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Research article

Simultaneous removal of atrazine and organic matter from wastewater using anaerobic moving bed biofilm reactor: A performance analysis

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ABSTRACT

In this study, an anaerobic moving bed biofilm reactor (AMBBR) was designed to biodegrade atrazine under mesophilic (32 °C) condition and then it was evaluated for approximately 1 year. After biofilm formation, acclimation, and enrichment of microbial population within the bioreactor, the effect of various operation conditions such as changes in the concentration of influent atrazine and sucrose, hydraulic retention time (HRT), and salinity on the removal of atrazine and chemical oxygen demand (COD) were studied. In optimum conditions, the maximum removal efficiency of atrazine and COD was 60.5% and 97.4%, respectively. Various models were developed to predict the performance of atrazine removal as a function of HRT during continuous digestion. Also, coefficients kinetics was studied and the maximum atrazine removal rate was determined by Stover - Kincannon model ($r_{max} = 0.223\ kg_{ATZ}/m^3d$). Increasing salinity up to 20 g/L NaCl in influent flow could inhibit atrazine biodegradation process strongly in the AMBBR reactor; whereas, the reactor could tolerate the concentrations less than 20 g/L easily. Results showed that AMBBR is feasible, easy, affordable, so suitable process for efficiently biodegrading toxic chlorinated organic compounds such as atrazine. There was no accumulation of atrazine in the biofilm and the loss of atrazine in the control reactor was negligible; this shows that atrazine removal mechanism in this system was due to co-metabolism.

1. Introduction

Many chemical xenobiotic compounds entering the environment are aromatic compounds that used in industry and agriculture frequently (Boopathy, 2017). These compounds are entered into soil and aquatic environment through various resources and because of their specific characteristics, they accumulate in the environment (Pérez-Leblic et al., 2012). Atrazine (2-chloro-4-

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ethylamino-6-isopropylamino-1,3,5-triazine) as a kind of s-triazine is one of the most commonly used herbicide in the worldwide (Baghapour et al., 2013). It's known as possible human carcinogens (Group 2B) and persistent organic pollutants (POPs) in the environment (Nasseri et al., 2014). Due to atrazine widespread use in several industrial and agricultural activity and physicochemical properties, is frequently detected in surface and subsurface water resources (Baghapour et al., 2013; Boopathy, 2017). United state environmental protection agency (EPA) and world health organization (WHO) have established the maximum contaminant level (MCL) of atrazine in drinking water as 3 and 2 μg/L, respectively. Similarly, in Iran the institute of standards and industrial research of Iran (ISIRI) has set a limit for atrazine as 2 μg/L (Baghapour et al., 2013; Nasseri et al., 2014).

In order to protect human health and environment from the adverse effects of atrazine, the effluents containing it needed to be treated with suitable methods (Baghapour et al., 2013). Atrazine treatment is mainly carried out by various physicochemical methods. These methods of treatment are pricey, complicated and produce a lot of toxic intermediates (Boopathy, 2017). The biological method breaks and transforms the complex compounds to simple compounds, such as carbon dioxide, water, nitrogen and etc. (Nasseri et al., 2014). Atrazine removal thru biological methods is also employed both aerobically as well as anaerobically, but very few studies have done on atrazine biodegradation in anaerobic conditions versus aerobic conditions (Boopathy, 2017; Nasseri et al., 2014). Aerobic bioprocesses have been used to biodegrade atrazine but they have high aeration cost, are complex for operation and maintenance, generate high amount of biomass which poses economic and environmental challenges (Baghapour et al., 2013; Derakhshan et al., 2016). In anaerobic treatment process, converts the organic pollution to a small quantity of sludge and can produce a large amount of biogas (Ghosh and Philip, 2004).

In recent decades, anaerobic moving bed biofilm reactors (AMBBRs) technology in wastewater treatment of municipal, industrial, and agricultural has been used successfully (Wang et al., 2009). In AMBBRs reactor, the growth of microorganisms occurs on moving solid carriers resulting in the formation of stable biofilm (Kawan et al., 2016; Kermani et al., 2008). The biofilm careers used in AMBBR usually are with density less than water and they can move in the fluid inside the reactor (di Biase et al., 2017; Kermani et al., 2008). The main advantage of AMBBRs along with increasing microbial activity is reduction of head losses, no filter channeling, operated easily, without biomass loss or temperature dependence, strong tolerance to loading impact and relatively smaller reactor (di Biase et al., 2017; di Biase et al., 2016; Kawan et al., 2016). Also, they have a simple design and not using a mechanical mixing, and therefore are economically affordable (di Biase et al., 2017; Wang et al., 2009). For those reasons, AMBBRs are appropriate for the treatment of a variety of wastewaters containing toxic and xenobiotic compounds (di Biase et al., 2017; Wang et al., 2009).

The main objective of this study was to evaluate the efficiency of AMBBR reactor as well as HRTs in atrazine and COD removal at different influent concentrations in order to examine the capability of this reactor in different loading rates. Secondly, we aimed to explore the effect of salinity on biodegradation of atrazine as a model compounded of chlorinated aromatic xenobiotic that are widely used in agriculture.

