ENVIRONMENTALLY BENIGN SYNTHESIS OF IRON AND SILVER NANOPARTICLES AND THE EXAMINATION OF THEIR APPLICATION POSSIBILITIES

PH.D. THESIS

ANDREA RÓNAVÁRI

SUPERVISORS:
DR. ZOLTÁN KÓNYA
DR. MÓNIKA KIRICSI

DOCTORAL SCHOOL OF ENVIRONMENTAL SCIENCES
DEPARTMENT OF APPLIED AND ENVIRONMENTAL CHEMISTRY
FACULTY OF SCIENCE AND INFORMATICS
UNIVERSITY OF SZEGED

SZEGED
2017

1. INTRODUCTION AND OBJECTIVES

Nowadays, one of the most important challenges in environment protection is the identification and disposal of chlorinated aliphatic hydrocarbon contamination of subsurface waters and geological materials. To achieve this goal, an innovative, environmentally, economically and socially acceptable technology is required. Owing to the scientific advancements of recent years, a method that fulfills these demands should be based on nanotechnology. The utilization of nanotechnology in the field of decontamination, just like in many other fields of life, leads to a massive acceleration of technological improvement, usually associated with significant advances in terms of cost and efficiency. Many publications have been released about the role of metal or metal oxide nanoparticles in environmental remediation.

In environmental practice, zero valent iron nanoparticles are frequently used for the *in situ* decontamination of groundwaters from halogenated hydrocarbons. One of the main obstacles to the widespread utilization of this technology is its high production costs, thus timely research activities focus on the development of an economically and industrially efficient method that could make nanosized materials even better candidates for environmental protection. Another requirement for a safe application of nanoscale iron is to elaborate its possible limitations, such as toxicity and impact on the ecosystem. For these reasons, we must examine how zero valent iron nanoparticles affect the organisms that live in the regions to be remediated, as well as the manner and scale of their effects on bacterial species' diversity.

Recently, several metal and metal oxide nanoparticles have been successfully synthesized at the Department of Applied and Environmental Chemistry at the University of Szeged. Linked to this research area, the aims of my doctoral work were to develop cost-, time- and energy-efficient methods for the preparation of zero valent iron nanoparticles, coupled with their in-depth characterization, as well as to analyze their applicability for the environmental remediation focusing on chlorinated aliphatic hydrocarbon contaminants and to understand their overall effects on chemical and biological systems. Furthermore, in order to expand and compare our findings on zero valent iron particles with a well-characterized system having already established standards, including exact reference values and well-defined parameters, we included silver nanoparticles into the analyses and carried out a detailed chemical and biological comparative examination. Since our silver nanoparticles were created by green synthesis methodologies using herbal extracts, we aimed to determine

their performance in different biological systems and to identify how the green material used for the synthesis can influence the physical, chemical and biological characteristics of the obtained nanomaterials.

2. EXPERIMENTAL

To obtain nanosized iron suspensions with semi-green synthesis methods aqueous solutions of iron(II) chloride, iron(III) chloride and iron(II) sulphate were reduced using sodium borohydride or sodium dithionite at room temperature with air or inert atmosphere. The molar ratios of the reactants were 1:1 (with sodium dithionite), 1:2 (with Fe(II) ion containing precursors and sodium borohydride) and 1:3 (with Fe(III) ion containing precursors and sodium borohydride). The suspensions were produced in 2500, 5000 and 10000 ppm concentrations. Water was used as a solvent in each case (tap water with the presence of air, deoxygenized ion exchanged water with inert – nitrogen – atmosphere), with the end volume of 250 mL. Vigorous stirring was maintained throughout the reactions; firstly, sodium hydroxide was added to the prepared iron salt solution (to insure basic environment), then the corresponding reducing agent was added to the mixture. The sodium borohydride solution was added with a speed of 6 drops/minute to control the formation of hydrogen gas, while the sodium dithionite solution was added into the mixture at once. Following their addition, the samples were stirred for 5 (for sodium borohydride) and 40 (for sodium dithionite) minutes and the end products were washed with water and ethanol. Our experiments were carried out with fresh samples, shortly after the synthesis reactions.

In order to observe the effect of polyvinylpyrrolidone (PVP) on nanoparticle morphology and reactivity, the synthesis methods were carried out in a similar way but using distilled water, inert atmosphere and PVP in the amount corresponding to 0.3% of the iron content of the sample.

