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Amoxicillin removal from aqueous solutions using submerged biological aerated filter

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ABSTRACT

Amoxicillin is widely used as an antibiotic in the modern medicine. Due to its chemical structure, polarity, activity level, antibiotic specifications, and environmental sustainability, Amoxicillin leaks into the ground waters, surface waters, and drinking water wells. Many physical and chemical methods have been suggested for removing Amoxicillin from aquatic environments. However, these methods are very costly and have many performance problems. In this study, biodegradation of Amoxicillin by submerged biological aerated filter was evaluated in the aquatic environment. In order to assess the Amoxicillin removal from the aquatic environment, this bioreactor was fed with synthetic wastewater based on sucrose and Amoxicillin at three concentration levels and four hydraulic retention times. The maximum efficiencies for Amoxicillin and soluble chemical oxygen demand removal were 50.7 and 45.7%, respectively. The study findings showed that Stover–Kincannon model had very good fitness in loading Amoxicillin in the biofilter ($R^2 > 99\%$). There was no accumulation of Amoxicillin in the biofilm and the loss of Amoxicillin in the control reactor was negligible. This shows that Amoxicillin removal from the system was due to biodegradation. It can be concluded that there was no significant inhibition effect on mixed aerobic microbial consortia. It was also observed that Amoxicillin degradation was dependent on the amount of Amoxicillin present in the influent and by increasing the initial Amoxicillin concentration, Amoxicillin biodegradation increased as well.

Keywords: Amoxicillin; Antibiotic; Biodegradation; Submerged aerated filter; Aquatic Environment

1. Introduction

With development of hygiene in human societies and subsequent increase of expectation of life, selling and consumption of pharmaceutical compounds for prophylaxis and treatment of diseases are rapidly increasing [1,2]. Today, nearly 3,000 pharmaceutical compounds [3,4] with natural or synthetic origins and different chemical structures are being used all over the world [5,6]. In addition, consumption of antibiotics has been reported to be between 100,000 and 200,000 ton per year around the world [7,8]. These compounds as environmentally hazardous materials [9] are able to

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change various ecosystems [10]. Recently, it is reported that these compounds have been found in surface water, underground water, and sewage treatment plant [11,12]. Possible sources of pharmaceuticals in the environment have been illustrated in Fig. 1.

Due to their chemical structure, polarity [2], hydrophilicity [15], low volatility [3], activity level, antimicrobial characterization, and environmental sustainability, pharmaceutical compounds lead to adverse effects for humans as well as other organisms [2,14]. Amoxicillin is one of the most common types of antibiotics that used in the modern medicine [10]. Massive production of this antibiotic was started since the World War II and has been continued to the present time [16]. Amoxicillin is one of the β-lactam components (penicillin family, a semi-synthetic antibiotic), which are the components of broad-spectrum antibiotics that are effective in many gram-positive and gram-negative microorganisms and are used to treat certain infections caused by bacteria [17–21], such as pneumonia, bronchitis, gonorrhea, as well as the infections of the ears, nose, throat, severe respiratory, gastrointestinal, urinary, dental infections, and skin. It is also used in a variety of animal foods [22,23]. Due to the appropriate oral absorption of Amoxicillin compared to other members of penicillin family, it has much consumption [21]. The side effects of Amoxicillin include nausea, vomiting, fatigue, malaise, abdominal pain, fever, pruritus, liver injury, and jaundice [24–26]. Due to insufficient removal of Amoxicillin in the conventional water and wastewater treatment plant, it is introduced into the surface water and groundwater which cause changes in aquatic ecosystems [4,16,27] and also causes bacterial resistance to these drugs and failure of treatment with antibiotics [8,15,17,27,28]. The physicochemical properties and the chemical structure of Amoxicillin are listed in Table 1 and Fig. 2, respectively.

In general, several mechanisms, such as adsorption, incineration, oxidation–reduction, photolysis, hydrolysis [3], and chemical degradation are available for removing Amoxicillin from contaminated water and wastewater [8,10,18,20,31]; however, these mechanisms are very costly and have many performance problems [5,20]. Biodegradation is an economically viable mechanisms [5] which may lead to complete degradation of Amoxicillin into simpler compounds, such as carbon dioxide, water, nitrogen, and organic materials. Biodegradation of Amoxicillin and other antibiotics is the most effective option for removing these pollutants from the environment [3,32,33]. Antibiotic biodegradation is a process which can occur in different environments, such as soils, sediments, surface and groundwater, and biological sludge [7,34–36]. Most organic xenobiotic compounds including pharmaceuticals [37] are potentially sensitive to one or more biological transformations [31]. Amoxicillin biodegradation depends on various factors, including environmental circumstances, external source of carbon and nitrogen, pH, temperature,

