ORIGINAL ARTICLE

Degradation of Amoxicillin by Bacterial Consortium in a Submerged Biological Aerated Filter: Volumetric Removal Modeling

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Abstract

Background: 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 groundwater, surface waters, and drinking water wells. Many physical and chemical methods have been suggested for removing Amoxicillin from aquatic environments. However, these methods are so costly and have many performance problems.

Methods: In this study, biodegradation of Amoxicillin by submerged biological aerated filter (SBAF) was evaluated in the aquatic environment. In order to assess the removal of Amoxicillin from the aquatic environment, this bioreactor was fed with synthetic wastewater based on sucrose and Amoxicillin at 3 concentration levels and 4 hydraulic retention times (HRTs).

Results: The maximum efficiency for Amoxicillin and Soluble Chemical Oxygen Demand (SCOD) removal was 50.8% and 45.3%, 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 removal of Amoxicillin from the system was due to biodegradation.

Conclusions: 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 in the influent and by increasing the initial Amoxicillin concentration, Amoxicillin biodegradation increased, as well.

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Keywords: Amoxicillin; Antibiotic; Biodegradation; Submerge Aerated Filter; Aquatic Environment

Introduction

Today, nearly 3,000 pharmaceutical compounds[1] with natural or synthetic origins and different chemical structures are being used all over the world.[2] Recently, these compounds have been diagnosed in surface water, underground water, and sewage treatment plants.[3,4] Due to their chemical structure, polarity,[5] hydrophilicity,[6] low volatility,[7] activity level, antimicrobial characterization and environmental sustainability, pharmaceutical compounds lead to adverse effects for human as well as other organisms.[5,8] Amoxicillin is one of the most common types of antibiotics used in modern medicine.[9] Due to the appropriate oral absorption of Amoxicillin compared to other members of penicillin family, it has many uses.[10] The side-effects of Amoxicillin include nausea, vomiting, fatigue, malaise, abdominal pain, fever, pruritus, liver injury and jaundice.[11,12] Due to insufficient removal of Amoxicillin in the conventional water and wastewater treatment plant, it is introduced into the surface water and groundwater, changes the aquatic ecosystems[1,13] and also causes bacterial resistance to these drugs and failure of treatment with antibiotics.[14,15] The physicochemical properties and the chemical structure of Amoxicillin are listed in Table 1 and Fig. 1, respectively.

In general, several methods, such as adsorption, incineration, oxidation-reduction, photolysis, hydrolysis,[7] reverse osmosis,[3] and chemical degradation are available for removing Amoxicillin from contaminated water and wastewater;[14,17] however, these methods are very costly and have many performance problems.[17,18] Biodegradation is an economically viable technology[19] which may lead to complete degradation of Amoxicillin and produce simple 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.[7,20] Gartiser *et al.* (2007)[21] 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*[22] and *Rhodococcus B30*,[23] has been proved in previous researches. Jelic *et al.* (2012)[24] investigated the effects of different factors on the efficiency of treatment of wastewaters bearing pharmaceutical compounds. The study showed that when hydraulic retention times (HRT) increased, the removal of pharmaceuticals significantly increased as well.

Most bacteria, like *E.coli*,[25] *Staphylococcus aureus*,[26] *Helicobacter pylori*,[15]and *Acinetobacter*[27] have shown bacterial resistance to antibiotics; therefore, removing Amoxicillin from the environment is a major problem. Amoxicillin in this study was selected on the basis of several criteria: high consumption by people and harm for environment 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).

