# Seasonal Monitoring of Microbial Activity Using Conventional Approaches in a Full-Scale Urban Biological Wastewater Treatment Plant

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**Research Article**

**Keywords:** Urban wastewater, Treatment plant, Microbial activity, Seasonal monitoring

**Posted Date:** November 29th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-2272580/v1

# Abstract

Activated sludge processes contains various groups of microorganisms with different metabolic properties, which are responsible for contaminants removal. Therefore, it is important to elucidate the general structure and functional properties of biomass in activated sludge processes. For this purpose, a full-scale domestic biological wastewater treatment plant in Tunceli (Turkey), Tunceli WWTP, was monitored to observe seasonal variations in process performance and biomass properties for a year. It was observed that nitrifying bacteria developed abundantly in the rainy and cool spring season as they were suppressed in summer because their large losses took placed due to an environment containing high alkalinity values. In September, aerobic heterotrophic, nitrify, denitrify, and anaerobic activities increased. It can be said that mature biomass contained young and active levels in an environment in which the sludge volume index (SVI) value increased to 196 mL/g. As a result of the improvement in the structural and functional properties of biomass, the nitrogen removal efficiency reached to 99%. Throughout whole study, the structural improvement observed in biomass reflected in its removal activity. The amount of biomass and removal activity decreased with the abundance of organic matter in the influent at the period in which biomass was closer to being categorized in the aged sludge class. The results showed that as the lowest mixed liquid volatile suspended solids (MLVSS) value of the year was 400 mg/L in November, MLVSS value reached the highest amount (1,400 mg/L) in December which aerobic heterotrophic activity accelerated with a decrease in organic matter level.


# Introduction

Direct discharge of urban wastewater into the environment causes significant risks to aquatic ecosystems and public health (Sibanda et al., 2015). Eutrophication, which develops because of an excess of nutrients, results in the deterioration of the natural balance (Abell et al., 2022). Biological systems are widely used all over the world in the treatment of urban wastewater (Czerwionka et al., 2012), because they produce effective and economical solutions. Also, their simple and sustainable operation brings forth the importance of biological systems (Akter et al., 2022). On the other hand, due to the sensitive nature of aquatic ecosystems and because of increasing social concerns, discharge standards are becoming stricter, therefore, it is necessary to optimize biological processes to produce a high qualified effluent.

Biological treatment processes are facilities that may differ depending on seasonal changes (Kim et al., 2021; Liang et al., 2020; Sun et al., 2021). Influent content is an important factor for the development of microbial activity in biological processes and is also influenced by seasonal conditions. Microbial activity, which determines the success of the treatment, develops in this direction. Therefore, characterizing the influent is very important in elucidating the structural and functional properties of the biological system (Czerwionka et al., 2012; Henze and Comeau, 2008; Wijaya and Soedjono, 2018; Zhang et al., 2020). In biological treatment processes, it is possible to achieve optimum removal at the optimum microorganism growth rate in an appropriate manner (Abel and Evivie, 2022; Subramaniam et al., 2022). Hydraulic retention time, biomass retention time, recycling rate, and aeration amount and time are important operating parameters in the optimization of biological processes (Capodici et al., 2019; Fan et al., 2017). Regulation of these parameters taking into account plant influent content and seasonal changes will significantly contribute to the optimization of a wastewater treatment plant.

The biomass present in biological treatment systems contains a combination of different groups of microorganisms that have different metabolic properties but that also complement each other (Dai et al., 2022). Researches on the contribution of these groups to the removal efficiency of full-scale biological treatment plants are quite limited (Kim et al., 2015). There is an increasing interest in studies in this area, including the observation of seasonal changes (Liang et al., 2020; Sun et al., 2021; Zhang et al., 2019). However, since the recent studies generally require advanced microbial and technological approaches, it is both difficult and quite expensive for the operators to monitor the facility and intervene in emergencies with these techniques. Therefore, it is very important to establish acceptable approaches between process performance and microbial properties, which can be easily applied in a full-scale treatment plant laboratory.

This study aims to examine the synergistic effect of microbial structure on treatment ability in full-scale conventional activated sludge processes with the approaches to be applied easily by operators. In this context, climatic conditions and influent composition were associated with biological behavior of Tunceli WWTP and the biological activities based on the changes observed in the ambient water of the aeration basin were examined and adopted as an approach. In this regard, we also aim to determine useful criteria for evaluating the temporal variation of the ambient water in the aeration basin. It is expected that the results obtained from the study will contribute to the operational optimization of urban biological wastewater treatment plants.

# Materials And Methods

Sewage system

Composition of a raw wastewater varies by depending on the singular and combined system structure of the sewage network (Henze and Comeau, 2008). The existing sewage network located in Tunceli (Turkey) has a combined system structure that carries rain and snow water as well as wastewater during rainy periods. Approximately 99% of domestic wastewater discharged in the city is collected through this network, and industrial wastewater is not taken into the network. Domestic wastewater is conveyed to the biological wastewater treatment plant (Tunceli WWTP) with the help of five pumping stations and eight pumping shafts in the network. At present, the population of the province is 33,000, and the total daily flow to the treatment plant is approximately 5,250 $m^3$.

Facility and operation

The treatment plant is within the boundaries of Tunceli, and the location of the facility is suitable for wastewater transmission. A satellite view of the facility's location can be seen in Fig. 1. There are three carousel-type oxidation ponds designed for carbon, nitrogen, and phosphorus removal. The ponds are operated with an extended aeration process, which provides activated sludge for the removal of biodegradable organic wastes under aerobic conditions. Together, aerobic and anoxic conditions are effective in carousel-type conventional activated sludge processes. Part with mechanical aerators is aerobic side, and long ducts without ventilation are suitable places for the development of anoxic levels (Balku, 2007). Depending on the floc structure, activities of anaerobic levels can also be observed there.

Volume of each aeration basin is 2,426 $m^3$. Hydraulic retention time (HRT) in the basins is approximately 17.4 h, and solid retention time (SRT) is approximately 24.4 days. There are three circular final settling ponds in the facility, and no pre-sedimentation ponds. Each pond has a diameter of 15 m and a volume of 446 $m^3$. HRT in the final settling ponds is approximately 3.1 h. The facility's pre-treatment unit consists of a mechanical screen, a venturi weir, and an aerated oil-sand trap. Then, a chemical coagulation unit is designed for phosphorus removal. With an operational revision, it acts as a kind of area for flow balancing. Even the recycled sludge from the final settling process is first taken into this pond and then sent to the aeration basins. This small-sized pond, which is the first mixed point of the biomass with the influent at the facility, acts as a preliminary biological unit. Excess sludge from the final settling process is dewatered in thickening and filter-press units.

## Sampling

Sampling for the monitoring of the WWTP was carried out every month for one year. The pre-treatment, aeration basin, and treatment plant effluent were chosen as sampling points. Three-point sampling allows periodic monitoring in its simplest form. Observation points can be seen in the treatment plant flow diagram in Fig. 2. The observation point at the entrance of the treatment plant allows one to monitor the pollution load of the facility; the observation point at the exit of the plant allows for monitoring of the biomass settling process. When the data taken from the observation points at the entrance of the facility and at the aeration basin's exit are evaluated together, the results for biological treatment efficiency can be obtained. The facility's influent is also the outlet of the sewage system. Because of this, it is possible to monitor the sewage system from this location.

## Field measurements and laboratory analysis

Temperature, pH, conductivity, oxidation-reduction potential (ORP), and dissolved oxygen (DO) measurements were performed at the wastewater treatment plant with a portable multi-parameter measuring device (YSI Professional Plus). Alkalinity, chemical oxygen demand (COD), orthophosphate ($PO_4^{3-}$), organic nitrogen, ammonia nitrogen ($NH_3$-N), sulfate ($SO_4^{2-}$), nitrite ($NO_2^-$), nitrate ($NO_3^-$), and the necessary solids analyses determining mixed liquid suspended solids (MLSS) with in the mixed liquid volatile suspended solids (MLVSS) were performed in Munzur University's Environmental Engineering Department Laboratory. The volume of settled sludge measurements for calculating the sludge volume index (SVI) values was made on-site at the treatment plant. Samples taken from the treatment plant were transported to the laboratory under cold transport conditions and stored at + 4 ºC. The analyses were started on the same day, completed within 3 days, and made in accordance with standard methods (APHA, 1998).

# Results And Discussion

Evaluation of On-Situ measurements

The temporal variations of On-Situ measurements performed at the treatment plant can be seen in Fig. 3. Temperature change affects significantly microbial activity in activated sludge processes (Kim et al., 2021; Sun et al., 2021; Xue et al., 2019). The temperature values (Fig. 3.a) observed in the treatment plant exhibited significant seasonal changes. They were between 15–20°C in spring and autumn, 20–25°C in summer, and 10–15°C in winter in the aeration basin.

