Degradation of atrazine by microbial consortium in an anaerobic submerged biological filter

Simin Nasseri, Mohammad Ali Baghapour, Zahra Derakhshan and Mohammad Faramarzian

ABSTRACT

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) (ATZ) is one of the components of S-triazine. Due to its certain characteristics, ATZ causes pollution in various ecosystems and has been of concern for its probable carcinogenic effects on humans. Researchers have used chemical and physical methods for removing ATZ from the environment. Although these methods are quick, they have not been capable of complete mineralization. Therefore, researchers are looking for methods with lower energy consumption and cost and higher efficiency. In this study, biodegradation of ATZ by microbial consortium was evaluated in the aquatic environment. The present study aimed to evaluate the efficiency of ATZ removal from aqueous environments by using an anaerobic submerged biological filter in four concentration levels of atrazine and three hydraulic retention times. The maximum efficiencies of ATZ and soluble chemical oxygen demand (SCOD) were 51.1 and 45.6%, respectively. There was no accumulation of ATZ in the biofilm and the loss of ATZ in the control reactor was negligible. This shows that ATZ removal in this system was due to biodegradation. Furthermore, the results of modeling showed that the Stover–Kincannon model had desirable fitness ($R^2 > 99\%$) in loading ATZ in this biofilter.

Key words | atrazine, biodegradation, environment, herbicide, microbial consortium

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ABBREVIATIONS

ASBF	Anaerobic Submerged Biological Filter
ATZ	Atrazine
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
EU	European Union
HDPE	High Density Polyethylene
HPLC	High-Performance Liquid Chromatography
HRT	Hydraulic Retention Time
ISIRI	Institute of Standards and Industrial Research of Iran
MCL	Maximum Contaminant Level
MSM	Mineral Salts Medium
MLSS	Mixed Liquor Suspended Solids
OLR	Organic Loading Rate
SCOD	Soluble Chemical Oxygen Demand
VOLs	Volumetric Organic Loads
WHO	World Health Organization

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INTRODUCTION

The herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) (ATZ) (Chena *et al.* 2011; Sagarkar *et al.* 2013) has been used throughout the world since 1959 for controlling weeds in agriculture (Baxter *et al.* 2013). Although many European countries have banned its use (Migeot *et al.* 2013), ATZ is still being used in other countries, such as China, the USA (Chena *et al.* 2011), and Iran (Rezaee *et al.* 2011; Dehghani *et al.* 2013). Nonetheless, it has been detected above the recommended levels

(0.1 ppb) throughout aquatic environments (Chena *et al.* 2011; Baghapour *et al.* 2013). Atrazine is a member of the S-triazine group herbicides and is a probable human carcinogen (Group 2B) (Chan & Chu 2003; Abigail & Das 2012). It is also considered as one of the 33 priority substances of major concern in European waters to be monitored under the Water Framework Directive by the European Commission (Chan & Chu 2003). According to the statistics of Iranian Plant Protection, 250 tons of this herbicide were used in 2008 and the average consumption was 1–5 kg per hectare (Rezaee *et al.* 2011).

Atrazine is resistant in the environment and, as a result, causes serious environmental problems. Moreover, it penetrates through the surface and subsurface water bodies due to its high mobility in soil, persistence, low vapor pressure, and its massive application (Tafoya-Garnica *et al.* 2009; Hunter & Shaner 2010; Wang & Xie 2012; Baghapour *et al.* 2013).

When people are exposed to ATZ at levels above the drinking water maximum contaminant level (MCL) for relatively short periods of time, they may face congestion of heart, lungs, and kidneys, low blood pressure, muscle spasm, weight loss, and damage to the adrenal gland, central nervous system, and the immune system (Ghosh & Philip 2006; Du *et al.* 2011; Sagarkar *et al.* 2013).

In some soils, ATZ is stable for more than four years (Lazorko-Connon 2011). Kannan *et al.* (2006) conducted a study on Lake Michigan and estimated the half-life of ATZ in surface water to be more than 14 years. Also, ATZ's half-life in groundwater has been reported to vary from 15 months to 20 years (Grover & Cessna 1991; Spalding *et al.* 2003). The United States Environmental Protection Agency (EPA) and European Union (EU) have established the maximum amount of herbicides in drinking water in the ppb range. EU has established the permissible limit for ATZ as 0.1 $\mu g\, l^{-1}$ (Farré *et al.* 2007; Getenga *et al.* 2009; Sagarkar *et al.* 2013). However, the EPA, World Health Organization (WHO), and Institute of Standards and Industrial Research of Iran (ISIRI) have established the MCL of ATZ in drinking water as 3, 2, and 2 $\mu g\, l^{-1}$, respectively (Katsumata *et al.* 2006, 2010).

