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Research article

Removal of ametryn and organic matter from wastewater using sequential anaerobic-aerobic batch reactor: A performance evaluation study

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ABSTRACT

The present study was aimed to investigate biodegradation of 2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine (ametryn) in a laboratory-scale anaerobic sequential batch reactor (ASBR) and followed by aerobic post-treatment. Co-treatment of ametryn with starch is carried out at ambient environmental conditions. The treatment process lasted up to 150 days of operation at a constant hydraulic retention time (HRT) of 24 h and an organic loading rate (OLR) of 0.21–0.215 kg-COD/m^3/d. Ametryn concentration of 4 and 6 mg/L was removed completely within 48–50 days of operation with chemical oxygen demand (COD) removal efficiencies > 85% at optimum reactor conditions. Ametryn acted as a nutrient/carbon source rather causing toxicity and contributed to methane gas production and sludge granulation in the anaerobic reactor. Biotransformation products of ametryn to cyanuric acid, biuret, and their further conversion to ammonia nitrogen and CO_2 are monitored during the study. Adsorption of ametryn on to reactor sludge was negligible, sludge granulation, presence of ANAMMOX bacteria, and low MLVSS/MLSS ratio between 0.68 and 0.72. The study revealed that ametryn removal occurred mainly due to biodegradation and co-metabolism processes. Aerobic post-treatment of anaerobic effluent was able to remove COD up to 95%. The results of this study exhibit that anaerobic-aerobic treatment is feasible due to easy operation, economic, and highly efficient.

1. Introduction

Advancement in agriculture sector encourages the use of agrochemicals like fertilizers, pesticides, herbicides, etc. to improve the crop production and thereby contribute to the accumulation of xenobiotic compounds which affect the environment and human. Herbicide 2-ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine (ametryn) is a phytotoxic aromatic organic herbicide mainly used to kill unwanted plants in various crop fields such as corn, sugarcane, pineapple, etc. (Peters et al., 2014). Ametryn is considered to be more toxic to dicots than monocots, and toxic risks have detected in terrestrial species that have depended on the grasses and broadleaf plants for their food (USEPA, 2010). Ametryn is ubiquitous in surface and groundwater due to its low soil sorption capacity. About 3.5 mg/L of ametryn was found in the agricultural runoff water (Sangami and Manu, 2017), wastewater treatment plants (Navaratna et al., 2016), and nearby the agricultural fields (Allan et al., 2017). Ametryn belongs to s-triazine group of herbicides, has less water solubility (209 mg/L at 25 °C), a melting point of 80 °C, and has pKa value of 4. Ametryn is known as an endocrine disruptor (Sanderson et al., 2000), aquatic ecosystem disruptor (Velisek et al., 2017), and can cause various health effects to human and animals (USEPA, 2010). Usage of such type of herbicides has been banned in the European Union (EU) since 2002 due to their environmental consequences (Liu et al., 2016). The prescribed permissible limit of ametryn in groundwater is 1.4 μg/L, and in the surface water is 14 μg/L (USEPA, 2010).

Biological treatment methods including membrane bioreactor (Navaratna et al., 2016), biodegradation using isolated bacterial strains (Szewczyk et al., 2018; Bhaskar et al., 2019), and various aerobic treatment processes are used to treat ametryn from water (Sandoval-Carrasco et al., 2013). The applicability of these methods is limited to a pilot-scale level as the adaptation of isolated bacterial strain to the real wastewater conditions is difficult and generates complex intermediate compound (Velisek et al., 2017). On the other hand, the aerobic treatment of ametryn has limitations due to the lack of molecular oxygen required for the ring cleavage (Boll, 2005). Aerobic treatment processes involve high operating cost, complex operation, and maintenance and generate high sludge, which poses economic and environmental challenges (Derakhshan et al., 2016).

The anaerobic sequential batch reactor (ASBR) is one of the novel additions to wastewater treatment engineering, wherein they can offer both suspended and attached growth type of treatment in a single

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reactor. Anaerobic treatment process converts the organic matter to less quantity of sludge and produces a high quantity of biogas (Ghosh and Philip, 2004). Under anaerobic reducing conditions, the aromatic compounds undergo dehalogenation, dechlorination, and demethylation reactions produce simple end products (Suflita et al., 1982). ASBR can generate less sludge due to endogenous decay (Li and Wu, 2014), and can provide long sludge retention time (Chin et al., 2005; Mahesh and Manu, 2019b). At long SRTs, the bacterial adaptation and development of the required metabolic pathway to degrade targeted pollutant is high (Koh et al., 2008). Anaerobic co-treatment of toxic compounds can be diluted with biodegradable organic compounds, which can enhance the biogas production and buffering capacity of the reactor (Xu et al., 2018). Starch is used as co-substrate as it is a simple organic compound which can be digested anaerobically through carbohydrate degradation cycle and it also contributes to the degradation of toxic compounds (Wang et al., 2018). This type of co-treatment can be suitable for the effluents containing toxic compounds in anaerobic batch reactors (ASBR). In ASBR the anaerobic/facultative bacteria attack on functional groups like methyl thio, isopropyl amino, and ethyl amino attached to ring in reductive steps and can use them as a carbon source, and the N-alkyl groups in the ametryn structure may serve as electron acceptors during the anaerobic processes and support rapid growth of the bacteria (Gibson and Harwood, 2002). Few studies have been reported to treat s-triazine type of herbicides like atrazine with 55–60% removal using anaerobic moving bed bioreactor (Ghosh and Philip, 2004; Derakhshan et al., 2018), and up to 22% for ametryn using ASBR (Mahesh and Manu, 2019a). This kind of anaerobic treatment systems requires more insight and development to remove ametryn more efficiently and effectively. The anaerobic effluent may contain the biodegradable organic compounds, mainly the TPs of starch and ametryn, which is removable in the aerobic post-treatment step. The anaerobic-aerobic integrated treatment strategy has been used to remove a different type of wastewater including wool dyeing effluents (Penha et al., 2005), dye wastewater (Abiri et al., 2017), herbicides like ametryn and dicamba (Mahesh and Manu, 2019a, 2019b). Existing biological treatment methods for removal of ametryn and other s-triazine compounds are limited, and few available studies reported a partial degradation through biotransformation. Due to ametryn recalcitrance in aerobic systems, it has to be combined with other pre-treatment processes and its degradation using isolated strains is also limited only to laboratory-scale studies. Thus complete removal of ametryn is environmentally significant, and therefore we have improved our previous removal technique by operating the reactor for a long operation period with gradual loading of ametryn.

