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Survey on efficiency of BF/AS integrated biological system in phenol removal of wastewater

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ABSTRACT

Due to the efficiency and flexibility of integrated biological systems, they have been widely developed for the treatment of various waste-waters in recent decades. Present study aims at revealing the efficiency of bio-filter and activated sludge (BF/AS) integrated biological system in phenol removal from wastewater. The cylindrical bioreactors (continuous flow, 12 L) are made from Plexiglas. Interlaced plastic discs were fixed inside the reactor to hold the biofilm. The effects of pH (6.5–8), phenol concentration (100–500 mg/L), nitrogen (30–80 mg/L), phosphorous (6–16 mg/L), glucose concentration (50–500 mg/L), MLSS concentration (1450–3500 mg/L) and hydraulic retention time (2–4.4 h) were evaluated. Results of the study showed that this system could remove 500 mg/L of phenol concentration and 4–4.5 kg COD/m^{3}d organic load in 4 h under favorable conditions. Pearson correlation coefficient between removal efficiency and phenol concentration was –0.446 ($P < 0.001$). By increasing phenol concentration, removal efficiency decreased. Appropriate COD/N/P for maximum efficiency was equal to 100/10/2. Dominant identified bacteria in the system include: *Pseudomonas aeruginosa*, *Pseudomonas alcaligenes*, *acetinobacter*, *morexella* and *Brevundiomonas vesicalaris*. Phenol decomposition was done according to the second-order kinetic reaction. Concentration difference (ΔA) to retention time difference (ΔT) ratio were 53.9, 69.5 and 100 for retention times of 2.2, 6 and 7 h, respectively. The BF/AS integrated biological system is highly efficient in removing phenol from aqueous and as an environment-friendly procedure could treat waste-waters containing average phenol concentrations in a relatively short period.

Keywords: Phenol; BF/AS; COD/N/P ratio; Biological system

1. Introduction

Phenol (C_2H_5OH), one of the toxic aromatic hydrocarbons, is a colorless white solid in pure state [1]. This compound and its derivatives are used in various industries such as resin production, dye production, pest fighting toxins, pharmacy, oil refinery, petrochemical industry, coal mines, steel and aluminum industries and some other industries [2,3]. According to classification of environmental protection agency (EPA), phenolic compounds were classified as the main pollutants [4]. These pollutants are compounds with well-known impacts such as being suspected of being carcinogenic and damaging to fetus at very strong toxicity [5]. Therefore, characterization, identification and determination of the amount of phenolic compounds in environment and especially in water resources and environmental monitoring are of great importance in their controlling and dispersion and reducing their impacts on environment. In order to remove the phenol from waste-waters, there are several methods including chemical oxidation [6], surface absorp-

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tion [7], and biological purification [8]. Among the methods mentioned, biological system is of more utility due to its main advantages such as compatibility with environment [9]. Also, in this method, no harmful chemicals are used; therefore, wastewater and resulted sludge from these processes have less harmful impacts on acceptor resources compared to chemical ones [10]. Bio-filter and activated sludge integrated biological system are two biological methods. Using bio-filters to control volatile compounds dispersion was considered as a novel technology. Basically waste-waters containing little biologically decomposable materials are suitable for biofiltration. Biological filter is an encapsulated box where a substrate was installed for microorganism's fixation. By growing these microorganisms, a thin layer known as biological cover was created on these surfaces [11].

It seems that in mixed media where both biologically decomposable materials and toxic and resistant materials to biological decomposition are presented, microorganisms could overcome resistance and decompose toxic and resistant molecules. Such compounds were utilized as an auxiliary substrate in various reactors such as anaerobic sludge bed with upright flow for removing phenol and chlorophenol [12,13]. According to previous studies, volatile fatty acids, sucrose [14], glucose [15–17], acetate [18] and similar compounds which are readily decomposable biologically are considered as appropriate substrates for carbon consumed by microorganisms and as effective factors in accelerating the action of microorganisms and decreasing set-up time and preparing reactors for decomposing more resistant materials. Especially, these strategies could considerably reduce reactor's residence time. In present study, phenol removal was evaluated under anaerobic conditions and at various glucose concentrations that have high biological decomposition ability. For these reasons, present study was conducted aiming at evaluating the efficiency of bio-filter and activated sludge (BF/AS) integrated biological system in removing phenol from wastewater and effect of phenol and glucose concentration on system efficiency.

