A comparison of anaerobic 2, 4-dichlorophenoxy acetic acid degradation in single-fed and sequencing batch reactor systems

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Abstract. This paper compares the practical limits of 2, 4-dichlorophenoxy acetic acid (2,4-D) degradation that can be obtained in two laboratory-scale anaerobic digestion systems; namely, a sequencing batch reactor (SBR) and a single-fed batch reactor (SFBR) system. The comparison involved synthesizing a decade of research conducted by the lead author and drawing summative conclusions about the ability of each system to accommodate industrial-strength concentrations of 2,4-D. In the main, 2 L liquid volume anaerobic SBRs were used with glucose as a supplemental carbon source for both acid-phase and two-phase conditions. Volatile fatty acids however were used as a supplemental carbon source for the methanogenic SBRs. The anaerobic SBRs were operated at an hydraulic retention time of 48 hours, while being subjected to increasing concentrations of 2,4-D. The SBRs were able to degrade between 130 and 180 mg/L of 2,4-D depending upon whether they were operated in the acid-phase or two-phase regime. The methanogenic-only phase did not achieve 2,4-D degradation however this was primarily attributed to difficulties with obtaining a sufficiently long SRT. For the two-phase SFBR system, 3.5 L liquid-volume digesters were used and no difficulty was experienced with degrading 100 % of the 2,4-D concentration applied (300 mg/L).

1. Introduction

With an estimated annual worldwide use of over 2.2 million tonnes [1]; it is not surprising that engineers and scientists are increasingly interested in the fate of pesticides, particularly since they have been found in the earth's water and soil environments. Over and above their known potential to specifically pollute the earth's water ways, there is an increasing concern about the pesticide impact on public health [2, 3]. In particular, one of the most ubiquitous pesticides is the organic herbicide 2, 4-dichlorophenoxy acetic acid (2,4-D). This pesticide is used to enhance agricultural productivity (mainly for crops of the cereal variety); however, it is also the active constituent in several herbicides used to manage broadleaf weeds.

Within the New Zealand context, agriculture is a major revenue earner with a third of all commodity exports arising from the agricultural-based sector [4]. Such industries rely heavily on pesticides with 2,4-D and/or others of the phenoxy herbicide category making up 68 % of all New Zealand herbicides [5]. It is hardly surprising therefore that 2,4-D contamination of water has been

reported not only in New Zealand but also around the world [6]. Pollution of aqueous environments however not only arises from surface run-off and aerial drift (i.e. crop spraying); it also arises from pesticide discharges originating from chemical manufacturing plants. In particular, it has been reported that concentrations of chlorinated organic compounds in industrial streams can range from ten to several hundreds of mg/L, depending upon the industry [7].

In recent years, biological processes have received increasing attention as both cost-effective and technically-efficient methods for the treatment of xenobiotic substances [8]. Notwithstanding, limited success has been achieved with conventional activated sludge systems, particularly regarding their ability to degrade certain pesticides [9]. Because of this, there exists a continual need to conduct further research into alternative biological options for the treatment of recalcitrant compounds [10, 11]. More specifically, no study to date has reported on the 2,4-D degradation efficiency of a sequencing batch reactor (SBR) system as compared to a single-fed batch reactor (SFBR) system under anaerobic conditions. Of major interest is their ability to accommodate industrial strength concentrations.

The prime function of this paper is to synthesize together (and comment on) several published studies that have recently examined the biodegradation potential of 2,4-D under anaerobic conditions. These disparate studies (conducted mainly over the last decade in the lead author's previous laboratory (the University of Auckland, New Zealand)) have built on each other to explore the practical upper limits of both SBR and SFBR systems anaerobically degrading 2,4-D. The anaerobic SBR work was mainly done using glucose as an extra carbon source; while, as part of this report, the SBR also explored the impact of phase separation (acid-phase vs. two-phase digestion) on 2,4-D removal.