In this context, the main objective of this study is to evaluate the removal efficiency of ametryn during the co-treatment with starch in the anaerobic and aerobic reactor. Secondly, to study the anaerobic sludge, the impact of ametryn on MLVSS/MLSS ratio and proposal of ametryn biodegradation pathway.

2. Materials and methods

2.1. Inoculum

Anaerobic sludge was collected from the sludge decant outlet of a UASB reactor from the wastewater treatment plant of Mangaluru City Corporation, and aerobic sludge had collected from sewage treatment plant located in the NITK campus. The sludge was characterized and used separately as inoculum in the anaerobic and aerobic reactors. Mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) concentration of the sludge was 13–13.2 and 9.2–9.3 g/L to anaerobic reactors and 3.8–4.1 and 2.6–3.4 g/L to aerobic reactors respectively.

2.2. Reactor installation and experimental protocol

Two sets of anaerobic and aerobic batch reactors with a working capacity of 2 L were operated to remove ametryn from influent water (Fig. 1S). The reactors inoculation with seed sludge and the remaining volume was filled up with synthetic water containing ametryn concentration of 0.1 mg/L, and COD of 2100–2200 mg/L. The previous study conducted by the author, the maximum removal of ametryn was obtained at higher room temperature range (i.e., 30–32.1 °C) and hence reactors were operated under room temperature in the present study. The reactors were acclimated to influent feed, as outlined in the procedure (Supplemental data). After achieving the reactors acclimation, the influent was mixed with ametryn concentration of 4–6 mg/L to mimic the agriculture runoff concentration (Sangami and Manu, 2017). Two anaerobic reactors were operated in batch mode, out of which using one reactor as control (R1), and the other reactor to treat ametryn (R2). Similarly, two aerobic reactors were operated to treat effluents from anaerobic reactors, tagged as R3 for control and R4 for the ametryn treating reactor.

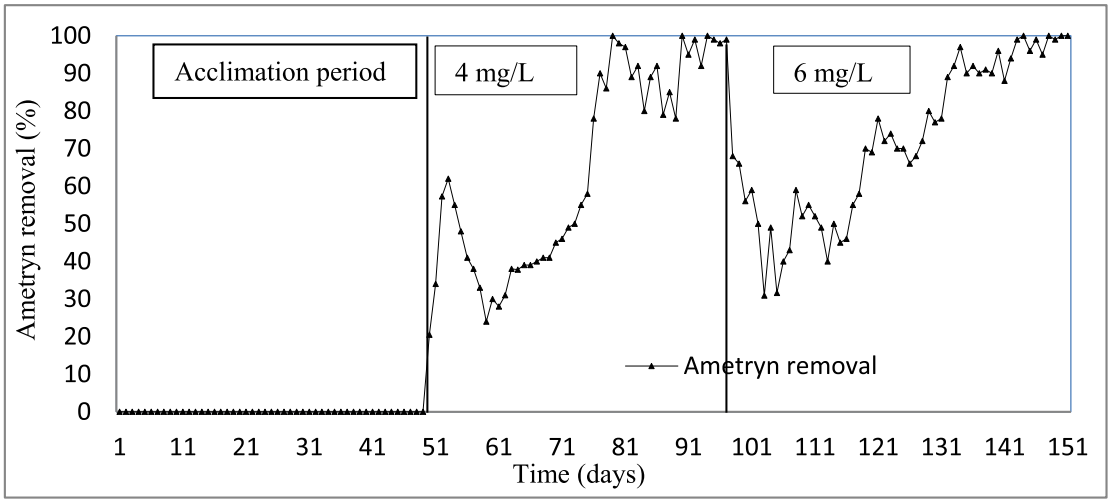
2.3. Analytical techniques

Transformation products (TPs) of ametryn were detected using gas chromatography with a high-resolution mass spectrometer (GC-HRMS, GC – Agilent 7890 and MS – Jeol (AccuTOF GCV)). Ametryn concentration was quantified using high-performance liquid chromatography (HPLC, Agilent Technologies, 1260) equipped with a diode array detector, and detailed HPLC procedure was adopted (Supplementary data). The maximum wavelength (λ_{max} = 224 nm) was measured using UV-VIS spectrophotometer (AU – 2701, Systronics). The sludge adsorption study was carried out as per the method developed by Weaver et al. (2004). ORP, pH, and temperature were measured using portable meters from Hanna instruments (edge®, Hanna Instruments). Bacterial surface morphology was studied using the scanning electron microscope (SEM – JSM, Jeol), and the sample coating was done using auto coater (JEOL, Smart coater).