In studies examining the microbial ecology of biological wastewater treatment plants, pH is reported as a strong factor affecting the microbial community structure and distribution (Zhang et al., 2019). The temporal variation of pH values measured at the treatment plant in Tunceli WWTP can be seen in Fig. 3.b. In general, the values measured at the influent, in the aeration basin, and at the effluent have different levels. The seasonal temperature-related conditions such as rainy, dry, and hot, or cold affected the pH values of the influent at the facility. It can be said that the microbial activity in the sewer lines played a part in changes observed in the influent during dry and hot periods. In the changes during the rainy months, the physical-chemical effects resulted from dilution were obvious. Influent pH values gradually decreased close to or in the summer months when precipitation gradually decreased and temperatures increased. The pH values measured in the aeration basin varied between 7.38 and 7.78. With investigating the temporal changes of the values, we observed that the pH values increased gradually towards the summer months. It could be said that heterotrophic activity was the driving force in the seasons. The pH values in influent and aeration basin were similar during certain months of the year and differ during others. In general, the changes were independent of each other. After the final settling process, they generally increased in the effluents. The effect of the rinsing process can also be mentioned as a value increase.

The temporal variation of the conductivity values measured in the treatment plant is given in Fig. 3.c. The values of influent conductivity decreased during rainy spring months as increased in dry summer months. As a result of microbial activities in the facility, conductivity values decreased at varying rates throughout the year. The temporal variation of DO values measured at the treatment plant can be seen in Fig. 3.d. In general, DO values of influent were lower than those of effluent. The seasonal precipitation and temperature factors have a significant effect on influent DO value change. High DO values measured at the effluent may be associated with the rinsing process in the final settling ponds. Therefore, the physical-chemical changes in the supernatant layer of the final settling tank, which is the biomass-liquid phase separation zone, were the primary driving force.

DO values measured throughout the year in the aeration basin operated under controlled conditions ranged from 2.1 to 4.0 mg/L. Adequate ventilation is reported to be a requirement in ensuring optimum development of aerobic nitrifying levels, in addition to aerobic heterotrophic levels. In ventilation, 2.0 mg/L is recommended as the lowest limit (Balku, 2007; Metcalf and Eddy, 2004). However, ventilation in treatment plants constitutes a significant part of energy costs (Capodici et al., 2019). In this respect, it should be carried out on achieving the optimum treatment level with constant ventilation throughout the year. Due to the different DO sensitivities of ammonium oxidizing bacteria and nitrite oxidizing bacteria, varying rates of ventilation strategies can also be considered (Li et al., 2021). The highest values of DO in the aeration basin were measured in the spring months. The values measured during this period were in the range of 2.5–4.0 mg/L. The results showed that rainfall waters feeding the sewage system were significantly effective and the lowest values of DO throughout the year were measured in the range of 2.0–2.7 mg/L during the dry and hot periods. DO values measured in autumn and winter were generally higher than 2.5 mg/L. Transporting of DO to the treatment plant with sewage water is advantageous. The effect of the high DO amount carried to the plant by the influent leads to the success of $NH_3$-N removal observed in the spring months (Kim et al., 2021). However, it is clear as a result of these studies that the effect of temperature dictates the development of the nitrification process during the winter months.

The temporal variation of the ORP values measured at the treatment plant is given in Fig. 3.e. ORP provides perhaps the most detailed data in monitoring oxic–anoxic processes (Eusebi et al., 2016). The aeration basin ORP change was independent of influent. At this point, the effect of aerators in the basin was obvious. Thus, the limits of the value change range were narrowed. In general, the dominant heterotrophic microbial activity in the aeration basin moved in tandem with the development of the positive potential. Various stages of microbial activity can be monitored from the changes in the ORP levels. Instantaneous measurements of the mixed liquid taken from the aeration tank can be expected to represent the ORP values at the time when microbial activities occur. After the biomass-liquid phase separation occurs in the final settling tank, the effect of microbial activities on ambient water is eliminated. Changes in this situation can be monitored in the plant effluent. In July, ORP value in the aeration tank showed a high decrease after final sedimentation.

Evaluation of microbial activity based on process performance

Heterotrophic microorganisms are effective in COD removal in activated sludge processes. Another measure of the activity of these microorganisms is alkalinity production. On the other hand, because autotrophic nitrifying microorganisms use alkalinity, alkalinity removal means functional activity of nitrifiers. In this respect, there is a vital connection between heterotrophic and autotrophic microorganisms in a secondary wastewater treatment process (Metcalf and Eddy, 2004). The observed changes in the relevant parameters in the aeration tank allow us to monitor the activities of these microbial groups. However, nitrogen and phosphorus removal occurs only with the joint activity of both groups. Therefore, the temporal variation of the proportional values of the relevant parameters will be used to determine what the shares of heterotrophs and autotrophs may be during nitrogen and phosphorus removal. The changes observed between the rates in the course of time are correlated one after the other. The difference in each ratio at consecutive times reflects the activity of the unrelated life level.

In order to facilitate the monitoring of autotrophic and heterotrophic activities, $(NH_3\text{-}N)_{removal}/(COD)_{removal}$, and $(NH_3\text{-}N)_{removal}/(Alkalinity)_{removal}$ values for the one-year study period are given in Table 1. In addition, $(O\text{-}phosphate)_{removal}/(COD)_{removal}$, and $(O\text{-}phosphate)_{removal}/(Alkalinity)_{removal}$ values can be seen in Table 1. The changes in metabolic activities based on $NH_3$-N and o-phosphate removal are presented in Table 2, which was created by using the values in Table 1. The values entered in the row for each month in Table 2 reflect the change associated with the previous month. Since March was the starting month, the line for this month was left blank. Values entered in the row for April reflect the observed changes in microbial activity between March and April. Similarly, the values entered in the row for May reflect the changes of microbial activity observed between April and May. Successive associations are maintained in this way. The (+) and (–) values in the Table 2 showed that the relevant metabolic activity increased and decreased, respectively.

Table 1
$(NH_3\text{-}N)_{removal}/(COD)_{removal}$ and $(NH_3\text{-}N)_{removal}/(Alkalinity)_{removal}$ and $(O\text{-}phosphate)_{removal}/(COD)_{removal}$, and $(O\text{-}phosphate)_{removal}/(Alkalinity)_{removal}$ of the working months values

| | $(NH_3\text{-}N)_{rem}/(COD)_{rem}$ | $(NH_3\text{-}N)_{rem}/(Alk)_{rem}$ | $(O\text{-}phosphate)_{rem}/(COD)_{rem}$ | $(O\text{-}phosphate)_{rem}/(Alk)_{rem}$ |
|---|---|---|---|---|
| March | 15% | 16% | 20% | 20% |
| April | 13% | 22% | 10% | 18% |
| May | 20% | 17% | 21% | 17% |
| June | 18% | 33% | 21% | 39% |
| July | 11% | 56% | 15% | 80% |
| August | 6% | 23% | 31% | 117% |
| September | 28% | 29% | 28% | 29% |
| October | 14% | 25% | 16% | 27% |
| November | 4% | 29% | 17% | 119% |
| December | 10% | 44% | 14% | 64% |
| January | 3% | 22% | 26% | 179% |
| February | 2% | 8% | 13% | 48% |

Table 2
Changes observed in monthly $NH_3$-N and o-phosphate removals of heterotrophic and nitrifying bacteria

| | **$NH_3$-N Removal** | | **O-phosphate Removal** | |
|---|---|---|---|---|
| | Heterotrophic | Nitrifying | Heterotrophic | Nitrifying |
| March | - | - | - | - |
| April | + 6 | − 2 | − 2 | − 10 |
| May | − 5 | + 7 | − 1 | + 11 |
| June | + 16 | − 2 | + 22 | constant |
| July | + 23 | − 7 | + 41 | − 6 |
| August | − 33 | − 5 | + 37 | + 16 |
| September | + 6 | + 22 | − 88 | − 3 |
| October | − 4 | − 14 | − 2 | − 12 |
| November | + 4 | − 10 | + 92 | + 1 |
| December | + 15 | + 6 | − 55 | − 3 |
| January | − 22 | − 7 | + 115 | + 12 |
| February | − 14 | − 1 | − 131 | − 13 |

As seen in Table 1, $(NH_3\text{-N})_{removal}/(\text{Alkalinity})_{removal}$ values in March and April were 16% and 22%, respectively. As generally known, alkalinity removal is associated with nitrifying metabolism. In nitrifying metabolisms, $NH_3$-N will be removed at approximately same amount. Therefore, the difference between values reflects the heterotroph $NH_3$-N utilization. Accordingly, the results show that the heterotroph $NH_3$-N removal increased in April. The $(NH_3\text{-N})_{removal}/(\text{COD})_{removal}$ values for March and April were 15% and 13%, respectively. In heterotrophic metabolism, where a similar amount of COD is removed, approximately the same amount of $NH_3$-N will be removed. Therefore, the difference between $NH_3$-N removal values reflects activity changes of autotrophic nitrifying bacteria. For example, the decrease in $NH_3$-N removal in April is due to slowing activity of autotrophic nitrifying bacteria.

