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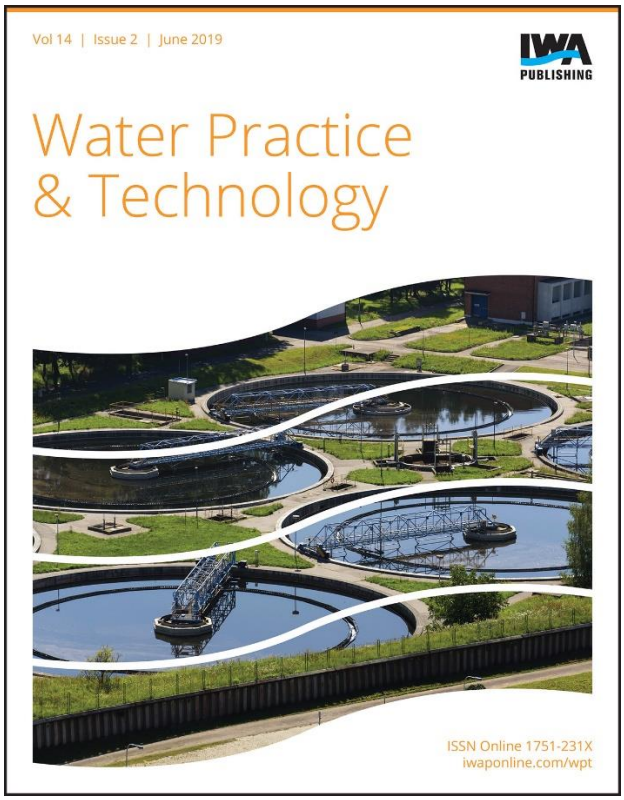

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Biodegradation of ametryn and dicamba in a sequential anaerobic-aerobic batch reactor: A case study

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Abstract

Agricultural runoff often contains persistent halogenated herbicide compounds like 2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine (ametryn) and 3,6-dichloro-2-methoxybenzoic acid (dicamba). These can enter the food chain through drinking water, causing serious effects for people and the environment. A sequential anaerobic reactor followed by an aerobic reactor was operated and investigated for herbicide removal efficiency at constant, three-day, hydraulic retention time (HRT) and organic loading rate (OLR) of 0.2025 kg-COD/m^3/d. The effect of the herbicides on anaerobic bacteria was evaluated based on total biogas production and bacterial activity, which indicated that there was no inhibition on the acclimated biomass. The sequential reactor pair removed 72% of ametryn and 78% dicamba, with COD removal efficiencies of 86% and 85% respectively. The different high-performance liquid chromatography (HPLC) peaks indicate that the compounds are biotransformed and this was confirmed by gas chromatograph high resolution mass spectrometry (GC-HRMS).

Key words: agriculture runoff, ametryn, biodegradation, biotransformation, dicamba, sequential anaerobic-aerobic batch reactor

INTRODUCTION

Agrochemicals like pesticides and herbicides are considered hazardous when discharged into the environment. Herbicides are used extensively to increase agricultural production by reducing the incidence of weeds in crops. Many pesticides and herbicides are persistent organic pollutants. They are released mainly during the wet season in runoff from the crop fields (Navaratna *et al.* 2016) and increase pollution levels downstream (Conte *et al.* 2016). Agricultural runoff can contain up to 500 mg/L of pesticide (Chiron *et al.* 2000), runoff from sugarcane fields, for instance, contained 3.5 mg/L of ametryn (2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine) and 93.7 mg/L dicamba (3,6-dichloro-2-methoxybenzoic acid) (Sangami & Manu 2017), the samples were analysed using the HPLC operating procedure shown in Table 1. India consumed around 0.5 kg/hectare of herbicide in 2014 (De *et al.* 2014), which is relatively little compared with the quantities consumed in Japan (15 kg/hectare), the United States (2) and the European Union (4) in 2001 to 2003 (OECD 2008). Herbicide application to crop fields is expected to increase in future due to lack of availability of labour, increased food needs, user awareness, and crop land expansion. The herbicides used and their derivatives have become a major concern in environmental engineering, as they enter fresh water bodies in agricultural runoff and increase toxicity in the water.

Ametryn belongs to the class III herbicide category (moderately toxic to fish, large mammals and humans), and is highly toxic to crustaceans and molluscs (Hurley 1998). It is weakly soluble in water (209 mg/L) and has low affinity to soil with a pKa of 4 (Frías *et al.* 2004). Dicamba is a well-known endocrine disrupting chemical, and exposure to it affects human eyes, thyroid, liver, kidney

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Table 1 | HPLC operating conditions

HPLC Parameter	Ametryn	Dicamba
Mobile phases and their ratio	58:42	50:50
Column temperature (°C)	25	35
Wavelength (nm)	223	274
Flow rate (mL/min)	1	0.75
Retention time (min)	8.882	1.382

Mobile phase = methanol: water; Sample volume = 20 μL; run time = 20 min; column name and size = RP- C18, 100*4.6 mm, 3.5 μ pore size.
Source: Sangami & Manu 2017.

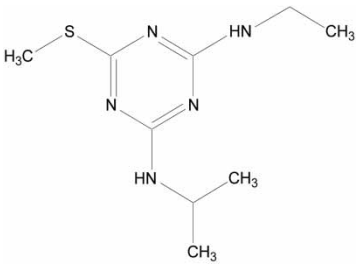
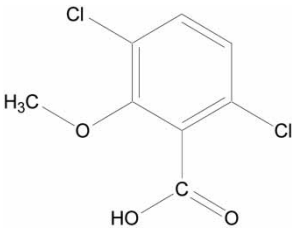
and nervous systems (USEPA 2006). It is weakly adsorbed on to soil with a pKa of 1.87 but is highly mobile (Comfort *et al.* 1992), and highly water soluble (Vencill 2002). The prescribed limits for ametryn and dicamba in surface waters are 14 (USEPA 2010) and 200 μg/L (Hamilton *et al.* 2003), respectively. The two herbicides' physical and chemical properties are shown in Table 2.

