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# A Study on the Absorption Well Sludge Potential in Promotion of Denitrification Process in Extended Aeration Reactor

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***Abstract:*** *When being present on the organic matters in the anoxic conditions, several bacteria do this conversion (denitrification). The current study aimed at the investigation of the effects of a useful and cost-effective method for nitrogen removal. In the current study, through creation of the anaerobic respiration conditions (anoxic) in the second line of aeration unit with a high SRT, and an MLVSS rate of 4000mg/l, in the HRT (Hydraulic Retention Time) of 8 to 36 hours, in the wastewater treatment plant with the Extended Aeration Activated Sludge by the use of heterotrophic bacteria (Pseudomonas and alcaligenes), was proposed and analyzed. Many of different heterotrophs are responsible for anaerobic denitrification, which have been identified in the sludge from the household waste wells (soak away). The findings show a decrease in COD and BOD by more than 93% and 96%, respectively, and nitrogen and phosphorus removal efficiency up to 90% and 87%. The nitrification was completely done. Among all the bacteria separated in the current study, the Pseudomonas with a rate of 36.2% and Alacaligenes with a rate of 29.8%, were identified as the dominant species. The results of the current study showed that the advantages of the use of mentioned method would lead to the removal of the organic matters and nutrients, and activity of denitrifying bacteria, besides a significant reduction in algae growth. According to the results of the analysis by the T-Test, with two independent groups and in the significance level of 5%, there is a significant different between the aerobic and anoxic groups in terms of NH4 and NO2 amount, while in the significance level of 10%, there is a significant difference between the two groups in NO3 amount.*

***Keywords:*** *Denitrification, Absorption Well, Wastewater Sludge.*

## INTRODUCTION

Nitrogen is one of the pollutants present in the wastewater, which can be found in organic and non-organic forms. The most stable form of nitrogen in the water and wastewater is the nitrate. In terms of the public health, nitrate can lead to the problems such as the Methemoglobinemia and cancer. According to Chan (2003), in the recent years, the nitrified compounds removal (denitrification) methods have been divided to physical and chemical methods, such as the Pambern et al. (2008) method, and the use of reducing metals, ion exchangers, reverse osmosis, and use of enzymes and electrodealysis. The physical and chemical methods are mainly expensive, and on the other hand, the use of biological methods has a low cost and does not produce any harmful residues. The biological methods for removal and conversion of different forms of nitrogen, like the other methods, are divided into conventional and modern methods. Among the conventional methods, the complete nitrification and nitrate denitrification can be named, and among the modern methods, the Short-Cut Nitrification or Partial Nitrification, the Anaerobic Ammonium Oxidation (Anammox), the Completely Autotrophic Nitrogen Removal Over Nitrite (Canon), the Oxygen-Limited Autotrophic Nitrification-Denitrification (Oland), the Single Reactor System for High Ammonia Removal Over Nitrite (SHARON), and the Side Stream Nitrification Process, can be named (Metcalf, 2003; Guo et al., 2008). Among the other modern nitrogen removal methods, the Side Stream Nitrification Process can be named (Neerackal et al., 2016). The side stream processes are those based on the production of the nitrifying agents under the proper conditions, and adding them to the main process of the activated sludge. To this purpose, a small tank is used besides the aeration, in which the nitrifying bacteria, under the proper conditions such as provision of ammonia from the liquid outlet of sludge digesters, fluid obtained from sludge digestion, or other ways such as manual addition grow in high amounts, and then are added to the main tank of activated sludge, or a separate nitrification tank (Szatkowska, 2007; EPA, 2007).

Therefore, the basis of the extensive research on the new biological methods for removal and conversion of the different forms of nitrogen from the wastewaters, including the wastewater containing high concentrations of ammonia, such as the liquid separated from the anaerobic digesters (Metcalf, 2003), and also, the sludge dewatering in the urban wastewater treatment plants is the economic issues and the reduction of treatment costs (Guo et al., 2008).

