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Research Paper:
Studying the Effluent Quality of Enhanced Modified Ludzack Ettinger-oxic Settling Anaerobic Process (E-MLE-OSA) for Treating Real Municipal Wastewater

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ABSTRACT

Background: Wastewater collection, treatment, discharge, additionally as reusing the treated wastewater in urban are critical factors for maintaining public health preventing contamination of water resources. This study aimed to investigate the performance of enhanced modified Ludzack Ettinger process-oxic settling anaerobic (MLE-OSA) process for treating real municipal wastewater in Sari wastewater Treatment, Sari City, Iran.

Methods: To combine the OSA process technique with the MLE system, the Sludge Holding Tanks (SHT) were implemented in the return sludge line of designed pilot studies which comprised 1) MLE, which was regarded as the control system similar; 2) MLE-OSA$_4$ with a 70-L SHT operated at 4-h Hydraulic Retention Time (HRT), and 3) MLEOSA$_6$ with a 107-L SHT operated at 6-h HRT. To start the process, the overflow effluent of the primary settling tank of the Sari wastewater treatment plant was used. After 45-60 days, the reactors reached a steady state. The parameters of Dissolved Oxygen (DO), Oxidation-Reduction Potential (ORP), temperature, pH, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD$_5$), Total Suspended Solids (TSS), and nutrients were analyzed.

Results: The results revealed that the average TSS, COD, BOD, phosphorus, nitrite, nitrate, and total nitrogen in MLEOSA$_4$ effluent decreased 20.1, 14.6, 2.97, 10, 51.1, 19.3, and 20.7%, respectively compared to MLE. Similarly, in MLE-OSA$_6$, the values decreased 34.2, 16.1, 14.6, 16.7, 69.6, 31.3, and 25.8%, respectively compared to the control process.

Conclusion: Therefore, using natural wastewater, the enhanced MLE-OSA process showed better performance in removing the study parameters and better quality for the effluent. Furthermore, the quality of effluent is following the Iran Department of Environment (IDE).

Keywords:
Municipal wastewater, Wastewater treatment, MLE process, OSA process, Nutrient removal, Enhanced MLE-OSA process

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1. Introduction

Wastewater collection, treatment, discharge, and reusing the treated wastewater in urban and industrial areas are key factors for maintaining public health, preventing contamination of water resources, and protecting the environment [1]. Thus, establishing a wastewater treatment plant per se cannot eliminate the environmental concerns; the performance of these treatment plants must constantly be assessed to achieve the promising environmental standards [2]. It is crucially important to optimally run the treatment to reach the environmental standards, which depend on the type of the applied process, wastewater quality, and operation conditions. Improper operating of the system leads to poor performance of the treatment processes that result in lower quality for effluent and contrasts with the expected quality standards. Thus, regular assessment and evaluation of the effluent quality and comparing it with the approved standards are indispensable [3-6]. According to the national standards of Iran, the concentrations of parameters like Biochemical Oxygen Demand (BOD_5), chemical oxygen demand (COD), Total Suspended Solids (TSS), Total Nitrogen (TN), NH_4^+, NO_2, NO_3, and Total Phosphorus (TP) are determined to be 30, 60, 40, 14, 2.5, 10, 50, and 6 m/L, respectively. However, releasing the wastewater treatment effluent to the surface water resources requires observing the Iran Department of Environment (IDE) standards. The pollutants removal efficiency of different processes is affected by the type of wastewater treatment [7, 8].

A study was conducted in the north of Italy to compare a full-scale modified system of the Activated Sludge-Anaerobic Side-Stream Reactor (AS-ASSR) using real wastewater with the previous conventional activated sludge configuration. The results demonstrated a 28% reduction in sludge production along with effective removal of substrate and nutrients compared with the control system. By inserting ASSR, all measured parameters were within the standard range in the effluent [2]. In the modified Conventional Activated Sludge System (CAS)- Oxic Settling Anaerobic (OSA) process, a laboratory-scale anaerobic tank was used to study the reduction of biological sludge using artificial sewage. The results revealed that COD concentration in the OSA process effluent was less than that of the control system due to excess substrate in the anaerobic tank. NH_4-N in the OSA process effluent increased because of denitrification, though in a long-term operation, both systems had similar removal efficiency for NH_4-N. Moreover, there was an increase in the concentration of PO_4-P in the OSA process effluent regarding the low Oxidation-Reduction Potential (ORP) level and phosphorus release after anaerobic conditions [5]. In another research, the performance of a pilot plant was monitored for 16 months which was operated with OSA technique in anaerobic/anoxic phase under Conventional Activated Sludge System (CAS) fed with real wastewater to reduce the excess biological sludge by changing the operational parameters. This study revealed that the effluent COD concentration with an average of 39 mg/L was less than the legal limit of effluent discharge to the receiving waters in Italy. Moreover, no considerable solid loss (TSS) was observed in the effluent. However, most of the time, the phosphorus concentration of the wastewater was higher than the influent, and poor performance was also observed regarding the removal of phosphorus [9]. To study the efficiency of sludge reduction and stability of the OSA process, we performed UNITANK and UNITANK-OSA on a pilot-scale using real wastewater. The results revealed that despite a slight decrease in Total Phosphorus (TP) contents, the quality of the effluent was not affected, and there was a 48% sludge reduction [10].

A study was conducted on the effect of Sludge Retention Time (SRT) as the main contributor of sludge reduction in OSA-based Sequencing Batch Reactor (SBR) systems by using artificial wastewater. It revealed that sludge reduction in this process did not significantly affect the efficiency of the nutrients removal and the effluent quality. However, it affected microbial activities and metabolic processes. With the removal percentage of about 93%, the COD concentration of the effluent was about 20 mg/L, and the amount of NH_4-N and NO_2-N in the effluent was insignificant, i.e., less than 0.1 mg/L. Lateral hydrolysis and acidification decreased the nitrification and denitrification activities, while it increased the phosphorus removal activities [11]. Several studies worked on employing the OSA process in lab-scale for evaluating the reduction of excess sludge in Conventional Activated Sludge System (CAS). These studies revealed that this process had good results in reducing the excess sludge production, COD removal, sludge disposal improvement, and removal efficiency of 19%-49% in dissolved phosphorus. It had no effects on the treatment efficiency of anaerobic conditions and effluent quality [5, 12]. According to the literature review and efficiency of the OSA process in reducing excess sludge, nitrogen, and phosphorus removal, we decided to apply this technique in the modified Ludzack Ettinger (MLE) process in the Sari wastewater treatment process (WWTP) to remove phosphorus and nitrogen up to the standard limits. Hence, this study was conducted to investigate the effluent quality of the enhanced MLE-OSA