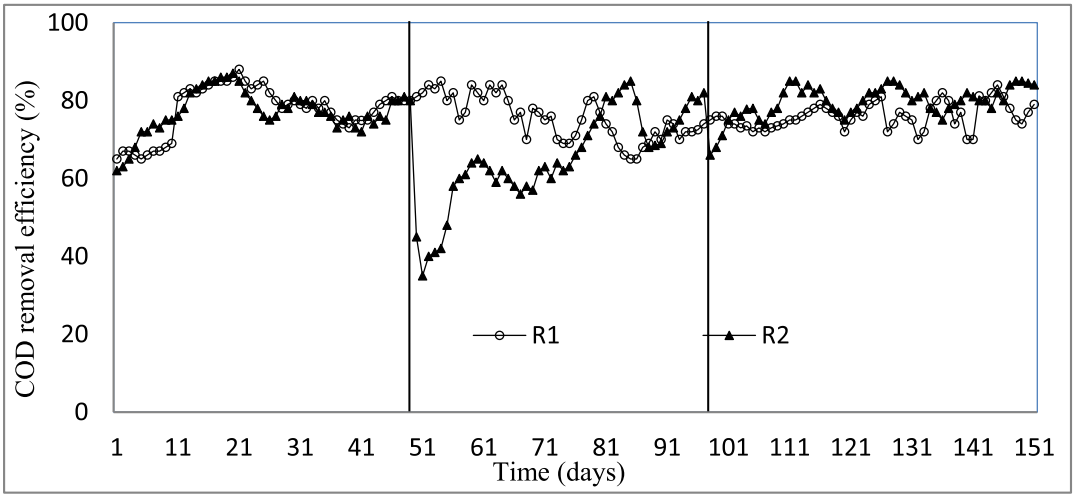
Wastewater quality parameters like chemical oxygen demand (COD, closed reflux method), alkalinity, ammonia nitrogen, MLSS, and MLVSS were measured as per the standard methods (APHA, 2005). Volatile fatty acid concentration from the reactor effluent was measured using the method developed by Baxter (2014).

2.4. Influence of pH, alkalinity, temperature, and ORP on ametryn treatment

Treatment processes in any biological systems mainly influenced by various parameters and anaerobic reactors were monitored for pH, alkalinity, temperature, and redox potential (ORP). pH was maintained in the reactor at the required range for methanogenic bacteria between 6.5 and 7.7 by adding $NaHCO_3$. Alkalinity was monitored during the study period as an inhibition indicator of the reactor, which was observed to be in the range 1650–2400 mg-$CaCO_3$/L. R2 reactor liquid temperature observed in the range 28.5–31.2 °C and R1 temperature was 28.2–30.8 °C. ORP is a parameter used to determine the nature of biochemical reactions that takes place in the reactor. Negative ORP value indicates the reducing reactions under anaerobic conditions, low ORP values < -320 mV may indicate a strong and efficient anaerobic condition (Van der Zee and Cervante, 2009). The measurement of ORP in the R1 and R2 reactors was done daily, during the study period, and it ranged from −200 to −310 mV, low ORP observed in R2 than R1 reactor.



(a)



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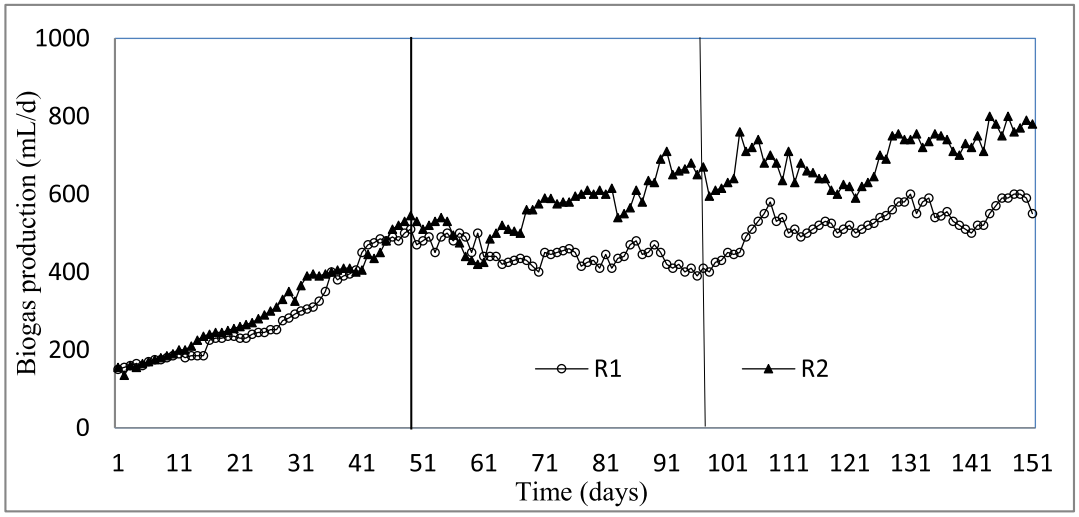

(c)

Fig. 1. Performance of ametryn treating reactor (R2) compared with control reactor (R1): (a) ametryn removal efficiency, (b) COD removal efficiency of R2 and R1 (c) Biogas production of R2 and R1 reactors.

3. Results and discussion

3.1. Acclimation of inoculum

Acclimation of seed sludge to the biodegraded ametryn and COD removal is an essential step in the anaerobic treatment process. The constant COD removal efficiencies greater than 80% for three consecutive days at stable operating conditions (constant pH, temperature, and alkalinity), that condition is said to be reactor stabilization (Manu and Chaudhari, 2002; Derakhshan et al., 2018). Anaerobic reactors are acclimated in 48 days, and Fig. 1a–c presents the performance of the reactors. These observations may indicate that the biomass in the R2 has acclimated and enriched to metabolize 0.1 mg/L of ametryn. Stabilization of bioreactors usually conducted to ensure constant biological activity and the time required to stabilize the reactors may vary from 25 to 40 days depending on reactor operating conditions, and characteristics of the substrates (Khorsandi et al., 2018). Some studies have reported different stabilization periods for various type of influent wastewater. Acclimation to atrazine in an anaerobic biodegradation study required 84 days (Nasseri et al., 2014); anaerobic biodegradation of pharmaceutical wastewater took 90 days (Oktem et al., 2008) and in our previous study, ASBR acclimation was achieved in 48 days for influent containing starch (Mahesh and Manu, 2019b).

