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Using Green Chemistry to Produce Supported Iron Nanoparticles from Oak Leaf Extract and Biochar

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Abstract

Zero Valent Iron Nanoparticles (nZVI) have recently received attention for their ability to be used for environmental remediation of contaminated water and soils. While these nanoparticles can remove toxic chemicals from the environment (e.g. nitrate), the environmental impact of their production needs to be considered in order to truly assess the degree of environmental remediation. Therefore, we are applying multiple principles of green chemistry to improve the synthesis of nZVI. Here we combine two methods of creating nZVI. 1) Oak leaf extract has been used as the reducing and capping agent in synthesizing nZVI (OL-nZVI). This is done by boiling oak leaves (*Quercus alba*) in water and mixing the resulting extract with iron (III) citrate solution. 2) nZVI has been previously made in our lab by supporting nZVI on biochar (BC-nZVI). Here, the biochar is created from milo seed (*Sorghum bicolor*) via pyrolytic carbonization and gasification; carbothermal reduction of iron (III) citrate then results in the production of nZVI. Thus, this study attempts to place OL-nZVI on milo seed biochar (OL-BC-nZVI) by supporting iron (III) citrate on biochar and then reducing it using the oak leaf extract. We expect that the biochar support will lead to improved chemical stability by shielding the nZVI from oxidation and active/basic media. Transmission electron microscopy (TEM), powder X-ray diffractometry (PRXD), surface area analysis, and monitoring of the chemical concentrations of Fe^0 and Fe^{2+} will be used to compare the physical attributes of the OL-BC-nZVI to those of BC-nZVI. Whereas the two are physically similar, the former is produced by a particularly green method because it uses sustainable, natural materials, water as the solvent, relatively low temperatures, and does not produce harmful byproducts. Future studies will include the efficacy and efficiency of biotic denitrification by OL-BC-nZVI with bacteria and *in situ*. Results will be discussed in this context.

Keywords: nZVI, Biochar, Green Chemistry

1. Introduction

Denitrification is a topic of concern in the agriculturally focused Midwestern United States, which has seen increased nitrate runoff to streams and rivers [1], [2], [3], [19], [20]. The U.S. Environmental Protection Agency (EPA) sets the safe limit of nitrate in drinking water at 10 mg/L NO_3-N; it is carcinogenic and toxic to both humans and animals [1], [2]. According to a report by EPA, more than 35% of lakes in the Unites States are contaminated by excess nutrients, which includes nitrates [23]. While wetlands and buffer zones can naturally denitrify runoff, they are not sufficient because of their limited retention time and capacity [21]. Thus, a pollution treatment program is necessary.

Large scale bioreactors are capable of denitrification when utilizing various substrates such as woodchips [24]. Our previous work suggests that combining zero valent iron nanoparticles with a denitrifying bacteria such as *Alcaligenes eutrophus* creates a suitable bioreactor. However, current methods of nZVI fabrication require significant energy

consumption and produce toxic byproducts [3], [4], [5], [6]. Here, a method of nZVI production that is cheap, renewable, and environmentally sustainable is investigated.

1.1 nZVI and Green Chemistry

Nanoparticles have high potential for environmental remediation [18]. nZVI particles have been shown to remove nitrates, [1], [2] antibiotics, [3] heavy metals, [4], [5] and other pollutants [6] from water or soil. nZVI particles are highly tailorable: their traits can be selected for longevity, durability, reactivity, size, and surface-to-volume ratio. Green methods seek to replace hazardous reducing agents like sodium borohydride or decrease energy requirements associated with conventional processes. The American Chemical Society's (ACS) principles of green chemistry [7] are invoked here to design environmentally-friendly production of nZVI particles suited for a bioreactor and *in situ* application for increased denitrification. Specifically, this project addresses the principles of less hazardous chemical synthesis, safer solvents and auxiliaries, use of renewable feedstocks, design for energy efficiency and environmental degredation, and inherently safer chemistry for accident prevention.