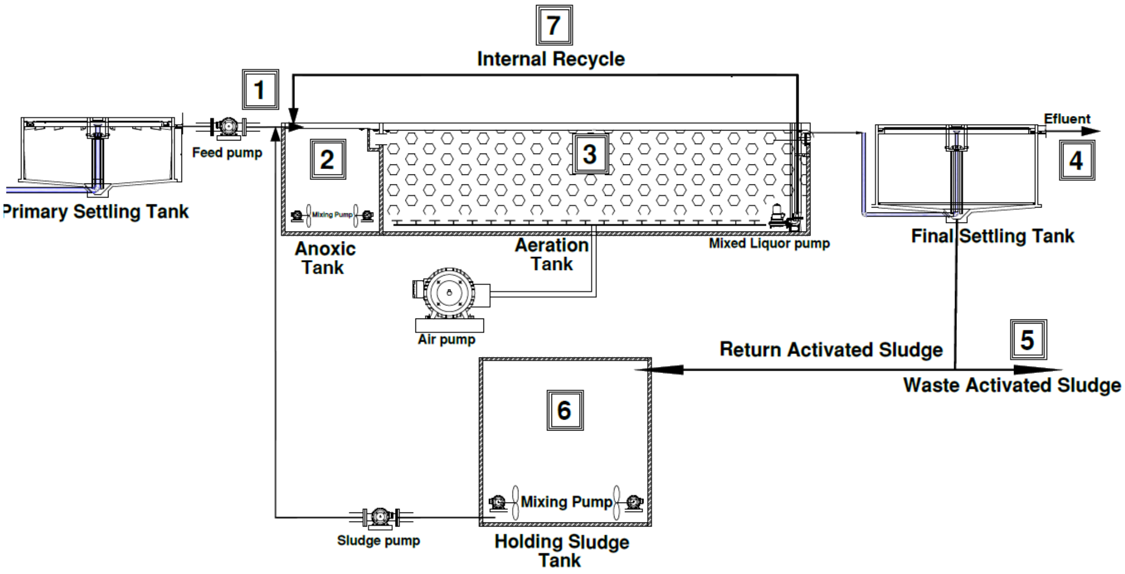

Figure 1. The general schematic of MLE-OSA pilot study

system, using real wastewater to remove organic matters and nutrients in the recycled effluent following the laws and limitations, as well as environmental requirements and health threats to protect the environment.

2. Materials and Methods

The enhanced MLE-OSA process was designed and operated in a pilot plant by placing an anaerobic/anoxic sludge holding tank (SHT) in the sludge return line of the MLE system (Figure 1). The sites where samples were taken are labeled with numbers.

Three pilots were used in the project according to the design criteria of Sari City wastewater treatment plant, including an anoxic reactor with a volume of 38.5 L, an aerobic reactor with a volume of 168 L, a sedimentation tank with a volume of 144 L, and MLE-OSA$_4$ and MLE-OSA$_6$ systems each equipped with sludge holding tanks with volumes of 70 and 107 L, respectively.

To supply real wastewater for pilot feeding, the overflow effluent of the primary settling tank of Sari WWTP was used. Its characteristics are presented in Table 1. The pH of the influent wastewater was within the neutral range, and the wastewater contained considerable amounts of organic matter, nitrogen, and phosphorus [8].

To start up the pilot, the contents of an anoxic and aeration tank of Sari WWTP with an Mean±SD mixed liquor suspended solids (MLSS) of 3000±35 mg/L were used. Peristaltic pumps were employed to supply accurate input flow (Qin) as much as 23 L/h, recirculation activated sludge (QRAS) with a percentage of 80%, as much as 18.4 L/h, and internal recirculation (IR) or mixed liquor recirculation (MLR) with 147%, as much as 32 L/h.

Table 1. Characteristics of wastewater used as influent of MLE-OSA pilot

Parameter	Min	Max	Mean±SD
COD	171	351	269±32
BOD$_5$	149	273	216±25
TN	30	53	41±4
TP-P	2.43	8.47	3.96±0.83
pH*	7.47	7.77	7.6±0.076

*All parameters units are presented in mg/L except pH.

COD: Chemical Oxygen Demand; BOD$_5$: Biochemical Oxygen Demand; TN: Total Nitrogen; TP-P: Total phosphorus.

To uniformly distribute the airflow and create a favorable condition for suspension, some micro-pore aerator stones were placed at the bottom of the aeration tank. To supply and maintain the oxygen concentration of the solution, as much as 2-3 mg/L, several aeration pumps with 25 L/m capacity were installed.

After setting up the pilot with urban wastewater for each condition, 45 to 60 days were determined for operation and maintenance to reach the steady-state condition. The results of all tests were acceptable, with less than 10% fluctuation in the period mentioned above. Four groups of tests were done at sampling stations: 1) control measures for controlling and maintaining the optimal condition of the environment and microorganisms' function, including temperature, dissolved oxygen, and pH; 2) operational parameters for maintaining optimal conditions, such as determining the quality of sludge and system performance including the amount of MLSS in aeration tank, Sludge Volume Index (SVI), and sludge retention time (SRT); 3) main parameters, such as the criteria for reaching to the steady-state condition, including COD, TSS, and effluent pH; and 4) determining nutrients, including nitrogen and phosphorus. All the mentioned parameters were performed according to the methods written in the 2014 edited book of "Standard Method for Examination of Water and Wastewater" [13].

After the pilot processes of MLE, MLE-OSA$_4$, and MLE-OSA$_6$ reached a steady-state condition, MLE as the control system was contrasted with the enhanced MLE-OSA system. To investigate the study objectives, modified action was taken using SHT with 70 L and 107 L capacities and HRT of 4 and 6 h in the return sludge line. Then, the physicochemical parameters set in another 45 days of operation and maintenance were analyzed daily and weekly to evaluate effluent quality for nutrient and organic matter removal, sludge production rate, sludge properties, and the performance of the modified MLE-OSA process in comparison with MLE. All the materials employed in the laboratory were purchased from Merck Co, Germany. The equipment contained an ORP meter (AZ Instrument Corp), pH meter (AQUALYTIC), Dissolved Oxygen (DO) meter (AQUALYTIC AL20Oxi), digital weighing scale (METTLER model PJ300), and spectrophotometer (Hach DR6000). After conducting the experiments, collecting data, and obtaining the results, the descriptive results were studied with descriptive statistics, such as mean, standard deviation, etc. Since the data followed a normal distribution, the ANOVA test was used. For comparing the means of parameters obtained from the system operation state, the Tukey test was employed with a 5% level of significance. All information, which was obtained from Grapher version 15, Graph pad version 6, Excel version 2014, and SPSS version 24, were meticulously analyzed.

3. Result and Discussion

In this research study, three processes were investigated, namely, MLE, MLE-OSA$_4$ with 4-h HRT, and MLE-OSA$_6$ with 6-h HRT, for urban wastewater treatment. The purpose was to evaluate and compare the effluent quality after 45 days of operation and maintenance. The results were compared with the standards of IDE and the design criteria of Sari WWTP. In the following parts, the findings are presented as follows.

Concentration Changes of BOD, COD, and TSS in the Effluent

The average concentration changes of BOD, COD, and TSS in the effluent in 45 days of operation and maintenance of MLE, MLE-OSA$_4$, and MLE-OSA$_6$ after reaching the steady-state condition can be observed in Figures 2, 3 and 4. According to Table 2, the F-value, P-value of less than 0.05, and analyzing the mean differences of BOD, COD, and TSS changes in the effluent in the three studied processes, the mean differences of COD and TSS concentration changes were statistically significant in the three processes. However, no significant differences were found in the mean concentration changes of BOD$_5$ of the effluent in all three processes.

