Biodegradation of agricultural herbicides in sequencing batch reactors under aerobic or anaerobic conditions

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ABSTRACT

This study investigated the biodegradability of the herbicides isoproturon and 2, 4-dichlorophenoxyacetic acid (2,4-D) in sequencing batch reactors (SBRs). Two laboratory-scale (2 L liquid volume) SBRs were employed: one reactor performing under aerobic and the other under anaerobic conditions. The aerobic SBR was operated at an ambient temperature (22 ± 2°C), while the anaerobic SBR was run in the lower mesophilic range (30 ± 2°C). Each bioreactor was seeded with a 3:1 mixture (by weight) of fresh sludge and biomass that had been previously exposed to both herbicides. The effect of herbicide concentration on either treatment process was explored at a hydraulic retention time (HRT) of 48 h, using glucose as a supplemental carbon substrate. Although no isoproturon degradation was observed in either system during the study, complete 2,4-D removal occurred after an acclimation period of approximately 30 d (aerobic SBR) and 70 d (anaerobic SBR). The aerobic reactor achieved complete 2,4-D utilization at feed concentrations up to 500 mg/L. A further increase to 700 mg/L, however, proved to be inhibitory since 2,4-D biodegradation was negligible. On the other hand, the anaerobic SBR was able to degrade 120 mg/L of 2,4-D, which corresponds to 40% of the maximum feed concentration applied. Moreover, glucose was consumed first throughout the experiment in a sequential utilization pattern relating to 2,4-D, with biodegradation of both substrates following closely first-order kinetics.

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1. Introduction

The extensive use of pesticides and their contamination potential have become major concerns in the field of environmental engineering and science. Although the benefits generated by the application of agro-chemicals are evident in terms of increased agricultural productivity and improved public health through disease control (e.g. malaria, yellow fever, and dengue), the presence of pesticide residues in the soil, water, and air has created potential risks from both a human and a natural environment perspective (DeLorenzo et al., 2001; Barron et al., 2003; Otto et al., 2007).

During the last decade, two of the most commonly used herbicides in agricultural areas include 2,4-dichlorophenoxy acetic acid (2,4-D—a phenoxy acid compound) and 3-(4-isopropylphenyl)-1,1-di-methylurea (isoproturon—a phenyl urea compound) (Buenrostro-Zagal et al., 2000; Cooke et al., 2004). Consequently, both herbicides have been frequently detected in surface and ground waters in many parts of the world (Dorigo et al., 2004; Mitchell et al., 2005; Macur et al., 2007).

Contamination of the aqueous environment could be the result of pesticide discharges from manufacturing plants, storage sites, accidental spills, and surface runoff. An environmental protection strategy from an engineering viewpoint may involve collection and treatment of pesticide-contaminated wastewater, which can be accomplished by a variety of physical, chemical, and/or biological processes.
In recent years, biological processes have been considered to be a cost-effective and environmentally sustainable alternative for the treatment of such wastewaters (Gogate and Pandit, 2004; He, 2006). However, conventional biological treatment systems (e.g. activated sludge) have shown limited success in removing potentially toxic substances, including pesticides (Meric et al., 2003).

Sequencing batch reactor (SBR) technology has recently become an attractive alternative option for the removal of various xenobiotic compounds from wastewaters (Tomei et al., 2004; Mohan et al., 2005). Most of the studies available regarding herbicide wastewater treatment, however, have focused on the treatment of a single compound (i.e. 2,4-D) either aerobically or anaerobically (Chin et al., 2005). Furthermore, although biodegradation of isoproturon has been observed to occur in natural environments such as soils or aquifers, albeit at very low concentrations within the ug/L range (Perrin-Ganier et al., 2001; Johnson et al., 2004), limited information is available on its biodegradation potential in bioreactors (Li et al., 2005).

The main purpose of this study was therefore to explore the biodegradability of isoproturon and 2,4-D in SBRs operated under aerobic or anaerobic conditions, using glucose as a supplemental carbon substrate. In addition, the effect of increasing herbicide concentration on both treatment processes was investigated in detail.