In general, several methods are available for removing ATZ from contaminated water and wastewater. However, these methods are very costly, have many performance problems, produce a lot of toxic intermediates, and cannot completely mineralize ATZ (Ghosh & Philip 2006; Abigail & Das 2012). Biodegradation is an economically viable technology which may lead to complete degradation and mineralization of ATZ and produce simple compounds, such as carbon dioxide, water, nitrogen, and organic materials. Biodegradation of ATZ and other herbicides is the most effective option for removing these pollutants from the environment (Wang & Xie 2012; Abigail & Das 2012). Herbicide biodegradation is a process which can occur in different environments, such as soils, sediments, surface and groundwater, and biological sludge (Izadi *et al.* 2009).

Wei *et al.* (2008) investigated the effects of hydraulic retention time (HRT) on the treatment efficiency of wastewaters bearing ATZ. The study showed that when HRT reached 24 h, ATZ removal significantly increased. A summary of some research performed on the microbial degradation of ATZ in anaerobic and anoxic conditions is presented in Table 1.

Yang *et al.* (2010) studied a simple consortium including two members of *Klebsiella* sp. A1 and *Comamonas* sp. A2 isolated from the sewage of a pesticide mill in China. In contrast to many other reported microorganisms, the consortium was insensitive to some commonly used nitrogenous fertilizers. Atrazine was completely mineralized in spite of the presence of urea, $(NH_4)_2CO_3$, and $(NH_4)_2HPO_4$ in the medium. Moreover, Wang & Xie (2012) studied ATZ removal from contaminated soil and water by *Arthrobacter* sp. and the results showed that adding an external source of carbon and nitrogen increased the bacterial growth and ATZ degradation rates. Furthermore, Chung *et al.* (1996) studied the anaerobic biotransformation of ATZ in wetland sediments receiving wastewater from a local mill by using various carbon and energy sources. About 20% of total ATZ was transformed and this reduction was assumed as the mineralization of ATZ to end products, such as NH_3 and CO_2.

In another study, Rezaee *et al.* (2011) examined ATZ removal by two *Pseudomonas* bacteria (*fluorescens* and *aeruginosa*) in three concentration levels of ATZ. The results showed that ATZ was significantly degraded by the *Pseudomonas* bacteria. During 48 h, 48.18, 72.6, and 91.5% of ATZ was degraded by *P. fluorescens*. Also, *P. aeruginosa*

Table 1 | The results of some previous studies on atrazine removal

Operating condition/Microorganism type	Reactor type	Performance		Initial conc. of atrazine (mg l^{-1})	Reference
		HRT	Atrazine removal (%)		
Anaerobic/mixed culture/co-metabolic process	Wood charcoal in fixed-bed reactor	38 weeks	20	10	Keerthinarayana & Bandyopadhyay (1997)
Anaerobic/mixed culture	Suspended growth	5 days 34 days 150 days	40 62 42	Wide range	Ghosh &Philip (2004)
Facultative anaerobic bacterium	Culture tubes	1 week	47	75	Jessee *et al.* (1983)
Anaerobic/mixed culture/co-metabolic process	Suspended growth	5 day	50	1–15	Ghosh *et al.* (2001)
Nocardioides and natural consortia	Culture tubes	3 days	50	10	Topp (2001)
Anoxic/Pure culture/M91-3	Biphasic column systems	6 days	60	22	Crawford *et al.* (1998)

degraded 19.08, 33.83, and 62.66% of ATZ in three concentration levels of 100, 200, and 300 mg l^{-1}, respectively. They also found that increasing the ATZ concentration led to higher degradation rates of the herbicide.

Anaerobic submerged biological filter (ASBF) is a type of attached growth system. In this system, high biomass concentration can be obtained in a reactor through cell immobilization by attachment to a surface. The advantages of this system include low sludge yield, not requiring electrical energy, the capability to be built and repaired with locally available materials, long service life, biogas production, resistant to organic and hydraulic shock loadings, etc. Due to the specific structure of ASBF, the common treatment problems, such as bulking and rising, do not exist in this system (Metcalf & Eddy 1991; Baghapour *et al.* 2011).

Most chemical pesticides, like atrazine, have shown carcinogenic and mutagenic effects and removing ATZ from the environment is a major problem. Up to now, researchers have done projects to control the transport and fate of ATZ in the soil and aquatic environments; however, since those methods are costly, produce hazardous byproducts, and have insufficient removal efficiency, biological methods seem more cost-effective. Therefore, the present study aims to remove ATZ from the aqueous environment at different concentration levels and HRTs by a consortium of microorganisms using ASBF.

MATERIALS AND METHODS

Biological filter set-up

The experiments were performed in 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 Figure 1. The model consisted of a Plexiglas column with 100 mm inside diameter as downflow ASBF. 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 other synthetic packing media. The most important physical properties of the media were porosity: 92%, specific area: 410 $m^2 m^{-3}$, and density: 186 kg m^{-3}. In addition, the physical specifications of the model were outside diameter: 160 mm, inside diameter: 100 mm, height: 60 cm, total volume: 4.7 l, and effective volume: 3.9 l. To prevent the interference effects of light (photocatalytic) and algae growth, the column was covered with 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.