2. Materials and methods

2.1. Startup and operation of bioreactors

In this pilot-scale study, the effects of the input atrazine concentration, HRT, and salinity were evaluated in an AMBBR reactor. As indicated in Fig. 1S, the AMBBR reactor is used that is made of Plexiglas (diameter = 20 cm, height = 50 cm, freeboard = 2 cm) with a working volume of 15 L. 70% of the reactor volume were filled with prepared media (diameter = 2 cm, height = 1 cm, and relative density = 0.98). The area of biofilm carriers available for pre-formed biofilm was about 410 m^2/m^3 (Baghapour et al., 2013; Nasseri et al., 2014). Continuous mixing reactor was done by a submerged pump installed in the reactor floor. Mixing time was adjusted for 2 to 8 times per hour based on organic loading rate increase during experiments. The duration of mixing lasted for 1.30 min. This duration could animate the bed in the reactor. Hydraulic retention times (HRTs) was set by controlling the flow rate of influent synthetic wastewater. To discharge the possible accumulated sludge, a drain valve was used at the bottom of the bioreactor. To control confounding variables, fluctuations in raw wastewater, and the system best operation, synthetic wastewater was used. pH level of raw sewage was set about 7.5 ± 0.1 using sodium bicarbonate (0.5 mol/L). Bicarbonate can act as a buffer to maintain pH and also as an electron receptor for hydrogenotrophic methanogenesis (Delgado et al., 2012; Paulo et al., 2003). The water needed for synthetic wastewater was provided from tap water. The synthetic wastewater had the following composition: $NaHCO_3$ = 20 mg/L, $MgSO_4 \cdot 7H_2O$ = 5 mg/L, KH_2PO_4 = 5 mg/L, $CaCl_2 \cdot 2H_2O$ = 5 mg/L, $(NH_4)_2HP_2O_4$ = 50 mg/L, $C_{12}H_{22}O_{11}$ variable (300–1100 mg/L), atrazine variable (0.1, 1 and 10 mg/L) and trace element were: $FeSO_4 \cdot 7H_2O$ = 0.2 mg/L, $ZnCl_2$ = 0.1 mg/L, $CoCl_2$ = 0.1 mg/L, $NiCl_2$ = 0.1 mg/L, $CuSO_4 \cdot 5H_2O$ = 0.001 mg/L, H_3BO_3 = 0.2 mg/L, $MnSO_4$ = 0.5 mg/L (Nasseri et al., 2014). According to previous studies, the maximum removal efficiency of atrazine biodegradation occurs at 32 °C (Baghapour et al., 2013; Nasseri et al., 2014). In this study, the temperature was set at 32 °C in the feeder tank by an electric heater. Although the compositions of synthetic wastewater were completely soluble in water, a small electric mixer was used to return sewage from the floor to the top of the tank in order to prevent quality changes in the wastewater happened due to storage. The electric mixer rotated all the wastewater every 15 min (Fig. 1S). The operational scheme of the system for 9 phases is presented in Table 1.

2.2. Preparing and installing the reactor

To set up the system and initiate biological adaptation stage, a filter column with an approximate volume of 15 L as pilot was seeded by the mesophilic anaerobic sludge digester bacteria collected from Shiraz urban wastewater treatment plant which had no operational problem, the concentration suspended solids was 30 g/L, and VSS/TSS ratio was 0.8; the remained space inside the bioreactor was filled with the synthetic wastewater made out of a chemical oxygen demand (COD) of 10,000 mg/L. For more acclimation of microorganisms at the presence of atrazine in the environment, the reactor was fed with synthetic wastewater (15 L) containing 0.1 mg/L atrazine. The effluent was re-circulated to the influent while the concentration of atrazine and COD were evaluated in the re-circulated solution. At the first startup of the reactor, organic loading rate (OLR) in AMBBR was 0.5 g COD/L d. Due to methanogenic bacteria grow more slowly than acidogenic bacteria, OLR should be reduced at the reactor startup until organic acids produced by the fermentation bacteria that have rapid growth do not decrease the buffering system (Nasseri et al., 2014). Afterwards, OLR was gradually increased until it reached 2 g COD/L d. It was assumed that when the concentration of COD in the re-circulated solution increased to more than 95% degradation, acclimation is attained. To enrich the population of the microorganisms capable of

Table 1
The operational scheme of the AMBBR (at 32 °C).

Phase	HRT (hours)	Initial Conc. of atrazine (mg/L)	Initial Conc. of COD (mg/L)
1	24	0.1	251.5 ± 17.83
2	24	1	249.6 ± 13.61
3	24	10	250.7 ± 12.37
4	24	0.1	502.1 ± 9.61
5	24	1	497.8 ± 10.23
6	24	10	500.8 ± 12.35
7	24	10	995.6 ± 8.62
8	24	1	1007.1 ± 8.14
9	24	0.1	995.3 ± 13.47

degrading atrazine, the recycle solution was replaced with a fresh one containing 0.1 mg/L atrazine, and as mentioned before the effluent was re-circulated to the influent. This procedure was repeated for 3 consecutive cycles to ensure atrazine -degrading and microbial population increase. To ensure the microbial activity in this phase, surface cultivation of mixed liquor suspended solids (MLSS) in the bioreactor was frequently performed in a mineral salt medium (MSM) solution containing atrazine. The MSM preparation method was conducted according to the previous study (Tafoya-Garnica et al., 2009). Also, to evaluate the growth of biofilm on media in this phase, scanning electron microscope (SEM) was applied. This stage lasted for 88 days. A brief explanation is brought in the Supplementary data. The external wall of the reactor was covered with the aluminum foil in order to avoid the confounding effect of light (photo-catalyst) and algae growth. In addition, a control pilot with the same physical characteristics of the main pilot was utilized to increase the accuracy and omit the effect of the confounding factors.

