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Effect of hydraulic retention time and aeration time on the performance and microbial diversity in an upflow aerobic/anoxic sequential bioreactor

Ali Almasi[a], Seyyed Alireza Mousavi[a,*], Zahra Bahman[b], Mohammad Reza Zolfaghari[b], Ali Akbar Zinatizadeh[c]

[a]*Department of Environmental Health Engineering, Kermanshah University of Medical Sciences, Kermanshah, Iran, Tel. +98 09181317314; email: alialmasi@yahoo.com (A. Almasi), Tel. +98 09188336569; Fax: +98 08318263048; emails: seyyedarm@yahoo.com, sar.mousavi@kums.ac.ir (S.A. Mousavi)*
[b]*Department of Microbiology, Islamic Azad University, Qom Branch, Qom, Iran, emails: bahman_zahra@yahoo.com (Z. Bahman), mreza.zolfaghary@gmail.com (M.R. Zolfaghari)*
[c]*Department of Chemical Engineering, Razi University, Kermanshah, Iran, Tel. +98 09188581130; email: aliazinatiz@yahoo.com*

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ABSTRACT

This study investigated the microbial diversity in a laboratory-scale upflow aerobic/anoxic sequential bioreactor (UAASB) with an alternate aeration. A bacteriological study was carried out, as a cultural-based technique, detecting dominant bacteria in the aerobic/anoxic conditions. Central composite design and response surface methodology were applied to investigate the effects of two operating parameters, namely hydraulic retention time (HRT) and aeration time (AT), on the performance of the UAASB. The HRT range of 12–36 h, and the AT range of 40–60 min/constant time of anaerobic conditions (1 h), were examined on the diversity and capability of system. Results of biological investigation showed that HRT and AT have significant effects on bacterial population and diversity, which also is function of organic loading value.

Keywords: Industrial wastewater; Aerobic, anoxic; Aeration time; Bacteria consortium

1. Introduction

Industrial wastewaters comprise different types of pollutants such as organic materials, heavy metals, solvents, dyes, and pesticides that have negative effects on water sources [1]. Therefore, the environmental protection agencies have made strict regulations all around the world to control discharge of industrial wastewater into receiving waters.

Different methods applied for treating industrial wastewater, physicochemical methods are broadly used [2]; these methods are associated with some drawbacks, such as low efficiency, waste brine disposal that implies a post treatment, high capital and operating cost. Researchers reported biological process as a most favorable approach to treat wastewater with suitable BOD_5/COD ratio, because of several advantages such as cost-effectiveness process, high potential for wastewater treatment, relatively easy to control, and high stability and reliability [3].

The efficiency of biological process is affected by several parameters—pH, dissolved oxygen (DO) concentration, carbon source, energy source, temperature, biomass density, diversity of micro-organisms. Among

*Corresponding author.

them, microbial community plays a key role to achieve successful process to treat complex pollutants [4]. Therefore, identification of microbial diversity is of great importance to improve reactor operation and performance [5].

Results of the new estimation of bacterial diversity in activated sludge bioreactors predict much higher numbers of up to ~4,500 (pyrosequencing technology) than previous studies 17–268 (16S rRNA gene clone library analyses) [6]. Researchers have noted that bacterial community in a bioreactor is directly dependent on environmental and operational factors (e.g. temperature, pH, retention time, DO concentration, etc.) [6–8]. Many studies have been carried out to find the correlations between the bacterial community diversity and the bioreactors' performance to improve the operation of the bioreactors [6].

However, the dominant bacteria in biological process of wastewater with high concentration of organic matter are heterotrophic but as mentioned with changing environmental and operational parameters, the community of bacteria will change. For example, at the high COD/N and low concentration of oxygen <2 mg/L in a system with mixed culture, the dominant bacteria are heterotrophic. But when the oxygen increased to 3 mg/L and wastewater was contained with low concentration of organic matter and high concentration of ammonium, the population of nitrifying bacteria will increase significantly. Nitrifying bacteria have different metabolic potentials based on the environmental and operational conditions [9,10].