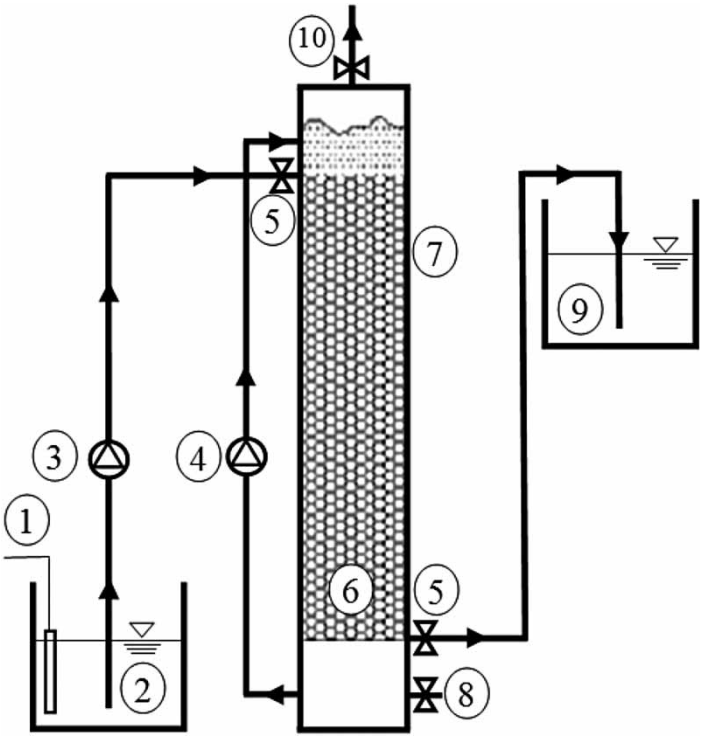

Figure 1 | Flow diagram of the physical model. 1. Temperature controller. 2. Reservoir of feed stock. 3. Peristaltic pump. 4. Return flow line. 5. Sampling ports. 6. Packing media. 7. Anaerobic Biological Filter. 8. Discharge sludge port. 9. Reservoir of outlet. 10. Exhaust of biogas.

Synthetic wastewater

The synthetic wastewater used for feeding the bioreactor was a mixture of sucrose and tap water with chemical oxygen demand (COD) of $1{,}000 \pm 15.7\ \mathrm{mg\,l^{-1}}$. pH fluctuations were controlled using $0.5\ \mathrm{mol\,l^{-1}}$ sodium bicarbonate. Table 2 shows the composition of wastewater used as the feed of the pilot reactor during the test period. Synthetic wastewater was injected into the top of the anerobic filter by a peristaltic pump. Based on the study by Abigail *et al.* (2012), the maximum removal efficiency of ATZ biodegradation occurs at 32 °C. Accordingly, in this study, the temperature was controlled at 32 ± 0.2 °C in the reservoir by an electric heater.

Table 2 | Chemical composition of synthetic wastewater

	Component	Concentration ($\mathrm{mg\,l^{-1}}$)
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	0.2
	$MnSO_4$	0.5
	$(NH_4)_2HP_2O_4$	50
	$C_{12}H_{22}O_{11}$	Variable (600–900)
Atrazine	Variable (0.01, 0.1, 1, and 10)	

Start-up and system operation

In order for the system to operate, the column was filled with synthetic wastewater with $10{,}000\ \mathrm{mg\,l^{-1}}$ COD. The initial seeding was done by using a 1 l mesophilic anaerobic sludge digester of the municipal wastewater treatment plant, Shiraz, Iran. At the first start-up of the reactor, organic loading rate (OLR) in ASBF was $0.5\ \mathrm{g\,COD\,l^{-1}d^{-1}}$. Because methane forming bacteria grow more slowly than acid

forming bacteria, OLR should be reduced at the reactor start-up until organic acids produced by the fermentation bacteria that have rapid growth do not decrease the buffering system. Afterwards, OLR was gradually increased until it reached 20 g COD $l^{-1}d^{-1}$. The whole start-up period lasted for 12 weeks. During this time, the wastewater inside the reactors was changed four times and pH, dissolved oxygen (DO), and temperature were measured as 7.5 ± 0.2, 0.05 mg l^{-1}, 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) and biofilm in the bioreactor was frequently done in a mineral salts medium (MSM) solution containing ATZ. The MSM preparation method was performed based on the study by Rezaee *et al.* (2011).

Experiments

After microbial adaptation was completed, continuous feeding was started. In order to assess the effect of HRT on the efficiency of the filter, wastewater with a strength of 1,000 mg l^{-1} was injected into the anerobic reactor by a peristaltic pump with different ATZ concentrations (since the range of ATZ concentrations is highly varied in the ecosystem and depends on different factors, four logarithmic levels of ATZ concentrations, i.e., 0.01, 0.1, 1, and 10 mg l^{-1}, 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 3.