Regarding the equations dominating the first and second phases of nitrification, it is revealed that the full nitrification requires the creation of large volumes of aeration for generation of a proper sludge age, provision of oxygen, and also, provision of sufficient alkalinity, which all require high costs (U.S.E.P.A, 2013). And generally, the reason behind removal of nitrate is the excessive growth of the algae and preparing the conditions for nitrification, as well as reduction in the dissolved oxygen, toxicity, and the corrosion (Ramaraj, Tsai and Chen, 2010).

The increase of these matters in water resources leads to the algal bloom phenomenon, and if continues, it would cause the reduction in water quality due to eutrophication and aging phenomenon (Abbaspoor, 1993). The nitrogen stabilizing blue-green algae stabilizes the nitrogen in the aerobic condition. In the anaerobic conditions, the nitrogen stabilizing process is done in the plant cells of blue-green algae. The nitrogen stabilizing photosynthetic bacteria need anaerobic conditions for growth (Wiesmann, 1994; Ip, Chew and Randall, 2001).

Nitrification (nitrate generating) and the denitrification (nitrate removal) are two biological processes, which are repeatedly used in conventional methods of biological removal of nitrogen, since they are complementary. The biological removal of nitrogen is done by the aid of these two aerobic and anoxic processes. Due to the nitrification, the ammonia is initially converted into nitrite by the help of oxidizing bacteria, and then it is converted from the nitrite to nitrate by the nitrite oxidizing bacteria. Nitrification, which is an aerobic process and in which, the oxygen acceptor is considered to be electron, is done by the aerobic autotroph bacteria (Scaglione et al., 2013; Guo et al., 2007). The denitrification can be expressed by the following equations:

(1) $2\ NH_4^+ + 3O_2 \rightarrow 2\ NO_2^- + 2\ H2O\ + 4H^+$

(2) $2\ NO_2^- + O_2 \rightarrow 2NO_3^-$

The overall reduction-oxidation reaction of the nitrification is also expressed by through the equation (3):

(3) $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$

According to the most common definition, denitrification is a process in which the nitrate is converted into N2, by the aid of nitrite, nitric oxide, and nitro oxide. From the biochemical point of view, denitrification is bacterial process in which the nitrogen oxide (in ion and gas forms), participates as the final acceptor of electron in the respiratory transfer of electron (Kessru et al., 2006). The electrons are transported from an electron donor (substrate, organic compounds) through different data transportation systems in order to convert to oxidized nitrogen. The released energy from the phosphoric process is saved in the ATP, to be used for cellular respiration by the denitrifier organisms. The reduction of nitrate in the denitrification process is elaborated in the equation (4) (Kessru et al., 2006; Foglar et al., 2005; Szatkowska, 2007; EPA, 2007; Metcalf, 2003; Guo et al., 2008; U.S.E.P.A, 2013; Ramaraj, Tsai and Chen, 2010; Abbaspoor, 1993; Wiesmann, 1994; Ip, Chew and Randall, 2001; Scaglione et al., 2013; Guo et al., 2007; Makoto, 2017):

(4) $2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$

Nitrogen generation in the denitrification process can be shown by the equations (5) and (6):

(5) $4(CH_2O) + 4NO_3^- \Rightarrow 4HCO_3^- + 2N_2O + 2H_2O$
(6) $5(CH_2O) + 4NO_3^- \Rightarrow H_2CO_3 + 4HCO_3^- + 2N_2 + 2H_2O$

Also, the denitrification process is done in the anoxic conditions, in which the optional heterotrophic bacteria play the main role. In this process, nitrate is the acceptor of electron, and the organic and non-organic matters are the electron donors (Hiatt, 2006).

**Absorption Well System:**

The wastewater disposal systems are divided into the urban wastewater treatment systems and the absorption well. However, unfortunately, due to the defection and weakness of the urban wastewater systems in the environment which lack a wastewater collection system, the absorption well method is still being used, which has environmental and health hazards.

**The Absorption Well:**

It is a dome-shaped well excavated in the permeable layer of soil, and its task is storing the waste and separating water from the wastewater by leakage in the permeable layers. In the environments which lack wastewater collection system, this type of wells is used. Usually, in a 5 to 20 years period, after it is filled, its wall becomes watered, or due to the rise of groundwater, the water permeation rate is lowered, leading to the wastewater overrun from the well, which itself leads to the environmental issues and diseases. It is usually discharged through the vacuum by private sector, being buried in the environments assigned by the municipality.