2.3. Experimental protocol

Effects of influent concentrations of atrazine (0.1 up to 10 mg/L) (Baghapour et al., 2013; Nasseri et al., 2014), COD (250–1000 mg/L) (Derakhshan et al., 2016; Ghosh and Philip, 2004), HRT (6–24 h) and salinity were investigated on the performance of the AMBBR reactor regarding atrazine biodegradation. In each phase of the experiment, the bioreactor operated until the steady-state performance was achieved. The operation was continued for 3–5 times. It was presupposed that steady-state condition is attained when changes of atrazine removal percentage remain below 2%. The HRT of 24, 12, and 6 h was considered in the current study for removing target contaminants. After microbial adaptation and enrichment, reactor operation continuous phase was launched feeding with synthetic wastewater containing 0.1 mg/L atrazine and COD 250 mg/L at an HRT of 24 h. The concentrations of COD, as well as influent and effluent atrazine, were monitored on a daily basis. Given that the range of atrazine concentrations is highly varied in the ecosystem and depends on different factors (Baghapour et al., 2013; Nasseri et al., 2014), 3 logarithmic levels of atrazine concentrations, i.e., 0.1, 1, and 10 mg/L were selected in order to examine a wide range of concentrations and atrazine removal efficiency by AMBBR bioreactor. After achieving stable conditions in each phase, sampling was done and parameters such as concentrations of influent atrazine (by HPLC) (Derakhshan et al., 2016), COD, volatile fatty acids (VFA), volatile suspended solids (VSS), and total suspended solids (TSS), pH, DO and temperature was investigated (APHA, 2005). The COD was colorimetrically determined (Model DR 5000 Spectrophotometer, Hach) following dichromate digestion. The pH was electrochemically measured (Model F-22, Horiba). Within each stage, sampling was conducted from the two influent and effluent points of the reactor and the tests were repeated at least twice. Samples taken from effluent and influent were centrifuged initially to remove suspended solids and then filtered through a cellulosic paper filter with a pore size of 0.45 μm, and the filtrate was analyzed. The mean of the obtained data was calculated. Sampling method and tests' implementation were performed according to the guidance provided by standard methods for the examination of water and wastewater book (APHA, 2005). Atrazine was extracted from samples by the liquid-liquid extraction method proposed by Ghosh and Philip (2004). Also, to check the buildup and absorption of atrazine in biofilms, the recommended technique by Derakhshan et al. (2016) was utilized. Detailed descriptions for sample analysis can be found in Supplementary data.

3. Results and discussion

3.1. Biomass acclimation in batch–feed mode

The acclimation of biomass to biodegraded atrazine and COD removal in anaerobic conditions was a substantial phase of the AMBBR operation. Results of the acclimation phase is summarized in Table 2. As can be seen, the time needed to achieve above 95% biodegradation of atrazine decreased from 31 days to 12 days, while its degradation efficiency increased from 98.38 to 99.15% during the four runs of the acclimation phase. These results clearly indicate that the microorganisms in the biomass were successfully acclimated and enriched to biodegrade 0.1 mg/L atrazine. Previous studies revealed that the extracellular enzymes (exoenzyme) of anaerobic microbe secretion in biological activity in response to demanding environmental conditions such as high organic loadings and exposure to toxic and POP. Extracellular polymeric substances (EPS) contains compounds such as humic acid, protein, amino acids, polysaccharides, structural components of cells, and products induced by cellular metabolism (Hoffman and Decho, 1999; Wingender and Jaeger, 2002). According to Louwe Kooijmans and Van Velsen (1986), a UASB reactor required a period of 180 days to start up at 25 °C. The acclimation time for anaerobic biodegradation of atrazine was reported by Nasseri et al. (2014) to be 84 d. Vijayaraghavan and Ramanujam (1999) reported the start-up time of 160 days for an anaerobic biofilter. Oktem et al. (2008) reported acclimation time for anaerobic biodegradation of pharmaceutical wastewater was about 90 d. Results showed that no biological accumulation in the obtained biofilm observed and the removal of atrazine rate in control reactor was negligible indicating that the removal of atrazine in this system was due to microbial activity.

3.2. AMBBR startup and effects of atrazine and COD concentration

After successful acclimation and enrichment, the operation of the AMBBR was changed from batch to continuous mode by continuously feeding a synthetic wastewater. Fig. 1 shows the

Table 2
Results of the biomass acclimation to atrazine biodegradation.

Acclimation run	Time (d)	OLR (g COD/L d)	COD removal efficiency (%)	ATZ degradation (%)
1st run	31	0.5	96.28	98.38
2nd run	27	1	96.52	98.57
3rd run	18	1.5	97.13	98.54
4th run	12	2	97.3	99.15

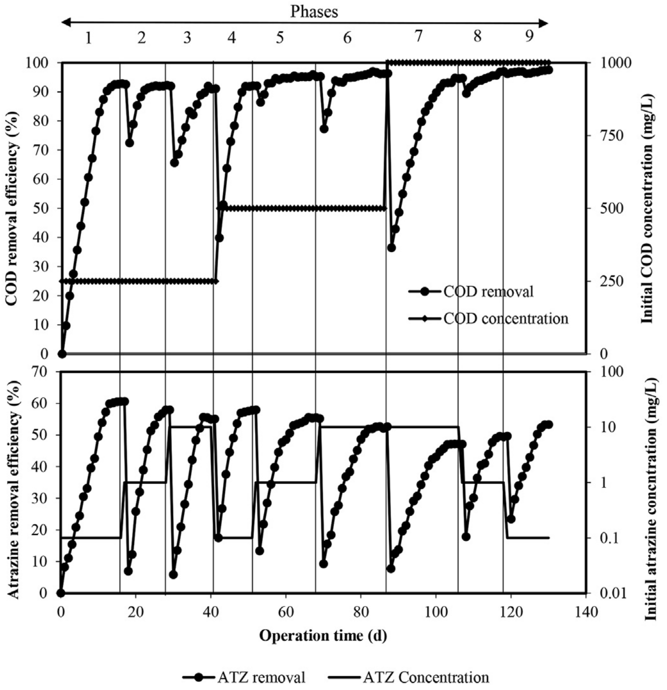

Fig. 1. Time-course biodegradation of atrazine and COD removal in the AMBBR at various inlet concentrations (atrazine = 0.1–10 mg/L; COD = 250–1000 mg/L, HRT = 24 h).