Sampling was regularly carried out with two times repetitions and when the column reached a steady state regarding ATZ residual and soluble COD, the efficiency of ATZ and SCOD removal was determined. When the difference between some consecutively measured values is less than the previously measured ones, it is the beginning of a steady state. By further sequential measurements, the mean and standard deviation of different parameters can be calculated. Steady-state condition for different parameters occurs almost simultaneously.

The parameters measured in this research were ATZ residual concentration, SCOD, BOD_5, pH, DO, and temperature. The first two parameters and the filter efficiency in ATZ 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 certain 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* (APHA 1998).

Table 3 | The operational scheme of the runs (at 32 °C)

Run	HRT (hours)	Initial conc. of atrazine (mg l^{-1})	Initial conc. of SCOD (mg l^{-1})	Initial conc. of BOD_5 (mg l^{-1})	DO (mg l^{-1})	pH
1	24	0.01	996.5 ± 19.70	398.56	0.016 ± 0.065	7.38
2	24	0.1	1,004.6 ± 12.71	341.57	0.018 ± 0.054	7.50
3	24	1	999.7 ± 12.30	309.91	0.015 ± 0.069	7.37
4	24	10	1,002.1 ± 12.61	230.48	0.014 ± 0.064	7.47
5	12	0.01	997.8 ± 10.45	448.99	0.017 ± 0.056	7.33
6	12	0.1	1,000.8 ± 15.05	320.27	0.018 ± 0.058	7.37
7	12	1	995.6 ± 5.62	298.69	0.013 ± 0.059	7.34
8	12	10	1,007.1 ± 8.14	241.70	0.016 ± 0.061	7.38
9	6	0.01	995.3 ± 14.31	428.01	0.021 ± 0.055	7.35
10	6	0.1	997.4 ± 14.19	349.11	0.013 ± 0.057	7.45
11	6	1	994.8 ± 9.35	288.51	0.019 ± 0.056	7.38
12	6	10	1,003.3 ± 8.66	210.99	0.018 ± 0.057	7.44

Atrazine extraction and determination

Atrazine was extracted from wastewater by the liquid–liquid extraction method suggested by Ghosh & Philip (2004). In addition, dichloromethane (sp. gr.1.32 with atrazine solubility of 28 g l^{-1} at 25 °C) was used as the extractant. The extraction efficiency by this method was 92 ± 0.88%. Atrazine was measured by high performance liquid chromatography (HPLC) (Model: UV-2487, Water, USA) using UV/VIS detector at a wavelength of 220 nm and using Dionex Summit P580, HPLC pump. Analysis was carried out according to the method reported by Yang *et al.* (2010). The analytes were filtered through a 0.22 μm nylon syringe filter. The concentration of ATZ 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 an 80–20% methanol gradient with water, the flow rate was 0.5 ml min^{-1}, and the peak retention time was 12 min. Before each run, the instruments were standardized with anticipated ATZ concentration range. For standardization of the instrument, six standards of ATZ 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 ATZ in biofilm, the method suggested by Ghosh & Philip (2004) was utilized.

Modeling

In almost all the studies, including the one by Baghapour *et al.* (2011), VOL is confirmed as the criterion for submerged filters design 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 Stover & Kincannon (1982). The original model assumed that the suspended biomass was negligible in comparison to the biomass attached to the media (Biglione *et al.* 2008; Tafoya-Garnica *et al.* 2009).

$$R_{ATZ} = R_{max}\frac{B_{ATZ}}{K + B_{ATZ}} \quad (1)$$

where R_{ATZ} is the volumetric ATZ removal, R_{max} is the maximum rate of volumetric ATZ removal, B_{ATZ} is the ATZ load per unit volume of the filter, and K is the constant of half velocity. All the parameters are in $kg_{Atrazine}\ m^{-3}d^{-1}$.

The values of B_{ATZ} and R_{ATZ} could be obtained using from the following equations:

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

$$R_{ATZ} = \frac{Q}{V}(C_i - C_e) \quad (3)$$

where C_i is ATZ concentrations in the influent ($kg_{Atrazine}\ m^{-3}$) and C_e is ATZ concentrations in the effluent ($kg_{Atrazine}\ m^{-3}$).

Using Equations (2) and (3) and Tables 3 and 4, values of B_{ATZ} and R_{ATZ} could be computed for various situations. The main values are presented in Table 5. As well, the values of K and R_{max} were obtained using the Curve Expert software and are presented in Table 6. Also, suggested equations of multivariable modeling were obtained by using MATLAB software presented in Table 7. Some graphs were drawn using MATLAB software.

RESULTS

During the system operation period, the HRT was reduced from 24 to 12 h and then to 6 h. According to the HRTs, the flow rate in the reactor was set at 0.1504, 0.3009, and 0.6018 l h^{-1}, respectively. The most important parameters monitored in the experiments were ATZ residual and SCOD and the means of the measured data are reported in this paper (Table 4). COD of the inflow wastewater was 1,000 ± 15.75 mg l^{-1} in all the situations. The trend of ATZ and SCOD removal is shown in Figures 2 and 3.