Table 1: Physicochemical properties of Amoxicillin[16]

IUPAC[1] 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[2]	26787-78-0
Molecular Weight	Amoxicillin: 365.40; Amoxicillin trihydrate: 419.41
Molecular width	1.32nm
Physical characteristics	Solid or liquid, white to off-white crystalline powder, Penicillin-type odor
Solubility in water	3430 mg/L water
Melting point	194°C
Boiling point	743.2°C at760mmHg
Flash point	403.3°C
pK_a[3]	3.39, 6.71, 9.41
Log KOW[4]	0.87

1. International Union of Pure and Applied Chemistry; 2. Chemical Abstract Services Registry Number; 3. Acid dissociation constants; 4. Octanol/water partition coefficient

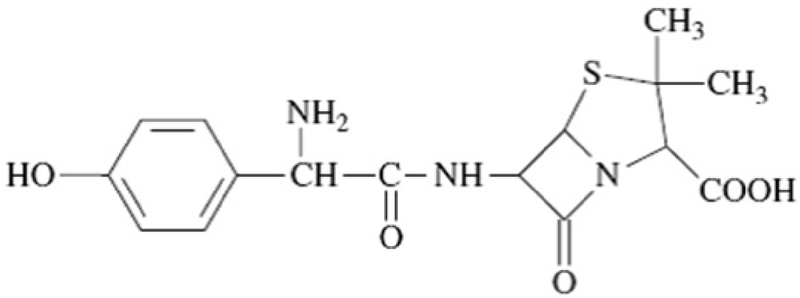

Figure 1: This figure shows the structure of Amoxicillin as a common antibiotic.

Materials and Methods

Chemicals and Reagents

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

Setup of Biological Filter

The experiments were performed in the pilot scale. The physical model was set up in the School of Health, Shiraz University of Medical Sciences, Shiraz, Iran. A simplified flow-diagram of the pilot plant is shown in Fig. 2. 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 cm 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 2 and 3, 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.

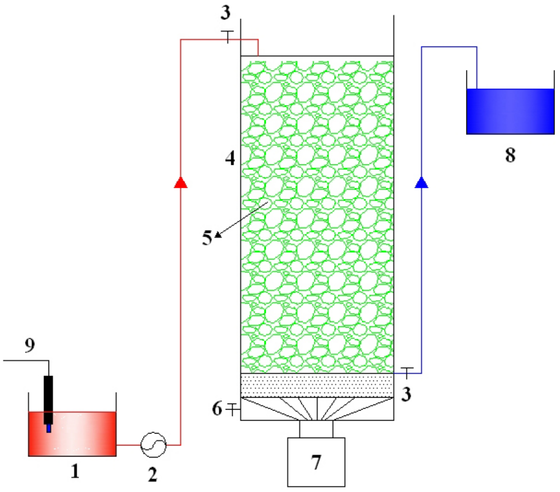

Figure 2: This figure shows the flow diagram and schematic figure of the physical model used in this study.
1. Reservoir of feed stock; 2. Peristaltic pump; 3. Sampling ports; 4. Biological aerated filter; 5. Packing media; 6. Discharge sludge port; 7. Air compressor; 8. Reservoir of outlet; 9. Temperature controller

Table 2: Physical properties of the media

Properties	Value and specification
Type media	Fixed bed (random packed)
Shape	Corrugated raschig rings
Material	HDPE[1]
Density (Kg/m^3)	186±2
Specific gravity	0.98
Porosity (%)	92
Specific area (m2/m3)	410
Thickness (micron)	350
Outside diameter (mm)	15
Inside diameter (mm)	12
Height (mm)	11-13

1. High Density Polyethylene (HDPE)

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.

Synthetic Wastewater

The synthetic wastewater used for feeding the bioreactor was a mixture of sucrose and tap water with COD of 1000±21.6 mg/L. pH fluctuations were controlled using 0.5 mol/L sodium bicarbonate. Table 4 shows the composition of wastewater used as the feed of the pilot reactor during the test period. Synthetic wastewater was injected to the top of the aerobic filter by a peristaltic pump. Based on the study by Zhaou *et al.*,[28] the maximum removal efficiency of biodegradation pharmaceutical compounds occurs at 32°C. Accordingly, in this study temperature was controlled in the reservoir at 32±0.2°C by an electric heater.