2. Materials and methods

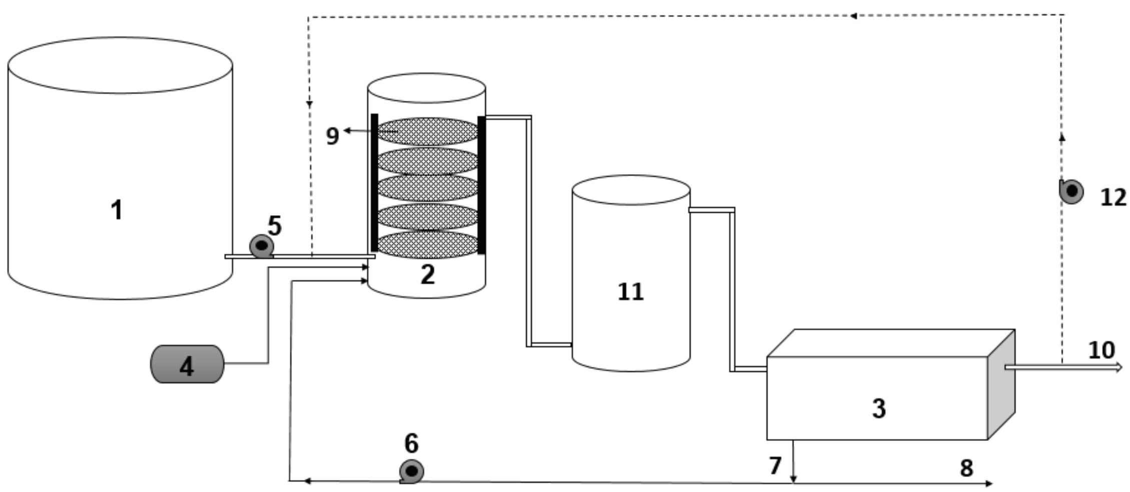
In order to conduct this empirical study, a bio-filter/activated sludge integrated with biological system was used in a series and pilot scale. The effective volume of each cylindrical reactor made of Plexiglas was about 12 L. Interlaced plastic discs were installed inside the bio-filter reactor to hold biofilm fixed. The system under study was aerobic and both reactors were equipped with diffuser aerators.

Hydraulic flow in the system was continuous and up-right. In order to inject solutions into the system, two bilateral diaphragm pumps were used. Watering capacity of the pumps was set between 0.1–2 ml/s.

Precipitation basin or clarifying pond consisted of a pond with dimensions, 20 × 30 × 90 cm (width × length × height). Flow diagram and the system design have been shown in Fig. 1.

In order to determine the amount of phenol probably released due to aeration from top of the biological reactor, total gases and vapor released from reactors were passed through impinger containing 0.1 N Na-OH solution via vacuum pump. This process continued for 14 h thanks to the capacity of vacuum pumps batteries. Sampling was conducted in three replications to ensure the accuracy of the results.

In order to adapt microorganisms to phenol, initially 0.1 mg/L of phenol along with dry milk solution was injected into the system and with the gradual increase in the concentration of phenol, dry milk concentration decreased, so within a month, phenol concentration reached 100 mg/l



1) Storage tank
2) Biofilter
3) Sedimentation Tank
4) Air Compressor
5) Pump
6) Return sludge pump
7) Sludge discharge
8) Excess sludge
9) Media
10) Effluent
11) Activated Sludge Reactor
12) Effluent recirculation pump

Fig. 1. Schematic of studied BF/AS combined system.

and within three months it rose to 500 mg/L. phenol was injected into the system as the carbon and energy source of microorganisms and nitrogen and phosphorous were added as primary products for nutrition. To evaluate the effect of nitrogen and phosphorous, the researchers considered COD/N/P ratio as 100/5/1 in the beginning of the study and then they changed their concentration.