The effects of ametryn and dicamba could be detrimental to living organisms over time if discharged untreated. Water scarcity, pollution of surface- and ground-waters, and stringent effluent discharge standards all demand more efficient and economically sound treatment methods. To date, several treatment methods have been developed, including both physico-chemical (Gao *et al.* 2009) and biological processes (Milligan & Häggblom 1999; Sandoval-Carrasco *et al.* 2013; González-Cuna *et al.* 2016; Navaratna *et al.* 2016). Biological treatment methods are commonly the most suitable alternatives used to treat pesticide contaminated wastewaters (Chin *et al.* 2005; Ghoshdastidar & Tong 2013).

The sequential batch reactor (SBR) is considered the most viable biological method for treating wastewaters with varying quality, quantity and temperature. SBRs perform well due to long solid retention times (Ndon 2007), and their ease of operation anaerobically, aerobically or in combination (Chin *et al.* 2005). They are more cost-effective than other treatment methods. It is reported that combined anaerobic-aerobic SBRs have shown good pollutant removal efficiencies in the treatment of domestic (Callado & Foresti 2001) and dye wastewaters (Manu & Chaudhari 2002), and the degradation of organic pollutants (Wang *et al.* 2014).

Under anaerobic conditions, the herbicides ametryn and dicamba can be transformed to less aliphatic hydrocarbons in the presence of electron donors produced during dehalogenation reactions (Milligan & Häggblom 1999). The dehalogenated metabolites become easier to oxidise to their respective end products in the subsequent aerobic reactor (Gaunt & Hester 1989). The reported studies have focused on the treatment of 2,4-dichlorophenoxyacetic acid using only aerobic or anaerobic SBR processing (Chin *et al.* 2005; Celis *et al.* 2008), and have not included ametryn and/or dicamba.

Table 2 | Physico-chemical properties of ametryn and dicamba

Properties	2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine (Ametryn - $C_9H_{17}N_5S$)	3,6-dichloro-2-methoxybenzoic acid (Dicamba- $C_6H_2Cl_2(OCH_3)CO_2H$)
Structure		
Molecular weight (g/mol)	227.35	221
Maximum solubility (mg/L)	209 at 25 °C	4,500 at 25 °C

Source: Sangami & Manu 2017.

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In this preliminary study an attempt has been made to investigate the performance of a sequential anaerobic-aerobic batch reactor (SAAR) for the treatment of influent concentrations (25 mg/L) of ametryn and dicamba in water, and to determine the biotransformation products (BTPs) formed during treatment.

MATERIALS AND METHODS

Chemicals

Analytical grade ametryn and dicamba were purchased from Sigma-Aldrich. High performance liquid chromatography (HPLC) solvents like methanol and Milli-Q water were purchased from MERCK, and starch and sodium bicarbonate from Hi-Media. The chemical structures of the two herbicides are shown in the Table 2. A stock herbicide solution was prepared by dissolving separately 250 mg of ametryn in 2 L and 250 mg of dicamba in 1 L freshwater. The influent to the anaerobic reactors was prepared using 200 ml of the ametryn and 100 ml of the dicamba stock solutions in two volumetric flasks containing 2 g/L of starch and 4 g/L of sodium bicarbonate solution. A trace metal solution was prepared using (g/L): $CoCl_2.6H_2O$ (1.613), $FeSO_4$ (8.39), $MgSO_4.7H_2O$ (5), H_3BO_3 (0.1), $ZnCl_2$ (0.0473), $CuSO_4.5H_2O$ (0.0782), $NiSO_4.6_2O$ (1.698), $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.54), $CaCl_2$ (7.776), and $MnCl_2.4H_2O$ (7.863).

Bacterial seed sludge and characterization

The anaerobic seed sludge for the SAAR was collected from the outlet of the up flow anaerobic sludge blanket (UASB) reactor of Mangaluru Municipal Corporation's sewage treatment plant (STP), at Kavoor, Mangaluru, India. Aerobic seed sludge (activated sludge) was collected from the sludge recycling unit of the STP at NITK campus, Surathkal, Mangaluru. The anaerobic and aerobic sludge was characterised for both mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the laboratory, and sieved to uniform solid-grain size (250 μm). The required quantity of sludge was inoculated directly but separately into the anaerobic and aerobic reactors.

Herbicide toxicity studies in anaerobic reactors

The methanogenic activity study was conducted following the protocol described by Isa *et al.* (1993). The reactors were tested separately for maximum initial concentrations of 25 mg/L of ametryn and dicamba, with starch as the carbon source and sodium bicarbonate ($NaHCO_3$) as buffering agent. The reactors were capped after each feed and connected to a gas-liquid displacement system to record the gas production rate. Displacement of 5% w/v potassium hydroxide (KOH) solution from the liquid bottle was collected and measured as methane gas. The methane yield was measured every two hours for 12 hours. The feeding, decanting and gas recording procedure was followed for three consecutive days. The maximum slope obtained on the graph of methane yield (quantity) versus time indicates the methanogenic activity of the sludge (kg-CH_4 – COD/kg.VSS/d).

Reactor set up and operation

The anaerobic and aerobic SBRs were designed to be operated manually – see Figure 1. Three anaerobic reactors were made out of 2.5 L glass bottles, with provision for collecting biogas. The bottles were 12.2 cm (diameter) by 28 cm (high), with a usable liquid volume of 2 L and 0.5 L free space. All the anaerobic reactors were inoculated with 9 g/L MLVSS of anaerobic seed sludge.