2. Materials and methods

2.1. Reactor operation and configuration

This research was conducted at the laboratory-scale, with the SBRs having total and liquid volumes of 3 L and 2 L, while the SFBRs had total and liquid volumes of 4 L and 3 L respectively. Ports were incorporated into each SBR for feeding, decanting, sample collection/wastage, nitrogen gas addition and biogas collection. To maintain anaerobic conditions, nitrogen gas was injected into the head space of the SBR, with bolted O-ring stoppers sealing the reactors. In contrast, sealing of the SFBRs was by a rubber cork, while a pipe throughout the cork connected to an anaerobic respirator system in order to monitor gas production.

Both systems were mixed by magnetic stirrers, while feeding and decanting (of the SBR) was by peristaltic pumps. When methane or two-phase digestion was desired in the SBR, heated coils controlled by a voltmeter held the temperature generally in the lower mesophilic range (32 ± 2^{o}C). When acid-phase conditions were desired, the pH was maintained between 4.5 and 5.0 by an aqueous solution of 0.05 N H_2SO_4. When methane or two-phase conditions were desired, 0.05 N NaOH was added to maintain the pH around 7.0.

The experimental program consisted of 10 runs which were broken down into two stages; namely, 7 SBR runs and 3 SFBR runs. Table 1 shows the experimental matrix as well as the operating conditions. Note that the mixed liquor volatile suspended solids (MLVSS) values are average values for the SBR while they are initial values for the SFBR. In selected runs, samples were sequentially extracted and measured for residual 2,4-D, in order to track the degradation behavior. Previous laboratory research had established that biological degradation was the main mechanism of 2,4-D removal, rather than biosorption [12]. In addition, a control reactor had definitively proven that there were no abiotic losses.

Runs 1 to 3 (table 1) explored the impact of increasing 2,4-D concentration in the acid-phase SBR while runs 4 and 5 examined one 2,4-D concentration only, in the methane-phase SBR. Runs 6 and 7 investigated two different 2,4-D concentrations in the SBR under two-phase anaerobic conditions, while runs 8 to 10 considered increasing 2,4-D concentrations in the two-phase conditions of the SFBR. Given that this research investigated industrial-strength concentrations of 2,4-D (i.e. 20 – 300 mg/L); a relatively long hydraulic retention time (HRT) of 48 hrs was chosen for the SBRs, while

nominal solids retention times (SRTs) were targeted at either 10 d (acid-phase SBRs) or in the 12-20 d range (methane and two-phase SBRs).

Table 1. Operating conditions of experimental design.

Run	2,4-D Feed [ ]	Phase	Temp (°C)	Length of Run (day)	MLVSS
1 – SBR	20	Acid	22 ± 2	129	2450 ± 440
2 – SBR	100	Acid	22 ± 2	33	2630 ± 110
3 – SBR	200	Acid	23 ± 2	26	2350 ± 60
4 – SBR	20	Methane	33 ± 2	116	3150 ± 766
5 – SBR	20	Methane	38 ± 2	42	2318 ± 262
6 – SBR	100	Two-phase	30 ± 2	62	4640 ± 309
7 – SBR	300	Two-phase	30 ± 2	31	4910 ± 273
8 – SFBR	100	Two-phase	32 ± 2	73	10,780 ± 330
9 – SFBR	200	Two-phase	32 ± 2	73	10,680 ± 170
10 – SFBR	300	Two-phase	32 ± 2	73	10,730 ± 270

2.2. Feed and biomass characteristics

The SBR reactors were seeded with a mixture (3:1 ratio by weight) of anaerobically digested sludge from a nearby domestic Auckland sewage treatment plant. Biomass was obtained from an on-going University of Auckland research program studying biomass exposure to 2,4-D. For the acid and two-phase SBRs (Runs 1-3, 6-7), the feed consisted of various concentrations of 2,4-D (table 1) and a target value of supplemental substrate; namely, 1000 mg/L of COD in the form of glucose. For the SBR methane phase (Runs 4,5), a target value of 1000 mg/L supplemental COD in the form of a VFA mixture (60 % acetate, 30 % propionate and 10 % butyrate) was used. The SFBRs were also seeded with digested sludge, however the feed was basically primary sludge with no supplemental carbon substrate added. In all cases, 2,4-D was added using small pellets (98 % purity) which had previously been dissolved in water. The concentrations of the 2,4-D stock solution, was about 500 mg/L (just to keep it well below the solubility limit of about 900 mg/L). In general, 2,4-D pellets dissolve very poorly even at concentrations well below the solubility limit.