3.2. Co-treatment process in the anaerobic reactor: effect of COD and ametryn loading

After successful acclimation of anaerobic reactors, the actual treatment process was carried out for 24 h HRT, 0.21–0.215 kg-COD/m^3/d of OLR and ambient reactor liquid temperature of 28.5–31.4 °C. Performance of anaerobic reactors during the treatment period of 150 days with influent ametryn concentration of 4 and 6 mg/L is depicted in Fig. 1a-c. Influent ametryn concentration was increased after observing the complete removal (100%) of 4 mg/L, considering the COD and other reactor components as constant. Ametryn removal efficiency on day 49 was observed to be 20.5% with a drop of COD removal efficiency from 80 to 45%, while the total gas production equal to 530 mL/day. The sudden decrease in the COD removal efficiency attributes to temporary shock to anaerobic biomass by the addition of ametryn. In our previous study, the reactor did not recover even after 30 days for high influent concentrations of 25 mg/L (Mahesh and Manu, 2019a), and Atrazine treatment study (Derakhshan et al., 2018). The higher VFA in the range 700–950 mg/L, and alkalinity 1850–2400 mg-$CaCO_3$/L, may indicate the toxicity condition in the reactor. From day 63 onwards the ametryn removal efficiency was increased gradually from 38%, and 100% removal efficiency was achieved on day 79 and again on day 90. Complete reduction of ametryn was observed within 50 days of operation, whereas membrane bioreactor (MBR) operated for about 214 days was able to remove up to 65% of 1–2 mg/L of ametryn (Navaratna et al., 2016).

Dehalogenation, dechlorination and demethylation reactions under reducing conditions supported the dissociation of ametryn to its primary metabolites and further reduced to simple end products such as methane and CO_2. The ametryn adsorption on reactor sludge was monitored with high priority throughout the study period. COD removal was also observed to be greater than 60%, and a maximum of 81% was achieved on day 95, total gas production was greater than the control by around 270 mL/d. Higher total gas production was an indication of ametryn conversion to its TPs, and ultimately to nitrogen, hydrogen, and carbon dioxide gases (Sene et al., 2010). High effluent COD (400–480 mg/L) from the R2 reactor indicates incomplete degradation of transformation products of ametryn and starch, similar observations were reported previously during the treatment of 2,4-d (Celis et al., 2008). Anaerobic sludge granulation, the appearance of ANAMMOX bacteria after 83 days of Ametryn introduction may indicate the conversion of ametryn to nitrogen source (Cook and Huetter, 1981). In our previous study, no such sludge granulation was observed in ASBR for 25 mg/L of ametryn over 30 days (Mahesh and Manu, 2019a).

The influent ametryn concentration was increased to 6 mg/L, the degradation pattern and reactor performance can be observed from 98th day [Fig. 1a-c]. Reduced COD removal may be attributed to the formation of high concentrations of VFA up to 1300 mg/L due to increased ametryn loading, which might have become non-degradable in the ASBR. VFA in the R1 reactor remained between 300 and 550 mg/L. Formation of long-chain fatty acids increases the VFA concentration and it became toxic to the sensitive methanogens (Shin et al., 2003). Another reason could be the appearance of high concentrations of ammonia nitrogen (range 60–75 mg/L) compared to < 20 mg/L of R1 reactor. These observations are reported in the case of atrazine and s-triazine treatment, but none of the studies have reported such observations for ametryn. As described in the cyanuric acid degradation pathway, production of nitrogen with increased ametryn concentration and excessive nitrogen formation might be another reason for the toxicity of methanogens in the sludge. Alkalinity observed was in the range of 1700–2400 mg-$CaCO_3$/L, and low biogas production up to 645 mL/day on 130th day. From 132nd day onwards the reactor was recovered, indicated by reduced ammonia nitrogen (20–35 mg/L), alkalinity (< 1200 mg-$CaCO_3$/L, high biogas production (> 730 mL/day). The reactor biomass recovered due to the biotransformation N-alkyl groups of ametryn at low initial concentrations, and over long operation periods, these observations are in line with their study involving atrazine removal (Derakhshan et al., 2018).

Further, complete mineralization was achieved from day 147 with greater COD removal efficiency of 85%. High biogas production up to 800 mL/d, and COD removal efficiency more than 85% was observed on the 150th day in the R2 reactor, whereas R1 was able to produce biogas of 590 mL/d and COD removal efficiency of 77%. Increased biogas production and COD removal efficiency of both R1 and R2 may indicate the enhanced biotransformation and follow by mineralization of ametryn and starch. It has been reported that high influent herbicide concentration to the bioreactors creates a greater chance of herbicide exposure for bacterial metabolism (Baghapour et al., 2013). At low concentrations in the anoxic MBR reactor and only about 46% of ametryn removal was achieved, indicates the poor bacterial adaptability and possible recalcitrance (Navaratna et al., 2016). Ametryn biodegradation studies using fungal and bacterial isolates have reported incomplete removal efficiencies (< 15%) with the formation of different ametryn metabolites (Szewczyk et al., 2018). However, the complete removal in the present study is mainly due to the anaerobic reducing reactions, bacterial adaptability, co-metabolism, and utilization of ametryn as carbon/nutrient source. The complete removal of ametryn indicated by the disappearance of intensity peak in HPLC, similar observations reported by Sánchez-Sánchez et al. (2013) and absorbance peak in the UV spectra (Fig. 2S). As per the author's knowledge, this is the first study to report the complete removal of 6 mg/L ametryn using ASBR without any pre-treatment.

3.3. Biodegradation of ametryn and pathway proposal

The major intermediate compounds produced were identified using GC-HRMS, and the biodegradation pathway is proposed, as shown in Fig. 2. The degradation pathway derived reveal the formation of intermediate compounds like n-ethyl-6-(methylsulfanyl)-1,3,5-triazin-2,4-diamin, deisopropylhydroxyatrazine, 2,4-dihydroxy-6-(N′-ethyl) amino-1,3,5-triazine, hydroxyatrazine, and n-isopropylammelide. These compounds further undergo enzymatic reactions and can produce CO_2 through the cyanuric acid pathway. The appearance of cyanuric acid and biuret in the MS analysis support these observations (Fig. 3). Intermediate compounds proposed are in agreement with the compounds produced during the biodegradation of s-triazine (Cook and Huetter, 1981), and atrazine (Derakhshan et al., 2018) studies.