1.2 Bacterial Denitrification

The following scheme summarizes the processes by which the proposed bioreactor functions:

$$NO_3^- + Fe^0 + H_2O \rightarrow NH_4 + \text{Denitrifying Bacteria} \rightarrow N_2(g) \quad (1)$$

$$NO_3^- + \text{Denitrifying Bacteria} \rightarrow N_2(g) \quad (2)$$

Certain bacteria such as *Alcaligenes eutrophus* naturally perform denitrification by reducing either nitrate or ammonia to nitrogen gas. Furthermore, *A. eutrophus* has been stimulated by the addition of Fe^0 to increase denitrification rates [2]. Scheme 1 shows the proposed nZVI (Fe^0) converting nitrate to ammonia, which then undergoes bacterial denitrification. This can be performed in two ways [2]:

$$NO_3^- + 2.82Fe^0 + 0.75Fe^{2+} + 2.25H_2O \rightarrow NH_4^+ + 1.19Fe_3O_4 + 0.5OH^- \quad (3)$$

$$4Fe^0 + NO_3^- + 7H_2O \rightarrow 4Fe^{2+} + NH_4^+ + 10OH^- \quad (4)$$

Fe^0 and ferrous oxides can also act as electron donors for bacteria, meaning that by adding iron to the media, bacterial denitrification rates increase, as depicted in Scheme 2 [2].

1.3 Green Chemistry Methods

To customize the nZVI production for bacterial denitrification and subsequent environmental remediation, a green reducing agent, capping agent, and support are necessary. As a precursor to the implementation of a bacterial bioreactor, we compare multiple methods of fabrication. Four reducing methods and two iron sources are considered in increasing order of desirability.

Table 1: Criteria used in the green chemical classification of reducing agents and iron complexes ranked from least to most

		Reducing Agents		Iron Complexes	
Least Green		**Thermal Reduction**	**Byproducts**: CO_2 **Energy Costs**: 800° C for 6 hours **Safety:** Furnace contains asbestos.	**Iron Chloride**[16]	**Byproducts:** Hydrogen chloride gas, chlorine, metal oxides. **Safety:** Considered hazardous by 2012 OSHA standards.
Poor		**Sodium Borohydride**[14]	**Byproducts:** Boron and sodium oxides, irritating gases and vapors. **Safety:** Considered hazardous by 2012 OSHA standards.		
Better		**Ascorbic Acid**[15]	**Reliability:** Often needs presence of $NaBH_4$ and is less tested. **Renewable:** via citrus fruits.	**Iron Citrate**[17]	**Safety:** Some irritation/hazardous concerns. **Renewable:** via citrus fruits.
Most Green		**Oak Leaf Extract**	**Safety:** Unregulated **Renewable:** via Oak trees.		

Thermal reduction releases CO_2 and has high energy requirements but is very reliable [13]. Sodium borohydride gives off toxic byproducts but is a well-documented nZVI reducing agent. Ascorbic acid is a reducing and capping agent that is renewable but has little reported success without the addition of $NaBH_4$[8], [9]. Oak leaf extract is renewable, safe, and has been used to successfully produce nZVI[10],[11]. It contains both reducing and capping agents in the form of phenolic and carbonyl compounds [4].

1.4 Oak Leaf Extract

The reducing capabilities of Oak Leaf Extract (OLE) have been quantified by Ferric Reducing Antioxidant Power (FRAP) and Folin-Ciocalteu assays in previous studies, indicating that oak leaves (*Quercus alba*) have the best reducing capabilities when compared to 25 other leaves [10]. Other leaves shown to reduce iron salts to iron zero include green teas, mulberries, pomegranate, and eucalyptus [1], [2], [10], [11]. GC-MS and IR analysis reports the presence of phytols, terpenoids, and polyphenol groups in many of the extracts which are responsible for reducing the iron [17]. We are based in the Midwest where oak trees are the most common out of the documented flora viable for nZVI production. The renewability and cost effectiveness of OLE means that it would be well suited for large scale synthesis of nZVI [18].