2. Materials and methods

2.1. Reactor configuration and operation

Two laboratory-scale SBRs were used in this study: one system was operated under aerobic conditions and the other under anaerobic conditions. Each reactor was made of 5-mm-thick Plexiglas cylinders, had an internal diameter of 100 mm, a total volume of 3 L, and an operating liquid volume of 2 L. Sealing of the reactors was achieved by bolted O-ring mounted Plexiglas stoppers. Five ports were installed in each SBR for feeding, decanting, sample collection and wastage, supply of air (aerobic) or nitrogen (anaerobic), and gas venting. Air was introduced to the aerobic system (designated as SBR1) through a submerged diffuser located at the bottom of the reactor. A dimmer was used to control the air flow and maintain a dissolved oxygen (DO) concentration higher than 2 mg/L throughout the experiment. In the anaerobic system (designated as SBR2), pressurized nitrogen gas was introduced to the headspace to ensure anaerobic conditions. In both reactors mixing was provided by magnetic stirrers while feeding and decanting were carried out by appropriately calibrated peristaltic pumps. An outline of the system configuration is presented in Fig. 1.

In order to provide optimum conditions for each process, the aerobic SBR was operated at an ambient temperature (22 ± 1°C), while the temperature in the anaerobic SBR was maintained in the lower mesophilic range (30 ± 2°C) with the use of a water bath. Based on previous studies on 2,4-D biodegradation (Mangat and Elefthinotis, 1999; Chin et al., 2005), a hydraulic retention time (HRT) of 48 h was selected for both systems, while the solids retention time (SRT) was maintained within the 12–15 d range. The operational sequence, on a 24 h basis, was identical in both reactors and included the following periods: (i) feeding, 15 min; (ii) reaction, 22 h 30 min; (iii) settling, 1 h; and (iv) decanting, 15 min.

2.2. Biomass and feed characteristics

The aerobic system (SBR1) was seeded with a mixture (at a 3:1 ratio by weight) of fresh waste activated sludge from the North Shore Wastewater Treatment Plant in Auckland, New Zealand, and of biomass that had been exposed to isoproturon and 2,4-D during previous studies conducted at the Environmental Laboratory, University of Auckland (Li et al., 2005). A similar approach was used for seeding the anaerobic system (SBR2); however, the fresh sludge was collected from the anaerobic digesters of the above-mentioned facility. The synthetic feed was made up with glucose (1000 mg/L) serving
### Table 1 – Summary of herbicide feed concentrations and duration of experimental stages

<table>
<thead>
<tr>
<th>ASBR</th>
<th>Stage</th>
<th>Duration (d)</th>
<th>Feed concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASBR</td>
<td></td>
<td>Isoproturon</td>
</tr>
<tr>
<td>1 and 2</td>
<td>Start-up</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>1 and 2</td>
<td>I-1</td>
<td>91</td>
<td>20</td>
</tr>
<tr>
<td>1 and 2</td>
<td>I-2</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>II-1</td>
<td>62</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>II-1</td>
<td>84</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>II-2</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>II-2</td>
<td>26</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>II-3</td>
<td>15</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>II-4</td>
<td>16</td>
<td>50</td>
</tr>
</tbody>
</table>

as supplemental carbon substrate, varied concentrations of the two herbicides investigated (Table 1), and appropriate amounts of nutrients required for either aerobic (Yoong and Lant, 2001) or anaerobic conditions. The initial volatile suspended solid (VSS) concentration in SBR1 was 3000 mg/L while in SBR2 it was 7000 mg/L.

During the start-up stage, no herbicides were added to the feed in order to minimize any potential toxic effects at the beginning of the experiment. This was followed by the addition of isoproturon only throughout stage I, while both herbicides (isoproturon and 2,4-D) were applied in stage II. In order to designate the different herbicide concentrations employed during the study, a numerical symbol was added to each stage as illustrated in Table 1. Regarding the origin of herbicides used, 2,4-D was in a form of small pellets (98% purity) manufactured by Aldrich Chemical Co. Inc., while isoproturon was in a form of a suspension concentrate (500 g/L) manufactured by Taranaki NuChem Ltd. The solubility limits in water at 20°C are 900 mg/L for 2,4-D and 60 mg/L for isoproturon.