Effluent TSS changes

The Mean±SD TSS changes in the effluent during 45 days after reaching the steady-state condition in MLE, MLE-OSA$_4$, and MLE-OSA$_6$ were respectively 7.4±1.96, 5.9±0.86, and 4.88±0.7 mg/L (Figure 2).

It is observed that the mean TSS of effluent in MLE-OSA$_4$ has had a 20.17% decrease in comparison to the MLE process. Similarly, it has decreased 34.19% in MLE-OSA$_6$ compared to the control system. The post hoc Tukey test (Table 1) illustrates that TSS in the effluent will be enhanced if there is an increase in the HRT in anoxic/anaerobic SHT. This outcome could be due to the release and distribution of intercellular polymers in SHT as an influential factor in bridging the biological clots and formation of appropriate flocs and improving the settleability [14, 15]. Different components are required for cells to make floc by clotting bacteria in the process of activated sludge. These components include bacterial fibrils, sticky polysaccharides, and poly hydroxy butyrate or starch granules for sticking together or clotting. They are naturally provided by increas-

Table 2. Investigating mean differences of TSS, COD, and BOD in Effluent (Eff) of studied processes in the pilot

Variable	Process	Days	Mean* (mg/L)	SD	F	P
TSS Eff	MLE	45	7.416^{C}	1.962	42.774	0.0009
	MLE-OSA$_4$	45	5.920^{B}	0.867		
	MLE-OSA$_6$	45	4.880^{A}	0.726		
COD Eff	MLE	45	14.278^{B}	4.075	6.417	0.002
	MLE-OSA$_4$	45	12.197^{A}	2.912		
	MLE-OSA$_6$	45	11.982^{A}	2.956		
BOD Eff	MLE	45	8.222	3.036	2.893	0.063
	MLE-OSA$_4$	45	7.977	2.416		
	MLE-OSA$_6$	45	7.022	1.924		

*The observed indices reveal the significance level of the means in groups based on Tukey's test.

A, B, C: 3 Categories or there are 3 different groups.

ing the mean cell residence time [16]. These findings are 96% in line with those of Velho et al., who worked on an enhanced process of AS-ASSR in full-scale during 270 days operation using real wastewater in contrast to conventional activated sludge system. The Mean±SD concentrations of TSS in the effluent in their study were 6.7±3.2 and 5.3±0.3, respectively [1]. Furthermore, the average concentration of TSS in the effluent of Anaerobic Oxic (AO) and Anoxic + Oxic Settling Anaerobic (A+OSA) processes reported in Zhou et al. is similar to the present study [14, 17].

Effluent COD changes

The Mean±SD effluent COD changes in 45 days of MLE, MLE-OSA$_4$, and MLE-OSA$_6$ processes were 14.27±4, 12.19±2.9, and 11.98±2.9 mg/L, respectively, after reaching the steady-state condition (Figure 3).

It was always less than 60 mg/L, which is the permitted limit based on IDE for discharging effluent to surface water resources. In other words, the mean changes of COD in the effluent in MLE-OSA$_4$ was 14.57% less

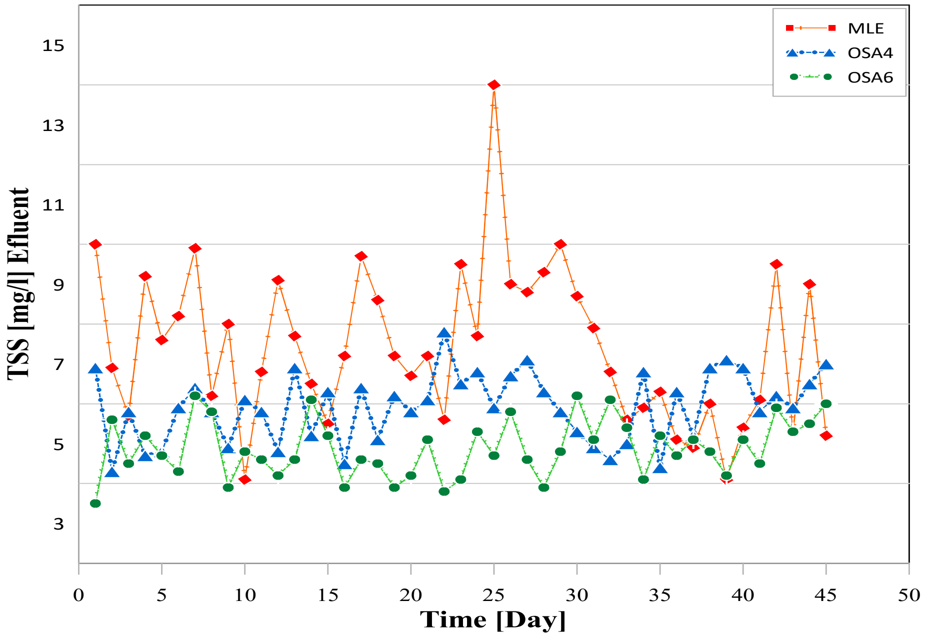

Figure 2. Concentration changes of tss in effluent of studied processes during the time

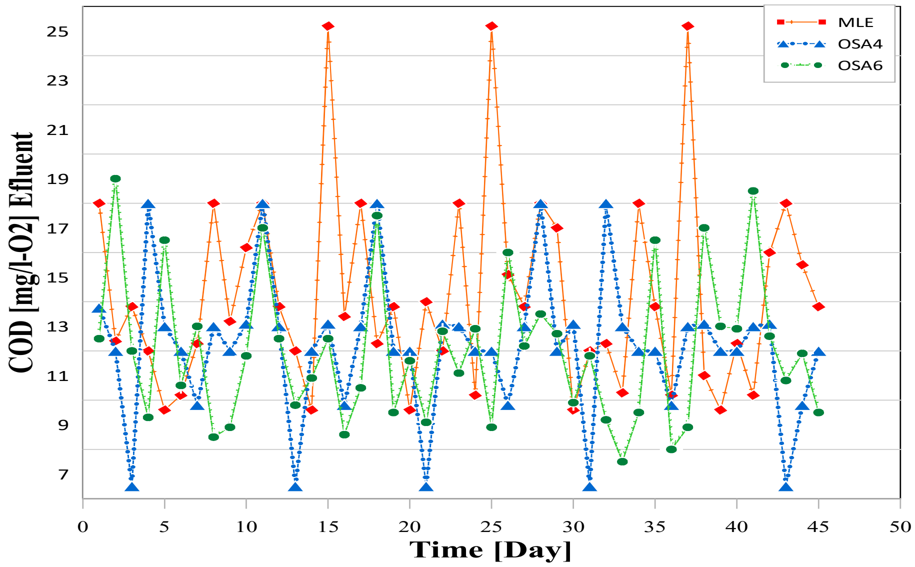

Figure 3. Concentration changes of Chemical Oxygen Demand (COD) in effluent of studied processes during the time

than the MLE process, and similarly, in the MLE-OSA$_6$ process, the value showed a 16.08% decrease compared to MLE. It is inferred that this decrease is due to the low ORP and increase in substrate distribution in SHT, which agrees with the findings of a similar study [18]. Velho et al. reported an Mean±SD COD concentration in effluent of CAS and AS+ASSR processes as 19±3 and 3±20 mg/L with a removal efficiency of 94% and 95%, respectively [1].