The temporal variation of $NH_3$-N and orthophosphate removal values for the study year can be seen in Fig. 4. Microbial activity has a wide range of $NH_3$-N removal levels throughout the year. The removal observed in April was 98%, one of the highest annual values. In terms of time period, the highest annual $NH_3$-N removal levels were observed during spring. At this point, the suitability of environmental conditions such as temperature and DO can be brought to the fore (Kim et al., 2021).

With considering the evaluations for o-phosphate removal in Table 2, it could be seen that o-phosphate consumption decreased based on heterotrophic and nitrified activities in April. O-phosphate removal levels varied between 91–97% throughout the study year. Microbial activity was balanced by high and narrowly varying levels of phosphorus removal. In other words, the biological phosphorus requirement remained high throughout the year. In this regard, phosphorus limitation is of upmost importance in plant operation. The decrease in the amount of influent o-phosphate in April was an important factor in the loss of removal.

The temporal variation of COD and alkalinity removal values is given in Fig. 5. The COD and alkalinity amounts determined in the influent and aeration tank during the whole year are given in Table 3. In terms of the time period, alkalinity removal associated with nitrifying activities has the highest values during the spring months. In March and April, COD removal was 95%, which was in line with the organic carbon requirement of heterotrophic metabolism. In May, removal decreased to 85%. In March and April, the MLVSS values in aeration tank were at a level of 800 mg/L as 570 mg/L in May. In activated sludge processes, aerobic heterotrophic microorganisms, which have rapid growth kinetics and constitute an important part of microbial density, play a role in high-level biomass amount changes (Metcalf and Eddy, 2004; Van Loosdrecht and Jetten, 1998). Nitrifying bacteria, autotroph microorganism, was characterized by its low growth rate, and therefore, low biomass production. In urban wastewater treatment plants, nitrifiers constitute approximately 5.3– 11.5% of total bacteria (Xia et al., 2018).

The SVI reached its highest value in March during the year, above the 250 mL/g, and the biomass was in the young sludge class. In April, the value decreased to 170 mL/g. It was observed that the biomass matured from March to April without a loss of mass. This was reflected in the nitrogen removal success in April. In parallel with the decrease in the amount of biomass in May, the SVI value also decreased to 130 mL/g, and it is understood that active mature elements have been removed from the structure. The temporal variation of MLVSS, SVI, and F/M values can be seen in Fig. 6.

Table 3
COD and alkalinity amounts present and removed during the treatment process

| | COD Values (mg/L) | | | Alkalinity Values (mg/L) | | |
|---|---|---|---|---|---|---|
| | Plant Influent | Removal | Aeration Basin | Plant Influent | Removal | Aeration Basin |
| March | 200 | 190 | 10 | 485 | 185 | 300 |
| April | 234 | 223 | 11 | 420 | 130 | 290 |
| May | 156 | 132 | 24 | 445 | 160 | 285 |
| June | 114 | 109 | 5 | 430 | 60 | 370 |
| July | 245 | 241 | 4 | 470 | 45 | 425 |
| August | 263 | 243 | 20 | 485 | 65 | 420 |
| September | 130 | 125 | 5 | 480 | 120 | 360 |
| October | 250 | 210 | 40 | 470 | 120 | 350 |
| November | 260 | 210 | 50 | 470 | 30 | 440 |
| December | 190 | 180 | 10 | 460 | 40 | 420 |
| January | 180 | 140 | 40 | 460 | 20 | 440 |
| February | 230 | 220 | 10 | 420 | 60 | 360 |

In the dry and hot period, which includes June, July, and August, alkalinity removal decreased significantly. The alkalinity removal observed in the summer months varied between 10–15%. In this respect, the significant change observed in alkalinity removal in June made it important within the framework of microbial status change. Following summer months, when heterotrophic activities increase, ambient water has higher alkalinity values. Considering seasonal changes, the high alkalinity values of the sewage environment water, namely, influent, can be emphasized here. In general, it could be said that the sewage ambient water was useful for the heterotrophic activities of the treatment plant during this period. The first significant increase in the amount of solids precipitated in the influent occurred in June. Biological flocs constituted a significant part of the precipitated solids. The flocs observed in the influent were an indicator of biological life in the sewer lines.

Although the amount of organic matter carried to the facility by influent decreased in June, the rate of COD removal was high in this month. There was a slight increase in the MLVSS and SVI values. Importantly, $NH_3$-N removal was considerably reduced in June. The removal, which was 86% in May, decreased to 57% in June. In the following summer months, $NH_3$-N removal remained approximately at the same level. In this regard, it appeared that microbial life was categorized in June to a status characterized by lower consumption of $NH_3$-N. The amount of $NH_3$-N measured at the outlet of the aeration basin increased periodically during the summer months. In this respect, it can be said that nitrifying life has incomplete nitrification processes.

In July, the amount and the rate of COD removal increased at the same time. During this month, the MLVSS value raised and reached 870 mg/L. The SVI value also increased to 170 mL/g. There was an increase in the amount of structurally improving biomass. It can be said that the microbial growth and structural improvement observed in July were related to the high rate of organic matter removal. In other words, there was rapid metabolic activity in the density of active and mature aerobic heterotrophic microorganisms. The rate of COD removal decreased in August, and the MLVSS value was 634 mg/L in this month. The F/M value was at a level of 41%. In this environment, where nutrient and organic matter abundance was available, microbial life reduced in mass. The SVI value decreased to 114 mL/g in August, and the biomass approached the aged sludge class. In general, F/M values gradually increased from the beginning to the end of the period.

Nitrification is the rate-limiting process in biological nitrogen removal systems (Kim et al., 2015). Nitrite is the first–step conversion product in the nitrification process. The conversion takes place by oxidation of $NH_4$-N to $NO_2$-N (Czerwionka et al., 2012). Figure 7.a shows the temporal variation of nitrite values of the facility influent and aeration basin. In general, the nitrite values of the treatment plant influent are smaller than the nitrite values of the aeration basin. Based on the nitrite values of the influent and aeration basin, nitrite production was observed in the ambient water of the aeration basin. Nitrite values gradually increased in certain periods, decreased in certain periods, and remained stable in certain periods in the aeration basin environment during the year. Nitrate is the second step conversion product in the nitrification process. The conversion takes place by oxidation of $NO_2$-N to $NO_3$-N (Czerwionka et al., 2012). Figure 7.b shows the temporal variation of nitrate values of the facility influent and aeration basin. As seen in Fig. 7.b, the nitrate values of the influent during the working year were higher than the nitrate values of the aeration basin, contrary to expectation. Therefore, we thought that the microbial activity in the sewage system realized under uncontrolled conditions. It has been observed that hydrolysis processes were the main mechanisms with high nitrate levels in sewer lines (Henze and Comeau, 2008; Metcalf and Eddy, 2004). In addition to the high level of influent nitrate values, the low level of nitrate values in the facility indicates intense denitrification activities. Besides, the low level of nitrification second step processes is another of the main reasons.

The temporal variation of $(NO_3)_{removal}/(COD)_{removal}$ and $(SO_4)_{removal}/(COD)_{removal}$ values can be seen in Fig. 8. It enables us to monitor the activities of denitrified and anaerobic metabolism within microbial association. The observed temporal variation indicated that the denitrified levels exhibited activity that can be characterized by certain periods. It is noteworthy that denitrified activities were low in the spring season, but the denitrifying activities observed in the following periods were high. Significant increases in activity over periods can be noted as a characteristic feature of the biological system. In the spring season, April exhibited such a feature.

During the working year, the influent sulfate values were higher than the values in the aeration basin. Influent sulfate concentration values were remarkable higher when anaerobic conditions prevailed in the sewer lines than values of the aeration basin in the dry-hot months. This can be explained by the hydrolysis processes present in sewer lines (Metcalf and Eddy, 2004). The significant decrease of the influent sulfate concentration values in the ambient water of the aeration basin, where aerobic conditions prevail, can be explained by the association of life in the same floc structure. This can be seen as a trace of anaerobic microbial life within the floc structure.

The lowest sulfate removal was observed in April during the operational year. Although aerobic heterotrophic activities developed during this month, nitrifying activities slightly decreased and denitrifying activities increased. As a measure of denitrifying activity development, nitrate removal was high in April. Remarkably, in this environment, sulfate removal was at the lowest level of the year. Sulfate removal increased in May. During this month, when the growth rate of aerobic heterotrophs slowed down and the growth rate of nitrifiers increased, the activities of denitrified levels decreased. In this environment, where nitrate–reducing metabolism slowed down, sulfate-reducing metabolism accelerated.

At the end of the dry and hot period, MLVSS value in September is very close to the August value, meaning that no biomass increase was observed. On the other hand, the COD removal reached 96%. In this month, although the efficiency of COD removal increased, the amount of removed COD decreased due to the decreasing organic matter concentration of the influent. In this environment, where the abundance of organic matter disappeared, the SVI value increased to 196 mL/g. It could be said that biomass included active young microorganisms in addition to mature. In September, alkalinity removal was 25%. After the summer months, the activities of the nitrification processes in line with the alkalinity requirement became visible again. In this respect, September, which is considered as the end of the dry and hot period, differed from other summer months. While the alkalinity value of the ambient water of the aeration basin dropped dramatically from 420 mg/L in August to 360 mg/L in September. This decrease indicates a change in microbial status. The suitability of the environmental conditions belonging to the transition month between seasons became effective in the observed changes.