Denitrifiers are responsible for elimination of nitrite and nitrate (nitrification production) to nitrogen gas, which for adequate performance need special environmental and operational conditions [11]. Special bacteria perform biological phosphorus removal with the ability to store excess amounts of phosphorous as polyphosphates in their cells at special conditions. So phosphorous variation could be taking place in the biological processes, thereafter enrichment for effective micro-organisms would be preferable.

The purpose of this research is to investigate the effect of hydraulic retention time (HRT) and aeration time (AT) on the microbial community and upflow aerobic/anoxic sequential bioreactor (UAASB) performance. In this regard, central composite design (CCD) and the statistical method of response surface methodology (RSM) were used to design the experiments and investigate the effects of these individual factors (HRT and AT). The method considers effect of one factor at a time, and neglects interactive effects of factors. Furthermore, polynomial models were developed, and analysis of variance (ANOVA) provided the statistical results and diagnostic checking tests to evaluate adequacy of the models.

2. Materials and methods

2.1. Activated sludge and wastewater

The bioreactor was inoculated with activated sludge from aeration tank of Faraman's industrial wastewater treatment plant (FIWTP), Kermanshah, Iran. The initial concentration of the mixed liquor suspended solids (MLSS) in the reactor was 6 g/L. The feed (raw wastewater) was providing from influent of FIWTP, its characteristics are shown in Table 1.

2.2. Experimental setup and performance

An UAASB with a working volume of 2.6 L was used in this study (Fig. 1). The height and diameter of this glass bioreactor were 122 and 5.2 cm, respectively. The aerobic condition was prepared by a fine air bubble diffuser, which was connected to air pump from bottom through an airflow meter with digital timer. The airflow was kept constant to provide an oxygen concentration >6 mg/L during all runs. The anaerobic process was performed at each run after AT by turn off the air pump according to Table 2 of experimental design. The UAASB was operated under room temperature (20 ± 2°C) at different HRT (12, 18, 24, 30, and 36) and AT (40, 45, 50, 55, 60 min) based on extensive review on previous research works. Before each run, all characteristics of raw wastewater were determined and the efficiency of system was accounted based on result of sampling after sedimentation of MLSS (30 min after turn off the air pump).

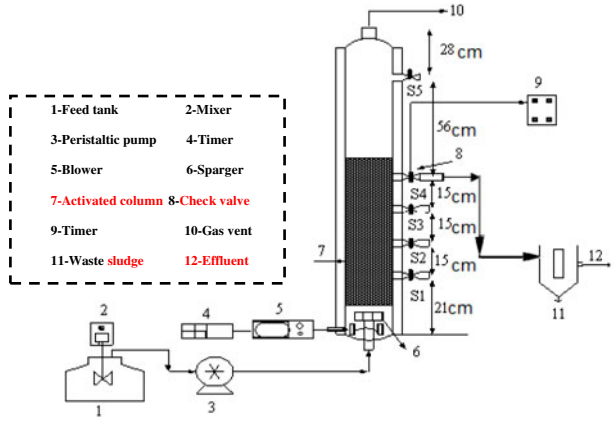

Fig. 1. Schematic of the experimental setup for the UAASB reactor with five sampling valves (S1–S5).

2.3. Analytical procedure

Analysis of COD, MLSS, and total Kjehldahl nitrogen (TKN), nitrate, total nitrogen (TN), total phosphorous (TP), sludge volume index (SVI), and settling velocity was performed following standard methods [12]. The DO concentration inside the reactor was determined using a DO probe (WTW DO Cell OX 330, electro DO probe, Germany), and turbidity was measured by a turbidity meter (model 2100 P, Hach Co., USA).