By substitution of the values of Table 6 into Equation (1), the results presented in Figures 4–7 were obtained and submerged filters could be designed using these diagrams. As VAL and VOL increased, the values of VAR and VOR increased as well and these relationships were not linear.

Table 4 | Effluent concentrations of atrazine and SCOD and efficiency of their removal from the bioreactor in the steady state at 32 °C

Run	Output conc. of atrazine (mg l^{-1})	Output conc. of SCOD (mg l^{-1})	BOD_5/SCOD	Removal efficiency (%)	
				Atrazine	SCOD
1	$0.0073 \pm 1 \times 10^{-5}$	556.1 ± 1.212	0.40	26.8	44.2
2	$0.0702 \pm 2.4 \times 10^{-6}$	571.6 ± 2.251	0.34	29.7	43.1
3	$0.6059 \pm 2 \times 10^{-5}$	583.8 ± 1.382	0.31	39.6	41.6
4	$4.89 \pm 3.7 \times 10^{-4}$	545.9 ± 1.679	0.23	51.1	45.6
5	$0.00821 \pm 5.1 \times 10^{-6}$	603.7 ± 3.162	0.45	17.9	39.5
6	$0.0783 \pm 1 \times 10^{-6}$	619.5 ± 2.977	0.32	21.7	38.1
7	$0.706 \pm 1.6 \times 10^{-5}$	618.3 ± 1.197	0.30	29.4	37.9
8	$6.41 \pm 3 \times 10^{-4}$	603.2 ± 1.469	0.24	35.9	40.1
9	$0.0091 \pm 2 \times 10^{-5}$	692.7 ± 3.283	0.43	9.2	30.4
10	$0.0809 \pm 5.2 \times 10^{-5}$	706.3 ± 1.825	0.35	19.1	29.2
11	$0.7789 \pm 1.7 \times 10^{-5}$	723.2 ± 0.617	0.29	22.1	27.3
12	$7.017 \pm 1.5 \times 10^{-4}$	691.3 ± 1.677	0.21	28.3	31.1

The number of repetitions in each run after steady state = 3.

Table 5 | Volumetric load and removal of atrazine and SCOD from the bioreactor at 32 °C

Run	B_{ATZ} ($kg_{Atrazine}$ m^{-3} d^{-1})	R_{ATZ} ($kg_{Atrazine}$ m^{-3} d^{-1})	B_{SCOD} (kg_{SCOD} m^{-3} d^{-1})	R_{SCOD} (kg_{SCOD} m^{-3} d^{-1})
1	9.2×10^{-6}	2.47×10^{-6}	0.920	0.40664
2	9.2×10^{-5}	2.73×10^{-5}	0.920	0.39652
3	9.2×10^{-4}	3.64×10^{-4}	0.920	0.38272
4	9.2×10^{-3}	4.70×10^{-3}	0.920	0.41952
5	1.84×10^{-5}	3.28×10^{-6}	1.840	0.7268
6	1.84×10^{-4}	3.99×10^{-5}	1.840	0.70104
7	1.84×10^{-3}	5.41×10^{-4}	1.840	0.69736
8	1.84×10^{-2}	6.60×10^{-3}	1.840	0.73784
9	3.68×10^{-5}	3.38×10^{-6}	3.680	1.11872
10	3.68×10^{-4}	7.01×10^{-5}	3.680	1.07456
11	3.68×10^{-3}	8.13×10^{-4}	3.680	1.00464
12	3.68×10^{-2}	1.04×10^{-2}	3.680	1.14448

Table 6 | K and R_{max} coefficients of the bioreactor at 32 °C for Stover–Kincannon model

	Atrazine	SCOD
R_{max}, (kg m^{-3} d^{-1})	0.0231	1.9623
K, (kg m^{-3} d^{-1})	0.0448	3.1304
R^2	0.997	0.988

DISCUSSION

Atrazine degradation potential of the mixed anaerobic consortium was evaluated under various ATZ concentrations and HRTs and the results are presented in Table 4. Results (Figures 2 and 3) showed that in this filter, the ATZ and SCOD removal efficiency increases with HRT. The findings of this study demonstrated that the solution containing ATZ was easily biodegraded and treated in an ASBF. Moreover, ATZ removal efficiencies were above 28% where high ATZ influent was introduced in the ASBF (runs 4, 8, and 12). The major part of the input ATZ was consumed during these runs as indicated by low effluent ATZ concentration (below $4.89 \pm 10^{-4} \times 3.7$ mg l^{-1}). The treatment efficiencies achieved at longer HRTs (24 h) in the ASBF fed with low, moderate, and high ATZ concentrations in the influent are summarized in Table 4. It is evident that in comparison to other HRTs, ATZ and SCOD removal efficiencies were increased at long HRTs due to the slight decrease in ATZ and organic loading rates in the ASBF. Based on the results obtained from Figures 4–7, the extent of ATZ loading rate was not highly effective in biological ATZ and organic removal efficiencies. When, the HRT was set at 24 h and the ASBF was operated at these conditions until