Usually, the wastewater, after entering the well, is dissolved by the anaerobic bacteria, and the amount of solid suspended particles in the wastewater is reduced. Simultaneous with this action, the excess water permeates the environment and it may reach the underground waters. Although there has been no studies conducted on the absorption wells bacteria, it is estimated that three phases as hydrolysis, acidification and methanization take place in it. Usually, the absorbing burial of this type of wastewater leads to the severe harms to the underground waters. Thus, in an agreement, the urban absorption well wastewater is transferred to the wastewater treatment plant by the private sector.

In the current study, wastewater from the absorption well in an average volume of $100m^3$ is transferred to the wastewater treatment plant by the private sector, discharged in a tank with a volume of $100m^3$ (figure 1).

There is the tolerance of difficult physical and chemical conditions of the tank among the most important attributes of a useful bacteria in wastewater biotechnology. Therefore, in the current study, after investigation and identification of the microorganisms in the absorption well, which were identified as the denitrification generating bacteria, they were used for improvement of nitrogen removal in the extended aeration treatment system without initial sedimentation. Many of different heterotrophs responsible for denitrification are anaerobic. The dissolved oxygen level is preferred to be zero, and the nitrate is used as electron acceptor (Neerackal, 2016; Kulkarni, 2016). Also, in the current study, the second and third lines of aeration are exploited in the anoxic form and by the use of inverter aeration in the conditions as 0.9 hour anaerobic (silent aeration) and 0.1 hour aerobic (figure 1). In this method, the idle phase of sequencing batch reactor method has been modeled. The provision of anoxic conditions (anaerobic), besides the aerobic condition, is inevitable for the denitrification process. In this case, the nitrate produced in aerobic phase (nitrification) can be removed in the anoxic phase (denitrification). Also, for completing the denitrification, the carbon source needed by the heterotrophic microorganisms must be provided for them. In the current study, instead of using an external carbon source, the sequent reduction and absorption well sludge have been used (U.S.E.P.A, 2013).

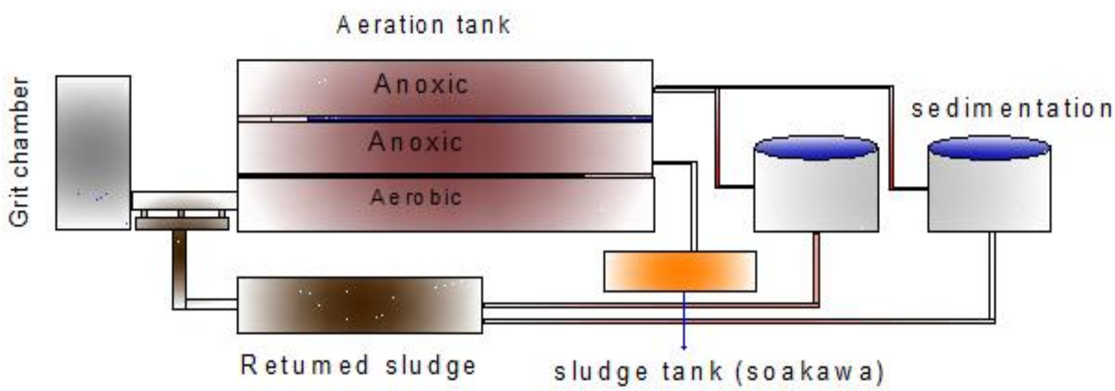


**Figure 1:** the flowchart of pilot design in the treatment plant

**Analysis and Calculation Methods:**

In the current study, all the samples of oxygen parameters needed for biochemical processes (BOD5), the oxygen needed for chemical processes (COD), the total nitrogen (TN), and MLSS and MLVSS indices were extracted based on the methods provided in the book "Standard Methods of Wastewater Treatment" (APHA, 2005). Also, for measurement of some forms of nitrogen including the nitrate, nitrite, and ammonia, the spectrophotometer device UV-VIS was used. The measurement of dissolved oxygen (DO) and the (PH) was done by using portable devices made by WTW and HACH companies, respectively.

