Removal of dissolved glyphosate from waters by adsorption under the influence of metals

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SINGAPORE

**REMOVAL OF DISSOLVED GLYPHOSATE FROM WATERS BY
ADSORPTION UNDER THE INFLUENCE OF METALS**

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Interdisciplinary Graduate School

Nanyang Environmental and Water Research Institute

**A thesis submitted to the Nanyang Technological University in partial
fulfilment of the requirement for the degree of
Doctor of Philosophy**

2019

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarized materials, and has not been submitted for a higher degree to any other University or Institution.

.....19 / 08 / 2019.....

Date

....

H. M. G. A. D. HERATH

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

31/07/2019
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Date

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PROFESSOR NG WUN JERN

Authorship Attribution Statement

This thesis contains material from 3 papers published in the following peer-reviewed journal(s) where I was the first author.

Chapter 4 is published as G. A. D. Herath, L. S. Poh, and W. J. Ng, "Statistical optimization of glyphosate adsorption by biochar and activated carbon with response surface methodology," *Chemosphere*, 2019. DOI: 10.1016/j.chemosphere.2019.04.078

The contributions of the co-authors are as follows:

- Professor Ng Wun Jern provided the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts. The manuscript was revised by Dr Dave Poh.
- I co-designed the study with Professor Ng Wun Jern and performed all the laboratory work at Residue, Resource and Reclamation lab in Nanyang Environmental and Water Research Centre. I analysed the data.
- All microscopy, including sample preparation, was conducted by me in the facility for analysis, characterization, testing and simulation.
- Professor Ng Wun Jern provide guidance in the interpretation of the measured data.

Chapter 6 is submitted as G. A. D. Herath and W. J. Ng, "Decomposition of glyphosate under the influence of iron loaded wood biochar," *Chemical Engineering Journal*, 2020.

The contributions of the co-authors are as follows:

- Professor Ng Wun Jern provided the initial project direction and edited the manuscript drafts.
- I prepared the manuscript drafts.
- I co-designed the study with Professor Ng Wun Jern and performed all the laboratory work at Residue, Resource and Reclamation lab in Nanyang Environmental and Water Research Centre. I analysed the data.
- All microscopy, including sample preparation, was conducted by me in the facility for analysis, characterization, testing and simulation.

- Professor Ng Wun Jern provide guidance in the interpretation of the measured data.

Chapter 7 is published as G. A. D. Herath and W. J. Ng, “Copper induced abiotic decomposition of glyphosate in aqueous solutions,” Chemosphere, 2020.

The contributions of the co-authors are as follows:

- Professor Ng Wun Jern provided the initial project direction and edited the manuscript drafts.
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H. M. G. A. D. HERATH

Summary

Glyphosate, which is founded in 1974 is the most widely used weed control product in the world. The International Agency for Research on Cancer (IARC) declared glyphosate as “probably carcinogenic” to humans in 2015. Research on potential toxicity has gained momentum because of the new findings revealing higher toxicity of glyphosate than originally reported. As a result, public perception that this was a rather benign herbicide is beginning to erode. Due to its toxicity, detection of glyphosate and finding effective methods to control the glyphosate concentration levels in surface waters in agricultural areas where the herbicide is used extensively became an immediate concern. Chronic kidney disease associated with glyphosate has been reported in agricultural areas such as Central province in Sri Lanka, sub-regions of El Salvador, Nicaragua, Costa Rica, and Andra Pradesh of India. Researches have reported the detection of glyphosate and heavy metals cations in the drinking waters of these areas. According to previous studies, glyphosate tends to form soluble or insoluble complexes with the metal cations in waters containing heavy metal cations such as iron, zinc, copper and magnesium. This thesis work discusses the removal of dissolved glyphosate from surface waters by adsorption using activated carbon and biochar under the influence of metals, namely ferrous, copper and zinc. During the adsorption experiments, glyphosate is degraded when in contact with Fe^{2+} and Cu/Cu^+ ions. By-products such as amino-methyl-phosphonic acid (AMPA), which are more persistent and more toxic than glyphosate are formed by the degradation of glyphosate. Findings emphasize the importance of exploration of glyphosate heterogeneous degradation as glyphosate removal techniques such as adsorption can impair the water quality due to the formation of toxic by-products.

The study is divided into two main parts. Firstly, commonly used adsorbent types are analysed focusing on the effect of surface area and pore distribution on the removal of glyphosate from surface waters. In this study, four coconut shell activated carbons, two wood activated carbons, and two porous polymers with different particle and pore sizes are tested. The finely powdered coconut shell based, and wood-based activated carbon types effectively remove the glyphosate. For coconut shell based activated carbon, the kinetics experiment data better fit with the pseudo-second-order model than the pseudo-first-order model; indicating chemisorption. Surface variations of activated carbon is analysed before and after glyphosate adsorption to analyse the effects of surface charge and ligands on glyphosate adsorption. Hydroxyl and carbonyl groups favour the removal of glyphosate from aqueous solution. Finely powdered coconut shell activated carbon is then compared with iron loaded wood biochar adsorbent for glyphosate removal from aqueous solution.

The adsorption data for the finely powdered coconut shell activated carbon and iron loaded wood biochar better fit the Freundlich isotherm model. The rate of glyphosate adsorption is found to follow the pseudo-second-order model. Box–Behnken design and percentage contribution with Pareto analysis techniques are used in surface response and efficiency calculations; modelling the process conditions and their effects. The pH of the solutions is regulated by buffering the solution during the adsorption process. Highest efficacy of glyphosate removal is obtained by optimizing parameters; operating pH, initial glyphosate concentration, temperature, adsorbent dose, and contact time. The conditions yielding the best removal for the aforementioned parameters are; *pH* 8.0, 0.2 mg/L, 50.0 °C, 11.4 g/L, 1.7 h for activated carbon, and *pH* 5.0, 0.7 mg/L, 50.0 °C, 12.3 g/L, 1.9 h for iron loaded wood biochar respectively. The maximum removal capacity and efficiency for the optimised conditions are; 0.0173 mg/g, 98.45% for activated carbon, and 0.0569 mg/g, 100.00% for biochar.

During the adsorption process with iron loaded wood biochar, with the reduction of dissolved glyphosate concentration, an increase in the AMPA concentrations in the samples is detected. Iron attached to the surface of wood biochar degraded glyphosate to form AMPA. Evidently, glyphosate is strongly adsorbed onto iron oxides, yet few studies focus on the following degradation. Therefore, as the second part of thesis, further studies are conducted to examine glyphosate decomposition in an aqueous medium when in contact with prevailing inorganic ions in agricultural areas, viz. ferrous, copper and zinc. Findings confirm; if there exist Fe^{2+} and Cu/Cu^+ ; it mediates the energy barriers opposing bond dissociation leaning towards C–N bond cleavage to form AMPA. Glyphosate degradation forming AMPA is a reduction reaction. Fe^{2+} and Cu/Cu^+ oxidise reducing glyphosate. Box–Behnken design and percentage contribution with Pareto analysis techniques are used respectively in surface response and efficiency calculations to determine the environmental conditions which induce glyphosate degradation with Fe^{2+} and Cu/Cu^+ . Parameters such as operating pH, initial glyphosate concentration, temperature, iron loaded biochar/ porous carbon dose, and contact time are investigated for their effect on the degradation efficiency. The conditions yielding the highest AMPA concentration are; *pH* 5.0, 50.0 mg/L, 50.0°C, 11.4 g/L, 24.0 h for iron loaded wood biochar, and *pH* 5.0, 35.9 mg/L, 50.0°C, 34.7 g/L, 24.0 h for copper respectively. At these conditions, for copper; 18.61% of degraded glyphosate is converted to AMPA and for iron loaded wood biochar; 26.1% of degraded glyphosate is converted to AMPA. Glyphosate is degraded into non-detectable levels when in contact for >24 h at the aforementioned optimised environmental conditions. The abiotic degradation pathway for glyphosate is studied. Porous carbon materials such as activated carbon and biochar catalyse the degradation. C–N and C–P bond cleavage can happen at the surface of the adsorbent, where the preference for cleavage rest on glyphosate and porous carbon material ratios. Intermediate identification on glyphosate adsorption reveal further reactions; a polymerisation of intermediates forming complex phosphonic acids (i.e. Bis-2-aminoethyl-aminomethyl phosphonic acid, Bis-phosphonomethyl-amino-phosphonomethyl-aminomethyl phosphonic acid) in addition to AMPA. Existence of zinc ions does not impact the degradation of glyphosate.

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Symbols and Abbreviations

°C	degree Celsius
®	registered trademark
™	trademark
µg	microgram
µL	microliter
µm	micrometre
AOPs	Advanced Oxidation Processes
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
AMPA	Amino-Methyl-Phosphonic Acid
ATR	Attenuated Total Reflection
BET	Brunauer-Emmett-Teller
Cd	Cadmium
CKDu	Chronic Kidney Disease (unknown)
cm	centimetre
CNS	Central Nervous System
cRfD	Chronic Reference Dose
DFT	Density Functional Theory
DR	Dubinín-Radushkevich
e.g.	for example
EPSPS	5-Enol-Pyruvoyl-Shikimate-3-Phosphate Synthase
et al.	and others
EXAFS	Extended X-ray Absorption Fine Structure
FE-SEM	field emission scanning electron microscope
FTIR	Fourier Transform Infrared Spectroscopy
g	gram
GAC	Granular Activated Carbon

GE-HT	Genetically Engineered- Herbicide Tolerant
GT	Glyphosate Tolerant
h	hour
HPLC	High Performance Liquid Chromatography
HT	Herbicide Tolerant
IUPAC	International Union of Pure and Applied Chemistry
K _d	Dissociation constant
LC/MS	Liquid Chromatography Mass Spectrometry
L	litre
m	meter
MAP	Mono Ammonium Phosphate
MF	microfiltration
mg	milligram
min	minute
mL	millimetre
mm	millimetre
NA	not applicable
NER	Non-Extractable Residues
NF	nanofiltration
nm	nanometre
NOM	Natural Organic Compounds
PAC	Powdered Activated Carbon
pH	potential of hydrogen ($-\log [\text{H}^+]$)
pK _a	acid dissociation constant ($-\log K_a$)
R ²	coefficient of determination
rpm	revolutions per minute
SEM	Scanning Electron Microscope
TR	Total Reflection
UV-Vis	Ultraviolet-Visible
WWTP	Wastewater Treatment Plant

List of Publications

Peer reviewed journals

- G. A. D. Herath, L. S. Poh, and W. J. Ng, "Statistical optimization of glyphosate adsorption by biochar and activated carbon with response surface methodology," Chemosphere, 2019
- G. A. D. Herath and W. J. Ng, "Copper induced abiotic decomposition of glyphosate in aqueous solutions," Chemosphere, 2020 (Under Review)
- G. A. D. Herath and W. J. Ng, "Decomposition of glyphosate under the influence of iron loaded wood biochar," Chemical Engineering Journal, 2020 (Under Review)

Conference papers with oral presentations

- G. A. D. Herath, and W. J. Ng, "Sorption and decomposition of glyphosate under the influence of Iron inhibited biochar" in 10th IWA Symposium on Waste Management Problems in Agro-Industries, Rhodes, Greece, 2019
- G. A. D. Herath, and W. J. Ng, "Decomposition of glyphosate in aqueous solutions under the influence of Copper and Zinc metals" in 10th IWA Symposium on Waste Management Problems in Agro-Industries, Rhodes, Greece, 2019
- G. A. D. Herath, R. D. Webster and W. J. Ng, "Removal of glyphosate using commercial grade biochar and activated carbon: Statistical optimization of adsorption parameters using response surface methodology," in Environmental Science and Technology, Houston, USA, P190-202. 2018 ISBN 978-1-5323-2264-8

CHAPTER 1

INTRODUCTION

1.1 Background

Glyphosate (N-phosphonomethyl-glycine) is a nonselective broad-spectrum systemic post-emergence organophosphate herbicide [1]. In 1974 glyphosate was identified for its herbicidal activity. For two decades, the volume sold was limited because glyphosate was only used in agricultural and non-agricultural areas when land managers wanted to kill all vegetation or to control late-season weeds [2]. In 1996, genetically engineered-herbicide tolerant (GE- HT) soybean, maize, and cotton varieties were approved for planting, this technological breakthrough dramatically increased the application of herbicide. Since 1974, the appraised global use of glyphosate is nearly 8.6 billion kilograms [3]. Approximately 90% of global glyphosate usage has been used after the introduction of glyphosate tolerant crops in 1996 [3]. More specifically, 56% of global usage of glyphosate is accountable for genetically developed glyphosate tolerant crops which two third of the volume has been consumed in America [3]. However, the application of glyphosate has increased by 15 million kilograms in the past years [4]. Considering this amounts as a whole, no herbicide or pesticide has achieved to such a rigorous and extensive spread in use [5].

Glyphosate is considered as the most commonly available and most widely used herbicide exist [6]. Glyphosate was previously considered to be a safe, environmentally friendly herbicide because of its rapid adsorption onto soil particles. Usually, glyphosate residues in agricultural soils are mobilised off-site by wind and water-erosion than by runoff or leaching [7]. But studies have found that glyphosate may become portable by water in soils with high phosphate levels. Since, glyphosate and inorganic phosphate have displayed to compete for the same active sites [8] when adsorbing to the soil, excessive amount of phosphorus applications can impact the bioavailability and transport potential of the

herbicide [9]. Plant material containing glyphosate is reverted to the soil during the vegetal cycle (via leaf senescence) and after harvest (as crop residues). The glyphosate herbicide in plant residue involves a lag in mineralization resulting in increase in half-life almost four-fold and the fraction of non-extractable residues increases compared to that of directly applied. These factors increase the amount of glyphosate concentration in the soil and in waters.

With multiple applications and with increase in volumes, intensity of levels of glyphosate and its primary metabolite amino-methyl-phosphonic acid (AMPA) [10] in the air [11], soil [12], surface water [13, 14] and ground waters [15, 16] has heightened at rising levels [17, 18]. According to Food Standards Agency, United Kingdom [19], from 2007– 2013 glyphosate residues have been found in 10–30 % of grain-based samples in considerable amounts. A study carried out in Argentina on soybean crops has revealed that glyphosate concentrations detected in crop residues and soils are in the ranges of 0.5 and 5.0 mg/kg, and in waters in the ranges of 0.10 to 0.70 mg/L [13]. Glyphosate concentrations detected in faster moving, more rapidly diluting water bodies, are generally lower approximately 10–15 µg/L [20–22] whereas higher glyphosate concentrations levels have been recorded in stagnant water bodies, such as wetlands or isolated ponds, [23] due to less dilution and dispersion.

Studies have reported it is toxic to humans at concentrations below levels applied in agriculture [24] and that there is a probable risk to the cardiac and respiratory systems and of contact dermatitis and eczema [25]. The International Agency for Research on Cancer (IARC) which is a part of World Health Organisation (WHO) has classified glyphosate as a “probable human carcinogen” due to its mutagenic, genotoxic, and carcinogenic effects [26]. To overcome the health risks associated with the utilization of glyphosate in agriculture, the removal of herbicide from soil and/or water is required.

Studies concerning glyphosate removal revealed that existing conventional processes in the traditional waste water treatment plant such as flocculation, coagulation and sedimentation are not entirely efficient because of the small size of the organic pollutant and low concentration levels in source water [27]. Studies has been conducted on the biodegradation of glyphosate [28], using algae [29, 30] and microorganisms [31]. On the other hand, a variety of developed technologies such as advance oxidation processes [32], coagulation

[33, 34], membrane technology [35], and adsorption [36] have been demonstrated more effective for the abatement of glyphosate from the aqueous phase. Adsorption has significant advantages over other methods because it is simple, environment friendly, and cost effective. Activated carbon [29], biochar [30], hydro char [31], carbon nanotubes [37], and hierarchically structured carbon [38] are some carbon-based materials extensively studied for their adsorption efficiency.

Adsorption was evidenced to be effective for removal of dissolved glyphosate in previous studies [39, 40]. Adsorption is often considered over other removal technologies for a number of reasons, viz. the process is adaptable to different applications, relatively selective for dissolved glyphosate, and sorbents are regarded as potentially non-hazardous to handle [41]. This makes adsorption an effective technique in glyphosate removal applications; coupled with the possibility of regenerating spent adsorbents economical [42]. Suitable sorbents can be found among the biochar and activated carbons available in the market. In terms of material cost, biochar is potentially more economical than activated carbon. Among the activated carbons, there can be powdered (PAC) and granular (GAC) activated carbons [43]. Activated carbons are, however, associated with high energy requirements during manufacture and are relatively more expensive. Biochar, a by-product of the pyrolysis process, typically costs less. The latter is further enhanced by biochar's source material – e.g. paper sludge [44], wood waste [36] and industrial residues [25].

Nowadays heavy metals are among the most important pollutants in source and treated water. Industrial uses of metals and other domestic processes (e.g. burning of fossil fuels, incineration of wastes, automobile exhausts, smelting processes and the use of sewage sludge as landfill material and fertiliser) have introduced substantial amounts of potentially toxic heavy metals into the atmosphere and into the aquatic and terrestrial environments [45]. The heavy metal ions are stable and persistent environmental contaminants since they cannot be degraded and destroyed. While many of the heavy metals are needed by plants at the micronutrient level, higher concentrations are known to produce a range of toxic effects. The presence of copper, zinc, cadmium, lead, mercury, iron, nickel and others metals, has a potentially damaging effect on human physiology and other biological systems when the tolerance levels are exceeded [46].

Amongst all the treatment processes, adsorption using sorbents is one of the most popular and effective processes for the removal of heavy metals from wastewater. The adsorption process offers flexibility in design and operation and in many cases produces treated effluent suitable for re-use, free of colour and odour [47]. In addition, because adsorption is sometimes reversible, the regeneration of the adsorbent with resultant economy of operation may be possible [48]. The removal of heavy metal ions using low-cost abundantly available adsorbents: agricultural wastes such as tea waste and coffee [49], hazelnut shells [50], peanut hull [51], red fir [52] and maple [53], sawdusts [54], pinus bark [55], different bark samples [56], palm kernel husk [57], coconut husk [58], peanut skins [59], modified cellulosic materials [60], chemically modified cotton [61], corncobs [62] and modified corncob [63], rice hulls [64], apple wastes [65], coffee grounds [66], bark [67], modified bark, wool fibers [68], tea leaves [69], and wool, olive cake, pine needles, almond shells, cactus leaves, charcoal [70], modified lignin [71], banana and orange peels [72], modified sugar beet pulp [73], modified sunflower stalk, palm fruit bunch [74], maize leaf and different agricultural by-products [75] were used and investigated.

Negative charge at phosphonate group and positive charge at amino group due to the zwitterionic behaviour [76] of the glyphosate herbicide induce strong metal chelating abilities resulting in formation of metal complexes with heavy metals, in soil and water at pH higher than 6.5 [77]. When water contains cations such as iron, copper, zinc, calcium and magnesium, glyphosate forms metal complexes. The complexation reduces the herbicidal activity and increases the half-life of glyphosate. The adsorption of the herbicide to soil and plants is strongly swayed by cations attachment [78]. Previous studies have demonstrated the increased risk of kidney diseases by glyphosate-metal complex detected in surface waters [79]. Strong evidence is found in Central province in Sri Lanka [79, 80], in sub-regions of the Pacific coasts of the Central America (CA) [28, 81], Costa Rica, Nicaragua [82], countries of El Salvador [83, 84], and from Andhra Pradesh of India [32]. Hence, glyphosate-metal complexation is becoming a severe public health problem.

Evidently, adsorption is an effective technique in glyphosate and heavy metal removal applications separately, yet few studies focus on the following glyphosate-metal complexation during the glyphosate adsorption process.

1.2 Problem statement

Studies carried out during the past two decades indicate that glyphosate has strong complexing properties [85]. It has three functional groups (amine, carboxylate, and phosphonate) which may coordinate with metal ions. Depending on the pH of the reaction medium, i.e. the degree of deprotonation, glyphosate may be coordinated as a tridentate ligand through the amine group nitrogen, the carboxylate group oxygen, and the phosphonate group oxygen or as tetradentate ligand when the phosphonate group coordinates through two oxygen atoms [86, 87]. Complexes formed with divalent cations Zn^{2+} and Cu^{2+} are more stable than those formed with trivalent cations [88]. Among the cations studied there is a stability order from; for divalent cations $\text{Zn} > \text{Cu} > \text{Ca} > \text{Mg}$ and for trivalent cations $\text{Co} > \text{Fe} > \text{Cr} > \text{Al}$ [88].

With the emergence of glyphosate concentration levels and heavy metal concentration levels in surface and treated waters, many researches have conducted on the removal of these micro pollutants. Technologies capable of moving contaminants from one phase (e.g., water) into another (e.g., solid) have been widely reported in the removal of emerging contaminants. Hence, adsorption processes have been extensively applied for the removal of glyphosate [89-91] and heavy metals [45, 47]. It is noteworthy that glyphosate is exposed not only to soil metallic ions [92, 93] but also to metals in storage bins [94] as well as metallic nozzles and mineralized water when spraying.

However, despite their great importance, theoretical or experimental studies addressing the influence of metal ions on the removal of dissolved glyphosate from waters by adsorption are scarce. This has been identified as the research problem in this study. Lack of information on the effect of glyphosate-metal complexation on the glyphosate adsorption process hinders the transfer of existing operational knowledge to identify design consequences when implementing a successful treatment method to remove dissolved glyphosate. In recent research, metal oxides and activated carbon loaded with metal ions have been experimented to increase the uptake of glyphosate during adsorption. Therefore, it is important to have a deeper understanding on influence of metal ions on the dissolved glyphosate to amplify the use of technology for the removal of glyphosate.

1.3 Aim and objectives

In the view of above gaps identified, the overall aim of this study is to holistically examine dissolved glyphosate removal method using adsorption with biochar to eliminate glyphosate herbicide in drinking waters under the influence of metals to examine the impact of competitive adsorption caused by the metals. The specific objectives are listed as follows:

- To examine the adsorption of glyphosate qualitatively and quantitatively.
 - Study the specific role of surface characteristics such as pore distributions, surface area, surface charge, existing ligands, in glyphosate adsorption
 - Study the adsorption kinetics and isotherm study for the selected adsorbents for glyphosate adsorption
- To evaluate the effect of influencing operating parameters (i.e. operating pH, temperature, initial glyphosate concentration, adsorbent dosage, contact time) in glyphosate uptake using response surface methodology and to optimise the parameters for a better performance
- To examine the effect of attached ferrous metal in the porous carbon adsorbent on the glyphosate adsorption,
 - Study the effect of influencing external parameters (i.e. operating pH, temperature, initial glyphosate concentration, porous carbon dosage, contact time with carbon) in glyphosate adsorption using response surface methodology
- To study the influence of prevailing inorganic ions, namely copper and zinc in water on the glyphosate adsorption onto porous carbon adsorbent,
 - Study the effect of influencing external parameters (i.e. operating pH, temperature, initial glyphosate concentration, porous carbon dosage, contact time with carbon and metal ions) in glyphosate degradation using response surface methodology

1.4 Scope of this study

Limits to the study research are noted from the start. The research is conducted for initial glyphosate concentration range from 0.2 – 50 mg/L covering the glyphosate levels detected in different studies on industrial and surface waters.

The nature of the waters in different areas is driven by what goes into the water. A purely domestic effluent characteristic mirrors the cultural tastes of the region; high salt and oil and grease predominate the diet, then the same will be true for the effluent. Also, different treatment systems produce different effluent characteristics; some systems do better nitrogen removal, or phosphorus removal. To complicate matters, industrial and commercial users discharge different organic and inorganic chemicals, i.e. metals, chlorinated compounds, phenols, plasticizers, and metals. Besides contamination, impurities can also cause adsorption of analytes (i.e. the substances which are to be analysed in the sample) leading to analytical results below the true concentrations of the analytes in the wastewater. As most wastewaters are not at all sterile, it can be assumed that biochemical processes (which are desired in biological treatment stages) will not stop when the wastewater sample is transferred to a bottle; i.e. nitrification (oxidation of ammonia to nitrite or nitrate if still oxygen is dissolved in the sample resulting in too low ammonia and too high nitrite, or nitrate concentrations determined), denitrification (reduction of nitrate or nitrite to nitrogen gas resulting in too low nitrate or nitrite concentrations determined) or oxidation of organic wastewater constituents (resulting in errors of organic sum parameters analysed in the sample). Synthetic wastewater and surface water samples were formulated for use in the project to reduce the number of variables.

Raman technique and surface-enhanced Raman scattering (SERS) spectroscopy was first used for glyphosate detection in aqueous solutions by Costa et al. [95, 96]. They reported the analytical application of SERS in the trace analysis of glyphosate on silver nanocubes aqueous suspensions and assigned the experimental Raman and SERS bands of glyphosate. In more recent studies, Sharma et al. developed a method for fabrication of SERS substrate with regular dendritic silver nanostructures on carbon coated Ag-Cu-grids, which can be used for glyphosate detection [97]. According to the analysis report of Microbe Inotech Laboratories, requested by Moms Across America (MAAM), glyphosate was detected, by enzyme-linked immune specific assay (ELISA), at concentration of 0.107–2.671 ppb [98].

However, in this study mass spectrometry is preferred over ELISA and surface-enhanced Raman scattering spectroscopy. Since the 1980s, glyphosate residue determination has been conducted by chromatographic methods, including gas or liquid chromatography (GC/LC) combined with mass spectroscopy (MS) [99]. LC–MS/MS has a high sensitivity and accuracy [100]. LC–MS/MS analyses for detection of glyphosate in various fields have been previously reported [101]. Glyphosate is a highly polar herbicide containing an amino-phosphonate structure. Using this property, many mass analysis methods based on derivatisation have been developed [102, 103], but their drawback is that they are time-consuming and the procedures for conversion steps are difficult. While several direct procedures without derivatisation have been recently reported [104, 105], a method for glyphosate detection using appropriate and accurate analysis is not yet reported. Therefore, an analytical method for determination of glyphosate by LC–MS/MS was developed and validated. The developed methods have simple procedures for sample preparation without any conversion steps.

Glyphosate forms complexes with metals stability order from; for divalent cations $\text{Zn} > \text{Cu} > \text{Ca} > \text{Mg}$ and for trivalent cations $\text{Co} > \text{Fe} > \text{Cr} > \text{Al}$. Glyphosate is exposed not only to dissolved metallic ions in waste waters [92, 93] but also to metals in storage bins [94] as well as metallic nozzles and mineralized water when spraying. In recent research, metal oxides and activated carbon loaded with metal ions have been examined for its ability to increase the uptake of glyphosate during adsorption.

Among the trivalent cations, Fe^{3+} was the most widely available stable metal ion in the environment. Most of the metals in storage bins as well as metallic nozzles used in handling water are made with copper and iron. Meanwhile, various types of magnetic porous polyimides containing Fe_3O_4 cores [106-108] and iron loaded porous carbon materials [109] have been reported in recent years in the application of removal of contaminants in wastewater. Thus, iron loaded biochar was used on glyphosate adsorption to have a deeper understanding on influence of Fe^{2+} metal ions on the dissolved glyphosate to amplify the use of technology on the removal of glyphosate.

Copper pollution has become an emerging environmental problem in agricultural areas [110]. Sprays containing copper have been applied as fungicides over 100 years [111] in vegetable crops, pome and stone fruit orchards and vineyards [112]. For example, 1.25 -

7.50 kg/ha of copper oxychloride is applied per season in vineyards [112, 113]. However, foliar application of these copper-containing fungicides leads to a significant input of copper to the environment through drift and dripping off excess [114]. During the long-term application, copper accumulates and retains in waters and soil [115-117]. Copper not only affects the quality and quantity of crops; it can be threatening to the animal and human health [78, 118].

Figure 1.1 Scope of the overall study

On the other hand, zinc is a vital element in plant growth [119] and has been widely applied in the agricultural industry. However, excessive amounts of zinc can be toxic especially to the aquatic bodies. Most agricultural soils contain zinc in the range of 10–300 mg/kg [120]

which is higher than copper and iron concentrations. Evidently, copper and zinc form the most stable glyphosate-metal divalent complexes. Hence, the effect of copper and zinc cations on glyphosate adsorption onto porous carbon-based adsorbents is examined.

