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Analysis of glyphosate degradation in a soil microcosm☆

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ABSTRACT

Glyphosate (GLP) herbicide leaching into soil can undergo abiotic degradation and two enzymatic oxidative or hydrolytic reactions in both aerobic and anaerobic conditions; biotic oxidation produces aminomethylphosphonic acid (AMPA). Both GLP and AMPA are phytotoxic. A comprehensive GLP degradation reaction network was developed from the literature to account for the above pathways, and fifteen experimental data sets were used to determine the corresponding Michaelis-Menten-Monod (MMM) kinetic parameters. Various sensitivity analyses were designed to assess GLP and AMPA degradation potential against O_2 (aq) and carbon (C) availability, pH, and birnessite mineral content, and showed that bacteria oxidized or hydrolyzed up to 98% of GLP and only 9% of AMPA. Lack of a C source limited the GLP cometabolic hydrolytic pathways, which produces non-toxic byproducts and promotes AMPA biodegradation. Low bacterial activity in O_2 (aq)-limited conditions or non-neutral pH resulted in GLP accumulation. Birnessite mineral catalyzed fast GLP and AMPA chemodegradation reaching alone efficiencies of 79% and 88%, respectively, regardless of the other variables and produced non-toxic byproducts. Overall, O_2 (aq) and birnessite availability played the major roles in determining the partitioning of GLP and its byproducts mass fluxes across the reaction network, while birnessite, C availability, and pH affected GLP and AMPA biodegradation effectiveness.

1. Introduction

Glyphosate (GLP) is the herbicide most globally used as for 2014, with 747 and 79 millions of applied kilograms in agricultural and non-agricultural soils, respectively (Benbrook, 2016). Likely, the commercial success of this herbicide lies in its high efficacy as a broad-spectrum systemic herbicide (Heap, 2017), its presumed safety to humans (APVMA, 2016; EFSA, 2015; EPA, 2016), and the production of genetically modified GLP-tolerant crops such as cotton, soybean, alfaalfa, corn, sugarbeets, and canola (Monsanto Company, 2017).

The current understanding of GLP degradation in the environment, and particularly in soil and surface waters, is relatively well founded. GLP biodegradation was initially observed in a GLP waste stream (Jacob et al., 1988), in industrial activated sludge (Balthazor and Hallas, 1986; Jacob et al., 1988), and in contaminated soils (Rueppel et al., 1977). Biological degradation has been reported and investigated in a number of studies, which have identified two major pathways for GLP breakdown. In one pathway, the carbon-phosphorus (C-P) lyase enzyme, which is relatively widespread amongst bacteria (Hove-Jensen et al., 2014), releases sarcosine (SRC) and phosphate (PO_4^{3-}). PO_4^{3-} is next used by bacteria to meet their metabolic requirements (Balthazor and Hallas, 1986), while excess PO_4^{3-} inhibits this pathway (Pipke and Amrhein, 1988b). In the second pathway, an oxidase breaks down the carbon-nitrogen (C-N) bond in GLP, and produces aminomethylphosphonic acid (AMPA), which conserves the C-P bond, and glyoxylate (GLX), which can be used by bacteria as a C source (Levering et al., 1984).

Chemical degradation appears to be weak against C-P bond (Moore et al., 1983), but recent studies have suggested that birnessite mineral-mediated GLP chemical degradation can be faster than biological degradation (Li et al., 2015; Paudel et al., 2015). Experimental tests have reported different levels of pesticides biodegradation rates depending on environmental factors, the most important of which being O_2(aq) availability (Shaler and Klecka, 1986; Nair and Schnoor, 1994; Stucki et al., 1995). A strain of the genus *Pseudomonas* has been reported to biodegrade GLP either to SRC or AMPA depending on P requirements (Jacob et al., 1988), but it is expected that also other genera possess a similar regulatory capability. O_2(aq) is required for GLP biodegradation