Startup and System Operation

The column was filled with synthetic wastewater of 10000 mg/L. In addition, seeding was provided by aerobic bacteria taken from an activated sludge system treating the pharmaceutical industry effluent. Total Solids (TS) concentration of the seed sludge was approximately 100 g/L, 95% of which was Total Volatile Solids (TVS). The air compressor was then turned on

Table 3: Physical properties of the reactor

Column	Outside diameter (mm)	Inside diameter (mm)	Height (cm)	V_t^{1} (L)	V_e^{2} (L)
SBAF	110	100	60	4.7	3.9

1. Total volume; 2. Effective volume

Table 4: Chemical composition of synthetic wastewater

Nutrients	Component	Concentration (mg/L)
	$NaHCO_3$	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)	

and the reactors started to work in a batch condition. In aerobic conditions, the mixed bacteria were stimulated to grow by adding oxygen and start producing 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 it was used for 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 days. During this time, the wastewater inside the reactors was changed four times and pH, Dissolved Oxygen (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 will be presented in the corresponding section. To ensure the microbial activity in this stage, surface cultivation of *Mixed Liquor Suspended Solids* (MLSS) 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.*[29]

Experiments

After the 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 strength of 1000 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, depending 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 5.

Sampling was regularly carried out with 2 times repetitions and when the column reached a steady state (When the difference between the measured values in consecutive measurements is less than a certain amount , it is the beginning of a steady state.) 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, DO, and temperature. The first two parameters, 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 the 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 examination of water and wastewater.

Amoxicillin Extraction and Determination

Amoxicillin was extracted from wastewater by

Table 5: The operational scheme of the runs (at 32°C)

Run	HRT (hrs)	Initial Conc. of Amoxicillin (mg/L)	Initial Conc. of SCOD (mg/L)	Initial Conc. 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	1005±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	1010±14.31	422.93	4.5±0.37	7.24
10	3	0.1	1004±14.19	358.76	4.4±0.41	7.33
11	3	1	1001±9.35	288.22	4.3±0.39	7.29
12	3	10	991±8.66	210.48	4.3±0.40	7.40

liquid–liquid extraction method suggested by Jen *et al.*[30] and Zhang *et al.*[31] In addition, 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 Chromatograph (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.*[32] and the analytes were filtered through a 0.22 µm nylon syringe filter (Albert). The concentration of Amoxicillin was determined with a reversed phase C_{18} column, 0.5µm, 4.6×250 mm (Spherisorb®, Water, USA). The injection volume was 20 µL, the column worked at room temperature, the mobile phase was Acetate ammonium (0.01 mol/L), acetonitrile (ACN) delivered at a constant flow rate of 0.5 ml/min was used as the mobile phase for gradient elution, and the 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°C until use. The standards were prepared by serial dilutions. To check the build-up of Amoxicillin in the biofilm and sludge, the method suggested by Matsuo *et al.* was utilized.[33]

Modeling

In almost all references, including Baghapour *et al.*,[34] 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 (equation 1). The Stover–Kincannon model was first proposed for a rotary biological contactor by Kincannon and Stover (1982).[35] The original model assumed that the suspended biomass was negligible in comparison to the attached biomass.

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

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 Kg_{AMX}/m^3d

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

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

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

C_i is Amoxicillin concentrations in the influent (Kg_{AMX}/m^3)
C_e is Amoxicillin concentrations in the effluent (Kg_{AMX}/m^3)
Q is inflow rate to the reactor (m^3/d)
V is reactor volume (m^3)

The effluent concentration of Amoxicillin and SCOD are presented in Tables 6 and 7 respectively. Using equations 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

Table 6: Effluent concentration of Amoxicillin (mg/L)

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

Table 7: Effluent concentration of SCOD (mg/L)

HRT	**Initial Amoxicillin concentration(mg/L)**			
(hrs)	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

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

of k and r_{max} were obtained using the software Curve Expert; the results are presented in Table 9 and for curve plotting MATLAB and Excel softwares were used.