**Testing Method:**

The sampling from absorption well sludge and investigation of the microbial structure in the samples, is provided in the tables (1) and (5). Also, the combined sampling from the activated sludge for determination of MLSS and MLVSS concentration, as well as the input parameters of biological unit are provided in table (2). In order to do the separation experiments and identify the microbes, the samples from the absorption well were chosen. To this purpose, the microbial separation and identification experiments were done on a sample which was collected from the absorption well sludge and transferred to the laboratory in 4 centigrade temperature (Honda et al., 1999). For culturing of heterotrophic bacteria, the extended and plate culturing methods, and for the counting of microbial cells with the extended culturing method and investigation of the morphology of colonies grown on the warm color culturing environment, and observation and identification of bacterial cells, the chemical test for differential diagnosis and PCR, the bacteria with denitrification capacity were identified (Lin et al., 2007). Then, the colonies of culturing environment were cultured on the agar nutrient culturing environment for purification of the culturing. For identification of the separated bacteria, the standard methods of bacteria identification were used (Murray et al., 1981).

**Table 1:** the attributes of absorption well sludge per unit of mg/l

| DO | PH | COD | BOD | MLVSS |
|---|---|---|---|---|
| 0.18 | 8.12 | 786 | 385 | 1318 |

**Table 2:** the attributes of input parameters of treatment plant per unit of mg/l

| DO | PH | COD | BOD | MLVSS | TN | TP |
|---|---|---|---|---|---|---|
| 0.3 | 7.82 | 415 | 220 | 3968 | 31 | 16.2 |

**Experimental Test:**

All the biological processes of nitrogen removal have an aerobic environment in which the biological nitrification takes place. Also, a little anoxic volume or time must be provided for the accomplishment of biological denitrification, so that the complete removal of nitrogen by the oxidation of ammonia nitrogen and reduction of nitrite and nitrate to nitrogen, takes place. As was mentioned, the establishment of denitrification phenomenon, besides the advanced treatment, requires an anaerobic unit and a sequent reduction, and generally, the generation of nitrogen removing bacteria is very low. The studies by Morlang (2009) shows that the MCRT sludge age is needed for the increase in nitrogenized bacteria, and since the cycle of the bacteria existing in the SMLSS is so extended and they are competing, there is not enough time for reproduction of nitrogen removing bacteria for coexisting in the aerobic environment. Among the factors for acceptance of wastewater absorption wells in the Wastewater Treatment Plant of Bandar Anzali, is the type of process (extended aeration) which makes the organic and hydraulic shocks uniform. Initially, a sedimentation tank with volume of $100m^3$ was built in the treatment plant, and the sludge absorbed by an 8KW sludge pump was directed towards the second line of aeration through the valve No.1 (tables 3 and 4). Each phase of treatment plant includes a grit chamber, aeration with a volume of $10000m^3$ in 3 reciprocating waste lines (each line having 4 aerations with inverter), two secondary sedimentation tanks, each with a volume of $2000m^3$, return sludge pumping station, and a UV disinfection system. Firstly, the input wastewater, after passing through the grit chamber, enters the aerated tank inlet mixed with the return sludge to be aerated in a successive form. After passing through the first line, the wastewater enters the second cycle and line, and is mixed with the absorption sludge by the pumping. In the current study, the considered variables included the DO, HRT, and the number of anoxic-aerobic secondary sub-cycles, which was implemented based on the optimum range, and modification of the dissolved oxygen in the range of 2mg in the first line, and 0.5mg in the second and third line was done manually (reduction in aeration cycle) or by the overflow control valve. Also, the enrichment of denitrifiers was done by the absorption well sludge, and regarding the cellular retention time (SRT), the final MLSS and MLVSS existing in the aeration reactor was in the 46 day, 5720, 3968 mg/l range. The time of process was permanent and the second and third lines of aeration were automatically without oxygen (off) for 0.9 hour, and it was on for 0.1 hour, so that the mixing act would lead to the movement of MLSS towards next part and line, and it is all stopped in the this process. In the current study, in order to investigate and control the denitrification process, the amounts of nitrate, nitrite, ammonia, alkalinity, PH, COD, temperature, and flow discharge were monitored.