To determine the effect of N and P on phenol removal efficiency in the system, 30, 40, 50, 60 and 80 mg/L of N and 6, 8, 10, 12 and 16 mg/L of P were added to phenol solution and its efficiency was evaluated.

In order to determine the relationship between efficiency changes by increasing various concentrations of glucose and dominant microbial species in the system at 0, 50, 250 and 500 mg/L concentrations, the dominant microorganisms were identified and determined. So, precipitated microorganisms were completely mixed in clarifier unit of the system and then 50 ml of it was transferred to laboratory and 0.001 ml was transferred into blood agar and EMB culture media by a sterile loop. To culture and isolate the existing bacteria in the sample, streak plate technique was used. Microorganisms that grew at the last dilution on the culture medium were recognized as the dominant microorganisms.

To determine kinetic order, initially various hydraulic retention times of 2.2, 6, 7 and 9 h and phenol concentration in effluent wastewater were measured. The ratio of changes in phenol concentration to changes in various hydraulic retention times was calculated after determining phenol concentration in effluent waste-waters, and then the log of the ratios was determined.

It should be noted that soluble oxygen content and system temperature in the system varied from 0.5 to 2 mg/L and from 21 to 35°C, respectively.

Results were analyzed using SPSS software Version 21 and correlation coefficients among the variables under study were analyzed via Pearson, Kendall and Spear-man correlation tests.

Table 1
Phenol removing efficiency by BF/AS combined system based on different concentrations of glucose (phenol, nitrogen and phosphorous concentrations 500, 50 and 10 mg/L, respectively)

Glucose (mg/L)	Phenol removal (%)		System total efficiency
	Bio-filter system	Activated sludge system	
0	23.9 ± 2.59	9.2 ± 4.01	33.4 ± 15.1
50	54.92 ± 19.6	7.5 ± 2.3	62.16 ± 1.3
250	28.75 ± 10.1	6.4 ± 9.03	35.9 ± 7.8
500	22.76 ± 1.8	2.1 ± 2	25.1 ± 2.7

Table 2
Recognized Bacteria at different glucose concentrations in BF/AS combined system(phenol concentration 500 mg/L)

Bacteria	Glucose Concentration (mg/L)			
	0	50	250	500
Escherichia coli	–	+	+	+
Pseudomonas aeruginosa	+	+	+	+
morexella	+	+	+	+
Brevundiomonasvesicalaris	+	+	–	–
Pseudomonas alcaligenes	+	+	–	–
acetinobacter	+	–	–	–