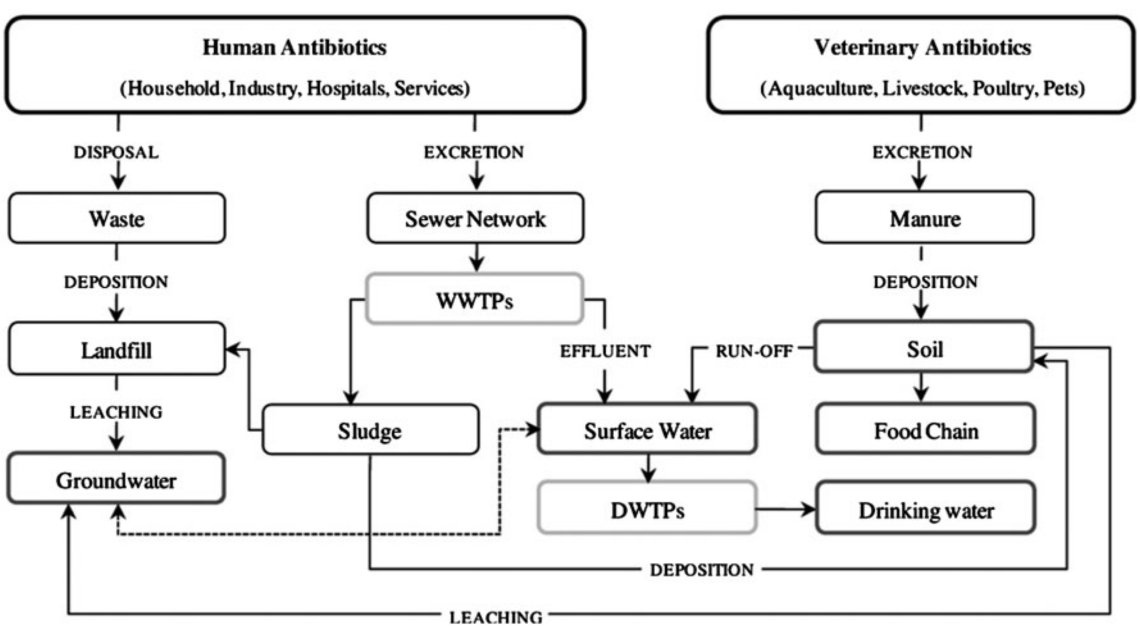

Fig. 1. Origin and principal contamination routes of human and veterinary antibiotics [13,14].

Table 1
Physicochemical properties of Amoxicillin [29,30]

IUPAC[a] name	α-amino-hydroxybenzylpenicillin
Synonyms	Amox; AMC; Amoxicillin trihydrate; Amoxicillin anhydrous; DAmoxicillin; p-hydroxyampicillin
Molecular formula	$C_{16}H_{19}N_3O_5S$
No. CASRN[b]	26787-78-0
Molecular weight	Amoxicillin: 365.40; Amoxicillin trihydrate: 419.41
Molecular width	1.32 nm
Physical characteristics	Solid or liquid, white to off-white crystalline powder, penicillin-type odor
Solubility in water	3,430 mg/L water
Melting point	194℃
Boiling point	743.2℃ at 760 mm Hg
Flash point	403.3℃
pK_a[c]	3.39, 6.71, 9.41
log KOW[d]	0.87

[a]International Union of Pure and Applied Chemistry.
[b]Chemical Abstract Services Registry Number.
[c]Acid dissociation constants.
[d]Octanol/water partition coefficient.

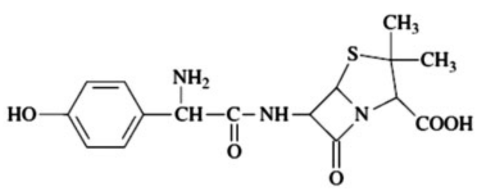

Fig. 2. Chemical structure of Amoxicillin [16].

microbial activity, and solids retention time (SRT) [3,34,37,38]. Gartiser et al. [39] reviewed the inherent biodegradability of 17 antibiotics in a combined test design based on the Zahn–Wellens test. According to the results, only Amoxicillin, Imipenem, and Nystatin showed certain ultimate biodegradation. Amoxicillin degradation by microorganisms, such as *Microcystis aeruginosa* [40] and *Rhodococcus* B30 [13], has been proved in previous researches. Jelic et al. [7] investigated the effects of different factors on the efficiency of treatment of wastewaters bearing pharmaceutical compounds in conventional wastewater treatment system. The study showed that increase in hydraulic retention times (HRTs) significantly increased pharmaceuticals removal as well. A summary of some researches performed on the microbial degradation of Amoxicillin is presented in Table 2.

In another research, Zhou et al. [41] examined Ampicillin and Aureomycin removal by two bioreactors in different HRTs levels. The results showed that these antibiotics were significantly degraded by biofilm airlift suspension reactor (BASR). During 12.58 h, 9.5 and 8.7%, respectively, of Ampicillin and Aureomycin were degraded by BASR. Also, in anaerobic baffled reactor (ABR), in HRT of 60 h 42.1% and 31.3%, and in HRT of 30 h, 16.4 and 25.9% of Ampicillin and Aureomycin were respectively degraded. They also found that BASR did not show effective COD removal in the presence of the two antibiotics under different HRTs.