During the monitoring, for fixing the amount of Mixed Liquor Volatile Suspended Solids (MLVSS) in the reactor, which is indicative of the existing active biomass, the amount of the return sludge to the system's input is calculated based on the equation (7).

(7) $(Q_r + Q_{in}) \times MLSS = Q_r \times SDI + Q_{in} \times SS_{in}$

In which, the $Q_r$ and $Q_{in}$ stand for return sludge flow and the input waste flow, respectively. The SDI is the sludge density index, and the MLSS is the Mixed Liquor Suspended Solids, and the $SS_{in}$ is the suspended solid matters in the input waste.

**Table 3:** the ratio of aborption well sludge to the aeration volume and input wastewater in the pilot design

| ratio of aborption well sludge to the aeration volume (percent) | Return sludge ratio (percent) | Ratio of absorption suldge discharge to the input watewater (percent) |
|---|---|---|
| 1 | 70 | 1-2 |

**Table 4:** the suggested optimal values for parameters and variables considered for pilot design

| $BOD^5$ Input Mg/l | | $BOD^5$ well Mg/l | DO First line Mg/l | HRT _ h | The percentage of aborption slugde and wastewater mixture | mlvss Mg/l | | PH | DO Second line Mg/l |
|---|---|---|---|---|---|---|---|---|---|
| Min. | Max. | | | | | Min. | Max. | | |
| 100 | 200 | 300 | 1-2 | 28 | 1% | 2500 | 4000 | 7 – 8.1 | o.5 |
| 200 | 300 | 500 | 2-3 | 30 | 1.5% | 3500 | 5000 | 7 -8.1 | 0.5 |
| 300 | 500 | 1000 | 3-4 | 36 | 2% | 4500 | 6000 | 7 –8.1 | 0.5-1 |

**Results:**

The results of the current study is indicative of nitrogen reduction in the output waste by the use of Pseudomonas bacterium with organic matters source, and also reduction and complete removal of algae in the output channel of waste.

The studies conducted by Christine et al. on the Pseudomonas shows that this bacterium has a high enzyme activity for reduction of nitrate. The studies done by Kessru et al. (2006), have indicated the ability of Pseudomonas bacteria and Alcaligenes for conversion of nitrate to nitrogen. The research by Fuglar et al. (2005) on the high concentrations of nitrogen and by the use of bacteria growth also ended in similar results. It was shown in their study that the initial concentration of nitrate affects the maximum growth of the microorganisms responsible for nitrate removal.

The studies conducted by Chan (2003) have also shown that a pH in the range of 6.5 to 8 cannot negatively affect the nitrate removal process, however, the highest percentage of nitrate removal (90%) was witnessed in the pH=7. These results are in lines with the results of Pambern et al. (2008). They found out that the increase in pH from 7.5 to 8.5 would lead to increase in nitrite concentration and a reduction in nitrite oxidation, and vice versa. In the other conventional activated sludge systems, the denitrification bacteria inevitably exit from the system, especially when the sludge age is very low (Mohammed et al., 2008). For a sludge age of above 50 days, the denitrification bacteria concentration is higher than the other activated sludge systems. Usually, the age lower than 50 days is not common in these systems. Therefore, the denitrification bacteria have a fairly proper compatibility with the other active heterotrophic bacteria in consumption of ammonia nitrogen (Jianlong and Ning, 2004).

The studies conducted by Chiu et al. (2007) on the denitrification and nitrate removal showed that the best nitrogen removal efficiency was obtained when the carbon-to-oxygen ration is 11.1. With the constant value of dissolved oxygen as 0.5mg/l, the total nitrogen (TN) removal efficiency in an SRT of the above 30 days reached up to be higher than 89%, since there have been higher carbon (the organic matter) as the electron donor, and lower nitrate as the electron acceptor. In a study conducted by Wang et al. (2008) also, it was revealed that

reducing the dissolved oxygen from 2.5 to 0.3mg/l increases the NAR (Nitrite Accumulation Ratio). Also, the proper range for dissolved oxygen in the Liang et al. (2011) study was reported to be up to 0.8mg/l in the anoxic conditions, while in the current study, an amount of 0.5mg/l in the anoxic conditions was exploited.
Based on the microbial examinations results, after initial culturing of the samples, high numbers of bacterial colonies grew on the plates in a 48-hour incubation, and through doing supplementary research, and purification of the generated colonies, form among the 12 species of separated bacteria, 4 species including the Pseudomonas, Alcaligenes, Shigella, and Moraxella were identified to have the highest percentage.