3. Results and discussion

3.1. Effect of glucose on phenol removal efficiency

Results of present study showed that phenol removing efficiency rose with increasing glucose concentration up to 50 mg/L, while at higher glucose concentrations, the removal efficiency reduced (Table 1). Since glucose is biodegradable and phenol is degraded slowly, so probably phenol is removed later. Then, the negative impact of glucose on BF/AS efficiency was observed. So, it could be said that in order to reach higher removal efficiency, it is essential to keep the glucose content low in waste-waters containing phenol. The delay phase of absence of glucose for phenol absorption was short. It seems that microorganisms have no tendency to absorb phenol in the presence of glucose. For this reason, with raising the concentration of glucose, the efficiency of the phenol absorption reduces. Although the consumption of glucose is affected by preventive properties of the phenol When the concentration of the glucose rises, the outlet concentration of phenol rises gradually. Complete decomposition of the phenol occurs in a shorter period of time in the absence of glucose. Pearson correlation coefficient among these variables was –0.419 ($P < 0.042$); in other words, increasing glucose concentration up to 50 mg/L increased removal efficiency and changing the glucose concentration reduced it. Also, the results of this study showed that the change in the concentration of glucose leads to changes in the microbial species in the system in a way that in zero concentration or absence of glucose, the bacteria *Pseudomonas aeruginosa, morexella, Brevundiomonas vesicalaris, Pseudomonas alcaligenes and acetinobacter* were recognized while in the presence of glucose and increasing its concentration, *Escherichia coli* were identified that dominated gradually at 250 and 500 mg/L. On the other hand, in the presence of glucose and increasing its concentration, no *acetinobacter* was recognized. In addition to *acetinobacter, Brevundiomonas vesicalaris* and *Pseudomonas alcaligenes* were removed from the system at 250 and 500 mg/L glucose and finally, *neisseria vivery bacteria* were not found at 500 mg/L glucose (Table 2). Tariqian et al. (2003) studied the effect of glucose as competitive substrate on phenol and 5- chlorophenol dissection by *pseudomonas putida*. Their results revealed that phenol dissection occurs according to Monad Equation in this condition and furthermore, in this process, glucose plays an important role as the raw matter for substrate growth [19].

3.2. Effect of mixed liquor suspended solids concentration on phenol removal efficiency

Results showed that there was direct and significant correlation between MLSS concentration and phenol

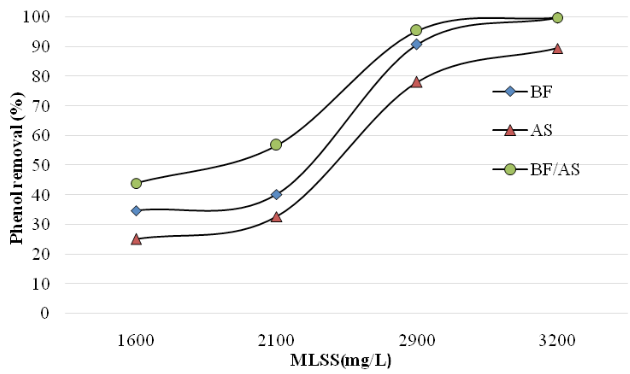


Fig. 2. Phenol removing efficiency at different MLSS concentration (initial increasing phenol concentration: 100 mg/L, pH: 7, Nitrogen: 50 mg/L, Phosphorous: 10 mg/L).

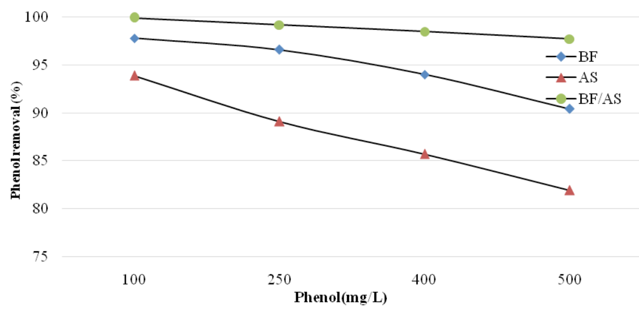


Fig. 3. Phenol removing efficiency of the studied system at various phenol concentrations (pH: 7, Nitrogen: 50 mg/L, Phosphorous: 10 mg/L, glucose concentration: 50 mg/L and hydraulic retention time: 4.4 h).

removal efficiency (Fig. 2). Pearson correlation coefficient between MLSS concentration and phenol removal efficiency was 0.569 in active sludge reactor and 0.680 ($P < 0.001$) for MLSS concentration in bio-filter reactor. In a study by Azimi et al. (2013) for the purpose of revealing the performance of active sludge combined with fixed state substrate in food industries waste-water treatment, results revealed that there was direct relationship between MLSS concentration and COD removal efficiency in a way that by raising concentration, the amount of COD removal rose [20]. They also found that MLSS concentration ranged from 1450 to 3500 mg/L which is the highest amount of COD removal in high MLSS concentration (3500 mg/L) being consistent with our results.