In September, $NH_3$-N removal reached the highest value of the year with 99%. This indicates the conditions in which the metabolic activities of nitrifying bacteria are highest. However, the nitrite value showed that the ammonium-oxidizing bacteria were dominant and a complete nitrification did not occur in September and in the following months. On the other hand, it was understood that the most intense denitrification and sulfate reduction activity of the working year were observed in this month, when considering the influent levels of nitrate and sulfate.

The alkalinity removal in October was quite similar to removal in September. Although nitrogen removal decreased, it is considered to be at a good level. September and October stand apart from other months in terms of their removal levels. Microbial life rearranged in September was a certain size and growth rate in October. In October, the MLVSS value increased to 740 mg/L. In this month, when organic matter abundance was noticeable, the SVI value decreased to 154 mL/g. In October, while the amount of COD carried to the facility by influent increased, the rate of removal decreased.

In November, the alkalinity value of the aeration basin ambient water increased. In this environment, alkalinity removal associated with nitrifying levels was significantly reduced. In the dominant habitat of heterotrophic levels, nitrogen removal was severely disrupted. The amount of COD transported to the facility with the influent in November and removed in the aeration basin was similar to the amount observed in October. The removal rate continued to decrease. In this month, the F/M value of 64% was the highest one of the year. The MLVSS value was 400 mg/L, the lowest value of the year. These data showed that active mass continued its vital activities by shrinking in an environment in which the abundance of organic matter growth continued from October to November.

The SVI value in November decreased slightly to 114 mL/g. The structural and functional changes observed in November were similar to those found in August. In both months, denitrifying activities increased at a high level in the mass, which decreased in food abundance. As of November, the nitrite amounts observed in the ambient water were the lowest values of the working year. At this juncture, the negative effect of the decrease in ambient water temperatures on nitrified life was observed (Sun et al., 2021).

The alkalinity removal observed in December, January, and February was the lowest of the year. In winter, the alkalinity removal associated with nitrifying activities varied between 5–15%. Aeration basin ambient water alkalinity values in December and January were 420 mg/L and 440 mg/L, respectively. In February, the alkalinity value of the microbial ambient water of the aeration basin was 360 mg/L. In December, $NH_3$-N oxidation increased to 39%. The removal levels for January and February were similar. $NH_3$-N removal levels for these months were 18% and 19%, respectively, which were the lowest values of the year. In winter, $NH_3$-N removal was in the 15–40% range. Similarly, Kim et al. (2021) also showed that the effect of the observed decrease in temperatures on nitrifying life was quite evident.

In December, the amount of COD carried to the facility by influent decreased. However, the rate of COD removal increased. In this month, the MLVSS was at its highest value in the working year at 1,400 mg/L. In December, the F/M value decreased sharply to 14%. The lowest value of the year was found in this month. The SVI value increased to 150 mL/g. In an environment where organic matter abundance decreased and biomass structurally improved, aerobic heterotrophic growth increased significantly. Similar situations were observed in June and September. Thus, this case could be noted as a characteristic feature of the biological system. In December, the amount of nitrogen carried to the plant by influent increased. The amount of nitrogen carried to the plant by influent was also high in July, when a significant increase in the MLVSS value was observed. In, the change in the proportional values of the o-phosphate and $NH_3$-N amounts measured in the ambient water of the aeration basin throughout the year is given in Fig. 9. The ratios of o-phosphate/$NH_3$-N in July and December, when aerobic heterotrophic activities increased, were close to each other. It was important to note that, the nitrogen removal activity increased at peaks of the ratios in April and September.

In January and February, the $NH_3$-N removal associated with heterotrophic microorganisms gradually decreased. $NH_3$-N oxidation for both months decreased remarkably. It was observed that phosphorus removal associated with heterotrophic levels increased at a high level in January and decreased at a high level in February. The high increase in phosphorus removal in January, when aerobic heterotrophic activities slowed down, indicated intense denitrifying activities. Similarly, the significant decrease in phosphorus removal in February, when aerobic heterotroph growth slowed down, indicated that the density of denitrifying bacteria decreased significantly. The increase and decrease of denitrifying activity in January and February, respectively, can also be seen in metabolic activities based on nitrate reduction. In these environments, metabolic activities based on sulfate reduction also increase and decrease, respectively.

Phosphorus removal associated with nitrifying levels greatly increased in January and nitrogen removal associated with nitrifying levels decreased significantly during this month. Nitrified life increased a decrease in heterotrophic activity in January. However, the significant decrease in nitrifying nitrogen consumption in this environment indicated a low efficiency of the growing mass. Phosphorus removal associated with nitrifying levels declined significantly in February. The decrease in removal may be associated with the decrease in the amount of phosphorus carried to the plant by influent during this month. There was a slight decrease in nitrified nitrogen consumption in February. In January, the SVI value decreased and was at its lowest during the year. The value was 80 mL/g, and the biomass was in the aged sludge class. In February, the value increased and approached 150 mL/g.

During the winter months, nitrifying activities decreased significantly. Denitrified activities continued to exist at a certain level. The level during winter was higher than in July and August. The existence of denitrified activities at a certain level can be explained by the low sensitivity of these levels to cold weather. In addition, nitrate fed into the plant by influent was an important source for denitrifying activities. As in other seasons, in this period, when the weather turned cold, biomass was characterized by certain structural and functional properties under the influence of climatic environmental conditions.

# Conclusions

Sewage ambient water showed significant changes depending on rainy-dry and hot-cold seasonal conditions. The observed effect of dilution was high during the rainy periods in spring. In this period, dissolved oxygen carried to the facility by precipitation water positively affected microbial activities. In addition, the positive effect of seasonal temperature values was important. Nitrogen removal increased during this period, when seasonal conditions were suitable for microbial life. It was disadvantageous when phosphorus restriction was perceived in the influent due to the dilution of precipitation waters. In an environment in which biomass reduced and rearranged, such as what occurred in May, when precipitation decreased, the structuring of nitrified levels was observed.

As a result of the studies carried out, seasonal changes come to the fore in the planning of treatment plant operating arrangements. In line with the dominant activities of certain microorganism groups in different seasons, the ambient water properties of the aeration basin are regulated. In this respect, the future operating arrangements necessary in months marked by seasonal transition characteristics will be crucial in terms of treatment success. It is important to note that nitrifying activity has a very important place in the success of nitrogen removal. The spring season, which has a generic effect on microbial life, is essential in the development of nitrifying levels—the highest efficiency in nitrogen removal was observed during spring. In summer, as the weather warms, heterotrophic activity becomes more dominant, and nitrifying activity is limited in this environment. In the winter months, the nitrifying activity is significantly interrupted by the decrease in temperature. This seasonal effect is perhaps one of the most important constraints of biological wastewater treatment plants that operate for nitrogen removal. In winter, considering the predominant activities of aerobic and facultative heterotrophs, operational arrangements can increase the nitrogen removal efficiency of these levels. During this period, when the DO level of the aeration basin is high, low and varying ventilation strategies can be emphasized. Studies on SRT and sludge removal will positively support the development of nitrified levels in addition to heterotrophic levels.

Microbial growth phases can be clearly traced through the structural and functional properties of biomass. The critical point in facility treatment optimization is the operating arrangements made in these phases. It was observed that there is a close relationship between the structural development of biomass and its metabolic activity. Over time and depending on the organic matter and nutrient content of the influent, structural improvement and deterioration observed in the biomass are common. In general, microbial metabolic activity is accelerated by decreasing the amount of organic matter carried by the influent. In this environment, in which the amount of biomass increases, mature and active microorganisms increase the success of nitrogen removal. When organic matter abundance is observed, microbial life decreases in mass. In these environments, where the rate of metabolic activity slows down, the biomass appears to have structurally recovered. Similar changes observed in different months can be noted as characteristic features of the biological system. Based on the observed changes, future arrangements made that focus on the recycling and sludge removal rate within the framework of SRT will be effective in maintaining the efficiency of the mature and active biomass. In addition, the changes in the DO requirement at different phases of microbial growth should be carefully considered.

Within the scope of European Union Urban Wastewater Treatment Directive (Directive 91/271/EEC, 1991), pH, COD, and total suspended solids effluent values constituted the discharge standards for all months. While the total nitrogen effluent values meet the discharge standards in March, April, May, and September, the effluent values exceeded the discharge standards in the remaining months. In this regard, operating regulations for nitrogen removal was of primary importance. As a result of the current study, the coagulation unit, which is located immediately after the entrance unit and designed for phosphorus removal but not used for this purpose, can be considered for use as a phosphorus dosing unit. Thus, nitrogen removal efficiency could be increased in this manner in periods when phosphorus was required.

# Declarations

**Acknowledgements** Thanks to the directors of Tunceli Municipality for allowing the on-site studies at the urban biological wastewater treatment plant, and Munzur University, where the laboratory studies were carried out, and special thanks to Prof. Dr. Halil Hasar and reviewers for their useful and constructive criticism.