2.4. Microbial community analysis

The microbial community analysis was accomplished before turn off aeration. The cultural-based identification and enumeration were carried out for heterotrophic bacteria, total coliforms, fecal coliform, fecal streptococci, *Staphylococci*, *Clostridium perferenjence*, nitrifyers, and denitrifyers according to standard methods for the examination of water and wastewater [12]. At different runs after achieving steady state, the granular sludge form each samples according to staining method was crushed by two slides surface and six stained slides according to the gram method were prepared. Furthermore, the enumeration and estimation of the viable bacterial cell was carried out at ratio of granule/sterilized dilution water equal 1, which diluted by 10–10 dilution factor. The prepared samples were cultured on selective culture media for isolation and identification according to novel technique that developed in this study. Some of the bacteria are very slow growers, are very hard to cultivate, and would not be counted on an HPC plate. Clostridia are severe anaerobes and hence would not be found in the population growing on the plate count media. The multifarious minimal media (e.g. R2A) lead to retrieval of more strains from diverse bacterial genera than the generally used PCA, and the results display that R2A medium can be more suitable when compared with PCA [13].

2.5. Experimental design and data analysis

The design of experiments software (DOE: version 6.0.6) was used to design the experiments and statistical analysis of data using CCD and RSM, which has the ability to eliminate errors systematically with an estimate of the experiment, minimize the number of experiments, and determine an empirical model based on the experiments performed [14,15]. The study was carried out to optimize operation conditions and to the study the interactive effects of experimental factors, namely "HRT" and "AT." The effects of operational parameters well known on the efficiency of biological system, which can be due to diversity of the microbial community in different operation conditions. The optimum regions for these factors could increase the capability of the system significantly because of balance creation between microbial communities of process. The experiments were appraised based on the CCD with a factorial matrix of 9 steady state runs (Table 2). The coded value term was used to represent the independent variables at three levels: low level (−1), central (0), and high level (+1). The responses that resulted from the variable interactions with dependent parameters included the concentrations of NH_4^+-N, NO_3^--N, NO_2^--N, BOD, COD, pH and the presence of density of different types of bacteria (total coliforms, fecal coliform, fecal *Streptococci*, *Staphylococcus*, *C. perferenjence*, nitrifiers, and denitrifiers) to fulfill an inclusive statistical analysis of reactor performance and microbial diversity via UAASB. The responses were measured online in all experiments. After accomplishing the experiments at a set value of independent variables (HRT and AT), the experimental data were used to develop an empirical model using ANOVA via the Design-Expert software. The significance of the variables was recognized based on the confidence levels above 95% ($p < 0.05$) in the polynomial model [14]. Furthermore, the ANOVA method was applied in the graphical analysis of data to accomplish the interaction between the variables and responses.

3. Results and discussions

3.1. Development of models and ANOVA

The achieved results for mentioned responses in the reactor were analyzed, and relation between two operating parameters in the UAASB (*A*: HRT and *B*: AT) and eight important process responses were mathematically modeled by RSM. Table 3 represents achieved models and pertinent ANOVA results. Data in Table 3 demonstrate that the models were significant at the 5% confidence level since P-values were less than 0.05. A high R^2 value, close to 1, is desirable and appreciable result. "Adjusted R^{2}" is R^2 adjusted for the number of terms in the model relative to the number of points in the design. An estimation of the fraction of overall variation in the data shows the accounted coefficients by the model. A measurement of the Predicted R^2 indicates the amount of variation in new data explained by the model. A reasonable agreement of Adj. R^2 with Pred. R^2 is necessary and difference should not be greater than 0.2 (20%). Therefore, achieved results reveal that the data fit the

models strongly. "Adequate Precision" compares the range of predicted values at the design points to the average prediction error (measure of signal to noise ratio). Moreover, as the requirement of the models, Adequate Precision should be greater than 4 in order to show that the noise is not contributing any error in the response surface, and the values according to Table 3 proved that the models did not have any significant error due to the noise. Hence, statistical analysis revealed adequacy of models, and developed models can be used to navigate the design space defined by the CCD.

3.2. Microbial community analysis

Table 3 summarizes the different kinds of micro-organisms that known in the system as heterotrophic bacteria, coliforms, *Colesteridium*, *Streptococcus* and *Staphylococcous*, nitrifiers, and denrtrifiers under the different operation conditions. Thus, they are dominant genera that are observed between micro-organisms in the HRTs and aeration regime in level of ≤0.05 of statistical analysis. Results depict that the maximum microbial communities of heterotrophic bacteria in both R2Agar and nutrient agar took place at the lowest density of bacteria when HRT was 12 h, and it was promoted when AT increased up to 60 min. It is also revealed that in the low and high ATs (40 and 60 min), microbial density was not efficiently reduced; however, it was revealed when AT was 60 min, the density increased slightly.