Acclimation allows the bacteria time to express the enzyme-inducing genes to induce the pesticide's biodegradation; and, as such, was defined as the time from the first application of 2,4-D (or the next increment in 2,4-D concentration where applicable) until the beginning of consistent 2,4-D removal. This was defined as removal over 10 %; that is, 2 or 3 % removal occasionally occurred, however this was generally ignored until the removal reached ≥ 10 %.

2.3. Analytical and sampling methods

The reactor contents, influent and effluent from each reactor were collected and measured at least three times per week. During in-depth track studies, sampling was done primarily on an hourly basis. Chemical parameters included glucose, 2,4-D, MLVSS, pH, temperature, and VFAs. All samples, except solids were centrifuged at 4,000 rpm for 8 min and the supernatant was filtered through a 0.2 μm nylon filter membrane and acidified to a pH of 2 with 9.8 % H_2SO_4, before further analysis. Solids were filtered using 55-mm-diameter glass microfiber filters (GF/C 1822 055). Glucose was analyzed by the American Chemical Society (ACS) method using anthrone as a reagent [13]. 2,4-D was measured by a Dionex® high performance liquid chromatograph (HPLC) equipped with a Microsorb® MV 100-5 C18 column (24 cm) and an UV/FIS detector (wavelength 240 nm, temperature 40°C, elution with acetonitrile/water (60/40) at 1.2 mL/min). VFAs (i.e. acetic, propionic, butyric, and valeric acids) were determined by a Hewlett-Packard® HP-6890 gas chromatograph (GC) using helium as carrier gas (inlet split at 5:1, temperature 240°C, split flow 9.0 mL/min at 22.16 psi, total flow 13.2 mL/min). The system was equipped with a EC-1000 column (30 cm) and an flame ionization detector (FID) (temperature 300°C, hydrogen flow 45.0 mL/min, air flow 400 mL/min,

constant makeup flow of nitrogen 32.0 mL/min). An 1 mmol VFA standard solution was used for calibration purposes.

All other parameters were measured according to Standard Methods for the Examination of Water and Wastewater [14].

3. Results and discussions

3.1. Degradation of 2,4-D: SBR reactor configuration

Table 2 indicates the percentage of degradation achieved during each run along with the amount of acclimation time needed to begin degradation. What is clear from the data is that a reasonably long time frame (i.e. 100 days) was needed before the acid phase anaerobic SBR showed any 2,4-D degradation (Run 1). However once acclimated, subsequent runs (2 and 3) quickly adjusted to the new, increased concentration; thereby shortening the acclimation period (11 and 7 days respectively). It can be seen however that Run 3 never achieved full degradation of the 200 mg/L of 2,4-D. It is reasonable therefore to suggest that at least for the conditions studied in this research, acid-phase digestion has an upper removal bound of around 130 mg/L of 2,4-D.

Table 2. Length of time needed for acclimation and % 2,4-D degradation.

Run	2,4-D Feed [ ]	Phase	Length of Acclimation	% 2,4-D Degraded
1 - SBR	20	Acid	100 days	100
2 - SBR	100	Acid	11 days	100
3 - SBR	200	Acid	7 days	65
4 - SBR	20	Methane	Unachieved	0
5 - SBR	20	Methane	Unachieved	0
6 - SBR	100	Two-phase	46 days	100
7 - SBR	300	Two-phase	12 days	59
8 – SFBR	100	Two-phase	25 days	100
9 – SFBR	200	Two-phase	25 days	100
10 - SFBR	300	Two-phase	30 days	100

When the SBR was operated in the methane phase (Run 4), it was observed that even a small concentration of 2,4-D (20 mg/L), experienced no degradation. From a reporting perspective, since the SBR never acclimated to the pesticide, the percent 2,4-D degradation achieved was 0 %. Similarly, Run 5 did not achieve 2,4-D acclimation (again 0 % degradation), even though to encourage 2,4-D degradation, it was operated at a higher temperature (table 1). Due to the apparent lack of success, Run 5 was terminated after 42 days (table 1).