Most bacteria, like *Escherichia coli* [42], Staphylococcus aureus [43], Helicobacter pylori [28], and Acinetobacter [44], have shown bacterial resistance to antibiotics; therefore, removing Amoxicillin from the environment is a major problem. Amoxicillin in this study was selected due to high consumption by the resident population for environmental and public health. Up to now, researchers have done projects to control the transport and fate of Amoxicillin in the soil and aquatic environments; however, since those methods are costly and have insufficient removal efficiency, biological methods seem more economical and cost-effective. Therefore, the present study aims to remove Amoxicillin from aqueous environment at different concentrations and HRTs by using submerged biological aerated filter (SBAF).

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used were of analytical grade and were purchased from Merck Co. (Germany). Amoxicillin standard was supplied by Sigma–Aldrich (USA). Dichloromethane was used as a solvent with an analytical reagent grade (99.5% purity). A stock solution of 30 mg/L Amoxicillin was prepared by dissolving 3 mg solid standard of Amoxicillin (99.9% purity) into 100 mL methanol. Besides, the working solutions were prepared by diluting the appropriate volume of the stock solution in methanol. The standard solution was stored in the freezer at −20℃. The stock solutions were prepared by dissolving the required amounts of chemicals in deionized water (Millipore Milli-Q). Except for Amoxicillin, all other stock solutions were autoclaved at 120℃ for 20 min and kept at 4℃. All the solutions were kept separately and were not mixed with other stocks in order to prevent precipitation. Amoxicillin solution was prepared (strength 0.01–10.0 mg/L) by dissolving a known quantity of Amoxicillin in distilled water and shaking it intermittently for at least 5 d. Cartridge Amoxicillin solution was covered with the aluminum foil and kept at 4℃ in dark in order to prevent photolytic degradation.

Table 2
The results of some previous studies on Amoxicillin removal

Operational condition	HRT	AMX removal efficiency	Reference
Upflow anaerobic sludge blanket (UASB)	23.2 h	21.6	[19]
Upflow anaerobic sludge blanket (UASB)	23.5 h	20.2	[20]
Novel micro-aerobic hydrolysis acidification reactor (NHAR)	9.3 h	20.4	
Cyclic activated sludge system (CASS)	14.9 h	68.2	
Biological contact oxidation tank (BCOT)	14.9 h	80.6	

2.2. Setup of biological filter

The experiments were performed in the pilot scale. The physical model was setup in the School of Health, Shiraz University of Medical Sciences, Shiraz, Iran. A simplified flow diagram of the pilot plant is shown in Fig. 3. The model consisted of a Plexiglas column of 100 mm inside diameter as downflow SBAF. The effective height of the filter and the free board were 55 and 5 cm, respectively. The column was filled with immobilized biofilm support of corrugated raschig rings with the same height and diameter. The rings were used as the biofilm support material because of their high porosity (up to 90%) and low price compared to the other synthetic packing media. The physical properties of the media and the physical specifications of the model are presented in Tables 3 and 4, respectively. To prevent the interference effects of light (photocatalytic) and algae growth, the column was covered by aluminum foil. Also, a control pilot was used in order to increase the accuracy of the project and eliminate the effects of the interfering factors.

Aeration was done from the bottom of the SBAF reactor by diffusers placed upside down. The amount of the injected air was chosen in such a way that oxygen would not be a limiting factor for the biological growth.

2.3. Synthetic wastewater

The synthetic wastewater used for feeding the bioreactor was a mixture of sucrose and tap water with COD of 1,000 ± 21.6 mg/L. The pH fluctuations were controlled using 0.5 mol/L sodium bicarbonate. Table 5 shows the composition of wastewater used as the feed of the pilot reactor during the test period. Synthetic wastewater was injected at the top of the aerobic filter by a peristaltic pump. Based on the study of Zhou et al. [41], the maximum removal efficiency of biodegradation pharmaceutical compounds occurs in at 32˚C. Accordingly, in this study, the temperature in the reservoir was controlled at 32 ± 0.2˚C by an electric heater.

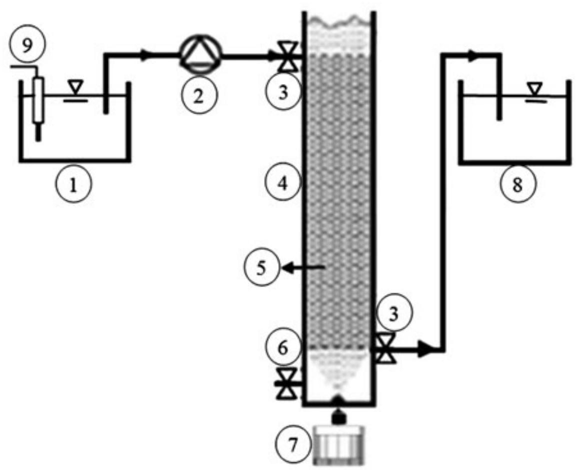

Fig. 3. Schematic figure of the physical model: (1) reservoir of feed stock, (2) peristaltic pump, (3) sampling ports, (4) biological aerated filter, (5) packing media, (6) discharge sludge port, (7) air compressor, (8) reservoir of outlet, and (9) temperature controller.