3.3. Effect of phenol concentration on phenol removal efficiency

Results showed that there was inverse relationship between the concentration of input phenol and removal efficiency and by increasing phenol concentration, removal efficiency reduced (Fig. 3). Results of Pearson correlation test between input phenol concentration and removal efficiency showed that correlation coefficient was –0.446 ($P < 0.001$). Almasi et al. [21] studied the effect of various phenol concentrations (100–400 mg/L) on the efficiency of waste-water fixation pond for phenol removal in petroleum refinery waste-water and they found that the highest phenol removal belonged to the lowest phenol concentration (100 mg/L). Also results of study by Nakhli et al. [22] demonstrated that the highest phenol removal was associated with the lowest phenol concentration (200 mg/L). In their study, phenol concentration was considered 200–1200 mg/L which was consistent with present study.

According to results, this system is able to receive organic load about 4–4.5 kg COD/m^{3}d in which nearly 100% of the input phenol could be removed. It should be stated that BF/AS integrated system could provide 90.8% phenol removal efficiency by receiving 8–9 kg COD/m^{3}d organic load. This high efficiency could be related to various reasons such as high concentration and microbial diversity. Since in this system, microorganisms' establishment is conducted in a combined method, so it is possible to provide more microorganisms in the system. On the other hand, microbial diversity in the system is high which leads to the presence of various microorganisms with different abilities for phenol removal in the system. Another reason for the high efficiency in the BF/AS system is physical state of the system and hydraulic flow type. So, this system was designed to decrease the possibility of blocking waste-water and air flowing inside the system in addition to providing high surface area for binding the microorganisms and; therefore, it is possible to provide the contact and accessibility of microorganisms with nutrients, minerals and oxygen in various parts of the system. Yong et al. [23] evaluated the capability of SBR biological system for infiltration of waste-water with phenol concentration of 1300 mg/L. The highest phenol removal efficiency in Yong et al. [23] was 97% in which the amount of loading into system was 3.12 kg BOD/m^{3}d, being consistent with our study.

3.4. Effect of pH on phenol removal efficiency

In present study, in order to study the correlation between pH and phenol removal efficiency and determine the appropriate pH for achieving maximal phenol removal in the system studied, the system efficiency was evaluated in 6.5–8 pH range with 0.25 intervals. Since affecting pH trend on phenol removal efficiency in above-mentioned range was not constant, therefore correlation between them was separately evaluated at 6.5–7 and 7–8 pH range. According to results, Pearson correlation coefficient among them in 6.5–7 was 0.936 and in 7–8 was 0.936 ($P < 0.001$). Therefore, according to results the most proper pH for maximal phenol removal in studied combined biological system was pH 7 and by increasing or decreasing pH in desired range, the efficiency considerably decreased (Fig. 4). Another important issue is the effect of pH on the system efficiency in the pH range tolerable by microorganisms. It is obvious that in pH amounts other than these range, the pH effect strongly increases and microorganism's life and consequently total system efficiency would be threatened. Change et al. [24] studied the capability of two yeast species, *Candida tropicalis* and Candida *tropicalis fosant* at various PHs and found that the highest phenol

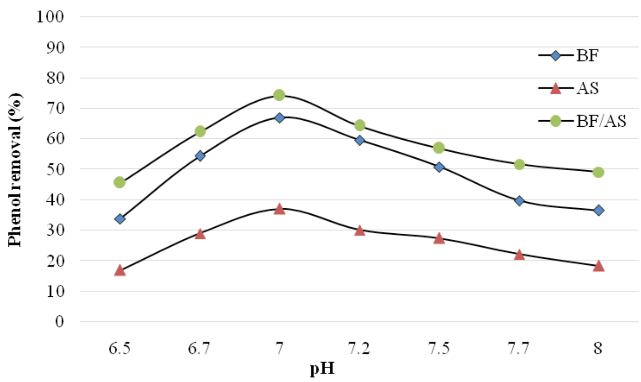


Fig. 4. Phenol removing efficiency at various pH values (initial increasing phenol concentration: 500 mg/L, Nitrogen: 80 mg/L, Phosphorous: 16 mg/L, glucose concentration: 250 mg/L).

removal by these yeasts occurred in pH 7 at 32°C which is in agreement with present study.