Run 6 operated as a two-phase digester in that the acid- and methane-phases were allowed to find their natural equilibrium in terms of co-existence. As can be seen, it took 46 days to acclimate (i.e. longer than the acid-phase only SBR (at an equivalent concentration of 100 mg/L)) but this was not surprising, since its initial starting concentration was so much higher. However, Run 6 performed better than the methane-only phase digesters (Run 4 and 5) which did not acclimate. This does suggest that an additional reason for the long acclimation period of Run 6 (i.e. as compared to Run 2) was the presence of a significant number of methanogenic bacteria. When the 2,4-D concentration was increased to 300 mg/L (Run 7), again a radically shortened acclimation period was experienced, even though the step-change in concentration was quite significant (i.e. the 200 mg/L concentration level was eliminated). As table 2 indicates, 100 % of the 2,4-D was removed at a concentration of 100 mg/L (Run 6); however, only 59 % was removed at the new concentration of 300 mg/L (Run 7). Figure 1 indicates that 59 % removal was a mean value for Run 7.

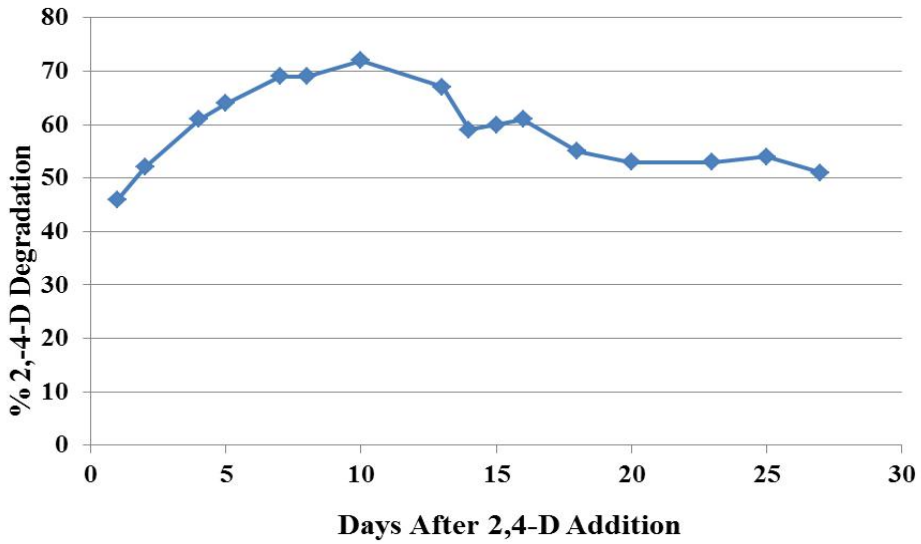

Figure 1. Example of Stability of 2,4-D Degradation: Run7.

3.2. Degradation of 2,4-D: SFBR reactor configuration

Runs 8, 9 and 10 (table 2) in the SFBR systems achieved 100 % degradation for each increment in concentration, taking approximately the same amount of time to acclimate in each case. A comparison of the SFBR to the SBRs is not quite valid here however; since certainly any initial decline in 2,4-D can be accounted for by dilution. That is, during each sampling day, a certain volume of liquid was taken from the 4 L reactors in order to measure VFAs, 2,4-D, and solids etc. Since it is impossible to do this for 73 days without running out of liquid, an equivalent amount of tap water had to be added to each reactor on sampling days. This effect however has been taken into account by applying appropriate "dilution factors" to normalize all data obtained.