Iron nanoparticles were also synthesized with the help of herbal – coffee, green tea and Virginia creeper (*Parthenocissus quinquefolia*) – extracts. The Virginia creeper extract was prepared in the following procedure: 5 grams of dried and ground leaves were added to 100 mL of distilled water and this "solution" was heated to 80 °C for 80 minutes, the product was filtered using a microfilter of 0.2 μm pore diameter after cooling and was stored at 4 °C until further use. Similar procedures were followed with the preparations of coffee and green tea extracts; 2 grams of coffee or 5 grams of green tea were added to 100 mL of distilled

water respectively. The heat treatment lasted for 5 minutes in the case of coffee and 20 minutes for green tea, on 80 °C in both cases.

These extracts were added in 1:1 volume ratios into 0.1 M iron(III) chloride solutions prepared with tap water. The reaction lasted for 24 hours at room temperature. The resulting particles were washed with distilled water and ethanol, and were applied freshly during the experiment.

The synthesis of silver nanoparticles was performed in a similar way. The above-explained coffee and green tea extracts were used with one main difference – distilled water was used in all cases. During the reaction procedure 0.1 M of aqueous silver nitrate solution was mixed with the respective herbal mixture in 1:1 volume ratio for 24 hours at room temperature. The resulting samples were washed with ion-exchanged water and kept on 4 °C until further use.

The characteristics of the prepared iron and silver nanoparticles were analyzed in several different methods. The chemical composition of the various nanoparticle samples were identified with X-ray diffractometry (XRD). The purity of the samples was determined with the help of energy dispersive X-ray spectroscopy (EDS), particle morphologies were observed with transmission electron microscopy (TEM). The reduction potential (ORP) and iron content of the particles were defined successfully. The degradation effectiveness of the nano iron suspensions were examined using a groundwater sample contaminated by volatile chlorinated hydrocarbons.

The testing of the chosen sample ($nZVI_S^D$) was done as part of a large scale commercial remediation project undertaken in 2014-2015 on the south-eastern part of the Great Hungarian Plain. Along with monitoring the changes in the chemical composition of the volatile chlorinated hydrocarbons using gas chromatography–mass spectrometry (GC-MS) and gas chromatography with flame ionization detector (GC-FID) in relation to the addition of iron nanoparticle samples, microbial diversity changes were observed in microcosm systems with DNA-based measurements (genomic DNA isolation, polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), quantitative PCR).

The quality and stability of the nano silver suspensions were analyzed with Fourier-transform infrared spectroscopy (FT-IR), inductively coupled plasma mass spectrometry (ICP-MS), dynamic light scattering (DLS) and ultraviolet-visible absorption spectroscopy (UV-VIS). The biological activity of the silver nanoparticles was determined, using different antimicrobial (agar-diffusion method, colony-forming units determination) and cytotoxicity (cell proliferation assay, crystal violet staining) experiments.

3. NOVEL SCIENTIFIC RESULTS

T1. Zero valent iron nanoparticles were synthesized using optimized environmentally benign green and semi-green synthesis methods.

1.1 XRD, EDS and TEM measurements confirmed that we successfully produced iron nanoparticles at room temperature and under ambient conditions by applying different initial iron salts (iron(II) and iron(III) sulphate, iron(III) chloride) and reducing agents (sodium borohydride and sodium dithionite), using tap water instead of resource intensive deoxygenated solvents.

1.2 The average particle size, crystal structure and reduction capacity of the produced iron nanoparticles were determined. Although the iron nanoparticles prepared in the semi-green way had smaller average diameter and larger reduction potential than the samples made with green synthesis, the latter ones also performed well in our experiments.

T2. We were the first to produce iron nanoparticles using Virginia creeper (*Parthenocissus quinquefolia*) extract.

2.1 The successful synthesis was confirmed by XRD, EDS, TEM and oxidation/reduction potential measurements.

2.2 Although the iron nanoparticles prepared using Virginia creeper extract had smaller average diameter than the coffee and green tea reduced samples, the reactivity of the former one did not outperform the latter ones' performance in our experiments.

T3. We proved that the method of iron nanoparticle preparation largely influenced the chemical properties of the obtained samples.

3.1 The average particle size, crystal structure and reduction capacity of the produced iron nanoparticles were determined. Although the iron nanoparticles prepared in the semi-green way had smaller average diameter and larger reduction potential than the samples made with green synthesis, the latter ones also showed good performance in our experiments.

3.2 It was confirmed that the iron nanoparticles reduced by sodium borohydride outperformed the iron nanoparticles synthesized by using sodium dithionite both in the measured oxidation/reduction potential values, and in the volatile chlorinated hydrocarbon reduction tests.

T4. It was demonstrated that the application of iron nanoparticles obtained by reducing ferrous(II) sulphate with sodium dithionite ($nZVI_S^D$) could serve as a sustainable, efficient and economical alternative in environmental remediation.