Zhou et al. concluded that the Mean±SD COD concentration in effluent of AO and A+OSA processes were respectively 41.8±13.6 and 41.10±6.8 mg/L so that the average COD removal efficiency in AO and A+OSA were close to each other (nearly 85.3%) [14]. Vitanza et al., in their study, demonstrated that the effluent COD concentration in the OSA process was less than the legal limit in Italy, with an average of 39 mg/L [9]. Similarly, Wang et al. reported that the effluent COD concentration in OSA-based Sequencing Batch Reactor (SBR) system was about 20 mg/L with 93% removal efficiency [11]. On the other hand, Ye et al. demonstrated that COD removal in modified CAS-OSA with anoxic/anaerobic tank with different retention time in SHT was not significant, and the change in COD removal efficiency from OSA and control system (2%-3%) was negligible [15]. Chen et al. detected the COD distribution in the SHT of the Membrane Bio Reactor - Oxic Settling Anoxic Membrane bioreactor (MBR-OSA) system, particularly in ORP values lower than -100 mV. However, it was found that this COD is consumed after sludge return to MBR. In other

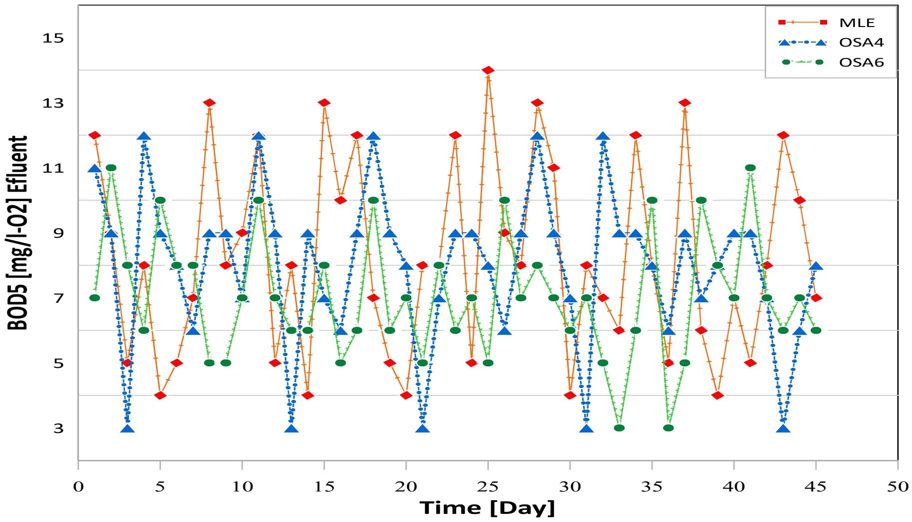

Figure 4. Concentration changes of Biochemical Oxygen Demand (BOD$_5$) in the effluent of studied processes during the time

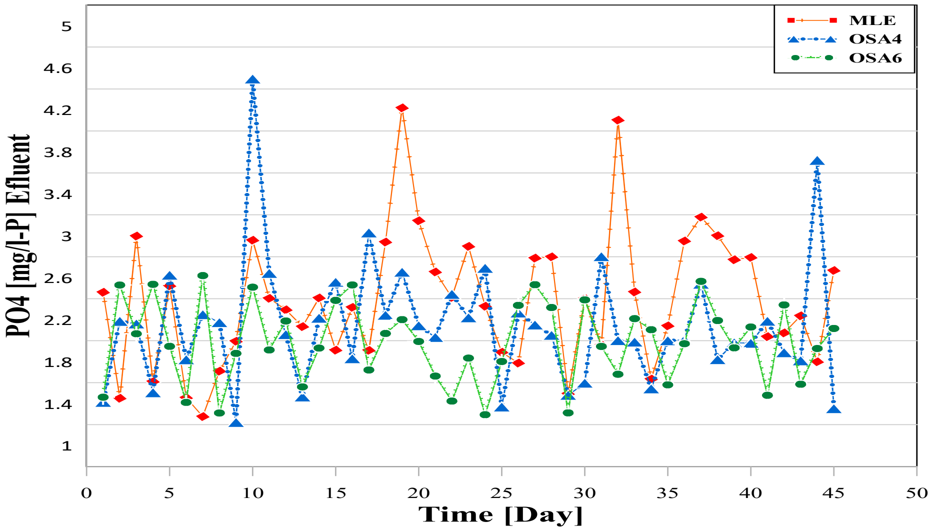

Figure 5. The concentration changes of PO_4-3 of effluent in the studied processes during the time

words, the OSA process leads to COD removal, and most of the excess produced COD in OSA is biodegradable and has the least effect on COD removal efficiency [19]. Generally, it can be inferred that when the sludge is exposed to low ORP in the anaerobic tank, it may be subject to a starvation condition which increases the substrate removal ability in the aeration tank with the presence of nutrients [19]. The study conducted by Oliveira et al. revealed that COD online release in an anaerobic tank might happen under stress condition, i.e., low ORP level and starvation in ASSR [20]. Although dissolved COD is released under the effect of organic matter cellular lysis and hydrolysis due to metabolism and destruction of sludge in SHT with low ORP, it provides a greater carbon source for increasing denitrification in an anoxic tank [19]. In their study, Foladori et al. demonstrated that hydrolysis and solubilization of non-bacterial material occur in the anaerobic condition in an ASSR-OSA system with full-scale. Also, there was a considerable increase in dissoluble biodegradable COD and NH_4^+-N in the anaerobic sludge treatment. In contrast, the destruction and lysis of bacterial cells mostly occur under aerobic conditions [21]. Another study was conducted by Demir et al., who compared the effluent COD in the enhanced CAS-OSA process and the control system. They found that COD concentration in the effluent of the OSA process (the reactor fed with the sludge from the anaerobic tank) was significantly lower than that of the control system due to the excess amounts of substrate. Nevertheless, installing an anaerobic tank will improve the COD removal with the same influent COD concentration [5]. However, it should be considered that substrate loading is a crucial factor affecting the performance of the system [11, 12] and achieving these results is related to the organic loading in the pilot. Since we used real wastewater in the current study, the organic loading constantly fluctuated.

Effluent BOD changes

The Mean±SD changes in BOD_5 concentration of effluent in 45 days of operation and maintenance of MLE, MLE-OSA_4, and MLE-OSA_6 after reaching the steady-state condition were 8.22±3, 7.97±2.4, and 7.02±1.9 mg/L, respectively with a falling trend (Figure 4).

In other words, the average BOD levels of effluent were 2.97% and 14.59% lower in MLE-OSA_4 and MLE-OSA_6 compared to the MLE process. Some of the influent BOD_5 produced in SHT due to the organic matter hydrolysis and sludge metabolism, and the decay was consumed and changed into the end products and energy for metabolism and cellular growth depending on the electron acceptability. The rest was a source of carbon supply for denitrification in anoxic reservoirs and holding tanks in this process. In other words, most of the dissolved compounds produced in the anaerobic reactor are biodegradable, and slight changes have been observed in the removal efficiency [22]. In line with the present study, Velho et al. concluded that the average BOD_5 concentration of effluent in CAS and AS+ASSR processes were similar (5±0.1 and 5±0.3 mg/L, respectively) with a removal efficiency of 96% and 97%, respectively [1].