Table 7 | Suggested equations of multivariable modeling

	Variable	Suggested equation	R^2
The effect of initial ATZ concentration and HRT on ATZ removal efficiency	x = initial ATZ concentration y = HRT	$f(x, y) = (1.213x) + (3.9y) + (-0.000198xy) + (-0.2329y^2) + (0.001483xy^2) + (0.005182y^3)$	0.96
The effect of initial ATZ concentration and HRT on SCOD removal efficiency	x = initial ATZ concentration y = HRT	$f(x, y) = (-2.244x) + (7.288y) + (0.2474x^2) + (-0.01598xy) + (-0.4416y^2) + (-0.0005446x^2y) + (0.0008222xy^2) + (0.008909y^3)$	0.99

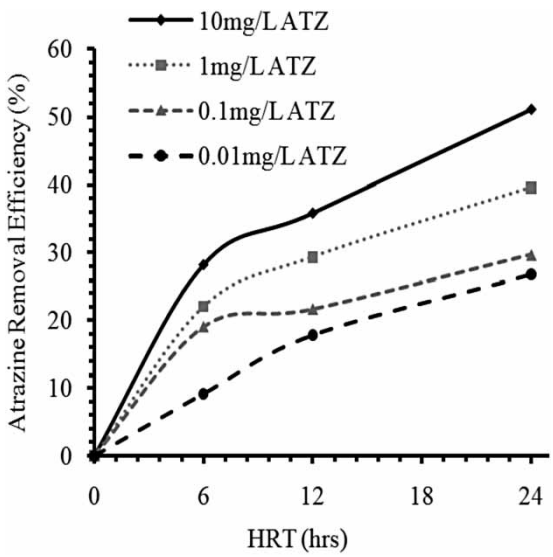

Figure 2 | The trend of atrazine removal in the bioreactor at 32 °C.

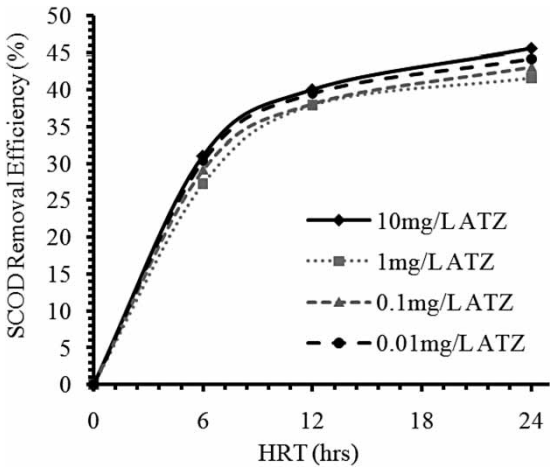

Figure 3 | The trend of SCOD removal in the bioreactor at 32 °C.

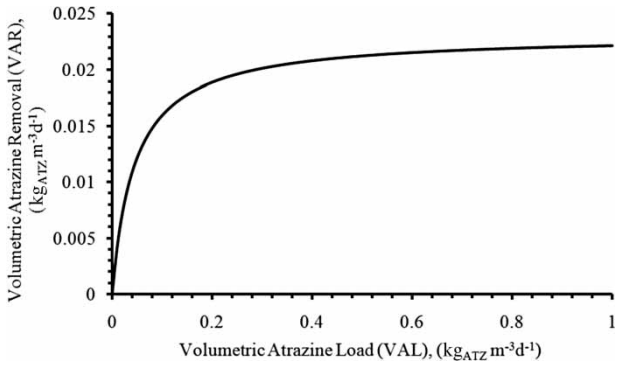

Figure 4 | Atrazine loading of the bioreactor in the range of 0 to 1 $kg_{Atrazine}\ m^{-3}\ d^{-1}$ at 32 °C.

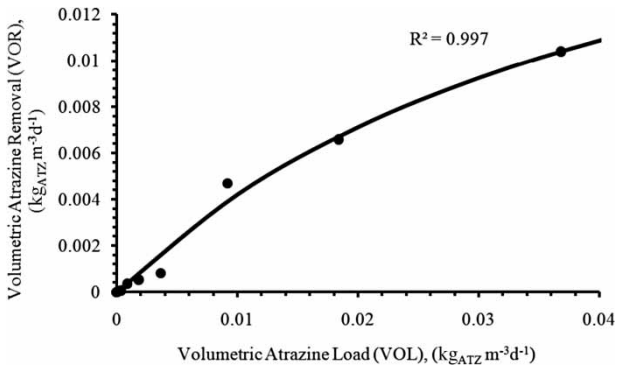

Figure 5 | Atrazine loading of the bioreactor in the range of 0 to 0.04 $kg_{Atrazine}\ m^{-3}\ d^{-1}$ at 32 °C.