variations in the influent and effluent COD and atrazine values and removal efficiencies of COD and atrazine over a 133 d experiment. Atrazine concentration was changed based on requirements and laboratory conditions without considering sucrose concentration and other components of wastewater. When the removal of the pollutants reached to maximum in stable condition, more atrazine was inserted by a synthetic wastewater. The concentrations of influent atrazine were 0.1, 1, and 10 mg/L and COD concentrations were 250, 500 and 1000 mg/L. As is clear from Fig. 1, the hydraulic retention time was set at 24 h. Then, a synthetic wastewater containing COD (250 mg/L) and atrazine (0.1 mg/L) and in loading rate of 0.36 kg_{COD}/m^3.d was used. Achieving stable condition, atrazine and COD removal efficiencies increased to more than 60% and 92%, respectively. In the next phase, a different concentration of synthetic wastewater was applied in order to evaluate the effect of influent atrazine on its removal in an anaerobic bioreactor. At the end of the first phase, 1 day following the changes in the influent wastewater compounds, impressive reduction in atrazine removal efficiency occurred (53%). However, atrazine and COD removal efficiencies increased over time and after continuing the operation of the anaerobic bioreactor. Sudden decrease in the atrazine removal at this phase can be attributed to atrazine addition and temporary shock to biofilms and the microorganisms available in the environment due to the sudden change in the concentration and composition of feeding solutions. This finding is in line with other studies, including Tay et al. (2000). After the first phase, the concentration of atrazine was increased to 1 mg/L from 17th day of anaerobic bioreactor operation up to 29th day. COD and HRT were kept constant while the operation of the reactor was continued to achieve steady state condition and atrazine removal reach to more than 50%. The same results were repeated for the third phase. However, no change was observed in COD efficiency removal following atrazine concentration increase demonstrating that COD removal is independent of influent atrazine concentration. This finding can be due to minor role of atrazine in COD that is consistent with results of previous studies, including Nasseri et al. (2014) and Ghosh and Philip (2004). In the fourth phase, sucrose concentration was increased to 500 mg/L from 41th day to 86th day while the concentration of influent atrazine was varied at HRT 24 h and loading of 0.71 kg_{COD}/m^3.d. The reactor operation was stopped when anaerobic bioreactor reached again to stable condition and the COD removal efficiency got to 95%. COD removal efficiency decrease because of loading increase. Dutta et al. (2014) with using anaerobic fluidized membrane reactor removed pharmaceuticals and organic matter from synthetic municipal wastewater. They observed that increasing the OLR reduces the removal efficiency. Nasseri et al. (2014) investigate performance of an anaerobic submerged biofilter and they reported that as the amount of OLR increases, the removal efficiency of the bioreactor was decreases which is in line with the findings of the study by Ghosh and Philip (2004). Atrazine removal efficiency was reduced to 57% under this condition revealing no significant change compared to previous phase. When the initial concentration of carbon source increased from 500 to 1000 mg/L, removal efficiency of atrazine reduced. This finding can be due to prevention of atrazine degrading enzymes secretion and production by microorganisms that these enzymes are secreted in terms of the lack of substrate and harsh environmental conditions (Hoffman and Decho, 1999; Wingender and Jaeger, 2002). Atrazine accumulation in sludge and biofilm was monitored at various time intervals and no accumulation was seen in the samples during the operation of the bioreactor. The average of COD and atrazine efficiency removal, when achieved steady-state condition at a 24 h HRT in the AMBBR bioreactor, is shown in Table 3.

As depicted in Table 3, atrazine efficiencies removal in stable condition in anaerobic reactor in all phases of operation for influent atrazine and COD, they were above 47%, and 91% respectively. Nasseri et al. (2014) atrazine biodegradation was analyzed in an anaerobic biofilter reactor, and at different atrazine loading rate that in optimal condition atrazine removal rate of 51% was reported. The standard deviation of less than 1% for atrazine and COD removal during steady-state condition indicated the satisfactory and uniform bioreactor performance and also the stability of operation conditions. Reduction in atrazine efficiency removal when influent atrazine concentrations increased can be attributed to the achievement of microorganisms to the maximum biodegradation of atrazine and the deterrent effect of the substrate on biomass activity in different investigated concentrations (Buttiglieri et al., 2011). The significant decrease in atrazine removal when atrazine concentrations increased to 10 mg/L in wastewater entering the bioreactor can be explained by possible inhibitory effects of atrazine on the activity of degrading microorganisms in

Table 3
Atrazine and COD removal efficiency in the steady state at 32 °C.

Initial ATZ Con. (mg/L)	Initial COD Con. (mg/L)	ATZ removal efficiency (%)	COD removal efficiency (%)
0.1	250	60.533 ± 0.29	92.065 ± 0.58
	500	57.734 ± 0.20	95.453 ± 0.41
	1000	52.935 ± 0.42	97.393 ± 0.83
1	250	57.508 ± 0.47	91.980 ± 0.98
	500	55.293 ± 0.39	94.647 ± 0.85
	1000	49.508 ± 0.22	96.717 ± 0.44
10	250	55.159 ± 0.29	91.329 ± 0.60
	500	52.450 ± 0.33	92.678 ± 0.93
	1000	47.115 ± 0.37	96.217 ± 0.69

the reactor under the operation conditions (El-Bestawy et al., 2013; Ghosh and Philip, 2004).