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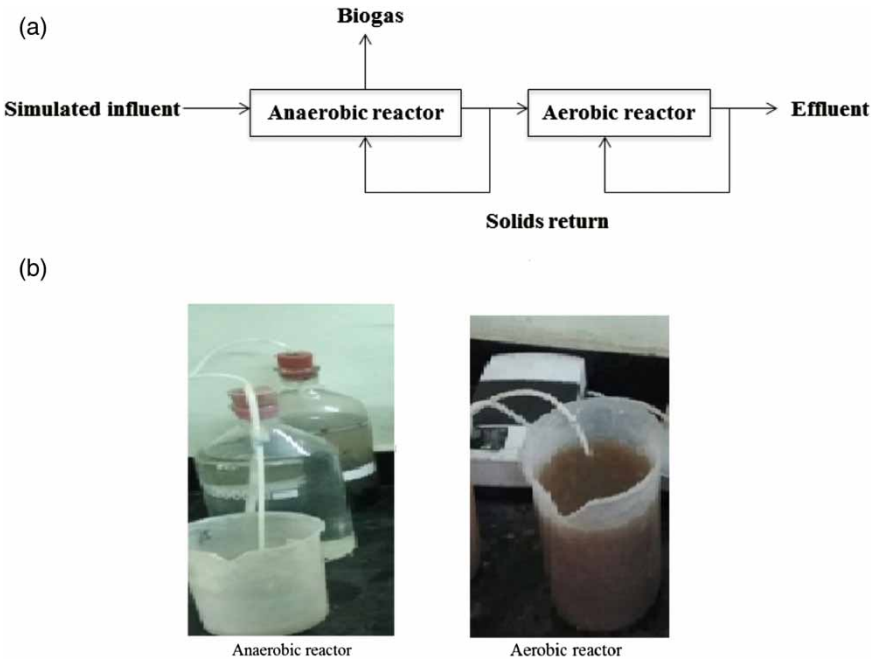

Figure 1 | (a) Flow diagram and (b) laboratory set-ups of anaerobic and aerobic SBRs.

Three aerobic reactors were made using 2.5 L plastic beakers of 13.7 cm internal diameter and 18 cm tall, with 2 L usable internal volume and 0.5 L free space. They were aerated using diffusers to maintain the dissolved oxygen (DO) level. All were inoculated with 2.5 g/L MLVSS of aerobic seed sludge.

One anaerobic and aerobic reactor set – set An1-A1 – was used as the control – i.e., with no herbicide. Two similar sets were used to treat herbicides, one for ametryn (An2-A2) and the other for dicamba (An3-A3). The reactors' daily operating cycle included processes like feeding (10 min), reaction (23 hrs), settling (30 min), and decanting (20 min) (Chin *et al.* 2005).

The synthetic influent to the anaerobic reactor was prepared separately to yield initial concentrations of 25 mg/L for ametryn and dicamba. The effluent from the anaerobic reactor was subsequently treated in the sequential aerobic reactor after 24 hours. The effluent samples from each sequential anaerobic and aerobic reactor set were analysed daily during the 60 day treatment period. Reactor performance was determined in terms of both herbicide and COD removal, as well as gas yield. Reactor stability was monitored by measuring alkalinity, reduction-oxidation potential (redox), pH, temperature and DO (aerobic reactors). The aerobic reactor DO concentration was maintained between 6.0 and 7.5 mg/L.

The herbicide and COD removal efficiencies were calculated using Equation (1),

$$(\eta) = (C_{\text{in}} - C_f)/C_{\text{in}} * 100 \tag{1}$$

where (η) is the removal efficiency (%), C_{in} – the concentration of herbicide or COD in the influent (mg/L), and C_f – the concentration of herbicide or COD in the reactor effluent (mg/L).

EXPERIMENTAL PROCEDURE

Reactor operation

The anaerobic reactors were operated with feed water containing 2 g-starch/L as the carbon source and 4 g-$NaHCO_3$/L as buffer, to maintain the reactor pH between 6.5 and 7.5. The aerobic reactors

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were operated using anaerobic reactor effluent as feed. All the reactors, three anaerobic and three aerobic, were operated for more than 28 days with similar feed and draw-off processes, while the bacterial biomass acclimatized to the controlled environment. The aerobic reactors attained quasi-steady state condition after 14 days, showing constant COD removal efficiency of 82% for three consecutive days. The anaerobic reactors took 26 days to attain steady state conditions. This confirmed that consistent biological activity was taking place in all reactors.

From day 28 onwards, An2 was fed with synthetic influent containing 25 mg/L of ametryn and An3 with 25 mg/L of dicamba. The OLR was maintained at 0.2025 kg-COD/m^3/d (2 g-starch/L and 25 mg-herbicide/L). The volumetric exchange ratio was maintained at 50% of volume and the HRT can be taken as 24 hours. The experiments were conducted at constant HRT of 24 hours throughout. The reactors' liquid temperatures were between 27 and 32.1 °C throughout treatment. Each run was carried out on a daily basis by decanting 1 L of supernatant and feeding with fresh influent.

The aerobic reactors A1, A2 and A3 were operated using the effluents from An1, An2 and An3, respectively, as feed. The OLR fluctuated between 0.02 and 0.038 kg-COD/m^3/d. 0.5 L of supernatant was decanted from the aerobic reactors before each fresh feed. The volumetric exchange ratio was maintained at 25%, and the aerobic reactor HRT was 48 hours. Details of influent quality are given in Table 3. The overall performance of the anaerobic-aerobic reactor pairs was evaluated at three-day HRT.