3.5. Effect of hydraulic retention time on phenol removal efficiency

Results showed that Pearson correlation coefficient between hydraulic retention time and phenol removal efficiency was 0.643. This implies that there is direct correlation between hydraulic retention time and phenol removal efficiency. Appropriate hydraulic retention time for achieving suitable infiltration and/or phenol removal from waste-water at 100 mg/L was 4.4 h (Fig. 5). Yung and Lant [23] studied the ability of SBR biological system for biological degradation of phenol at high loading and concentration. In their study, the amount of phenol loading was 3.12 mg / m^3d (equal to 2.1 g COD/MLVSS g·d), hydraulic retention time of 10 h, microbial retention time of 4 d and phenol concentration of 1300 mg/L and COD removal efficiency of 97%. Boylaqua et al. [24] studied the phenol removal using the enzymatic and aerobic biological integrated system and reported that the input phenol concentration in the system was 420 mg/L and the required hydraulic retention time for obtaining 99% removal efficiency was 48 h which is consistent with present study.

3.6. Effect of Nitrogen (N) and Phosphorous (P) concentrations on phenol removal efficiency

Results showed that increasing N concentration up to 50 mg/L, raised the efficiency but changing the concentration, decrease or increase in N concentration, reduces the phenol removal efficiency (Fig. 6). Results of Pearson correlation coefficients for N and phenol removal efficiency at 30–50 mg/L was 0.883 ($P < 0.0002$) and at 60–80 mg/L was –0.894 ($P < 0.003$). Since changes in *P* concentration are simultaneous with change in N concentration, all correlation coefficients are similar.

Since almost 100% of the phenol used in present study is decomposable by present microorganisms, so their BOD and COD indexes are almost equal. Also, considering the phenol concentration in the solution entered into sys-

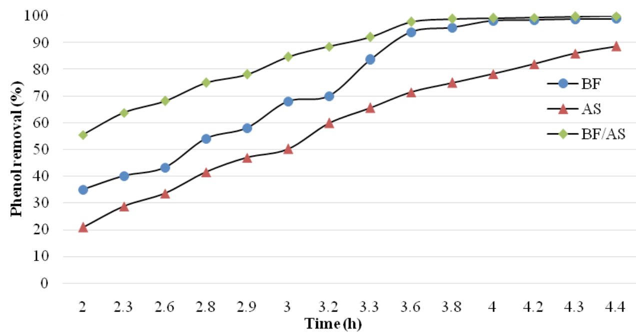


Fig. 5. Phenol removing efficiency at various hydraulic residence times (initial increasing phenol concentration: 100 mg/L, pH: 7, Nitrogen: 50 mg/L, Phosphorous: 10 mg/L and glucose concentration: 50 mg/L).