2.4. Startup and system operation

The column was filled with synthetic wastewater with COD of 10,000 mg/L. In addition, seeding was

Table 3
Physical properties of the media

Properties	Value and specification
Type media	Fixed bed (random packed)
Shape	Corrugated raschig rings
Material	HDPE[a]
Density (kg/m^3)	1868 ± 2
Specific gravity	0.98
Porosity (%)	92
Specific area (m^2/m^3)	410
Thickness (micron)	350
Outside diameter (mm)	15
Inside diameter (mm)	12
Height (mm)	11–13

[a]High density polyethylene (HDPE).

Table 4
Physical properties of the reactor

Column	Outside diameter (mm)	Inside diameter (mm)	Height (cm)	V_t[a](L)	V_e[b](L)
SBAF	110	100	60	4.7	3.9

[a]Total volume.
[b]Effective volume.

provided by aerobic bacteria collected from an activated sludge system of the pharmaceutical industry effluent treatment plant. Total solids concentration of the seed sludge was approximately 100 g/L, and 95% of which was total volatile solids. The air compressor was then turned on and the reactors were operated in a batch condition. In aerobic conditions, the mixed bacteria are stimulated to grow by supplying oxygen and hence produce enzymes which can oxidize or degrade the target pollutant. The sludge was fed with wastewater for a month to make the system acclimatized with the changed environment and was used for the further experiments. During this period, very low concentrations of Amoxicillin were added for further acclimatization of the microorganisms with the operational conditions.

The bacterial adaptation stage lasted for about 25 d. During this time, the wastewater inside the reactors was changed four times and pH, DO, and temperature were measured as 7.3 ± 0.2, 4.3 mg/L, and 32 ± 0.2°C, respectively. Reduction of soluble chemical oxygen demand (SCOD) was also measured daily. The results of the measurements are presented in the corresponding section. To ensure the microbial activity in this stage, surface cultivation of mixed liquor suspended solids in the bioreactor was frequently done in a mineral salts medium (MSM) solution containing Amoxicillin. The MSM preparation method was performed based on the study by Rezaee et al. [45].

Table 5
Chemical composition of synthetic wastewater

	Component	Concentration (mg/L)
Nutrients	$NaHCO_3$	20
	$MgSO_4.7H_2O$	5
	KH_2PO_4	5
	$CaCl_2.2H_2O$	5
	$FeSO_4.7H_2O$	0.2
	$ZnCl_2$	0.1
	$CoCl_2$	0.1
	$NiCl_2$	0.1
	$CuSO_4.5H_2O$	0.001
	H_3BO_3	0.02
	$MnSO_4$	0.5
	$(NH_4)_2HP_2O_4$	50
	$C_{12}H_{22}O_{11}$	Variable (600–900)
Amoxicillin	Variable (0.01, 0.1, 1 and 10)	

2.5. Experiments

After microbial adaptation was completed, the continuous feeding was started. In order to assess the effect of HRT on the efficiency of the filter, wastewater with COD of 1,000 mg/L was injected to the aerobic reactor by a peristaltic pump with different Amoxicillin concentrations (since the range of Amoxicillin concentrations is highly varied in the ecosystem and depends on different factors, four logarithmic levels of Amoxicillin concentrations; i.e. 0.01, 0.1, 1, and 10 mg/L, were selected in this study) and various discharges corresponding to different HRTs and different volumetric organic loads (VOLs) in the filter. The operational scheme of the system for 12 phases (runs) is presented in Table 6.

Sampling was regularly carried out with two times repetitions and when the column reached a steady state (when difference between the measured values in consecutive measurements is less than the amount of before time, it is the beginning of a steady state then with sequential measurements extracted the mean and standard deviation of different parameters. Steady state condition for different parameters will occur almost simultaneously) regarding Amoxicillin residual and soluble COD, the efficiency of Amoxicillin and SCOD removal was determined.

The parameters measured in this research were Amoxicillin residual concentration, SCOD, BOD_5, pH, dissolved oxygen (DO), and temperature. The first two parameters and the filter efficiency in Amoxicillin and substrate removal could be obtained in each run. In addition, at a specified HRT, pH, DO, and temperature were measured every day. To obtain rates of $BOD_5/SCOD$, BOD_5 measurements were carried out at each run. These parameters were included in the list of measurements just to be sure about the proper operation of the system and stability of the reactors. Unless otherwise specified, the analyses of various parameters were done as the procedures suggested in standard methods for the examination of water and wastewater.

2.6. Amoxicillin extraction and determination

Amoxicillin was extracted from wastewater by liquid–liquid extraction method suggested by Jena et al. [46] and Zhang et al. [47]. In addition,

Table 6
The operational scheme of the runs (at 32°C)