Analytical methods

The herbicide concentrations were measured using HPLC (Agilent 1260, USA), reverse phase – C18 column (100*4.6 mm, 3.5 μm pore size) and diode array detector (DAD). Ametryn and dicamba were analysed in HPLC using the operating procedure outlined (Table 1). Herbicides can be detected in HPLC when methanol and water are used as mobile phases at different ratios during detection of specific compounds, the solvents are allowed to flow continuously into the instrument detector through capillary tubes at a fixed flow rate. The detector wavelength, temperature, and flow rate were set to the specific values in Table 1. The instrument read the specified values for calibration, after which the samples containing herbicide were injected into the mobile phase through the injector. The sample was carried to column, which separates the different molecular species based on their physical and chemical properties, and passes them to the detector. The detector generates liquid chromatograms having intensity peaks for particular retention times. Keeping the sample volume (20 μL) and wavelength (ametryn = 223 and dicamba = 274 nm) constant, the best calibration curve for the standard herbicide solutions was established in the HPLC by varying the mobile phase ratios (methanol and water), flow rate and temperature. Finally, the sample with unknown herbicide content was injected and the chromatogram obtained compared with the standard curve to calculate the herbicide concentration.

The BTPs were analysed using GC-HRMS [GC – Agilent 7890 and MS – Jeol (AccuTOF GCV)]. The maximum wavelength (λ_{max}) value for the standard herbicide solution and reactor samples was determined using a UV spectrophotometer (AU-2701, Systronics, India). DO was measured in the aerobic reactor using a portable meter (Henna Instruments, India), by dipping the electrode tip in the reactor

Table 3 | Reactor influent characteristics

Parameter reactor	pH	Alkalinity (mg-$CaCO_3$/L)	COD (mg/L)	Redox (mV)	Temperature (°C)	DO (mg/L)
Anaerobic – i.e., An1, An2, An3	7.5–8.2	1,900–2,100	1,700–2,100	10 to 20	25–27.5	7–8
Aerobic – i.e., A1, A2, A3	5.6–7.3	1,800–2,400	160–1,200	−105 to −275	28–31.5	0–1

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liquid and pH, temperature, and redox were measured in the reactors with another calibrated meter (edge® dedicated pH/ORP Meter, Hanna Instruments, India).

The influent and effluent samples from the reactors were centrifuged at 5,000 rpm (Remi, R – 24, Remi Electrotechnik, India) for 20 minutes and filtered through 0.2 μm paper. The reactor sludge was centrifuged, autoclaved and re-suspended using 20 mL of 100% methanol, and mixed on a mechanical shaker (150 rpm) for 24 hours, as per Weaver *et al.* (2004), and the liquid sample processed as above. Parameters like alkalinity, COD, MLSS, and MLVSS were measured using APHA standard methods (2005).

RESULTS AND DISCUSSION

Reactor performance

Experiments in the control reactors (An1 and A1)

Control reactor performance was monitored by measuring COD removal efficiency and gas production – Figure 2(a). Effluent parameters including redox, alkalinity, pH and temperature were monitored throughout the study period. Reactor pH was always between 6.6 and 7.7, which is within the acceptable range for a methanogenic reactor (Ross 1992). An1 alkalinity and redox are shown in Figure 2(b); the alkalinity ranged from 1,900 to 2,600 mg-$CaCO_3$/L, the higher concentrations possibly arising from conversion of sulphite to sulphide in the reactor (McCartney & Oleszkiewicz 1991). COD removal exceeded 90% after five days of operation but fell to some extent (to 60%). This may have been caused by the presence of sodium salts and sulphides in the feed, which could inhibit methanogen activity. The issue was overcome by adding a 2 ml/L solution of trace metals to create favourable conditions for and strengthen the methanogens (Manu & Chaudhari 2002). After this the COD removal efficiency consistently exceeded 80%.

The reactor's redox potential was between approximately −250 and −300 mV, the level required for anaerobic reactions occurring under reducing conditions (Van der Zee & Cervantes 2009). The 24-hour average methane gas production in the control reactor (An1) was in the range 300 to 350 mL/d (data not shown), and the total gas yield (including methane and other biogas components) averaged 550 to 710 mL/d (Figure 2(a)). Gas production variation is directly proportional to reactor temperature, higher temperatures (32.1 °C) favouring anaerobic degradation processes. Treatment of the anaerobic effluent in the aerobic reactor (A1) improved the total COD removal efficiencies to more than 95%, perhaps by oxidation of volatile fatty acids to water and CO_2 through the β-oxidation pathway (Gaunt & Hester 1989). The performance of A1 and its effluent alkalinity are shown in Figure 2(c).

Experiments in An2 (ametryn)

The reduction in ametryn and COD concentrations in An2, and the gas production rate are shown in Figure 3. The maximum ametryn removal efficiency achieved was 22% on day 54. Acute initial toxicity was indicated by the reduced reactor performance, when the data are compared with the control. The toxicity was overcome after day 45, with increased COD and ametryn removal, and higher biogas production. The maximum ametryn removal during treatment is shown by the HPLC results as arising from biotransformation, not degradation. The spectrophotometer wavelength scan reported lower absorbance intensity at 223 nm (Sandoval-Carrasco *et al.* 2013). The GC-HRMS analysis confirmed the generation of BTPs such as esters and fatty acids. The An2 pH was between 6.9 and 7.5, which is favourable for methanogenesis. Reactor temperature in An2 was higher than in the