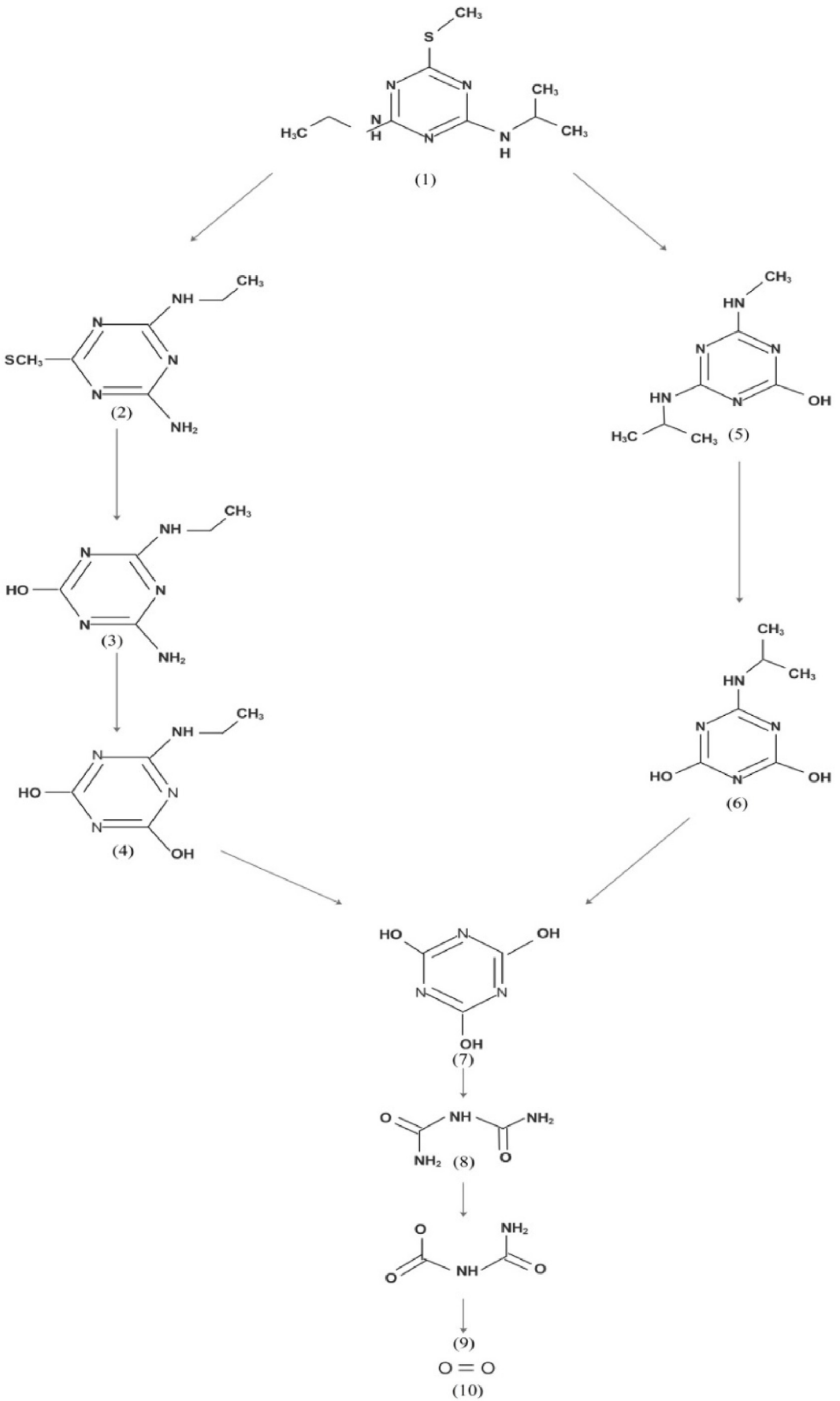

Fig. 2. Proposal of ametryn biodegradation pathway. (1) – ametryn, (2) - n-ethyl-6-(methylsulfanyl)-1,3,5-triazin-2,4-diamin, (3) – deisopropylhydroxyatrazine, (4) – 2,4-dihydroxy-6-(N′-ethyl)amino-1,3,5-triazine, (5) – hydroxyatrazine, (6) – n-isopropylammelide, (7) – cyanuric acid, (8) – biuret, (9) – allophanate, (10) – CO_2.

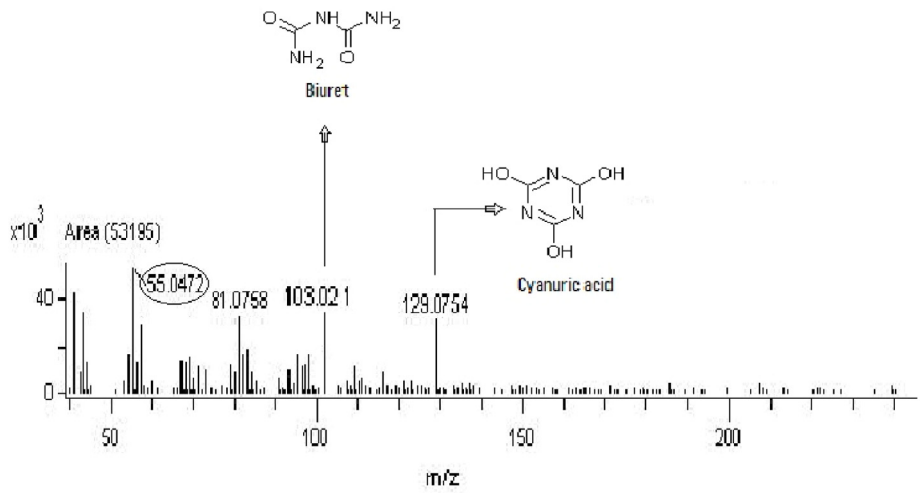

Fig. 3. GC-HRMS result obtained for R2 effluent showing the cyanuric acid, biuret, and fatty acids.

