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Review report on PhD Thesis of MSc. Nhung H. A. Nguyen entitled “Biological effects of iron-based nanomaterials evaluated from single species to complex microbial communities“

MSc. Nguyen submitted the doctoral thesis dealing with the study of iron-based nano- and microparticles and their effects on individual microorganisms and microbial communities. This topic is very important and interesting, especially due to the potential use of these materials in large scale environmental technologies, especially for remediation of contaminated soils, groundwater or surface water. As mentioned in the title, the main interest of this work was focused on complex studies of impacts of various iron-based nano- and micromaterials (both magnetite and zero valent iron) on Gram-positive and Gram-negative microorganisms and microalgae, both in the form of single species and microbial communities.

In general, the thesis is written in a very good way, in good English, and contains enormous amount of experimental data. In fact, majority of presented results was published in high quality journals. The thesis is based on 6 papers in impacted journals and one NanoRem report. It is also important to say that MSc. Nguyen has also studied the toxicity and other properties of other types of nanomaterials, including graphene oxide, silver nanoparticles and electrospun membranes.

The Background and aims part covers basic information about the planned and performed research, including information about the iron based nano- and micromaterials used and methods for their characterization, types of bacteria and microalgae tested and methods employed for the study of toxic effects. It can be clearly seen that a really complex characterization of a wide variety of iron based materials using various biological system was performed.

The Results and discussion section summarizes in the complex way the results presented in published papers. Also this part is very well written, it is accompanied with

graphs and photos. Numerous effects of iron-based materials have been observed and properly discussed.

As already mentioned, majority of presented results was already published in high quality impacted papers. I have just a few general questions connected with the presented thesis, namely:

- On page 16 you mention the term „superparamagnetic behaviour“. Can you explain what type of materials exhibits superparamagnetism?
- In the first paper, in the Chapter 2.1. (ZVI particles) you write: „The nZVI particles had a mean diameter of 160 nm, a maximum size of 540 nm“. Similar statements can be often seen in many papers, however, what is the most often used characterization of nanomaterials, as to their size? Do you really work with “true” nanoparticles?
- Magnetic (fluid) hyperthermia, mentioned e.g. in Paper 5, is a promising procedure for the treatment of cancer diseases. In your opinion, could this or similar procedure be applied in real remediation processes or for biofilm removal?
- Comparing both naked and surface modified iron-based nano- and micromaterials, which type (taking into account their price, efficiency, effect on living structure, type of reaction products etc.) is better suited for real (large-scale) remediation applications?
- Magnetic iron oxide particles often exhibit peroxidase-like activity. In your opinion, can this property be of interest in remediation processes?

In my opinion, the reviewed thesis fulfils all requirements posed on theses aimed for obtaining PhD degree. Due to the scientific novelty, an extensive range of research, excellent presentation and discussion of the obtained research and great publication activity this thesis is ready to be defended orally without any changes, in front of respective committee. After successful defence I would like also to propose to award the PhD degree to MSc. Nguyen.

České Budějovice, July 18th, 2018



Biological effects of iron-based Nanomaterials evaluated from single species to complex microbial communities

Referee's Comments

The thesis is well put together. Clearly, Nhung's work has generated a nice collection of peer-reviewed papers which support the quality of her work.

The thesis is written in English and I find no obvious grammatical errors although Nhung is not a native English speaker. This is an additional quality of the thesis.

The thesis gives a good summary of the findings published in the peer reviewed papers and a conclusion. There are 4 omissions which must be addressed:

1. The NP were chosen because of their bio-applications. This needs to be expanded. Which applications? Are they used as anti-bacterial solutions? If not, is the release of the NP accidental? In the thesis, this seems to be taken for granted that the reader knows about the use of these NP.
2. The NP were well-characterised but the physico-chemical characteristics of the NP are not used in an analysis to explain the observed differences in responses caused by the various NP. We are not any wiser as to why there are such differences.
3. The thesis needs a better discussion chapter. The discussion should address:
 - a. The limitations in the current work
 - b. The recommendation – which NP and in which functionalisation can be used commercially, alternatively, which characteristics had to be eliminated in order to reduce toxicity.
 - c. The scope for future work.
4. For the characterisation of the various NP, I think an analysis on the ability of the NP to produce oxidant radicals in a cell free system would be useful. This would be linked to the surface area, as measured by BET, to obtain a measure of the surface reactivity of the materials. A summary table of the NP characteristics would be useful (Table 4.2 in Chapter 4).

In conclusion, there are enough materials here for a very good PhD.

Overall, the scientific methods and the results are impressive and they have been peer-reviewed. However one is left wondering what is precisely the purpose of doing all these tests?

Firstly, what is the commercial use for the chosen materials? Are they popular? The justification for the choice of NP must be clearly stated.

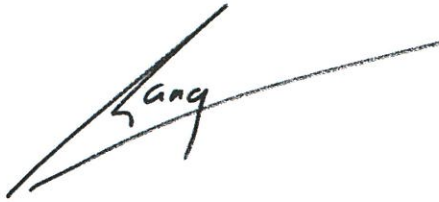
Secondly, with all the results, can we go further than just asserting that there are differences in the materials regarding their toxicity? What cause the difference in toxicity? The comprehensive characterisation data were not used extensively and there is room here for another publication. In the discussion chapter, there are some statements alluding to this but it would be nice to actually do the data analysis.

I recommend that the candidate be awarded with the PhD for her work. It is clear that the results of her work are of use for Nanotoxicology even if she does not clearly explain it.

In the defence of the thesis, the candidate should be prepared to address the issues raised here.

Prof Lang Tran

Institute of Occupational Medicine, Edinburgh (UK)

A handwritten signature in black ink, appearing to read 'Lang', is written over a long, thin, slightly curved horizontal line.

2nd August 2018