**Author Contributions Statement** Deniz Demirbilek: investigation, assessment, methodology, data processing, and original draft preparation. Ubeyde İpek: methodology, data interpretation, and supervision. Ülkü Yetiş: methodology, data interpretation, and conceptualization. All authors have read and agreed to the published version of the manuscript.

**Funding** This work has not been funded by any institution or organization.

**Data availability statement** All of the material in this work is owned by the authors and no permissions are required.

**Competing Interest Statement** The authors declare no competing interests.

# References

1. Abel, E.S., & Evivie, S.E. (2022). Effect of varying environmental conditions on the growth and viability of selected microorganisms using conventional cultures. Journal of Applied Sciences and Environmental Management, 26(3), 393–397. https://dx.doi.org/10.4314/jasem.v26i3.4
2. Abell, J.M., Özkundakci, D., Hamilton, D.P., & Reeves, P. (2022). Restoring shallow lakes impaired by eutrophication: Approaches, outcomes, and challenges. Critical Reviews in Environmental Science and Technology, 52(7), 1199–1246. https://doi.org/10.1080/10643389.2020.1854564
3. Akter, S., Suhan, M.B.K., & Islam, M.S. (2022). Recent advances and perspective of electrocoagulation in the treatment of wastewater: A review. Environmental Nanotechnology, Monitoring & Management, 17, 100643. https://doi.org/10.1016/j.enmm.2022.100643
4. APHA, (1998). *Standard methods for the examination of water and wastewater*. 20th ed., American Public Health Association, Washington, D.C.
5. Balku, S. (2007). Comparison between alternating aerobic–anoxic and conventional activated sludge system. Water Research, 41, 2220–2228. doi: 10.1016/j.watres.2007.01.046
6. Capodici, M., Corsino, S.F., Trapani, D.D., Torregrossa, M., & Viviani, G. (2019). Effect of biomass features on oxygen transfer in conventional activated sludge and membrane bioreactor systems. Journal of Cleaner Production, 240, 118071. https://doi.org/10.1016/j.jclepro.2019.118071
7. Czerwionka, K., Makinia, J., Pagilla, K.R., & Stensel, H.D. (2012). Characteristics and fate of organic nitrogen in municipal biological nutrient removal wastewater treatment plants. Water Research, 46, 2057–2066. https://doi.org/10.1016/j.watres.2012.01.020
8. Dai, H., Sun, Y., Wan, D., Abbasi, H.N., Guo, Z., Geng, H., Wang, X., & Chen, Y. (2022). Simultaneous denitrification and phosphorus removal: A review on the functional strains and activated sludge

processes. Science of the Total Environment, 835, 155409. https://doi.org/10.1016/j.scitotenv.2022.155409

9. Directive 91/271/EEC, (1991). *Concerning urban waste water treatment*. Official Journal of the European Communitie, No: L 135/40.
10. Eusebi, A.L., Panigutti, M., & Battistoni, P. (2016). Reduction of biological sludge production applying an alternating oxic/anoxic process in water line. *Water Environment Research*, 88, 483–489. doi:10.2175/106143015X14212658614432
11. Fan, H., Liu, X., Wang, H., Han, Y., Qi, L., & Wang, H. (2017). Oxygen transfer dynamics and activated sludge floc structure under different sludge retention times at low dissolved oxygen concentrations. Chemosphere, 169, 586–595. https://doi.org/10.1016/j.chemosphere.2016.10.137
12. Henze, M., & Comeau, Y. (2008). Wastewater characterization. In: Henze, M., Van Loosdrecht, M.C.M., Ekama, G.A. and Brdjanovic, D. (Eds.), *Biological wastewater treatment: principles modelling and design*. ISBN: 9781843391883. IWA publishing, London, UK.
13. Kim, J.O., Cho, K.H., Ligaray, M., Jang, H.M., Kang, S., & Kim, Y.M. (2015). Monitoring influential environmental conditions affecting communities of denitrifying and nitrifying bacteria in a combined anoxic-oxic activated sludge system. International Biodeterioration & Biodegradation, 100, 1–6. https://doi.org/10.1016/j.ibiod.2015.01.016
14. Kim, T., Hite, M., Rogacki, L., Sealock, A.W., Sprouse, G., Novak, P.J., & LaPara, T.M. (2021). Dissolved oxygen concentrations affect the function but not the relative abundance of nitrifying bacterial populations in full-scale municipal wastewater treatment bioreactors during cold weather. Science of the Total Environment, 781, 146719. https://doi.org/10.1016/j.scitotenv.2021.146719
15. Li, S., Li, J., Yang, S., Zhang, Q., Li, X., Zhang, L., & Peng, Y. (2021). Rapid achieving partial nitrification in domestic wastewater: Controlling aeration time to selectively enrich ammonium oxidizing bacteria (AOB) after simultaneously eliminating AOB and nitrite oxidizing bacteria (NOB). Bioresource Technology, 328, 124810. https://doi.org/10.1016/j.biortech.2021.124810
16. Liang, H., Luo, X., Ye, D., Yan, J., & Luo, L. (2020). Seasonal succession of bacterial communities, metabolic potential and ecological functions in soy sauce wastewater treatment system. International Biodeterioration & Biodegradation, 154, 105083. https://doi.org/10.1016/j.ibiod.2020.105083
17. Metcalf & Eddy, (2004). *Wastewater engineering: Treatment and reuse*. Fourth Edition, McGraw-Hill, New York.
18. Sibanda, T., Selvarajan, R., & Tekere, M. (2015). Urban effluent discharges as causes of public and environmental health concerns in South Africa’s aquatic milieu. Environmental Science and Pollution Research, 22(23), 18301–18317. doi: 10.1007/s11356-015-5416-4
19. Subramaniam, L.S., Lutpi, N.A., Wong, Y.S., Ong, S.A., Rahmat, N.R., & Siripatana, C. (2022). Synergistic effect between iron and food/microorganism (F/M) ratio in biological wastewater treatment. In *Proceedings of the 3rd International Conference on Green Environmental Engineering and Technology* (pp. 455–460). Springer, Singapore.

20. Sun, C., Zhang, B., Ning, D., Zhang, Y., Dai, T., Wu, L., Li, T., Liu, W., Zhou, J., & Wen, X. (2021). Seasonal dynamics of the microbial community in two full-scale wastewater treatment plants: Diversity, composition, phylogenetic group based assembly and co-occurrence pattern. Water Research, 200, 117295. https://doi.org/10.1016/j.watres.2021.117295
21. Van Loosdrecht, M.C.M., & Jetten, M.S.M. (1998). Microbiological conversions in nitrogen removal. Wat. Sci. Tech. 38, 1–7. https://doi.org/10.1016/S0273-1223(98)00383-7
22. Wijaya, I.M.W., & Soedjono, E.S. (2018). Physicochemical characteristic of municipal wastewater in tropical area: Case study of Surabaya City, Indonesia. Earth and Environmental Science, 135. doi:10.1088/1755-1315/135/1/012018
23. Xia, Y., Wen, X., Zhang, B., & Yang, Y. (2018). Diversity and assembly patterns of activated sludge microbial communities: A review. Biotechnology Advances, 36, 1038–1047. https://doi.org/10.1016/j.biotechadv.2018.03.005
24. Xue, J., Schmitz, B.W., Caton, K., Zhang, B., Zabaleta, J., Garai, J., Taylor, C.M., Romanchishina, T., Gerba, C.P., Pepper, I.L., & Sherchan, S.P. (2019). Assessing the spatial and temporal variability of bacterial communities in two Bardenpho wastewater treatment systems via Illumina Miseq sequencing. Science of the Total Environment, 657, 1543–1552. https://doi.org/10.1016/j.scitotenv.2018.12.141
25. Zhang, L., Shen, Z., Fang, W., & Gao, G. (2019). Composition of bacterial communities in municipal wastewater treatment plant. Science of the Total Environment, 689, 1181–1191. https://doi.org/10.1016/j.scitotenv.2019.06.432
26. Zhang, B., Ning, D., Yang, Y., Van Nostrand, J.D., Zhou, J., & Wen, X. (2020). Biodegradability of wastewater determines microbial assembly mechanisms in full-scale wastewater treatment plants. Water Research, 169, 115276. https://doi.org/10.1016/j.watres.2019.115276