Szewczyk et al. (2018), claims the formation of 2-hydroxy atrazine, ethyl hydroxylated ametryn, s-demethylated ametryn, and deethylametryn, and Bhaskar et al. (2019), have reported 2 – acetamido – 4 (isopropylamino) – 6 – (methylthio) – s triazine) and 2 – amino – 4 (ethylamino) – 6 – (methylthio) – s triazine as the intermediate compounds during biodegradation of ametryn. These intermediate compounds are of great concern to the environment due to their toxic risks, which need to be removed completely (Velisek et al., 2017). However, in the present study, the major TPs identified were cyanuric acid, biuret, and long-chain fatty acids such as 9-Octadecenal, and oleic acid. Formation of long-chain fatty acids were mainly the anaerobic fermentation products of starch, whereas cyanuric acid and biuret may be the TPs of ametryn. The degradation of cyanuric acid to biuret, ammonia nitrogen, CO_2, and the CO_2 contributed to the methanogenesis process (Cook et al., 1985; Sene et al., 2010). Therefore, successive reduction of ametryn to a different type of intermediate compounds and finally to carbon/nitrogen source thus contributed to high methane gas production.

The biogas production was measured during the treatment period, before and after the herbicide introduction in both the reactors (Fig. 2c). Methane gas production was measured using the 5% w/v KOH solution displaced from the gas-liquid displacement system (on regular intervals of 10 days). Methane gas production in the R2 reactor was higher than the R1 by 280–350 mL/d (i.e., 35–41% v/v). High methane yield indicates rich nutrient condition prevailing over continued operation due to the conversion of ametryn to nitrogen or carbon source, leading to enhanced methanogenesis in R2 (Cook and Huetter, 1981). Biogas production varied concerning ametryn and COD removal efficiencies, and it is in correlation with variation in MLVSS.

3.4. Anaerobic sludge characterization: MLVSS and ametryn adsorption

Ametryn adsorption concerning the variation in MLVSS concentration between the R1 and R2 reactor throughout treatment is tabulated in Table 1. Around 2.30 mg/g.MLVSS of ametryn was adsorbed on to reactor sludge till day 70, and later, there was no adsorption detected. No ametryn was detected in the sludge extract due to desorption from the sludge, biotransformation, and also due to the high dissociation constant (pKa) value of ametryn (Frías et al., 2004). Other reasons are also responsible for poor adsorption of ametryn on to sludge, the low octanol/water participation coefficient (log K_{ow}) of 2.83 (Navaratna et al., 2010), and low log D value of 2.60 of ametryn (Wick et al., 2011). Ametryn was adsorbed initially during the present study, and no adsorption of ametryn was detected over the long operation. Navaratna et al. (2016) report similar findings from their ametryn treatment study using MBR. MLVSS was varying significantly from day 49–80 for about 6.4–9.2 g/L, which indicates slight toxicity inhibited by the herbicide. The toxic inhibition on the anaerobic biomass leads to deterioration of granules and poor sludge quality. Biomass regenerated over the continued operation, and MLVSS was found to be > 9.6 g/L, which was greater than the MLVSS concentration of R1 reactor (by 0.3–0.4 g/L).

The sludge stabilization ratio (MLVSS/MLSS) was observed in the range of 0.67–0.82 in the R2, and in R1 reactor, it was 0.67–0.72 (Table 1). Increase in MLVSS/MLSS ratio up to 0.82 in the R2 reactor indicate a significant reduction in SRT, and further, reduced MLVSS/MLSS ratio of 0.67–0.77 contributed to high SRT (Derakhshan et al., 2018). The SRT and MLVSS/MLSS ratios are inversely proportional to each other. Impact of SRT on the reactor performance was reported previously by Metcalf and Eddy (1991). The impact of SRT on dicamba removal was studied previously, wherein a long operation period contributed to high SRT in the reactor (Mahesh and Manu, 2019b). There was a low SRT (35–50 days), during the first 10–20 days of ametryn introduction indicates poor sludge quality due to slight toxicity. High SRT observed during the long treatment period, about 100–150 days during the acclimatization period and 150–180 days on 98th day and 170–210 days on 150th day. Long operation period promoted the active biomass growth in the presence of ametryn, which has improved the sludge quality and contributed to long SRT. Long SRT of 180 days reported in the MBR treatment and excessive sludge toxicity of influent ametryn dose (2.76 mg/L) demanded the wasting of sludge to maintain the required SRT (Navaratna et al., 2016). ASBR in the present study is found to be very effective for yielding high SRT and fast recovery for herbicide toxicity than in other studies (Koh et al., 2008; Wang et al., 2018).

Anaerobic sludge granulation with increased MLVSS concentration > 9.8 g/L indicates the adaptability of anaerobic bacteria and ametryn acts as a nutrient source. These observations, including granulation and the size of granules, greatly influence the reactor performances (Gao et al., 2011). Anaerobic sludge granulation during this period may be the significant indication of active biomass growth; sludge obtained from R1 and R2 reactor is depicted in Fig. 4. The seed sludge to both the anaerobic reactors contained grains size < 250 μm at the time of start-up, the size of the granules varied from 0.2 to 0.5 mm in size in R2 reactor from the day 70–150 (i.e., 32 days after 4 mg/L of ametryn introduction). There was no granulation observed in the control reactor throughout the study period. The granulation was observed from 70th day and sized up to 0.5 mm till the day 80. Further, granule size was reduced to 0.3 mm on day 81 after increasing the ametryn concentration to 6 mg/L and again reached to 0.5 mm from 150 days onwards.