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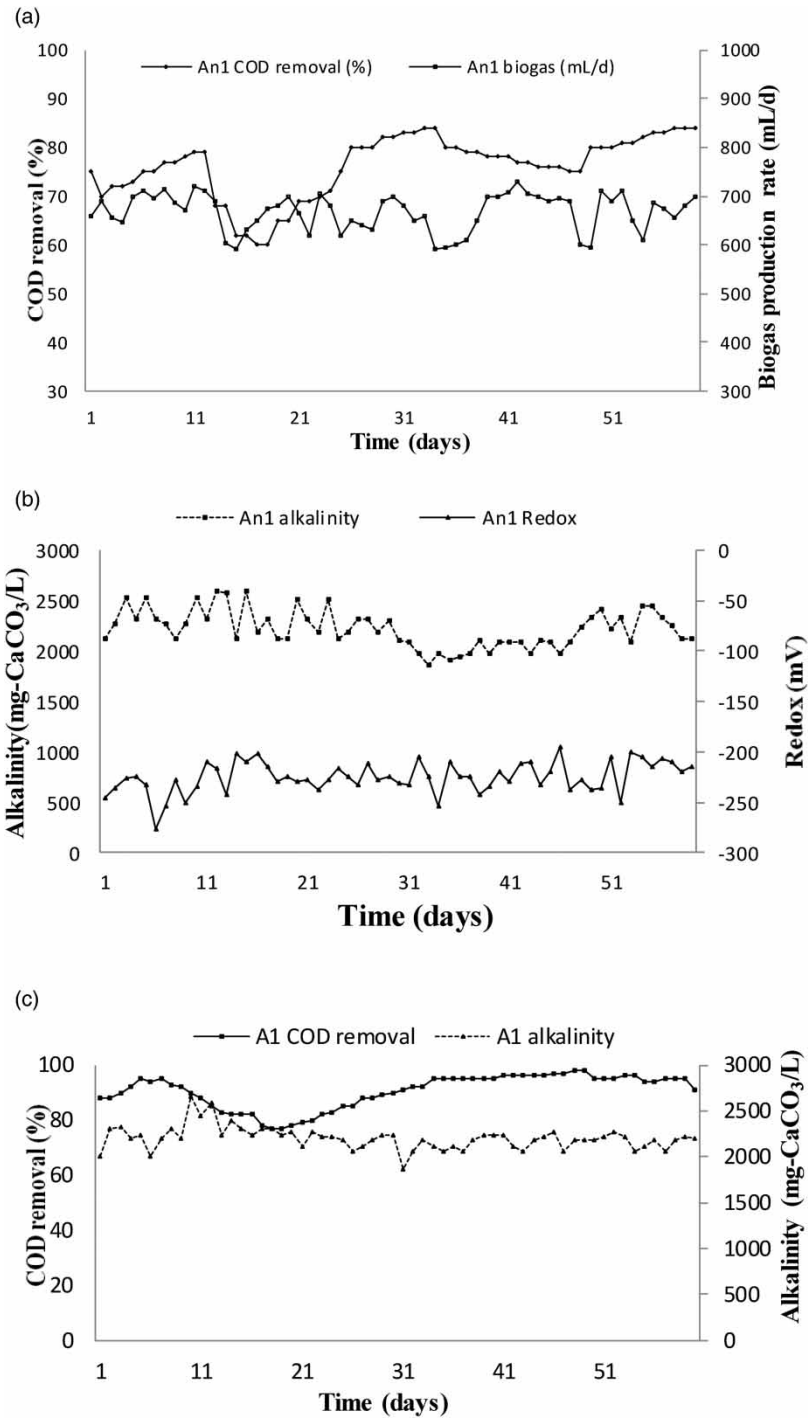

Figure 2 | (a) Anaerobic control (An1) reactor performance; (b) effluent alkalinity and redox potential and, (c) COD removal and effluent alkalinity (An1-A1).

control (An1) at between 30.3 and 31.3 °C. At the higher temperatures, higher redox values of between −250 and −280 mV were recorded, indicating that the reactor was performing better, which was confirmed by its higher gas yield and better COD removal efficiency. The An2 redox and alkalinity performances are shown in Figure 4. The effluent alkalinity obtained for low COD removal and methane yield between days 37 and 41 indicates slightly toxic condition arising from the formation of VFAs, but no other toxic inhibitions were reported. The low proportional removal of COD may also indicate undegraded organic compounds in the effluent (González-Cuna *et al.* 2016).

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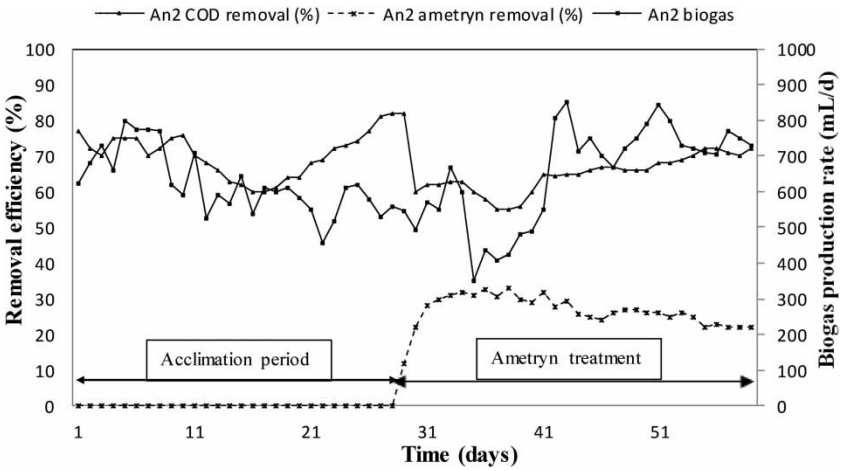

Figure 3 | Performance of anaerobic reactor treating ametryn (An2).

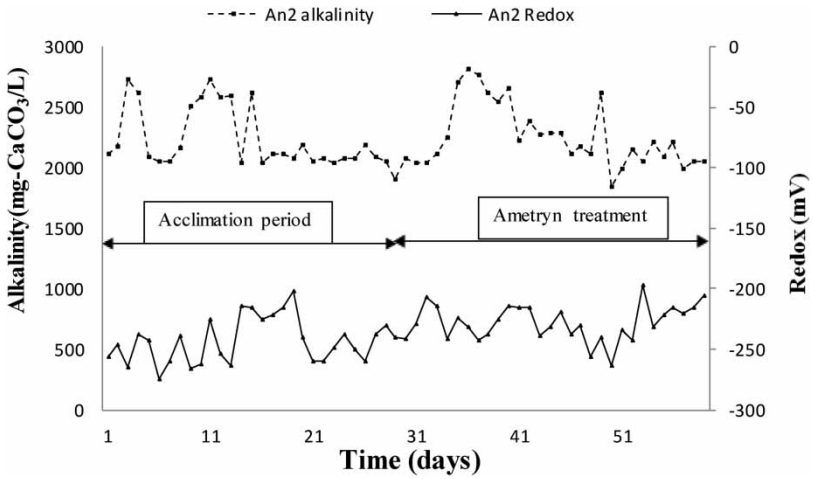

Figure 4 | Alkalinity and redox potential of An2.