# Figures


## Figure 1

Urban wastewater treatment plant satellite view

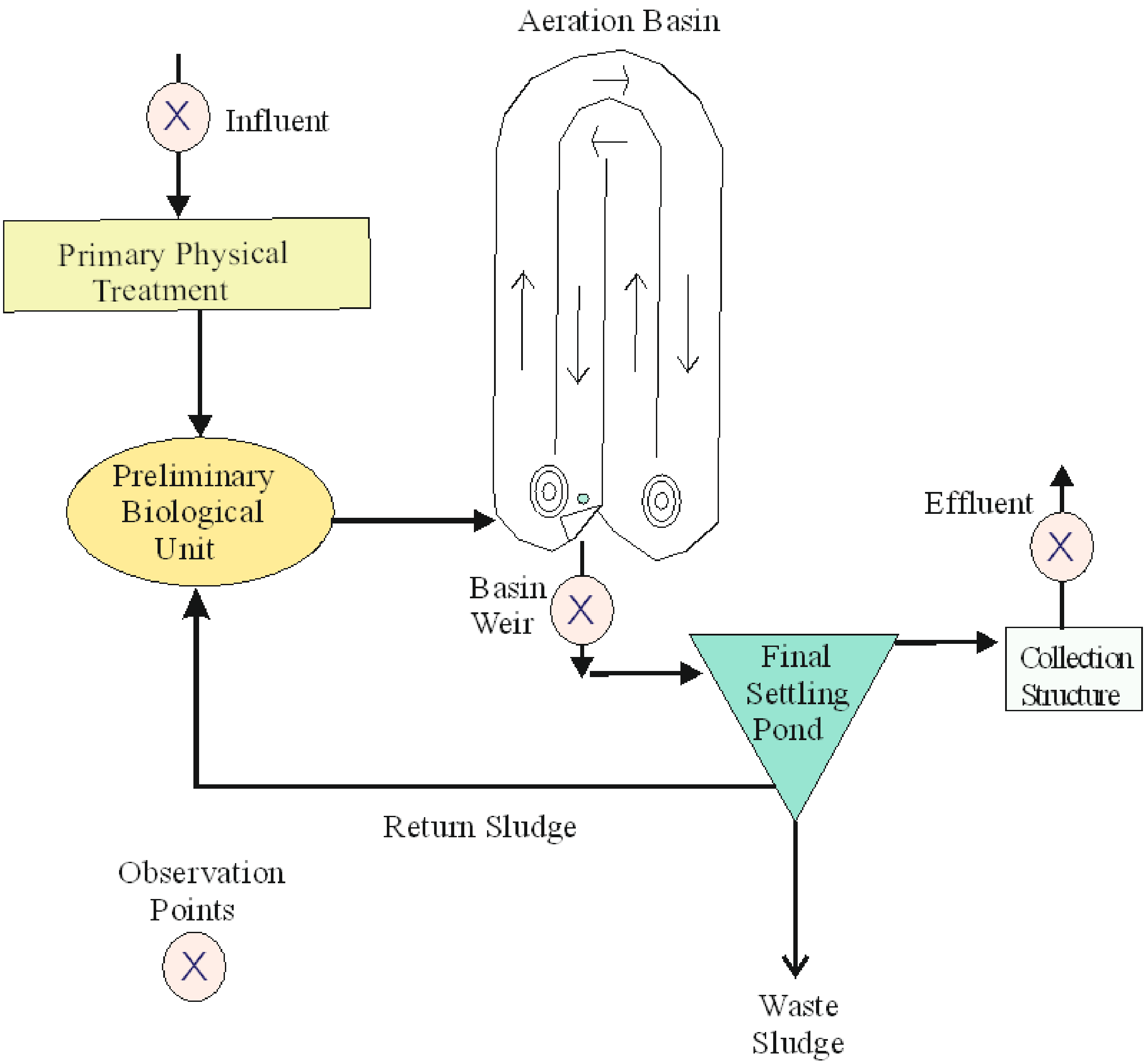
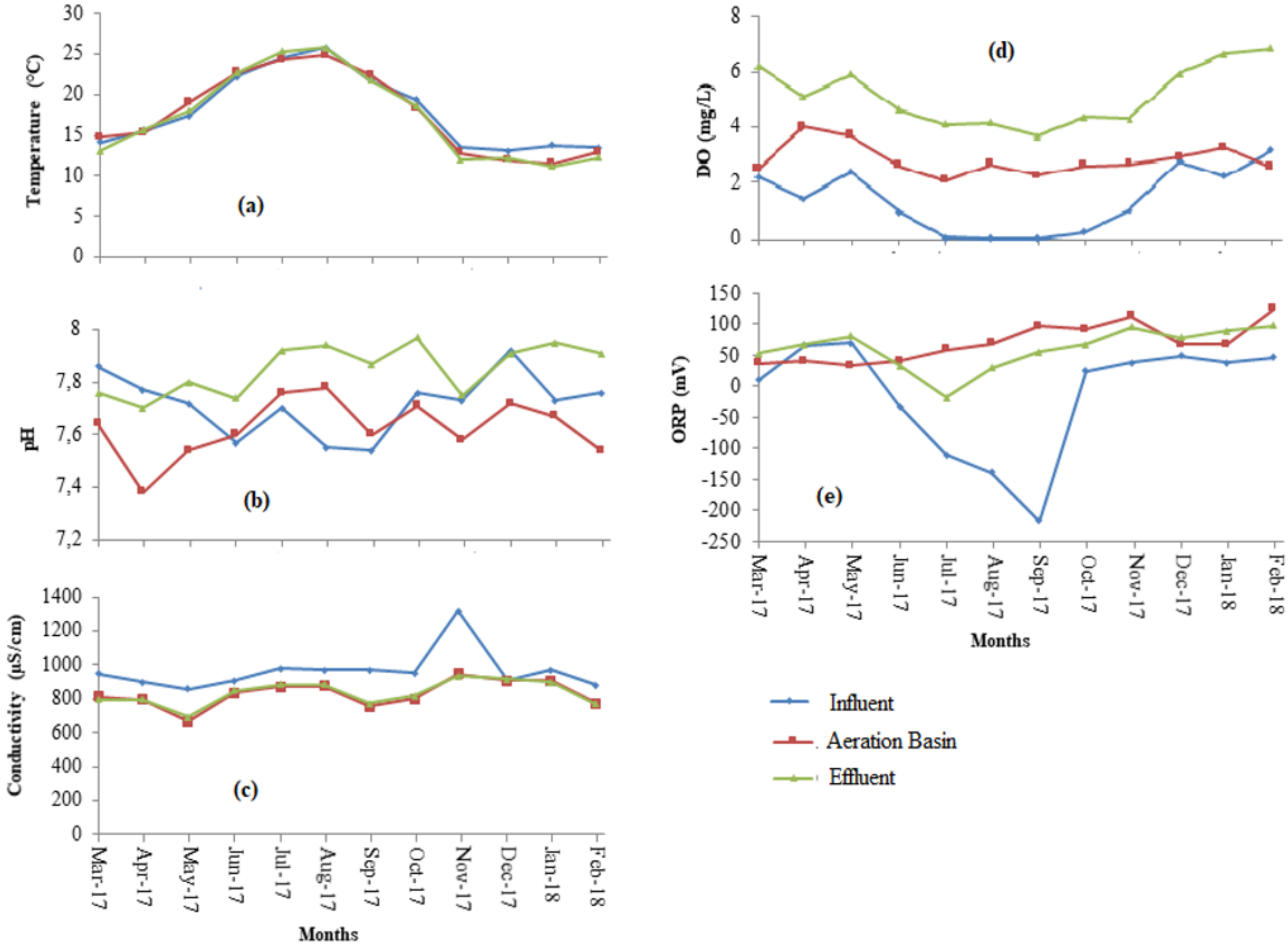



**Figure 2**

Observation points for monthly periodic monitoring studies




## Figure 3

On-Situ measurements in WWTP. (a) Temperature, (b) pH, (c) Conductivity, (d) DO, (e) ORP