Concentration Changes of Phosphorus in the Effluent

Mean±SD change of phosphorus concentration in the effluent processes were 2.386±0.636, 2.147±0.603, and 1.978±0.387 mg PO_4^{-3}-P/L, respectively after 45 days of operation and maintenance of MLE, MLE-OSA_4, and MLE-OSA_6 (illustrated in Figure 5 and Table 3). The mean differences of PO_4^{-3} of effluent in the studied

Table 3. The concentration level of PO_4^{-3} of effluent in the studied processes

Variable	Process	Days	Mean* (mg/L)	SD	F	P
	MLE	45	2.386 B	0.636		
PO_4^{-3} Eff	MLE-OSA_4	45	2.147 AB	0.603	5.939	0.003
	MLE-OSA_6	45	1.987 A	0.387		

*The observed indices reveal the significance level of the means in groups based on the Tukey test.

MLE: Modified Ludzack Ettinger; OSA: Oxic Settling Anaerobic.

processes considering the F-value and P-value of less than 0.05, presented in Table 2, demonstrated that the observed difference was statistically significant. Furthermore, the Tukey test showed that the mean difference of phosphorus in the effluent was statistically significant between MLE and MLE-OSA_6. However, no significant differences were found between MLE-OSA_4 and MLE-OSA_6 for the mean phosphorus concentration in the effluent and similarly between MLE and MLE-OSA_4.

Effluent phosphorus concentration changes

The Mean±SD changes in the concentration level of phosphorus in the effluent in 45 days of operation and maintenance of MLE, MLE-OSA_4, and MLE-OSA_6 after reaching the steady-state condition were 2.38±0.6, 2.14±0.6, and 1.98±0.38 mg/L, respectively in PO_4^{-3}-P and followed a descending order. In other words, the average phosphorus level in the effluent of MLE-OSA_4 compared to that of the MLE process decreased 10%, and in MLE-OSA_6, it decreased up to 16.7%. The results of the ANOVA test and post hoc Tukey with the P value 0.05 emphasized a greater decrease in the effluent phosphorus average in MLE-OSA_6. Therefore, the mean differences of effluent phosphorus in MLE-OSA_6 in contrast to MLE were significant. However, the difference in the mean value of effluent phosphorus between MLE-OSA_4 and MLE-OSA_6 and between MLE-OSA_4 and MLE was not statistically significant. Other studies also demonstrate that the OSA process can contribute to removing phosphorus. The phosphorus in eukaryotes and prokaryotes can be stored in the intracellular volutin granules as

Table 4. The amount of NH_4+, NO_2-N, NO_3-N, and TN in Effluent (Eff) in the studied processes

Variable	Process	Days	Mean* (mg/L)	SD	F	P
	MLE	45	0.383 ^{A}	0.091		
NH_4^+-N Eff	MLE-OSA_4	45	0.682 ^{B}	0.095	230.937	0.0009
	MLE-OSA_6	45	0.749 ^{C}	0.075		
	MLE	45	1.809 ^{C}	2.404		
NO_2-N Eff	MLE-OSA_4	45	0.884 ^{B}	0.499	91.687	0.0009
	MLE-OSA_6	45	0.549 ^{A}	0.276		
	MLE	45	9.073 ^{C}	2.404		
NO_3-N Eff	MLE-OSA_4	45	7.324 ^{B}	0.991	32.29	0.0009
	MLE-OSA_6	45	6.236 ^{A}	1.340		
	MLE	45	11.377 ^{B}	2.367		
TN Eff	MLE-OSA_4	45	9.022 ^{A}	0.864	37.517	0.0009
	MLE-OSA_6	45	8.437 ^{A}	1.538		

*The observed indices reveal the significance level of the means in groups based on Tukey's test.

MLE: Modified Ludzack Ettinger; OSA: Oxic Settling Anaerobic.

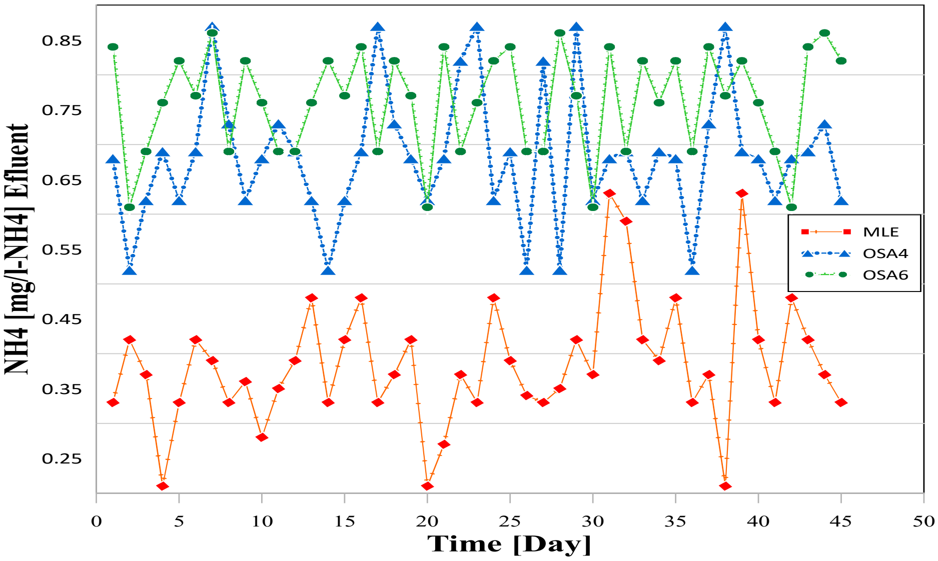

Figure 6. Concentration changes of ammonia nitrogen effluent in the studied processes during the time

polyphosphate. Unlike nitrogen and carbon, phosphorus cannot be removed from the wastewater in the form of gas; therefore, it would be removed by discharging some sludge as excess sludge. The phosphorus in effluent from activated sludge is approximately 90% of orthophosphate [16, 23, 24]. The selective process of phosphorus removal includes an anaerobic phase during which the stored phosphate is released into the dissolution simultaneously with energy release. Then, in the aerobic phase, microorganisms consume the excess phosphorus, and the energy produced in this stage is stored in the phosphorus bonding of polyphosphates as the future energy source. After applying the OSA technique, the process of Enhanced Biological Phosphorus Removal (EBPR) or excess absorption of phosphorus higher than cellular need is also performed by bacteria. EBPR process contributes to the absorption and secretion of orthophosphate by polyphosphate-accumulating organisms (PAOs) through recirculation of sludge between aerobic and anaerobic phases; phosphorus is removed by disposal of orthophosphate-rich sludge. The primary structure of EBPR consists of an anaerobic tank and an aerobic tank with a recirculation line between them [4, 25]. The OSA includes a redox condition similar to the EBPR network, which encourages selecting high-polymeric inorganic polyphosphates (polyP) bacteria [4, 17].

