaneous nitrification de-nitrification and phosphorus removal (SNDPR) could be the promising treatment process for simultaneous nitrogen and phosphorus removal.

Likewise, the treatment plant has been operating since 2002, hence there needs to be proper maintenance and upgrading of the system. Although the plant facility has been running for the last 20 years, the data analysis was done based on the last five years' data. The influent flow rate, BOD and TP concentration in influent and effluent wastewater, and Nitrogen level in the effluent were the only data provided by the treatment plant. It is quite difficult to evaluate the treatment process without examining all the physiochemical parameters associated with the wastewater. The data collection was carried out by random sampling from the treatment plant. It could have been better if the data collection was based on a time-proportional or quantity-proportional sampling system. Enhanced biological phosphorus removal (EBPR) can be a good alternative but data such as flow rate, BOD, COD, TSS, ammonia, VFAs, VSS, pH, DO; etc. needs to be analyzed to find out the effectiveness of the EBPR system. Phosphorus is a finite but crucial resource that cannot be substituted with any other element. For this reason, initiatives to recycle phosphorus in wastewater should be done shortly. Moreover, there needs to be an investigation and perform further experiments to improve mass balance in the system.

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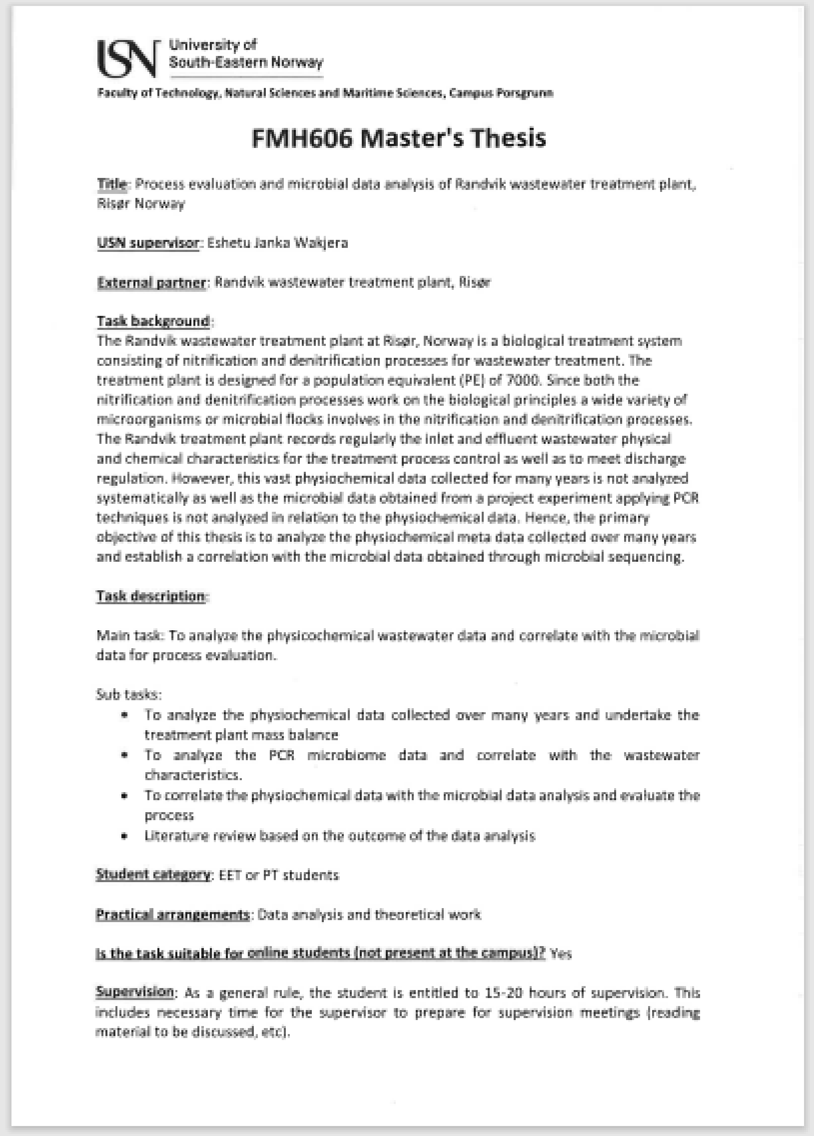
Appendices

Appendix A	**Master thesis task description**
Appendix B	**Physiochemical data of the Randvik wastewater treatment plant provided by the Risør Municipality**

Appendix A Master thesis task description



University of
South-Eastern Norway

Faculty of Technology, Natural Sciences and Maritime Sciences, Campus Porsgrunn

FMH606 Master's Thesis

Title: Process evaluation and microbial data analysis of Randvik wastewater treatment plant, Risør Norway

USN supervisor: Eshetu Janka Wakjera

External partner: Randvik wastewater treatment plant, Risør

Task background:
The Randvik wastewater treatment plant at Risør, Norway is a biological treatment system consisting of nitrification and denitrification processes for wastewater treatment. The treatment plant is designed for a population equivalent (PE) of 7000. Since both the nitrification and denitrification processes work on the biological principles a wide variety of microorganisms or microbial flocks involves in the nitrification and denitrification processes. The Randvik treatment plant records regularly the inlet and effluent wastewater physical and chemical characteristics for the treatment process control as well as to meet discharge regulation. However, this vast physiochemical data collected for many years is not analyzed systematically as well as the microbial data obtained from a project experiment applying PCR techniques is not analyzed in relation to the physiochemical data. Hence, the primary objective of this thesis is to analyze the physiochemical meta data collected over many years and establish a correlation with the microbial data obtained through microbial sequencing.