Ametryn adsorption onto the reactor sludge was investigated because the solids were retained throughout the study. The sludge had adsorbed around 35 mg/mg-MLVSS of herbicide by day 40. No further adsorption was found as the process continued, however, possibly due to ametryn's high pKa value (Navaratna *et al.* 2016). The 80% COD removal efficiency and methane yields of around 14% (compared to the control) suggest that the methanogens adapted suitably and were important in the treatment process.

Sequential anaerobic-aerobic treatment of ametryn in A2

In A2, 72% ametryn removal efficiency was achieved between days 55 and 58, with COD removal efficiency of 86% – Figure 5. Comparison with the 92% COD removal in the control reactor indicates that a portion of intermediate organic compounds was not digested by the aerobic bacteria initially. After an initial lag, the aerobic reactor's performance improved and became stable, with constant COD removal efficiency. The reactor sludge contained no trace of ametryn. The HPLC and UV spectra reports for the effluent indicate extensive degradation of ametryn metabolites. The GC-HRMS analysis showed that the metabolites formed during anaerobic treatment were oxidised to their end products in the aerobic phase. The anaerobic BTPs (different fatty acids) contain more

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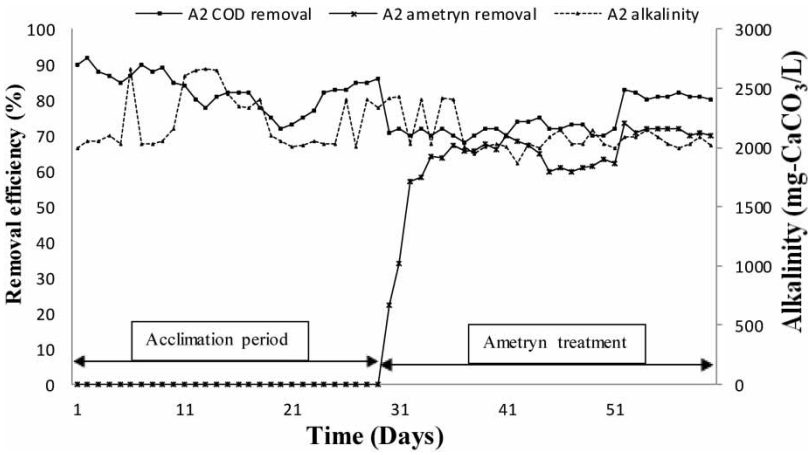

Figure 5 | Performance of aerobic reactor (An2-A2).

carbon atoms than functional groups (halogens and alkyls), which can be oxidised to CO_2 and water through the tricarboxylic acid cycle (Ratledge 1992). Oxidative reduction of fatty acids through β-oxidation produces acetyl-Coenzyme A by successive loss of C_2 units.

Experiments in An3

The maximum dicamba and COD removal efficiencies reported, 58 and 72%, were achieved on day 56, along with high total gas production – Figure 6. The pH in An3 was between 6.8 and 7.8, and the temperature between 30.3 and 32.1 °C. Variations in effluent alkalinity and reactor redox potential were observed, and are shown in Figure 7. In practice, the stability parameters remained within the required ranges, like those in the control reactor. Dicamba degradation in the reactor was monitored using HPLC, the changed peak sets in the chromatogram indicating dicamba BTP formation. The high COD removal efficiency and methane yield exceeding that in the control reactor by 12 to 14% suggest that dicamba was processed predominantly by the methanogenic bacteria. The maximum dicamba removal efficiency may arise from the formation of more oleic acid groups as BTPs, and possibly the degradation and adsorption of oleic acid onto the sludge leading to high CH_4 yields (Pereira *et al.* 2002). No dicamba was adsorbed onto the reactor sludge, so there were no related peaks in the chromatogram, probably because of the compound's high water solubility – 4,500 mg/L – and its low soil sorption capacity (Magga *et al.* 2008).

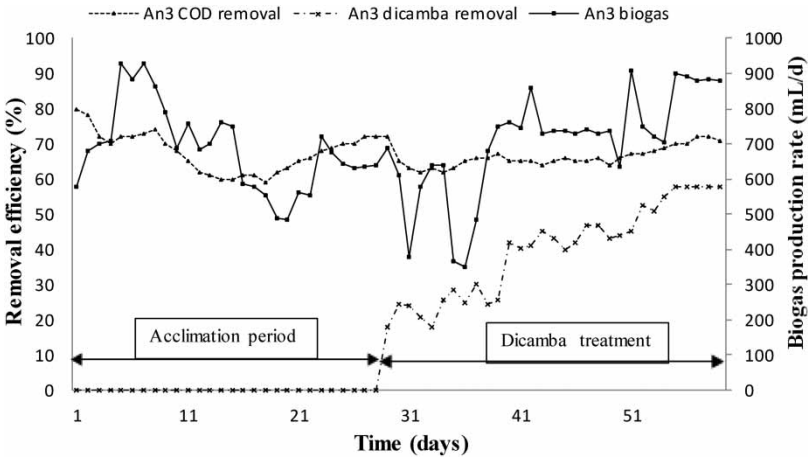

Figure 6 | Performance of anaerobic reactor treating dicamba (An3).