1.5 Milo Seed Biochar

Supporting the nZVI on biochar (nZVI@BC) prevents aggregation [3] thus increasing reactivity. Biochar can be made from renewable materials; milo seeds (*Sorghum bicolor*) are used here. Additionally, the post-reduction nZVI@BC is easier to separate from the reaction mixture than nZVI and can be re-pyrolized for reuse and mitigation of secondary contamination [22]. The nZVI@BC produced by the four methods of reduction shown above are characterized by

transmission electron microscopy (TEM) and powder X-ray diffractometry (PRXD), and the presence of zero valent iron, nanoparticle size, and uniform adsorption is measured.

2. Methods

Chemicals were used as received (Fisher Scientific, Hampton, NH). Elemental analysis was determined by Inductively Coupled Plasma (ICP) at the Microanalysis Laboratory of the School of Chemical Sciences, University of Illinois (Urbana-Champaign, IL). Transmission Electron Micrographs (TEM) were collected on a JEOL 2100 (Center for Microanalysis of Materials, University of Illinois, Urbana-Champaign, IL) or a Hitachi 7000 (Southern Illinois University School of Medicine, Springfield, IL) on 300 mesh gold grids with Formvar™ prepared by grinding and sonicating samples in water.

Table 2: Sample Overview: contents of each sample prepared

Sample ID	Reducing Agent	Iron Complex	Biochar
3A[a]	Thermal Reduction	1.42 ± 0.5 g Iron Citrate	6.25 ± 0.5 g
3D	80.0 ± 0.5 mL 1.65 M $NaBH_4$	2.703 ± 0.5 g $FeCl_3$	5.00 ± 0.5 g
3B[a]	75.0 ± 0.5 mL 0.98 M $NaBH_4$	1.42 ± 0.5 g Iron Citrate	6.25 ± 0.5 g
5B	10.0 ± 0.5 mL 0.1 M Ascorbic Acid	0.612 ± 0.5 g $FeCl_3$	------
5A	10.0 ± 0.5 mL 0.1 M Ascorbic Acid	0.676 ± 0.5 g Iron Citrate	------
3E	510.0 ± 0.5 mL OLE	2.703 ± 0.5 g $FeCl_3$	5.00 ± 0.5 g
4G	10.0 ± 0.5 mL OLE	0.227 ± 0.5 g Iron Citrate	1.00 ± 0.5 g

Samples in order of increasing green chemistry classification, first by reducing agent (Thermal<$NaBH_4$<Ascorbic Acid<OLE), then by iron complex ($FeCl_3$ < iron chloride).

[a] Amount of iron complex calculated using 2% adsorption, based on prior results.[13]

2.1 Biochar Preparation [12]

Milo seeds were baked in air at 160° C for 24 hours, stirred, baked for another 24 hours at 220° C, and cooled. An iron tube was plugged with glass wool, a rubber stopper, and a connector tube to an oxygen tank. The seeds were packed into the tube, an oxygen flow was established, and an incandescent thermal zone was initiated at the top of the tube with a propane torch. The tube burned through (ideal burn rate of 1 cm/5 min), 5 wt% DI water was added, and the tube was burned again. 15 wt% DI water was added, and the solution was transferred to a Vycor tube, then burned. Thus a porous carbon structure was created as a support for nZVI.

Iron was adsorbed to the previously prepared biochar by combining 62.5 grams of $FeCl_3$, 125 mL of DI water, and 6.25 grams of biochar in a capped glass bottle which was shaken for 24 hours at 200 RPM. The product was vacuum filtered through Grade 1 Whatman filter paper and left to dry for 48 hours.

2.3 Oak Leaf Extract Preparation [10]

Oak leaves were gathered on the UIS campus, dried at room temperature for a week, ground in a coffee grinder, sifted through a 4 mm sieve, spread onto a glass tray, and dried at 50ºC for 48 hours. Once cooled, 3.7 grams of leaves were placed in a 250 mL Erlenmeyer flask with 100 mL of DI water and a stir bar. The flask was placed in a 1 L bath of 80ºC water for 20 minutes with stirring. The solution was vacuum filtered with Grade 1 Whatman filter paper and the filtrate (OLE) was retained for further use.