Table 1
Ametryn adsorption on the R2 reactor sludge, and characterization of MLSS, MLVSS, MLVSS/MLSS ratio and their comparison between R1 and R2 reactors.

Sampling period (days)	Ametryn adsorbed (mg/g.MLVSS)	MLVSS concentration (g/L)		MLSS concentration (g/L)		MLVSS/MLSS	
Reactor	R2	R1	R2	R1	R2	R1	R2
0–48	–	9.2 ± 1	9.3 ± 1	13	13.2	0.71	0.71
49–60	2–2.3	9.3	6.4–6.7	13.1	8–8.7	0.7	0.77–0.87
61–71	1.4–2	9–9.35	7.2–8.1	12.7–13.1	9.4–10	0.7–0.71	0.76–0.81
72–82	< 1	9.2–9.3	8.8–9.2	12.8–13.3	11.8–13.2	0.69–0.71	0.7–0.74
83–97	0	9.1–9.3	9.2–9.3	12.7–13.8	12. 8–13.5	0.67–0.71	0.68–0.71
98–108	1.3–2	9.2–9.3	8–8.2	12.1–12.8	10 ± 0.5	0.68–0.72	0.8–0.82
109–119	1–1.2	9.1–9.4	9–9.2	12.5–13.1	11.6–11.9	0.71–0.72	0.77
120–130	< 1	9.2–9.3	9.2–9.4	12.8–13.1	12.8–13	0.7–0.72	0.71–0.72
131–141	0	9.1–9.3	9.3–9.5	12.6–13	12. 9–13	0.7–0.71	0.73
142–152	0	9.2–9.4	9.3–9.6	12.7–13.1	12.9	0.72	0.72–0.74

3.5. Morphology of the biomass in the R2 reactor

SEM analysis indicated the appearance of ANAMMOX (anaerobic ammonium oxidation) bacteria in the anaerobic granule. Fig. 5 shows the SEM images obtained for the anaerobic sludge of control (R1) and ametryn treating reactor (R2) on the 150th day. The rod/oval-shaped bacteria particularly of cocci, diatoms were identified as ANAMMOX bacteria (Cao et al., 2016; Khan et al., 2014), and presence of cocci bacterial community might have metabolized the ametryn, the same bacteria that was detected dominantly during the atrazine biodegradation (Sánchez-Sánchez et al., 2013). From this study, it is clear that the activated biomass obtained from treatment plants have the capability of ametryn biodegradation. SEM image of R1 sludge indicates no such distinct bacterial appearance when compared to R2 reactor.

3.6. Influence of pH, alkalinity, ORP, and temperature on the anaerobic reactor performance

The pH in the reactor was maintained in the neutral range of 6.6–7.7 as required for methanogenic treatment (Pirsaheb et al., 2018), by using 4 g/L of sodium bicarbonate. There was no much deviation in the required pH level. Alkalinity in the influent was in the range of 850–1300 mg-$CaCO_3$/L, whereas the effluent contained 1800–2400 mg-$CaCO_3$/L for R1 and 1650–2500 mg-$CaCO_3$/L R2. High alkalinity reported at low COD removal rates may be due to the accumulation of inorganic substrates like sulfates, nitrates, causing toxicity on biomass (Manu and Chaudhari, 2002). The anaerobic condition exhibit negative ORP value and reducing reactions in the R2 reactor indicated by low ORP values indicates that ametryn acted as an electron acceptor, the functional groups had attacked by the methanogens (Gibson and Harwood, 2002). ORP in the R2 and R1 reactors varied between −230 and −310 mV and −200 to −280 mV respectively. Low ORP values in the R2 compared to R1 reactor indicate highly active biomass in R2, which is in agreement with studies reported by Manu and Chaudhari (2002). Low ORP values indicated the limited redox reactions leading to low dicamba removal efficiencies, and addition of a redox mediator enhanced the treatment efficiency > 15% reported previously in Mahesh and Manu (2019b). However, the addition of redox mediator was not required in this study as the ORP level was at the required range, and maximum removal efficiency was achieved within 40–50 days of operation. Temperature plays a crucial role in anaerobic degradation processes (Chae et al., 2008) and at high ambient temperature ranges, 30–31.4 °C, maximum reactor performance was observed. During the treatment period, the anaerobic experimental reactor temperature varied between 28.5 and 31.4 °C, while the control reactor temperature was observed to be lower than R2 reactor by 0.4 ± 0.1 °C.

3.7. Post-treatment in the aerobic reactor

The aerobic SBR operated as a post-treatment to anaerobic effluent, and the performance of aerobic control (R3) and aerobic ametryn treating reactor (R4) is depicted in Fig. 6. The combined MBR/UV/GAC study for removal of 5 mg/L of ametryn was able to remove about 61% (Navaratna et al., 2016), whereas in the present study 100% removal was achieved in the ASBR reactor alone. Anaerobic biotransformation products mainly constituted by long and short-chain fatty acid due to the fermentation of starch can be oxidized by aerobic/facultative bacteria (Gaunt and Hester, 1989). Long-chain fatty acids lose carbon atom by the β-oxidation pathway produces acetyl-CoA, and further oxidized to CO_2 via the tricarboxylic acid cycle (Ratledge, 1992). Initially, there was a low COD removal efficiency in the R4 reactor, maybe due to the toxicity of fatty acids. Reduction in sludge toxicity observed, after the commencement of VFA biodegradation (Gaunt and Hester, 1989).