As shown in Figure 1.1, the scope of the whole study is mainly divided into two main sections; (1) adsorption behaviour of glyphosate with carbon-based adsorbents (2) the influence of prevailing inorganic ions, namely ferrous, copper and zinc in the glyphosate adsorption onto porous carbon-based adsorbents.

The results of this study could bring in a novel insight into the condition of drinking water in agricultural areas. More importantly, the results of this study could help obtain comprehensive knowledge; on the removal mechanism of glyphosate by adsorption in a complex water matrix and on the mechanism of glyphosate degradation by hybrid Fe/biochar system. The overall work to be conducted in this research will provide theoretical and practical guidance for drinking water process treatment design.

1.5 Research significance

One intended outcome of the study is to holistically examine the feasibility of glyphosate removal with biochar adsorption to eliminate herbicide in drinking water under the influence of metals to examine the impact of competitive adsorption caused by the metals. On a practical level, a second intended outcome of the study is to apply the knowledge gained during the study, in drinking water treatment process design. Especially in agricultural areas where the water contains Fe^{2+} , Cu/Cu^+ ions and glyphosate; implementation of a drinking water treatment solution with carbon adsorbents as a potentially low-cost, highly efficient and flexible solution. Further, a third intended outcome contributes to the determination of dissolved glyphosate concentration levels by LC–MS/MS. The method implemented in the study have simple procedures for sample preparation without any conversion steps and quantify four organic compounds; glyphosate (N-phosphonomethyl glycine), AMPA (Amino-methyl-phosphonic acid), glufosinate (2-Amino-4-hydroxymethyl-phosphonyl butanoic acid), glyphosine (N, N-bis-phosphonomethyl glycine). Finally, a further concern in the research are; (1) the study only investigated five operating conditions; initial glyphosate concentration, operating pH, temperature, metal ions/ biochar dosage and contact time on adsorption processes. Future attempts could include other operational factors, (2) Ferrous, copper and zinc metal cations

were used in this study to study their effects on the glyphosate adsorption process. However, various inorganic cations exist in wastewaters and surface waters which should be checked, (3) The study can be expanded to observe the influence of inorganic ions on glyphosate to provide a holistic picture regarding the fate and distribution of glyphosate in the environment and help to evaluate the retention and potential accumulation of these toxic compounds.

1.6 Thesis outline and chapter summary

The whole thesis can be divided into eight chapters, and the organization is as follows:

Chapter 1 describes the background of the study and gives a general overview on the research to provide a rationale regarding the need of this sort of study particularly highlighting the negative impacts of the emergence of glyphosate in plants, soils, waters and ultimately in human health.

A comprehensive literature review is presented in Chapter 2, pertaining to the findings in the following areas; the occurrence and harmfulness of glyphosate herbicide, traditional water treatment technologies used in glyphosate removal, especially highlighting advanced oxidation process in degradation and activated carbon in adsorption of glyphosate, suggesting a possible hybrid process for the treatment of glyphosate in waters. The framework of the thesis is also provided.

Chapter 3 provides the methods and materials which were employed in the study. Further, a detailed explanation of the experimental setup, analytical method and data analysing techniques are provided.

The results and the analysis of glyphosate adsorption onto coconut shell based activated carbon and wood-based biochar are presented from Chapter 4 to Chapter 5. Chapter 4 comprises with the effect of adsorbent surface characteristics in glyphosate adsorption. Different methods to improve the glyphosate removal; in particular, the enhancement of surface characteristics such as surface area, pore distribution, surface charge and the existence of surface ligands on glyphosate adsorption is studied. Chapter 5 presents a study conducted on the effect of adsorption process operating parameters in glyphosate adsorption. A comparison between the use of coconut shell based activated carbon and

ferrous loaded wood-based biochar in glyphosate removal and different factors affecting the removal of glyphosate are investigated to ascertain the optimised operation conditions for the adsorption process. Adsorption kinetics and isotherm studies have been conducted for a better understanding of the process.

Chapter 6 constitutes the analysis of glyphosate degradation due to attached ferrous in wood-based biochar. Liquid chromatography-mass spectrometry (LC/MS) technique is used to determine the degradation intermediates. Several conditions affecting the degradation is studied analysing the change in the concentration of by-products with the change in each condition. Chapter 7 summarise the extended analysis done in line with chapter 6 to understand the effect of other inorganic metal ions such as copper and zinc on dissolved glyphosate. A preliminary mechanism for glyphosate degradation process is investigated by adding different metal cations. The degradation pathways are built based on the intermediates detected and previous reports.

Finally, chapter 8 summarises the whole thesis work, concluding overall findings to provide recommendations for the future.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to glyphosate and occurrence of glyphosate in aquatic environment

2.1.1 History of glyphosate

In 1950, a Swiss chemist Dr Henri Martin discovered glyphosate [121]. After identified it's herbicidal activity, glyphosate was commercialized as Roundup [122] in 1974. Roundup® (Monsanto) and Touchdown® (TD; Syngenta) are the most common herbicides containing glyphosate [2].

For two decades, glyphosate's nonselective behaviour limited its application that it was only used to kill all the vegetation (e.g.; between the orchards and viticulture, at industrial yards such as train, pipeline, and powerline rights of way) or to control late-season weeds [5]. Genetically engineered-herbicide tolerant (GE- HT) soybean, maize, and cotton varieties [5] was introduced and approved for planting in the U.S in 1996 followed by alfalfa and sugar beets HT species in 2005 and 2008 respectively[5]. This technological breakthrough dramatically increased the application of glyphosate herbicide.

Figure 2.1 The structural formula of glyphosate

2.1.2 Chemistry behind glyphosate molecule

Glyphosate as shown in Figure 2.1, which is called N(phosphonomethyl) glycine or as 2-[(phosphonomethyl)amino] acetic acid is a broad-spectrum systemic herbicide and crop desiccant, which is nonselective, post emergence, and widely use in agriculture [123]. It belongs to chemical group of amino acids. Glyphosate particle mass is around 280.8 yg/particle, volume of one particle is 0.165 nm³ and spherical diameter of a particle is 0.681 nm. Glyphosate similar to its precursor glycine, consists zwitterionic behaviour by showing both negative and positive charge at phosphonate group and amino group respectively [76]. Typical half-life of herbicide is 47 days in soil and 92 days in water.

2.1.3 Glyphosate dissociation constant

Glyphosate acid dissociation constant sequence is as shown in Figure 2.2 [124]. When pH is low glyphosate occupies a net positive charge and adsorbs to organic matter in soil. When pH is increasing, the net charge increases [76].

Figure 2.2 Glyphosate acid dissociation constants [124]

2.1.4 Phytotoxic effect

Glyphosate is absorbed from roots and rhizomes by plant tissues and translocated through the phloem of the plant, to inhibit activity of specific enzymes such as shikimate-3-phosphate-5-enolpyruvyl (EPSP), while interrupting the aromatic amino acid synthesis such as phenylalanine, tyrosine, and tryptophan [43, 125, 126]. Usually, EPSP enzyme catalyses the plant reactions which transfer phosphoenolpyruvate (PEP) to shikimate-3-phosphate (S3P) resulting generation inorganic phosphate(P_i) [76]. Glyphosate, forms a ternary complex with EPSP enzyme and S3P, which is responsible for herbicide activity [127, 128] while inhibiting EPSP synthase in a reversible reaction, under competitive PEP and uncompetitive S3P [129, 130]. Due to this transport phenomenon through the system, any plant which is treated with glyphosate slowly dies within days or weeks. Glyphosate half-life inside plants is 35 days [131]. Any part of the plant which was not accessed during this 35 days survives [76]. Herbicidal activity of glyphosate is summarised in Figure 2.3.

Figure 2.3 Reaction pathway of Shikimate-3-phosphate (S3P) [76]

2.1.5 Impact of phosphate fertilizer on sorption of glyphosate by soil

Usually, glyphosate residues in agricultural soils [132-134] are mobilised off-site by wind and water-erosion than by runoff or leaching [135]. There are several studies which have focused on the interactions between phosphate and glyphosate in soil [136]. As glyphosate and inorganic phosphate have displayed to compete for the same active sites [8] when adsorbing to the soil, excessive amount of phosphorus applications can impact the bioavailability and transport potential of the herbicide [137]. Cadmium can be detected in phosphate fertilizers resulting Cd accumulation in soil [138] with continuous application of

phosphate fertiliser. When this Cd meets the glyphosate, Cd-Glyphosate complex is formed. Cd-Glyphosate complexes holds onto the surface of the negatively charged soil colloids. Munira et al [135], has examined the impact of field-aged phosphate (Mono Ammonium Phosphate (MAP)) concentration, fresh phosphate co-application and cadmium concentration on glyphosate sorption in soil. Co-applications of MAP also has impacted the available glyphosate sorption sites. But the impact by MAP on glyphosate adsorption was less than the impact by phosphate [135]. This signifies that glyphosate may become portable by water in moderately acidic to slightly alkaline soils containing high phosphate levels [139].

Apart from phosphate, increase in the soil pH decrease the glyphosate sorption by soil [140]. The effect of temperature on the sorption has not been studied extensively and has assumed to be insignificant [141].

2.1.6 Impact of plant residues on fate of glyphosate in soils

Incompliance with the soil conservation practices in agriculture, plant materials were reverted to soil. Thus, during (via leaf senescence) vegetal cycle and (as crop residues) after harvest glyphosate which was inhibited in plant materials were returned back to soil [142, 143]. Noteworthy amount of foliar herbicides [144, 145] absorbed by leaves of plants of both weed and crops [146] return back by this method. This ancient method of soil conservation practice is widely practised even today at crops which grow glyphosate tolerant varieties [41, 147-149]. As glyphosate is applying at tillage systems as a weed controlling method [150], there is a risk of additional supplement of glyphosate to soils through the addition of glyphosate tolerant plant residues by this practice as glyphosate is not degraded by most glyphosate tolerant plants [44, 151-153].

Tillage directly affect the remaining plant fragmentation and dispersion. Tillage indirectly affect the ecological conditions such as moisture and temperature of soil which drives the decomposition of plant residues [142, 154, 155]. Buried materials are more vulnerable to microbial attack due to improved soil-residue contact [122, 156]. The application of crop remaining onto soil vividly improves the dynamics of residue decomposition [123, 157, 158]. Researches on soil incubation of glyphosate with oilseed rape, maize [144] and soybean cells [145] have presented that the decomposition of the herbicide in plant residue is different from that directly applied on bare soil. Usually, all the glyphosate in soil

degrades within 80 days. The glyphosate herbicide in plant residue involves a lag in mineralization and the fraction of non-extractable residues increases compared to that of directly applied as described in Figure 2.4. The amounts of residues, mainly comprises of the metabolite Amino-Methyl-Phosphonic acid (AMPA), and Non-Extractable residues (NER) [146]. According to Figure 2.4, mineralization of glyphosate in plant residues has decreased, almost four-fold resulting in increase in half-life compared to the glyphosate which was directly applied. The portion of non-extractable and extractable residues has increased up in two and three folds respectively. Addition to that, the perseverance of glyphosate in soil increases when plant residue distribution was less homogeneous.

Figure 2.4 Summarization of fate of glyphosate in soil with direct application and through plant residue decomposition [146]

2.2 Distribution of glyphosate in aquatic waters in acidic farmlands

2.2.1 Quantifying the usage of glyphosate

Glyphosate is applied in various forms. I.e. ammonium salt, dimethylammonium salt, isopropyl amine salt, diammonium salt, or potassium salt [121].

2.2.1.1 Glyphosate usage in the United States

Glyphosate was the seventh most widely applied pesticide in U.S prior to the introduction of genetically engineered (GE) crops. According to the Environmental Protection Agency (EPA) data [5] as showed in Table 2.1 12.5 million kg of herbicide glyphosate has been applied in agriculture sector in 1995. Within 1996-2002 with GE-HT crops gained market share, application of glyphosate in agricultural sector in U.S. rose rapidly, reaching 36 million kg by 2000. From 1974–2014, totally 1.37 billion kgs of glyphosate was applied for agricultural purposes in the United States. Non-agricultural uses also have risen but, less dramatically. Figure 2.5 shows the amount of glyphosate applied in soya been market.

Table 2.1 Use of glyphosate in United State: 1974 – 2014 [5]

	1974	1982	1990	1995	2000	2005	2010	2012	2014
Glyphosate Use (1000 kg)	635	3538	5761	18,144	44,679	81,506	118,298	118,753	125,384
Agricultural	363	2268	3357	12,474	35,720	71,441	106,963	107,192	113,356
Non-agricultural	272	1270	2404	5670	8958	10,065	11,335	11,562	12,029
Glyphosate use (1000 lb)	1400	7800	12,700	40,000	98,500	179,690	260,804	261,807	276,425
Agricultural	800	5000	7400	27,500	78,750	157,500	235,814	236,318	249,906
Non-agricultural	600	2800	5300	12,500	19,750	22,190	24,989	25,489	26,519
Share agricultural (%)	57.1	64.1	58.3	68.8	79.9	87.7	90.4	90.3	90.4
Share non-agricultural (%)	42.9	35.9	41.7	31.3	20.1	12.3	9.6	9.7	9.6

Figure 2.5 Exponential increase in application glyphosate with introduction of Genetically Engineered - soya bean to the market [5]

2.2.1.2 Global glyphosate usage

Global agricultural use of glyphosate also expanded from 1996 with the introduction of GE-HT crops as depicted in Table 2.2. To be more precise, total glyphosate volume utilized by farmers rose up by 14.6-fold, to 747 million kg in 2014 from 51 million kg in 1995.

Table 2.2 Global usage of glyphosate 1994-2014 (Agricultural and non-Agricultural) [5]

	1994	1995	2000	2005	2010	2012	2014
Glyphosate use (1000 kg)	56,296	67,078	193,485	402,350	652,486	718,600	825,804
Agricultural	42,868	51,078	155,367	339,790	578,124	648,638	746,580
Non-agricultural	13,428	16,000	38,118	62,560	74,362	69,962	79,224
Glyphosate use (1000 lb)	124,112	147,882	426,561	887,030	1,438,485	1,584,242	1,820,585
Agricultural	94,508	112,608	342,525	749,108	1,274,546	1,430,002	1,645,927
Non-agricultural	29,604	35,274	84,036	137,922	163,940	154,240	174,658
Share agricultural (%)	76	76	80	84	89	90	90
Share non-agricultural (%)	24	24	20	16	11	10	10

Totally, 8.5 billion kg of glyphosate which is accountable for 71.6 % of total herbicide usage, have been applied worldwide over the last decade as shown in Table 2.2. Glyphosate is account for about one-fourth of global herbicide trades (see Table 2.3).

Table 2.3 Change in the share of total glyphosate as the active ingredient with decades [5]

	Total use (million kg)	Increase from previous period	Share of total use 1974–2014 (%)
1974	3.2	NA	0.0
1975–1984	130.5	127	1.5
1985–1994	387.3	257	4.5
1995–2004	1909	1522	22.3
2005–2014	6133	4224	71.6
Total	8563		

2.2.1.3 Factors driving the upward usage of glyphosate

The dominant factor for the expansion has been the commercialisation of GE-HT crops. Attractive and falling price per kilogram of active ingredient, rapid expansion in the number of crops registered on the use of glyphosate, new application technique and timing such as introduction of conservation tillage and no-tillage systems, and incorporation of glyphosate creatively; i.e. Using as a desiccant to accelerate the harvest of small grains are the other

factors which has been affected the expansion in usage of glyphosate. Table 2.4 explains the amounts of glyphosate applied on different crops types.

Table 2.4 The amount of glyphosate uses on GE-HT crops and other crops [5]

	2010	2011	2012
Cotton	8.6	11.8	9.5
North America	5.64	6.99	6.32
Rest of world	3.00	4.8	3.1
Maize	47.7	65.6	70.2
North America	26.1	28.5	31.0
Rest of World	21.63	37.1	39.2
Soybeans	223.7	239.1	265.1
North America	41.9	42.0	43.6
Rest of world	181.7	197.1	221.5
Canola	13.7	16.5	18.6
North America	0.4	0.3	0.5
Rest of world	13.3	16.2	18.1
Global use on HT crops	293.7	333.0	363.4
Global use on All crops	578.1	616.8	648.6
Percent use on HT crops (%)	51	54	56

2.2.2 Rise of glyphosate levels in environment

With multiple applications, and with increase in volumes applied intensity of levels of glyphosate and its primary metabolite AMPA [10] in the air [11], soil [12], surface water [13, 14] and ground waters [15, 16] has heightened at rising levels [17, 18] (see Table 2.5). According to Food Standards Agency, United Kingdom [19], from 2007– 2013 glyphosate residues have been found in 10–30 % of grain-based samples in considerable amounts. A study carried out in Argentina on soybean crops has revealed that glyphosate concentrations detected in crop residues and soils are in the ranges of 0.5 and 5.0 mg/kg, and in waters in the ranges of 0.10 to 0.70 mg/L [13]. Glyphosate concentrations detected in faster moving, more rapidly diluting water bodies, are generally lower approximately 10–15 µg/L [20-22] whereas higher glyphosate concentrations levels have been recorded in stagnant water bodies, such as wetlands or isolated ponds, [23] due to less dilution and dispersion. Table 2.6 shows glyphosate and AMPA concentrations levels in rain waters.

Table 2.5 Reported levels of residual topsoil concentrations (ug/kg) of glyphosate and AMPA and time from the last application [159]

Treatment	Time from the last application (TLA) (d)	Glyphosate concentration (um/kg)		AMPA concentration (um/kg)	
		High	Low	High	Low
		contamination level	contamination level	contamination level	contamination level
Glyphosate	1	1502.3	386.7	518.9	458.0
Control	1	41.4	<LD	22.3	<LD
Glyphosate	8	1082.8	37.0	727.5	485.7
Control	8	<LD	<LD	33.5	<LD
Glyphosate	10	886.5	34.7	732.8	40.0
Control	10	<LD	<LD	36.9	20.0
Glyphosate	14	489.6	79.0	789.7	299.3
Control	14	<LD	<LD	<LD	<LD
Glyphosate	40	316.3	206.7	796.2	581.7
Control	40	<LD	<LD	<LD	<LD
Glyphosate	80	190.5	40.0	958.6	491.2
Control	80	<LD	<LD	18.1	<LD
Glyphosate	188	186.8	140.9	1052.1	895.2
Control	188	5.0	<LD	7.0	<LD

LD: limit of detection

2.2.3 Glyphosate's chronic exposure level

According to United States Environmental Protection Agency (US-EPA) the highest level of glyphosate concentration allow for human consumption in water is 0.7 mg/L [160].US EPA glyphosate daily Chronic Reference Dose (cRfD) for human exposure is 1.75 milligrams per kilogram of bodyweight (mg/kg bodyweight/day). European Union's (EU) regulated cRfD for human exposure for glyphosate is 0.3 - 0.5 mg/kg/day which is 3.5-times lower than EPA's limit. Antoniou M. et al [161] has amassed cogent evidence supporting the need for a five times diminution to the EU cRfD limit changing it to 0.1 mg/kg/day.

Table 2.6 Glyphosate and AMPA concentrations levels in rain waters [162]

Research work	Location	Samples	% detection	Glyphosate conc. (mg/L)		% detection	AMPA conc. (mg/L)	
				max	median		max	median
Scribner et al. (2007) [163]	USA	14	86	1.1	0.274	86	0.47	0.113
Battaglin et al. (2014) [16]	USA	85	70 .6	2.5	0.11	71 .8	0.48	0.04
Chang et al. (2011) [11]	Mississippi 2007	11	73	1.9	0.2	73	0.3	0.1
	Mississippi 2008	19	68	1.6	0.15	74	0.48	<0.1
	Lowa 2007	14	71	2.5	0.2	36	0.2	<0.1
	Lowa 2008	24	63	1.8	0.1	50	0.24	<0.1
	Indian -a 2004	12	92	1.1	0.14	92	0.47	<0.1
Van Stempvoort et al. (2016) [164]	Canad -a	15	86 .7	0.135	0	26 .7	0.019	0

2.3 Impact of glyphosate on ecosystem and human health

2.3.1 Effect of glyphosate on vertebrates and other animals

The enzyme EPSPS which is inhibited by glyphosate in plants is not an essential enzyme for vertebrates [165, 166], and fishes i.e. zebrafish [167]. Thus, glyphosate hasn't been indicated as a common threat to animal species [168]. Yet, some ecological studies comprise probable effects on soil microbial communities and earthworms [169-171], crustaceans [172], monarch butterflies [173], and honeybees [174] by glyphosate. More recently, Hernández-Plata et al [175] has revealed that rats expose to glyphosate, have low locomotor activity extracellular dopamine (DA) levels in striatum and binding to D1-DA (subtype of the dopamine) receptor in the nucleus accumbens [175]. When glyphosate was

orally administrated by pregnant rats, there was an ability to cross the placenta [175, 176], and to decrease in glutamate uptake by glial cells in the hippocampus, thus leading to glutamate excitotoxicity [177]. Also long exposure to glyphosate based herbicides induced neurobehavioral alterations [178], and altered the activity of brain enzymes [179] in rats.

2.3.2 Effect of glyphosate on human health

In Paraguay and rural regions of South America where farming is common and where glyphosate is heavily used, an alarming trend of birth defects, other facial defects including microcephaly, anencephaly, and cleft palates [10, 180] has started to surface. Increasing rates of cyclopia at endemic levels has been found in communities in coca plantations Colombia from epidemiological studies between year 2004 and 2008 [181, 182]. Glyphosate can permeate the human placenta and thus can cause glyphosate induced teratogenesis [176] and other potential adverse impacts in cell development. Glyphosate can severely affect the metabolic processes and especially, kidney and liver [79, 80, 177, 183-197]. Pesticides are hypothesized as the main factor linked up with neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases [198, 199] via their oxidative stress mechanisms [200]. Pesticides affect the central nervous system (CNS) which is highly susceptible to free radical damages [201]. It has been extensively verified that exposure to glyphosate (either the commercial formulation or the active ingredient) leads to oxidative stress in several tissues, in body together with the brain [177, 202-205]. There also have been evidence of cases reported where people who were extensively exposed to glyphosate been diagnostics with Parkinsonian syndrome [206, 207].

2.4 Glyphosate and metal hybrid system

2.4.1 Glyphosate - metal complex formation

Glyphosate, when apply straight to the soil shows low activity. Soil constituents, especially non-phytotoxic product such as CO_2 , PO_4^{3-} and NH_3 [208] largely attract and adsorb glyphosate. Then glyphosate is degraded due to microbiological activity [209]. As discussed previously, glyphosate competes with inorganic phosphate for adsorption sites in soil such as organic matter [210] and amorphous hydroxides of iron and aluminium [211], and ferric oxides [212]. Connected glyphosate tend to sediment by a mechanism similar to inorganic phosphate [78, 213].

Glyphosate herbicide possesses two functional groups amino and carboxylate which bonds with metal ions, especially near neutral pH where they are deprotonated [87] placing in a privileged position among other herbicides due to its ability to coordinate as a tridentate ligand. When water is hard containing cations such as iron, zinc, calcium and magnesium glyphosate form soluble or insoluble complexes with cations which are not absorbed by plants. Therefore, glyphosate adsorption to soil can be intensely subjective to the presence of dissociated cations [214]. Formation of metal complexes was increased with the increase of pH [109, 215]. This complexation reduce the effectiveness [76] of glyphosate as an herbicide. The adsorption of glyphosate to soil, bio-degradation, photo-degradation processes of glyphosate is modified [76, 216] with the formation of soluble or insoluble metal complex. Figure 2.6 depicts the usual and modified degradation process of glyphosate.

Figure 2.6 Degradation pathway of glyphosate in hard water and normal water [79]

2.4.1.1 Change in glyphosate phytotoxicity with metal complex formation

Complexation with monovalent cations, such as potassium, ammonium, and sodium to increases the overall phytotoxicity of glyphosate while di and trivalent cations decreases the phytotoxicity [217] of the herbicide. Foliar absorption of glyphosate by weed plants

was affected when glyphosate is bonded with di valent cation like iron, calcium, manganese, magnesium and zinc [218, 219]. There were some exceptions such as; the absorption of glyphosate in corn and soybeans [3] was increased when treated with calcium chloride. In addition, the translocation of glyphosate in wheat was increased when mixed with iron and manganese [218, 220].

2.4.1.2 Understanding the herbicide metal complex

As, glyphosate metal complexation can be complicated by different metal oxidation states which could result a range of possible geometries [221], for a deeper understanding of the complex, theoretical calculations should be used [88]. Usually, to study the interaction of metals with biomolecules discrete Fourier transform (DFT) methods have been progressively incorporated [88, 222, 223] as DFT method could effectively analyse the effect of electronic correlations in a larger system [224].

2.4.2 Structural aspects of the formed glyphosate metal complexes

One molecule of glyphosate bonds with one metal ion forms glyphosate-metal 1:1 complex. The bonds are made between metal ion and the three donor groups of glyphosates; amino, carboxylate and phosphonate resulting in two chelates five-membered rings. In the glyphosate-metal 1: 2 complex, two molecules of glyphosate and one atom of metal ion, bonds to form a distorted octahedral shape [76, 88] as shown in Figure 2.7.

Figure 2.7 Glyphosate metal complex structure (a) one molecule of glyphosate and metal (1:1 complex), and (b) two molecules of glyphosate and metal (2:1 complex) [76]

In Figure 2.8, a) two glyphosate molecules with two protonated amino group ($-\text{NH}_2^+$) (blue) and two Hydrogen atoms (white) binds with metal, b) two glyphosate molecules, one with protonated amino group ($-\text{NH}_2^+$) and the other with amino group ($-\text{NH}-$) bonds with metal (green), c) two glyphosate molecules with amino groups ($-\text{NH}-$) coordinate with metal (green), and d) two glyphosate molecules with amino groups ($-\text{NH}-$) bonds with metal (green) with three bonds. In Figure 2.9, a) two glyphosate molecules with protonated amino group ($-\text{NH}_2^+$) (blue) and two Hydrogen atoms (white) bonds with metal (green), b) two glyphosate molecules with amino group ($-\text{NH}-$) bond with metal (green) with three bonds.

Figure 2.8 2:1 glyphosate metal complex in octahedral formation [88]

Figure 2.9 2:1 glyphosate metal complex in tetrahedral formation [88]

2.4.3 Stability constant and solubility product of glyphosate metal complexes

Table 2.7 summarises stability constants of some glyphosate metal complexes. For both the systems 1:1 and 2:1, thermodynamic stability order is $Mg < Ca < Al < Cr < Fe < Co < Cu < Zn$. Di-valent cation complexes are more stable than tri-valent cation complexes. Among the divalent cations' stability order is from; $Mg < Ca < Cu < Zn$ and for trivalent cations; $Al < Cr < Fe < Co$. The complex 2:1 shows better stability at EPSP synthase active sites [88].

Table 2.7 Stability constants of the complexes [87, 225]

Cu(II)	Zn(II)	Mn(II)	Ca(II)	Mg(II)
11,92	8,4	5,53	3,25	3,25

2.4.4 Impact of glyphosate metal complexes

Studies have reported prolong health effects such as kidney diseases [28, 32, 79-81, 83, 84] that can induce when glyphosate is coupled with metals in hard water. Strong evidence supporting this hypothesis can be found in Central province in Sri Lanka [79, 80], in sub-regions of the Pacific coasts of the Central American (CA) [28, 81], Costa Rica, Nicaragua [82], countries of El Salvador [83, 84], and from Andra Pradesh of India [32]. Unfortunately, no study is available focusing on the effects of hardness-glyphosate complex combination to animal or human health.