Run	HRT (h)	Initial con. of Amoxicillin (mg/L)	Initial con. of SCOD (mg/L)	Initial con. of BOD_5 (mg/L)	DO (mg/L)	pH
1	12	0.01	992 ± 19.70	398.56	4.3 ± 0.38	7.32
2	12	0.1	996 ± 12.71	342.37	4.4 ± 0.44	7.38
3	12	1	994 ± 12.30	305.61	4.3 ± 0.36	7.30
4	12	10	995 ± 12.61	235.91	4.5 ± 0.40	7.39
5	6	0.01	998 ± 10.45	448.10	4.5 ± 0.37	7.32
6	6	0.1	998 ± 15.05	232.31	4.4 ± 0.39	7.44
7	6	1	1,005 ± 5.62	299.71	4.3 ± 0.40	7.34
8	6	10	998 ± 8.14	237.85	4.4 ± 0.34	7.33
9	3	0.01	1,010 ± 14.31	422.93	4.5 ± 0.37	7.24
10	3	0.1	1,004 ± 14.19	358.76	4.4 ± 0.41	7.33
11	3	1	1,001 ± 9.35	288.22	4.3 ± 0.39	7.29
12	3	10	991 ± 8.66	210.48	4.3 ± 0.40	7.40

Dichloromethane (sp. gr. 1.32) was used as the extractant. The extraction efficiency by this method was 93 ± 0.78%. Amoxicillin was measured by high performance liquid chromatography (HPLC) (Model: UV-2487, Water, USA) using UV–vis detector at a wavelength of 230 nm and using Dionex Summit P580, HPLC pump. Analysis was carried out according to the method reported by Zazouli et al. [48] and the analytes were filtered through a 0.22 μm nylon syringe filter. The concentration of Amoxicillin was determined with a reversed phase C_{18} column, 0.5 μm, 4.6 × 250 mm (Spherisorb, Water, USA). The injection volume was 20 μL, the column working at room temperature; the mobile phase was acetate ammonium (0.01 mol/L); and acetonitrile (ACN) delivered at a constant flow rate of 0.5 mL/min were used as the mobile phase for gradient elution and peak retention time was 12 min. Before each run, the instruments were standardized with anticipated Amoxicillin concentration range. For standardization of the instrument, six standards of Amoxicillin were prepared in advance and stored in an amber bottle in the refrigerator at 4℃ until use. The standards were prepared by serial dilutions. To check the buildup of Amoxicillin in the biofilm and sludge, the method suggested by Matsuo et al. was utilized [49].

2.7. Modeling

In almost all references, including Baghapour et al. [50] and Coskun et al. [6], it is confirmed that the criterion for submerged filters design is the (VOL) and the rate of substrate removal is obtained from hyperbolic relations, such as Stover–Kincannon function (Eq. 1). The Stover–Kincannon model was first proposed for a rotary biological contactor by Kincannon and Stover [51]. The original model assumed that the suspended biomass was negligible in comparison to the attached biomass.

$$r_{\text{AMX}} = r_{\max}\frac{B_{\text{AMX}}}{k + B_{\text{AMX}}} \quad (1)$$

where r_{AMX} is the volumetric Amoxicillin removal, $r_{\max}$ is the maximum rate of volumetric Amoxicillin removal, B_{AMX} is the Amoxicillin load per unit volume of the filter, and k is the constant of half velocity. All the parameters are in $\text{Kg}_{\text{AMX}}/\text{m}^3\text{d}$.

The values of B_{AMX} and r_{AMX} could be obtained from the following equations:

$$B_{\text{AMX}} = \frac{Q}{V}C_i \quad (2)$$

$$r_{\text{AMX}} = \frac{Q}{V}(C_i - C_e) \quad (3)$$

C_i is the Amoxicillin concentrations in the influent ($\text{Kg}_{\text{AMX}}/\text{m}^3$).

C_e is the Amoxicillin concentrations in the effluent ($\text{Kg}_{\text{AMX}}/\text{m}^3$).

Q is the inflow rate to the reactor (m^3/d).

V is the reactor volume (m^3).

Using Eqs. (2) and (3) and Tables 6 and 7, values of B_{AMX} and r_{AMX} could be computed for various situations. The main values are presented in Table 8. The values of k and $r_{\max}$ were obtained by using the

Table 7
Effluent concentration of Amoxicillin (mg/L)

HRT (h)	Initial Amoxicillin concentration (mg/L)			
	0.01	0.1	1	10
3	$0.0088 \pm 1 \times 10^{-4}$	$0.0799 \pm 1 \times 10^{-3}$	$0.7689 \pm 1 \times 10^{-3}$	$7.069 \pm 1 \times 10^{-4}$
6	$0.0081 \pm 5 \times 10^{-4}$	$0.0774 \pm 1 \times 10^{-4}$	$0.6959 \pm 1 \times 10^{-3}$	$6.334 \pm 1 \times 10^{-3}$
12	$0.0074 \pm 1 \times 10^{-4}$	$0.0692 \pm 1 \times 10^{-3}$	$0.5979 \pm 1 \times 10^{-4}$	$4.919 \pm 1 \times 10^{-3}$

Table 8
Volumetric load and removal of Amoxicillin and SCOD from the bioreactor at 32°C