**Table 5:** the percentage of the bacteria separated from the abruption well sludge

| Pseudomonas | Alacaligenes | shigella | moraxella |
|---|---|---|---|
| 36.2% | **29.8%** | **12.3%** | **21.7%** |

The bacteria, especially the Gram-negative bacteria, are among the main components of activated sludge flocks. According to the research, Hundreds of strains of bacteria are present in activated sludge, a small fraction of which are detectable by culture-based techniques (Portune, Prez and Varez, 2014). Among the total population of the cultivable bacteria identified in the current study, the Pseudomonas with 36.2 and Alcaligenes with 29.8% were the dominant species. Based on the microbial examinations, all the microorganisms identified in the current study were inactive (Portune, Prez and Varez, 2014).
In the recent studies, Akin & Ugurlu investigated the effects of presence of an anaerobic environment on phosphorus biological removal process in the SBR reactor. In this study, the phosphate concentration changes were investigated through measurement of filtered input and output waste from the aerobic and anaerobic environments. In this study, the highest removal rate was obtained in the aerobic environment with a retention time of 12 hours, which was 87%, that actually confirms the hypothesis of the organic absorption in phosphorus biological mechanism.
Based on the t-test of two independent groups, in the 5% significance level, there were no significant differences in NH4 and NO2 between the aerobic and anoxic groups, however, there is a significant difference in NO3 between the aerobic and anoxic groups in the 10% significance level. In fact, it can be said that there is a significant difference between all the parameters between the aerobic and anoxic groups in the significance level of 10%.
In the 0.05 significance level, there is significant and reverse correlation between the time and ammonia parameter, as with the increase in time, the amount of ammonia is significantly reduced.
In the 0.05 significance level, there is significant and reverse correlation between the time and nitrate parameter, as with the increase in time, the amount of nitrate is significantly reduced.
In the 0.05 significance level, there is a significant and reverse correlation between the time and nitrite parameter, as with the increase in time, the amount of nitrite is reduced.
The analysis of the results is indicative of the reduction in COD and BOD to 93 and 96%, respectively, and the nitrogen and phosphorus removal efficiency to 90 and 87%, respectively. The nitrification was done completely, and in the MLSS concentrations above 6000 mg/l, the denitrification has been done better, which can be due to the formation of larger microbial clots, and consequently, the probability of emergence of anaerobic environments on the center. In a reactor's cycle, the two aerobic and anoxic time intervals are considered to be consecutive, so that the nitrate generated in the aerobic phase, is removed in the anoxic phase, and the carbon needed for the heterotrophic bacteria is provided for them through the re-entering of the feed (absorption sludge) in the anoxic conditions; however, the best performance of the reactor was obtained in the minimum loading of nitrogen and organic matters. In this state, the amounts of ammonia, nitrate, and nitrite in the input waste was -15.1, -5.6, and 3.1mg/l, and in the reactor's output, the minimum

possible amounts were -0.1, -0.6, and 0.043mg/l, respectively. The system's performance in removal of nitrate, nitrite, and ammonia have been provided in figure based on the different retention times (2).