Results

During the system operation period, the HRT was reduced from 12 to 6 hours and then to 3 hours. According to the HRTs, the flow rate in the reactor was set at 0.32, 0.65, and 1.3 L/hr, 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 6 and 7). COD of the inflow wastewater in all situations was 1000±21.6 mg/L. The trend of Amoxicillin and SCOD removal is shown in Figs. 3 and 4.

By substitution of the values of Table 9 into Eq. 1, the results presented in Figs. 5 and 6 are obtained and submerged filters could be designed using these diagrams. The relationship among Amoxicillin concentration, HRT and efficiencies of removal Amoxicillin and SCOD is displayed in Figs. 7 and 8. Also, BOD_5/COD ratio in Effluent at 32^{o}C is presented in Table 10.

At the initial Amoxicillin concentrations of 0.01, 0.1, 1 and 10 mg/L, Amoxicillin removal efficiency was

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

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

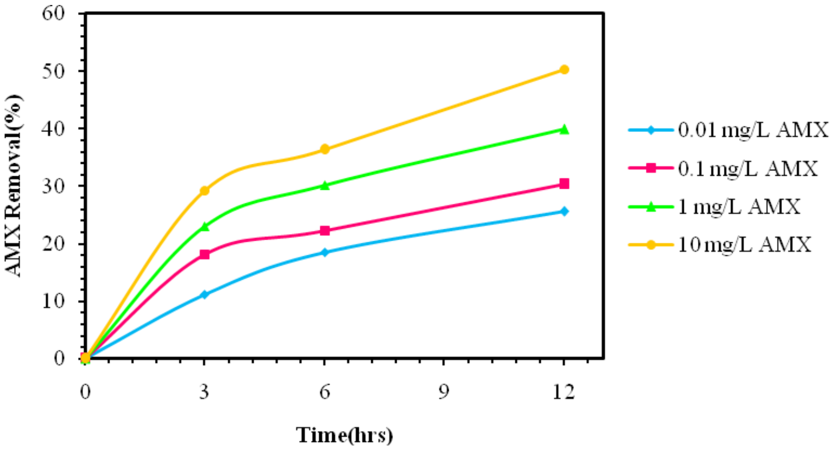

Figure 3: This figure shows the relationship curves for Amoxicillin removal and HRT in different initial concentrations of Amoxicillin at 32°C.

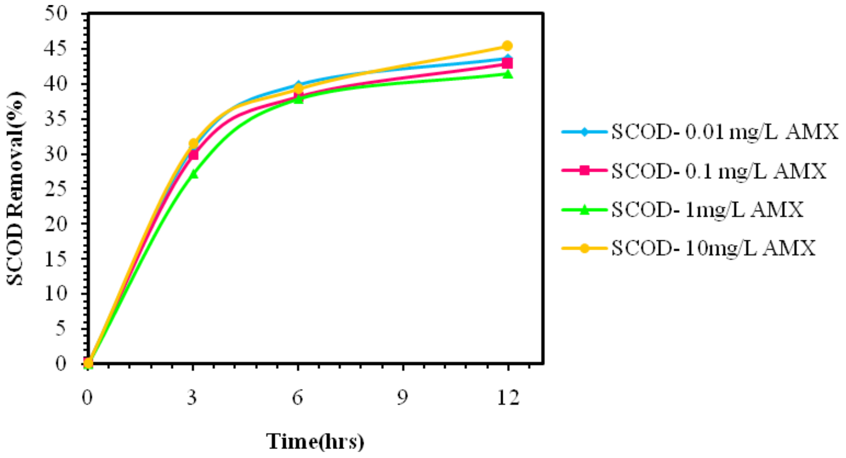

Figure 4: This figure shows the relationship curves for SCOD removal and HRT in different initial concentrations of Amoxicillin at 32°C.