Run	B_{AMX} (Kg_{AMX}/m^3d)	r_{AMX} (Kg_{AMX}/m^3d)	B_{SCOD} (Kg_{SCOD}/m^3d)	r_{SCOD} (Kg_{SCOD}/m^3d)
1	1.84×10^{-5}	4.765×10^{-6}	1.84	1.3781
2	1.84×10^{-4}	5.648×10^{-5}	1.84	1.3211
3	1.84×10^{-3}	7.396×10^{-4}	1.84	1.3468
4	1.84×10^{-2}	9.347×10^{-3}	1.84	1.3855
5	3.68×10^{-5}	6.881×10^{-6}	3.68	2.5649
6	3.68×10^{-4}	8.316×10^{-5}	3.68	2.4766
7	3.68×10^{-3}	1.118×10^{-3}	3.68	2.5060
8	3.68×10^{-2}	1.343×10^{-2}	3.68	2.6091
9	7.36×10^{-5}	8.243×10^{-6}	7.36	4.8060
10	7.36×10^{-4}	1.472×10^{-4}	7.36	4.2099
11	7.36×10^{-3}	1.700×10^{-3}	7.36	4.3792
12	7.36×10^{-2}	2.156×10^{-2}	7.36	4.5043

software Curve Expert software and are presented in Table 9 and for curve plotting use of MATLAB and Excel software.

3. Results

During the system operation period, the HRT was reduced from 12 to 6 h and then to 3 h. According to the HRTs, the flow rate in the reactor was set at 0.32, 0.65, and 1.3 L/h, respectively. The most important parameters monitored in the experiments were Amoxicillin residual and SCOD and the means of the measured data are reported in this paper (Tables 7 and 10). COD of the inflow wastewater in all situations was 1,000 ± 21.6 mg/L.

By substituting the values of Table 9 into Eq. (1), results presented in Figs. 4 and 5 are obtained and submerged filters could be designed using these diagrams. Relationship between Amoxicillin concentration and HRT, and removal efficiencies of Amoxicillin and SCOD illustrated in Figs. 6 and 7.

At the initial Amoxicillin concentrations of 0.01, 0.1, 1, and 10 mg/L, Amoxicillin removal efficiency were 11.2, 20, 23.1, and 29.3%, respectively, after 3 h. After 6 h, however, Amoxicillin removal efficiency in the reactor reached 18.6, 22.4, 30.3, and 36.6%, respectively. Finally, after 12 h, Amoxicillin removal in reactor was 25.8, 30.5, 40.2, and 50.7% at the initial Amoxicillin concentrations of 0.01, 0.1, 1 and 10 mg/L, respectively (Table 11). In steady state conditions at HRT of 3 h and the initial Amoxicillin concentrations of 0.01, 0.1, 1, and 10 mg/L, the average SCOD removal was 31, 30.1, 27.3, and 31.3%, respectively. Besides, at the HRT of 6 h and the initial Amoxicillin concentrations of 0.01, 0.1, 1, and 10 mg/L, SCOD removal efficiency was 40.1, 38.4, 38.6, and 39.2%, respectively. Finally, the average SCOD removal efficiency was 44.3, 43.1, 41.7, and 45.7% at HRT of 12 h and the initial Amoxicillin concentrations of 0.01, 0.1, 1, and 10 mg/L, respectively. In all the cycles of the operation, SCOD removal efficiency and effluent BOD_5/SCOD were more than 30 and 0.40%, respectively.

Table 9
k and r_{max} coefficients of the bioreactor at 32 °C for Stover–Kincannon model

	Amoxicillin	SCOD
$r_{max,}$ (kg/m^3d)	0.0566	20.5800
k, (kg/m^3d)	0.1125	25.8460
R^2	0.996	0.999

Table 10
Effluent concentration of SCOD (mg/L)

HRT (h)	Initial Amoxicillin concentration (mg/L)			
	0.01	0.1	1	10
3	695.71 ± 2.251	707.97 ± 0.616	726.79 ± 3.283	688.87 ± 1.212
6	604.96 ± 1.196	619.99 ± 1.675	620.96 ± 1.825	597.97 ± 1.469
12	556.95 ± 1.382	565.66 ± 2.977	583.82 ± 3.161	543.91 ± 1.678

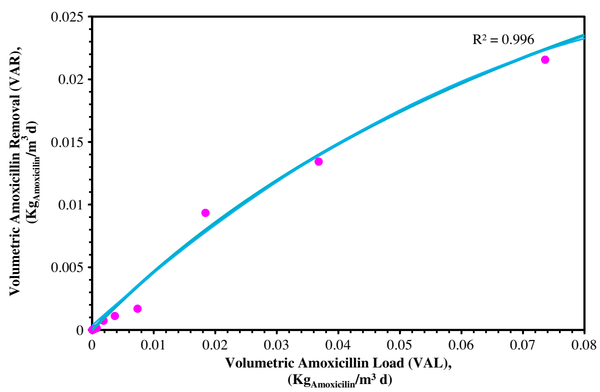

Fig. 4. Amoxicillin loading of the bioreactor in the range 0–0.08 Kg_{AMX}/m^3d at 32°C.