4.1 It was confirmed that iron nanoparticles obtained by reducing ferrous(II) sulphate by sodium dithionite ($nZVI_S^D$) showed similarly high performance in the measured oxidation/reduction potential values, and in the volatile chlorinated hydrocarbon reduction tests as sodium borohydride-reduced iron nanoparticles.

4.2 It was demonstrated that the $nZVI_S^D$ sample produced by semi-green synthesis proved to be capable of reducing volatile chlorinated hydrocarbons based on the performed field test.

T5. It was verified that the initial iron salts and reducing agents used in iron synthesis do not only define the reactivity and morphology of the produced nanoparticles, but also influence the biological activity of the iron particles (their impact on anaerobic bacteria).

5.1 It was ascertained that the introduction of nanoiron (in 0.1 g/L concentration) had an impact on the microbial composition of the microcosm systems and on the dechlorinating processes. Our tests revealed that the iron nanoparticles, prepared by any combination of the used reducing agents and iron salts, applied in a 0.1 g/L concentration reduced the size of the *Dehalococcoide*s population after an initial inhibitory effect. The relative amount of 16S rDNS and dehalogenase genes containing bacteria also decreased with the addition of any nanoscale iron while the total mass of microbes increased. The quantity of sulphate reducing bacteria increased and the amount of methanogenic bacteria decreased in the presence of the nanoiron samples reduced by sodium dithionite. Methanogenesis was observed only in case of the iron samples reduced with sodium borohydride as revealed by the dechlorinating tests.

5.2 It was demonstrated – in line with the literature data – that following an initial inhibitory effect, surviving anaerobic bacterial populations of microcosm systems managed to achieve a similar composition like that of the initial microflora, which had a proven reductive dehalogenation activity.

T6. It was proved that the green entity used for the nanomaterial synthesis can largely define the physical, chemical and biological characteristics of the obtained nanoparticles, therefore it is recommended to have a circumspect selection of the green extracts, and a comprehensive screen of the products should be carried out prior their applications to delineate their behavior in the presence of living systems.

6.1 Silver nanoparticles were also successfully synthesized using coffee and green tea extracts which was certified by XRD, EDS, TEM measurements. The comprehensive chemical and biological characteristics of the obtained nanoparticles were investigated. It was found that both AgNPs proved to be effective in the examined concentrations against nearly all the tested microbes; however, GT-AgNPs performed always markedly better in toxicity and antimicrobial screens than C-AgNP counterparts. However, only C-AgNP particles were biocompatible with the tested HeLa and NIH/3T3 cells, showing no mammalian cytotoxicity, which renders C-AgNPs as attractive potential candidates for further applications.

6.2 Surprisingly, in every biological test we performed, in contrast to the literature data, we found that bigger sized GT-AgNPs resulted to be more effective than smaller C-AgNPs. In fact, ICP-MS measurements verified that ~3.5 times more silver ions can be released from GT-AgNPs than from C-AgNPs, which might be the direct consequence of the thick matrix, where C-AgNPs seem to be completely embedded.

6.3 We successfully managed to extend the interesting observations we made with iron nanoparticles to silver nanoparticles, a reference material with defined features, which are well described in the literature. Our most important experience-based finding was that the reducing agent applied for nanoiron or nanosilver production can largely define not only the size and shape of the nanoparticles but also their behaviour in biological systems.

4. PUBLICATIONS RELATED TO THE PRESENT THESIS

1. **Impact of the morphology and reactivity of nanoscale zero-valent iron (NZVI) on dechlorinating bacteria.**
   Rónavári A., Balázs M., Tolmacsov P., Molnár Cs., Kiss I, Kukovecz Á., Kónya Z.
   Water Research, 2016, 95:165-173
   DOI: 10.1016/j.watres.2016.03.019
   $IF_{2015/2016}$ = 5.991 Independent citations: 4

2. **Environmentally benign synthesis methods of zero valent iron nanoparticles.**
   Kozma G., **Rónavári A.**, Kónya Z., Kukovecz Á.
   ACS Sustainable Chemistry & Engineering, 2016, 4(1):291–297
   DOI: 10.1021/acssuschemeng.5b01185
   $IF_{2015/2016}$ = 5.267 Independent citations: 4

3. **Biological activity of green-synthesized silver nanoparticles depends on the applied natural extracts: a comprehensive study.**
   Rónavári A., Kovács D., Igaz N., Vágvölgyi C., Boros IM., Kónya Z., Pfeiffer I., Kiricsi M.
   International Journal of Nanomedicine, 2017, 12:871-883
   DOI: 10.2147/IJN.S122842
   $IF_{2015/2016}$ = 4.320 Independent citations -