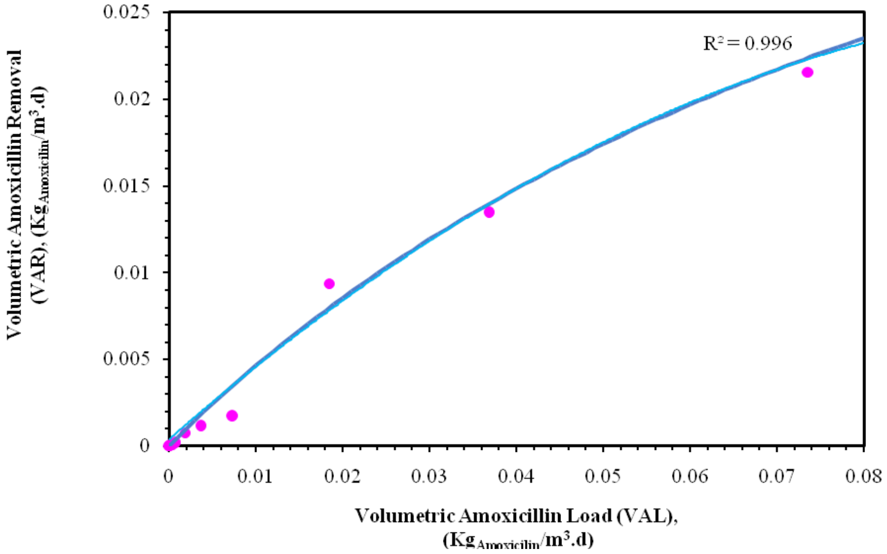

Figure 5: This figure shows the Stover–Kincannon model for Volumetric Amoxicillin Load (VAL) and Volumetric Amoxicillin Removal (VAR) in the range of 0 to 0.08Kg_{AMX}/m^3d at 32°C.

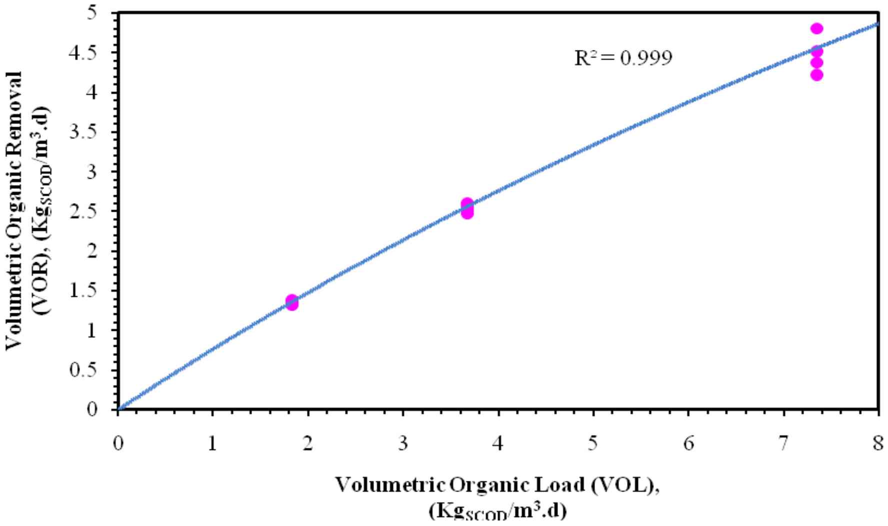

Figure 6: This figure shows the Stover–Kincannon model for Volumetric Organic Load (VOL) and Volumetric Organic Removal (VOR) in the range of 0 to 0.08Kg_{AMX}/m^3d at 32°C.