The percentage of phosphorus in the activated sludge is about 1%-3%, whereas it is 6%-7% when the EBPR process is used. This process is rather inexpensive and can remove phosphorus in low concentrations. It also reduces the costs of chemicals and sludge disposal, which are associated with the chemical disposal of phosphorus [24]. Two groups of bacteria are used; both fermentative bacteria and polyphosphate (polyP) or polyphosphate accumulators as phosphorus accumulating organisms [26]. Fermentative bacteria are facultative anaerobe, while polyP bacteria are solely aerobic. The key point of EBPR is exposing PAO bacteria in both aerobic and anaerobic conditions [16, 24]. Sludge passage or periodic circulation through SHT contributes to the growth of phosphorus accumulating organisms capable of biologically removing the phosphorus [1, 27-30]. Velho et al. reported that adding ASSR can affect the mechanism of biological phosphorus removal. They reported that the average concentration of TP effluent in CAS and AS+ASSR processes with an 8% daily return of the activated sludge mass in ASSR was 1.1±6 and 1.4±0.7 mg/L with the removal efficiency of 60% and 66%, respectively. This finding agrees with the rising trend of removal efficiency in the current study, which were 31%, 36%, and 39% [1].

Wang et al. studied the concentration of effluent phosphorus in four different reactors of SBR-OSA with diverse SRTs and found that it was less than 0.35 mg/L. They attributed it to enhancing microbial community, optimized organic components, and increased PAOs [11]. In another study by Ye et al., they demonstrated that TP removal efficiency in the enhanced process of CAS-OSA increased 19% when the anoxic/anaerobic was located in SHT with different retention times. The process was dependent on substrate loading and phosphorus absorption in biomass [15]. Goel and Noguera et al. in two different studies showed the enhanced biological phosphorus removal in the SBR-OSA process. They revealed that EBPR SBR-ASSR had a greater removal (97%) than the control EBPR SBR (84%), despite increasing

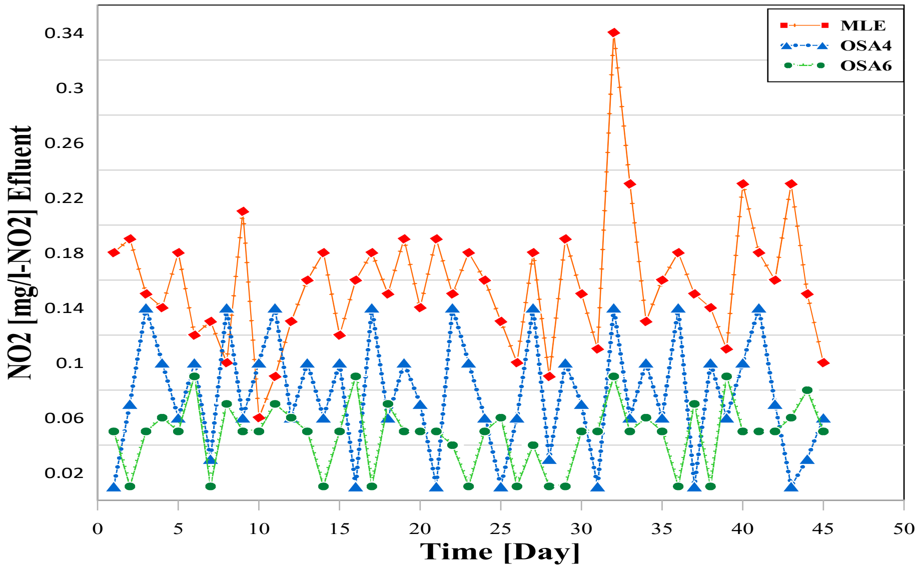

Figure 7. Concentration Changes of Nitrite Effluent in the Studied Processes During the Time

phosphate distribution in the anaerobic phase [4, 27]. Vitanza et al. employed the OSA technique in the anoxic/anaerobic phase in CAS using real wastewater with Return Activated Sludge (RAS), 25% of influent. They reported the poor performance of phosphorus removal. Given the operational problems of sludge disposal from the pilot tank, it was periodically removed from SHT, which was in contrast to the primary principles of the EBPR technique (enhancing excess sludge phosphorus using PAOs). Therefore, the influent phosphorus concentration level was usually high because of the higher influent phosphorus level from the holding tank and decreased cellular absorption in the aeration tank [9]. Sun et al. found that the effluent quality in UNITANK-OSA lacked a considerable effect, though TP concentration had slightly increased [10]. Wang et al. investigated microbial community structure to discover the effect of an anaerobic tank in the OSA process on the performance and biological phosphorus removal efficiency [11]. The total phosphorus in the aerobic sludge of the OSA process was twice as much as the reference process. The results revealed that the accumulation of biological phosphorus led to a greater phosphorus removal efficiency in the OSA system. Therefore, phosphate accumulating

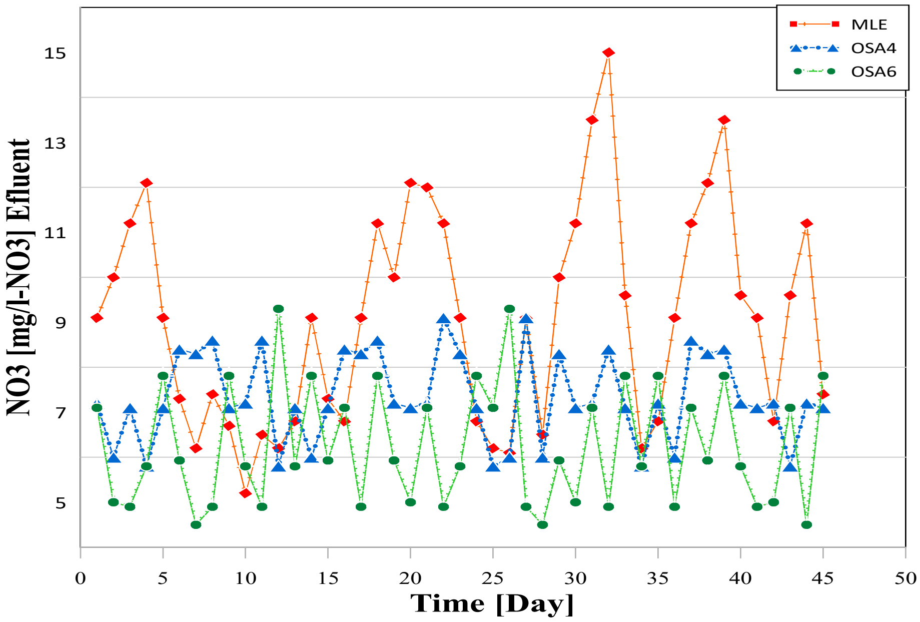

Figure 8. Concentration changes of nitrate effluent in the studied processes during the time