5. CONFERENCE PRESENTATIONS RELATED TO THE PRESENT THESIS

1. **Applications of iron nanoparticles for soil remediation.**
   Rónavári A., Kozma G., Kónya Z., Kukovecz Á.
   PhD Student Conference in Environmental Sciences, Budapest, 2013 (oral presentation)

2. **Comparison of the reactivity and the effect of different nanoscale zero-valent iron on microbial populations in trichloroethylene contaminated groundwater.**
   Rónavári A., Balázs M., Németh A., Rutkai E., Urbán G., Tolmacsov P., Kiss I., Kukovecz Á., Kónya Z.
   Workshop on Functionalized Surfaces and Nanocomposites, Joint Meeting of WG2-WG3-WG4 of COST Action CM1101, Szeged, 2013 (poster presentation)

3. **Comparison of the reactivity and the effect of different nanoscale zero valent iron on microbial populations in trichloroethylene contaminated groundwater.**
Rónavári A., Balázs M., Németh A., Rutkai E., Urbán G., Tolmacsov P., Kiss I., Kukovecz Á., Kónya Z.
XI. Environmental Analytical and Technology Conference - Innovative environmental diagnostic technologies and methods for healthier human environment, Hajdúszoboszló, 2013 (oral presentation)

4. **The effect of different nanoscale zero-valent iron on microbial populations in cis-1,2-dichloroethylene contaminated groundwater.**
Rónavári A., Balázs M., Németh A., Rutkai E., Urbán G., Tolmacsov P., Kiss I., Kukovecz Á., Kónya Z.
Power of microbes in Industry and Environment, Primosten, Croatia, 2013 (poster presentation)

5. **Assessing the application and impact of different nanoscale zero-valent irons on microbial populations.**
Rónavári A., Balázs, M., Németh, A., Rutkai, E., Urbán, G., Tolmacsov, P., Kiss, I., Kukovecz, Á., Kónya, Z.
I. Innovation in Science Doctoral Student Conference, Szeged, 2014 (poster presentation)

6. **Investigation of the reactivity and the effect of different nanoscale zero valent iron on microbial populations in cis-, dichlotoethylene (cDCE) contaminated groundwater.**
Rónavári A., Balázs M., Homa M., Németh A., Rutkai E., Urbán G., Tolmacsov P., Kiss I., Kukovecz Á., Kónya Z.
16th Danube-Kris-Mures-Tisza (DKMT) Conference Environment and Health, Arad, Romania, 2014 (poster presentation)

7. **Remediation by nZVI: impact on the soil microbial community.**
Rónavári A., Balázs M., Rutkai E., Tolmacsov P., Kiss I, Kukovecz Á., Kónya Z.
XVIII. International Symposium on Gnotobiology, Saint Petersburg, Russia, 2014 (poster presentation)

8. **Groundwater remediation using environmentally benign zero valent iron nanoparticles.**
Kozma G., **Rónavári A.**, Kukovecz Á, Kónya Z.
The International Bioscience Conference and the 6th International PSU – UNS Bioscience Conference (IBSC), Novi Sad, Serbia, 2016 (poster presentation)

9. **Impact of nanoscale zero valent iron on the soil microbial community: the role of morphology and reactivity.**
Rónavári A., Balázs M., Tolmacsov P., Molnár Cs., Kiss I, Kukovecz Á., Kónya Z.
The International Bioscience Conference and the 6th International PSU – UNS Bioscience Conference (IBSC), Novi Sad, Serbia, 2016 (poster presentation)

10. **Biological activity of silver nanoparticles prepared by coffee and green tea extracts.**
Rónavári A., Igaz N., Kovács D., Kónya Z., Pfeiffer I., Kiricsi M.
Annual Meeting of the Hungarian Society for Microbiology and XI. Fermentation Colloquium, Keszthely, 2016 (poster presentation)

6. OTHER PUBLICATIONS

1. **Effect of DNA polymerases on PCR-DGGE patterns.**
Balázs M., **Rónavári A.**, Németh A., Bihari Z., Rutkai E., Bartos P., Kiss I., Szvetnik A.
International Biodeterioration & Biodegradation, 2013, 84:244-249
DOI: 10.1016/j.ibiod.2012.05.011
IF_{2013} = 2.235 Independent citations: 11

2. **Structure and stability of pristine and Bi and/or Sb decorated titanate nanotubes.**
Rónavári A., Buchholcz B., Kukovecz Á., Kónya Z.
Journal of Molecular Structure, 2013, 1044:104-108
DOI: 10.1016/j.molstruc.2012.12.008
IF_{2013} = 1.599 Independent citations: 3