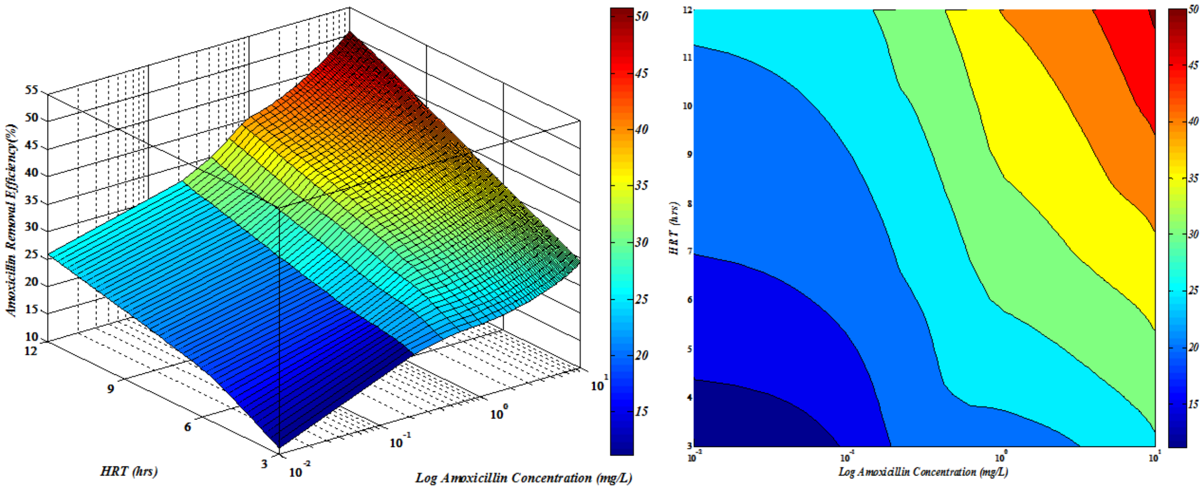

Figure 7: This figure shows the three dimensional model fitted for Amoxicillin concentration, HRT and Amoxicillin removal efficiency at 32°C.

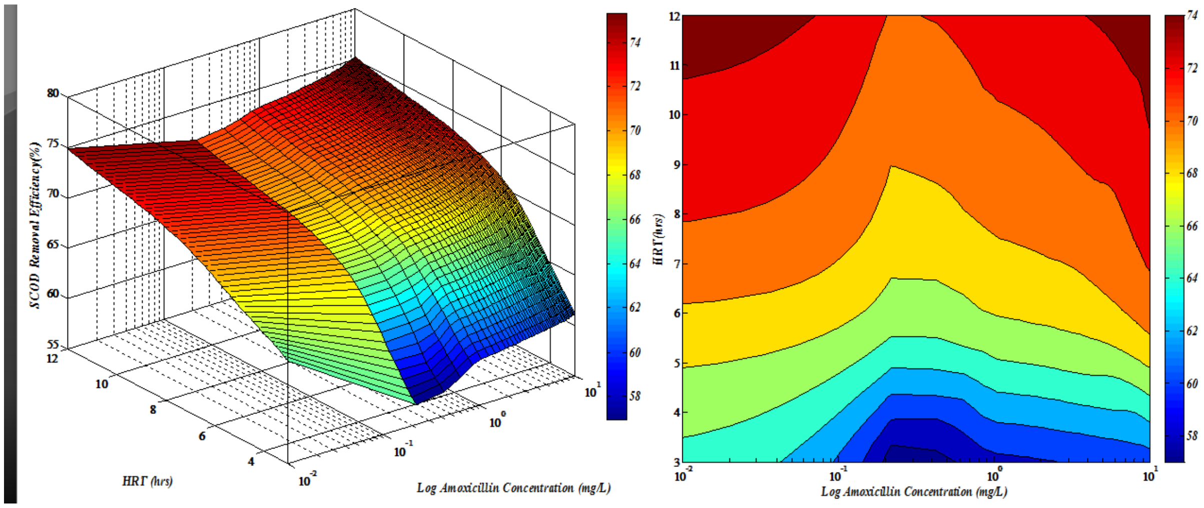

Figure 8: This figure shows the three dimensional model fitted for Amoxicillin concentration, HRT and SCOD removal efficiency at 32°C.