2.5 Technologies for glyphosate removal

2.5.1 Overview of organic micro pollutant removal performance

As described in last section, there is already evidence on the occurrences of glyphosate and its metabolites in drinking water sources. However, since the molecular sizes of glyphosate is quite small and their concentrations in water are very low, the conventional water treatment process (coagulation, flocculation and sedimentation) had been demonstrated not effective in removing glyphosates. Adams et al. [226] researched on the effective removal of frequently occurred micropollutants under the existing conditions at the traditional wastewater treatment plant (WWTP). The results showed that the coagulation/ flocculation/ sedimentation process at conventional WWTP with iron salts and alum was ineffective for the removal for

most of the micropollutants. Meanwhile, the author pointed out that adsorption with powdered activated carbon (PAC), and oxidation with chlorine and ozone, and reverse osmosis processes were all presented to be effective in antibiotics removal under the same conditions.

Boyd et al. [227] examined 12 types of EDCs/PPCPs (endocrine disrupting chemicals and pharmaceuticals and personal care products) at two water treatment plants in USA and Canada. The results showed that the conventional water treatment process of coagulation/flocculation/ sedimentation plus 2 mg/L PAC addition did not effectively remove these micro organic pollutants. Several other technologies have been suggested, tested on to remove EDCs/PPCPs, such as adsorption, advanced oxidation processes, biodegradation and membrane filtration. Table 2.8 compared the removal efficiency of a different treatment methods for a range of existing EDCs/PPCPs types. According to the Table 2.8 activated carbon adsorption, advanced oxidation, and high-pressure membrane are some efficient ways to eliminate these micro pollutants. Although advanced oxidation processes have an excellent performance, there are still some challenges with that technology in practice, such as, the by-products problems and high investment. Tight membranes (like nanofiltration (NF) and reverse osmosis (RO)) have a good rejection of micro organic pollutants but they require high pressure for applications, hence their operational cost is great as well. Biodegradation is usually used in wastewater treatment process. Low-pressure driven membrane techniques (e.g. microfiltration (MF) and ultrafiltration (UF)), together with the pretreatment of coagulation and/or adsorption, have been considered a promising strategy of removing these micro organic contaminants without chemical changes. The published literature shows adsorption processes as a pre-treatment method can remove glyphosate [39, 40]. Adsorption distinguish it from other removal technologies as a non-hazardous, environmentally friendly [41], economical, flexible(in design), highly selective and efficient technique and at the same time the adsorbents can be regenerated [42]. Furthermore, incorporation of an adsorbent is convenient to be combined with the existing facilities to deal with the urgent problematic water sources.

Table 2.8 Estimation of EDCs/PPCPs removal efficiency by individual processes

Group	Classification	Activated carbon	Bio char	O ³ / AOPs	UV	Cl ₂ /ClO ₂	Coagulation/ flocculation	Nano filtration	Reverse osmosis	Degradation (Biodegradation -B/ Photo degradation -P/ Activated Sludge -AS)
Endocrine disrupting chemicals (EDCs)	Industrial chemicals	E	E	G-F	E	P	L-P	E	E	G E (B)
	Inorganics	L-P	F	P	P	P	P	G	E	L-P
	Organometallics	E-G	E-G	E-L	F-P	F-P	L-P	E-G	E	E-L
	Metals	G	G	P	P	P	G-F	G	E	P (B), E (AS)
	Pesticides	E	E	E-L	E	E-P	P	G	E	E (P)
	Steroids	E	E	E	E	E	P	G	E	E-L (B)
Pharmaceuticals and personal care products (PPCPs)	Antibiotics	G-F	E	E-L	G-F	G-P	L-P	E	E	E (B), E-G (P)
	Antidepressants	E-G	E-G	E-L	G-F	F-P	L-P	E-G	E	E-G
	Anti-inflammatory	E	E-G	E	E	F-P	P	E-G	E	E (B)
	A ray contrast media	E-G	E-G	E-L	G-F	F-P	L-P	E-G	E	E (B and P)
	Lipid regulators	E	E	E	G-F	F-P	P	E-G	E	P (B)
	Psychiatric control	E-G	E-G	E-L	G-F	F-P	L-P	E-G	E	E-G

Key; E, excellent (>90%); G, good (90-70%); F, fair (70-40%); L, low (40-20%); and P, poor (<20%)

2.5.2 Advanced oxidation process in treating glyphosate

Micro organic pollutants such as undesired pharmaceuticals and other nonbiodegradable compounds are partially degraded and removed at the conventional WWTPs, which typically comprised on biological processes. Considerable proportion of the micropollutants escape and enter into aquatic environment [228, 229] untreated. For instance certain compounds such as analgesics and anti-tumour agents failed to be degraded using biological treatment methods [36, 90, 91] and are adsorbed onto sewage sludge [230]. This sewage sludge can potentially be harmful to wildlife. Therefore, rigorous exertions need to be added at the wastewater treatment process. The application advanced treatment processes [37, 38, 231] of highly efficient and cost-effective especially when the wastewater is recycled and reused, are encouraged when working towards building strategies for a sustainable future. A number of advanced physical or chemical techniques including activated carbon adsorption [43, 109, 133, 213, 215, 232-237], membrane separation and advanced oxidation processes have been tested for effective removal of emerging micro organic pollutants.

2.5.3 Adsorption process in treating glyphosate

2.5.3.1 Removal by carbons

Hierarchically structured porous materials such as activated carbons [238-240] or carefully manufactured biochar possess some exclusive properties such as convenient accessibility to active sites, high surface area, enhanced mass diffusion and transport due to their structural, component, porosity, morphological level uniqueness. Introduction of hierarchical characteristics (multi-level pores) to a material extensively enhance its performance as an adsorbent [241] compared with single-sized porous materials [242, 243]. According to some theoretical calculations, hierarchically porous structures [244-246] can reduce the diffusion limitations, such as resistance [247, 248] and enhance diffusion rate of compounds by shortening the diffusion pathways [249].

Activated carbon adsorption processes are one of the most frequently used treatment technologies in drinking water treatment due to its low initial cost and convenient application. Activated carbon owns a highly microporous structure, which could remove contaminants from water by either physical or chemical adsorption, and the former usually

governs majority of the removal efficiency. Factors, such as the porous and chemical structure of the carbon, molecular dimensions, compound properties, and other solution characteristics, could affect the uptake of compounds onto activated carbon. Powder activated carbon (PAC) effectively removes some pesticides, herbicides many problematic organic pollutants, taste and odor compounds. The US EPA has categorized packed-bed granular activated carbon (GAC) as the best available technology considering its effectiveness in treating numerous regulated organic micro pollutants.

Both PAC and GAC have been proven to be an efficient process to remove a variety of micropollutants and ecotoxicity in municipal water treatment industry in practice [140, 250, 251]. As to glyphosate, the adsorption by activated carbons has been largely improved due to the existence of amine groups in the structure [252]. Ternes et al. [27] investigated the riddance of selected pharmaceuticals in deionized (DI) water and groundwater by GAC adsorption in batch adsorption experiments and pilot scale test. In the batch experiment, they found that the glyphosate with initial concentration of 100 $\mu\text{g/L}$ can be efficiently removed with a carbon dosage of less than 0.2 g/L. The suspensions were mixed for 24 h before analyzing the results. They reported that the occurring of natural organic substances could compete for adsorption sites with micropollutants. The K values in groundwater were smaller than those in deionized water for all compounds, which indicated that sorption efficiencies were always affected by occurrence of natural organic compounds.

Yoon et al. [253] examined the removal of three estrogenic compounds on several PAC in batch adsorption tests with a contact time of 24 h, also indicated the influence on the adsorption of the target compounds when natural organic compounds (NOM) were presented in solution. Although activated carbon was capable of the removal of all organic contaminants, its removal capacity is limited by the contact time. Both above stated studies conclude that the adsorption with PAC or GAC usually takes 24 hours to ensure the adsorption capacity is fully complete. Thus, the challenge is that, when PAC is applied in water treatment plants, the contact time is usually 2 hours or less, which is not sufficient enough to obtain the maximum adsorption capacity of the adsorbent, especially when PAC is used as the pre-treatment for water treatment, the contact time could be even shorter. With low adsorption kinetic values activated carbon always requires a long contact time to ensure full utilization of the adsorption capacity. Otherwise a high carbon dosage or a large contact reactor are needed which will offset the advantage of small footprint of membrane filtration and increase the cost of treatment process.

Another solution is to find out a kind of adsorbent with faster adsorption kinetics. Najm et al. [254] reported that smaller the particle size of PAC, faster the adsorption kinetics. Several restudies about adsorption of organics in aqueous environment with both submicron-sized powdered activated carbon (SPAC) and PAC were published recently. Matsui et al. [255] ground the commercially available PAC to get SPAC, and the particle size of activated carbon was reduced from 33 μm to 0.8 μm and 3.8 μm . They found SPAC removed organic micro pollutants much faster than PAC. Adsorption of micro pollutants with SPAC of 0.8 μm could reach 80% (by UV_{260}) of equilibrium within only 1 min of contact time. On the contrary, PAC could only reach 35% (by UV_{260}) at 60 min. The SPAC was also effective at increasing its adsorption capacity for NOM but not for the smaller molecule phenol. Hence, pulverization has improved the adsorption of the small molecular organic pollutants in both pure water and natural water. Furthermore, SPAC was less sensitive to the competition from NOM. Table 2.9 summarise the research work on glyphosate adsorption onto activated carbon and biochar.

Table 2.9 Related work on glyphosate adsorption on activated carbon and biochar

Carbon source	Adsorption conditions	Glyphosate detection method	Conclusion	Remarks	Reference
Rice husk derived and Dendro wood biochar	Glyphosate conc.: 20 mg/L	Ultraviolet–visible spectroscopy; 10 ml of aliquot was mixed with 1% ninhydrin and 1% sodium molybdate. 0.2 ml of filtered sample was kept in a water bath at 85-95 °C for 12 min/ 70–100 °C for 3-4 min, Ruhemann’s purple colour's absorbance was measured at 570 nm	Adsorption is strongly pH dependent	Both physisorption and chemisorption mechanisms govern the glyphosate adsorption	Herath et al, 2016 [90]
	Adsorbent dose: 0.5 g/L				
	pH: optimum based on edge exp.				
	Contact time: 30 – 240 mins				
	Shaker: 3000 rpm for 15 mins				
Dendro wood biochar	Glyphosate conc.: 5 - 100 mg/L	Ultraviolet–visible spectroscopy	Adsorption is strongly pH dependent	Chemisorption govern the adsorption; functional groups: - phenolic, amine, carboxylic and phosphate	Mayakaduwa et al, 2016 [91]
	Adsorbent dose: 1.0 g/L				
	pH: 3.0 - 8.0				
	Contact time: 4 h				
	Shaker: 100 rpm		Maximum adsorption; 44 mg/g (within the pH range of 5 – 6)		

Carbon source	Adsorption conditions	Glyphosate detection method	Conclusion	Remarks	Reference
Hardwood biochar	Glyphosate conc.: 0.02 – 10.02 mg/L	Liquid scintillation counting (LSC); 1ml aliquot of the filtered solution is mixed with 5ml of scintillation cocktail. The ¹⁴ C amount is quantified.	Adsorption/ desorption is strongly affected by surface chemistry and physical constraints	Desorption was not affected by CaCl ₂ solution	Hall et al, 2018 [256]
	Adsorbent dose: 10.0 g/L			Desorption is improved by 86% with a K ₂ PO ₄ solution	
	Contact time: 24 h				
Forest soil	Glyphosate conc.: 5–30 mg/L	Ultraviolet–visible spectroscopy	Maximum glyphosate adsorption; 161.29 mg/g (at pH 12, initial glyphosate conc. 25.74 mg/l, contact time 119.99 min and adsorbent dose 0.05 g/L)		Sen et al, 2017 [257]
	Adsorbent dose: 0.05–1.5 g/L				
	pH: 2.0 – 14.0				
	Contact time: 5–120 min				
	Shaker: 24h after 48hrs shaking		Removal efficiency; 87.8%		

Carbon source	Adsorption conditions	Glyphosate detection method	Conclusion	Remarks	Reference
Rice husk derived biochar	Glyphosate conc.: 20 mg/L	Ultraviolet–visible spectroscopy	Maximum removal; 82.0% (at pH 4) Adsorption capacity; decreased with increasing pH	Adsorption occurred by physisorption mechanism	Herath et al, 2016 [89]
	Adsorbent dose: 0.5 g/L				
	pH: 3.0 - 9.0				
	Contact time: 5–120 min				
	Shaker: 3000 rpm for 15 mins				
Palm biochar supported Nano-zero-valent iron	Glyphosate conc.: 5–40 mg/L	HPLC coupled with fluorescence detection	Maximum adsorption capacity 80 mg/g at pH=4	Adsorption was chemical and surface process	Jiang et al, 2018 [140]
	Adsorbent dose: 0.5 g/L			At low concentrations, coexisting pollutants didn't influence the adsorption	
	pH: 2.0 - 7.0				
	Contact time: 0–3750 min				
	Coexisting pollutants (Sulfamethazine (SMT), Cd (II) and H ₂ PO ₄ ⁺); 0–20 mg/L				

2.5.3.2 Comparison on the existing carbon adsorbent materials

In Table 2.10 and 2.11 existing carbon-based adsorbents are extensively compared.

Table 2.10 Comparison between Biochar and Activated carbon

Activated Carbon	Biochar
<ul style="list-style-type: none"> • Widely available and used in wastewater treatment plants • Made from biomass, coal, coconut shell-, and wood • Ideal for removing contaminants from water [258, 259] • Costly to make and required post modification to activate 	<ul style="list-style-type: none"> • Requires less investment as biochar is the by-product of pyrolysis process • Less carbonized than activated carbon [260] • More hydrogen and oxygen remain – saves the additional cost in post modification step • Can be used as a soil amendment [261] simultaneously as biochar comprises of different nutrient compounds

Table 2.11 Comparison between Biochar and Hydro-char

Biochar	Hydro-Char
<ul style="list-style-type: none"> • Produce at high temperatures (600-700°C) • Stable [136, 262] • Dominated by aromatics • Well-organized C layers [263, 264] • Fewer H and O functional groups due to dehydration and deoxygenation of the biomass • Lower ion exchange capacities 	<ul style="list-style-type: none"> • Produce at lower temperatures (300-400°C) • Less stable • Diversified organic characters, dominated by alkyl moieties, including aliphatic and cellulose type structures and • Contain more C-O and C-H functional groups • Hydro chars due to their different chemical properties are not qualified for European Biochar Certificate (EBC) standardization

Properties of biochar [136, 261, 265] mainly governed by the type of feedstock and on pyrolysis conditions such as; temperature, reaction time, reactor type, and heating rate [266]. Those pyrolysis conditions significantly effect on biochar chemical composition, pore structure, aromaticity [267]. When reaction time and temperature of the reactor is increased, percentage carbon in the char increases while percentage oxygen and surface hydrophilicity decreases [268].

2.5.3.3 Modification and performance enhancement

Figure 2.10 Schematic diagram of biochar modification [269]

Figure 2.11 The methods used in enhancing performance of engineered biochar

Modifications enhance char's sorption ability by increasing the surface areas and through addition of new sorption sites with specific surface functional groups converting surface more conducive to electrostatic attraction. Modification is creatively engineered based on the type of the treated contaminants (i.e., anionic/ cationic, inorganic/ organic, polar/ non-polar, hydrophilic/ hydrophobic), environment, remediation goals. Modifications are done to enhance surface properties. Figure 2.10, and Figure 2.11 explains the respective modifications used in different applications.

2.6 Research statement

2.6.1 Research framework

In view of the aforementioned issues, the overall objective of this research is to study the application of readily available, cheap and effective adsorbent to remove glyphosate herbicide in drinking water under the influence of metals. First, the effect of physical characteristics such as pore distributions, surface area and chemical characteristics such as surface charge, existing ligands, loaded ferrous metal were scrutinised qualitatively and quantitatively, in the glyphosate adsorption. Commercially available promising two adsorbent types; activated carbon and ferrous loaded biochar were analysed in terms of adsorption kinetics and adsorption isotherms to compare and rate the effectiveness of them in glyphosate adsorption. The influencing parameters in uptake is optimised for a better performance. It was observed that ferrous in biochar degrade the glyphosate molecule. The influence of prevailing inorganic ions, namely copper and ferrous in degradation of dissolved glyphosate; formation of by-products and the degradation pathway was examined. The results to be obtained from this research expected to contribute to influence in behavioural analysis of glyphosate with metal ions and on the treatment of dissolved glyphosate. Research framework is summarised in Figure 2.12.

Figure 2.12 Schematic framework of the study

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and materials

Glyphosate (N-(phosphonomethyl) glycine) (purity 96%), Copper(II) chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) ($\geq 99.99\%$ trace metals basis), Copper(I) chloride (CuCl) (anhydrous, $\geq 99.99\%$ trace metals basis), Copper powder (99.999% trace metals basis) and AMPA (99% pure) were purchased from Sigma-Aldrich, Singapore. Commercial grade coconut shell activated carbon was purchased from Puritas (Pvt) Ltd, Sri Lanka. Other activated carbons used, and wood biochar were purchased from Sigma-Aldrich, Singapore. All reagents and adsorbents used were of analytical grade and used without further purification. MilliQ ultra-pure ($18.2 \text{ M}\Omega \text{ cm}$) water was used in preparing the samples.

3.1.1 Iron loaded wood biochar

The iron loaded wood biochar was synthesized by the following procedure [270]. 10 g of the obtained wood biochar purchased from Sigma-Aldrich, Singapore was mixed with 500 ml of 7.3 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 7.23 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in a glass tube. This mixture was heated at 50°C to form a stable suspension. The pH was raised to 11 by adding 0.05 M NaOH solution, dropwise. After mixing for 60 min, the organic residues were removed by washing with deionized water (DI) until the pH of the washing solution reached 7.0. The fine powdered modified biochar (FMBC) was vacuum-filtered and then dried overnight at 50°C in a hot air oven [271].

3.2 Adsorbent characterization

Adsorbent characterisation was done by means of qualitative, quantitative and spectroscopic analysis.

3.2.1 Elemental composition and total ash content

Elemental composition of the carbons was determined with the Vario EL Cube elemental analyser, (Elementar, Germany). Total ash content of the adsorbent was determined by heating 1 g samples to 900 °C for 2 h in a muffle furnace and then weighing the residual ash content.

3.2.2 Pore size analysis

The specific surface area and pore size distribution were determined with the QUADRASORB™ gas sorption surface area and pore size analyser (Quantachrome Instruments, USA) in micropore analysis mode.

3.2.3 Zeta potential analysis

Activated carbon is full of surface functions, most of them are ionized. Activated carbon, when derived from biomass, often consist of ashes. These ashes are salts of alkali and alkaline earth metals, so when in water many Na⁺, K⁺, OH⁻ ions are produced. Hence, increases the surface conductivity.

When an electric field is applied to any charged particle, it will be influenced by that field. The extra movement that particles exhibit as a result of the electric field is called the electrophoretic mobility. Its typical units are $\mu\text{m}\cdot\text{cm} / \text{V}\cdot\text{s}$ (micrometer centimeter per Volt second) since it is a velocity [$\mu\text{m}/\text{s}$] per field strength [V/cm]. The electrophoretic mobility is the direct measurement from which the zeta potential can be derived using the complete Henry function $F(\kappa a)$.

$$U_E = \frac{2\epsilon z f(\kappa a)}{3\eta}$$

Where U_E is the electrophoretic mobility, η is the viscosity, ϵ is the dielectric constant, z is the zeta potential and $f(\kappa a)$ is the henry function. Zeta potential measurements are a useful and efficient method in evaluating the adsorption.

From a theoretical viewpoint, the electric potential of a dispersed particle in the interfacial double layer is known as zeta potential. Zeta potential of a particle is highly dependent on the dispersed solution pH. The electrostatic interaction between adsorbate and the surface

of adsorbent has a great effect on adsorption capacity. As glyphosate is negatively charged at most pH levels, when the zeta potential of carbon is higher, the electrostatic attraction between adsorbent and adsorbate greatly increase the adsorption capacity. Samples were prepared by diluting 1 mg adsorbent particles in 100 mL of 10^{-3} M NaCl solutions. pH of the samples was adjusted with diluted HNO₃ or NaOH solutions. Electrophoretic light scattering (ELS) was performed on a Malvern ZEN2600 Nano ZS zeta potential analyser (Malvern Panalytical Ltd, United Kingdom) based on He–Ne laser with a wavelength of 632.8 nm. An applied DC bias voltage of across cell was 80 V for the ELS measurements analysis. Water was used as dispersant and count rate and temperature of the system were set at 67.9 kcps and 25 °C respectively. In ELS, the raw data obtained was the electrophoretic mobility; typically, in units of $\mu\text{m/s}$ per V/cm or shorter in $\mu\text{mcm/Vs}$.

3.2.4 Inductively coupled plasma mass spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry (ICP-MS) is used to detect several metals and non-metals at low concentrations levels.

Table 3.1 Metals and non-metals detected using ICP-MS during the study

Element	Fe	Cu	Zn	Al	Mg	P	S
First							
ionisation							
efficiency	96	90	75	98	98	33	14
(M⁺/M⁺⁺+M)							
x 100%							
Most							
abundant	56	63	64	27	24	31	32
isotope							
Detection	1 $\mu\text{g/L}$	1 $\mu\text{g/L}$	1 $\mu\text{g/L}$	1 $\mu\text{g/L}$	1 $\mu\text{g/L}$	1 mg/L	1 mg/L
limit	– 50	– 50	– 50	– 50	– 50	-50	- 50
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L

The ion concentrations in the aqueous samples and in carbons, were measured utilizing the ICP-MS Agilent 5700 series instrument (Agilent Technologies, U.S.A) for the elements in Table 3.1 (More information was provided in Figure A1). The analysis of respective metals

and non-metals in the carbon adsorbents were done in accordance with US-EPA method 3052 (US-EPA-Method-3052, 1996). First, 0.10 ± 0.01 g of the carbon adsorbent samples were digested with a mixture of 8 mL of 60% HNO_3 , 1 mL of 50% HF, and 1 mL of 30% H_2O_2 in a microwave digester (Ethos One-Milestone) at 180 ± 5 °C for 20 min. The digested samples were diluted to total volume of 50 mL with deionised water and refrigerated ($<7^\circ\text{C}$) prior to analysis.

3.2.5 X-ray Photoelectron Spectroscopy

The surface chemistry of different membranes was studied by the Kratos XPS System-AXIS His-165 Ultra - X-ray photoelectron spectroscopy equipment (Shimadzu, Japan) with a monochromatic 'Al 1486.6 eV K_α ' - X-ray source. 40 eV energy and step size of 0.05 eV with a pass was injected at the high-resolution scans when examining the peaks. Spectra was calibrated to compensate for the charging effects, with graphitic carbon as the reference at a binding energy (BE) of 284.6 eV. The XPS results were collected and analysed using Casa XPS software.

3.2.6 Attenuated total reflection –Fourier Transform Infrared Spectroscopy

To investigate the surface chemistry, Fourier Transform Infrared spectroscopy were measured by an IRPrestige-21 FTIR spectrometer (Shimadzu, Singapore) equipped with attenuated total reflection (ATR) accessory. The spectra were collected within the wave number range from 400 to 4000 cm^{-1} . All measurements were carried out at room temperature.

3.2.7 Scanning Electron Microscopy and Energy Dispersive X-Ray Spectroscopy

Scanning Electron Microscope (SEM) Merlin with Cryo Workstation (Fision, USA) with Image Analyzer (Q500 Quantimax, USA) was used to observe the surface structure and morphology of the biochar and activated carbon. The carbon samples were fractured in liquid nitrogen; the surface and cross-sections of the carbons were studied. A computer image analysis software was used to calculate the surface porosity (Uyanik et al., 2010). The existence of loaded ferrous in biochar was detected by Energy Dispersive X-Ray Spectroscopy (EDX).

3.3 Analytical methods

Chromatographic analysis was done using LC-MS/MS (Agilent 6460 LC/MS QQQ, Agilent Technologies, U.S.A.) with an autosampler and a heat-controlled oven using a Zorbax SB-C18 column (2.1 x 100 mm, 3.5 μ m particle size) (Agilent Technologies, Singapore). A new LC-MS/MS method was developed to determine the glyphosate concentration with optimal parameters of mass spectrometry for each compound, including fragments of target compounds, cell acceleration voltage, collision energy (CE), dwell, fragmentor and polarity summarized in Table 3.2. The column temperature was maintained at 40 $^{\circ}$ C. The injection volume was 10 μ L, and the mobile phases were 1% formic acid in DI water (A) and methanol (100% LC-MS grade) (B) with the following linear-gradient flow program. The elute gradient was started at 99% A and 1% B compositions, which was held for 3 min. Then B composition was rose linearly in 10 secs to reach 10% A and 90% B. This condition was maintained for 2 min. The respective peaks occur during this time interval. The mobile phase of A and B composition was turned back to its initial condition in 10 secs soon after the ramping and was held for 2.8 min for re-equilibration of the column and system whilst maintaining a constant flow rate of 0.2 mL/min, total chromatography run time was 8 min per sample. Mass spectrometry investigation was conducted using (Mass Hunter Workstation Software, Quantitative analysis, Version B.07.00, Agilent Technologies) in multiple reaction monitoring modes.

The LC/MS instrument setup was consisted of positively and negatively operated an electrospray ionization (ESI) source, with respect to the analytes. The operating conditions were as follows: auxiliary gas (GS₂) 70 psi, atomization air pressure (GS₁) 60 psi, electrospray voltage (IS) 5500 V, electrospray voltage (IS) 4500 V collision chamber inlet voltage (EP) 10.0 V (ESI+ mode), collision chamber inlet voltage (EP) 5.0 V (ESI mode), ion source temperature (TEM) 600 $^{\circ}$ C and curtain gas (CUR) 35 psi. Mass Hunter Workstation analysing software version B.07.00/7.0457.0 (Agilent Technologies, U.S.A.) was used in controlling the system and during data acquisition, and data analysis steps.

Table 3.2 LC-MS/MS equipment parameters for the analysed compounds

	Active substrate	Precursor Ion	Product Ion	Dwell (V)	Fragmentor	Collision Energy (CE) (V)	Cell Accelerator voltage (V)	Polarity
Glyphosate	Quantification	168	81	80	76	12	7	Negative
	Confirmation	168	63	80	76	24	7	Negative
AMPA	Quantification	110	79	80	102	32	7	Negative
	Confirmation	110	63	80	102	16	7	Negative
Glyphosine	Quantification	262	122	80	92	16	7	Negative
	Confirmation	262	79	80	92	40	7	Negative
Gluphosinate	Quantification	180	95	80	112	12	7	Negative
	Confirmation	180	85	80	112	16	7	Negative

3.4 Batch adsorption

Glyphosate was dissolved by sonication in 0.01M NaCl prepared with milliQ water to prepare the stock solutions respective to Table A1, Table A2, Table A3 and Table A4. pH of the solutions was regulated by buffering during the adsorption process. 1 M NaOH and 1 M HCl solutions were used to regulate the initial pH of solutions respective to Table A1, Table A2, Table A3 and Table A4. 40 mL of the glyphosate stock solution each were added to 50 mL centrifuge tubes and weighed quantities of activated carbon or biochar (respective to Table A1, Table A2, Table A3 and Table A4) added. All experiments were conducted with mixing at 150 rpm on an incubating shaker at pre-determined temperatures respective to Table A1, Table A2, Table A3 and Table A4. After a pre-determined contact period (respective to Table A1, Table A2, Table A3 and Table A4), the tubes' contents were filtered using 0.22 µm syringe filters and the filtered samples were transferred to amber coloured vials. The samples were tested using LC-MS/MS (Agilent 6460 LC/MS QQQ,

Agilent Technologies, Singapore) with Zorbax SB-C18 (2.1 x 100 mm, 3.5 µm particle size) (Agilent Technologies, Singapore) column. Samples were prepared in triplicate while each sample was tested in duplicate to assure reproducibility.