Task description:

Main task: To analyze the physicochemical wastewater data and correlate with the microbial data for process evaluation.

Sub tasks:

- To analyze the physiochemical data collected over many years and undertake the treatment plant mass balance
- To analyze the PCR microbiome data and correlate with the wastewater characteristics.
- To correlate the physiochemical data with the microbial data analysis and evaluate the process
- Literature review based on the outcome of the data analysis

Student category: EET or PT students

Practical arrangements: Data analysis and theoretical work

Is the task suitable for online students (not present at the campus)? Yes

Supervision: As a general rule, the student is entitled to 15-20 hours of supervision. This includes necessary time for the supervisor to prepare for supervision meetings (reading material to be discussed, etc).

Supervisor (date and signature): 25/01/2023

Student (write clearly in all capitalized letters): SANDESH KUIKEL

Student (date and signature): 25/01/2023

Appendix B Physiochemical data of the Randvik wastewater treatment plant, Risør

2018

Sampling Date (Day of Year)	Influent flowrate m^3/day	BOD$_5$ (Influent) mg/l	BOD$_5$ (Effluent) mg/l	Total Phosphorus (Influent) mg/l	Total Phosphorus (Effluent) mg/l	Total Nitrogen (Effluent) mg/l
16	1591	140	1.5	5.3	0.67	9.1
45	1240	120	1.5	4.6	0.68	
72	803	290		9.2	0.88	5.7
109	912	210	1.5	8.7	0.44	
166	935	220	4.3	7.2	1.5	10
191	1461	260	4.2	8	1.7	11
242	1016	42	1.5	1.6	0.19	
264	1220	72		1.6	0.32	9.6
298	899	69	1.5	4.1	0.13	
331	969	260		3.5	0.11	8.3
341	1894	160	3.6	5.8	0.28	

2019

Sampling Date (Day of Year)	Influent flowrate m^3/day	BOD$_5$ (Influent) mg/l	BOD$_5$ (Effluent) mg/l	Total Phosphorus (Influent) mg/l	Total Phosphorous (Effluent) mg/l	Total Nitrogen (Effluent) mg/l
15	1033	130	1.5	5.9	0.4	2.7
38	2952	72	1.5	2.4	0.45	
72	2386	160	3.9	6.9	0.17	26
94	939	210	3.2	11	0.31	
123	996	200	1.5	7.3	0.31	2
170	1308	180	1.5	4.3	0.88	
185	1216	180	1.5	7.1	0.46	4
246	986	150	1.5	4.6	0.55	
284	1703	59	1.5	3.8	0.61	5.8
297	1240	120	1.5	4.5	0.21	
323	2632	91	7.7	2.2	0.45	3.8
340	1265	120	1.5	4.8	0.077	

2020

Sampling Date (Day of Year)	Influent (m^3/day)	BOD_5 (Influent) mg/l	BOD_5 (Effluent) mg/l	Total Phosphorous (Influent) mg/l	Total Phosphorous (Effluent) mg/l	Total Nitrogen (Effluent) mg/l
21	1359	66	3.1	3.9	0.49	20
59	1250	210	3.2	9.1	0.56	
70	2363	180	3.7	7	0.49	16
93	1017	140	3.7	6.7	0.65	
141	830	600	3.1	13	0.58	16
170	794	230	1.5	8.5	0.69	
184	1346	300	3.2	5.6	0.24	39
254	1138	220	1.5	5.1	0.098	
287	2309	250	3	6.8	0.42	43
316	1096	270	3	6.1	0.53	
329	1304	170	3	7.3	0.38	26
339	2907	310	3	6	0.19	

2021

Sampling Date (Day of Year)	Influent flowrate (m^3/day)	BOD_5 (Influent) mg/l	BOD_5 (Effluent) mg/l	Total Phosphorus (Influent) mg/l	Total Phosphorus (Effluent) mg/l	Total Nitrogen (Effluent) mg/l
12	1200	210	3	10	0.91	30
50	1100	260	4	9	0.8	
71	900	150	17	6.8	1.6	31
104	966	120	3	6.4	1.2	
126	1600	120	6	3.6	2.2	30
165	1650	190	4	9.8	2.8	
188	1650	170	6	6.3	2.1	42
225	1200	190	4	7.5	0.2	
253	1234.71	180	4	6.2	0.16	17
278	1399	240	1.5	7.1	1.3	
314	1770	100	1.5	4	0.4	5
341	910	87	4	2.9	0.32	

2022

Sampling Date (Day of Year)	Influent flowrate (m^3/day)	BOD_5 (Influent) mg/l	BOD_5 (Effluent) mg/l	Total Phosphorus (Influent) mg/l	Total Phosphorus (Effluent) mg/l	Total Nitrogen (Effluent) mg/l
11	2600	80	1.5	4.5	0.51	8.4
45	1560	310	4	7.5	1.3	
70	1920	100	10	5.9	0.42	33
110	972	93	4	4.1	0.27	
125	955	140	4	6.4	0.34	27
144	1101	210	4	9	0.36	
158	1378	240	3	7	0.16	6.8
207	1514	150	3	6.4	1.8	
270	3326	42	4	2.4	1.3	6.2
285	1009	55	5	3	1.9	
313	3271	130	1.5	5	0.75	9.6
346	835	120	4	5.4	0.85	