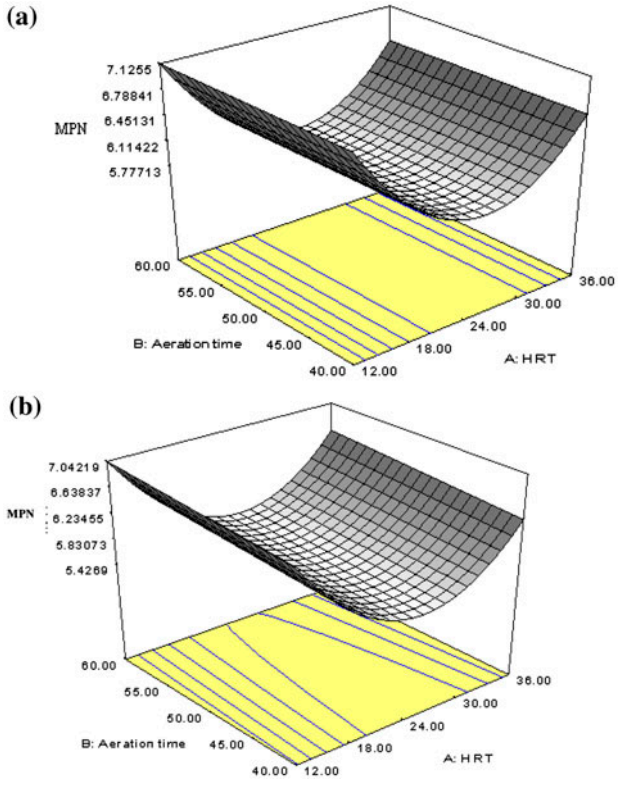

Fig. 2. Design-expert plots: response surface plot of microbial communities for total coliform (a) and fecal coliform (b).

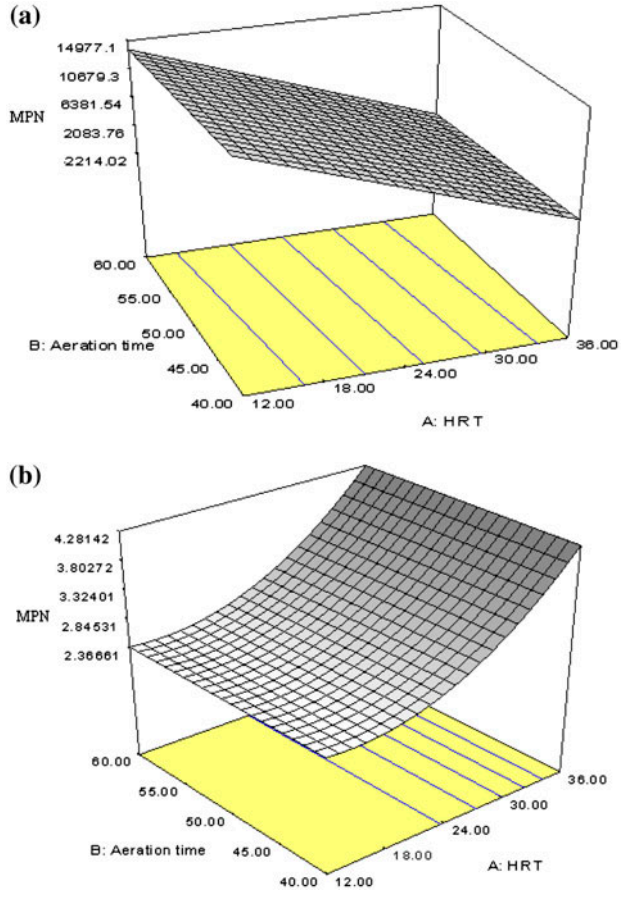

Fig. 3. Design-expert plots: response surface plot of residuals for microbial communities as denitrifying bacteria (a) and nitrifying bacteria (b).