3.5 Response surface experimental design

During the study, the Box-Behnken method was used to optimise operating parameters – such as; pH, initial glyphosate concentration (mg/L), temperature (°C), adsorbent dose (g/L), and contact time (h) to maximise removal capacity and efficiency. n-factor Box-Behnken method was chosen according to the number of continuous variables needed to be analysed. Box-Behnken method is selected because it is an independent quadratic design which does not contain any embedded factorials or fractional factorials. The method represents a box wherein each factor is varied at three levels - midpoints of the edges and at the centre as - 1, 0 and +1 - indicating low, medium and high values. The series of tests and levels of independent variables with coded and un-coded values for glyphosate adsorption and glyphosate degradation with ferrous and copper are in Table A1, Table A2, Table A3 and Table A4. For 5 independent parameters, 18 centre-points and 46 base runs were required to build the box. As each sample was in triplicate, the total number of runs required for the analysis was then 138. Table A2, Table A3 and Table A4 shows the design tables for 5, 6 and 7 continuous variables respectively. The sample solutions which were used in the adsorption and degradation tests were prepared to match the conditions at each of these points and the final concentration of glyphosate and generated AMPA concentrations in the solution after experiment for each set of conditions was then measured and recorded.

The data obtained were fitted with an empirical second-order polynomial model as follows:

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \sum_{j=1}^n \beta_{ij} x_i x_j + \sum_{i=1}^k \beta_{ii} x_{ii}^2 + \varepsilon \quad (1)$$

where β is the regression coefficient for the intercept. The empirical models calculated for activated carbon and biochar were used in plotting 3D surface plots and contour plots and analysed for the adsorption behaviour. The operation parameters were ranked according to the significance of each factor on the overall adsorption process using Perturbation analysis. To evaluate the accuracy of the model, Pareto analysis and variance (ANOVA) [257] tests were performed.

CHAPTER 4

GLYPHOSATE ADSORPTION: EFFECTS OF ADSORBENT SURFACE CHARACTERISTICS

4.1 Background

Glyphosate is considered as the most commonly available and most widely used herbicide exist [6]. With multiple applications and with increase in volumes, intensity of levels of glyphosate and its primary metabolite amino-methyl-phosphonic acid (AMPA) [10] in the air [11], soil [12], surface water [13, 14] and ground waters [15, 16] has heightened at rising levels [17, 18]. On the other hand, a variety of developed technologies such as advanced oxidation processes [32], coagulation [33, 34], membrane technology [35], and adsorption [36] have been demonstrated effective for the abatement of glyphosate from the aqueous phase. Adsorption has significant advantages over other methods because it is simple, environment friendly, and cost effective. Activated carbon [29], biochar [30], hydro char [31], carbon nanotubes [37], and hierarchically structured carbon [38] are some carbon-based materials extensively studied for their adsorption efficiency. However, different surface characteristics of adsorbent play a significant role in the adsorption process. In current researches, porous char's surface area modifications and addition of new sorption sites with specific surface functional groups to enhance char's sorption ability are trending. The objective is to understand the specific surface characteristics which favour the removal efficiency of glyphosate from aqueous solution. Inline to that objective, four coconut shell activated carbons, two wood activated carbons and two porous polymers with different particle distribution and surface area were tested with glyphosate to analyse the characteristics such as surface area and pore distribution on dissolved glyphosate

adsorption. Adsorption kinetic models were utilized in assessing the adsorbents. Secondly, the aforementioned coconut shell activated carbon was analysed before and after glyphosate adoption to analyse the surface charge and existing ligands which favour the glyphosate adsorption. The study comprehends the effects of adsorbent's physical and chemical characteristics on glyphosate uptake.

4.2 Effect of physical characteristics

4.2.1 Adsorbent characterization

4.2.1.1 BET micropore analysis

Table 4.1 Pore structural properties of adsorption materials

Adsorbent type	Particle size	Surface Area (m ² /g)		Pore Volume (cc/g)			Pore Radius D _p (r) (Å)		
		Multi Point BET (Brunauer–Emmett – Teller method)	DFT Cumulative	HK (Horvath – Kawazoe method)	SF (Saito and Foley method)	DFT Cumulative	HK (Horvath – Kawazoe method)	SF (Saito and Foley method)	DFT Cumulative
Coconut shell AC 1	> 1.18 mm	607.60	612.40	0.4161	0.4133	0.3926	1.84	1.75	4.29
Coconut shell AC 2	1.18 mm - 600 um	996.49	649.60	0.3899	0.3473	0.3699	3.39	5.58	5.38
Coconut shell AC 3	600 um - 212 um	1068.39	873.30	0.3963	0.3658	0.3379	3.44	5.86	5.38
Coconut shell AC 4	< 212 um	1108.42	972.90	0.4323	0.3874	0.4181	2.91	5.08	8.44
Wood AC 1	< 5 mm	286.50	377.20	0.1208	0.0695	1.4880	1.84	2.26	18.14
Wood AC 2	< 212 um	991.90	946.50	0.4575	0.4233	0.7848	1.84	1.75	8.44
Porous polymer 1	100 um - 80 um	612.00	459.70	0.2494	0.1752	0.7429	1.84	2.26	8.07
Porous polymer 2	80 um - 50 um	525.90	394.50	0.2104	0.1440	0.6997	1.84	2.26	8.44

Volume of a glyphosate particle is 0.165 nm³ and spherical diameter is 0.681 nm. So, a variety of adsorption materials with different surface area, pore volume and pore radius were tested.

The data on surface area and pore structural properties of the selected adsorbents were tested and stated in Table 4.1. The coconut shell based activated carbons was pulverised and sieved to get four submicron-sized coconut shell based activated carbons with different pore distributions and same surface functional groups. Coconut shell based activated carbon surface area has increased with the reduction of particle size. Two (a granular type and a finely powdered submicron-sized) wood based activated carbons, and two porous polymers were also selected.

4.2.1.2 SEM and EDX analysis

Figure 4.1 SEM images of (a) Coconut shell AC 1; (b) Coconut shell AC 2; (c) Coconut shell AC 3; (d) Coconut shell AC 4

SEM images of the four, coconut shell activated carbons with different particle distribution and surface area which were used in the analysis on the effect of different pore sizes are as shown in Figure 4.1. The SEM images of carbon's surface morphology and shape, size of the pores has been depicted in Figure 4.1. The results for the EDX analysis of the surface of carbons is illustrated in Figure 4.2, which proved conclusively that majority of the surface is consisted

of C and O atoms. Figure 4.3 and Figure 4.4 summarise the SEM and EDX analysis on two wood activated carbons and two porous polymers which were also used in the adsorption tests.

Figure 4.2 EDX analysis of (a) Coconut shell AC 1; (b) Coconut shell AC 2; (c) Coconut shell AC 3; (d) Coconut shell AC 4

Figure 4.3 SEM images of (a) Wood AC 1; (b) Wood AC 2; (c) Porous polymer 1; (d) Porous polymer 2

Figure 4.4 EDX analysis of (a) Wood AC 1; (b) Wood AC 2; (c) Porous polymer 1; (d) Porous polymer 2

4.2.2 Adsorption behaviour

In this experiment 40 mL volume samples of glyphosate 30 mg/L initial concentrations were mixed with fixed adsorbent dosage of 1 g/L. Solutions with each adsorbent were maintained at 1, 2, 4, 8, 16, 24, and 48 h to measure the glyphosate adsorption rate onto the surfaces of the adsorbents. Sorption kinetics were studied in order to analyse the effect of pore distributions

on glyphosate uptake. The residue glyphosate concentration variation for each adsorbent is depicted in Figure 4.5.

4.2.2.1 Adsorption kinetics

Figure 4.5 Equilibrium concentrations, C_e (mg/L) of (a) glyphosate and (b) AMPA

Figure 4.6 Sorption kinetics for glyphosate removal by adsorbents of different pore sizes: (a) pseudo-first order (b) pseudo-second order kinetic models; Conditions: adsorbent dosage= 1 g/L, pH = 6.0 ± 0.1 , initial concentration = 30 mg/L, T = $35 \pm 1^\circ\text{C}$

The experimental data on the sorption of glyphosate onto four coconut shell activated carbons, two wood activated carbons and two porous polymers; kinetic studies using pseudo-first order model and pseudo-second order model are shown in Figure 4.6. The sorption of glyphosate onto coconut shell activated carbon was fast, where the rest of the adsorbents followed relatively a slower process. The coconut shell activated carbon showed fast kinetics due to high affinity of imprinted cavities which allows the glyphosate molecules to easily access to the binding sites on the adsorbent [272], in comparison with that of other adsorbents. To better understand the sorption kinetics of glyphosate on carbon and polymer adsorbents, the sorption kinetic data were further described using intraparticle diffusion model.

4.2.2.2 Pseudo-first/second order model

Table 4.2 Sorption kinetic constants obtained by different models

Adsorbent type		Coconut shell AC 1	Coconut shell AC 2	Coconut shell AC 3	Coconut shell AC 4	Wood AC 1	Wood AC 2	Porous polymer 1	Porous polymer 2
Pseudo-first order model	Q_e	0.762	0.753	0.866	0.863	0.405	0.847	0.193	0.232
	k_1 (1/h)	0.220	0.470	0.208	0.207	0.061	0.286	0.073	0.123
	R^2	0.884	0.910	0.928	0.931	0.947	0.767	0.834	0.855
Pseudo-second order model	Q_e	0.628	0.548	0.5701	0.979	0.404	0.621	0.391	0.536
	k_2 (1/h)	1.898	2.687	2.564	0.854	1.491	2.267	1.026	0.595
	R^2	0.970	0.962	0.995	0.979	0.784	0.957	0.973	0.540

As shown in Figure 4.6, the experiment data are better fitted the pseudo-second order model than the pseudo-first order model for coconut shell activated carbon, for wood activated carbon and for one porous polymer adsorbent. The R^2 value for pseudo-second order model

is higher than that for pseudo-first order model, indicating multilayer chemisorption. All the calculated data for both pseudo-second order model and pseudo-first order model is recorded in Table 4.2.

4.2.2.3 Intraparticle diffusion model

In this study, due to the porous structure of the selected adsorbents the intraparticle pore diffusion model based on the Fick's Law was employed to describe the adsorption kinetics which is depicted in Figure 4.7.

Figure 4.7 Pore diffusion model; Conditions: adsorbent dosage= 1 g/L, pH = 6.0 $\pm$ 0.1, initial concentration = 30 mg/L, T = 35 $\pm$ 1°C

The model is fairly deviated from the experimental data for polymer-based adsorbents. But the coconut shell activated carbon fitted the model well indicating that the glyphosate sorption onto activated carbon can be controlled by the intraparticle pore diffusion mechanism. According to the adsorption behaviour calculations, finely powdered coconut shell activated carbon (Coconut shell AC 4) and the finely powdered wood based activated carbon (Wood AC 2) types effectively removed aqueous glyphosate. Several studies about adsorption of organics in aqueous environment with both submicron-sized activated carbon

and granular activated carbon were published recently. They have found submicron-sized activated carbon removed natural organic compounds much faster than granular activated carbon. Furthermore, submicron-sized activated carbon was less sensitive to the competition from natural organic compounds. The experimental data in this study confirms the above hypothesis. The coconut shell activated carbon (Coconut shell AC 1) was pulverised to get activated carbons with different pore distributions (Coconut shell AC 2, Coconut shell AC 3, Coconut shell AC 4). The pulverized submicron-sized coconut shell activated carbon showed better adsorption efficiency of the aqueous glyphosate. Hence, pulverization has improved the adsorption of the small molecular organic pollutants. Surface area in the range of 1108.42-991.90 m²/g and pore radius in the range of 2.9130-1.8380 Å is favourable for a better glyphosate removal.

4.3 Effect of surface characteristics

4.3.1 Fourier Transform Infrared Spectroscopy study

According to the adsorption behaviour with pore structural properties, finely powdered coconut shell activated carbon (Coconut shell AC 4) effectively removed aqueous glyphosate. The adsorption behaviour with surface characteristics, for effective removal of aqueous glyphosate is studied. The FTIR spectroscopy data of the virgin and after adsorption test glyphosate-loaded coconut shell activated carbon are shown in Figure 4.8. A sharp absorption peak correspond to the stretching vibrations of carbonyl double bond ($\nu_{\text{C=O}}$) at 1715 cm⁻¹ and a weak peak corresponding to carbon-oxygen single bond ($\nu_{\text{C-O}}$) in carboxyl groups at 1248 cm⁻¹ is observed in the virgin coconut shell activated carbon [273]. In the FTIR spectrum after adsorption test glyphosate-loaded coconut shell activated carbon, both above absorption peaks showed an obvious shift in the peak position. Both carbon-oxygen single bond bands and carbonyl double bond band has shifted to different frequencies. Representing the complexation of glyphosate to C-O and C=O bonds, the carbon-oxygen single bond band shifts to a lower frequency at 1242 cm⁻¹; whereas the carbonyl double bond stretching band exhibits a clear shift to a lower frequency at 1618 cm⁻¹. These results suggest a typical carboxylic adsorption.

Figure 4.8 Activated carbon FTIR analysis on functional groups

Another notable change can be observed on the surface of activated carbon, in C = O with respect to carboxylic acid, C = C/C $\equiv$ C, C – H, C – O with respect to esters, and C – H with respect to ketones. The peak respective to sp^3 carbons occurs around 2960 - 2850 cm^{-1} in a very small range which is subjected to change in its position depending on the subgroup it's attached with; such as terminal alkene, aldehyde or ketone. There are two components to be checked in determining the carboxylic acid. 1. The peaks which occur respective to O – H occurs around 3500 - 3600 cm^{-1} and 2. The peaks which occur at 1250-1700 cm^{-1} respective to C= O stretch. Together these peaks indicate that carboxylic acid is available in the surface of activated carbon in before adsorption before washing, before adsorption after washing and after adsorption stages. According to data, the intensity of carboxylic acid increases after adsorption process. Alkynes have variable and sharp peaks around 2260-2000 cm^{-1} which can be distinguished clearly in the surface of activated carbon before adsorption process which is not available after adsorption process. Aldehydes have a strong peaks respect to C = O around 1740-1720 cm^{-1} and C – H around 2900-3000 cm^{-1} which depict that aldehydes can be found on the surface of activated carbon before adsorption tests. The C – H stretch at 2981 cm^{-1} with C = O stretch

at 1752 cm^{-1} and C - O at 2555 cm^{-1} in the surface of activated carbon indicate of formation of saturated esters during the adsorption process.

4.3.2 X-ray Photoelectron Spectroscopy study

Figure 4.9 XPS analysis of activated carbon (a) C1s before adsorption test (b) O1s before adsorption test (c) C1s after adsorption test (d) O1s after adsorption test

The XPS wide scan spectra of the virgin and after adsorption test glyphosate-loaded coconut shell activated carbon are shown in Figure 4.9. The high resolution C1s and O1s spectra of the virgin and glyphosate-loaded carbon are shown in Figure 4.9. The wide scan XPS spectra shows that the main elements in the virgin adsorbent C and O. The C1s spectrum of virgin carbon comprises four peaks with binding energy of 284.69, 285.82, 286.40 and 288.47 eV, identified via deconvolution. These peaks can be assigned to C-C, C-O-H, C=C and C-H, representing the different chemical status of carbon atoms in the carbon [274, 275] attributed to alcohol, ether, and carboxyl groups. After the glyphosate adsorption, the peak of C=O bond shows a clear shift to a higher binding energy at 288.87 eV. This result reveals that the uptake of glyphosate. Additionally, the peak of C-O-H bond

also exhibits a small shift to a higher frequency after adsorption, indicating the involvement of alcohol groups in the uptake of glyphosate.

In the O1s spectrum of the virgin adsorbent, different functional groups decomposed into several component peaks overlapped on each other is shown in Figure 5.9. The peaks at binding energy of 532.21 eV can be assigned to the C-O or O-H (alcohol or ether groups) and 531.41 can be assigned to the C=O (carboxyl groups) [274, 275]. It is observed that the binding energy has shifted to the higher energy at 531.91 and 532.51 eV, for both C=O and C-O peaks respectively, due to the decrease of the electron density of the O atoms bound with glyphosate. From these results, it can be confirmed that both hydroxyl groups and carboxyl are associated with the uptake of glyphosate.

4.3.3 Energy Dispersive X-ray spectroscopy study

Figure 4.10 EDX analysis of activated carbon (a) before adsorption, (b) after adsorption

The results for the EDX analysis of the coconut shell adsorbent before and after adsorption are as illustrated in Figure 4.10, which proved conclusively that majority of the adsorbent is consist of C and O atoms. It contains little amount of Al in the surface. In Figure 5.8 The zeta potential of the activated carbon suspension is given as a function of pH. The data suggest that the solids are negatively charged all over the pH range with a decrease in the

negative charge in the 8-9 pH range. In neutral solutions the net charge could be negative as a result of the neutrally charged surface is balanced by the values of the negative charges on the edges. In alkaline solutions the OH⁻ groups are adsorbing on the edges leaving the particle with a net negative charge. Addition of aluminium reduces the negativity due to high positive charge Al³⁺ and high surface activity leading to the observed increase in the value of the zeta potential.

4.4 Conclusions

Both pseudo-first order and second-order models described the adsorption kinetics well. According to adsorption kinetics data, finely powdered wood-based activated carbon and coconut shell based activated carbon reached equilibrium quickly. The uptake with polymer adsorbents were slow. The batch sorption experiments revealed that finely powdered coconut shell based, and wood-based activated carbon types were effective for glyphosate sorption. Surface area in the range of 1108.42-991.90 m²/g and pore radius in the range of 2.9130-1.8380 Å is favourable for a better glyphosate removal. Pulverization improved adsorption of the glyphosate with coconut shell based activated carbon. In the XPS study, the peak of the C=O bond shows a clear shift to higher binding energy at after adsorption tests indicating the glyphosate adsorption. The FTIR study revealed that the surface hydroxyl and carbonyl groups played an important role in the sorption of glyphosate. The powdered form of carbons with surface hydroxyl and carbonyl groups could be a promising adsorbent for efficient removal of glyphosate from aqueous solution.

CHAPTER 5

GLYPHOSATE ADSORPTION: EFFECTS OF ADSORPTION PROCESS OPERATION PARAMETERS

5.1 Background

Different adsorption process operation parameters also affect the adsorption process. The major factors affecting glyphosate removal with adsorption process include; operating pH, initial glyphosate concentration, temperature, adsorbent dose, and contact time. This study is comparing a coconut shell activated carbon (Coconut shell AC 4 which performed well in Chapter 4: Glyphosate adsorption: effects of adsorbent surface characteristics) with ferrous loaded wood biochar for glyphosate removal from aqueous solutions under various experimental conditions. Lack of information on the effect of glyphosate-metal complexation on the glyphosate adsorption process hinders implementation of a successful treatment method to remove dissolved glyphosate. In recent research, metal oxides and activated carbon loaded with metal ions have been experimented to increase the uptake of glyphosate during adsorption. Meanwhile, various types of magnetic porous polyimides containing Fe_3O_4 cores [106-108] and iron loaded porous carbon materials [109] have been reported in recent years in the application of removal of contaminants in wastewater. Thus, iron loaded biochar was used with coconut shell activated carbon on glyphosate adsorption to have a deeper understanding on influence of Fe^{2+} metal ions on the dissolved glyphosate to amplify the use of technology on the removal of glyphosate. The traditional method of varying one variable while keeping the rest constant can be time-consuming and it does not account for the effect the variables have on each other [33]. Statistical optimisation using response surface methodology can possibly provide a better approach to building the model

[276, 277], designing the experimental plan and in evaluating and optimising the experimental data [34]. Response surface methodology is a statistical technique, in which the three factorial Box-Behnken method is applied to determine the treatment configuration which is potentially low-cost but with high efficiency when operated with the selected pH, initial glyphosate concentration, temperature, adsorbent dose, and contact time. The data were fitted to a second-order polynomial model and statistically validated by analysing the percent contribution with Pareto Analysis techniques and performing the variance test. Together these form a novel operational parameters combination. The objectives of this study are; (1) to develop a method which can be used to quickly identify the combination of operating parameters to improve removal efficiency of glyphosate from aqueous solution and (2) to understand the influence of Fe^{2+} metal ions on the dissolved glyphosate adsorption.

5.2 Characterization of adsorbent material

According to the previous study, surface area in the range of 1108.42-991.90 m^2/g and pore radius in the range of 2.9130-1.8380 Å is favourable for a better glyphosate removal. The data on surface area and pore structural properties (see Table 5.1) indicated coconut shell activated carbon surface area and pore volume were twice that of ferrous loaded wood biochar's. The activated carbon and biochar SEM images are shown in Figure 5.1. The surface of the activated carbon was smooth, and its pores were within the micropore range (see Table 5.1). Biochar had an irregular multilayer lamellar structure.

Figure 5.1 SEM images of (a) coconut shell activated carbon; (b) ferrous loaded wood biochar; before adsorption

Table 5.1 Pore structural properties and elemental composition of adsorption materials

Micropore analysis								
	Surface Area		Pore Volume (cc/g)			Pore Radius D _v (r)		
	(m²/g)					(Å)		
	MultiPoint							
	BET	DFT	HK	SF	DFT	HK	SF	DFT
	(Brunauer– Emmett– Teller method)	Cumulative	(Horvath- Kawazoe method)	(Saito and Foley method)	Cumulative	(Horvath- Kawazoe method)	(Saito and Foley method)	Cumulative
Coconut shell activated carbon	1108.43	972.90	0.43	0.39	0.42	2.91	5.08	4.30
Ferrous loaded wood biochar	594.20	561.90	0.26	0.21	0.34	2.96	1.75	4.30
Elemental analysis								
	C %	H %	N %	S %	O %	Ash Content %		
	(wt./wt.)	(wt./wt.)	(wt./wt.)	(wt./wt.)	(wt./wt.)	(wt./wt.)		
Coconut shell activated carbon	90.80 ± 0.87	1.91 ± 0.28	0.22 ± 0.00	0.99± 0.00	4.58	1.49 ± 0.26		
Ferrous loaded wood biochar	72.71 ± 0.50	2.10 ± 0.17	0.52 ± 0.10	0.99 ± 0.00	15.97	8.70 ± 0.18		
Electrophoresis mobility analysis (Temp. @25 °C)								
	Electrophoretic Mobility (µmcm/Vs)				Conductivity (mS/cm)			
Coconut shell activated carbon	-1.30 ± 0.43				11.51 ± 0.31			
Ferrous loaded wood biochar	-1.26 ± 0.78				11.54 ± 0.69			

Surface physical and chemical properties of the carbons on adsorption has been vigorously discussed in chapter 4.

5.3 Adsorption behaviour

5.3.1 Adsorption kinetics

Kinetic data was fitted to the pseudo-first-order model, pseudo-second-order model and Weber-Morris internal particle diffusion model, to elucidate the adsorption process and rate limiting step. The equations used are as follows;

$$\text{pseudo first order model; } \log(q_e - q_t) = \log(q_e) - \frac{k_1 \times t}{2.303} \quad (2)$$

$$\text{pseudo second order model; } \frac{t}{q_t} = \frac{1}{k_2 \times q_e^2} + \frac{t}{q_e} \quad (3)$$

$$\text{Weber-Morris internal particle diffusion model; } q_t = k_{id} \times t^{0.5} + c_i \quad (4)$$

where k_1 , k_2 and k_{id} were pseudo-first-order kinetic (min^{-1}), pseudo-second-order kinetic ($\text{g/mg} \times \text{min}$) and internal particle diffusion ($\text{mg/g} \times \text{min}^{-0.5}$) constants. q_e and q_t are adsorption capacities at time t and at equilibrium (refer Figure 5.2 for the pseudo-first order and pseudo-second-order kinetic plots).

Figure 5.2 Plots of (a) Pseudo-first-order model and (b) Pseudo-second-order model for coconut shell activated carbon and ferrous loaded wood biochar for the adsorption of glyphosate

Table 5.2 Kinetic parameters and isotherm constants for the adsorption of glyphosate onto coconut shell activated carbon and biochar

Kinetic model	Carbon type	Parameters				
		Co (mg/L)	q _e (mg/g)	k _i (1/min)	R ²	
Pseudo-first-order model	Coconut shell activated carbon	10	0.7669	0.1114	0.8997	
	Ferrous loaded wood biochar	10	0.8664	0.1471	0.7982	
		Co (mg/L)	q _e (mg/g)	k ₂ (g/mg*min)	R ²	
Pseudo-second-order model	Coconut shell activated carbon	10	0.7669	0.5812	0.9976	
	Ferrous loaded wood biochar	10	0.8664	0.1581	0.9997	
Isotherm model	Langmuir			Freundlich		
	K _L (L/mg)	Q _m (mg/g)	R ²	K _F (mg ⁿ⁺¹ *g*L ⁿ)	n	R ²
Coconut shell activated carbon	0.0861	1.0549	0.0610	0.0941	1.0873	0.8110
Ferrous loaded wood biochar	0.0451	1.1645	0.1238	0.2558	0.9772	0.9567
Weber-Morris intraparticle diffusion model	I		II		III	
	Y (I)	R ²	Y (II)	R ²	Y (III)	R ²
Coconut shell activated carbon	0.085x + 0.4372	0.9024	0.0536x + 0.5052	0.9995	-	-
Ferrous loaded wood biochar	0.0994x + 0.6907	1	0.0507x + 0.7187	1	0.0198x + 0.7728	0.9672

In this experiment 40 mL volume samples of glyphosate 10 mg/L initial concentrations were mixed with fixed adsorbent dosage of 1 g/L. Solutions with each adsorbent were maintained at 1, 2, 4, 8, 16, 24, and 48 h to measure the glyphosate adsorption rate onto the surfaces of the adsorbents. Sorption kinetics were studied in order to analyse the effect of pore distributions on glyphosate uptake. The adsorption rate for glyphosate with coconut shell activated carbon and ferrous loaded wood biochar was determined. Biochar was significantly faster in

reaching equilibrium compared to activated carbon. The times taken for equilibrium to be reached were 15 h and 24 h for biochar and activated carbon respectively. Similar equilibrium times have been reported for biochar derived from rice husk [89].

Figure 5.3 (a) Weber-Morris intraparticle diffusion plot; (b) Adsorption isotherms of coconut shell activated carbon and ferrous loaded wood biochar for the adsorption of glyphosate

Initially, rapid adsorption could be observed in the system due to electrostatic attraction and the availability of active sites on the surface. Saturation of available adsorption sites in the ferrous loaded wood biochar with time then led to an equilibrium state. According to the obtained values in Table 5.2, pseudo-second-order model's regression coefficient was better ($R^2 > 0.99$) than that for the pseudo-first-order model. This suggested the adsorption could have involved chemical reaction [26]. It was also noted that for coconut shell activated carbon, k_2 (pseudo-second-order kinetic constant) was inversely related to the initial glyphosate concentration (C_0) 10 mg/L. k_2 decreases as the initial solution concentration increases, since it takes a longer time to reach equilibrium. This could have happened due to intermolecular collisions between glyphosate molecules in the solution because of the high concentrations and which then delayed molecules reaching the active sites on the adsorbent [278]. For the activated carbon, the Weber-Morris particle diffusion curve (see Figure 5.3(a)) could be divided into two sections when considering the linear segment. None of the lines passed through the origin and so indicating there were two rate-limiting steps (see Table 5.2 for the respective equations). The phenomenon is explained as below based on the results by the kinetic and isotherm data. In the first step, the rate was

limited by external film diffusion where molecules crossed the boundary layer to reach the adsorbent surface. In the second step, the rate dropped due to inter-particle resistance arising from surface fouling at the sorbent's interface with the sorbate solution. In ferrous loaded wood biochar, three rate controlling steps were observed (see Figure 5.3(a)). The third step with the slower removal rate would have been due to pore diffusion [279].

The absence of this step in activated carbon suggested the biochar had pores which were smaller in size compared to activated carbon's [279]. An increase of c_i (the intercepts of the Weber-Morris intraparticle diffusion model's equations I, II and III in Table 5.2) with time indicated an increase in the boundary layer thickness with time. This suggested the need to consider resistance in the external film at high glyphosate concentrations.