3.3. Effect of HRT on the AMBBR performance

Ensuring the cost-effectiveness and economic feasibility of the operation phase of a bioreactor is essential. Thus, the volume of the reactor and its compactness effectively undermines the economic aspects of the operation phase (Metcalf and Eddy, 1991). The effects of different HRTs between 6 and 24 h on the performance of the AMBBR were investigated in order to determine the optimum level that results showed in Fig. 2. Over 47 d (from Day 131 to Day 177 of the operation), the HRT was lowered stepwise from 24 h to 6 h while the atrazine and COD concentration were kept fixed at 0.1 and 200 mg/L respectively. This led to an increase from 0.14 to 0.57 g_{ATZ}/m^3d loading rate. Although the reduction in HRT from 24 to 12 h reduced the removal of atrazine from 57% to 11% and COD from 97% to 70%. AMBBR reactor performance was recovered again and reached to stable conditions and removal efficiency of more than 56% after about 17 days. In the following phase of operation, HRT was reduced to 6 h and the effluent was monitored. As is clear from Fig. 2a, atrazine and COD removal rates were very sharp after following the change in HRT and decreased to 10% and 58% respectively. However, continued operation of the reactor demonstrated that the bioreactor can quickly restore its performance and the removal of atrazine and COD after steady-state conditions and 23 days following hydraulic shock exertion reached to more 55% and 96%, respectively. Recovery in the performance of AMBBR reactor regarding biodegradation of atrazine and COD after reducing HRT specifies that AMBBR reactor is resistant against loading rates and hydraulic shocks happened by increasing the flow rate. Following HRT increase, COD and atrazine removal efficiencies augmented that can be due to increase in contact time and availability of biological substrate resulted in methanogens activity increase. Similar results are reported by Nasseri et al. (2014) and Ghosh and Philip (2004) regarding atrazine degradation increase with the increases of HRT. Chung et al. (1996) investigated atrazine removal in wetland sediments at anaerobic condition by using various carbon and energy sources. They showed that approximately 20% of total atrazine was transformed and this reduction was assumed as the mineralization of atrazine to end products, such as NH_3 and CO_2. According to Fig. 2a, it can be concluded that the removal of atrazine and COD was more than 57% and 97% respectively at an HRT of 24 h. The removal means of atrazine in stable condition at a retention time of 12 and 6 h was decreased slightly (56%, 55%). The reactor needed longer time for recovery and retrieval to reach steady-state performance when HRT was shorter. Mainly because the reduction in contact time between the biomass and substrate reduced the time for the transfer of atrazine and contaminants from fluid volume into the biomass in the bioreactor. Accordingly, the concentration of effluent pollutants increased and therefore the efficiency and percentage of removal of decreased. In shorter retention times, the contact time between substrate and biomass was lessened, and thus biodegradation rates also reduced. In addition, the decreased in atrazine removal by HRT reduction due to reduced contact time between atrazine molecules and biomass suggesting biokinetics of atrazine biodegradation by microorganisms participated in the process. With respect to Fig. 2b, COD and atrazine removal in stable condition during the operation was invariable with a standard deviation of less than 0.4%. This uniformity can be attributed to the nature of the bioreactor used in the current study posting abundant sticking biomass adapted well for biodegradation of pollutants. Moreover, the structure and properties of bioreactor not only maximized the contact between biomass and substrate but also led to the reduction of the hydraulic shortcuts which subsequently resulted in a very uniform

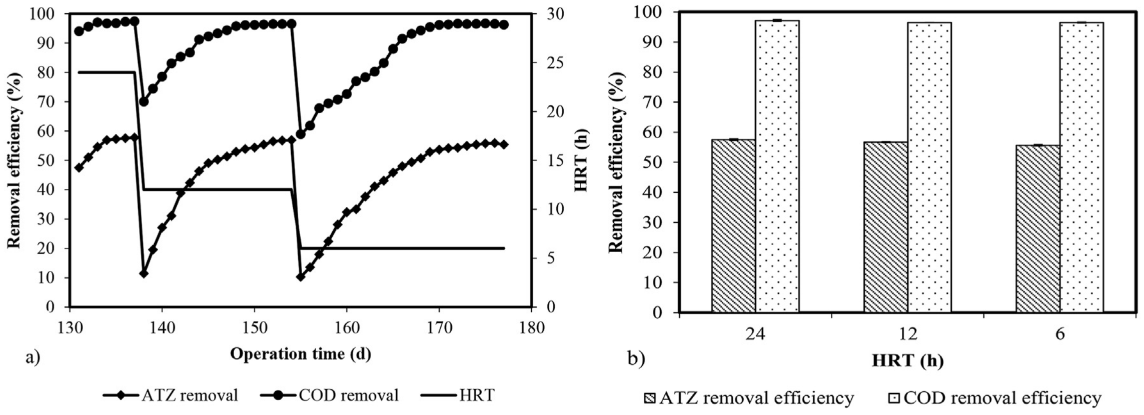

Fig. 2. Biodegradation of atrazine and COD removal in the AMBBR at various HRT (atrazine = 0.1 mg/L; COD = 500 mg/L): (a) time-course and (b) mean steady-state results.

distribution of contaminants in the reactor (Ghorbanian et al., 2014; Mazioti et al., 2015). With regard to obtained results, it could be supposed that variations in HRT in defined range are effective on atrazine and COD efficiency removal which has been approved by the previous studies and can support the accuracy of experiments and AMBBR bioreactor correct performance. The results experimental that the HRT is a controlling parameter in the decomposition of organic matter. Many sources hold that the setup of an anaerobic reactor is a complex process and depends on various factors such as temperature, pH, sludge type chosen for initial seeding, characteristics of wastewater and the presence of toxic compounds in it, nutrients, HRT, and loading rate. In the anaerobic system, appropriate HRT provides enough time for microorganisms and pollutants contact within the fluid volume and increases the removal efficiency of pollutants and TSS by influencing the rate of biogas production and the flow speed (di Biase et al., 2016; Oktem et al., 2008; Schmidt and Ahring, 1996). Ruiz et al. (1998) evaluated the efficiency of UASB reactor in removal of COD and TSS at 20 °C and reported that COD and TSS removal efficiencies augmented from 53% to 73% and 63%–80%, respectively, by increase of HRT from 4 h to 8 h. Zhang et al. (2013) investigated the performance of an anaerobic system at 10 h HRT and achieved COD efficiency more than 90%.