bacteria suggests the possibility of removing phosphate more than usual in aerobic conditions [31]. Demir et al. compared the effluent PO_4-P in an enhanced CAS-OSA process with that of a control system. They concluded that the increase in the effluent phosphorus concentration in the OSA process from 4 to 6.8 mg/L was due to maintaining sludge at an OPR level under -250 mV and without any external food as well as phosphorus release after anaerobic conditions [5]. To prevent leakage of phosphate to the effluent, they enhanced the configuration of the system, used metabolic behavior of POAs in EBPR process, considered the sludge recirculation ratio, initial ratio of COD/P, and ORP maintenance. It should also be considered that a lower ORP level leads to a decrease in sludge and an increase in phosphate release in anaerobic conditions [1, 4]. Because of the periodical passage of sludge in anaerobic conditions where EBPR is repeated, polyP bacteria get stressed (low level of ORP and starvation condition in ASSR), which leads to an increase in organic phosphorus in the biomass and increase in phosphate removal through removed sludge and decrease in effluent phosphorus removal in OSA system. The sludge phosphorus percentage is normally 1%-3%, while the activated sludge phosphorus level in the EBPR system is 6%-7% [10, 24]. Henze et al. found out that the average sludge phosphorus in enhanced CAS-OSA is 0.07 mgP/mgVSS, which is 3 times greater than CAS processes, i.e., 0.02 mgP/mgVSS [32]. Therefore, placing the sludge holding tank leads to an increase in PAOs growth. However, a high phosphorus removal rate cannot be obtained without an effective anaerobic releasing process, i.e., an enhanced OSA process [33].

Changes of ammonia nitrogen, nitrite, nitrate, and total nitrogen of the effluent

Figures 6, 7, 8 and 9 illustrate the mean concentration changes of ammonia nitrogen, nitrite, nitrate, and total nitrogen in the effluent in 45 days of operation and maintenance of MLE, MLE-OSA$_4$, and MLE-OSA$_6$ processes after reaching the steady state. Based on the F value and P-value 0.05, stated in Table 4, the analysis of the mean differences of NH_4^+, NO_2-N, NO_3-N, and TN in the effluent reveals that the differences were significant. Moreover, the Tukey test shows that the mean differences of ammonia nitrogen, nitrite, and nitrate were statistically significant in the three processes. On the other hand, the mean differences of TN concentration changes in the effluent of MLE-OSA$_4$ and MLE-OSA$_6$ compared to the MLE process were not statistically significant.

Effluent ammonia nitrogen changes

A slight increase in the effluent ammonia nitrogen in the enhanced MLE-OSA system in 45 days after reaching the steady-state was because of anaerobic metabolism of nitrifiers and increase in cellular lysis in SHT [34]. Since the standard level of ammonia nitrogen amounts in the effluent is 2.5 mg/L, this slight increase does not inhibit achieving the world discharge standards. Cellular decay and lysis, as well as ammonification in anaerobic conditions and endogenous conditions in SHT in 4-h and 6-h of HRT, has led to protoplasm decay, adding ammonium ion to the dissolution. Consequently, by sludge return from anoxic tank to aeration tank, some ammonium ion is oxidized and changed into nitrate (biological nitrification) under the cellular assimilation process and by nitri-

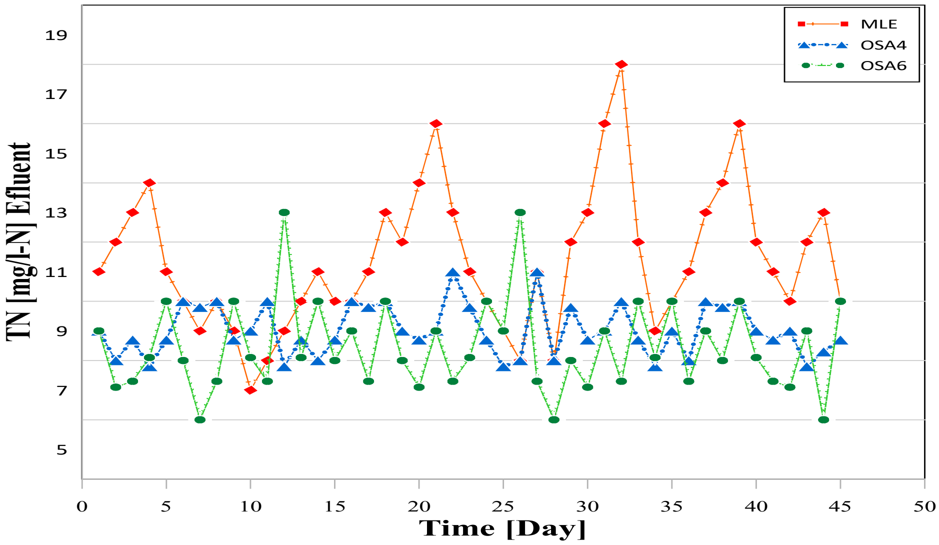

Figure 9. Concentration changes of TN effluent in the studied processes during the time

Table 5. The standards for effluent (discharge into surface water resources) and measured values in the studied processes

Parameter / Type of Process	TSS	COD	BOD_5	PO_4^{-3}-P	TN-N	NH_4^+-NH_4	NO_3-NO_3	Reference
Iran standard	40	60	30	6	14	2.5	50	Iran IDE
Sari Wastewater Treatment Plant*	40	50	30	**	14	2	48.71	Design principles
MLE	7.41	14.27	8.3	2.39	11.37	0.49	40.16	Present study
MLE-OSA_4	5.92	12.19	7.97	2.15	9.02	0.87	32.41	Present study
MLE-OSA_6	4.88	11.98	7.02	1.99	8.43	0.98	27.59	Present study
AS+ASSR	6.7±0.3	20±3	5±0.3	1.4±0.7	9±3	0.63	-	[1]
SBR-OSA	-	22	-	0.25-0.32	9.7-10.9	0.1-0.12	42.5-47.8	[11]

*The information is set according to the system design principles.

** Phosphate removal as a pollutant is not considered in the design principles.

MLE: Modified Ludzack Ettinger; OSA: Oxic Settling Anaerobic; AS: Activated Sludge; ASSR: Activated Sludge-Anaerobic Side-Stream Reactor; SBR: Sequencing Batch Reactor.

fiers, and the rest was removed through the effluent [35]. Zhou et al. worked on conventional activated sludge with anoxic/anaerobic phases (A+OSA) to decrease sludge and remove nitrogen. They reported that Mean±SD effluent ammonia nitrogen was 7.4±8.5 (NH_4-N) in A+OSA, which was greater than the AO process, i.e., 5.3±8 (NH_4-N). This result highlights a lower efficiency of nitrification in AO equipped with OSA, which can be attributed to nitrifiers' cellular decay in the anaerobic reactor (SHT) [14]. However, for denitrification in an anoxic tank, the average TN removal in A+OSA is greater than AO because this system has more access to carbon resources from cellular lysis and hydrolysis reaction [17]. Similarly, Demir et al. found out that an increase in ammonia nitrogen removal in the enhanced CAS-OSA effluent was due to denitrification. However, COD and NH_4^+-N distribution as biodegradable compounds produced in SHT normally yield a slight intervention in the efficiency of ammonia nitrogen removal [36]. In the long run, both systems displayed similar removal efficiency for NH_4^+-N [5]. Velho et al. reported that the Mean±SD concentrations of effluent ammonia nitrogen in CAS and AS+ASSR were 0.55±0.11 and 0.49±0.14 mg/L with a similar removal efficiency of 98% associated with NH_4^+-N release in the anaerobic tank and quick consumption of it in aerobic conditions [1, 22]. Wang et al. revealed that effluent NH_4^+-N concentration in OSA-based SBR was quite small and less than 0.1 mg/L. They reported that inorganic nitrogen removal would increase if cellular retention time increased [11]. Ye et al. revealed that effluent NH_4+ in enhanced CAS-OSA was not considerably affected by placing the anoxic-anaerobic tank in SHT in different HRT [15].