Table 10: BOD_5/COD in Effluent at 32°C

HRT	**Initial Amoxicillin concentration(mg/L)**			
(hrs)	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

11.2%, 20%, 23% and 29.3%, respectively after 3 hrs. After 6 hrs, however, Amoxicillin removal efficiency in the reactor reached 18.7%, 22.6%, 30.4%, and 36.5%, respectively. Finally, after 12 hrs, Amoxicillin removal in the reactor was 25.9%, 30.7%, 40.2%, and 50.8% 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 hrs and the initial Amoxicillin concentrations of 0.01, 0.1, 1, and 10 mg/L, the average SCOD removal was 30.9%, 29.8%, 27.2%, and 31.3%, respectively. Besides, at the HRT of 6 hrs and the initial Amoxicillin concentrations of 0.01, 0.1, 1, and 10 mg/L SCOD removal efficiency was 39.9%, 38.2%, 37.9%, and 39.1%, respectively. Finally, the average SCOD removal efficiency was 43.6%, 42.8%, 41.5%, and 45.3% at HRT of 12 hrs and the initial Amoxicillin concentrations of 0.01, 0.1, 1, and 10 mg/L, respectively (Table 12). In all the cycles of the operation, SCOD removal efficiency and effluent BOD_5/SCOD were more than 27% and 0.42, respectively.

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 5 and 6. The findings of this study demonstrated that the solution containing Amoxicillin was biodegraded and treated in a submerged

Table 11: Amoxicillin removal efficiency (%)

HRT	Initial Amoxicillin concentration(mg/L)			
(hrs)	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	Initial Amoxicillin concentration(mg/L)			
(hrs)	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

biological aerated filter. Moreover, Amoxicillin removal efficiencies were above 35% where 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 hrs) in the SBAF fed with low, moderate, and high Amoxicillin concentrations in the influent are summarized in Table 11. 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 at 12 hrs 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 positively affects the SBAF performance. This can be due to the increase of the probability of the contaminants' exposure with microbial consortium and increase of SRT, which is consistent with the results obtained by Jelic *et al.* (2012) and Zhaou *et al.* (2006). 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. A summary of some research performed on the microbial degradation of Amoxicillin is presented in Table 13.

Comparison of the results of the previous studies (Table 13) and the present one shows that this system has a great 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 a high concentration 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 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 non-biodegradability 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 0.42 in all the experiments. Moreover, significant changes were observed in BOD_5/COD ratios by increasing the HRT.

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 in order to trace concentrations. The results

Table 13: The results of some previous studies on Amoxicillin removal

Operational condition	HRT	AMX removal efficiency	Reference
Up-flow anaerobic sludge blanket (UASB)	23.2 hrs	21.6	36
Up-flow anaerobic sludge blanket (UASB)	23.5 hrs	20.2	
Novel micro-aerobic hydrolysis acidification reactor (NHAR)	9.3hrs	20.4	17
Cyclic activated sludge system (CASS)	14.9 hrs	68.2	
Biological contact oxidation tank (BCOT),	14.9 hrs	80.6	

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 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*.

Conclusion

The present study investigated the ability of a SBAF to remove Amoxicillin from aqueous environment. The SBAF was operated at 3 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 treatment of Amoxicillin from aqueous solutions. There was no significant inhibition effect on mixed aerobic microbial consortia. Amoxicillin degradation depended on the strength of wastewater and the amount of Amoxicillin in the influent and HRTs. Also, Stover-Kincannon model described the Amoxicillin degradation in aquatic environment more desirably using a SBAF.

Authors' Contributions

Mohammad Ali Baghapour, Mohammad Reza Shirdarreh and Mohammad Faramarzian carried out the research entitled "Degradation of Amoxicillin by Bacterial Consortium in a Submerged Biological Aerated Filter: Volumetric Removal Modeling", participated in the sequence alignment, and drafted the manuscript. All the authors read and approved the final manuscript.

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