The result of RSM in Fig. 2(a) also demonstrates that different values of HRT have various effects on the bacterial growth rate; results show similar condition for both responses, namely "total coliform (TC) and fecal coliform (FC)." Moreover, an increase in the HRT caused a decrease in the value of TC and FC gradually, however, in the HRT more than 30 h, the value of TC and FC tented to increase. In the effluent, fecal coliforms were in the range of 4 Log (CFU mL^{-1}), while no one was detected in effluent. Khan et al. confirmed that the percentage removal of the TC and FC concentration in an upflow anaerobic sludge blanket followed by aerobic post treatment depends on different variables, specially HRT [16].

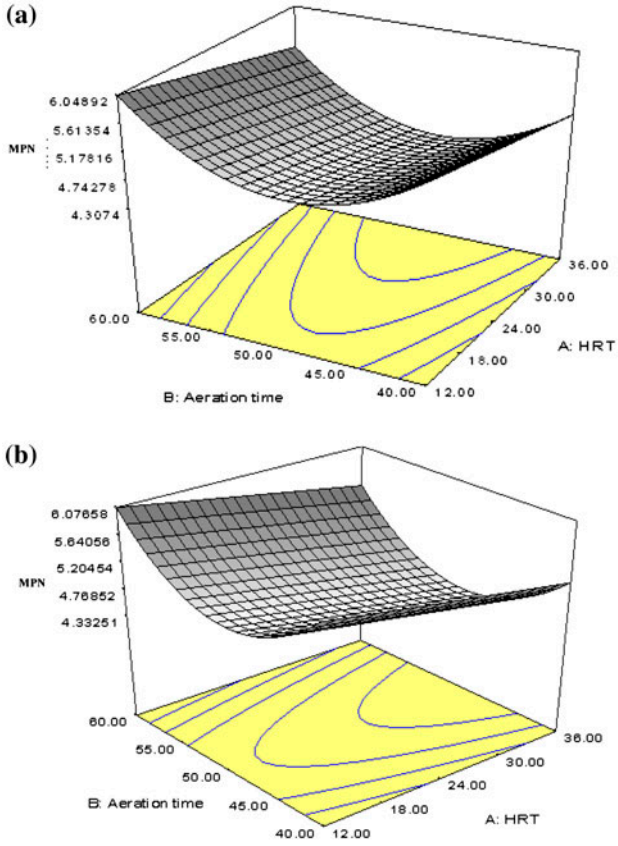

Fig. 4. Design-expert plots: response surface plot of residuals for microbial communities as *Clostridium* (a) and *Staphylococci* (b).