2.4 Thermal Reduction of Sample 3A

To prepare sample 3A 7.18 grams of the previously prepared biochar with adsorbed iron was placed into small alundum boats which were slid into a ceramic tube. The ceramic tube was plugged with stoppers penetrated by glass tubing to allow for gas flow. The terminal end of the tubing was affixed to a mineral oil bubbler to allow for flow control. The apparatus was put into a tube furnace and purged with argon gas for 30 minutes, after which time the furnace was heated to 800 degrees Celsius and left at temperature for six hours. Afterwards, the apparatus was cooled under argon gas for another 30 minutes and the sample was collected and stored under anaerobic conditions.

2.5 Sodium Borohydride Reduction of samples 3D and 3B

To prepare sample 3D, 5 grams of the previously prepared biochar was placed into a 500 mL round bottom flask with 300 mL of DI water and sonicated for one hour. Then, a solution of 2.7 grams $FeCl_3$ in 20 ml DI water was added to the flask and sonicated for an additional hour while purging with argon. A solution of 5 grams $NaBH_4$ in 80 mL DI water was then added dropwise at 90ºC for four hours and then cooled, all under argon. The product was vacuum filtered through Grade 1 Whatman paper, rinsed with DI water and ethanol, and then vacuum dried at 50ºC for 36 hours.

To prepare sample 3B, 6.25 grams of the prepared biochar with adsorbed iron citrate was suspended in 200 mL DI water. Then 75 ml of a 0.1 M sodium borohydride solution was added dropwise to the suspension at 90 ºC for 4 hours while under argon. The suspension was then cooled, vacuum filtered through Grade 1 Whatman paper, rinsed with DI water, and put into a vacuum oven at 70 ºC for 24 hours.

2.6 Ascorbic Acid Reduction of samples 5A and 5B

Figure 1: Reaction products of 0.1 M iron citrate (sample 5A), iron chloride (sample 5B), and iron nitrate (left to right) mixed in a 1:5 volume ratio with 0.1 M ascorbic acid. Sample 5A produced a colored solution and nanoparticles were visible after centrifuging the product.

Aqueous solutions of ascorbic acid, iron citrate, iron chloride, and iron nitrate (0.1 M) were prepared. 2 mL of the corresponding iron solution were added to each of three 20 mL screw-cap vials, and then 10 mL of the ascorbic acid solution was added dropwise to each. The vials were then capped and set on the shaker platform at 200 rpm for one hour. They produced the solutions above (Fig. 1), only sample 5A was colored and when centrifuged produced a small amount of black solid. PXRD and TEM data was collected for sample 5A.

2.7 Oak Leaf Extract Reduction of samples 3E and 4G

To prepare sample 3E, 2.703 grams of $FeCl_3$ was placed into a round bottom flask with 5 grams of pure biochar and 100 mL of DI water. This suspension was sonicated for 1 hour under argon. 600 mL of OLE was added dropwise to the suspension after sonication over the course of four hours. The reaction chamber was kept at 90 ºC and was under

argon. The product was cooled under argon, vacuum filtered, rinsed with both DI water and ethanol, [1] and then vacuum dried at 50 °C for 24 hours.

To prepare sample 4G, one gram of the previously prepared biochar with iron citrate already adsorbed was placed in a vial where 10 mL of OLE was added dropwise. The vial was then shaken for one hour.

Three vials were prepared without biochar, using the stock iron salt solutions made for the ascorbic acid experiment. Again, two mL of the corresponding iron solution was measured out, however this time 6 mL of OLE was used as the reducing agent. Each solution turned black (Fig. 2) and when centrifuged, solid was recovered. However, none of these samples were tested further.