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along both pathways; therefore, anaerobic conditions can switch off GLP break down. Also P inhibition on aerobic hydrolysis may act as a switch favoring aerobic oxidation, and yet $O_2(aq)$ variability may allow only partial aerobic degradation. Laboratory tests showed that GLP can be biodegraded rapidly also by microorganisms never exposed to this xenobiotic (Sviridov et al., 2015), often in a cometabolic reaction consuming an additional C source. Additionally, bacteria capable to scavenge P from AMPA have shown enhanced GLP biodegradation to AMPA (Hove-Jensen et al., 2014). However, AMPA biodegradation generally occurs at a very low rate, likely because AMPA has to be acetylated before P can be scavenged (Hove-Jensen et al., 2014). After long exposures to GLP, bacteria have been shown to adapt to use GLP as a C and N source simultaneously (Sviridov et al., 2012). GLP and its catabolic intermediates can potentially be completely decomposed to end products useful to microorganisms (Wang et al., 2016). However, unfavorable pH levels can slow down microbial activity resulting in a lower GLP biodegradation efficiency (Carlisle and Trevors, 1988) and lengthening GLP persistence in the environment.

This work aims to: (1) introduce a detailed GLP soil degradation reaction network that integrates the most recent degradation pathways and provide the corresponding set of Michaelis-Menten-Monod (MMM) kinetic parameters for specific microbial strains; and (2) elucidate and quantify the combined effect of aqueous $O_2(aq)$ concentration, C source, pH, and birnessite availability on GLP degradation, and the fluxes along each pathway of the reaction network. To these aims, laboratory experiments surveyed from the literature were used in task (1). The sensitivity of the GLP degradation reaction network and emergence of response patterns were tested in task (2). The analyses were carried out using the general purpose multi-component bioreactive numerical model BRTSim (based on Maggi, 2015), but the developed reaction network and its corresponding MMM kinetic parameters can be easily integrated in other mechanistic models (e.g., TOUGHREACT (Xu et al., 2011), MODFLOW-PHT3D (Prommer et al., 2001), and HYDRUS (Yu and Zheng, 2010)) to predict GLP and its metabolites dynamics in soil, and can support best pesticide management practices.

2. Methods

2.1. Integrated GLP degradation pathways

In soil, GLP degradation involves biotic and abiotic processes. The former are mediated by three microbial functional groups along two pathways, P1 and P2, which are characterized by oxidative reactions, either cometabolic or not, and cometabolic hydrolysis reactions, respectively (Fig. 1). The latter are oxidations catalyzed by Mn ions contained in minerals such as birnessite. The two biotic pathways can be concurrent or not depending on C and P availability, and include intermediate reactions that have been identified only recently and are described below. The end products of GLP aerobic degradation are CO_2, formaldehyde CH_2O, and NH_3, while acetate and CH_4 are end products in anaerobic conditions. Integration and accounting of all chemical and biological species and corresponding reactions in the network of Fig. 1 are described in detail below, while the extended list of biochemical reactions within the network are available in the Supporting Information.

Pathway P1. starts with GLP aerobic oxidation to AMPA and GLX mediated by Agrobacterium radiobacter and Achromobacter Group V D (Mcauliffe et al., 1990) (pathway P1R1, Fig. 1); the same reaction is mediated by Flavobacterium sp. GD1 (Balthazor and Hallas, 1986), Ochrobactrum anthropi GPK 3 (Sviridov et al., 2012), and Pseudomonas sp. LBr (Jacob et al., 1988) in the presence of an additional C source as co-substrate (pathway P1R1s, Fig. 1). While GLX is part of metabolic pathways within the cell (Levering et al., 1981; Mcauliffe et al., 1990; Jacob et al., 1988), AMPA can be hydrolyzed to methylamine (MTH), PO_4^{3-}, and H^+ in aerobic conditions by *Arthrobacter atrocyaneus* ATCC 13752 (Pipke and Amrhein, 1988a) and *Flavobacterium* sp. GD1 (Balthazor and Hallas, 1986) (pathway P1R2s, Fig. 1). Balthazor and Hallas (1986) and Talbot et al. (1984) proposed an alternative biodegradation pathway for AMPA in the presence of pyridoxal phosphate and pyruvate to phosphonoformaldehyde and alanine; this reaction was observed in *Ochrobactrum anthropi* GPK 3, and the same bacteria also hydrolyzed phosphonoformaldehyde to formaldehyde, PO_4^{3-}, and H^+ (Sviridov et al., 2012). Despite this alternative pathway for AMPA was shown to occur in laboratory conditions, its occurrence in field is uncertain and was not explicitly accounted for in our analytical work. Alternatively, AMPA can adsorb onto the birnessite mineral surface $((Na_{0.3}Ca_{0.1}K_{0.1})(Mn^{3+}, Mn^{4+})_2O_4 \cdot 1.5H_2O)$ and be chemically oxidized by Mn ions to MTH, PO_4^{3-}, and H^+ (Li et al., 2015) (pathway P1R2c, Fig. 1). MTH can be either oxidized by *Arthrobacter* P1 (Levering et al., 1984) to CH_2O and NH_3 in aerobic conditions, or hydrolyzed aerobically by *Methanosarcina barkeri* (Hippe et al., 1979) to CO_2, CH_4, and NH_3 (pathway P1R3a and P1R3b, respectively, Fig. 1). Note that CH_2O was considered to be a model C source to all microbial functional groups while a number of organic compounds may have an equivalent function (Levering et al., 1981).