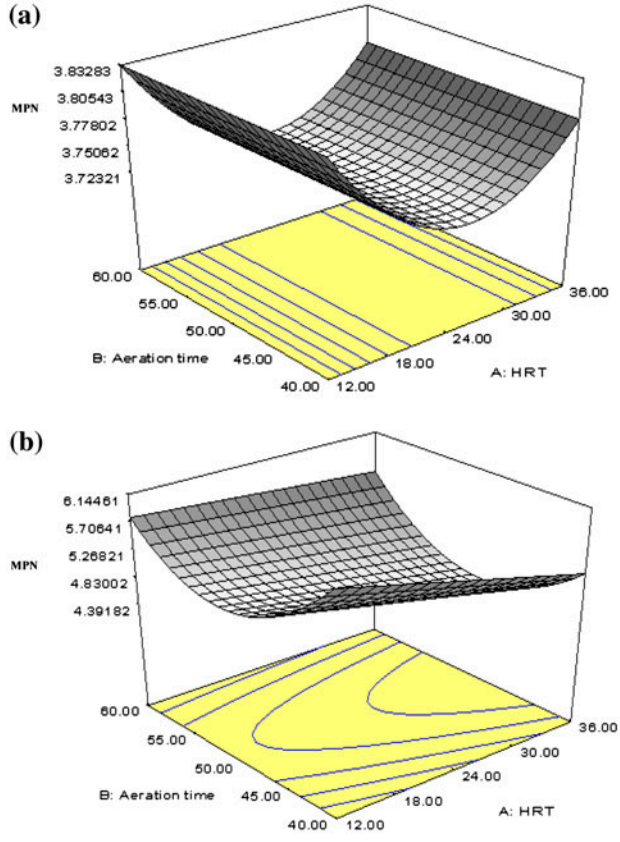

Fig. 5. Design-expert plots: response surface plot of residuals for microbial communities as bacterial biomass (a) and *Streptococcus* bacteria (b).

Fig. 2 shows that HRT was the important factor affecting bacterial population according to the obtained equation from bacterial data analysis. The low density of micro-organisms probably can be attributed to a decrease in F/M ratio inside the reactor at higher retention time. An increase in the biomass of the HRT 36 h is due to decomposition of micro-organisms, which can be source of food and subsequently regrowth of micro-organism.

The population of nitrifier bacteria increased as function of gradually increasing HRT. However, short HRT caused degradation of entire biodegradable organic material by heterotrophic bacteria, lack of food during operation of system at long HRT led to endogenous respiration phase resulting in a decrease in the bacterial population. Fig. 3(b) shows decrease in the denitrifiers population significantly due to increase in the HRT as function of enough organic carbon source for this type of bacteria, which can be a limiting factor for denitrification process in low concentration. According to Fig. 3, the different ATs did not show significant effect on denitrification process, but the oxygen concentration as one of the controlling factors in denitrification process can limit this process due to severe effect on denitrifying bacteria and reduce the efficiency of system. Mota and co-workers have confirmed that ammonia-oxidizing population is affected by operational conditions; among them, oxygen levels not only has effect on nitrification rate but also the species level of AOB [17], that are in agreement with a number of previous studies [18–20]. Nogueira et al. investigated the effect of HRT on the nitrifying and heterotrophic population dynamics in biofilm reactors that results reviled; minor effect of the HRT on the diversity of nitrifying bacteria in the biofilm. Furthermore, combined nitrification and carbon removal under oxygen limiting conditions could be accomplished in the biofilm reactor with low HRT but failed in the reactor with high HRT. This unexpected finding was caused by the formation of a thick heterotrophic layer on top of the nitrifying biofilm in the latter reactor that limited the nitrifiers oxygen supply. Thus, extension of the HRT is not always sufficient to develop combined nitrification and organic carbon elimination in biofilm reactors [21].

Table 1
Characteristics of Faraman's industrial estate wastewater

Parameters (mg/l)	Amount	SD[a]
TCOD	1,050	±150
BOD_U	380	±75
BOD_5	300	±20
nbCOD	750	±150
TN	250	±50
TP	51	±5
TSS	220	±140
pH[b]	6.2	±0.7

[a]SD: Standard deviation.
[b]No unit.

Fig. 4 demonstrates that increase in the AT more than 50 to 65 min caused fast growth of bacteria. The achieved result for *Clostridium* population, as in comparison to heterotrophic bacteria in terms of undesirable food availability led to resistance by capsuling; therefore, the effect of HRT on them is negligible. Fig. 5 shows the effect of HRT values on streptococcus density as slightly and concentration of this type of bacteria decreased with an increase in the HRT. On the other hand, results show significant effect of AT on the growth of streptococcus, which in low AT (40 min), the concentration was high and with an increase in the AT until 50 min, the concentration decreased.