steady-state conditions were reached, the ATZ and SCOD removal efficiencies were increased up to 51.1 and 45.6%, respectively (Table 4). Also, Figures 8 and 9 showed that in this filter, the ATZ and SCOD removal efficiency completely depend on HRT and initial ATZ concentration. Therefore, it can be concluded that decreasing ATZ as well as organic

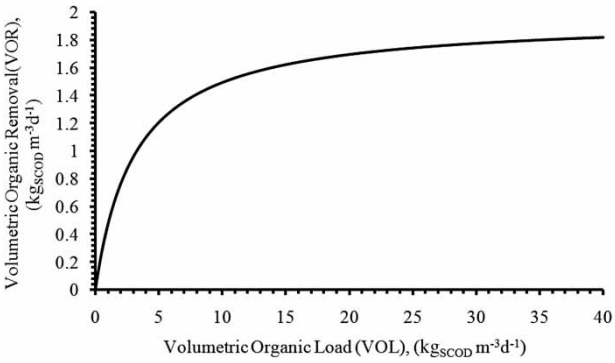

Figure 6 | Organic loading of the bioreactor in the range of 0 to 40 $kg_{SCOD}\ m^{-3}\ d^{-1}$ at 32 °C.

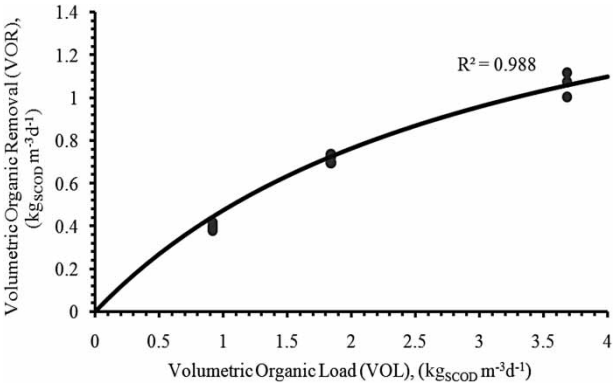

Figure 7 | Organic loading of the bioreactor in the range of 0 to 4 $kg_{SCOD}\ m^{-3}\ d^{-1}$ at 32 °C.

loading positively affect the ASBF performance. This can be due to the increase of the probability of the contaminants exposure with microbial consortium, which is consistent with the results obtained by Ghosh & Philip (2004) and Rezaee *et al.* (2011). Measurement of COD is important regarding the effluent discharge standards and COD represents the treatment potential of the reactor. In this study, ASBF showed an acceptable SCOD removal efficiency in all the experiments. As well, ATZ revealed no adverse effects on SCOD removal up to the concentration of 10 $mg\ l^{-1}$. However, SCOD reduction was reduced by 2–4% when ATZ concentration was increased to 0.1 and 1 $mg\ l^{-1}$, which is in agreement with the results of the study by Ghosh & Philip (2004). Since ASBFs have not been used for removing pesticides and this is a new method for ATZ removal, no research has been conducted on this issue. However, a number of similar studies are presented in Table 1. Keerthinarayana & Bandyopadhyay (1997) with anaerobic sediment batch bioreactor/dextrose as the external carbon source reached 20% efficiency at an initial ATZ concentration of 1 $mg\ l^{-1}$ within 38 weeks. Moreover, Jessee *et al.* (1983) conducted an experiment in test tube and reached 47% efficiency using facultative anaerobic bacterium during 1 week. Crawford *et al.* (1998) also used the initial ATZ concentration of 21.6 $mg\ l^{-1}$ and pure culture *M91-3* using fixed film batch column system under anoxic