3.4. Effect of atrazine loading rate

In order to compare the effect of influent atrazine concentration and HRT on biodegradation of atrazine in the anaerobic reactor, the removal of atrazine was examined after stable condition. Then, it was calculated as a function of loading rate based on influent atrazine concentrations in constant HRT and HRTs at the constant concentration of influent atrazine that is presented in Fig. 3. According to Fig. 3, atrazine removal after achieving steady-state in the AMBBR reactor was between 47 and 60% for loadings of 0.14–0.57 g_{ATZ}/m^3d based on regular changes in the initial concentration of influent atrazine and HRTs. The researchers found that anaerobic reactor is able to decompose concentrations and high levels of atrazine efficiently in various loading rates. The results presented in Fig. 3 clearly shows that high removal rate of AMBBR reactors in the biodegradation of atrazine is associated with high loading rates. Therefore, atrazine loading rate is defined when the maximum of influent atrazine decomposed in the AMBBR reactor (loading was about 0.57 g_{ATZ}/m^3d). The results also suggested that influent atrazine and organic matter concentrations, and HRT affected AMBBR reactor performance in different ways and the anaerobic reactor is more sensitive to HRT changes than influent atrazine and organic matter (COD) concentrations. The most important step in designing a bioreactor is determining the time needed to achieve the level of pollutant removal affecting the size parameter and economic aspects of the treatment process severely (Metcalf and Eddy, 1991). Therefore, AMBBR reactor aimed at removing atrazine and COD should be designed and operated based on HRT instead of influent atrazine concentrations. Findings from this study indicated that in the AMBBR bioreactor, increasing HRT is an effective strategy for removal of contaminants such as atrazine which decompose slowly. HRT increase provides more time for atrazine molecules to be exposed to the microorganisms and consequently improves and enhances the level of biodegradation. Finally, it seems that atrazine removal depends on the ability of biomass for metabolizing the available substrate. However, the removal of atrazine in the AMBBR reactor in this study was higher than previous studies, for example, the removal of atrazine was 10% higher than anaerobic biofilter in similar concentrations. Comparing the results of this study with other studies implies that AMBBR bioreactor can biodegrade chlorinated herbicides such as atrazine more than other biological systems (Chung et al., 1996; Ghosh and Philip, 2004; Nasseri et al., 2014). AMBBR bioreactor better performance compared to other bioreactors can be attributed to the presence of dense and mixed environment of microorganisms that favorably accustomed and adapted for atrazine degradation as well as to the geometric design of bioreactors. Among various strategies, the increase of loading was much more efficient in terms of HRT reduction than concentration increase of influent pollutants (Ejhed et al., 2018; Ghosh and Gopal, 2010). However, a summary of the performance of the anaerobic bioreactor for atrazine removal, found in the bibliography, is presented in Table 1S in the supplementary data.

3.5. Effect of atrazine and organic matter concentration on volatile fatty acid production

The effect of atrazine and organic matter concentration on the production of VFA in AMBBR reactor is shown in Fig. 4. As it is clear from Fig. 4, lower organic matter (COD) concentration revealed an increase in the production of VFA when different COD concentrations were examined that can be due to substrate short contact with population of microorganisms available in the anaerobic system. Substrate short exposure with microbial population

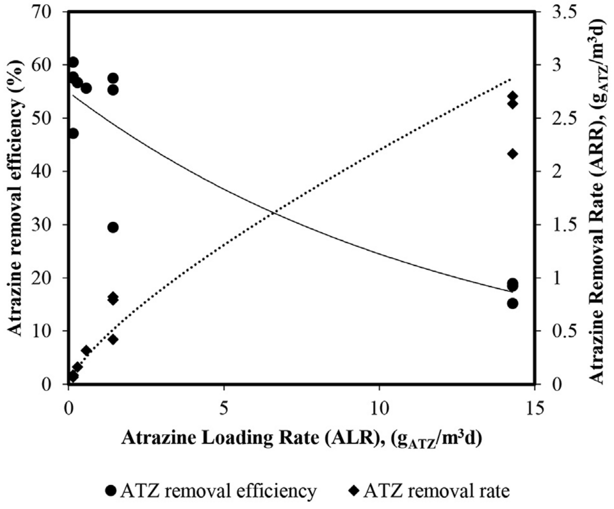

Fig. 3. Effect of atrazine loading rate applied based on the change of atrazine.

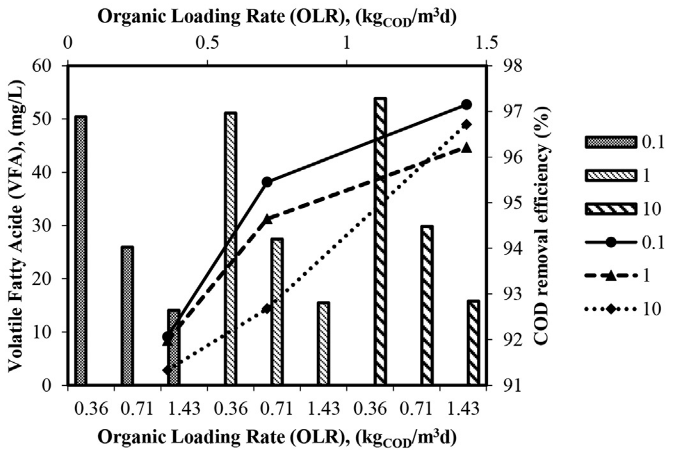

Fig. 4. Effect of initial atrazine and OLR on the VFA production in AMBBR reactor.

increased production and accumulation of intermediate products such as VFA. Fig. 4 presents the effect of atrazine initial concentration on the VFA production. It can be concluded that the production of VFA increases with increase of influent atrazine concentration because higher concentrations of atrazine cause higher loadings and result in higher production of VFA in AMBBR reactor. This finding can be attributed to high concentrations of toxic pollutants in the environment, the disproportion between pollutants volume and microorganisms' exposure time to this volume of toxic substances, incomplete biodegradation process, and production of intermediate products (Nkemka and Murto, 2010; Tay et al., 2000). High concentrations of atrazine can also temporarily disrupt bacterial metabolism and inhibit the activity of the microbial population (Pathak and Dikshit, 2012; Vijayaraghavan and Ramanujam, 1999). Accordingly, the effluent concentration of VFA increased that is in line with results of other studies (Gupta et al., 2016). Effluent concentration of VFA in the entire process of experiment was less than 73 mg/L.