3.3. Analysis of reactors function and comparison

Table 2 shows the results of 9 runs for both variables according to design of experiments for BOD_5, COD, TP, and NO_3 as responses in experimental work. According to mentioned results in Table 2, the maximum BOD_5 and COD removal took place when HRT and AT were 12 h and 60 min, respectively. The percentage of BOD_5 removal was reduced when HRT and AT increased up to 24 h and AT decreased to

Table 2
CCD for the study of two experimental variables: *I* and HRT, and the achieved experimental and analytical results

Run	Factor *A*: HRT (h)	Factor *B*: AT (min)	BOD removal (%)	TCOD removal (%)	TP removal (%)	N-NO_3 production (mg/l)
1	12	40	94	62	33	20.11
2	12	60	97	75	17	41
3	18	50	93	60	−0.1	36.29
4	24	45	84	50	−0.09	36.74
5	24	50	86	51	−0.1	36.34
6	24	55	87	55	−0.13	42.43
7	30	50	86	48	−0.45	59.14
8	36	40	93	42	−0.35	65.57
9	36	60	94	41	−0.38	73.7

Table 3
The developed models and ANOVA results

Response	Modified with significant terms	Probability	R^2	Adj. R^2	Adeq. precision	SD	CV	Press
Heterotroph nutrient agar	$7.55 - 0.34\,A + 1.12\,A^2$	=0.0004	0.79	0.75	9.70	0.31	3.95	1.57
Hetetrotroph R_2A	$7.84 - 0.4\,A + 1.19\,A^2$	<0.0001	0.92	0.91	17.64	0.19	2.27	0.53
Coliform	$5.56 - 0.22\,A + 0.12 + 1.14\,A^2$	<0.0001	0.94	0.93	18.65	0.15	2.51	0.37
Nitrifies	$2.75 + 0.95\,A + 0.58\,A^2$	=0.0002	0.81	0.77	11.71	0.34	11.40	0.55
Denitrifier	$6{,}381.54 - 7{,}960.00\,A - 635.56\,B$	=0.0010	0.75	0.70	11.58	3,087.94	48.39	1.604E+008
Staphilococcous	$4.67–0.33\,A + 0.15\,B + 0.93\,B^2$	=0.0014	0.80	0.74	10.41	0.27	5.46	1.48
Stereptococcus	$4.73 - 0.33\,A + 0.91\,B^2 + 0.18\,AB$	=0.0003	0.86	0.82	13.29	0.21	4.25	1.87
Biomass concentration	$3.72 - 0.2\,A + 0.88\,A^2$	<0.0001	0.86	0.83	12.27	0.018	0.49	6.221E-003
Clostridium	$3.23 - 0.22\,A + 1.5\,A^2$	=0.0004	0.79	0.75	9.70	0.31	3.95	1.57

45 min. However, BOD_5 removal at HRT beyond 24 h increased again. On the other hand, the COD removal was efficiently reduced with an increase in the HRT.

The produce of nitrate through nitrification process depends on many operational and environmental factors described in previous researches works [6,9]. This process is predominantly performed by two chemolithoautotrophic groups of bacteria, ammonia-oxidizing bacteria (AOB) that convert ammonia to nitrite, which is widely accepted as the rate-limiting step and nitrite-oxidizing bacteria (NOB) that are responsible for the conversion of nitrite to nitrate [22]. The HRT and AT as two important operation parameters play key role in this study in which, with an increase in the HRT and AT, the produce of nitrate increased from 20 mg/L at HRT:12 h and AT:40 min to 73.7 mg/L at HRT:36 h and AT:60 min. Results confirmed domination of heterotrophic bacteria on nitrifying bacteria during run reactor at low HRT and AT due to lack of sufficient oxygen and reaction time for nitrifying bacteria. Table 2 presents the Po_4^{3-}-P concentration in influent, effluent and Po_4^{3-}-P removal efficiency during the whole operation period in the aerobic/anoxic bioreactor. The average effluent concentration was 66.47 mg/L when Po_4^{3-}-P removal efficiency was 33% in HRT of 12 h.

4. Conclusion

The results showed that the efficiency of UAASB to remove organic matter, nitrogen components, and phosphorous is significantly influenced by both factors HRT and AT. The developed models with high correlation based on the experimental results of CCD and RSM were useful to understand the direct effect of HRT and AT on the performance of UAASB. The optimum operational conditions in order to have a maximum pollutant removal rate with more than 75% removal of total COD was achieved when the HRT and AT were 12 h and 60 min, respectively. The nitrate accumulation was observed throughout the experiments according to Table 1. This study contributed to a better understanding of the role of HRT and AT ratio on the system.

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