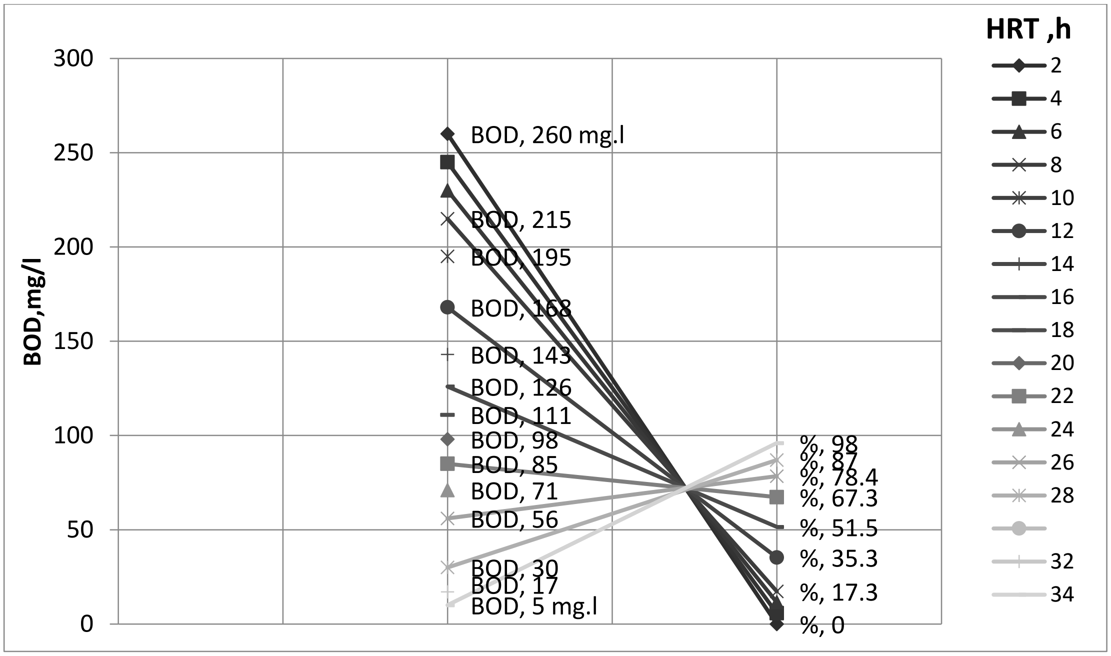


**Figure 2:** the system performance in BOD removal in different hydraulic retention

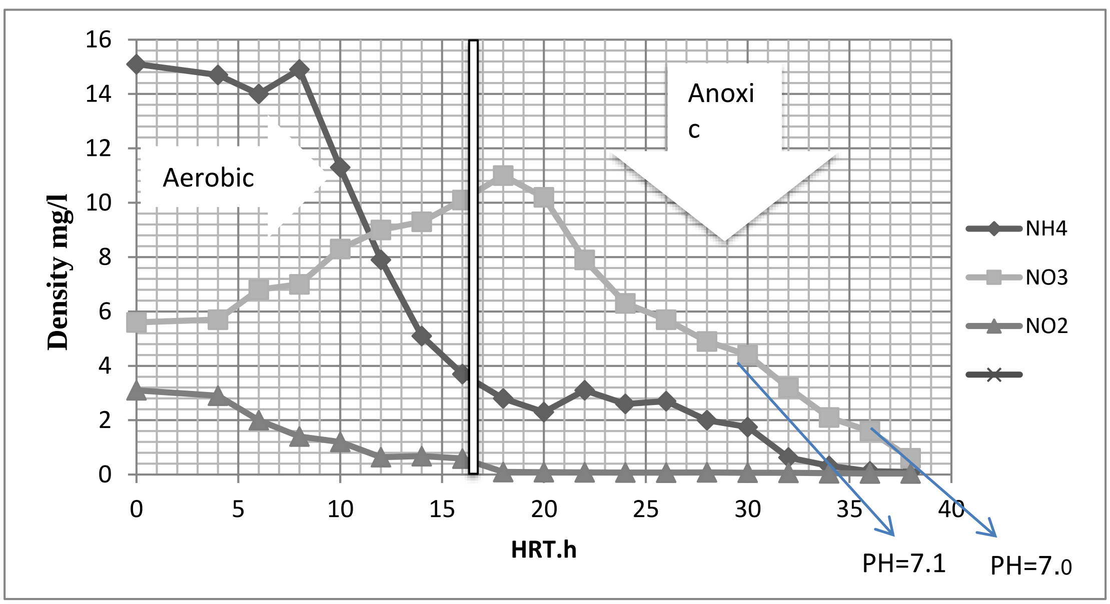


**Figure 3:** the change in nitrate, nitrite, and ammonia in the first and second cycles of extended aeration

## Conclusion

In the current study, after investigation and identification of the microorganism in the absorption well, which were known as the denitrification generating bacteria, were investigated for nitrogen removal in a without initial sedimentation extended aeration treatment system. Also, through creation of anoxic conditions in the aeration unit with high SRT, and MLVSS rate of 4000mg/l, in the hydraulic retention time (HRT) of 8 to 36 hours, the water treatment by the use of the extended aeration activated sludge, and by the aid of heterotrophic bacteria (Pseudomonas and Alcaligenes) was investigated. Many of different heterotrophs which are responsible for anaerobic denitrification, prefer the dissolved oxygen level to be zero, and use the nitrate as the electron acceptor. These bacteria can be activated in an island form, in an organic matter-rich environment and without the presence of oxygen, which have been identified in the soakaways. Also, in the current study, the second and the third lines of aeration were exploited in an anoxic form and by the use of inverter aeration with the capability of round modification in the condition as 0.9 hour of anoxic and 0.1 hour of aerobic conditions. In this method, the idle phase of SBR has been modeled. The provision of anoxic conditions (anaerobic), besides the aerobic condition, is inevitable for denitrification process. In this case, the nitrate produced in aerobic phase (nitrification) can be removed in the anoxic phase (denitrification). Also, for completing the denitrification, the carbon source needed by the heterotrophic microorganisms must be provided for them. In the current study, instead of using an external carbon source, the sequent reduction and absorption well sludge have been used.

Also, if the oxygen is being supplied by the input wastewater or the return cycle, the biologically dissolvable organic matters are consumed in an aerobic manner, therefore, its amount is reduced for the use of denitrification as the electron donor; thus, through sending the absorption well sludge to the second line in the anoxic conditions, the biomass reduction effect was decreased.

Alcaligenes: this bacterium is a heterotrophic nitrifying bacterium, mostly found in the sludge and soil. It is present in the continual culturing with different concentrations of oxygen, the growth rate, and in the presence of different compounds of mineral nitrogen (E.W et al., 1991; Makoto, 2017).