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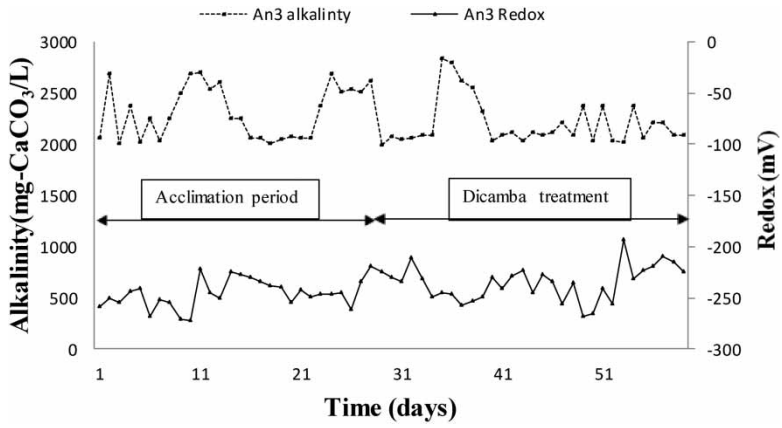

Figure 7 | Alkalinity and redox potential of An3.

Sequential anaerobic-aerobic treatment of dicamba in A3

The effluent from An3 (anaerobic) was treated further in A3 (aerobic) to remove dicamba's BTPs. A3's low COD removal efficiency compared to A1 indicates that these BTPs are recalcitrant to aerobic treatment initially. Up to 78% of the dicamba BTPs were removed, with 85% COD removal – Figure 8. The increased BTP removal efficiency in the later stages may be attributed to the 48-hour HRT, as HRT is important in biological treatment (Wang *et al.* 2014). The anaerobic effluent contained high concentrations of oleic acid, whose mineralization, to water and CO_2, would have lowered the COD concentration in A3. Gradual adaptation and development of anaerobic biomass over operating periods of 100 days was reported during the treatment of phenoxy acetic acid herbicide (Chin *et al.* 2005). The UV spectra obtained for the influent, and An3 and A3 effluents, in the trial reported here showed up to 78% removal of dicamba.

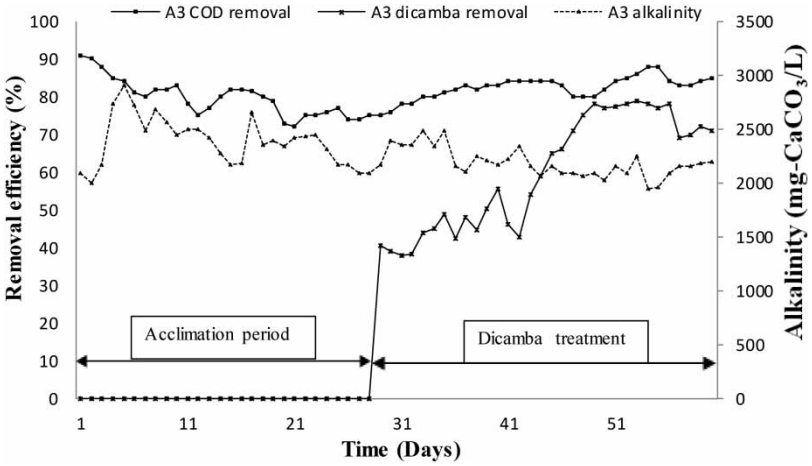

Figure 8 | Performance of aerobic reactor (An3-A3).

CONCLUSIONS

Laboratory-scale SAARs were set up in the study and used, separately, to treat the herbicides ametryn and dicamba. The study was short-term, lasting 30 days after acclimation, to determine herbicide removal efficiency at a combined three-day, anaerobic-aerobic HRT. The OLR of 0.2025 kg-COD/m^3/d, including either ametryn or dicamba, did not inhibit the anaerobic biomass. Higher total gas

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production from the herbicide-treating reactors than the control indicates that anaerobic bacteria were able to use herbicides as their carbon source. The influent and effluent HPLC chromatographs indicated the formation of BTPs, which was confirmed by GC-HRMS. Ametryn formed complex BTPs during the process, some of which might be toxic to the biomass. The chosen influent herbicide concentration caused slight inhibition and it was recovered gradually over continued operation. Herbicide removal efficiency increased generally due to formation of BTPs in the anaerobic reactors and subsequent mineralization in the aerobic reactors.

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REFERENCES

APHA 2005 *Standard Methods for the Examination of Water and Wastewater*, 21st edn. APHA-AWWA-WEF, Washington, DC.

Callado, N. H. & Foresti, E. 2001 Removal of organic carbon, nitrogen and phosphorus in sequential batch reactors integrating the aerobic/anaerobic processes. *Water Science and Technology* **44**(4), 263–270.

Celis, E., Elefsiniotis, P. & Singhal, N. 2008 Biodegradation of agricultural herbicides in sequencing batch reactors under aerobic or anaerobic conditions. *Water Research* **42**, 3218–3224.

Chin, H., Elefsiniotis, P. & Singhal, N. 2005 Biodegradation of 2,4-dichlorophenoxyacetic acid using an acidogenic anaerobic sequencing batch reactor. *Journal of Environmental Engineering and Science* **4**, 57–63.

Chiron, S., Fernandez-Alba, A., Rodriguez, A. & Garcia-Calvo, E. 2000 Pesticide chemical oxidation: state-of-the-art. *Water Research* **34**(2), 366–377.

Comfort, S. D., Inskeep, W. P. & Macur, R. E. 1992 Degradation and transport of dicamba in a clay soil. *Journal of Environmental Quality* **21**(4), 653–658.

Conte, L. O., Schenone, A. V. & Alfano, O. M. 2016 Photo-Fenton degradation of the herbicide 2,4-D in aqueous medium at pH conditions close to neutrality. *Journal of Environmental Management* **170**, 60–69.

De, A., Bose, R., Kumar, A. & Mozumdar, S. 2014 *Targeted Delivery of Pesticides Using Biodegradable Polymeric Nanoparticles*. Springer, New Delhi, India.