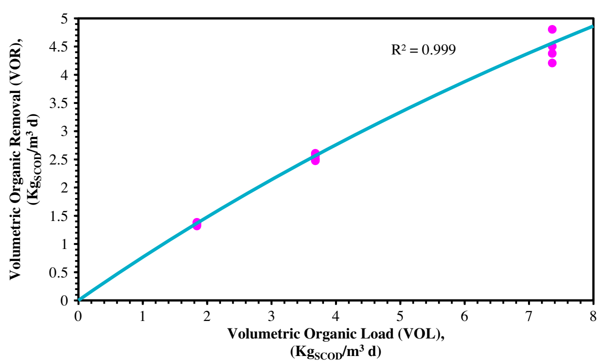

Fig. 5. Organic loading of the bioreactor in the range 0–8 Kg_{SCOD}/m^3d at 32°C.

4. Discussion

Based on the results, Amoxicillin degradation potential of the mixed aerobic consortium was evaluated under various Amoxicillin concentrations and HRTs and the results are presented in Tables 7 and 11. The findings of this study demonstrated that the solution containing Amoxicillin was biodegraded and treated in SBAF. Moreover, Amoxicillin removal efficiencies were above 35% when high Amoxicillin influent was introduced in the SBAF (runs 3, 4, and 8). The major part of the input Amoxicillin was consumed during these runs as indicated by low-effluent Amoxicillin concentration (below $4.92 \pm 2 \times 10^{-2}$ mg/L). The treatment efficiencies achieved at longer HRT (12 h) in the SBAF fed with low, moderate, and high Amoxicillin concentrations in the influent are summarized in Table 7. It is evident that in comparison with other HRTs, Amoxicillin and SCOD removal efficiencies were increased at long HRT due to the slight decrease in Amoxicillin and organic loading rates in the SBAF. However, the extent of Amoxicillin loading rate was not highly effective in biological Amoxicillin and organic removal efficiencies. Afterwards, the HRT was set to 12 h and the SBAF was operated at these conditions until steady state conditions were reached. The Amoxicillin and SCOD removal efficiencies were increased up to 50.7 and 45.7%, respectively (Tables 11 and 12). Therefore, it can be concluded that decreasing Amoxicillin as well as organic loading rates positively affect the SBAF performance. This can be due to the increase of the probability of the contaminants's exposure with microbial consortium and increase of SRT, which is consistent with the results obtained by Jelic et al. [7] and Zhou et al. [41]. Measurement of COD is important regarding the effluent discharge standards and COD represents the treatment potential of the reactor. In this study, SBAF showed acceptable SCOD removal efficiency in all experiments. Besides, Amoxicillin revealed no adverse effects on SCOD removal up to the concentration of 10 mg/L. However, SCOD reduction was reduced by 2–6% when Amoxicillin concentration was increased to 0.1 and 1 mg/L. Comparing the results of the previous studies (Table 2) with the present one show that this system has high ability for removing Amoxicillin from aqueous solutions. There was no accumulation of Amoxicillin in the biofilm and the loss of Amoxicillin in the control reactor was negligible. This shows that Amoxicillin removal from the system was due to biodegradation. High degradation rate of Amoxicillin at comparatively high Amoxicillin concentration might be due to the effect of concentration gradient. At high concentration

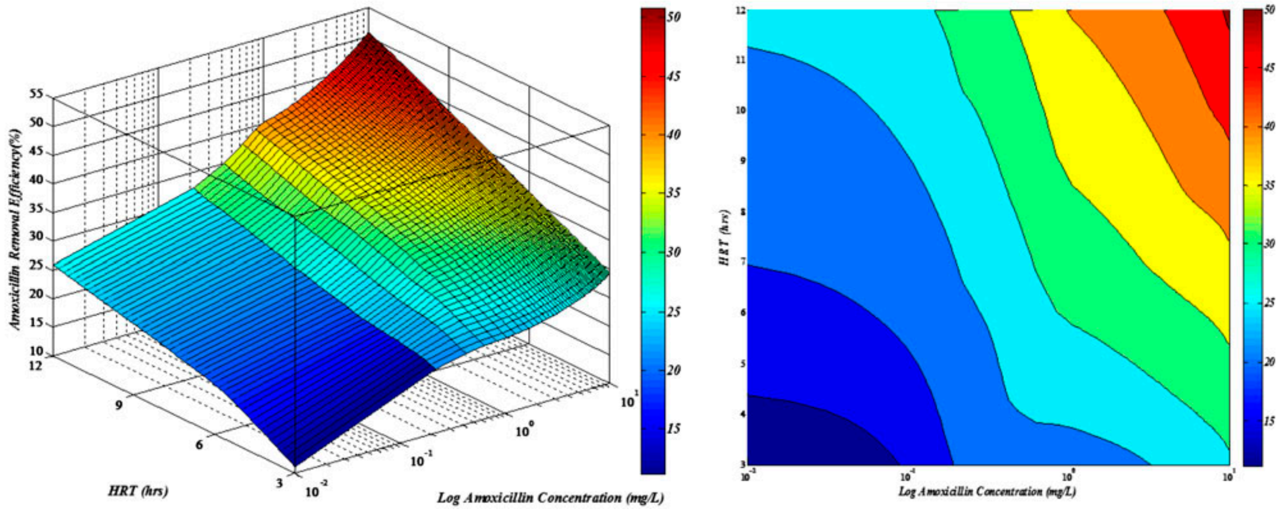

Fig. 6. Relationship between Amoxicillin concentration, HRT, and efficiency of removal Amoxicillin.