The use of this bacterium in ammonia removal treatment has been less reported. This bacterium shows a significant ammonia removal ability with a high power and a great amount of removal (Makoto, 2017).

Pseudomonas is among the most effective denitrifying bacteria which reduces the nitrate with a high efficiency in the wastewater environment through anaerobic respiration and in the anoxic conditions, and converts it into nitrogen (Jorge et al., 2006).

Microorganisms such as Pseudomonas Alcaligenes have many advantages of the use of biological nitrogen methods due to having the ability to convert the nitrogen into nitrogen gases (Zhao et al., 2010a).

The external parameters of the design, both quantitatively and qualitatively, showed that the proper and continuous use of this process would lead to the decrease in TN and TP, balancing the powers in the bacterial coexistence. Also, the algae growth in the aeration unit and output channels of treatment plant was totally stopped, and through the creation of the anoxic conditions, it has led to the growth of optional and anaerobic bacteria, and creation of dominant conditions.

Among the objectives of the current study were the flexibility of extended aeration and coexistence of the nitrifying and denitrifying bacteria in the same tank for growth and reproduction. The simultaneous growth of these microorganisms can be easily done through sequencing the aeration time and reaction in the reactor with a proper schedule. The simultaneous removal of both nitrogen and phosphorus is possible by these bacteria (Kulkarni, 2013).

Regarding the structure of microbial population of activated sludge and activity of the microorganisms related to the nature and type of the available hydrocarbons, the nutrient compounds and other environmental conditions such as PH, temperature, dissolved oxygen, the system mixture, and structure of biological unit, the identification of the activated microbial structure in the treatment system can be used for updating the design of treatment systems and exploiting them.

Since a large amount of the costs in an urban wastewater treatment plan are allocated to supplying the air needed for reactors, a considerable save in the costs can be fulfilled regarding the anoxic conditions in the second and third lines of the extended aeration.

Most of algae can absorb all forms of dissolved nitrogen, especially if they are accustomed to the presence of nitrogen. In the anoxic condition, the growth of biofilms would be limited, which is among the reasons behind separation of biofilm from the aeration reactor's wall and output channel in the pilot period (Toet et al., 2005)

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