OPPONENT'S REVIEW ON DIPLOMA THESIS

František Jiřík, BSc: **The evaluation of the photochemical activity of cyanochelin 1026 and its photolytic fragments**

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The review is written in English in keeping with the thesis.

The present work is a contribution to characterization of properties and function of a member of a class of cyanobacterial siderophores, cyanochelins. The object of the study was a compound previously identified by Dr. Hrouzek's laboratory, cyanochelin 1026.

The student performed isolation, purification and analysis of the photochemical reactivity of the compound with respect to its activity in Fe^{3+} reduction. In the course of the work, the author appears to have mastered a number of important analytical laboratory techniques such as liquid chromatography, absorption spectroscopy and mass spectrometry. The novelty, extent and scientific content of the present work are beyond doubt and the work is admissible as a MSc thesis.

At the same time, there are several issues I would like to point out.

I find the page count of the thesis impressive. For instance, just the introduction covering topics ranging from the life history of cyanobacteria to chemistry, synthesis and function of siderophores is quite elaborate and detailed and alone runs at about thirty pages. However, the sheer extent makes such a text rather difficult to proofread, apparently. This leads to a number of uncorrected errors both in typesetting and general language use. I will pinpoint a few, I hope as least some of this will be useful in the future.

The text contains numerous cases of merged words, at least one per page, on average; perhaps the worst affected part is the declaration of authorship (I cite):

"Prohlašuji, žejsemautoremtéto kvalifikační práce a žejsem ji vypracoval(a) pouze s použitímpramenů a literaturyuvedených v seznamupoužitýchzdrojů."

Now, while I assume that this is some kind of an artifact due to doc to pdf conversion or something like that, it does decrease the overall impression of the work, making it look, well, sloppy.

Of the several instances of the use of "despite", almost all seem to me to be incorrect, in linking 'despite' to a verb instead a noun. This occasionally complicates the understanding, e.g. (p31):

"Despite the photoreactivity of cyanochelin m/z1026 iron (III) complex was confirmed by preliminary test with photometric method using bathophenanthroline disulfonic acid, there is no knowledge about the kinetic [sic] of the reaction."

I suppose the correct sentence should read something like

"Despite the photoreactivity of cyanochelin m/z1026 iron (III) complex 'having been' confirmed..." as in "Although the photoreactivity [...] was confirmed, there is no knowledge". Not that the "complex was confirmed despite the photoreactivity".

There are also occasional errors like (p13): "A recently discovered Aetokthonostatin produced by, which was found to cause...": produced by what?

Now, putting these minor problems aside, I would like to comment on a couple of issues that I have with the scientific content of the work. I assume these will be clarified during the defense of the thesis (queries for the defense are labeled as **Q_** in the following). These are rather technical things which I find appropriate to comment on, because the present thesis seems strongly 'analytically inclined', thus I would expect the issues like reliability of calibration, detection limits and reaction mechanisms discussed in detail.

Despite the page count there are hardly any raw data presented. Although HPLC data and absorption spectra are mentioned, not a single absorption spectrum or a chromatogram is shown. It is my strong opinion that original data, at least illustrative, should be shown in a MSc thesis (or a journal publication, for that matter). Actually, page 42 announces: "The measured absorption spectra are presented in the result section." But no absorption spectra are anywhere to be found (considering myself primarily an optical spectroscopist, I yearn for a wavelength axis, of course). Plus, this is a qualification thesis, hence I would like to be able to evaluate the ability of the student to handle real data.

Since a significant portion of the presented data is in the form of calibration curves, seeing the source data would be helpful and it would simplify orientation in the discussion, in particular in the part dealing with the attempts at quantification of the iron ions. A single wavelength OD value often does not provide sufficient information to see the problem.

Q1. Can you please show some representative HPLC chromatograms and a DAD absorption spectrum of cyanochelin?

As for the calibration data, they frequently cover concentration up to hundreds μM, e.g. Fig.37, but the actual region of interest extends well below 50 μM and this is often poorly sampled, making the fitted calibration lines in my opinion less reliable (e.g. why put the last measured points from Fig.34 to zero as in Fig.23. How do the calibration data look like?). On the other hand, figure 17 is much better.

In this context I would like to specifically mention the plot in Fig.37 with the OD reading below 0.5 because the instrument used (Shimadzu 2600 or 2700) is in my experience perfectly capable of measuring several orders of magnitude smaller OD. But the plot in Fig.37 does not inspire much confidence in that. Now I have never worked with BPDS but the

involvement of peptides, buffers and metal ions indeed suggests that the assay will be difficult to optimize, as the thesis attests. Raw data might show better why this is the case and if something went wrong. What about the offset in Fig.35? Shouldn't OD go to zero at 0 μM?

Q2. Can you please show the absorption spectra that went into making of the plot in Fig.37?

Q3. Regarding the indicator used, BPDS, literature says that its extinction coefficient at 535 nm in Fe^{2+} complex is ~22000 1/(M.cm), Cowart (1993). Shouldn't then 100 μM Fe^{2+} have OD of ~2 rather than ~1 as shown?

I would like to stress that I do not dispute the importance of the determination of the order of reaction of photodegradation of cyanochelin, nor the finding that it is zero-order per se. But:

Q4. What is the possible explanation for the zero-order kinetics? How far in time does the reaction actually follow the zero order?

Hence, **Q5**: Can you please plot the photoreaction data (Fig.23, or better, Fig.34) along with the respective zero-order kinetics? Why is the applied method of determination of the rate constant better that just fitting the concentration vs. time data (Fig. 34) with the appropriate model, i.e. a straight line for zero-order, exponential for 1st order, etc.? This certainly deserves a discussion, rather than just a statement that it is so.

Q6. Maybe I have missed something but I am not sure how was the illumination done. Was the sample in a plastic tube as suggested by the sentence "The photolysiswas performed in one stock solution in 5 ml Eppendorf tube.", rather than, say, a spectroscopic cuvette?

In summary I **do recommend** the present MSc thesis of Bc. František Jiřík for defense, with the suggested grade **2 (very good)**.

České Budějovice, January 9, 2023 David Bína