Pathway P2. starts with GLP aerobic cometabolic hydrolysis to SRC, PO_4^{3-}, and H^+ mediated by *Achromobacter* sp. MPS 12A (Sviridov et al., 2012), *Arthrobacter* sp. GLP-1 (Pipke et al., 1987), *Arthrobacter atrocyaneus* ATCC 13752 (Pipke and Amrhein, 1988a), *Arthrobacter* sp. GLP-1/Nit-1 (Pipke and Amrhein, 1988b), *Pseudomonas* PG2982 (Moore et al., 1983), and *Streptomycete* StC (Obojska et al., 1999) (pathway P2R1s, Fig. 1). Similarly to AMPA, also GLP can undergo chemical degradation after adsorption onto birnessite mineral surface and, depending on the GLP-to-birnessite mass fraction ratio, different byproducts can be produced in varying fractions. Here, it was assumed that SRC, PO_4^{3-}, and H^+ were the only byproducts along this pathway, but also AMPA and other uncharacterized phosphate-containing chemicals were found in small amounts (Li et al., 2015) (pathway P2R1c, Fig. 1). Next, SRC can be oxidized either to glycine (GLY) and formaldehyde by *Pseudomonas Ovalis* in aerobic conditions (Appleyard and Woods, 1956), or to MTH, CO_2, and acetate by *Eubacterium acidaminophilum* using formate as the e^- donor in anaerobic conditions (Hormann and Andreesen, 1989) (pathway P2R2a and P2R2b, respectively, Fig. 1). Also in this case, acetate was converted into an equivalent number of CH_2O moles, and the overall reaction was rewritten to account for the use of CH_2O as a model C source in place of formate. MTH can be either oxidized or hydrolyzed via P1R3a in aerobic conditions, or in anaerobic conditions via P1R3b; GLY can be aerobically oxidized to CO_2, NH_3, and H_2O by *Pseudomonas Ovalis* (Appleyard and Woods, 1956), or anaerobically hydrolyzed to acetate and NH_3 by *Clostridium purinolyticum* (Därre and Andreesen, 1982) (pathways P2R3a and P2R3b, respectively, Fig. 1).

Three microbial functional groups were identified in the reaction network of Fig. 1. GLP and AMPA biodegraders were named B_{HYO} GLP Hydrolyzer and Oxidizer bacteria, while the MTH, SRC, and GLY byproducts were metabolized by B_{AER} Aerobic heterothropic bacteria and B_{ANAER} Anaerobic heterothropic bacteria depending on microorganisms tolerance towards $O_2(aq)$ levels. Both GLP oxidation and hydrolysis, as well as AMPA hydrolysis, were modeled as cometabolic reactions (Balthazor and Hallas, 1986; Jacob et al., 1988; Moore et al., 1983), and an additional oxidative reaction independent from CH_2O was implemented (Mcauliffe et al., 1990) to accurately represent experimental results (See SI 0.1).

The two pathways summarized above, including sources,