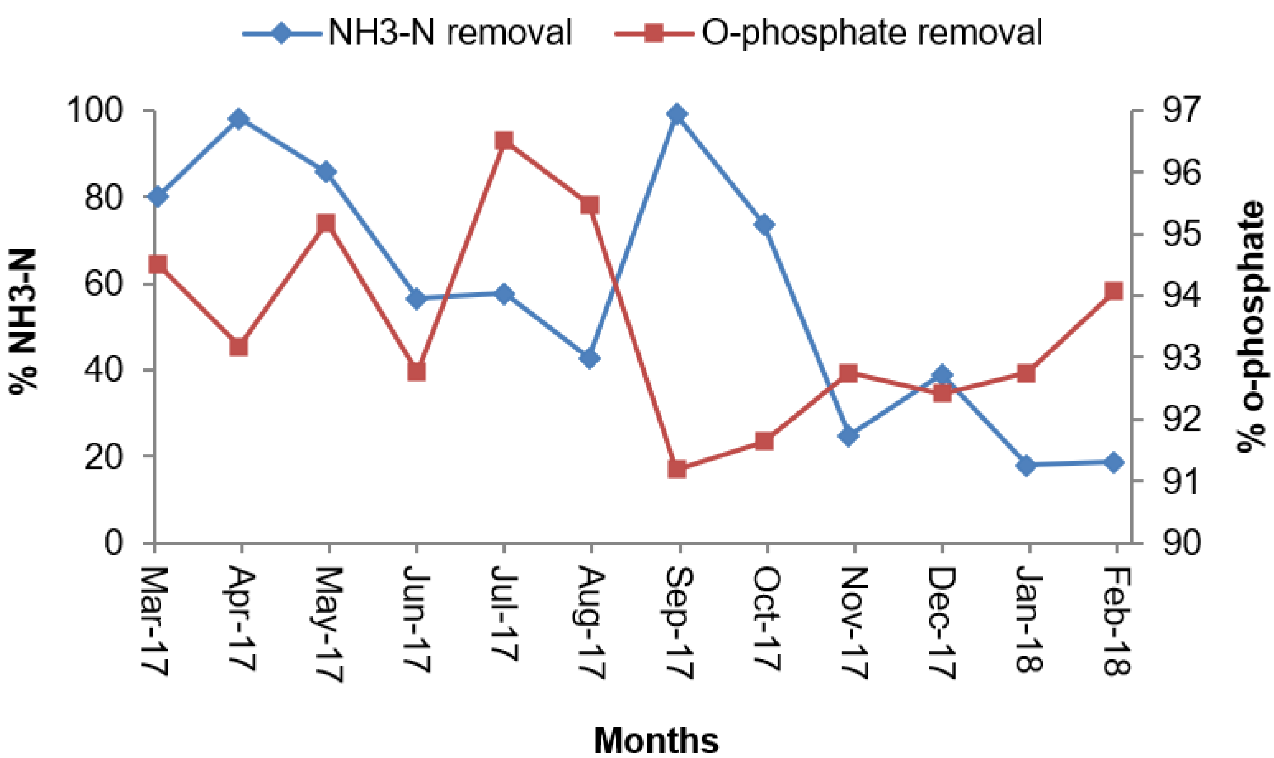



**Figure 4**

Temporal variation of $NH_3$-N and o-phosphate removal values

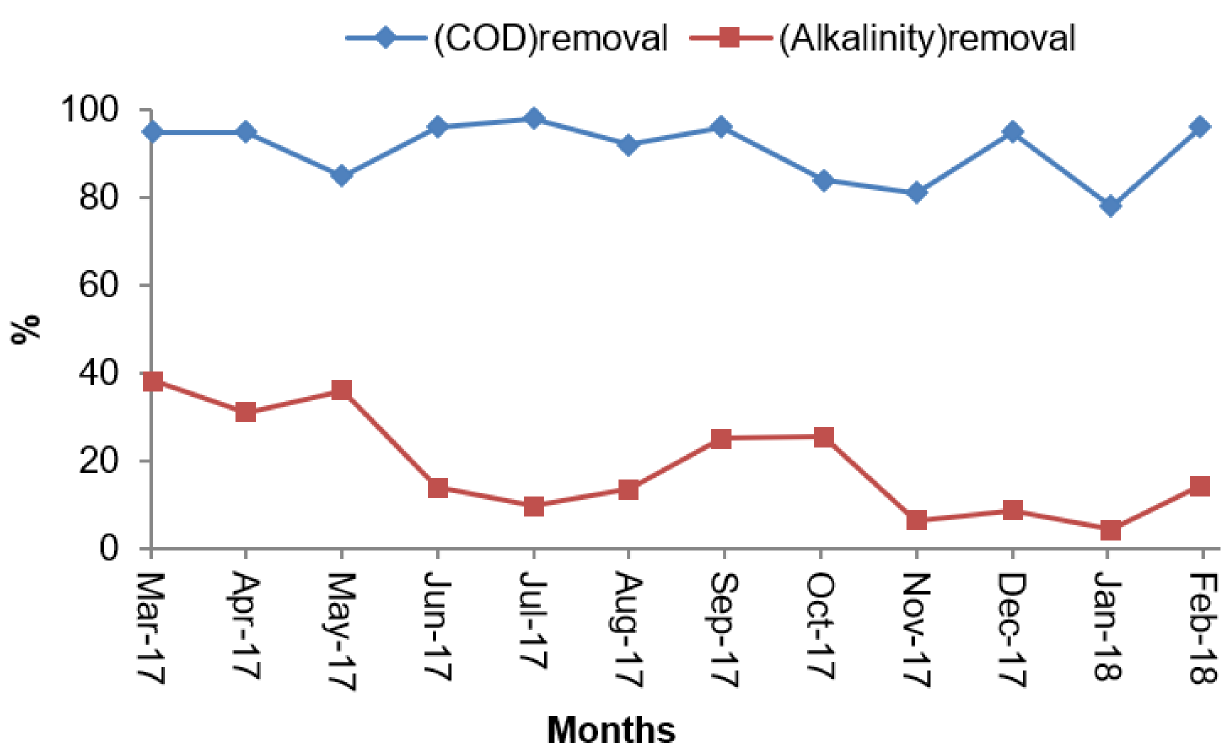



**Figure 5**

Temporal variation of COD and alkalinity removal values

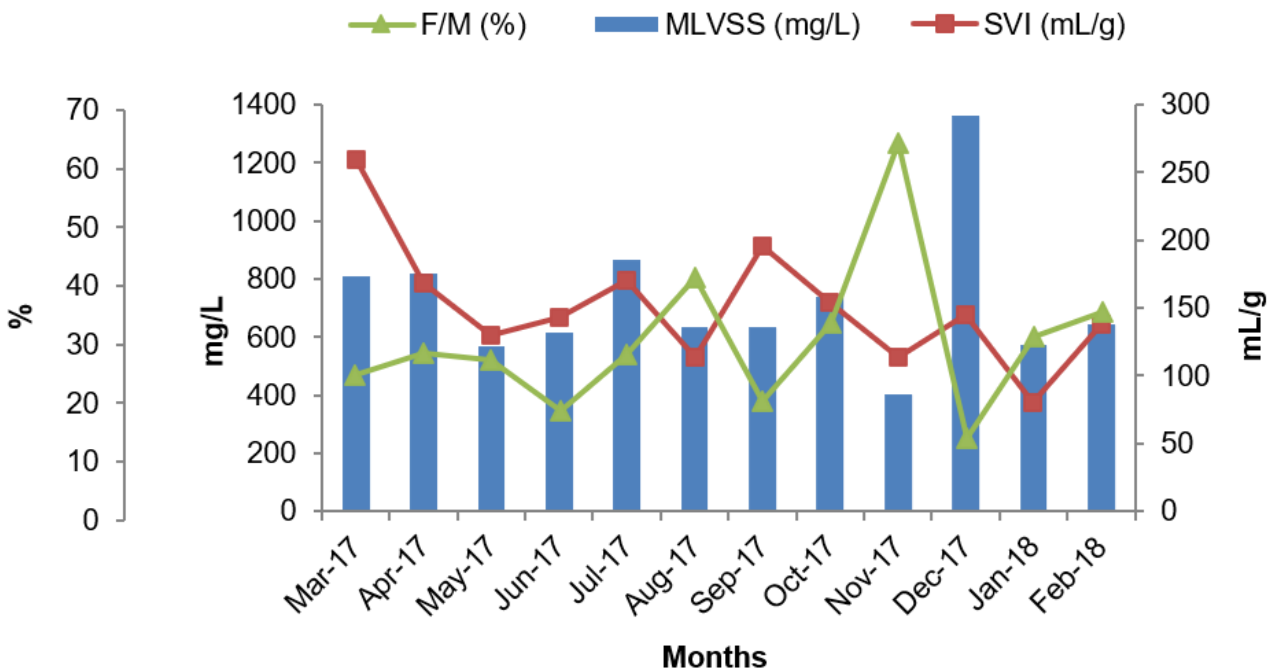
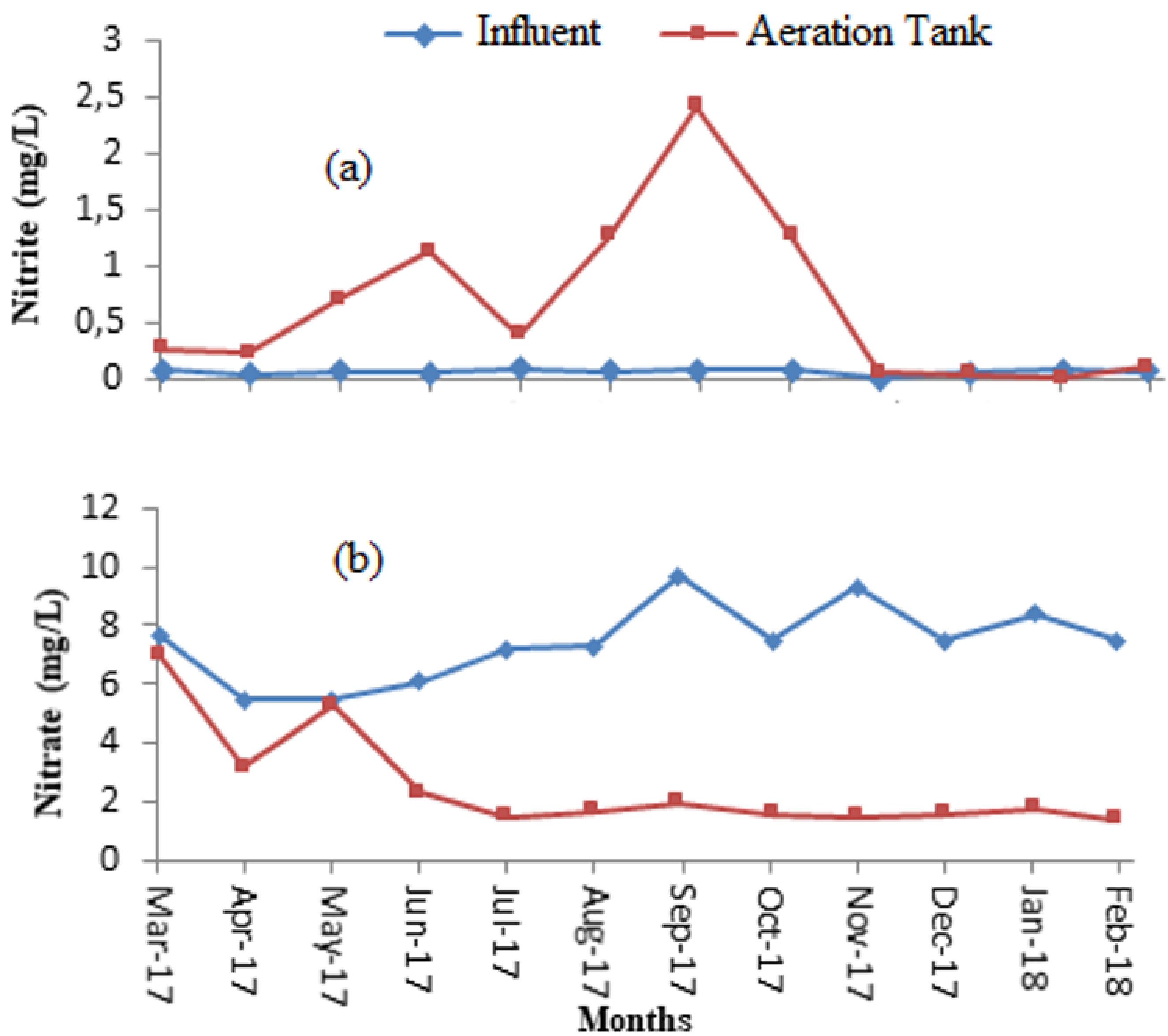