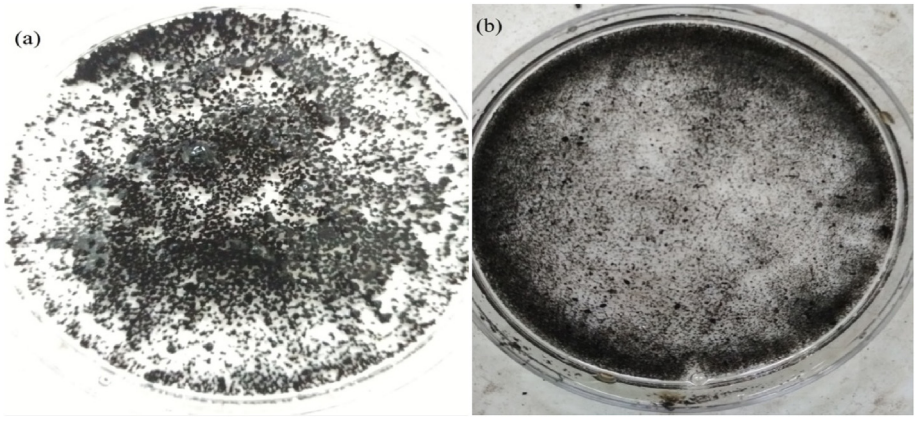

Fig. 4. Images of anaerobic sludge obtained on day 150 from the anaerobic reactors, (a) ametryn treating reactor (R2), and (b) control reactor (R1).

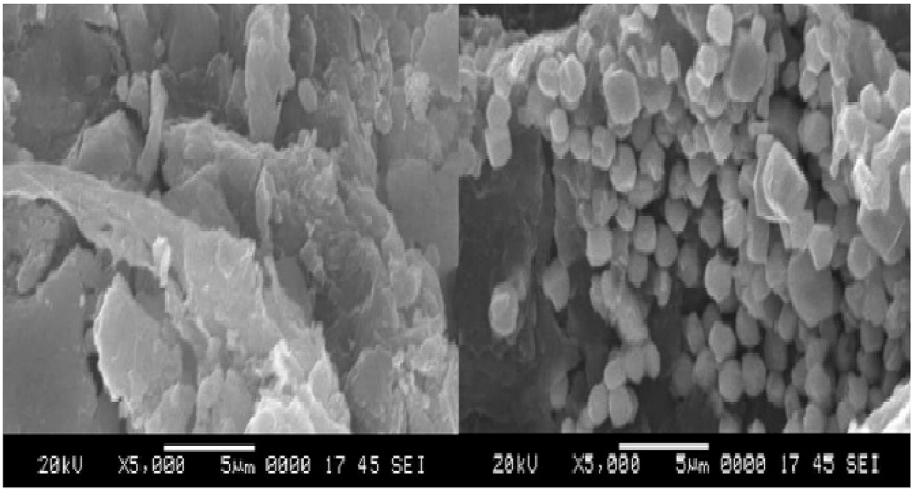

Fig. 5. Scanning electron microscope images reactor sludge, (a) control (R1), and (b) ametryn treating reactor (R2) obtained on 150th day.

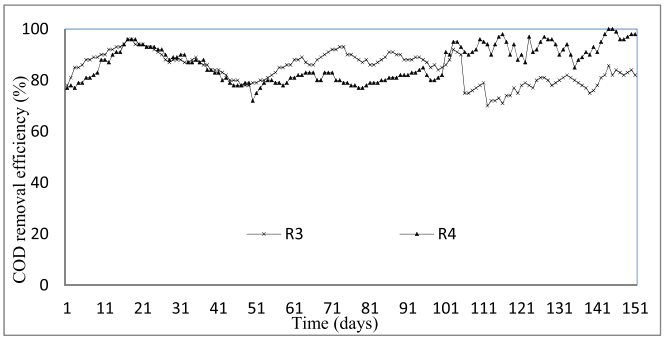

Fig. 6. COD removal efficiency of aerobic reactors, control (R3) compared with reactor treating the ametryn metabolites (R4).

Inhibitions in the aerobic reactor lasted after 10–15 days of operation due to low influent ametryn and TPs concentrations. Long term operation of more than 90 days was required to reduce the toxicity influent dicamba concentration of 60 mg/L (Mahesh and Manu, 2019b). Anaerobic metabolites of ametryn and starch were removed, indicated by the COD removal efficiencies greater than 95% and low VFA in the range 55–80 mg/L. Most of the previous studies on the aerobic treatment of refractory organic compounds have resulted in limited treatment efficiency, the formation of either recalcitrance or loss of biomass (Manu and Chaudhari, 2002; Sandoval-Carrasco et al., 2013). In the present study, the anaerobic TPs did not cause such a negative effect on the aerobic biomass due to mineralization of most of the compounds in the anaerobic step. HPLC and GC-HRMS results also supported the complete mineralization of ametryn. Thus a sequential anaerobic-aerobic system can be a novel addition to remove ametryn and its metabolic by-products from water. The treatment method is environmentally acceptable, economically viable, and can be used in large scale treatment plants to remove toxic compounds like ametryn and other halogenated compounds.

4. Conclusions

A laboratory-scale anaerobic and aerobic sequential batch reactor was operated for about 150 days to evaluate the removal efficiency of herbicide ametryn at a constant HRT (24 h), and OLR (0.21–0.215 kg-COD/m^3/d). The results of this study revealed that the co-treatment process in ASBR was able to remove 4–6 mg/L of ametryn completely over the continued operation of 48–50 days. Ametryn was mineralized in the anaerobic reactor mainly through biodegradation, and adsorption of ametryn on to the reactor sludge was negligible over long operation period. Ametryn acted as a nutrient source instead of causing toxicity and which has allowed the growth of ANAMMOX bacteria, sludge granulation leading to low MLVSS/MLSS ratio of 0.68–0.72. The sequence of biochemical reactions within ASBR was able to transform ametryn to long-chain fatty acids, VFA, cyanuric acid, biuret, and finally to a nitrogen source. Proposal of ametryn biodegradation pathway revealed the conversion of ametryn to ammonia nitrogen and then to nitrogen/carbon source. Sequential treatment of anaerobic effluent in the aerobic reactor enhanced the biodegradation of anaerobic metabolites to greater efficiencies. The anaerobic-aerobic treatment technique can solve the issues associated with water pollution caused by ametryn and such type of herbicides used in agriculture.

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Appendix A. Supplementary data

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