5.3.2 Adsorption isotherms

The adsorption equilibrium data were fitted to Langmuir and Freundlich isotherm models. The equations used are as follows:

$$\text{Langmuir model; } Q_e = \frac{Q_m \times K_l \times C_e}{1 + K_l \times C_e} \quad (5)$$

$$\text{Freundlich model; } Q_e = K_f \times C_e^{\frac{1}{n}} \quad (6)$$

where K_l and K_f are respectively Langmuir constant (L/mg), Freundlich constant ((mgⁿ⁺²*Lⁿ). $\frac{1}{n}$ represent the adsorption intensity. C_e and C_s are equilibrium concentration (mg/L) and the solubility limit (mg/L) respectively. Adsorption isotherms for the two adsorbents are plotted in Figure 5.3(b). Adsorption of glyphosate on both ferrous loaded wood biochar and coconut shell activated carbon were well described by the Freundlich isotherm ($R^2 > 0.8$). This indicated the adsorption was a multilayer process. Biochar had performed better than activated carbon with a maximum capacity of 0.2558 mgⁿ⁺²*Lⁿ and with $\frac{1}{n} > 1$ (see Table 5.2 for Langmuir and Freundlich models).

5.4 Response surface methodology

During the study, the Box-Behnken method was used to optimise operating parameters such as; pH, initial glyphosate concentration (mg/L), temperature (°C), adsorbent dose (g/L), and contact time (h) to maximise removal capacity and efficiency. The series of tests and levels

of independent variables with coded and un-coded values for glyphosate adsorption with coconut shell activated carbon and ferrous loaded wood biochar are in Table A1. For the aforementioned 5 independent parameters, 18 centre-points and 46 base runs were required to build the box. As each sample was in triplicate, the total number of runs required for the analysis was then 138. The sample solutions which were used in the adsorption tests were prepared to match the conditions at each of these points and the residue glyphosate concentrations in the solution after experiment for each set of conditions was then measured and recorded. Response surface calculation is further explained under Chapter 3: Materials and methods.

5.4.1 Effect of initial glyphosate concentration

Figure 5.4 RSM plots. Contour plot and the 3D surface graph of glyphosate removal (a) coconut shell activated carbon; (b) ferrous loaded wood biochar; showing the effect of contact time and initial glyphosate concentration

The combined effect of initial glyphosate concentration and contact time when adsorbent dosage, pH and temperature were fixed at 25.5 g/L, 6.5, and 35.0 °C for ferrous loaded wood biochar and coconut shell activated carbon is shown in Figure 5.4. The percentage removal of glyphosate had increased when the initial glyphosate concentration was increased from 0.2 mg/L to 20.0 mg/L. This was the capacity effect. When glyphosate molecules concentration in solution increased, the number of effective collisions of

glyphosate molecules with the adsorbent increased, leading to an increase in uptake. However, when the initial concentration was further increased to 50.0 mg/L, the removal percentage dropped. This indicated exhaustion of available surface on the sorbent. Similar behaviour when glyphosate was adsorbed onto forest soil had been observed [257], with the attendant decrease in k_{id} (distribution coefficient) - suggesting a depletion of active sites at higher concentrations limiting further sorption.

5.4.2 Effect of sorbent dose

Figure 5.5 RSM plots. Contour plot and the 3D surface graph of glyphosate removal (a) coconut shell activated carbon; (b) ferrous loaded wood biochar; showing the effect of contact time and adsorbent dosage

The effect of contact time and adsorbent dosage on glyphosate removal when other parameters such as initial glyphosate concentration, pH and temperature were fixed at 25.1 mg/L, 6.5 and 35.0 °C is shown in Figure 5.5. The percentage removal of glyphosate increased with increasing adsorbent dosage until 30.0 g/L. The active sites and hence surface area suitable for the removal of glyphosate is proportional to the added adsorbent amount. Thus, with the increase of adsorbent dose, the active sites and surface area suitable for adsorption of glyphosate increases [280]. But glyphosate removal amount was not improved when the sorbent dose increased above 30 mg/L. This could be due to two reasons: (1) because other operating parameters were not optimised to fully utilize the adsorption

capacity of adsorbent available at the higher adsorbent dosage in comparison to lower adsorbent dosage [281] and (2) due to the interactions between char particles there may aggregate and so reducing the available adsorption sites [282].

5.4.3 Effect of pH

Figure 5.6 RSM plots. Contour plot of glyphosate removal (a) coconut shell activated carbon; (b) ferrous loaded wood biochar; showing the effect of contact time and operating pH, Contour plot of glyphosate removal (c) coconut shell activated carbon; (d) ferrous loaded wood biochar; showing the effect of contact time and operating temperature

pH is one of the crucial factors which can affect the adsorption process [283]. In Figure 5.6 (a and b), the synergistic effect of pH and contact time when initial glyphosate concentration, dosage and temperature were fixed at 25.1 mg/L, 25.5 g/L and 35.0 °C is depicted. Angularly shaped contours reveal pH had affected the adsorption of glyphosate by both ferrous loaded wood biochar and coconut shell activated carbon. In coconut shell activated carbon, maximum removal was recorded at the highest pH of 8.0, with uptake increasing with pH increase. Glyphosate has four main acid dissociation constants, pK_a 2.0,

2.6, 5.6, and 10.6 and is negatively charged from pH 4.5 onwards (see Figure 5.7 for the dissociation of glyphosate). Previous research [257] had argued protonation of functional groups on the surface of activated carbon would transform the active sites to be more positively charged when pH was increased. This would lead to attraction of the glyphosate molecules through electrostatic interactions [257]. The increase in adsorption observed with pH increase in activated carbon in this study was consistent with reports in the literature [257]. Figure 5.8 shows the zeta potential of the activated carbon at various pH levels. The zeta potential dropped as pH increased, until pH 6.5. From pH 6.5, zeta potential of activated carbon increased as pH increased. Thus, as the pH of the solution increased from 6.5, the negative charge on the glyphosate molecule and the positive charge on the activated carbon surface increased. This induced attraction between the adsorbent and adsorbate which resulted in a rise in removal of glyphosate with activated carbon at higher pH levels.

Figure 5.7 Dissociation of glyphosate in 0.005 mg/L solution with the variation of pH
(modelled using Visual MINTEQ)

Ferrous loaded wood biochar performed well at lower pH levels. A similar observation was reported by Herath et al. [89] for adsorption of glyphosate with rice husk derived biochar

where adsorption capacity had significantly dropped as pH increased. In contrast to coconut shell activated carbon, zeta potential of ferrous loaded wood biochar decreased with increase of pH (refer to Figure 5.8) and this resulted in glyphosate uptake dropping as pH increased. Thus, the removal of glyphosate with ferrous loaded wood biochar was higher at pH 5.

Figure 5.8 Zeta potential of the coconut shell activated carbon and ferrous loaded wood biochar measured at different pH level

5.4.4 Effect of temperature

Temperature has been reported to play an important role in the adsorption process [284] and glyphosate adsorption is exothermic [233]. In Figure 5.6 (c and d) the synergistic effect of temperature and time on the uptake of glyphosate at fixed initial glyphosate concentration, adsorbent dosage and pH of 25.1 mg/L, 25.5 g/L and 6.5 respectively is shown. Overall adsorption by both coconut shell activated carbon and ferrous loaded wood biochar were better at temperatures above 40 °C. In biochar, comparing with activated

carbon, the effect of temperature was significant. When observing the 3D plots (see Figure 5.9), biochar had higher removal efficiency at higher temperatures. The adsorption kinetics of biochar indicated occurrence of chemical adsorption. Glyphosate reacted with biochar to result in a number of by-products (this shall be reported in chapter 6 of this thesis). Due to these chemical reactions at the adsorbent - adsorbate interface during adsorption, better removal had resulted at higher temperatures.

Figure 5.9 3D surface graph of glyphosate removal (a) activated carbon; (b) ferrous loaded wood biochar; showing the effect of contact time and pH, 3D surface graph of glyphosate removal (c) activated carbon; (d) ferrous loaded wood biochar; showing the effect of contact time and temperature

5.4.5 The collective effect of all variables on the removal capacity

Optimisation of the parameters using response surface methodology provided five plots demonstrating the effect of individual factors on adsorption by ferrous loaded wood biochar and coconut shell activated carbon (see Figure 5.10) The derived empirical equations for coconut shell activated carbon and ferrous loaded wood biochar which were used in the response surface study are as Eq. (7) and (8). F-values and P-values obtained from analysis of variance (ANOVA) calculations and Pareto analysis (see Figure 5.11) were used in evaluating the quality of the applied model. P-value < 0.01 for both activated carbon model

and biochar model entailed that the coefficients in the models are jointly significant, validating the quality of the models used in the response surface calculations.

Figure 5.10 Optimisation the effect of contact time, operating pH, temperature, initial glyphosate concentration, adsorbent dose for (a) coconut shell activated carbon and (b) ferrous loaded wood biochar

For coconut shell activated carbon;

$$\begin{aligned}
 \text{Glyphosate conc. (mg/L)} = & 16.74 - 0.8174 * \text{Contact time (h)} + \\
 & 0.968 * \text{Initial glyphosate conc. (mg/L)} - 0.754 * \text{Adsorbent dosage (mg/L)} + 0.358 \text{ pH} - \\
 & 0.0449 \text{ Temperature (C)} + 0.033466 * \text{Contact time (h)} * \text{Contact time (h)} + 0.00112 * \text{Initial g} \\
 & \text{lyphosate conc. (mg/L)} * \text{Initial glyphosate conc. (mg/L)} + 0.01364 * \text{Adsorbent dosage (mg/L)} \\
 & * \text{Adsorbent dosage (mg/L)} - \\
 & 0.0306 * \text{pH} * \text{pH} + 0.000521 * \text{Temperature (C)} * \text{Temperature (C)} - \\
 & 0.01430 * \text{Contact time (h)} * \text{Initial glyphosate conc. (mg/L)} - \\
 & 0.00273 * \text{Contact time (h)} * \text{Adsorbent dosage (mg/L)} - \\
 & 0.01561 * \text{Initial glyphosate conc. (mg/L)} * \text{Adsorbent dosage (mg/L)} + \\
 & 0.00459 * \text{Contact time (h)} * \text{pH} + 0.001460 * \text{Contact time (h)} * \text{Temperature (C)} - \\
 & 0.00460 * \text{pH} * \text{Temperature (C)}
 \end{aligned} \tag{7}$$

For ferrous loaded wood biochar;

$$\begin{aligned}
 \text{Glyphosate conc. (mg/L)} = & 12.08 - 0.319 \text{ Contact time (h)} + \\
 & 0.766 * \text{Initial glyphosate conc. (mg/L)} - 0.807 * \text{Adsorbent dosage (mg/L)} + 0.11 \text{ pH} \\
 & + 0.0421 \text{ Temperature (C)} + 0.01645 * \text{Contact time (h)} * \text{Contact time (h)} - \\
 & 0.00174 * \text{Initial glyphosate conc. (mg/L)} * \text{Initial glyphosate conc. (mg/L)} \\
 & + 0.01505 * \text{Adsorbent dosage (mg/L)} * \text{Adsorbent dosage (mg/L)} - 0.0153 * \text{pH} * \text{pH} - \\
 & 0.001786 * \text{Temperature (C)} * \text{Temperature (C)} - \\
 & 0.00318 * \text{Contact time (h)} * \text{Initial glyphosate conc. (mg/L)} - \\
 & 0.00326 * \text{Contact time (h)} * \text{Adsorbent dosage (mg/L)} - \\
 & 0.01415 * \text{Initial glyphosate conc. (mg/L)} * \text{Adsorbent dosage (mg/L)} - \\
 & 0.0050 \text{ Contact time (h)} * \text{pH} - 0.00231 \text{ Contact time (h)} * \text{Temperature (C)} + \\
 & 0.00861 \text{ pH} * \text{Temperature (C)}
 \end{aligned} \tag{8}$$

Table 5.3 Optimum values of the process parameters for the maximum process efficiency

Process parameter	Contact time (h)	Initial glyphosate concentration (mg/L)	Adsorbent dosage (g/L)	Operating pH	Operating temperature (°C)	Maximum removal capacity (mg/g)	Adsorption efficiency (%)	Corresponding difference removal capacity (mg/g)
Coconut shell activated carbon	Best conditions	1.7	0.2	11.4	8.0	50.0	0.0173	98.45
	Normal conditions	0.5	0.2	12.4	6.5	30.0	0.0129	79.85
								0.0044
Ferrous loaded wood biochar	Best conditions	1.9	0.7	12.3	5.0	50.0	0.0569	100.0
	Normal conditions	0.5	0.2	12.1	6.5	30.0	0.0089	53.65
								0.0480

A maximum removal capacity of 0.0173 mg/g and efficiency of 98.45% was determined for coconut shell activated carbon at pH 8.0, 0.2 mg/L initial glyphosate concentration, 50.0 °C, 11.4 g/L adsorbent dose, and 1.7 h contact time. For ferrous loaded wood biochar, optimized parameters were pH 5.0, 0.7 mg/L initial glyphosate concentration, 50.0 °C, 12.3 g/L adsorbent dose, and 1.9 h contact time for 0.0569 mg/g maximum removal capacity and 100.00% efficiency. If in field applications, the operating temperature is 25 °C – 30 °C,

adsorbent amount added could be increased by 8% to increase the adsorption efficiency by 13%. According to the Perturbation plot, the relative importance of parameters influencing glyphosate removal is as follows;

initial glyphosate concentration > adsorbent dose > contact time > temperature > pH

Figure 5.11 Pareto charts of the standardised effects of (a), (b) activated carbon and (c), (d) ferrous loaded wood biochar (response is glyphosate final concentration (mg/L), $\alpha = 0.05$)

This study indicated that higher temperature and lower pH was better for glyphosate removal by ferrous loaded wood biochar. The optimal initial glyphosate concentrations calculated for coconut shell activated carbon and biochar were 0.2 mg/L and 0.7 mg/L respectively (see Table 4.3). However, in practice, the initial glyphosate concentration would likely be determined by the situation onsite. At a wastewater treatment facility where the influent glyphosate concentrations were likely higher compared to the situation at a water treatment plant, biochar could be positioned upstream for coarse adsorption and activated carbon positioned downstream to polish.

Table 5.4 Response surface regression calculations of the model

Analysis of Variance for coconut shell activated carbon					
Source	Degrees of freedom	Adjusted sums of squares	Adjusted mean square of the error	F-Value	P-Value
Model	9	2890.27	321.14	20.53	0.002
Linear	3	2176.33	725.44	46.37	0
Contact time (h)	1	48.49	48.49	3.1	0.139
Initial glyphosate conc. (mg/L)	1	1007.67	1007.67	64.41	0
Adsorbent dosage (mg/L)	1	1120.17	1120.17	71.6	0
pH	1	0.3759	0.3759	6.46	0.052
Temperature (C)	1	0.7527	0.7527	12.94	0.016
Square	3	278.9	92.97	5.94	0.042
Contact time (h)*Contact time (h)	1	46.02	46.02	2.94	0.147
Initial glyphosate conc. (mg/L) *Initial glyphosate conc. (mg/L)	1	1.78	1.78	0.11	0.75
Adsorbent dosage (mg/L) *Adsorbent dosage (mg/L)	1	247.58	247.58	15.82	0.011
pH*pH	1	0.0175	0.0175	0.3	0.607
Temperature (C)*Temperature (C)	1	0.0507	0.0507	0.87	0.394
2-Way Interaction	3	435.03	145.01	9.27	0.017
Contact time (h)*Initial glyphosate conc. (mg/L)	1	70.03	70.03	4.48	0.088
Contact time (h)*Adsorbent dosage (mg/L)	1	2.47	2.47	0.16	0.708
Initial glyphosate conc. (mg/L) *Adsorbent dosage (mg/L)	1	362.54	362.54	23.17	0.005
Contact time (h)*pH	1	0.0262	0.0262	0.45	0.532
Contact time (h)*Temperature (C)	1	0.2648	0.2648	4.55	0.086
pH*Temperature (C)	1	0.0428	0.0428	0.74	0.43
Error	5	78.22	15.64		
Lack-of-Fit	3	78.2	26.07	2371.13	0
Pure Error	2	0.02	0.01		
Total	14	2968.49			

Analysis of Variance for ferrous loaded wood biochar					
Source	Degrees of freedom	Adjusted sums of squares	Adjusted mean square of the error	F-Value	P-Value
Model	9	1920	213.333	11.28	0.008
Linear	3	1297.6	432.532	22.86	0.002
Contact time (h)	1	3.93	3.933	0.21	0.668
Initial glyphosate conc. (mg/L)	1	385.79	385.79	20.39	0.006
Adsorbent dosage (mg/L)	1	907.87	907.874	47.99	0.001
pH	1	0.3979	0.3979	2.73	0.159
Temperature (C)	1	5.4952	5.4952	37.76	0.002
Square	3	317.2	105.734	5.59	0.047
Contact time (h)*Contact time (h)	1	10.08	10.078	0.53	0.498
Initial glyphosate conc. (mg/L)	1	4.27	4.274	0.23	0.655
*Initial glyphosate conc. (mg/L)					
Adsorbent dosage (mg/L) *Adsorbent dosage (mg/L)	1	301.17	301.171	15.92	0.01
pH*pH	1	0.0044	0.0044	0.03	0.869
Temperature (C)*Temperature (C)	1	0.5961	0.5961	4.1	0.099
2-Way Interaction	3	305.2	101.733	5.38	0.051
Contact time (h)*Initial glyphosate conc. (mg/L)	1	3.46	3.46	0.18	0.687
Contact time (h)*Adsorbent dosage (mg/L)	1	3.53	3.533	0.19	0.684
Initial glyphosate conc. (mg/L)	1	298.21	298.207	15.76	0.011
*Adsorbent dosage (mg/L)					
Contact time (h)*pH	1	0.0311	0.0311	0.21	0.663
Contact time (h)*Temperature (C)	1	0.6652	0.6652	4.57	0.086
pH*Temperature (C)	1	0.1502	0.1502	1.03	0.356
Error	5	94.59	18.918		
Lack-of-Fit	3	94.33	31.443	240.03	0.004
Pure Error	2	0.26	0.131		
Total	14	2014.59			

5.5 Conclusions

Both ferrous loaded wood biochar and coconut shell activated carbon indicated multilayer adsorption of glyphosate. Higher temperatures (above 40 °C) resulted in better removal efficiency for both coconut shell activated carbon and ferrous loaded wood biochar, indicating the influence of other mechanisms in addition to physical adsorption, i.e. chemical adsorption. Adsorption efficiencies of glyphosate were better at lower pH levels for biochar, and at higher pH levels for activated carbon. This has implications for treatment plants in determining the level of pH of the influent to be adjusted. It is proposed to use biochar to remove glyphosate at the roughing stage; or for treatment of wastewaters contaminated with higher concentrations. Activated carbon can then positioned at the polishing stage.

CHAPTER 6

DECOMPOSITION OF DISSOLVED GLYPHOSATE DURING THE ADSORPTION PROCESS WITH FERROUS LOADED WOOD BIOCHAR

6.1 Background

In recent years, a growing number of emerging organic contaminants such as herbicide in the chemical industry and agricultural industry are discharged into the environment. Literature reported that these organic contaminants widely distributed in many aquatic environments [5]. Additionally, they were detected in the human body, including liver, reproductive and central nervous systems which might be damaged after long-time exposure [285]. Glyphosate can permeate the human placenta and thus can cause glyphosate induced teratogenesis [176] and other potential adverse impacts in cell development. Glyphosate can severely affect the metabolic processes and especially, kidney and liver [286]. Moreover, Glyphosate herbicide possesses two functional groups amino and carboxylate which bonds with metal ions, especially near neutral pH where they are deprotonated [87] placing in a privileged position among other herbicides due to its ability to coordinate as a tridentate ligand. The complexation reduces the herbicidal activity and increases the half-life of glyphosate. The adsorption of the herbicide to soil and plants is strongly swayed by cations attachment [78].

In recent years, novel treatment processes for removal has been extensively studied, such as biodegradation [285, 287], adsorption [233, 236, 288] and photocatalytic degradation [289-291]. Among them, activated carbon adsorption processes are one of the most frequently used treatment technologies in drinking water treatment due to its low initial cost and convenient application [240, 252]. Along with the complexity of organic

composition in real water, it is necessary to develop novel adsorbents which are capable of removing various contaminants from wastewater simultaneously. Adsorbents with surface area in the range of 1108.42-991.90 m²/g and pore radius in the range of 2.9130-1.8380 Å. Those have been identified as the most is favourable physical structural properties for a better glyphosate removal. In recent research, metal oxides and activated carbon loaded with metal ions have been experimented to increase the uptake of glyphosate during adsorption. Meanwhile, various types of magnetic porous polyimides containing Fe₃O₄ cores [106-108] and iron loaded porous carbon materials [109] have been reported in the application on the removal of contaminants in wastewater. Thus, in the previous study, iron loaded biochar was used with coconut shell activated carbon on glyphosate adsorption to have a deeper understanding on the influence of Fe²⁺ metal ions on the dissolved glyphosate to amplify the use of technology on the removal of glyphosate. As anticipated, ferrous loaded wood biochar performed better than coconut shell activated carbon indicating the influence of other mechanisms in addition to physical adsorption, i.e. chemical adsorption. Evidence suggested that during the adsorption experiments recorded in chapter 5, Fe²⁺ metal ions in the wood biochar tend to degrade dissolved glyphosate; forming by-products such as amino-methyl-phosphonic acid (AMPA), which are known to be more persistent [292] and more toxic [23] than glyphosate. Findings emphasize the importance of the exploration of glyphosate heterogeneous degradation, as glyphosate removal techniques such as adsorption can impair the water quality due to the formation of toxic by-products.

Several studies proclaimed degradation of glyphosate driven by biotic pathways mediated by bacteria and fungi [293], catalytic wet oxidation and photolysis [93, 294, 295]. Photocatalytic processes have been found effective in removing glyphosate in water [296]. Glyphosate has been evidenced to be sturdily adsorbed on iron oxides, but none has reported of probable degradation when in contact with iron. This study aims to investigate the degradation of dissolved glyphosate in contact with iron-loaded biochar. The influences of iron on the degradation of glyphosate are evaluated by two different methods. Firstly, the half-life of glyphosate degradation was measured in the presence and absence of iron cations. Additional experiments were also carried out to investigate the effect of the different forms of iron (i.e. Fe metal powder, Fe²⁺, Fe³⁺) on degradation. Intermediate identification was done using liquid chromatography-mass spectrometry and the reaction mechanism was identified.

Secondly, the major factors affecting degradation process for glyphosate include the initial glyphosate concentration, the biochar dosage, temperature, and pH was analyzed. In this study, the variations of residue AMPA concentration is used in response surface calculations due to several reasons; (1) AMPA is considered to be more persistent [292] and more toxic [23] than glyphosate and (2) AMPA is the main by-product of the degradation process. The pH of the solutions was regulated by buffering during the process. Box–Behnken design and percentage contribution with Pareto analysis techniques were used respectively in surface response and efficiency calculations to model the process conditions.

6.2 Characterization of ferrous loaded biochar material

Figure 6.1 XPS analysis of ferrous loaded biochar (a) C1s before contact with glyphosate (b) O1s before contact with glyphosate (c) C1s after contact with glyphosate (d) O1s after contact with glyphosate

XPS and EDX analysis was conducted on the ferrous loaded wood biochar to examine the quantity and the position of ferrous inside it. As Figure 6.1 and Figure 6.2 confirms, the existence of 2.1% weight percentage and 0.48% atomic percentage of ferrous on the surface of the biochar.

Figure 6.2 EDX analysis of ferrous loaded biochar before contact with glyphosate

6.3 Evidence of degradation of glyphosate in contact with ferrous

Figure 6.3 FTIR analysis on functional groups of ferrous loaded biochar before and after contact with glyphosate

Biochar was analysed before and after contact with glyphosate using Fourier Transform-Infrared Spectroscopy (FTIR) in Figure 6.3 and Figure 6.4. The observed N – H stretches occurring around 3500 - 3200 cm^{-1} together with C – N at 1100 cm^{-1} confirms of availability of amine at the surface of biochar after contact due to existence of glyphosate and AMPA on the biochar surface. Especially, O – H ligands were found in the surface of biochar which have been proven to attract carboxylic acids such as glyphosate. After glyphosate is attracted to O – H functional groups, at the surface biochar has induced a possible reduction of glyphosate. During the reduction process, a cleavage at the C – N bond has taken place to produce AMPA and a polymerisation of intermediates to form phosphonic acids in the process of degradation. This phenomenon is confirmed by residue glyphosate and residue AMPA concentrations recorded during the process.

Figure 6.4 FTIR analysis on functional groups of glyphosates when in contact with different forms of iron and ferrous loaded biochar

Cyclic voltammetry was used to analyse the electrochemical properties of glyphosate in aqueous solution. Figure 6.5 illustrate the cyclic voltammogram obtained in 0.1 M glyphosate aqueous solution. The voltage was changed over the pattern 0.7 V, -0.50 V, 0.75 V, 0.7 V with a scan rate of 0.15 V/s and potential was measured between working and the reference electrodes, while the current is measured between working and counter electrodes.

As Figure 6.5 depicts, during the initial forward scan, a reducing potential was applied to generate a cathodic current assuming that dissolved glyphosate is reducible. A cathodic peak at -0.42 V was attributed to the reduction of the glyphosate molecule at the negative scan, which confirms its tendency to be reduced. Re-oxidization of the reduced glyphosate was observed during the reverse scan resulting an anodic current of $18 \mu\text{A}$. When analyzing the difference between the two peak potentials;

$$\Delta E_p = E_{pa} - E_{pc} \approx 0.07 > 0, \text{ and } i_{pa}/i_{pc} \neq 1 \quad (9)$$

Figure 6.5 Cyclic voltammograms of 0.1 M glyphosate (0.7 V, -0.50 V, 0.75 V, 0.7 V)
Conditions: working electrode, 1 mm Pt planar disk; reference electrode, Ag/AgCl wire in 3 M KCl; counter electrode, Pt-wire; potential scan rate, 0.15 V/s

Eq. (9) depict that glyphosate wave is not 100% reversible which hints that system involves dissociation or association during the electron transfer. However, these observations explain that glyphosate is reducible while being dissolved in water and that the redox reaction is not reversible. The reaction is a redox reaction as the oxidation number of

phosphorus (P) in glyphosate drops from (+5) to (+3) when transforming from glyphosate to AMPA.

According to Jaisi et al [297], who suggests that C – N and C – P bond cleavage can happen at the surface of catalyst; provided that the preference for cleavage rest on glyphosate and catalyst ratios. There is a possibility of abiotic degradation pathway for glyphosate given that if there exist an ionic species to mediate the energy barriers opposing bond dissociation [297]. When examine Figure 6.4, Figure 6.5 and Figure 6.6, Fe^{2+} behaves similar to ferrous loaded biochar suggesting that Fe^{2+} in biochar is oxidising to reduce glyphosate. The ferrous in biochar would lean towards C–N bond cleavage to form AMPA. Suggested possible degradation pathway for glyphosate in the presence of water where the C – P [293, 298, 299] or C – N [297] bonds are cleaved by Fe^{2+} is as follows (see Figure 6.7).

Figure 6.6 (a) glyphosate concentration and (b) AMPA concentration change in contact with different forms of iron and ferrous loaded biochar

Figure 6.7 Glyphosate to AMPA conversion reaction

6.4 Glyphosate degradation efficiency and kinetic study

Figure 6.8 Glyphosate degradation efficiency and AMPA generation rate when in contact with ferrous loaded biochar

In Figure 6.8 and Figure 6.9 experiment 40 mL volume samples of glyphosate 30 mg/L initial concentrations were mixed with fixed porous carbon dosage of 1 g/L. The residue glyphosate concentration and the generated AMPA concentration are measured. The degradation of glyphosate when in contact with biochar is presented in Figure 6.8. As shown in Figure 6.8, the amount of AMPA generated is not equivalent to the amount of glyphosate removed which indicate that glyphosate 1. has been converted to other by-products apart from

AMPA or 2. produced AMPA has been adsorbed by biochar. Glyphosate which turned out to be the most resistant when in contact with inorganic ions in waters, was able to be significantly removed by application of ferrous loaded biochar. Nearly, 90% of the initial concentration levels can be degraded within 24 h in this study. The Fe/Biochar system exhibited excellent removal ability for the removing of dissolved glyphosate.