Frías, S., Sánchez, M. J. & Rodrıguez, M. A. 2004 Determination of triazine compounds in groundwater samples by micellar electrokinetic capillary chromatography. *Analytica Chimica Acta* **503**(2), 271–278.

Gao, N. Y., Deng, Y. & Zhao, D. 2009 Ametryn degradation in the ultraviolet (UV) irradiation/hydrogen peroxide (H_2O_2) treatment. *Journal of Hazardous Materials* **164**(2), 640–645.

Gaunt, P. & Hester, K. W. 1989 A kinetic model for volatile fatty acid biodegradation during aerobic treatment of piggery wastes. *Biotechnology and Bioengineering* **34**(1), 126–130.

Ghoshdastidar, A. J. & Tong, A. Z. 2013 Treatment of 2,4-D, mecoprop, and dicamba using membrane bioreactor technology. *Environmental Science and Pollution Research* **20**, 5188–5197.

González-Cuna, S., Galíndez-Mayer, J., Ruiz-Ordaz, N., Murugesan, S., Piña-Escobedo, A., García-Mena, J. & Santoyo-Tepole, F. 2016 Aerobic biofilm reactor for treating a commercial formulation of the herbicides 2,4-D and dicamba: biodegradation kinetics and biofilm bacterial diversity. *International Biodeterioration & Biodegradation* **107**, 123–131.

Hamilton, D. J., Ambrus, A., Dieterle, R. M., Felsot, A. S., Harris, C. A., Holland, P. T. & Wong, S. S. 2003 Regulatory limits for pesticide residues in water (IUPAC technical report). *Pure and Applied Chemistry* **75**(8), 1123–1155.

Hurley, P. M. 1998 Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. *Environmental Health Perspectives* **106**(8), 437.

Isa, M. H., Farooqi, I. H. & Siddiqi, R. H. 1993 Methanogenic activity test for study of anaerobic processes. *Indian Journal of Environmental Health* **35**, 1–8.

Magga, Z., Tzovolou, D. N., Theodoropoulou, M. A., Dalkarani, T., Pikios, K. & Tsakiroglou, C. D. 2008 Soil column experiments used as a means to assess transport, sorption, and biodegradation of pesticides in groundwater. *Journal of Environmental Science and Health Part B* **43**(8), 732–741.

Manu, B. & Chaudhari, S. 2002 Anaerobic decolorisation of simulated textile wastewater containing azo dyes. *Bioresource Technology* **82**, 225–231.

McCartney, D. M. & Oleszkiewicz, J. A. 1991 Sulfide inhibition of anaerobic degradation of lactate and acetate. *Water Research* **25**, 203–209.

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doi: 10.2166/wpt.2019.027

Milligan, P. W. & Häggblom, M. M. 1999 Biodegradation and biotransformation of dicamba under different reducing conditions. *Environmental Science &Technology* **33**, 1224–1229.

Navaratna, D., Shu, L. & Jegatheesan, V. 2016 Evaluation of herbicide (persistent pollutant) removal mechanisms through hybrid membrane bioreactors. *Bioresource Technology* **200**, 795–803.

Ndon, U. J. 2007 Analysis and kinetics of the sequencing batch reactors. *Environmental Engineering Science* **24**(4), 505–514.

OECD 2008 *Environmental Performance of Agriculture at A Glance*. Organisation for Economic Co-operation and Development. http://www.oecd.org/greengrowth/sustainable-agriculture/40953155.pdf/ (accessed on 20 November 2017).

Pereira, M. A., Pires, O. C., Mota, M. & Alves, M. M. 2002 Anaerobic degradation of oleic acid by suspended and granular sludge: identification of palmitic acid as a key intermediate. *Water Science and Technology* **45**(10), 139–144.

Ratledge, C. 1992 Microbial oxidations of fatty alcohols and fatty acids. *Journal of Chemical Technology & Biotechnology* **55**(4), 399–400.

Ross, W. R. 1992 *Anaerobic Digestion of Waste-Water Sludge: Operating Guide*. Water Research Commission. Water Institute of Southern Africa, Pretoria, South Africa.

Sandoval-Carrasco, C. A., Ahuatzi-Chacón, D., Galíndez-Mayer, J., Ruiz-Ordaz, N., Juárez-Ramírez, C. & Martínez-Jerónimo, F. 2013 Biodegradation of a mixture of the herbicides ametryn, and 2,4-dichlorophenoxyacetic acid (2,4-D) in a compartmentalized biofilm reactor. *Bioresource Technology* **145**, 33–36.

Sangami, S. & Manu, B. 2017 Fenton's treatment of actual agriculture runoff water containing herbicides. *Water Science and Technology* **75**(2), 451–461.

USEPA 2006 *Re-registration Eligibility Decision for Dicamba and Associated Salts*. Available at: https://archive.epa.gov/pesticides/reregistration/web/pdf/dicamba_red.pdf (accessed on 15 August 2017).

USEPA 2010 *Re-registration Eligibility Decision (RED) for Ametryn*. Available at: https://archive.epa.gov/pesticides/reregistration/web/pdf/ametryn_red.pdf (accessed on 8 December 2017).

Van der Zee, F. P. & Cervantes, F. J. 2009 Impact and application of electron shuttles on the redox (bio) transformation of contaminants: a review. *Biotechnology Advances* **27**(3), 256–277.

Vencill, W. K. 2002 *Herbicide Handbook*, 8th edn. Weed Science Society of America, Lawrence, USA.

Wang, D., Ji, M. & Wang, C. 2014 Degradation of organic pollutants and characteristics of activated sludge in an anaerobic/anoxic/oxic reactor treating chemical industrial wastewater. *Brazilian Journal of Chemical Engineering* **31**(3), 703–713.

Weaver, M. A., Zablotowicz, R. M. & Locke, M. A. 2004 Laboratory assessment of atrazine and fluometuron degradation in soils from a constructed wetland. *Chemosphere* **57**(8), 853–862.