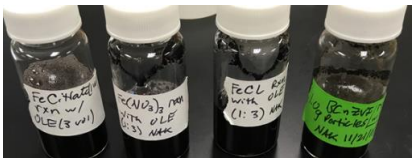

Figure 2: Reaction products of of 0.1 M iron citrate, iron nitrate, and iron chloride (left to right) mixed in 1:3 volume ratio with OLE. The last vial is sample 4G contained 1 gram of the previously prepared biochar with adsorbed iron and was reacted with 10 mL OLE.

3. Results

3.1 Presence of Iron

A qualitative iron determination was performed on all samples, a known iron control (not pictured), an acid blank, and a biochar control. Sample 4G had iron clearly visible to the naked eye (reddish rust spots) and as such was omitted from this test. The rest of the samples were extracted grinding up 0.4 g of sample and combining with 2 ml concentrated nitric acid before being allowed to sit for 48 hours. After extraction, the samples with any biochar in them (including the control) had become bubbly on top with sample 3B being the most noticeably thick. The liquid portion of each sample was brought to 20 ml with deionized water and then 5 drops of each were placed into a multiwell ceramic plate. To each well 2 drops of 0.1 M KSCN was added. A blood red coloration indicates the presence of iron.

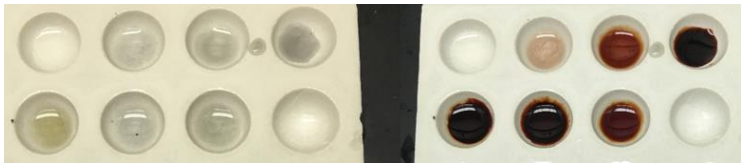

Figure 3: Samples before KSCN Addition (L) and after (R). Top left to right Acid Blank, Biochar Control, 5B, 3B. Bottom left to right 3C, 3A, 3E.

3.2 PXRD and TEM Imaging of Samples

PXRD patterns were collected on a Rigaku (Tokyo) MiniFlex+ (Co K_α, λ = 2.29 Å). The peak in the graph associated with Sample 3A demonstrates crystalline iron (Fe(110) for body-centered cubic structure, PDF#99-000-1743). All other samples do not indicate this peak. TEM images were collected using either a JEOL 2100 (3A, 3B) or Hitachi 7000 (3D, 3E, 4G).

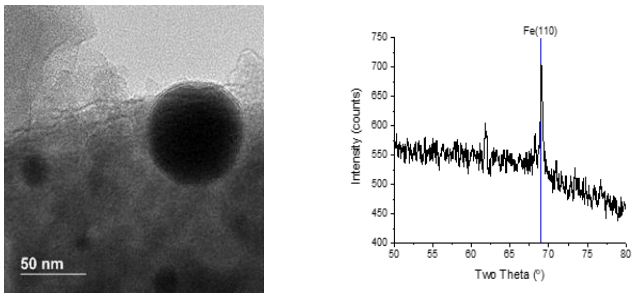

Figure 4. TEM (L) and PXRD (R) of 3A (iron citrate + biochar, shaken, thermal).

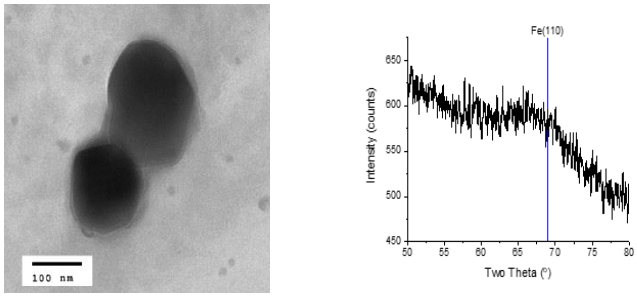

Figure 5. TEM (L) and PXRD (R) of 3D (iron chloride + biochar, sonicate, sodium borohydride).