6.5 Optimisation of glyphosate degradation process

Figure 6.9 Glyphosate degradation and AMPA generation with; (a) initial glyphosate concentration, (b) ferrous loaded biochar dosage, (c) temperature, and (d) pH when in contact with ferrous loaded biochar

The major factors affecting degradation process for glyphosate include the initial glyphosate concentration, the biochar dosage, temperature, and pH is analysed in Figure

6.9. According to the data, higher initial glyphosate concentrations, biochar dosage and temperature favors the AMPA generation. Lower pH conditions promoted the AMPA generation.

The traditional method of varying one variable while keeping the rest constant (see Figure 6.9) can be time-consuming and it does not account for the synergistic effect the variables have on each other [33]. Statistical optimisation using response surface methodology can possibly provide a better approach to building the model [276, 277], designing the experimental plan and in evaluating and optimising the experimental data [34]. During the study, the Box-Behnken method was used to optimise operating parameters – such as; pH, initial glyphosate concentration (mg/L), temperature (°C), porous carbon dose (g/L), and contact time (h) to maximise removal capacity and efficiency. 5-factor Box-Behnken method was chosen according to the number of continuous variables needed to be analysed. The series of tests and levels of independent variables with coded and un-coded values for glyphosate degradation with ferrous loaded wood biochar are in Table A2. For the aforementioned 5 independent parameters, 18 centre-points and 46 base runs were required to build the box. As each sample was in triplicate, the total number of runs required for the analysis was then 138. The sample solutions which were used in the degradation tests were prepared to match the conditions at each of these points and the resulted AMPA concentrations in the solution after experiment for each set of conditions was then measured and recorded. Response surface calculation is further explained under Chapter 3: Materials and methods.

6.5.1 Effect of initial glyphosate concentration

The combined effect of initial glyphosate concentration and contact time when ferrous loaded biochar dosage, pH and temperature fixed at 25.5 g/L, 6.5, and 35.0 °C is shown in Figure 6.10. To study the effect of initial concentration of glyphosate, the range of initial glyphosate concentration was chosen at 0.2 mg/L – 50 mg/L. Figure 6.10(a) shows the AMPA generation without the influence of iron metal cations by using activated carbon with similar performance as biochar which doesn't contain iron. Figure 6.10(b) shows the AMPA generation with the influence of iron-loaded biochar. Experiments showed that the apparent reaction rate constant decreased with the increasing initial concentration of glyphosate. The increased initial glyphosate could occupy more active sites on the porous

carbon surface, thus suppress the contact between glyphosate and ferrous molecules. The decreased degradation rate also suggested that there could be a competition between the intermediates and glyphosate for the active sites on the surface of biochar [300]. The effect of initial organic pollutant concentration has also been proved by Chen and Ray [301]. Their reports found out that the reciprocal initial degradation rate decreased with reciprocal initial concentration increased from 10 ppm to 120 ppm.

Figure 6.10 RSM plots. Contour plot and the 3D surface graph of AMPA generation with (a) activated carbon and (b) ferrous loaded biochar; showing the effect of contact time and initial glyphosate concentration presenting the influence of Fe in glyphosate degradation

6.5.2 Effect of ferrous loaded biochar dosage

Gogate and Pandit [302] pointed out that the use of excessive catalyst may reduce the amount of energy being transferred into the medium due to the opacity offered by the catalyst particles. The effect of contact time and biochar dosage on glyphosate removal when other parameters such as initial glyphosate concentration, pH and temperature were fixed at 25.1 mg/L, 6.5 and 35.0 °C is shown in Figure 6.11. To investigate the effect of biochar loading on degradation of glyphosate, a series of biochar dosage ranging from 0.2 - 50 g/L was studied. In this study, the biochar first attracts the glyphosate molecules and

adsorb the molecules providing a platform for the reaction acting as the catalyst for the reaction. As shown in Figure 6.11, the rate constant increased with increasing dosage of biochar and later levelled off at the higher dosage of biochar. The increase of degradation efficiency can be explained as being due to the increase in total surface area of the catalyst, namely number of active sites available for the degradation reaction as the dosage of catalyst increased. This interpretation is relatively consistent with the experiments conducted by Wong and Chu [303]. However, with further increase in catalyst loading, the number of active sites on the biochar surface would almost become constant due to the loss in surface area occasionally by agglomeration at a high solid concentration. In addition, above a certain level the amount of substrate molecules would not be sufficient to fill the surface-active sites of biochar. Hence further addition of biochar did not lead to the enhancement of the degradation rate. The results were in good agreement with those reported in the literature [304, 305].

Figure 6.11 RSM plots. Contour plot and the 3D surface graph of AMPA generation with (a) activated carbon and (b) ferrous loaded biochar; showing the effect of contact time and porous carbon dosage presenting the influence of Fe in glyphosate degradation

6.5.3 Effect of pH

In Figure 6.12, the synergistic effect of pH and contact time when initial glyphosate concentration, biochar dosage and temperature were fixed at 25.1 mg/L, 25.5 g/L and 35.0 °C is depicted. To study the effect of pH, the range of pH was chosen at 6 – 8 to analyse the conditions which are similar to the waters in acidic farmlands. pH is a governing factor in the catalytic wet oxidation process. Several studies report the influencing trends of pH effect for different compounds degradation [306-308]. It can be seen that the degradation of glyphosate by ferrous is strongly pH-dependent. Acidity favour the formation of AMPA. In the controlled system, change in the pH levels on the degradation of glyphosate is insignificant.

Figure 6.12 RSM plots. Contour plot and the 3D surface graph of AMPA generation with (a) activated carbon and (b) ferrous loaded biochar; showing the effect of contact time and pH presenting the influence of Fe in glyphosate degradation

6.5.4 Effect of temperature

In Figure 6.13 the synergistic effect of temperature and time on the uptake of glyphosate and AMPA generation at fixed initial glyphosate concentration, biochar dosage and pH of

25.1 mg/L, 25.5 g/L and 6.5 respectively is shown. To investigate the effect of temperature on degradation of glyphosate, a series of temperatures ranging from 20 - 50°C was studied. AMPA generation is very much temperature-dependent that higher temperatures favour the degradation process.

Figure 6.13 RSM plots. Contour plot and the 3D surface graph of AMPA generation with (a) activated carbon and (b) ferrous loaded biochar; showing the effect of contact time and temperature presenting the influence of Fe in glyphosate degradation

6.5.5 The collective effect of all variables on the glyphosate degradation and AMPA formation

Optimisation of the parameters using response surface methodology provided five plots demonstrating the effect of individual factors on the degradation process (see Figure 6.14). The derived empirical equations for AMPA generation with activated carbon and iron-loaded biochar which were used in the response surface study are as Eq. (10) and (11). According to the data, higher initial glyphosate concentrations, biochar dosage and temperature favour the AMPA generation. Lower pH conditions promoted the AMPA generation. The higher AMPA concentrations were obtained when the operating conditions were at; operating pH 5.0, initial glyphosate concentration 0.7 mg/L, operating temperature 50.0 °C, biochar dosage 12.3 g/L, and contact time 1.9 h. At the best-operating conditions,

56.9% of glyphosate is degraded and 26.1% of degraded glyphosate is converted to AMPA. F-values and P-values obtained by analysis of variance (ANOVA) calculations (see Table 6.1) and Pareto analysis (see Figure 6.15) were used in evaluating the quality of the applied model. P-value < 0.01 for AMPA generation for both activated carbon and biochar models (see Table 6.1) entailed that the coefficients in the models are jointly significant, validating the quality of the models used in the response surface calculations.

Figure 6.14 Optimisation the effect of contact time, initial glyphosate concentration, ferrous loaded biochar dosage, operating pH, temperature for AMPA formation under the effect of ferrous

For activated carbon;

$$\begin{aligned} \text{AMPA conc. (ug/L)} = & -28.4 + 6.53 \text{ Contact time (h)} + 5.22 \text{ Init. Glyphosate conc. (mg/L)} \\ & - 1.72 \text{ activated carbon dosage (g/L)} + 13.99 \text{ pH} + 3.80 \text{ Temperature (C)} + 0.0836 \text{ Contact} \\ & \text{time (h)*Contact time (h)} - 0.0446 \text{ Init. Glyphosate conc. (mg/L)*Init. Glyphosate conc.} \\ & \text{(mg/L)} + 0.0499 \text{ activated carbon dosage (g/L)* activated carbon dosage (g/L)} + 0.1845 \\ & \text{Contact time (h)*Init. Glyphosate conc. (mg/L)} - 0.2419 \text{ Contact time (h)* activated carbon} \\ & \text{dosage (g/L)} - 0.0502 \text{ Init. Glyphosate conc. (mg/L)* activated carbon dosage (g/L)} + \\ & 0.0766 \text{ Contact time (h)*Temperature (C)} - 0.503 \text{ pH*Temperature (C)} \end{aligned} \quad (10)$$

For ferrous loaded biochar;

$$\begin{aligned} \text{AMPA concentration (ug/L)} = & 668.1 + 31.88 \text{ Contact time (h)} - 0.66 \text{ Init. Glyph. Conc.} \\ & \text{(mg/L)} + 4.85 \text{ Biochar dosage (g/L)} - 221 \text{ pH} + 2.58 \text{ Temperature (C)} - 0.666 \text{ Contact time} \\ & \text{(h)*Contact time (h)} - 0.0084 \text{ Init. Glyph. Conc. (mg/L) *Init. Glyph. Conc. (mg/L)} - 0.1036 \\ & \text{Biochar dosage (g/L) * Biochar dosage (g/L)} + 21.71 \text{ pH*pH} + 0.1882 \text{ Temperature} \\ & \text{(C)*Temperature (C)} + 0.7209 \text{ Contact time (h)*Init. Glyph. Conc. (mg/L)} - 0.2099 \text{ Contact} \\ & \text{time (h)* Biochar dosage (g/L)} + 0.0513 \text{ Init. Glyph. Conc. (mg/L)* Biochar dosage (g/L)} - \\ & 2.149 \text{ Contact time (h)*pH} + 0.5116 \text{ Contact time (h)*Temperature (C)} - 1.881 \\ & \text{pH*Temperature (C)} \end{aligned} \quad (11)$$

Figure 6.15 Pareto charts of the standardised effects of (a), (b) activated carbon and (c), (d) ferrous loaded biochar (response is AMPA concentration (ug/L), $\alpha = 0.05$)

Table 6.1 Response surface regression calculations of the model

Analysis of Variance for AMPA formation with activated carbon					
Source	Degrees of freedom	Adjusted sums of squares	Adjusted mean square of the error	F-Value	P-Value
Model	9	431159	47907	47.21	0
Linear	3	344839	114946	113.28	0
Contact time (h)	1	77760	77760	76.63	0
Init. Glyph Conc. (mg/L)	1	156104	156104	153.85	0
porous carbon dosage (g/L)	1	110975	110975	109.37	0
pH	1	473.3	473.3	3.35	0.08
Temperature (C)	1	7715.1	7715.1	54.66	0
Square	3	16727	5576	5.49	0.006
Contact time (h)*Contact time (h)	1	3451	3451	3.4	0.08
Init. Glyph Conc. (mg/L) *Init. Glyph Conc. (mg/L)	1	5636	5636	5.55	0.029
porous carbon dosage (g/L) * porous carbon dosage (g/L)	1	6635	6635	6.54	0.019
2-Way Interaction	3	69594	23198	22.86	0
Contact time (h)*Init. Glyph Conc. (mg/L)	1	23320	23320	22.98	0
Contact time (h)* porous carbon dosage (g/L)	1	38786	38786	38.22	0
Init. Glyph Conc. (mg/L) * porous carbon dosage (g/L)	1	7488	7488	7.38	0.013
Contact time (h)*Temperature (C)	1	1456.3	1456.3	10.32	0.004
pH*Temperature (C)	1	1025.6	1025.6	7.27	0.013
Error	20	20294	1015		
Lack-of-Fit	16	20180	1261	44.31	0.001
Pure Error	4	114	28		
Total	29	451453			

Analysis of Variance for AMPA formation with biochar					
Source	Degrees of freedom	Adjusted sums of squares	Adjusted mean square of the error	F-Value	P-Value
Model	9	1687937	187549	41.21	0
Linear	3	1262091	420697	92.44	0
Contact time (h)	1	419762	419762	92.23	0
Init. Glyp Conc. (mg/L)	1	813990	813990	178.85	0
porous carbon dosage (g/L)	1	28339	28339	6.23	0.021
pH	1	34081	34081	13.84	0.001
Temperature (C)	1	344830	344830	140.05	0
Square	3	32880	10960	2.41	0.097
Contact time (h)*Contact time (h)	1	6127	6127	1.35	0.26
Init. Glyp Conc. (mg/L) *Init. Glyp Conc. (mg/L)	1	198	198	0.04	0.837
porous carbon dosage (g/L) * porous carbon dosage (g/L)	1	28581	28581	6.28	0.021
pH*pH	1	17623	17623	7.16	0.015
Temperature (C)*Temperature (C)	1	13235	13235	5.38	0.032
2-Way Interaction	3	392966	130989	28.78	0
Contact time (h)*Init. Glyp Conc. (mg/L)	1	355921	355921	78.2	0
Contact time (h)* porous carbon dosage (g/L)	1	29214	29214	6.42	0.02
Init. Glyp Conc. (mg/L) * porous carbon dosage (g/L)	1	7830	7830	1.72	0.205
Contact time (h)*pH	1	11482	11482	4.66	0.044
Contact time (h)*Temperature (C)	1	65040	65040	26.41	0
pH*Temperature (C)	1	14330	14330	5.82	0.026
Error	20	91024	4551		
Lack-of-Fit	3	80375	26792	42.77	0
Pure Error	17	10648	626		
Total	29	1778961			

6.6 Intermediate identification and pathway determination

6.6.1 Degradation with Fe loaded biochar

Glyoxylate, Amino-methyl-phosphonic acid (AMPA) (Figure 6.16(a)), Bis-2-aminoethyl-amino-methyl phosphonic acid (Figure 6.16(b)), Amino-phosphonomethyl-amino-methyl phosphonic acid (Figure 6.16(c)), Hydroxymethyl-phosphonomethyl-amino-methyl phosphonic acid (Figure 6.16(d)), 4-Bis-phosphonomethyl-amino-1-butanol (Figure 6.16(e)), Hexylamino-bis-methylene bisphosphonic acid (Figure 6.16(f)), and Bis-phosphonomethyl-amino-phosphonomethyl-amino-methyl phosphonic acid (Figure 6.16(g)) were identified as the degradation intermediates during the degradation reaction of glyphosate (see Table 6.2 for the retention time, mass-to-charge ratio and structure of the detected compounds). The reaction pathway for the aqueous glyphosate degradation with ferrous (Fe^{2+}) in the wood biochar and for the reaction follows is developed as per Figure 6.17. Fe^{2+} degrades glyphosate to form AMPA through C – N bond cleavage. The formed AMPA further reacts to result Amino-phosphonomethyl-amino-methyl phosphonic acid. Amino-phosphonomethyl-amino-methyl phosphonic acid is converted to either Bis-phosphonomethyl-amino-methyl phosphonic acid or Hydroxymethyl-phosphonomethyl-amino-methyl phosphonic acid based on the sequence of reactions. Later is further converted to Bis-2-aminoethyl-amino-methyl phosphonic acid, 4-Bis-phosphonomethyl-amino-1-butanol and Hexylamino-bis-methylene bisphosphonic acid.

The degradation of glyphosate process initiates via the in-situ oxidation of ferrous (Fe^{2+}) reducing the phosphorus atom in glyphosate molecule. This attack leads to the generation of a series of phosphonic acids through polymerisation. However, along the reaction process, the area of the peak representing amino-methyl-phosphonic acid (AMPA) kept decreasing while the area of peaks such as Amino-phosphonomethyl-amino-methyl phosphonic acid kept increasing constantly. The equilibrium point of the compounds is changing with contact time and porous carbon dosage.

Table 6.2 Identified intermediates of glyphosate- ferrous loaded biochar reaction by LC/MS analysis

Parent		Intermediates		
Compound	Name	T _{ret} (sec)	m/z	Structure
Glyphosate	Glyoxylate	66.9	71
	Amino-methyl-phosphonic acid (AMPA)	75.4	96.9
Glyphosate	Bis-2-aminoethyl-amino-methyl phosphonic acid	75.4	195
	Amino-phosphonomethyl-amino-methyl phosphonic acid	75.4	217
	Hydroxymethyl-phosphonomethyl-amino-methyl phosphonic acid	75.4	233

Parent Compound	Intermediates			
	Name	T _{ret} (sec)	m/z	Structure
	4-Bis-phosphonomethyl-amino-1-butanol	66.9	273.1
	Hexylamino-bis-methylene bisphosphonic acid	74.5	288
Glyphosate	Bis-phosphonomethyl-amino-phosphonomethyl-amino-methyl phosphonic acid	66.9	403.1

Figure 6.16 LC/MS chromatograms of the intermediates in the reaction of glyphosate-ferrous loaded biochar

Figure 6.17 Reaction mechanism aqueous glyphosate with Fe (II) cation

6.7 Conclusion

The abiotic degradation of glyphosate was examined when dissolved glyphosate was treated using ferrous loaded wood biochar. Fe^{2+} mediates the energy barriers opposing bond dissociation leaning towards C–N bond cleavage to form AMPA; Fe^{2+} oxidise reducing glyphosate. Different factors affecting the degradation process for the Fe/Biochar system were investigated. The higher AMPA concentrations were obtained when the operating

conditions were at; operating pH 5.0, initial glyphosate concentration 0.7 mg/L, operating temperature 50.0 °C, biochar dosage 12.3 g/L, and contact time 1.9 h. The glyphosate degradation percentage and glyphosate: AMPA conversion was 56.9% and 26.1% respectively. At the best-operating conditions, glyphosate was degraded to non-detectable levels within 24 h. Overall, the degradation of glyphosate was significant at lower pH, and at temperatures above 40 °C. Further analysis on the degradation of glyphosate to AMPA revealed a polymerisation of intermediates forming complex phosphonic acids (i.e. Bis-2-aminoethyl-aminomethyl phosphonic acid, Amino-phosphonomethyl-aminomethyl phosphonic acid, Hydroxymethyl-phosphonomethyl-aminomethyl phosphonic acid, Hexylimino-bis-methylene bisphosphonic acid, Bis-phosphonomethyl-amino-phosphonomethyl-aminomethyl phosphonic acid) addition to AMPA. These phosphonic acids are known to be more persistent and more toxic than glyphosate.

Various types of magnetic porous polyimides containing Fe₃O₄ cores [106-108] and iron loaded porous carbon materials [109] have been reported in the application on the removal of contaminants in wastewater. According to this study, dissolved glyphosate is degraded when in contact with iron loaded biochar. Fe²⁺ metal ions in biochar amplify the degradation of dissolved glyphosate. Moreover, degradation of glyphosate by Fe²⁺ ions is catalysed porous carbons. This phenomenon has not been observed prior to this study. Degradation products were identified and quantified with a special focus on AMPA concentration fluctuations during the degradation of glyphosate in the presence of Fe²⁺ ions at different operating conditions. This degradation pathway carries both scientific and environmental significance. Especially in agricultural areas where the water contains Fe²⁺ ions and glyphosate; implementation of a drinking water treatment solution with carbon adsorbents may degrade glyphosate through different pathways in resulting relatively toxic by-products.

CHAPTER 7

DECOMPOSITION OF GLYPHOSATE IN AQUEOUS SOLUTIONS UNDER THE INFLUENCE OF COPPER METAL IONS

7.1 Background

Copper pollution has become an emerging environmental problem in agricultural areas [110]. Sprays containing copper have been applied as fungicides over 100 years [111] in vegetable crops, pome and stone fruit orchards and vineyards [112]. Copper was the main component in Bordeaux mixture (a mixture of copper, sulfate and lime), which has been used to control mildew in orchards for more than a century. 1.25 - 80 kg/ha of copper oxychloride is applied per season in vineyards [112, 113]. Copper not only affects the quality and quantity of crops; it can be threatening to the animal and human health [78, 118]. Foliar application of these copper-containing fungicides leads to a significant input of copper to the environment through drift and dripping off excess [114]. During the long-term application, copper accumulates and retains in waters and soil [115-117]. Further, copper possesses good catalysis ability, is prone to oxidation, and affects the behaviour of coexisting pesticides in the soil and water.

On the other hand, zinc is a vital element in plant growth [119] and has been widely applied in the agricultural industry. However, excessive amounts of zinc can be toxic especially to the aquatic bodies. Most agricultural soils contain zinc in the range of 10–300 mg kg⁻¹ [120] which is higher than copper and iron concentrations.

The interactions of organic compounds and metals in the environment are an emerging concern [309-311]. It is noteworthy that glyphosate is exposed not only to soil metallic ions

[93, 94] but also to metallic nozzles, metallic storage bins and mineralized water during spraying. However, there are few studies on the influence of copper and zinc on the fate of glyphosate in the environment [309, 312]. Hence, the effect of copper cations and zinc cations on glyphosate degradation is examined to facilitate the understanding of the potential interactions between glyphosate and copper and to determine an effective method to remediate glyphosate contaminated environment.

The influences of copper on the degradation of glyphosate is evaluated by two different methods. Firstly, the half-life of glyphosate degradation measured in the presence and absence of copper ions. Additional experiments were also carried out to investigate the effect of the different copper isomers on degradation. Intermediate identification was done using liquid chromatography-mass spectrometry and the reaction mechanism was identified. The degradation of glyphosate into AMPA is been reported to take place by biotic pathways [12, 313]. Strong interaction with metals could trigger glyphosate degradation into AMPA. AMPA is (1) the main degradation product of glyphosate [94, 298, 314], (2) toxic to some plant species [298, 314-316] and (3) is known to be more persistent and more toxic than glyphosate [317]. Secondly, the synergistic effect of major environmental factors such as; operating pH, initial glyphosate concentration, temperature, and contact time with copper on the glyphosate degradation and AMPA generation was studied. The effect of introducing a carbon-based porous material such as activated carbon to an aquatic system containing glyphosate and copper is examined. Statistical optimisation using response surface methodology was used in building the model [276, 277], designing the experimental plan and in evaluating and optimising the experimental data [34].

7.2 Glyphosate degradation process

The degradation of glyphosate in aqueous solutions was investigated. The main factor hypothesized was that glyphosate is degraded by copper. However, in the environment, glyphosate usually is degraded through photolysis, photo-oxidant and photo-isomerization [124]. As there are highly reactive groups such as amino, carbonyl and phosphonic in the structure of glyphosate, photolysis is possibly the main degradation path of glyphosate in the environment. The influence of heavy metals on its degradation cannot be ignored. In this experiment 40 mL volume samples of glyphosate 10 mg/L initial concentrations were mixed with different dosages of copper metal, Cu^+ ion and Cu^{2+} ion concentrations. The

residue glyphosate concentration and the generated AMPA concentration are measured. To have a better understanding of the different functional mechanism of degradations, the experiments were carried out in the decontaminated synthesised water systems to avoid the interference of different external factors such as minerals and microorganisms. However, glyphosate exhibited abiotic degradation when in contact with Cu metals and Cu^+ ions. When glyphosate is degrading AMPA was generated. The main reaction identified as below (see Figure 7.1). The measured generated AMPA concentration is used as the indicator for the degradation when analysing the external conditions affecting the glyphosate degradation process.

Figure 7.1 Glyphosate to AMPA conversion reaction

7.3 Evidence of degradation of glyphosate in contact with copper

7.3.1 Intermediate identification and pathway determination with copper metal

Chromatographic analysis was done using LC-MS/MS to identify the reaction intermediates. Three intermediates named Formyl-iminobismethyl-ene bisphosphonic acid (Figure 7.2 (a)), Bis-phosphonomethyl-aminomethyl phosphonic acid (Figure 7.2 (b)), and 3-2-Methylprop-2-enoylamino-propyl-phosphonomethyl-aminomethyl phosphonic acid (Figure 7.2 (c)) were identified during the degradation of glyphosate with copper metal (see Table 7.1 for the retention time, mass-to-charge ratio and structure of the detected compounds). The reaction mechanism for the aqueous glyphosate degradation with copper metal (Cu) and for the reaction follows is developed as per Figure 7.3. Copper metal degrades glyphosate to form AMPA through C – N bond cleavage. The formed AMPA

further reacts to result in Formyl-iminobismethyl-ene bisphosphonic acid, Bis-phosphonomethyl-aminomethyl phosphonic acid and 3-2-Methylprop-2-enoylamino-propyl-phosphonomethyl-aminomethyl phosphonic acid in a sequence of reactions.

Table 7.1 Identified intermediates of glyphosate-copper metal reaction by LC/MS analysis

Parent Compound	Intermediates			
	Name	T _{ret} (min)	m/z	Structure
Glyphosate	Glyoxylate	1.230	71
	Amino methyl phosphonic acid (AMPA)	1.230	96.9
	Formyl-iminobismethyl-ene bisphosphonic acid	1.230	226.2
	Bis-phosphonomethyl- amino-methyl phosphonic acid	5.331	293.2
	3-2-Methylprop-2-enoylamino-propyl-phosphonomethyl- amino-methyl phosphonic acid	1.231	326

Figure 7.2 LC/MS chromatograms of the intermediates in the reaction of glyphosate-copper metal

Figure 7.3 Reaction mechanism aqueous glyphosate with copper metal

7.3.2 Intermediate identification and pathway determination with copper Cu(I) ions

Figure 7.4 LC/MS chromatograms of the intermediates in the reaction of glyphosate-copper Cu(I) ions

Table 7.2 Identified intermediates of glyphosate- Cu(I) ion reaction by LC/MS analysis

Parent Compound	Intermediates			
	Name	T _{ret} (min)	m/z	Structure
Glyphosate	Glyoxylate	1.230	71
	Amino-methyl-phosphonic acid (AMPA)	1.230	96.9
	Hydroxy-phosphonyl-methyl-phosphonomethyl-amino-methyl phosphonic acid	6.204	265.2
	Bis-phosphonomethyl-amino-methyl phosphonic acid	5.661	293.2

Parent Compound	Intermediates			
	Name	T _{ret} (min)	m/z	Structure
Glyphosate	5-Dimethylamino-1,3,3-trimethylcyclohexyl-methyl-phosphonomethyl-amino-methyl phosphonic acid	7.158	380.9
	4-Bis-phosphonomethyl-amino-butyl—phosphonomethyl-amino-methyl phosphonic acid	1.150	453

Hydroxy-phosphonyl-methyl-phosphonomethyl-aminomethyl phosphonic acid (Figure 7.4 (a)), Bis-phosphonomethyl-aminomethyl phosphonic acid (Figure 7.4 (b)), 5-Dimethylamino-1,3,3-trimethylcyclohexyl-methyl-phosphonomethyl-aminomethyl phosphonic acid (Figure 7.4 (c)), and 4-Bis-phosphonomethyl-amino-butyl—phosphonomethyl-aminomethyl phosphonic acid (Figure 7.4 (d)) were identified by LC/MS analysis during the glyphosate degradation and polymerisation process with Cupreous(I)/ Cu⁺. Table 7.2 depicts the retention time, mass-to-charge ratio and structure of the detected compounds during the glyphosate degradation and polymerisation process with Cu (I)/ Cu⁺. The reaction mechanism for the aqueous glyphosate degradation with Cu⁺ and for the reaction follows is developed as per Figure 7.5. Cu (I)/ Cu⁺ degrades glyphosate to form AMPA through C – N bond cleavage. The formed AMPA further reacts to result in Hydroxy-phosphonyl-methyl-phosphonomethyl-aminomethyl phosphonic acid.

Hydroxy-phosphonyl-methyl-phosphonomethyl-aminomethyl phosphonic acid is further converted to Bis-phosphonomethyl-aminomethyl phosphonic acid. Bis-phosphonomethyl-aminomethyl phosphonic acid is converted to either 5-Dimethylamino-1,3,3-trimethylcyclohexyl-methyl-phosphonomethyl-aminomethyl phosphonic acid or 4-Bis-phosphonomethyl-amino-butyl—phosphonomethyl-aminomethyl phosphonic acid depending on the sequence of reactions.