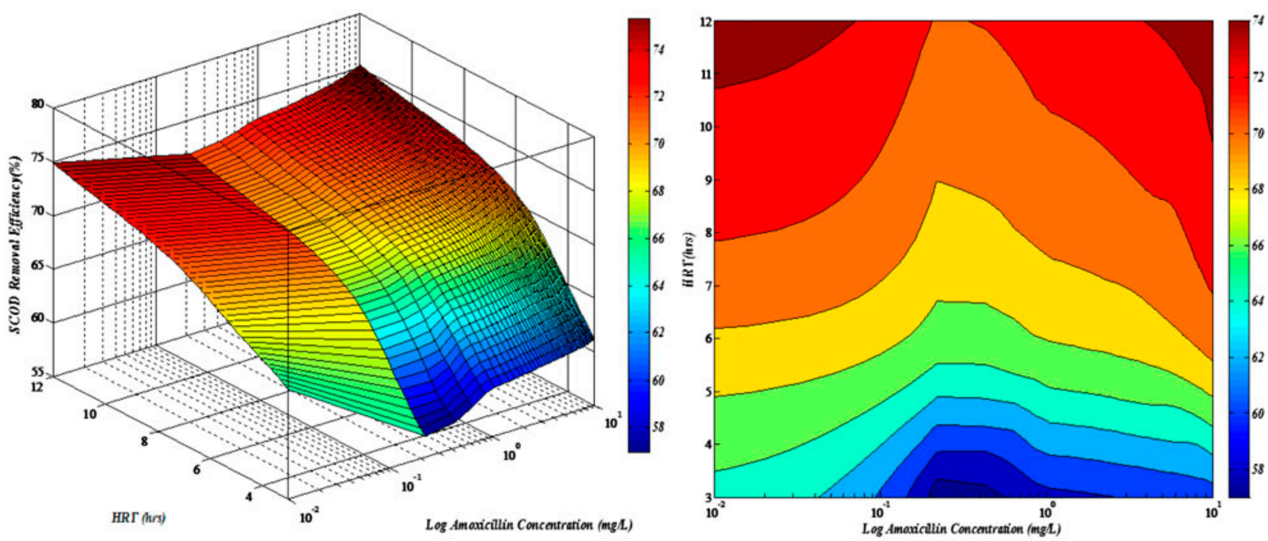

Fig. 7. Relationship between Amoxicillin concentration, HRT, and efficiency of removal SCOD.

gradient, the pollutant has a higher chance to be exposed to and/or penetrate through the cell which is essential for biodegradation. BOD_5 is a measure of the oxidation occurring due to microbial activity. The BOD_5/COD ratios are the commonly used indicators of biodegradability improvement where a value of zero indicates nonbiodegradability and an increase in the ratio reflects biodegradability improvement. In this study, the SBAF was able to increase the BOD_5/COD ratio to more than 0.40 in all the experiments. Moreover, significant changes were observed in BOD_5/COD ratios by increasing the HRT (Table 13).

Co-metabolic process is used for bioremediation of most persistence contaminants, such as Amoxicillin. In co-metabolic processes, by utilizing primary carbon source or nitrogen source, microbes produce enzymes or cofactor during microbial activities which are responsible for degradation of the secondary substrates (Amoxicillin). Also, the contaminants degrade in this process to trace concentrations. The results obtained from SBAF showed that the co-metabolic process was quite effective in removing Amoxicillin from the aqueous environment. Overall, the results of the modeling showed that Stover–Kincannon model

Table 11
Amoxicillin removal efficiency (%)

HRT (h)	Initial Amoxicillin concentration (mg/L)			
	0.01	0.1	1	10
3	11.2	20	23.1	29.3
6	18.6	22.4	30.3	36.6
12	25.8	30.5	40.2	50.7

Table 12
SCOD removal efficiency (%)

HRT (h)	Initial Amoxicillin concentration (mg/L)			
	0.01	0.1	1	10
3	31.0	30.1	27.3	31.3
6	40.1	38.4	38.6	39.2
12	44.3	43.1	41.7	45.7

Table 13
BOD_5/COD in effluent at 32°C

HRT (h)	Initial Amoxicillin concentration (mg/L)			
	0.01	0.1	1	10
3	0.52	0.46	0.41	0.39
6	0.62	0.56	0.53	0.48
12	0.61	0.57	0.52	0.49

had a very good fitness ($R^2 > 99\%$) in loading Amoxicillin in this biofilter, which is in line with the findings of Coskun et al. [6].

5. Conclusion

The present study investigated the ability of a SBAF to remove Amoxicillin from aqueous environment. The SBAF was operated at three different aerobic retention times in order to determine the optimum retention time for the highest Amoxicillin and COD removal. Finally, aerobic mixed biofilm culture was observed to be suitable for the treatment of Amoxicillin from aqueous solutions. There was no significant inhibition effect on mixed aerobic microbial consortia. Amoxicillin degradation depends on the strength of wastewater and the amount of Amoxicillin in the influent and HRTs. Also, Stover–Kincannon model more desirably described the Amoxicillin degradation in aquatic environment using a SBAF.

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