3. **Ion exchange defines the biological activity of titanate nanotubes.**
Rónavári A., Kovács D., Vágvölgyi Cs., Kónya Z., Kiricsi M., Pfeiffer I.
Journal of Basic Microbiology, 2016, 56(5):557-565
DOI: 10.1002/jobm.201500742
$IF_{2015/2016}$ = 1.585 Independent citations: 1

4. **Hydrodynamic chronoamperometric determination of hydrogen peroxide using carbon paste electrodes coated by multiwalled carbon nanotubes decorated with MnO_2 or Pt particles.**
Anojčić J., Guzsvány V., Vajdle O., Madarász D., **Rónavári A.**, Kónya Z., Kalcher K.
Sensors and Actuators B: Chemical, 2016, 233:83-92
DOI: 10.1016/j.snb.2016.04.005
$IF_{2015/2016}$ = 4.758 Independent citations: 5

7. OTHER CONFERENCE PRESENTATIONS

1. **Performance of DNA polymerases.**
Balázs M., Szvetnik A., Németh A., **Rónavári A.**, Kiss I.
Power of Microbes in Industry and Environment, Malinska, Croatia, 2010
(poster presentation)

2. **Effect of DNA polymerases on DGGE patterns.**
Balázs M., Németh A., **Rónavári A.**, Bihari Z., Kiss I., Szvetnik A.
BioMicroWorld 2011 - IV International Conference on Environmental, Industrial and Applied Microbiology, Torremolinos, Malaga, Spain, 2011 (poster presentation)

3. **Performance of DNA polymerases.**
Balázs M., Szvetnik A., Németh A., **Rónavári A.**, Kiss I.
15th International Biodeterioration and Biodegradation Symposium, Vienna, Austria, 2011 (poster presentation)

4. **Investigation of effects of different DNA Polymerases on DGGE patterns in an artificial microbial consortium.**
Németh A., Balázs M., **Rónavári A.**, Rutkai E., Urbán G., Kiss I., Szvetnik A.
5th International Symposium on Biosorption and Bioremediation, Prague, Czech Republic, 2012 (poster presentation)

5. **Synthesis, characterization and spectroscopic properties of pristine, Bi- and Sb-decorated titanate nanotubes.**
   Rónavári A., Buchholcz B., Kukovecz Á., Kónya Z.
   31st European Congress on Molecular Spectroscopy, Cluj-Napoca, Romania, 2012 (oral presentation)

6. **Structure and stability of pristine, Bi and/or Sb decorated titanate nanotubes / Bi és/ vagy Sb-nal dekorált titanát nanocsövek szerkezete és stabilitása.**
   Rónavári A., Buchholcz B., Kukovecz Á., Kónya Z.
   Annual meeting of the Hungarian Society for Microscopy, Siófok, 2013 (oral presentation)

7. **Hydrodynamic chronoamperometric determination of hydrogen-peroxide by carbon paste electrodes modified with different nanomaterials.**
   Zbiljić J., Guzsvány V., **Rónavári A.**, Kukovecz Á., Kónya Z., Kalcher K.
   20th Young investigators seminar on analytical chemistry, Maribor, Slovenia, 2013 (abstract)

8. **Synthesis, structure and stability of pristine, Bi and/or Sb decorated titanate nanotubes.**
   Rónavári A., Buchholcz B., Kukovecz Á., Kónya Z.
   International research and practice conference nanotechnology and nanomaterials, Bukovel, Ukraine, 2013 (poster presentation)

9. **Synthesis, structure and stability of pristine, Bi and/or Sb decorated titanate nanotubes.**
   Rónavári A., Buchholcz B., Kukovecz Á., Kónya Z.
   2nd International Summer School for young scientists "NANOTECHNOLOGY: from fundamental research to innovations", Bukovel, Ukraine, 2013 (oral presentation)

10. **Antimicrobial activity of ion-exchanged titanate nanotubes.**
    Rónavári A., Kovács D., Vágvölgyi Cs., Kónya Z., Kiricsi M., Pfeiffer I.
    18th Danube-Kris-Mures-Tisza (DKMT) Euroregional Conference on Environment and Health, Novi Sad, Serbia, 2016 (poster presentation)

8. SCIENTOMETRIC DATA

Peer-reviewed papers total:	7	out of this, related to the topic of thesis: 3
Cumulative impact factor:	25.755	out of this, related to the topic of thesis: 15.578
Independent citations total:	28	out of this, related to the topic of thesis: 8