3.6. *Effect of salinity on biodegradation of atrazine in the AMBBR*

The effect of salinity on atrazine biodegradation was examined in AMBBR reactor from 178th day up to 210th day. The effect of NaCl concentration ranging from 0.5 to 20 g/L on atrazine degradation with concentration of 0.1 mg/L, COD with concentration of 500 mg/L, and HRT set as 24 h was explored and the findings are depicted in Fig. 5. As can be seen, anaerobic reactor showed no sensitivity against salinity up to concentration of 5 mg/L since no change was seen in removal of atrazine in steady-stead condition. The mean of atrazine removal efficiency was more than 54% with 0.05–5 g/L concentrations. Increasing the concentration of NaCl to 10 g/L in the feeding solution, a reduction in the removal efficiency of atrazine was observed (27%) just three days after changing the salinity level. However, the system could revive itself again after 5 days and the rate of atrazine removal reached to more than 55% in stable condition. When NaCl concentration was 20 g/L, the means of atrazine removal efficiencies reduced suddenly to less than 28% one day following the changes in the influent synthetic wastewater. Fig. 5 indicates that AMBBR system is not able to tolerate the salinity with the concentration of 20 g/L. During the reactor operation, NaCl concentration was re-reduced to 0.5 g/L in order to examine the recovery reactor ability. Atrazine removal efficacies reached immediately to more than 31% two days following salinity reduction. These results indicate that the AMBBR reactor is able to revive itself under NaCl shock loading conditions. Accordingly, we can conclude that methanogenic and acetogenic bacteria can have high salinity tolerance and continue eliminating stable organic pollutants; therefore, they are recommended for saline industrial and agricultural wastewater treatment containing atrazine (Lefebvre and Moletta, 2006).

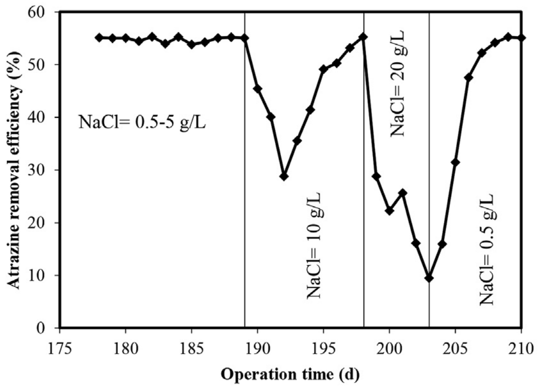

Fig. 5. The effect of salinity (0.5–20 g/L NaCl) on biodegradation of atrazine in the AMBBR (atrazine = 0.1 mg/L; COD concentration: 500 mg/L; HRT = 24 h).

3.7. *Sludge stabilization ratio*

The sludge stabilization ratio (VSS/TSS) varied from 0.53 to 0.77 in the AMBBR reactor. Fig. 6 shows the variation in VSS/TSS versus loading rates. The low VSS/TSS in the AMBBR can be attributed to greater solid retention. The effect of solid retention time (SRT) on sludge stabilization has been proven previously and VSS/TSS is inversely related to SRT (Metcalf and Eddy, 1991).

3.8. *Modeling*

Mathematical and experimental models are used to determine the relation between variables in order to evaluate experiments results. Moreover, these models are used to monitor and predict the performance of treatment unit and to optimize built a plant in laboratory scale (Derakhshan et al., 2017a, 2017b). Considering the design processes of biofilm, the removal rate of the substrate in the biofilm process is changed from limiting transfer reaction rate of the substrate from biofilm until limiting kinetics enzymes for substrate utilization and depends on the concentration of mass. Simplified models consist of a small number of variables and can be used to determine the reaction kinetics (Mann and Stephenson, 1997; Vanhooren et al., 2003). Among the models that are widely used to determine the reaction kinetics of biofilms in fixed and moving bed are including First order (Cheyns et al., 2010), Second Order (Grau) and Stover-Kincannon (Derakhshan et al., 2018). A brief explanation is brought in the Supplementary data. Using the Curve Expert software, X and Y data can be modeled using a toolbox of linear regression models or nonlinear regression models, thus coefficients can be derived. The first order, Grau and Stover-Kincannon models for atrazine removal in AMBBR has been shown in Fig. 7.

According to the value of correlation coefficient which was 0.826, 0.835 and 0.988, respectively, it can be concluded that first order and Grau models cannot be applied for predicting AMBBR's performance with high degree of precision. Stover-Kincannon model were obtained with correlation coefficients of 0.988 for atrazine removal, which shows a good degree of precision and can be used in the design of the AMBBR reactors. The values of k and

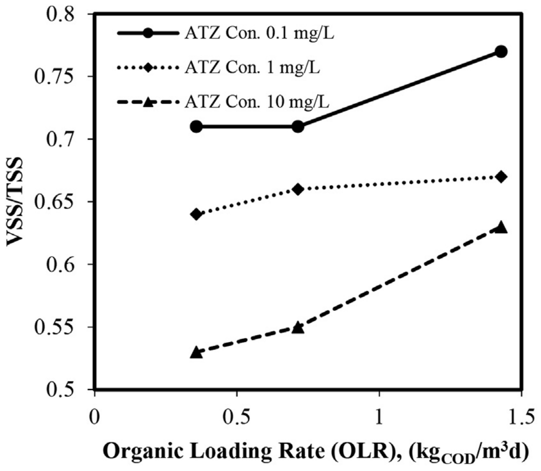

Fig. 6. Characteristics of sludge in AMBBR reactor and sludge stabilization ratio.

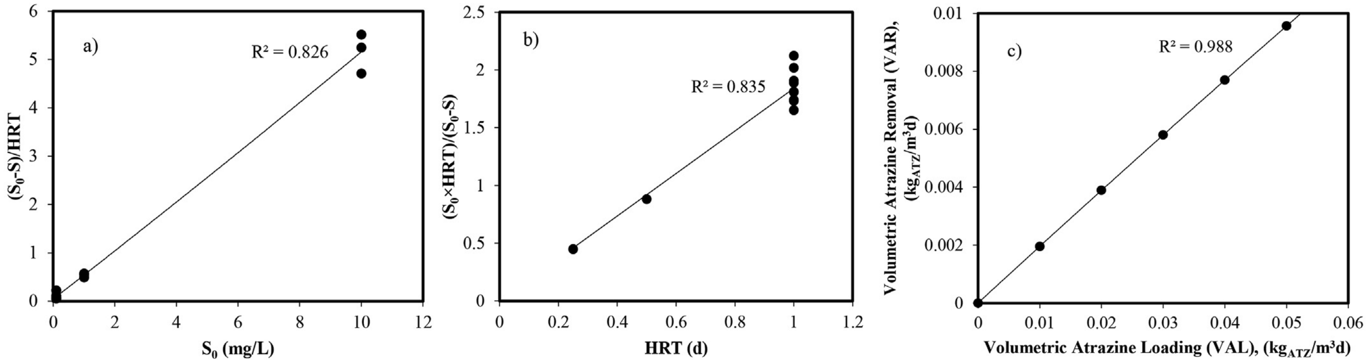

Fig. 7. Modeling of atrazine biodegradation in AMBBR: a) First order model; b) Grau model; c) Stover-Kincannon model.