The SFBR appeared to be a better system than the SBRs since it removed 100 % of the 300 mg/L 2,4-D concentration, whereas, the SBR only removed 59 % (table 2). However, this comparison may not be strictly valid for two reasons. Firstly, the SFBRs experienced a lower total amount of 2,4-D added, since they were fed 300 mg/L of 2,4-D only at the start of the experiment. In contrast, the SBRs were fed that amount on a daily basis as part of the SBR feeding regime. Undoubtedly, the SBR's continual exposure to incoming 2,4-D may have caused cumulative stress to the bacteria , despite the fact that they were actively-growing in response to the daily influx of glucose. Secondly, it has to be acknowledged that the SFBRs carried a much higher solids loading (table 1). As such, what might appear to be inhibition in the SBR might have been overcome by just straight adaption in the SFBR, simply because of the larger concentration of bacteria present.

3.3. Specific carbon consumption rates: Effect of 2,4-D concentration

Specific rates are not strictly applicable to an SFBR system primarily because it is considered as a "single-cycle" operation. However, rates can be readily calculated for the SBR system and; as such, Table 3 shows the specific carbon consumption rates for each of the SBR runs. As can be seen, the specific glucose consumption rate for Runs 1 to 3 seem to be independent of the 2,4-D concentration. However, the specific 2,4-D consumption rate clearly increased substantially between the 20 mg/L and 100 mg/L 2,4-D concentration. Increasing the 2,4-D concentration further to 200 mg/L however did not seem to increase the specific 2,4-D consumption rate. As such, the existence of a "rate" plateau suggests a possible limitation in the biomass' ability to degrade 2,4-D.

As mentioned, Runs 4 and 5 (operating in the methanogenic phase) did not remove 2,4-D, thus no specific 2,4-D rates are calculable. The lack of removal may be attributed to the potential toxicity of

2,4-D on methanogenic bacteria; however, in addition, at times, the SRT dropped perilously low (5 to 8 days) which meant there was difficulty in keeping the biomass in the system. The observed increase in the specific VFA utilisation rate between Runs 4 and 5, is likely due to the fact that Run 5 was operated at the higher temperature. Finally, as can be seen, the specific glucose consumption rate dropped substantially from Run 6 to Run 7 which indicates a negative effect on the glucose degradation pattern (caused likely by inhibition associated with the increased concentration of 2,4-D). However once again, the 2,4-D consumption rate reached a plateau which explains the upper bound on the 2,4-D removal (180 mg/L).

Table 3. Specific rates for carbon consumption.

Run	2,4-D Feed [ ]	Phase	Supplementary Substrate (mg/mg VSS·d)	2,4-D Rate (mg/mg VSS·d)
1 – SBR	20	Acid	2.45 ± 0.29	0.007 ± 0.001
2 – SBR	100	Acid	2.73 ± 0.22	0.047 ± 0.006
3 – SBR	200	Acid	2.74 ± 0.33	0.048 ± 0.005
4 – SBR	20	Methane	0.04 ± 0.003	0.000
5 - SBR	20	Methane	0.07± 0.002	0.000
6 - SBR	100	Two-phase	0.32 ± 0.03	0.015 ± 0.003
7 - SBR	300	Two-phase	0.19 ± 0.03	0.018 ± 0.004

3.4. Pattern of sequential carbon consumption: SBRs and SFSBRs

Figure 2 plots the % carbon removed for Run 2 of the SBRs as an example of the sequential pattern obtained in terms of carbon removal. That is, in an acid-phase anaerobic digester fed with both glucose and 2,4-D, complete degradation of both carbon substrates occurred in a sequential manner when the 2,4-D concentration was 100 mg/L or less. This pattern was consistently observed throughout this study, and implies sequential utilization known as "diauxic growth", a hallmark of systems treating toxic substances in the presence of readily biodegradable substrates [15, 16].