Effluent nitrite changes

The average effluent nitrite in MLE-OSA_4 compared to that of the MLE process decreased 51.13%, and the decrease for MLE-OSA_6 was 69.65% compared with MLE (Figure 7).

Similarly, Wang et al. reported that NO_2-N concentration in the effluent in OSA-based SBR with different HRT was relatively small and less than 0.1 mg/L, indicating the appropriate nitrification and denitrification for nitrogen removal [11]. Furthermore, Datta et al. stated similar levels of NO_2-N, NO_3-N, and NH_3-N in the effluent of control systems of BNR SBR and BNR SBR-ASSR [4, 37].

Effluent nitrate changes

The average effluent nitrate in MLE-OSA_4 compared to that of the MLE process decreased 19.27%, while the decrease for MLE-OSA_6 was 31.26% compared with MLE (Figure 8).

It was a reasonable outcome of placing SHT and a favorable condition for denitrification and enhancing PAOs. In other words, denitrification rate in Return Activated Sludge (RAS) is added to the denitrification rate of the internal recirculation (IR: Mixed Liquid Recirculation) stream, and this is the reason beyond increasing denitrification rate in the OSA process [38]. Chen et al.

in a study found that NO_3-N in MBR-OSA effluent (11-25 mg/L) was less than that of MBR (34 mg/L) and nitrate concentration in MBR-OSA effluent had a reverse relationship with ORP in OSA system (-250 mV to 100 mV). It shows that OSA has activated denitrification, and its efficiency was enhanced in anaerobic conditions [19]. Similarly, Demir et al. compared the results of NO_3-N in the effluent of enhanced CAS-OSA with a control system and found out that in the OSA process, there was a considerable decrease in NO_3-N concentration which could be attributed to denitrification [5]. Wang et al., in their study on OSA-based SBR system with different HRTs, showed that effluent NO_3-N concentration was less than international standards of effluent removal to receiving water indicating appropriate nitrification and denitrification for nitrogen removal [11].

Effluent total nitrogen changes

The average total nitrogen in the effluent of MLE-OSA_4 and MLE-OSA_6 decreased to 20.7% and 25.85%, respectively, compared to MLE. The falling trend in enhanced MLE-OSA with 4-h and 6-h HRT was because of the biological nitrification and denitrification conditions (Figure 9).

Velho et al. found that the average effluent TN concentrations in CAS and AS+ASSR processes were 9±3 and 9±2 mg/L, respectively, with a removal efficiency of 78% [1]. Moreover, the study conducted by Zhou et al. revealed that effluent TN in the enhanced A+OSA process was (21.9±10.3 mg/L) less than that of the control system (28.7±11.3) [17]. In the A+OSA process, the COD is released from cellular lysis and organic matter hydrolysis in SHT and so produces more carbon resources for denitrification in the anoxic tank. The average TN removal in AO and A+OSA systems were reported at 43.4% and 56.8%, respectively, due to decreased nitrogen oxides out of denitrification, COD release in SHT, and decline in the ratio of COD/TN. Therefore, the A+OSA process had a higher nitrogen removal efficiency than AO [17]. On the other hand, Ye et al. reported that effluent TN in enhanced CAS-OSA was not considerably affected when placing an anoxic/anaerobic tank in different retention times. In other words, removal efficiency in the CAS process was about 30%, and in the enhanced system, there was a 0%-9% decrease [15]. Foladori et al. found that the growing decline in TN is because of denitrification in the anoxic phase [39]. Wang et al. also revealed that effluent TN concentrations in OSA-based SBR system was less than 11 mg/L and stated that long SRT and the conditions in the holding tank were contributed to a slight increase in TN removal [11]. However, studies done by Vitanza et al. [9], Datta et al. [37],Troiani et al. [40] and Ye et al. [15] revealed that the OSA process had no negative effect on nitrogen removal. In other words, through an endogenous process in SHT and release of biodegradable carbon cBOD, transmitting it through return flow to the anoxic reactor, and increase in BOD: TKN ratio leads to increase in nitrate removal efficiency in the denitrification process.

Comparison of effluent quality and discharge standards to surface water resources

Table 5 presents the quality of effluent produced in the processes of this study compared to the standards of IDE for effluent releasing to surface water resources. Furthermore, a comparison is made based on the design principles of Sari WWTP and similar studies.

According to Table 4, all effluent quality indices measured in the present study are less than the standards and requirements of IDE for receiving surface water resources. Among the processes mentioned above, MLE-OSA_6 shows smaller values, except for a slight increase in ammonia nitrogen in the enhanced MLE-OSA process. Overall, in all cases, the values were less than the standards for effluent discharge.

4. Conclusion

The findings of this study demonstrated that the enhanced systems of MLE-OSA have a better function in removing pollutants. Moreover, compared to the standards of effluent discharge to the surface receiving water resources, the Iranian Department of Environment, and the principles for designing Sari WWTP, the quality of the effluent was more efficient through the MLE process. Therefore, placing the SHT enhances the effluent quality and does not intervene in measured parameters. Another finding of this study was the slight increase in effluent ammonia nitrogen level in the enhanced MLE-OSA process after reaching the steady-state condition. Since the standard for effluent ammonia nitrogen is 2.5 mg/L, this slight increase does not prevent achieving the world standards for effluent quality.

Study suggestions

Due to the continuous discharge of contaminants through effluent to receiving river, it is recommended to update and enhance the systems to advanced ones such as MLE-OSA, Anaerobic/Anoxic/Oxic (A_2O), University of Cape Town (UCT), and Vir ginia Initiative Plant (VIP).

Developing biological treatment systems can produce safe effluent according to the world standards criteria. Exploiting effluent through constructing transmission utilities and pumping for agricultural, natural resources, and industrial usage can compensate treatment costs economically and solve the issues related to the water crisis and environmental hazards.

Studying the processing of sludge cake produced by enhanced systems as fertilizer in agriculture, natural resources, and green space is highly recommended in future studies because it is rich in nitrogen and phosphate, and also the farming lands of our country, especially the north, face shortage of phosphate. It is recommended that IDE reconsider the limited amounts of phosphorus in discharging effluent of WWTPs to the receiving water and sync it with international environmental organization standards.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by Ahvaz Branch of Islamic Azad University.

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Authors' contributions

Conceptualization and performed the study design, literature review, experiments: Behzad Nikpour; Data analysis and manuscript edition: Reza Jalilzadeh Yengejeh; Involved in the study design, check & editing and project administration: Afshin Takdastan and Amir Hessam Hassani; Manuscript preparation and edition: Mohammad Ali Zazouli and Behzad Nikpour.

Conflict of interest

The authors declared no conflict of interest.

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