Figure 7.5 Reaction mechanism aqueous glyphosate with Cu(I) cation

7.4 External conditions affecting the glyphosate degradation process

Effect of external environmental conditions such as initial glyphosate concentration, pH, temperature and contact time on the degradation of glyphosate is analysed. Recently, activated carbon-based porous carbons were suggested as a better remediation method for the removal of glyphosate in the affected area. Thus, a carbon-based coconut shell activated carbon was added to examine the combined effect of carbon-based porous material with copper in the degradation of glyphosate. Response surface methodology was used in optimising the conditions to attain the conditions resulting in the highest AMPA concentrations. A series of tests and levels of independent variables with coded and uncoded values for glyphosate degradation with Cu metal and Cu⁺ ions are in Table A3. For 6 independent parameters, 18 centre-points and 54 base runs were required to build the box. As each sample was in triplicate, the total number of runs required for the analysis was then 162. The sample solutions which were used in the degradation tests were prepared to match the conditions at each of these points. Final AMPA concentration and the residue glyphosate concentrations were measured and compared for each condition combination. Response surface calculation is further explained under Chapter 3: Materials and methods.

7.4.1 Effect of initial glyphosate concentration

The combined effect of initial glyphosate concentration and contact time when pH and temperature fixed at 6.5 and 35.0 °C is shown in Figure 7.6. To study the effect of the initial concentration of glyphosate, the range of initial glyphosate concentration is chosen between 0.2 mg/L – 50 mg/L. Figure 7.6(a) shows the AMPA generation without the influence of copper metal cations and Figure 7.6(b) shows the AMPA generation with the influence of copper metal cations. AMPA generation without the influence of copper metal cations is considered the controlled test for the hypothesis. AMPA generation with copper cation is 3 times higher than the controlled test. AMPA concentration increases gradually with the increase of initial glyphosate concentration. When increasing the initial glyphosate concentration, AMPA generation efficiency dropped in the experiment (b) after 30 mg/L initial glyphosate concentration. This can be due to the capacity effect; with the increase of the number of glyphosate molecules in the solution, the number of effective collisions with copper cations can drop.

Figure 7.6 RSM plots. Contour plot and the 3D surface graph of AMPA generation; (a) without copper metal and (b) with copper metal showing the effect of contact time and initial glyphosate concentration presenting the influence of copper in glyphosate degradation

7.4.2 Effect of pH

In Figure 7.7, the synergistic effect of pH and contact time when initial glyphosate concentration and temperature were fixed at 25.1 mg/L and 35.0 °C is depicted. To study the effect of pH, the range of pH was chosen from 6 – 8. Figure 7.7(a) shows the AMPA generation without the influence of copper metal cations and Figure 7.7(b) shows the AMPA generation with the influence of copper metal cations. pH is a governing factor in the catalytic wet oxidation process. Several studies report the influencing trends of pH effect for different compounds degradation [306-308]. In this study, the effect of pH on glyphosate degradation under copper cation is significant that AMPA generation was decreased by 43.8% when pH was increased from 5.0 to 8.0. A significant impact of pH can be observed on the glyphosate degradation without copper. In the controlled system, lower pH levels have favoured the degradation of glyphosate. Glyphosate aqueous dissociation is strongly affected by the pH of the solution (see Figure 5.7). The rates at which glyphosate is hydrolysed and the equilibrium level of the hydrolysed glyphosate concentration in the solution

is lower at lower pH levels 5-6. Under normal basic conditions glyphosate remains protonated, so that it would not involve in reduction reaction to result the by-products. Oxidation tendency of the oxidant; copper is much higher in acidic condition than in basic condition.

Figure 7.7 RSM plots. Contour plot and the 3D surface graph of AMPA generation; (a) without copper metal and (b) with copper metal showing the effect of contact time and pH presenting the influence of copper in glyphosate degradation

7.4.3 Effect of temperature

In Figure 7.8 the synergistic effect of temperature and time on the AMPA generation at fixed initial glyphosate concentration and pH of 25.1 mg/L and 6.5 respectively is shown. To investigate the effect of temperature on degradation of glyphosate, a series of temperatures ranging from 20 – 50 °C is studied. Figure 7.8(a) shows the AMPA generation without the influence of copper metal cations and Figure 7.8(b) shows the AMPA generation with the influence of copper metal cations. AMPA generation is very much temperature-dependent. Higher temperatures favour the degradation process. The AMPA generation at 50 °C in the ‘with copper’ system is nearly 5 times greater than ‘without copper’ system.

Figure 7.8 RSM plots. Contour plot and the 3D surface graph of AMPA generation; (a) without copper metal and (b) with copper metal showing the effect of contact time and temperature presenting the influence of copper in glyphosate degradation

7.4.4 Effect of carbon-based porous material dosage

The effect of contact time and carbon-based porous material dosage on glyphosate removal when other parameters such as initial glyphosate concentration, pH and temperature were fixed at 25.1 mg/L, 6.5 and 35.0 °C is shown in Figure 7.9. Activated carbon dosage ranging from 0.2 - 50 g/L was added to aqueous samples containing copper and glyphosate to investigate the effect of carbon-based porous material dosage on the degradation of glyphosate. The AMPA generation variation when activated carbon is acting along and with copper, is analysed. Figure 7.9(a) shows the AMPA generation with activated carbon without the influence of copper metal cations and Figure 7.9(b) shows the AMPA generation with activated carbon with the influence of copper metal cations. AMPA concentration in both with and without copper, dropped with the increase of activated carbon suggesting probable adsorption of AMPA [236]. According to Figure 7.9(a), activated carbon has not generated AMPA in the absence of copper. Instead, the naturally occurred AMPA level existed in equilibrium with glyphosate was removed due to adsorption by activated carbon. In Figure 7.9(b), the combined effect of copper and activated carbon on AMPA concentration fluctuations is depicted pictorially. AMPA

concentration was lower when carbon dosage is lower than 10 g/L and higher than 30 g/L. Highest AMPA generation is reported when the carbon dosage ranges from 10 – 30 g/L. First, carbon adsorbs the glyphosate resulting in a drop in the AMPA concentration. Then, glyphosate C – N bond cleavage happened at the surface of porous carbon [309] facilitating the glyphosate degradation process. Porous carbon has catalysed glyphosate degradation with copper. Glyphosate and porous carbon ratio delimit the degradation efficiency [240]. Thus, activated carbon-based adsorbents were not recommended as a remediation method for the removal of glyphosate for the areas where water is affected by glyphosate and copper.

Figure 7.9 RSM plots. Contour plot and the 3D surface graph of AMPA generation (a) with activated carbon and (b) activated carbon together with copper metal; showing the effect of contact time and porous carbon dosage in glyphosate degradation

7.4.5 The collective effect of all variables on the degradation process

The major factors affecting degradation process for glyphosate include the initial glyphosate concentration, the dosage of carbon-based porous material (activated carbon), temperature, and pH and their synergistic effect with copper is depicted in Figure 7.10. Optimisation of the parameters using response surface methodology provided five plots demonstrating the effect of individual factors on degradation process (see Figure 7.11) The derived empirical equations for glyphosate degradation and AMPA generation which were

used in the response surface study are; Eq. (S1) and (S2). At the aforementioned conditions in Figure 7.11, 24.73% of glyphosate is degraded and 18.61% of degraded glyphosate is converted to AMPA after in contact with copper for 24 h. It is observed that glyphosate can be degraded 100% to form several by-products if the respective conditions are maintained for a longer time (>24 h). Degradation is higher at acidic waters with pH 5.8. High temperatures and high initial glyphosate concentrations resulted high AMPA concentration. The addition of porous carbons has favoured the degradation.

For glyphosate degradation;

Final glyphosate concentration (mg/L) = 2.8 - 0.457 Contact time (h) + 1.088 Init. Glyph. conc. (mg/L) - 0.736 Porous carbon dosage (g/L) - 0.13 pH + 0.330 Temperature (C) + 0.28 Copper weight (mg/L) + 0.02036 Contact time (h)*Contact time (h) - 0.000042 Init. Glyph. conc. (mg/L) *Init. Glyph. conc. (mg/L) + 0.011478 Porous carbon dosage (g/L) * Porous carbon dosage (g/L) - 0.012 pH*pH - 0.00405 Temperature (C)*Temperature (C) + 0.1170 Copper weight (mg/L)*Copper weight (mg/L) - 0.01493 Contact time (h)*Init. Glyph. conc. (mg/L) - 0.00378 Contact time (h)* Porous carbon dosage (g/L) + 0.0253 Contact time (h)*pH - 0.00220 Contact time (h)*Temperature (C) - 0.0094 Contact time (h)*Copper weight (mg/L) - 0.01353 Init. Glyph. conc. (mg/L) * Porous carbon dosage (g/L) - 0.0097 Init. Glyph. conc. (mg/L) *pH - 0.00352 Init. Glyph. conc. (mg/L) *Temperature (C) + 0.0024 Init. Glyph. conc. (mg/L)*Copper weight (mg/L) + 0.0127 Porous carbon dosage (g/L)*pH - 0.00036 Porous carbon dosage (g/L)*Temperature (C) + 0.0019 Porous carbon dosage (g/L)*Copper weight (mg/L) + 0.0066 pH*Temperature (C) - 0.113 pH*Copper weight (mg/L) - 0.0042 Temperature (C)*Copper weight (mg/L) (12)

For AMPA generation;

AMPA concentration (um/L) = -1112 + 13.6 Contact time (h) - 28.33 Init. Glyph. conc. (mg/L) + 14.41 Porous carbon dosage (g/L) + 463 pH - 32.4 Temperature (C) + 133.1 Copper weight (mg/L) - 1.256 Contact time (h)*Contact time (h) - 0.0332 Init. Glyph. conc. (mg/L) *Init. Glyph. conc. (mg/L) - 0.3167 Porous carbon dosage (g/L)* Porous carbon dosage (g/L) - 33.9 pH*pH + 0.420 Temperature (C)*Temperature (C) - 6.39 Cu added weight (mg/L)*Cu added weight (mg/L) + 0.610 Contact time (h)*Init. Glyph. conc. (mg/L) - 0.084 Contact time (h)* Porous carbon dosage (g/L) - 0.16 Contact time (h)*pH + 0.662 Contact time (h)*Temperature (C) - 1.24 Contact time (h)*Copper weight (mg/L) - 0.0465 Init. Glyph. conc. (mg/L) * Porous carbon dosage (g/L) - 0.08 Init. Glyph. conc. (mg/L) *pH + 1.262 Init. Glyph. conc. (mg/L) *Temperature (C) - 0.704 Init. Glyph. conc. (mg/L)*Copper weight (mg/L) + 0.67 Porous carbon dosage (g/L)*pH - 0.088 Porous carbon dosage (g/L)*Temperature (C) - 0.435 Porous carbon dosage (g/L)*Copper weight (mg/L) - 1.00 pH*Temperature (C) - 1.2 pH*Copper weight (mg/L) - 1.28 Temperature (C)*Copper weight (mg/L) (13)

Figure 7.10 Glyphosate degradation efficiency and AMPA generation rate when in contact with copper metal; synergistic effect of (a) contact time, (b) initial glyphosate concentration, (c) activated carbon dosage, (d) pH and (e) temperature with added copper

Figure 7.11 Optimisation the effect of contact time, initial glyphosate concentration, porous carbon dose, operating pH, temperature for glyphosate degradation and AMPA formation under the effect of copper

Figure 7.12 Pareto charts of the standardised effects of (a) Final glyphosate concentration (mg/L) and (b) Final AMPA concentration (um/L) ($\alpha = 0.05$, only 30 effects is shown)

Table 7.3 Response surface regression calculations of the model

Analysis of Variance for glyphosate degradation					
Source	Degrees of freedom	Adjusted sums of squares	Adjusted mean square of the error	F-Value	P-Value
Model	36	21431908	595331	19.56	0
Blocks	1	190	190	0.01	0.937
Linear	7	14697351	2099622	68.99	0
Contact time (h)	1	1210946	1210946	39.79	0
Init. Glyph. conc. (mg/L)	1	7454395	7454395	244.94	0
Porous carbon dosage (g/L)	1	343189	343189	11.28	0.001
pH	1	14362	14362	0.47	0.494
Temperature (C)	1	5505672	5505672	180.9	0
Copper weight (mg/L)	1	891	891	0.03	0.865
Square	7	2227182	318169	10.45	0
Contact time (h)*Contact time (h)	1	812493	812493	26.7	0
Init. Glyph. conc. (mg/L) *Init. Glyph. conc. (mg/L)	1	11439	11439	0.38	0.541
porous carbon dosage (g/L) * porous carbon dosage (g/L)	1	975890	975890	32.07	0
pH*pH	1	157154	157154	5.16	0.026
Temperature (C)*Temperature (C)	1	240573	240573	7.9	0.006
Copper weight (mg/L) *Copper weight (mg/L)	1	39774	39774	1.31	0.256
2-Way Interaction	21	4507185	214628	7.05	0
Contact time (h)*Init. Glyph. conc. (mg/L)	1	509818	509818	16.75	0
Contact time (h)* porous carbon dosage (g/L)	1	9433	9433	0.31	0.579
Contact time (h)*pH	1	128	128	0	0.948
Contact time (h)*Temperature (C)	1	217909	217909	7.16	0.009
Contact time (h)*Copper weight (mg/L)	1	20346	20346	0.67	0.416
Init. Glyph. conc. (mg/L) * porous carbon dosage (g/L)	1	12882	12882	0.42	0.517
Init. Glyph. conc. (mg/L) *pH	1	140	140	0	0.946
Init. Glyph. conc. (mg/L) *Temperature (C)	1	3552800	3552800	116.74	0

Analysis of Variance for glyphosate degradation					
Source	Degrees of freedom	Adjusted sums of squares	Adjusted mean square of the error	F-Value	P-Value
Init. Glyph. conc. (mg/L) *Copper weight (mg/L)	1	29526	29526	0.97	0.327
porous carbon dosage (g/L) *pH	1	9638	9638	0.32	0.575
porous carbon dosage (g/L) *Temperature (C)	1	16589	16589	0.55	0.462
porous carbon dosage (g/L) *Copper weight (mg/L)	1	10889	10889	0.36	0.551
pH*Temperature (C)	1	8098	8098	0.27	0.607
pH*Copper weight (g/L)	1	328	328	0.01	0.918
Temperature (C)*Copper weight (mg/L)	1	35652	35652	1.17	0.282
Error	87	2647770	30434		
Lack-of-Fit	77	2523068	32767	2.63	0.048
Pure Error	10	124702	12470		
Total	123	24079678			

Analysis of Variance for AMPA formation					
Source	Degrees of freedom	Adjusted sums of squares	Adjusted mean square of the error	F-Value	P-Value
Model	36	12124.1	336.78	39.98	0
Blocks	1	0.1	0.09	0.01	0.919
Linear	7	9022.7	1288.95	153.03	0
Contact time (h)	1	634.2	634.23	75.3	0
Init. Glyph. conc. (mg/L)	1	3249.8	3249.84	385.84	0
Porous carbon dosage (g/L)	1	5058.6	5058.57	600.58	0
pH	1	1.9	1.91	0.23	0.635
Temperature (C)	1	47.1	47.1	5.59	0.02
Copper weight (mg/L)	1	0.7	0.74	0.09	0.768
Square	7	1596.4	228.06	27.08	0
Contact time (h)*Contact time (h)	1	213.4	213.41	25.34	0
Init. Glyph. conc. (mg/L) *Init. Glyph. conc. (mg/L)	1	0	0.02	0	0.963

Analysis of Variance for AMPA formation

Source	Degrees of freedom	Adjusted sums of squares	Adjusted mean square of the error	F-Value	P-Value
porous carbon dosage (g/L) *	1	1281.7	1281.73	152.17	0
porous carbon dosage (g/L)	1	0	0.02	0	0.96
pH*pH	1	0	0.02	0	0.96
Temperature (C)*Temperature (C)	1	22.5	22.47	2.67	0.106
Copper weight (mg/L) *Copper weight (mg/L)	1	13.3	13.31	1.58	0.212
2-Way Interaction	21	1504.9	71.66	8.51	0
Contact time (h)*Init. Glyph. conc. (mg/L)	1	305.1	305.14	36.23	0
Contact time (h)* porous carbon dosage (g/L)	1	18.9	18.94	2.25	0.137
Contact time (h)*pH	1	3.2	3.17	0.38	0.541
Contact time (h)*Temperature (C)	1	2.4	2.41	0.29	0.594
Contact time (h)*Copper weight (mg/L)	1	1.2	1.16	0.14	0.711
Init. Glyph. conc. (mg/L) * porous carbon dosage (g/L)	1	1090.5	1090.5	129.47	0
Init. Glyph. conc. (mg/L) *pH	1	2.1	2.09	0.25	0.62
Init. Glyph. conc. (mg/L) *Temperature (C)	1	27.6	27.63	3.28	0.074
Init. Glyph. conc. (mg/L) *Copper weight (mg/L)	1	0.3	0.34	0.04	0.841
porous carbon dosage (g/L) *pH	1	3.5	3.46	0.41	0.523
porous carbon dosage (g/L) *Temperature (C)	1	0.3	0.29	0.03	0.854
porous carbon dosage (g/L) *Copper weight (mg/L)	1	0.2	0.2	0.02	0.877
pH*Temperature (C)	1	0.4	0.35	0.04	0.838
pH*Copper weight (g/L)	1	2.8	2.76	0.33	0.569
Temperature (C)*Copper weight (mg/L)	1	0.4	0.38	0.05	0.832
Error	87	732.8	8.42		
Lack-of-Fit	77	723.1	9.39	9.67	0
Pure Error	10	9.7	0.97		
Total	123	12856.9			

F-values and P-values obtained from the analysis of variance (ANOVA) calculations (see Table 7.3) and Pareto analysis (see Figure 7.12) were used in evaluating the quality of the applied model. P-value < 0.05 for glyphosate degradation model and P-value < 0.01 for AMPA generation model (see Table 7.3) entailed that the coefficients in the models are jointly significant, validating the quality of the models used in the response surface calculations.

7.5 Conclusions

The influence of copper and zinc cations on the degradation of dissolved glyphosate was studied. Existence of zinc metal cations did not impact the degradation of glyphosate. Glyphosate exhibited abiotic degradation when in contact with Cu/ Cu⁺ ions. The efficiency of the abiotic degradation of AMPA increased with increasing initial concentrations of glyphosate. The external conditions yielding the best glyphosate degradation when in contact with copper were; solution pH 5.85, initial glyphosate concentration 35.92 mg/L, temperature 50.0 °C, added copper metal dosage 2.13 g/L, contact time 24.0 h. At the aforementioned conditions 24.73% of glyphosate degradation and 18.61% of AMPA: glyphosate conversion resulted. AMPA generation was increased by 43.8% when pH was increased from 5.0 to 6.5. Addition of porous carbons such as activated carbon and biochar catalyse the glyphosate degradation by copper. C – N bond cleavage happened at the surface of porous carbon. This work demonstrates the potential degradation of dissolved glyphosate in contact with copper ions at the presence of porous carbons such as activated carbon. A similar fate of glyphosate can be expected in farmland waters and wastewaters, at the presence of copper cations. Thus, carbon-based adsorbents were not recommended as a remediation method for the removal of glyphosate for the areas where water is affected with both glyphosate and copper. Previous experimental studies on glyphosate interactions with heavy metals have not considered the potential abiotic degradation pathway of dissolved glyphosate in contact with copper ions which is revealed in this work. This study further reveals a novel heterogeneous degradation mechanism of accumulated glyphosate in waters which can further impair the water quality due to the formation of toxic and persistent by-products.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

Emergence of organic micro pollutants in surface waters and groundwaters has become an issue in drinking water treatment process. Amongst all the treatment technologies, adsorption by activated carbon and biochar has been considered as a flexible, convenient, and economical solution for organic micro pollutant removal. The overall objective of this study was to analyse the removal of dissolved glyphosate herbicide using carbon-based adsorbents under the influence of inorganic metals. This chapter sums up the outcomes of the research work outlined in the previous chapters and suggestions for future work.

An analytical method was developed to analyse glyphosate and three main by-products of glyphosate; AMPA (Amino-methyl-phosphonic acid), glufosinate (2-Amino-4-hydroxymethyl-phosphonyl butanoic acid), glyphosine (N, N-bis-phosphonomethyl glycine in aqueous medium using liquid chromatography-mass spectrometry (LC-MS/MS). The experimental design and analysing techniques were sensitive, reliable, and repeatable.

8.1.1 Study 1: effect of adsorbent surface characteristics in glyphosate adsorption

In this study, four coconut shell activated carbons, two wood activated carbons and two porous polymers with different particle distribution and surface area were tested with glyphosate to analyse the effect of surface area and pore distribution on dissolved glyphosate adsorption. Both pseudo-first order and second-order models described the adsorption kinetics well. According to adsorption kinetics data, finely powdered wood-based activated carbon and coconut shell based activated carbon reached equilibrium quickly. The uptake with polymer adsorbents was slow. The batch sorption experiments revealed that finely powdered coconut shell based, and wood-based activated carbon types

were effective for glyphosate sorption. Pulverization improved adsorption of the glyphosate. According to XPS analysis, the peak of the C=O bond shows a clear shift to higher binding energy at after adsorption tests indicating the glyphosate adsorption. The FTIR study revealed that the surface hydroxyl and carbonyl groups played an important role in the sorption of glyphosate. The powdered form of carbons with surface hydroxyl and carbonyl groups could be a promising adsorbent for efficient removal of glyphosate from aqueous solution.

8.1.2 Study 2: effect of adsorption process operating parameters in glyphosate adsorption

In the previous study, six commonly used adsorbent types have been evaluated for the removal of dissolved glyphosate especially focusing the effect of particle distribution and surface area of the adsorbent on the uptake. However, that doesn't conclude the effect of influencing operating parameters (i.e. operating pH, temperature, initial glyphosate concentration, adsorbent dosage, contact time) on the glyphosate adsorption. This study compares a coconut shell activated carbon with ferrous loaded wood biochar for glyphosate removal from aqueous solutions under various experimental conditions. Both ferrous loaded wood biochar and coconut shell activated carbon indicated multilayer adsorption of glyphosate. Higher temperatures (above 40 °C) resulted in better removal efficiency for both activated carbon and biochar, indicating the influence of other mechanisms in addition to physical adsorption, i.e. chemical adsorption. Adsorption efficiencies of glyphosate were better at lower pH levels for biochar, and at higher pH levels for activated carbon. This has implications for treatment plants in determining the level of pH of the influent to be adjusted. It is proposed to use biochar to remove glyphosate at the roughing stage; or for treatment of wastewaters contaminated with higher concentrations. Activated carbon can then positioned at the polishing stage.

8.1.3 Study 3: decomposition of glyphosate during the adsorption experimentations under the influence of iron loaded wood biochar

During the adsorption experiments recorded in previous study, glyphosate was revealed to be degraded when in contact with ferrous loaded wood biochar; forming by-products such as amino-methyl-phosphonic acid (AMPA), which are known to be more persistent and more toxic than glyphosate. Findings emphasize the importance of the exploration of

glyphosate heterogeneous degradation, as glyphosate removal techniques such as adsorption can impair the water quality due to the formation of toxic by-products. The abiotic degradation of glyphosate was examined when dissolved glyphosate was treated using ferrous loaded wood biochar. Fe^{2+} mediates the energy barriers opposing bond dissociation leaning towards C–N bond cleavage to form AMPA; Fe^{2+} oxidise reducing glyphosate. Different factors affecting the degradation process for the Fe/Biochar system were investigated. The higher AMPA concentrations were obtained when the operating conditions were at; operating pH 5.0, initial glyphosate concentration 0.7 mg/L, operating temperature 50.0 °C, biochar dosage 12.3 g/L, and contact time 1.9 h. The glyphosate degradation percentage and glyphosate: AMPA conversion was 56.9% and 26.1% respectively. At aforementioned conditions, glyphosate was degraded to non-detectable levels within 24 h. Overall, the degradation of glyphosate was significant at lower pH, and at temperatures above 40 °C. Further analysis on the degradation of glyphosate to AMPA revealed a polymerisation of intermediates forming complex phosphonic acids (i.e. Bis-2-aminoethyl-aminomethyl phosphonic acid, Amino-phosphonomethyl-aminomethyl phosphonic acid, Hydroxymethyl-phosphonomethyl-aminomethyl phosphonic acid, Hexylimino-bis-methylene bisphosphonic acid, Bis-phosphonomethyl-amino-phosphonomethyl-aminomethyl phosphonic acid) addition to AMPA.

8.1.4 Study 4: decomposition of glyphosate in aqueous solutions under the influence of copper and zinc metals ions

In the fourth study, the influence of copper and zinc cations on dissolved glyphosate was studied. Glyphosate exhibited abiotic redox degradation when in contact with Cu/ Cu^+ ions. The efficiency of the abiotic degradation of glyphosate is increased with the increase of initial glyphosate concentrations level. The external conditions yielding the best glyphosate degradation when in contact with copper were; solution pH 5.85, initial glyphosate concentration 35.92 mg/L, temperature 50.0 °C, added copper metal dosage 2.13 g/L, contact time 24.0 h. At the aforementioned conditions, 24.73% of glyphosate degradation and 18.61% of AMPA to glyphosate conversion resulted. AMPA generation was increased by 43.8% when pH was increased from 5.0 to 6.5. Addition of porous carbons such as activated carbon and biochar catalyse the glyphosate degradation by copper. C–N bond cleavage happened at the surface of porous carbon. This work demonstrates the potential degradation of dissolved glyphosate in contact with copper ions. A similar fate of

glyphosate can be expected in farmland waters and wastewaters, at the presence of copper cations. Heterogeneous degradation of accumulated glyphosate in waters can further impair the water quality due to the formation of toxic and persistent by-products. Existence of zinc metal cations did not impact the degradation of glyphosate.

In summary, this study investigates the applicability of carbon-based porous carbon for dissolved glyphosate removal to eliminate the herbicide in drinking water under the influence of metals to examine the impact of competitive adsorption caused by the metals. This study reports abiotic degradation during the glyphosate adsorption experiments, when in contact with Fe^{2+} and Cu/Cu^+ ions; forming toxic and persistent by-products. This phenomenon has not been observed prior to this study. Several studies have declared degradation of glyphosate driven; by biotic pathways mediated by bacteria and fungi and by photocatalytic and catalytic wet oxidation. This work for the first time, reports that dissolved Fe^{2+} and Cu/Cu^+ ions induce the degradation of glyphosate when in contact with porous carbons. Glyphosate degradation proceeds through cleavage of the first C–N bond position forming AMPA, which is known to be more persistent and more toxic than glyphosate .

Degradation products were identified and quantified with a special focus on AMPA concentration fluctuations during the degradation of glyphosate in the presence of Fe^{2+} and Cu/Cu^+ ions at different operating conditions. Experimental results allow commenting on the relative significance of the degradation pathway and the potential for further investigation into the factors that govern the preferential selection of pathways that introduce fewer toxic products to the environment. This degradation pathway carries both scientific and environmental significance. The results of this study can be used in drinking water treatment process design. Especially in agricultural areas where the water contains Fe^{2+} and Cu/Cu^+ ions and glyphosate; implementation of a drinking water treatment solution with carbon adsorbents may degrade glyphosate through different pathways in resulting relatively toxic by-products.