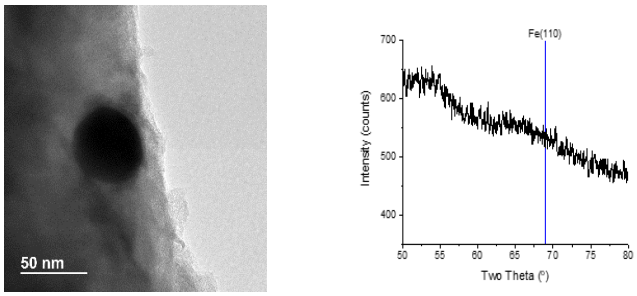

Figure 6. TEM (L) and PXRD (R) of 3B (iron citrate + biochar, shaking, sodium borohydride).

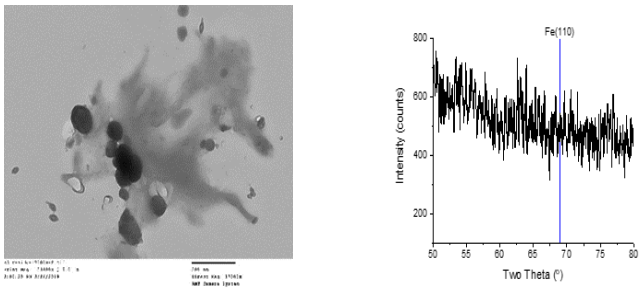

Figure 7. TEM (L) and PXRD (R) of 5B (iron chloride, shaking, ascorbic acid).

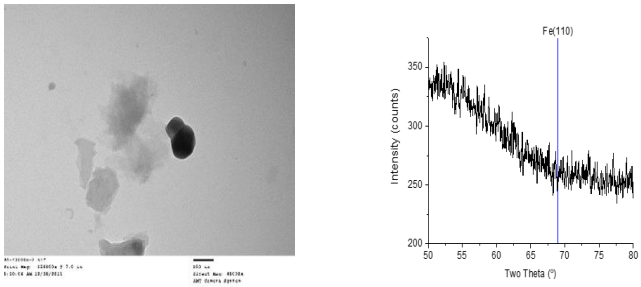

Figure 8. TEM (L) and PXRD (R) of 5A (iron citrate, shaking, ascorbic acid).

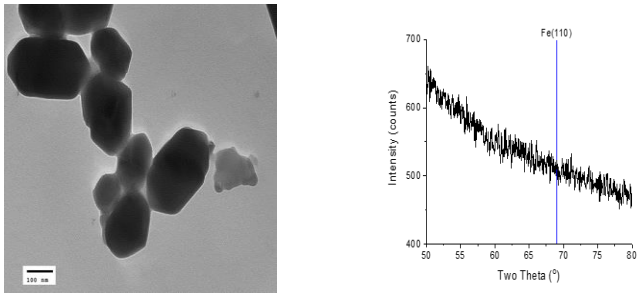

Figure 9. TEM (L) and PXRD (R) of 3E (iron chloride, sonication, OLE).

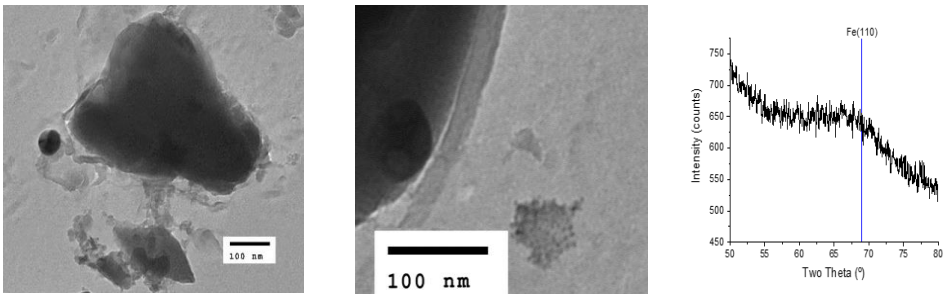

Figure 10. TEM (left, center) and PXRD (right) of 4G (iron citrate, shaking, OLE).