#### 2.3. Sampling and analytical methods

The influent, reactor content, and effluent from each reactor were analyzed three times per week. During track studies sampling was done mostly on an hourly basis, or as frequently as required. Influent samples were withdrawn from the feed bottles, effluent samples were taken after decanting, while reactor samples were withdrawn during the reaction period. The parameters determined included glucose, isoproturon, 2,4-D, total organic carbon (TOC), VSS, pH, temperature, DO (SBR1 only), and volatile fatty acids (VFAs) (SBR2 only). All samples, except those used for VSS determination, were centrifuged at 4000 rpm for 8 min and the supernatant was subsequently filtered through a 0.2 μm nylon filter membrane and acidified to a pH of 2 with 9.8% sulfuric acid, before further analysis.

For glucose analysis a procedure adopted by the American Chemical Society (ACS) was followed using anthrone as a reagent (ACS, 2000). TOC was analyzed by a Shimadzu model TOC-VCSH total organic carbon analyzer. Isoproturon and 2,4-D were measured by a Dionex high-performance liquid chromatograph equipped with a Microsorb MV 100-5 C18 column (24 cm) and a 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 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 an EC-1000 column (30 cm) and an flame ionization detector (temperature 300°C, hydrogen flow 45.0 ml/min, air flow 400 ml/min, constant makeup flow of nitrogen 32.0 ml/min). A 1 mmol VFA standard solution was used for calibration purposes. All other parameters were determined according to Standard Methods (1998). Details for all analytical tests performed can be found elsewhere (Celis, 2005).

### 3. Results and discussion

#### 3.1. Biomass acclimation to herbicides

Before proceeding with the addition of herbicides, each SBR was fed with 1000 mg/L of glucose as the sole organic carbon source for a period of 20 d (start-up stage). This practice was incorporated to allow for biomass build-up, minimize any problems due to toxic effects, and improve the operational stability of the bioreactors. It should be noted that a similar approach was followed in other studies using SBRs to explore the biodegradation potential of toxic organic chemicals such as 4-chlorophenol and 3-nitrobenzoic acid (Hu et al., 2005), and 4-nitrophenol (Tomei et al., 2004). During the start-up stage, both bioreactors achieved the expected level of performance evidenced by the complete biodegradation of glucose, stable biomass (expressed as VSS) content, and neutral pH conditions.

Following the start-up stage, 20 mg/L isoproturon was added to the feed to each SBR on operation day 21 (stage I-1, Table 1). Although glucose was completely consumed within each daily cycle, no isoproturon degradation was detected in either system during the 91 d of operation at this stage. Since one of the triggering mechanisms for the microbial degradation of a xenobiotic compound is the “toxic pressure” that the pollutant exerts on the biomass to induce subsequent enzymatic modifications (Pandey and Jain, 2002; Singh and Ward, 2004), it was decided to increase the isoproturon concentration in the feed to 50 mg/L to improve the percentage of “herbicide carbon” available, from 3% to 8%. Operation at the higher isoproturon concentration (stage I-2) for 40 d revealed no measurable degradation of the herbicide in either SBR, despite complete utilization of glucose and the lack of any signs of process instability or inhibitory effects. Another study has also reported that no isoproturon biodegradation (at a feed concentration of 20 mg/L) was observed in anaerobic batch reactors treating primary sludge, despite the simultaneous complete utilization of 2,4-D at concentrations up to 300 mg/L (Li et al., 2005).

The inability of biomass to degrade isoproturon could be attributed to the chemical behavior of the herbicide (i.e. low solubility in water, approximately 60 mg/L at 20°C), which
limits it bioavailability (Cooke et al., 2004). It has been also postulated that nitrogen-containing organic compounds, such as isoproturon, may not biodegrade easily in systems with high nitrogen availability in the form of nutrient salts (Perrin-Ganier et al., 2001; Singh and Ward, 2004). Since both synthetic feeds contained a large amount of ammonium salts (i.e. 325 mg/L ammonium sulfate in SBR1; 2200 mg/L ammonium bromide in SBR2), this may have also contributed to the lack of isoproturon biodegradation.

The addition of 100 mg/L of 2,4-D on day 152 of operation marked the onset of stage II-1 in both systems. After exposure to 2,4-D for approximately 3 weeks (i.e. days of operation 152–173), SBR1 exhibited the first signs of biodegradation. Removal of 2,4-D improved steadily until complete degradation (i.e. <1.0 mg/L in the effluent based on the detection limit) was accomplished several days later (Fig. 2). On the other hand, the first evidence of 2,4-D biodegradation in SBR2 was detected 46 days after the addition of the pesticide (day of operation 197). In a fashion similar to that observed under aerobic conditions, the percent removal pattern increased gradually, albeit at a much slower rate, until complete removal was achieved approximately 1 month later (Fig. 3). It is worth mentioning that a remarkable improvement in 2,4-D removal (from 44% to 93%) was achieved within 2 d (i.e. between days of operation 220 and 222). Moreover, no isoproturon degradation was observed during this stage as well.