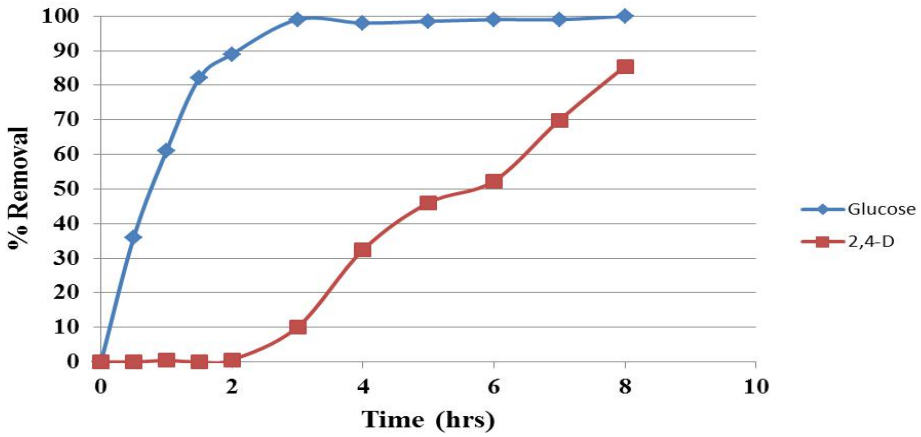

Figure 2. Example of sequential carbon degradation pattern in SBR: Run2.

Further to this, in Runs 6 and 7, VFAs were generated, with acetic and propionic acids being the major species (making up at least 90 % of the total VFAs). This pattern of speciation is characteristic of wastewaters low in protein and high in carbohydrate [17]. In Run 6, the VFAs were totally degraded within the first 8 hrs of the cycle, representing a stable relationship between acidogens and methanogens in the reactor. However, when the 2,4-D concentration was increased to 300 mg/L, a modest rise in total VFAs was observed followed by a 4 hr prolongation for total VFA consumption.

This confirms the deterioration in the performance of the anaerobic SBR at the largest 2,4-D concentration.

Figure 3 indicates the biodegradation pattern of the 2,4-D inside the SFBRs. It can be seen that after an acclimation period of between 25 and 35 days (a function of the amount of 2,4-D added), complete biodegradation of 2,4-D was accomplished.

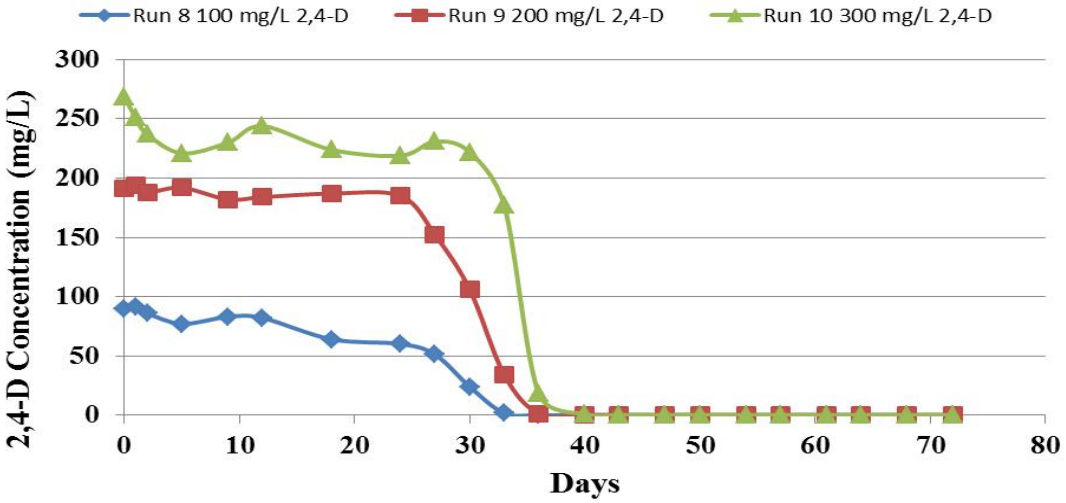

Figure 3. 2,4-D Biodegradation pattern: Runs 8-10.

4. Conclusions

This study indicated that after biomass acclimation, anaerobic biodegradation of industrial-strength concentrations of 2,4-D was accomplished in both the SBRs (except under methanogenic-only conditions) and the SFBRs. Under the experimental conditions of this research, the SBRs degraded between 130 and 180 mg/L of 2,4-D depending upon whether the reactors were operating in acid-phase or two-phase conditions. In contrast, the SFBRs degraded 100 % of the 2,4-D up to a concentration of 300 mg/L.

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