The figures above show three cases where nanoparticles were successfully supported on the Milo seed biochar: samples 3D, 3E, and 5A. In Fig. 4, there is an iron nanoparticle 50 nm diameter embedded in the Milo seed biochar support. This particle was reduced via thermal reduction and showed a clear peak on its PXRD graph corresponding to zero valent iron. The base salt was iron citrate and the adsorption method was shaking. In Fig. 5, the particles are agglomerated and free of any support, approximately 100 nm in diameter. Fig. 6 shows a 50 nm diameter particle embedded in a Milo seed biochar support. In Fig. 10, particles are associated with biochar, and a particle of 50 nm diameter is embedded in a Milo seed biochar support with a thin layer capping the support and enclosed particle.

Nanoparticles were also produced without biochar support. Fig. 7 and Fig. 8 also shows large particles of iron among what is likely residue of the ascorbic acid reducing agent. In Fig. 9, there are larger particles (approximately 200 nm width) agglomerating with no evidence of biochar support.

4. Discussion

4.1 Iron Analysis Results

In the PXRD graphs only one of the samples (3A) had a crystalline peak corresponding to Fe^0 ($2\theta = 65.5^o$) in the PXRD patterns. Other patterns did not show significant peaks corresponding to any sort of iron and this can be attributed to either very small crystals, amorphous material, or not enough sample in the X-ray beam. All samples were shown to contain some form of iron through the KSCN determination (Fig. 3). There was a false positive however in the biochar control, though the coloration was far lighter than the blood red hue of the other samples. The iron present in the samples can be visualized in the TEM results that showed 50 nm nanoparticles for samples 3A (thermal reduction of iron citrate), 3B (sodium borohydride reduction of iron citrate), and 4G (oak leaf extract reduction of iron citrate). Assuming all samples successfully created iron nanoparticles, and because the 50nm iron particles in 3A produce a crystalline powder pattern, the other iron particles lacking this crystalline powder pattern are likely amorphous. The methodology of rapidly precipitating iron at low temperatures did not provide enough energy for the iron atoms to arrange into a crystalline structure and resulted in amorphous nanoparticles. This energy difference between thermal reduction and the OLE reduction was one of the original motivations for this project, as it aligns with one of the principles of green chemistry (design for energy efficiency).
The PXRD results, while noisy, hint at the possibility that various ferrous oxides were present. The broad humps correspond to the diffraction angles expected for FeO(OH) (samples 5A and 3B), $Fe(OH)_3$ (samples 3E and 3D), and Fe_3O_4 in 3D (not shown). This may not mean an absence of zero valent iron, as impure nZVI can have an iron oxide coating [19], [20]. While ferrous oxides can provide electrons to bacteria for denitrification purposes [2], they require the presence of Fe^0 to convert nitrate to ammonia. The overall excessive noise seen in the PXRD graphs was itself a notable result. In the future a greater amount of sample could be prepared. With more sample present there would be a higher likelihood of the X-rays interacting with any zero valent iron that may be present, thus leading to a higher signal-to-noise ratio.

4.2 Adsorption

Adsorption of the nanoparticles onto the support is essential for practical use of the nZVI. The biochar support stabilizes the nanoparticles, but the Fe(0) concentration must be enough to provide electrons for the denitrification process of the proposed bioreactor. The two samples that produced TEM images of unsupported, unknown material, 3D (sodium borohydride reduction of iron chloride), and 3E (oak leaf extract reduction of iron chloride) were both the product of Fe(III) adsorption via sonication, suggesting that shaking for 24 hours is a more suitable method of iron adsorption onto the Milo seed biochar. This also suggests that much of the iron originally intended to be in solution with samples 3D and 3E was lost.

4.3 Iron Salt Complexes

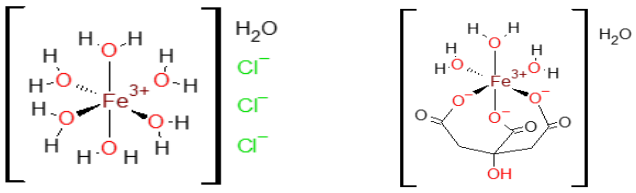

Figure 11. Structures of iron(III) chloride (left) and iron(III) citrate (right).