It is apparent that a substantially longer acclimation period (i.e. approximately 70 d) was necessary for the anaerobic biomass to accomplish complete biodegradation of 2,4-D compared to the aerobic one (i.e. approximately 30 d), which can be mainly attributed to the slower metabolism and the specialized functions (e.g. acidogens vs. methanogens) of the anaerobic consortia (Mohan et al., 2005). It has been reported that previous exposure of the biomass to a xenobiotic compound may reduce the corresponding acclimation period and accelerate the biodegradation rate of the compound in the system (Sinton et al., 1986). For instance, other investigations on 2,4-D biodegradation employing similar treatment systems without previous exposure of the biomass to the herbicide have reported longer acclimation periods ranging between 45 and 80 d under aerobic conditions (Orhon et al., 1989; Mangat and Elefniotis, 1999) and approximately 100 d under anaerobic conditions (Chin et al., 2005). It is apparent that the mixture of “pre-exposed” biomass and fresh sludge used in this study (see Section 2.2) reduced the acclimation period required for 2,4-D biodegradation by a remarkable margin in both bioreactors.

### 3.2. Effect of 2,4-D concentration

Following the complete removal of 100 mg/L of 2,4-D in both systems during stage II-1, the biodegradation potential of each SBR was explored through incremental changes in the concentration of the herbicide in the feed. In this respect, three additional concentrations of 2,4-D were applied to SBR1 (i.e. 300, 500, and 700 mg/L; stages II-2 to II-4) and one to SBR2 (i.e. 300 mg/L; stage II-2) (Table 1).

The aerobic reactor exhibited complete 2,4-D removal at feed concentrations of 300 and 500 mg/L. It is of note that this was achieved within the first 24-h cycle after each change, indicating that there was no need for biomass “adaptation” to the higher concentration applied. In contrast, an increase in 2,4-D from 500 to 700 mg/L resulted in no herbicide degradation and only partial (approximately 70%) glucose utilization (stage II-4, Fig. 4). To further elucidate bioreactor behavior, the specific consumption rates for glucose and 2,4-D were calculated from the corresponding track studies and the results have been summarized in Table 2. Taking into account the stability of reactor VSS indicated by the low standard deviation values (less than 10% of the mean), variations in the specific rates can be mainly attributed to biomass performance. In SBR1, an increase in 2,4-D concentration within the 100–500 mg/L range resulted in a remarkable decrease in the glucose consumption rate, which denotes an adverse effect on the glucose degradation pattern, despite the complete utilization of both carbon substrates. The 2,4-D specific rate...
increased gradually with an increase in concentration up to 500 mg/L, which reflects the ability of the biomass to degrade the herbicide. However, at 700 mg/L, the 2,4-D consumption rate was negligible and the glucose rate was drastically reduced due to the inhibitory effect of the herbicide.

In the anaerobic SBR, an increase in 2,4-D concentration from 100 to 300 mg/L resulted in partial degradation of the herbicide, averaging approximately 40% (stage II-2, Fig. 5). In a fashion similar to that observed in the aerobic system, the glucose consumption rate was reduced at the higher 2,4-D concentration applied (Table 2). The 2,4-D specific rate, however, appeared to have reached a plateau. The representative examples of the utilization profiles illustrated in Fig. 4 (SBR1) and Fig. 5 (SBR2) indicate that glucose was the preferred substrate since it was consumed completely before the onset of 2,4-D degradation, which implies a sequential utilization pattern. This pattern, also known as “diauxic growth”, has been observed in systems treating a potentially toxic substrate in the presence of a readily biodegradable one (Adour et al., 2005; Chin et al., 2005). This is due to the repression of enzymes that degrade a less rapidly metabolized carbon source when a more rapidly metabolized one is available (Chang and Alvarez-Cohen, 1995).