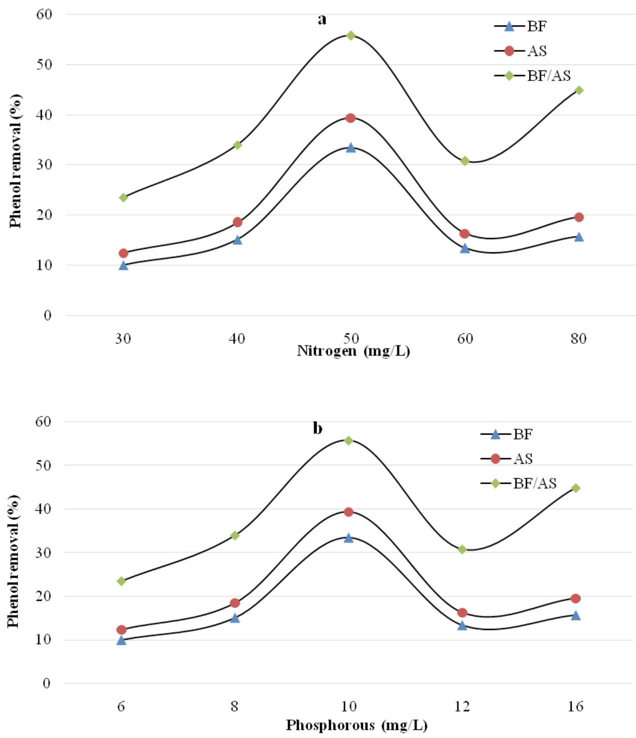


Fig 6. Phenol removing efficiency at a) Various Nitrogen concentrations and b) phosphorous concentrations (initial increasing phenol concentration: 500 mg/L, pH: 7, hydraulic retention time: 4.4 h).

tem, 500 mg/L, in maximal efficiency, phenol/N/P ratio is 100/24/4.8 and because of the production of 2.4 mg/L COD from each mg of phenol, so the maximal efficiency COD/N/P ratio was determined 100/10/2.

3.7. Determining evaporated phenol in BF/AS integrated system

In regard with aeration in the system under study, the possibility of phenol evaporation was evaluated. In order

Table 3
Evaporated phenol amount in BF/AS combined system

Reactor	Temperature	DO	*Q*	Phenol removing efficiency	Evaporated phenol	Total removed phenol	Evaporate phenol/total removed phenol
	°C	mg/L	mL/s	%	mg/d	mg/d	
BF/AS separately	30	1.85	0.81	70	0.081	27004	0.00031

Table 4
Phenol concentration changes to residence time ratio

Residence time (t)	Effluent phenol concentration (*A*)	Concentration difference (ΔA)	Residence time difference (ΔT)	$-(\Delta A/\Delta T)$	Mean *A*	Log-($\Delta A/\Delta T$)	Mean Loa (*A*)
h	mg/l	mg/l	h				
2.2	480	–205	3.8	53.9	377.5	1.73	2.58
6	275	–69.5	1	69.5	240.2	1.84	2.38
7	205	–200	2	100	110	2	2.04

to estimate the evaporated phenol amount from surface of the system, in 1 to 3 cases, evaporated phenol content from BF/AS integrated system was measured and the results are presented in Table 3. As it is seen in Table 3, the amount of evaporated phenol from BF/AS Integrated system was insignificant, 0.0006% of total removed phenol from the system.

3.8. Determining reaction's kinetic order in BF/AS biological system

According to results in Table 4, phenol degradation in this system is done according to second-order kinetic reaction. So, by raising phenol concentration, degradation rate declined exponentially. No report has been presented up to now in regard with determining the kinetic order of the phenol decomposition process in the BF/AS integrated biological system, but Hirta and colleagues [25] developed research floating platform (attached and floating growth) in order to determine the kinetic order of the phenol decomposition process in the BF/AS integrated biological system. The results of this study revealed that the phenol removal process in this system is conducted according to the kinetic zero-order reaction. In one sense, the speed of phenol removal in this system is independent of phenol concentration.

4. Conclusion

In general, results showed that BF/AS integrated biological system can remove phenol in high concentration(500 mg/L) and organic load (4–4.5 kg COD/m^{3}d) and within short time of (4.4 h) with high efficiency after the adaptation of microorganisms. Factors including pH, phenol concentration, N and P concentration, MLSS, glucose and hydraulic retention time have significant effect on the system efficiency. Also, the MLSS concentration had the highest impact on phenol removal efficiency.

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