Comparing the structures of iron(III) chloride and iron(III) citrate (Fig. 11), the citrate ligand of iron(III) chloride is bound to the iron at three points and so is harder to remove, a phenomenon known as the chelate effect [25]. While reaction with ascorbic acid produced a colorless solution with iron chloride (5B) vs. a yellow solution for iron citrate (5A), their TEM images both show 50nm particles. The expected chelate effect does not seem to hinder to the production of nZVI, so the greener iron citrate will be used in the future.

5. Conclusion

A green method of nZVI@BC production was explored by reducing iron citrate with thermal reduction, sodium borohydride, ascorbic acid, and oak leaf extract, with the latter being the most environmentally friendly procedure. Nanoparticles were all comparable in size (approximately 50nm). However, the iron was not consistently embedded in the BC in all trials. The Fe(III) failed to adsorb onto the BC by sonication as shown by TEM images, however the KSCN iron determination indicates the presence of iron in all samples. Another possible method for producing nZVI@BC would be to reduce the iron to form nanoparticles before being adsorbed onto the BC. The future goal of this project is to use the nZVI@BC in a bioreactor of denitrifying bacteria. nZVI@BC from carbothermal reduction has been shown to enhances bacterial denitrification, as seen in our previous work [13] summarized in Appendix A, and the literature indicates that nZVI from $NaBH_4$ can do the same[2]. The amorphous state of the nZVI from green chemistry procedures should be addressed in future studies to measure their effectiveness *in situ*. The nZVI@BC *via* OLE should also be tested for its anti-microbial properties. If it proves effective, the nZVI reduced by OLE and supported on Milo seed biochar will be a cheap and green denitrification system.

6. Acknowledgments

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7. References

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Appendix A: Denitrification Rates

As previously reported by our group[13], Milo seed biochar-supported iron nanoparticles from carbothermal reduction of iron(III) nitrate enhances denitrification rates of *Pseudomonas aeruginosa* in culture media (Fig. 12). In sealed bottles, degassed under argon, 85mL of low nutrient broth with 126 mg/L NO_3-N were incubated with nothing added, 0.1 mL of bacteria culture added, 0.37 g of nZVI@BC added, or both added. The mixture of both removed >80% of NO_3 in 100 hours.

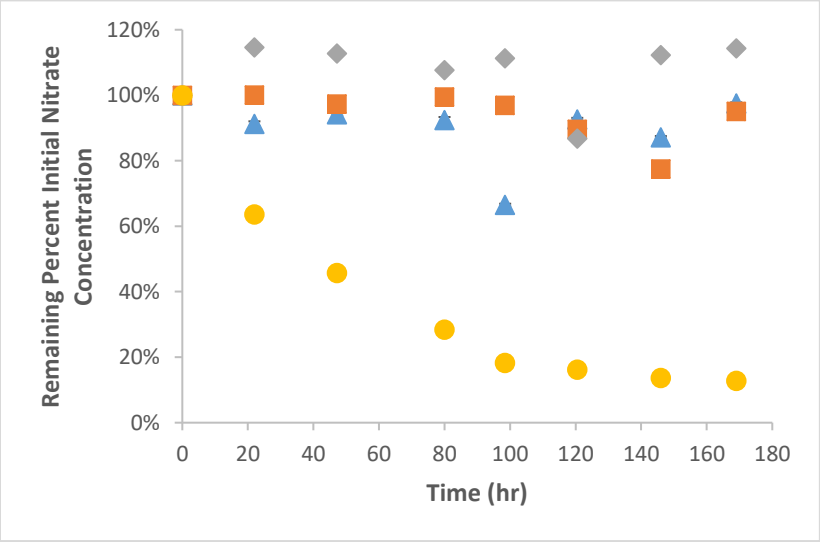

Figure 12: Percent initial nitrate concentration (126 mg-N/L) remaining in anaerobic batch reactor with neither bacteria nor nZVI added (▲), bacteria only (■), nZVI only (◊), or bacteria and nZVI (○) [13].