**Figure 6**

Temporal variation of aeration basin MLVSS, SVI, and F/M values




### Figure 7

Temporal variation of oxidized nitrogen values of facility influent and aeration basin, (a) nitrite values and (b) nitrate values

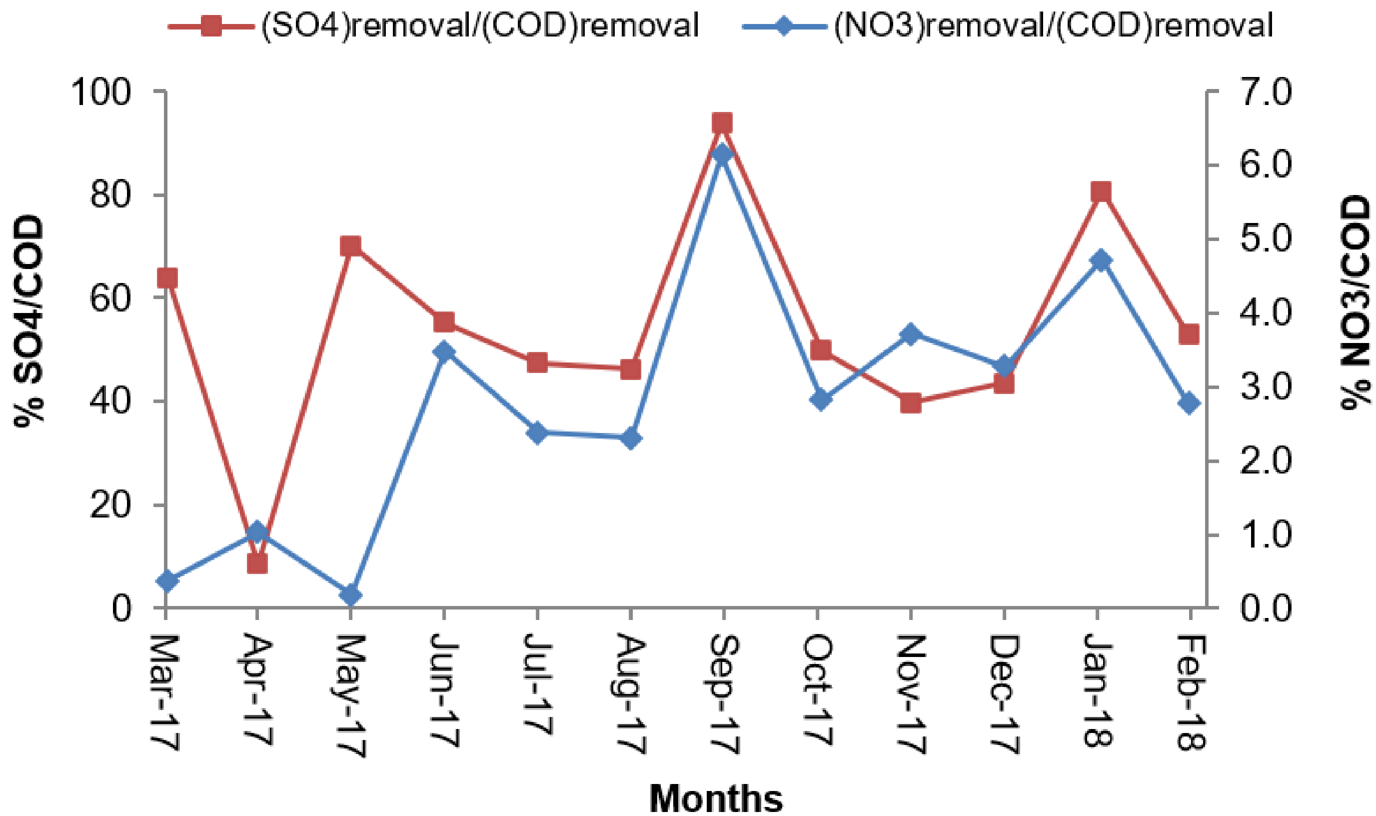



**Figure 8**

Temporal variation of $(NO_3)_{removal}/(COD)_{removal}$ and $(SO_4)_{removal}/(COD)_{removal}$ values

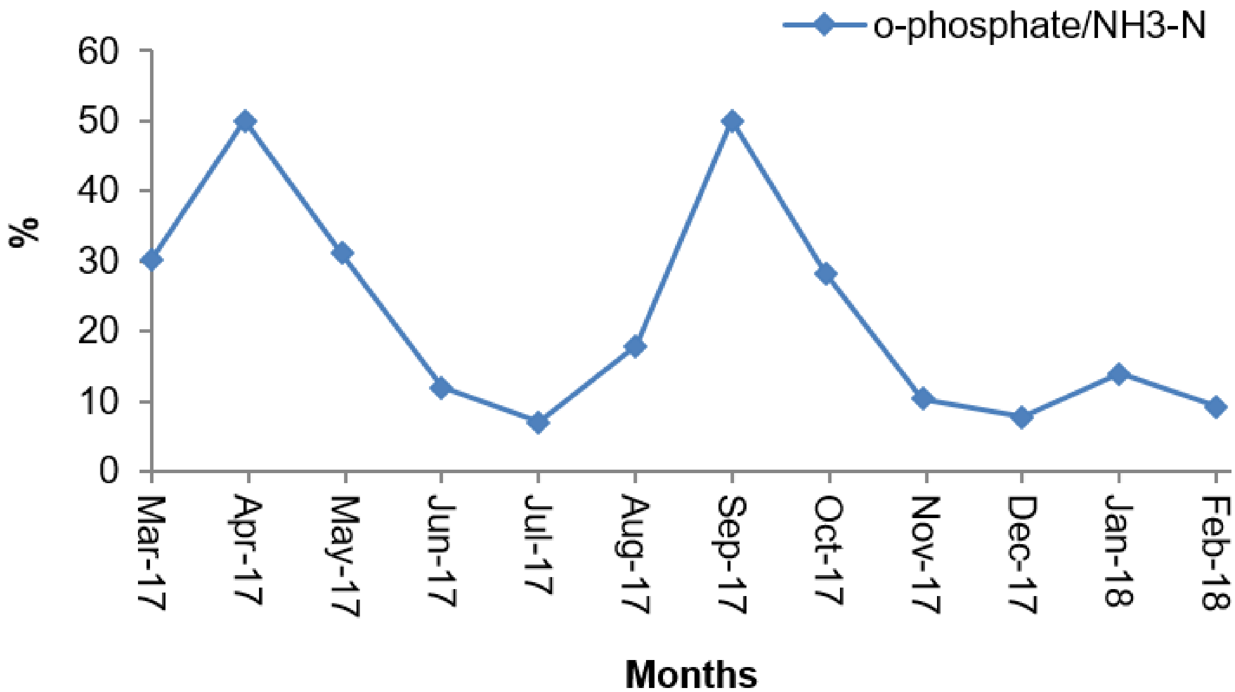



**Figure 9**

Change of proportional values of orthophosphate and $NH_3$-N amounts in aeration basin ambient water