8.2 Recommendations

Based on the present study, some limitations have been identified and corresponding recommendations are proposed for the future:

1. Several small molecular weight organic contaminants were identified during the qualitative study conducted on the by-products, formed during the degradation process. Yet, the study mainly quantified only four organic compounds; glyphosate (N-phosphonomethyl glycine), AMPA (Amino-methyl-phosphonic acid), glufosinate (2-Amino-4-hydroxymethyl-phosphonyl butanoic acid), glyphosine (N, N-bis-phosphonomethyl glycine) with the LC/MS analytical method. The results could have been more comprehensive if more compounds were taken into consideration.
2. The present study only investigated five operating conditions; initial glyphosate concentration, operating pH, temperature, metal ions/ biochar dosage and contact time on adsorption and degradation processes. Future attempts could include other factors; i.e. the presence of other solutes, physiochemical properties of adsorbents in the analysis.
3. Ferrous, copper and zinc metal cations were used in this study to study their effects on the glyphosate degradation process. However, various inorganic cations exist in wastewaters and surface waters compared to synthetic water samples which could lower the process efficiency. The degradation process for different mixtures of micro-organic/inorganic compounds existing in agricultural wastewater should be checked.
4. High turbidity, the existence of multiple constituents (i.e. co-existence of different organic pollutants and co-existence of organic pollutants and microorganisms) could affect the dynamics of the whole process. Future works are suggested to conduct using agricultural waters for a better understanding. Other operational conditions should be investigated for their influence on degradation.
5. The study can be expanded to observe biotic degradation under the influence of inorganic ions. The biomonitoring together with inorganic ions, will (1) provide a holistic picture regarding the fate and distribution of glyphosate in the environment; and (2) help to evaluate the retention and potential accumulation of these toxic by-products.
6. Adsorption is used as a pre-treatment process at conventional wastewater treatment process. Within the studied intervals, glyphosate was degraded to various intermediates. These by-products formation can happen at the pre-treatment step at the conventional wastewater treatment plant and can be toxic and inhibit the growth of microorganisms which are used later in aerobic and anaerobic digestion steps. A risk assessment needs

to be conducted on the toxicity of these by-products on the microorganisms used in wastewater treatment plants.

7. Further analysis on the degradation of glyphosate to AMPA revealed a polymerisation of intermediates forming complex phosphonic acids addition to AMPA. AMPA formed during degradation is considered more persistent and more toxic than glyphosate. However, there were more compounds which need to be tested for their toxicity, for a clearer understanding of the contaminants. The lack of toxicity data limits the environmental risk assessment of the overall study.

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Appendix

FIGURE A1: Elements determined by ICP–MS with its isotope abundance and most abundant isotope

TABLE A1: Experimental variables and levels in Box-Behnken design for glyphosate removal in real and coded (-1, 0, +1) values

Design Summary						Centre points:				18	
Factors:						5				Replicates:	
										3	
Base runs:						46				Total runs:	
										138	
Base blocks:						1				Total blocks:	
										1	
Sample No.	Block No.	Initial glyphosate conc. (mg/L)		Adsorbent dosage (g/L)		Contact time (h)		pH		Temperature (°C)	
1	1	0.2	-1	1	-1	12.25	0	6.5	0	35	0
2	1	50	1	1	-1	12.25	0	6.5	0	35	0
3	1	0.2	-1	50	1	12.25	0	6.5	0	35	0
4	1	50	1	50	1	12.25	0	6.5	0	35	0
5	1	25.1	0	25.5	0	0.5	-1	5	-1	35	0
6	1	25.1	0	25.5	0	24	1	5	-1	35	0
7	1	25.1	0	25.5	0	0.5	-1	8	1	35	0
8	1	25.1	0	25.5	0	24	1	8	1	35	0
9	1	25.1	0	1	-1	12.25	0	6.5	0	20	-1
10	1	25.1	0	50	1	12.25	0	6.5	0	20	-1
11	1	25.1	0	1	-1	12.25	0	6.5	0	50	1
12	1	25.1	0	50	1	12.25	0	6.5	0	50	1
13	1	0.2	-1	25.5	0	0.5	-1	6.5	0	35	0
14	1	50	1	25.5	0	0.5	-1	6.5	0	35	0
15	1	0.2	-1	25.5	0	24	1	6.5	0	35	0
16	1	50	1	25.5	0	24	1	6.5	0	35	0

Sample No.	Block No.	Initial glyphosate conc. (mg/L)		Adsorben t dosage (g/L)		Contact time (h)		pH		Temperatur e (°C)	
17	1	25.1	0	25.5	0	12.25	0	5	-1	20	-1
18	1	25.1	0	25.5	0	12.25	0	8	1	20	-1
19	1	25.1	0	25.5	0	12.25	0	5	-1	50	1
20	1	25.1	0	25.5	0	12.25	0	8	1	50	1
21	1	25.1	0	1	-1	0.5	-1	6.5	0	35	0
22	1	25.1	0	50	1	0.5	-1	6.5	0	35	0
23	1	25.1	0	1	-1	24	1	6.5	0	35	0
24	1	25.1	0	50	1	24	1	6.5	0	35	0
25	1	0.2	-1	25.5	0	12.25	0	5	-1	35	0
26	1	50	1	25.5	0	12.25	0	5	-1	35	0
27	1	0.2	-1	25.5	0	12.25	0	8	1	35	0
28	1	50	1	25.5	0	12.25	0	8	1	35	0
29	1	25.1	0	25.5	0	0.5	-1	6.5	0	20	-1
30	1	25.1	0	25.5	0	24	1	6.5	0	20	-1
31	1	25.1	0	25.5	0	0.5	-1	6.5	0	50	1
32	1	25.1	0	25.5	0	24	1	6.5	0	50	1
33	1	0.2	-1	25.5	0	12.25	0	6.5	0	20	-1
34	1	50	1	25.5	0	12.25	0	6.5	0	20	-1
35	1	0.2	-1	25.5	0	12.25	0	6.5	0	50	1
36	1	50	1	25.5	0	12.25	0	6.5	0	50	1
37	1	25.1	0	1	-1	12.25	0	5	-1	35	0
38	1	25.1	0	50	1	12.25	0	5	-1	35	0

Sample No.	Block No.	Initial glyphosate conc. (mg/L)		Adsorben t dosage (g/L)		Contact time (h)		pH		Temperatur e (°C)	
39	1	25.1	0	1	-1	12.25	0	8	1	35	0
40	1	25.1	0	50	1	12.25	0	8	1	35	0
41	1	25.1	0	25.5	0	12.25	0	6.5	0	35	0
42	1	25.1	0	25.5	0	12.25	0	6.5	0	35	0
43	1	25.1	0	25.5	0	12.25	0	6.5	0	35	0
44	1	25.1	0	25.5	0	12.25	0	6.5	0	35	0
45	1	25.1	0	25.5	0	12.25	0	6.5	0	35	0
46	1	25.1	0	25.5	0	12.25	0	6.5	0	35	0

TABLE A2 Experimental variables and levels in Box-Behnken design for 5 continuous experimental variables in real and coded (-1, 0, +1) values

Design summary (for 5 continuous experimental variables)		Centre points: 18	
Factors:	5	Replicates:	3
Base runs:	46	Total runs:	138
Base blocks:	1	Total blocks:	3

Sample No	Block No.	A	B	C	D	E
1	3	1	0	0	1	0
2	3	-1	1	0	0	0
3	3	0	0	-1	0	1
4	3	0	-1	0	0	-1
5	3	0	0	0	-1	-1
6	3	0	0	1	1	0
7	3	1	0	0	0	1
8	3	0	0	0	1	-1
9	3	0	-1	1	0	0
10	3	-1	-1	0	0	0
11	3	0	1	1	0	0
12	3	1	0	0	-1	0
13	3	0	1	-1	0	0
14	3	0	-1	0	-1	0
15	3	0	0	0	-1	1
16	3	0	0	1	0	-1
17	3	-1	0	0	0	1
18	3	-1	0	0	1	0
19	3	-1	0	-1	0	0
20	3	0	0	0	1	1
21	3	1	1	0	0	0
22	3	0	-1	0	1	0
23	3	0	0	0	0	0
24	3	1	0	-1	0	0
25	3	-1	0	1	0	0
26	3	0	-1	0	0	1
27	3	0	0	-1	1	0
28	3	-1	0	0	-1	0

Sample No	Block No.	A	B	C	D	E
29	3	0	0	0	0	0
30	3	0	0	0	0	0
31	3	0	0	1	-1	0
32	3	0	1	0	0	-1
33	3	1	0	0	0	-1
34	3	0	0	0	0	0
35	3	1	0	1	0	0
36	3	0	1	0	-1	0
37	3	0	0	-1	0	-1
38	3	0	0	-1	-1	0
39	3	0	0	0	0	0
40	3	0	0	1	0	1
41	3	1	-1	0	0	0
42	3	0	1	0	1	0
43	3	-1	0	0	0	-1
44	3	0	0	0	0	0
45	3	0	1	0	0	1
46	3	0	-1	-1	0	0
47	2	1	0	0	1	0
48	2	0	1	1	0	0
49	2	-1	0	0	1	0
50	2	-1	0	0	-1	0
51	2	0	0	0	0	0
52	2	0	0	1	0	-1
53	2	0	-1	0	0	1
54	2	0	0	0	0	0
55	2	1	0	0	0	1
56	2	0	-1	0	1	0
57	2	0	0	0	0	0
58	2	0	0	0	0	0
59	2	0	0	0	0	0
60	2	0	0	-1	1	0
61	2	0	0	1	-1	0
62	2	0	0	0	0	0
63	2	-1	0	0	0	1
64	2	-1	-1	0	0	0
65	2	0	-1	0	0	-1
66	2	1	0	1	0	0
67	2	0	0	-1	-1	0
68	2	1	0	0	0	-1
69	2	1	0	0	-1	0

Sample No	Block No.	A	B	C	D	E
70	2	-1	0	0	0	-1
71	2	-1	1	0	0	0
72	2	1	-1	0	0	0
73	2	0	-1	0	-1	0
74	2	0	0	-1	0	-1
75	2	1	1	0	0	0
76	2	0	1	-1	0	0
77	2	1	0	-1	0	0
78	2	0	0	0	1	-1
79	2	0	-1	-1	0	0
80	2	0	0	0	1	1
81	2	0	1	0	1	0
82	2	0	0	0	-1	-1
83	2	0	0	0	-1	1
84	2	0	0	-1	0	1
85	2	-1	0	1	0	0
86	2	0	1	0	0	1
87	2	0	0	1	1	0
88	2	0	-1	1	0	0
89	2	0	1	0	0	-1
90	2	0	0	1	0	1
91	2	-1	0	-1	0	0
92	2	0	1	0	-1	0
93	1	0	0	1	-1	0
94	1	0	-1	1	0	0
95	1	1	0	0	0	1
96	1	0	0	1	0	1
97	1	0	1	1	0	0
98	1	-1	-1	0	0	0
99	1	0	1	-1	0	0
100	1	0	0	-1	0	-1
101	1	-1	1	0	0	0
102	1	1	0	0	1	0
103	1	0	0	0	-1	-1
104	1	-1	0	0	0	-1
105	1	0	0	0	0	0
106	1	1	0	-1	0	0
107	1	0	0	0	1	1
108	1	0	0	0	0	0
109	1	0	0	0	0	0
110	1	0	0	0	0	0

Sample No	Block No.	A	B	C	D	E
111	1	0	-1	0	-1	0
112	1	0	0	0	0	0
113	1	0	-1	0	0	1
114	1	0	1	0	0	-1
115	1	0	-1	-1	0	0
116	1	-1	0	0	1	0
117	1	-1	0	-1	0	0
118	1	1	0	1	0	0
119	1	0	-1	0	1	0
120	1	0	0	-1	-1	0
121	1	1	0	0	-1	0
122	1	0	0	0	0	0
123	1	0	0	1	1	0
124	1	1	0	0	0	-1
125	1	-1	0	1	0	0
126	1	1	-1	0	0	0
127	1	-1	0	0	0	1
128	1	0	-1	0	0	-1
129	1	1	1	0	0	0
130	1	0	1	0	0	1
131	1	0	0	0	1	-1
132	1	0	0	-1	1	0
133	1	0	0	-1	0	1
134	1	-1	0	0	-1	0
135	1	0	0	0	-1	1
136	1	0	0	1	0	-1
137	1	0	1	0	1	0
138	1	0	1	0	-1	0

TABLE A3 Experimental variables and levels in Box-Behnken design for 6 continuous experimental variables in real and coded (-1, 0, +1) values

Design summary					Centre points:	18	
(for 6 continuous experimental variables)							
Factors:	6				Replicates:	3	
Base runs:	54				Total runs:	162	
Base blocks:	1				Total blocks:	3	
Sample No	Block No.	A	B	C	D	E	F
1	2	0	0	1	1	0	-1
2	2	-1	0	0	-1	1	0
3	2	0	-1	-1	0	1	0
4	2	-1	-1	0	-1	0	0
5	2	0	-1	-1	0	-1	0
6	2	1	0	0	1	1	0
7	2	1	1	0	-1	0	0
8	2	0	1	0	0	-1	-1
9	2	0	1	1	0	-1	0
10	2	0	0	0	0	0	0
11	2	-1	0	1	0	0	1
12	2	0	0	-1	1	0	1
13	2	0	0	1	1	0	1
14	2	0	0	-1	1	0	-1
15	2	0	0	1	-1	0	1
16	2	0	0	0	0	0	0
17	2	-1	-1	0	1	0	0
18	2	0	1	0	0	1	-1
19	2	1	0	-1	0	0	1
20	2	0	0	0	0	0	0
21	2	0	1	-1	0	1	0
22	2	-1	0	0	-1	-1	0
23	2	0	0	1	-1	0	-1
24	2	0	1	1	0	1	0
25	2	0	-1	0	0	-1	-1
26	2	0	-1	0	0	1	1
27	2	1	0	1	0	0	1
28	2	1	0	0	-1	1	0
29	2	0	-1	0	0	-1	1
30	2	0	1	0	0	-1	1
31	2	0	-1	1	0	-1	0

Sample No	Block No.	A	B	C	D	E	F
32	2	1	0	0	1	-1	0
33	2	-1	0	0	1	-1	0
34	2	1	0	1	0	0	-1
35	2	0	1	-1	0	-1	0
36	2	-1	1	0	1	0	0
37	2	0	0	-1	-1	0	-1
38	2	0	1	0	0	1	1
39	2	1	1	0	1	0	0
40	2	0	0	-1	-1	0	1
41	2	-1	0	1	0	0	-1
42	2	0	0	0	0	0	0
43	2	-1	1	0	-1	0	0
44	2	-1	0	-1	0	0	-1
45	2	1	0	-1	0	0	-1
46	2	-1	0	-1	0	0	1
47	2	0	-1	1	0	1	0
48	2	0	0	0	0	0	0
49	2	0	0	0	0	0	0
50	2	-1	0	0	1	1	0
51	2	1	-1	0	-1	0	0
52	2	0	-1	0	0	1	-1
53	2	1	-1	0	1	0	0
54	2	1	0	0	-1	-1	0
55	1	-1	0	-1	0	0	1
56	1	-1	-1	0	1	0	0
57	1	0	0	0	0	0	0
58	1	0	1	0	0	1	1
59	1	0	1	1	0	-1	0
60	1	0	-1	1	0	1	0
61	1	0	0	0	0	0	0
62	1	1	0	0	-1	1	0
63	1	0	-1	-1	0	1	0
64	1	0	1	-1	0	1	0
65	1	0	-1	0	0	-1	1
66	1	0	0	1	1	0	-1
67	1	1	0	0	1	-1	0
68	1	-1	0	-1	0	0	-1
69	1	0	0	1	1	0	1
70	1	-1	0	0	-1	-1	0
71	1	-1	0	1	0	0	-1
72	1	0	-1	0	0	1	1

Sample No	Block No.	A	B	C	D	E	F
73	1	0	0	1	-1	0	1
74	1	-1	0	0	1	-1	0
75	1	0	1	-1	0	-1	0
76	1	0	0	1	-1	0	-1
77	1	1	0	-1	0	0	1
78	1	0	0	0	0	0	0
79	1	0	0	-1	1	0	1
80	1	0	0	-1	1	0	-1
81	1	0	-1	-1	0	-1	0
82	1	0	0	-1	-1	0	-1
83	1	0	-1	0	0	-1	-1
84	1	1	0	0	1	1	0
85	1	-1	1	0	-1	0	0
86	1	0	-1	1	0	-1	0
87	1	1	0	1	0	0	-1
88	1	0	0	-1	-1	0	1
89	1	-1	1	0	1	0	0
90	1	1	1	0	-1	0	0
91	1	1	0	0	-1	-1	0
92	1	-1	0	0	-1	1	0
93	1	0	0	0	0	0	0
94	1	0	-1	0	0	1	-1
95	1	0	0	0	0	0	0
96	1	0	1	0	0	-1	-1
97	1	1	-1	0	-1	0	0
98	1	1	0	1	0	0	1
99	1	-1	0	1	0	0	1
100	1	-1	0	0	1	1	0
101	1	0	1	1	0	1	0
102	1	-1	-1	0	-1	0	0
103	1	1	0	-1	0	0	-1
104	1	1	-1	0	1	0	0
105	1	0	0	0	0	0	0
106	1	0	1	0	0	1	-1
107	1	1	1	0	1	0	0
108	1	0	1	0	0	-1	1
109	3	0	0	1	1	0	-1
110	3	0	1	0	0	-1	-1
111	3	-1	0	1	0	0	-1
112	3	0	-1	-1	0	-1	0
113	3	0	0	-1	1	0	-1

Sample No	Block No.	A	B	C	D	E	F
114	3	1	0	1	0	0	-1
115	3	0	-1	0	0	-1	1
116	3	0	-1	1	0	1	0
117	3	-1	-1	0	1	0	0
118	3	0	-1	1	0	-1	0
119	3	0	1	-1	0	-1	0
120	3	-1	0	1	0	0	1
121	3	1	0	0	-1	-1	0
122	3	0	-1	-1	0	1	0
123	3	1	0	0	-1	1	0
124	3	-1	0	0	1	-1	0
125	3	0	1	1	0	-1	0
126	3	0	1	0	0	-1	1
127	3	0	0	-1	-1	0	1
128	3	1	1	0	-1	0	0
129	3	0	0	0	0	0	0
130	3	-1	1	0	-1	0	0
131	3	0	1	0	0	1	-1
132	3	0	0	-1	-1	0	-1
133	3	0	0	0	0	0	0
134	3	1	0	0	1	-1	0
135	3	1	-1	0	-1	0	0
136	3	0	0	0	0	0	0
137	3	-1	-1	0	-1	0	0
138	3	0	0	-1	1	0	1
139	3	0	-1	0	0	1	1
140	3	0	1	-1	0	1	0
141	3	0	0	0	0	0	0
142	3	1	0	-1	0	0	-1
143	3	-1	0	-1	0	0	1
144	3	-1	0	0	1	1	0
145	3	-1	1	0	1	0	0
146	3	0	0	1	1	0	1
147	3	0	0	0	0	0	0
148	3	0	0	0	0	0	0
149	3	0	-1	0	0	1	-1
150	3	1	1	0	1	0	0
151	3	-1	0	0	-1	-1	0
152	3	0	1	1	0	1	0
153	3	0	0	1	-1	0	1
154	3	1	-1	0	1	0	0

Sample No	Block No.	A	B	C	D	E	F
155	3	0	1	0	0	1	1
156	3	0	0	1	-1	0	-1
157	3	1	0	0	1	1	0
158	3	0	-1	0	0	-1	-1
159	3	-1	0	0	-1	1	0
160	3	1	0	-1	0	0	1
161	3	1	0	1	0	0	1
162	3	-1	0	-1	0	0	-1

TABLE A4 Experimental variables and levels in Box-Behnken design for 7 continuous experimental variables in real and coded (-1, 0, +1) values

Design summary (for 7 continuous experimental variables)		Centre points:		18
Factors:	7	Replicates:	3	
Base runs:	62	Total runs:	186	
Base blocks:	1	Total blocks:	3	

Sample No	Block No.	A	B	C	D	E	F	G
1	3	-1	-1	0	1	0	0	0
2	3	0	0	0	0	0	0	0
3	3	0	0	0	-1	-1	-1	0
4	3	1	1	0	1	0	0	0
5	3	0	1	-1	0	0	-1	0
6	3	-1	1	0	1	0	0	0
7	3	0	1	0	0	-1	0	-1
8	3	-1	0	1	0	1	0	0
9	3	0	0	0	0	0	0	0
10	3	0	0	0	1	1	-1	0
11	3	0	0	0	0	0	0	0
12	3	0	0	-1	-1	0	0	1
13	3	1	-1	0	-1	0	0	0
14	3	1	1	0	-1	0	0	0
15	3	0	-1	0	0	-1	0	1
16	3	-1	0	0	0	0	-1	-1
17	3	0	-1	-1	0	0	-1	0
18	3	0	0	0	1	-1	-1	0
19	3	1	0	0	0	0	-1	1
20	3	-1	0	0	0	0	1	-1
21	3	0	1	-1	0	0	1	0
22	3	0	0	0	-1	1	-1	0
23	3	0	0	0	-1	-1	1	0
24	3	0	0	0	1	1	1	0
25	3	0	0	1	1	0	0	-1
26	3	-1	0	-1	0	1	0	0
27	3	1	0	1	0	1	0	0
28	3	0	0	0	0	0	0	0
29	3	0	1	0	0	1	0	-1

Sample No	Block No.	A	B	C	D	E	F	G
30	3	1	0	0	0	0	-1	-1
31	3	-1	-1	0	-1	0	0	0
32	3	0	0	-1	1	0	0	-1
33	3	0	-1	1	0	0	1	0
34	3	0	0	-1	1	0	0	1
35	3	1	0	0	0	0	1	1
36	3	0	0	1	-1	0	0	1
37	3	0	0	1	1	0	0	1
38	3	0	1	0	0	1	0	1
39	3	1	0	0	0	0	1	-1
40	3	-1	0	-1	0	-1	0	0
41	3	0	1	0	0	-1	0	1
42	3	0	1	1	0	0	-1	0
43	3	-1	0	0	0	0	-1	1
44	3	0	0	0	1	-1	1	0
45	3	0	0	0	0	0	0	0
46	3	0	-1	1	0	0	-1	0
47	3	0	-1	0	0	1	0	1
48	3	-1	0	0	0	0	1	1
49	3	0	0	0	-1	1	1	0
50	3	-1	0	1	0	-1	0	0
51	3	0	-1	0	0	-1	0	-1
52	3	1	0	-1	0	1	0	0
53	3	1	0	-1	0	-1	0	0
54	3	0	0	-1	-1	0	0	-1
55	3	1	-1	0	1	0	0	0
56	3	1	0	1	0	-1	0	0
57	3	0	0	1	-1	0	0	-1
58	3	0	1	1	0	0	1	0
59	3	-1	1	0	-1	0	0	0
60	3	0	-1	0	0	1	0	-1
61	3	0	-1	-1	0	0	1	0
62	3	0	0	0	0	0	0	0
63	2	-1	0	0	0	0	1	1
64	2	0	1	1	0	0	1	0
65	2	0	0	0	0	0	0	0
66	2	-1	0	-1	0	-1	0	0
67	2	-1	0	1	0	-1	0	0
68	2	1	0	0	0	0	-1	-1
69	2	1	1	0	-1	0	0	0
70	2	0	0	-1	1	0	0	-1

Sample No	Block No.	A	B	C	D	E	F	G
71	2	-1	-1	0	-1	0	0	0
72	2	0	0	0	-1	1	1	0
73	2	1	0	0	0	0	-1	1
74	2	1	0	1	0	1	0	0
75	2	1	0	0	0	0	1	-1
76	2	-1	0	1	0	1	0	0
77	2	0	-1	0	0	1	0	1
78	2	1	1	0	1	0	0	0
79	2	1	0	-1	0	1	0	0
80	2	1	0	0	0	0	1	1
81	2	-1	0	0	0	0	1	-1
82	2	0	1	0	0	-1	0	1
83	2	0	0	1	1	0	0	-1
84	2	0	0	0	0	0	0	0
85	2	1	-1	0	1	0	0	0
86	2	0	-1	1	0	0	-1	0
87	2	1	0	1	0	-1	0	0
88	2	1	-1	0	-1	0	0	0
89	2	1	0	-1	0	-1	0	0
90	2	0	0	0	0	0	0	0
91	2	0	0	0	1	1	1	0
92	2	0	0	1	1	0	0	1
93	2	0	0	0	1	1	-1	0
94	2	0	0	1	-1	0	0	-1
95	2	0	0	0	1	-1	-1	0
96	2	0	1	0	0	1	0	-1
97	2	0	0	1	-1	0	0	1
98	2	-1	-1	0	1	0	0	0
99	2	-1	1	0	-1	0	0	0
100	2	0	0	0	-1	1	-1	0
101	2	0	1	0	0	1	0	1
102	2	0	0	0	0	0	0	0
103	2	0	1	0	0	-1	0	-1
104	2	-1	1	0	1	0	0	0
105	2	0	0	-1	-1	0	0	-1
106	2	0	0	0	-1	-1	1	0
107	2	0	0	0	0	0	0	0
108	2	0	1	1	0	0	-1	0
109	2	0	0	0	1	-1	1	0
110	2	0	-1	1	0	0	1	0
111	2	0	-1	0	0	-1	0	1

Sample No	Block No.	A	B	C	D	E	F	G
112	2	0	0	0	0	0	0	0
113	2	0	-1	-1	0	0	1	0
114	2	0	0	-1	-1	0	0	1
115	2	-1	0	-1	0	1	0	0
116	2	0	0	-1	1	0	0	1
117	2	0	-1	0	0	-1	0	-1
118	2	0	0	0	-1	-1	-1	0
119	2	0	1	-1	0	0	1	0
120	2	0	-1	0	0	1	0	-1
121	2	0	-1	-1	0	0	-1	0
122	2	-1	0	0	0	0	-1	1
123	2	-1	0	0	0	0	-1	-1
124	2	0	1	-1	0	0	-1	0
125	1	1	0	0	0	0	-1	-1
126	1	0	0	0	-1	-1	1	0
127	1	0	-1	0	0	1	0	-1
128	1	0	0	0	-1	-1	-1	0
129	1	-1	1	0	-1	0	0	0
130	1	1	0	1	0	1	0	0
131	1	0	1	1	0	0	1	0
132	1	1	-1	0	-1	0	0	0
133	1	0	0	0	-1	1	-1	0
134	1	-1	0	0	0	0	1	-1
135	1	0	0	0	1	-1	1	0
136	1	0	1	-1	0	0	-1	0
137	1	0	0	-1	1	0	0	-1
138	1	0	0	-1	-1	0	0	-1
139	1	0	0	0	0	0	0	0
140	1	0	0	0	1	1	-1	0
141	1	1	0	-1	0	1	0	0
142	1	0	-1	1	0	0	1	0
143	1	0	-1	-1	0	0	-1	0
144	1	0	1	0	0	1	0	1
145	1	0	1	1	0	0	-1	0
146	1	1	0	-1	0	-1	0	0
147	1	-1	0	1	0	-1	0	0
148	1	0	0	0	1	1	1	0
149	1	0	0	0	1	-1	-1	0
150	1	-1	-1	0	1	0	0	0
151	1	0	0	1	-1	0	0	1
152	1	1	0	0	0	0	1	-1

Sample No	Block No.	A	B	C	D	E	F	G
153	1	-1	0	-1	0	1	0	0
154	1	0	0	0	0	0	0	0
155	1	0	0	1	1	0	0	-1
156	1	0	-1	0	0	-1	0	-1
157	1	0	0	1	1	0	0	1
158	1	0	0	0	0	0	0	0
159	1	0	-1	1	0	0	-1	0
160	1	0	0	0	0	0	0	0
161	1	0	0	0	-1	1	1	0
162	1	0	1	-1	0	0	1	0
163	1	0	-1	0	0	1	0	1
164	1	0	-1	0	0	-1	0	1
165	1	0	0	0	0	0	0	0
166	1	-1	0	0	0	0	-1	-1
167	1	1	1	0	1	0	0	0
168	1	0	1	0	0	1	0	-1
169	1	1	0	1	0	-1	0	0
170	1	0	0	0	0	0	0	0
171	1	0	1	0	0	-1	0	-1
172	1	-1	0	1	0	1	0	0
173	1	0	-1	-1	0	0	1	0
174	1	0	0	-1	1	0	0	1
175	1	-1	0	-1	0	-1	0	0
176	1	-1	-1	0	-1	0	0	0
177	1	1	1	0	-1	0	0	0
178	1	1	0	0	0	0	1	1
179	1	1	-1	0	1	0	0	0
180	1	-1	0	0	0	0	-1	1
181	1	1	0	0	0	0	-1	1
182	1	-1	0	0	0	0	1	1
183	1	0	0	1	-1	0	0	-1
184	1	0	1	0	0	-1	0	1
185	1	0	0	-1	-1	0	0	1
186	1	-1	1	0	1	0	0	0