r_{max} coefficients were determined 0.338 and 1.716 kg_{ATZ}/m^3d, respectively. Cheyns et al. (2010) reported that although that the transfer of herbicide in soil usually are described by equations of the first order, Monod model for biodegradation of atrazine has successfully a better fit with their data and also they held that the relationship between substrate concentration and microbial growth can be better described using kinetics Monod in water and soil. In a study by Katz et al. (2001) revealed that the kinetic coefficients of atrazine removal follow Monod equation. In their study, k_s and r_{max} were 10.16 ± 3.87 mg/L and 1.7 ± 0.72 g_{ATZ}/g_{VSS}.d, respectively. Comparing the results of previous studies with this study, we can conclude and use AMBBR bioreactor is more capable of removing atrazine from aquatic and Stover & Kincannon model has the better fit ($R^2 > 0.98$) for removing atrazine from aqueous environments. In a nutshell, this model is preferred for simulating the process of removing atrazine from aqueous environments.

3.9. Morphology of the biomass in the AMBBR

SEM image of biofilm grown on biofilm carrier is shown in Fig. 8. Sampling was conducted to probe biofilm characteristics 107 days following operation when atrazine efficiency removal with the influent concentration of 10 mg/L was 47.10% and HRT reached 24 h.

According to Fig. 8, various species of microorganisms grew on the media. Fig. 8 shows surface shape of biofilm carriers before the set-up phase. As can be seen, there are some cracks on surface of anaerobic biofilm formed on the media providing an internal porous structure. Previous studies have reported similar results indicating that the methanogen can create denser and flat biofilm using carbon source than acid-forming microorganisms. The structure and porous nature of anaerobic biofilm can provide a possibility for penetration and diffusion of nutrients and substrate into biofilm and egression of produced biogas. Biofilm is a matrix of metabolic activity of cells and extracellular compounds. Fig. 8 reveals that the increase in the density of biofilm microorganisms was a result of both cloning activity and dense growth and proliferation of microbial populations. Those colonized microorganisms had occurred vast majority of media surface that can be due to a combination of microbial layers with particles containing them (Schmidt and Ahring, 1996). Furthermore, high efficiency in pollutants removal and the formation of the desired biomass on the moving biofilm carrier can be due to anaerobic conditions inhibiting the growth and activity of filamentous bacteria that reduces removal rate (di Biase et al., 2016). On the other hand, the secretion and release of extracellular polymers by microorganisms can have an effective role in the stability of biofilms against hydraulic stresses (Wingender and Jaeger, 2002).

4. Conclusions

An anaerobic moving bed biofilm reactor (AMBBR) was developed and investigated for COD removal and biodegradation atrazine as a model of chlorinated compounds. Generally, it can be implied that AMBBR bioreactors have an excellent efficiency in removing organic materials and atrazine. In this study, despite the use of an inexpensive biofilm carrier, the means of atrazine and COD removal were desirable during all the procedures. Moreover, it was observed that bioreactor achieves faster to the steady-state

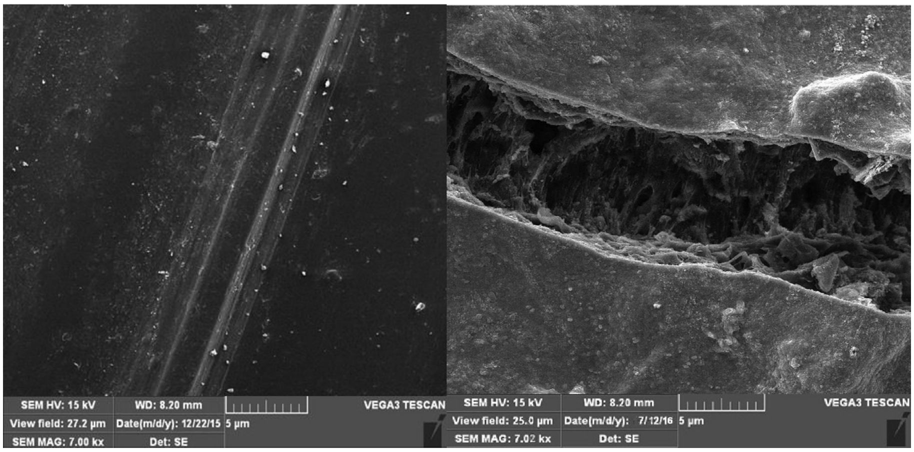

Fig. 8. SEM images of a sample of virgin surfaces of media (left) and media after biofilm formation (right).

condition by increasing HRT. Atrazine biodegradation in the AMBBR was influenced more negatively by HRT than by inlet atrazine concentration. Also, The AMBBR could efficiently recover following different shock loading conditions. Finally, anaerobic mixed biofilm culture was suitable for the treatment of atrazine from aquatic environments and atrazine removal mechanism in this system is co-metabolism.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2017.12.081.

Nomenclature

Q	Inflow Rate (L/d)
S_0	Influent substrate concentration (mg/L)
V	Reactor Volume (L)
k_s	Half saturation constant (mg/L)
S	Effluent substrate concentration (mg/L)
k_1	First order Kinetic constant (1/d)
HRT	Hydraulic retention time (day or hour)
C_i	Atrazine concentrations in the influent (kg/m^3)
C_e	Atrazine concentrations in the effluent (kg/m^3)
r_{ATZ}	volumetric atrazine removal (kg_{ATZ}/m^3d)
B_{ATZ}	volumetric atrazine loading (kg_{ATZ}/m^3d)
rmax	Maximum substrate removal rate (kg_{ATZ}/m^3d)
VSS	Volatile suspended solid (mg/L)
X	Concentration of suspended biomass (mg VSS/L)

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