Furthermore, it was observed that VFAs (i.e. acetic, propionic, n-butyric, and n-valeric acids) were generated in SBR 2, with acetic and propionic acids being the predominant ones and accounting together for over 90% of the total amount. The VFA speciation pattern obtained in this study is typical of wastewaters high in carbohydrate and low in protein content (Maharaj and Elefsiniotis, 2001; Oktem et al., 2006). Under stable operating conditions (i.e. stage II-1), the VFAs were completely consumed within the first 8h of the reaction period, indicating a well-balanced acidogenic–methanogenic activity in the system (Fig. 6). However, when the 2,4-D concentration increased to 300 mg/L (stage II-2), a slight increase in total VFA concentration and a remarkable delay in VFA consumption became apparent. This provides additional evidence of the decline in the overall performance of the anaerobic bioreactor at the highest 2,4-D concentration applied.

Finally, a TOC mass balance revealed that the effluent of the aerobic SBR in all stages, expect the last one (stage II-4), contained less than 10 mg/L of “residual” TOC (i.e. excluding the contribution of the isoproturon, which was not degraded). This indicates that there were practically no metabolic intermediate products accumulating in the bioreactor. Even during stage II-4, when the system was underperforming, the combined glucose, 2,4-D, and isoproturon concentrations accounted for 95% of the effluent TOC, which suggests that the inhibitory effect observed cannot be attributed to the accumulation of potentially toxic metabolites. Regarding the anaerobic SBR, the residual TOC concentration in the effluent fluctuated between 40 and 70 mg/L, throughout the study, showing no apparent trend. In addition, no VFAs were detected at the end of each cycle, and therefore it is possible that the residual TOC was due to the presence of other intermediate products of anaerobic metabolism. However, since there was no apparent increase in the residual effluent TOC concentration during stage II-2, it is unlikely that intermediate metabolites have played an important role with respect to the reduced performance of the system.

### 3.3. Kinetics of glucose and 2,4-D biodegradation

The kinetic rate constants associated with glucose and 2,4-D utilization were calculated based on a number of track studies, and the results have been summarized in Table 3. Overall, biodegradation of glucose and 2,4-D, under either aerobic or anaerobic conditions, can be closely approximated by first-order kinetics, indicated by the relatively small standard deviation values calculated for each run, leading to an average coefficient of variance of 14% (glucose) and 19% (2,4-D). It is evident that the kinetic rates of glucose and 2,4-D in the aerobic SBR remained practically unchanged during stages II-1 and II-2 (i.e. 2,4-D feed concentration up to 300 mg/L). A further increase in the herbicide concentration resulted in a sharp decline in the corresponding rates, with minimal values obtained during the last stage. Similarly, both rates in the anaerobic SBR were drastically reduced at the higher herbicide concentration applied (stage II-2). In summary, the kinetic rate trends observed also reflect the inhibitory effect of 2,4-D at higher concentrations on reactor performance, as mentioned previously.

The microbial consumption of glucose (either as a single carbon source or as a co-substrate) has been generally reported to follow a first-order kinetic model (Das et al., 2002; Jianlong et al., 2002; Chin et al., 2005). On the other
hand, biodegradation of xenobiotic chemicals has been linked to a number of different kinetic models including zero-order, first-order, second-order, and non-exponential models (Četkauskaitė et al., 1998; López et al., 2007; Ziagova and Liakopoulou-Kyriakides, 2007). It is also interesting to note that the utilization of 2,4-D (as well as that of other related chlorinated compounds) appears to follow a first-order reaction in several occasions (Soulas and Lagacherie, 2001; Atuanya and Chakrabarti, 2004).

### 4. Conclusions

This study revealed that following biomass acclimation, 2,4-D biodegradation was accomplished under both aerobic and anaerobic conditions. However, isoproturon removal was not detected in either SBR throughout the experiment. In the aerobic system, complete 2,4-D utilization was observed at feed concentrations up to 500 mg/L, while a further increase to 700 mg/L resulted in no herbicide removal. In contrast, the anaerobic SBR was able to degrade approximately 120 mg/L of 2,4-D (i.e. 40% of the maximum concentration applied). Glucose, serving as a supplemental carbon substrate, was readily consumed in a sequential utilization pattern with respect to 2,4-D. Overall, SBR technology proved to be a promising strategy for the treatment of herbicide-contaminated wastewater.

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