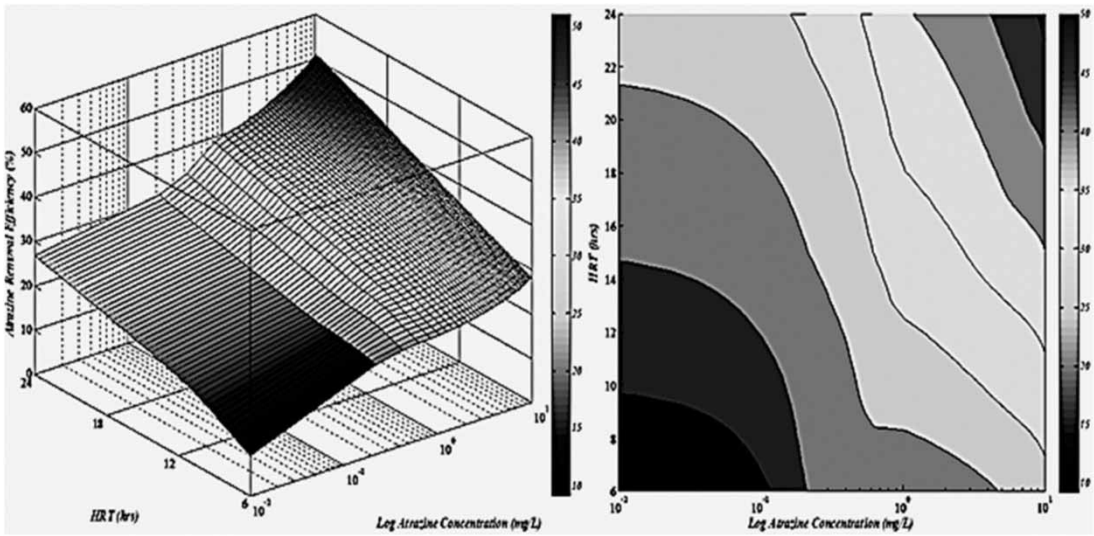

Figure 8 | The effect of initial atrazine concentration and HRT on atrazine removal efficiency.

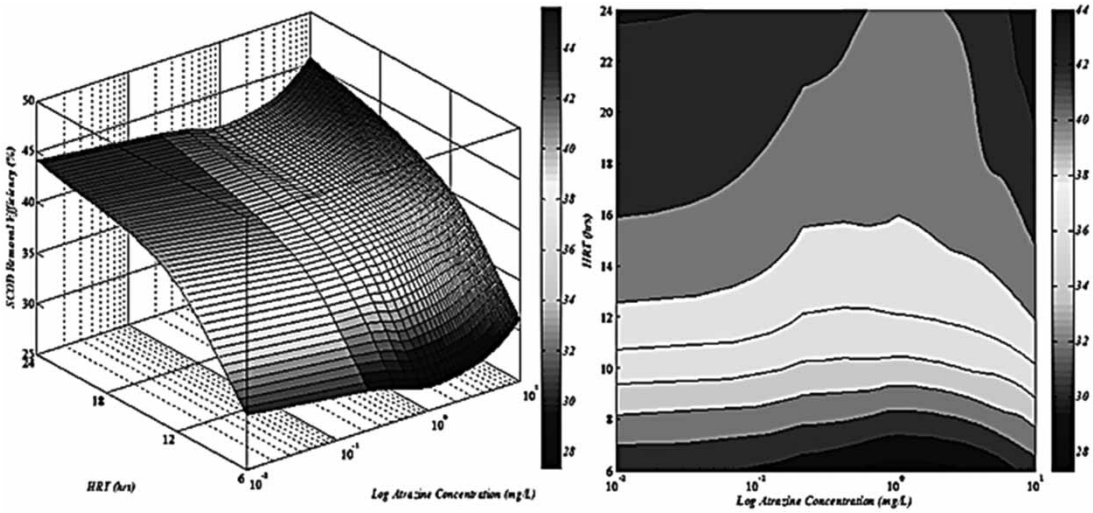

Figure 9 | The effect of initial atrazine concentration and HRT on SCOD removal efficiency.

conditions reaching 60% efficiency within 6 days. Ghosh & Philip (2004) with sequential mode of operation and dextrose as the external carbon source at the initial ATZ concentration of 1 mg l^{-1} reached 40% efficiency in 5 days. Also, in batch reactor with no external carbon and nitrogen, they reached 42% efficiency in 150 days. Comparison of the results of the previous studies to those of the present one shows that this system has a high capability for removing ATZ from aqueous solutions. There was no accumulation of ATZ in the biofilm and the loss of ATZ in the control reactor was negligible. This shows that ATZ removal from the system was due to biodegradation. High degradation rate of ATZ at comparatively high ATZ concentrations 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 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 ASBF was able to increase the BOD_5/COD ratio to more than 0.21 in all the experiments. Moreover, significant changes were observed in BOD_5/COD ratios by increasing the HRT.

Co-metabolic processes are used for bioremediation of most persistence contaminants, such as ATZ. By utilizing primary carbon or nitrogen sources in co-metabolic processes, microbes produce enzymes or co-factor during microbial activities which are responsible for degradation of the secondary substrates (toxic compounds, ATZ).

The results obtained from ASBF showed that the co-metabolic process was quite effective in removing ATZ from the aqueous environment. Additional nitrogen sources (ammonium phosphate) also showed no adverse effects on ATZ degradation. Similar results were also reported by Yang *et al*. Overall, the results of the modeling showed that the Stover–Kincannon model had a very good fitness ($R^2 > 99\%$) in loading ATZ in this biofilter, which is in line with the findings of the study by Cheyns *et al*. (2010).

CONCLUSION

The present study investigated the ability of an ASBF to remove ATZ from an aqueous environment. The ASBF was operated at three different anaerobic retention times in order to determine the optimum retention time for the highest ATZ and COD removal. Finally, anaerobic mixed biofilm culture was observed to be suitable for treatment of ATZ from aqueous solutions. There was no significant inhibition effect on mixed anaerobic microbial consortia. Atrazine degradation depended on the strength of wastewater and the amount of ATZ in the influent and HRTs. Also, the

Stover–Kincannon model more desirably described ATZ degradation in aquatic environment using an ASBF.

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