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The effect of ferrofluid and iron salts upon *Pseudomonas Aeruginosa growth*

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Experimental investigation on the iron impact upon some bacteria growth was carried out aiming to compare the effects of various sources of iron ions. Turbidimetric assay of the cell density in P. aeruginosa liquid culture media following the addition of iron aliquots delivered by iron salts and oxides was performed to get quantitative information on this human pathogen sensitivity to ferric and ferrous ions. The feature of iron scavenger of P. aeruginosa was revealed as resulted from the stimulatory influence of relatively low iron concentrations, for magnetite and ferrous sulfate while nonsignificant uptaken of iron from ferric chloride was found.

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1. Introduction

Pseudomonas bacteria are known as iron scavengers able to collect this element - from an environment where its level is lowering- in the form of siderophores – complex iron chelates known also as pyoverdines. Considering the iron abundance on the Earth (the forth chemical species) as well as the intensive industrialization of last century it seems more and more that iron loading became an environmental issue, some times referred as magnetic contamination [1-2]. *Pseudomonas aeruginosa* is only one example of microorganism able to uptake the iron ions, that was chosen for this study due to its wide spreading into various ecological nishes – including human body [3-4] where it may interfere with iron, mainly at the level of liver and the spleen -target organs for colloidal iron. The bacteria virulence might be correlated to the iron availability in the culture medium [5] while some siderophores may have antimicrobial activity as previously reported [6]).

The interest in the study of the effect of colloidal magnetite- supplied in the form of a ferrofluid -upon an ubiquitous bacterial colonizer of the human body is sustained also by the medical utilization of some ferrofluids (as contrast agents in NMR investigation [7-8] or drug carriers in cancer therapy [9-10]). Literature reports mention the fluorescent emission of *Pseudomonas* cells –related to the siderophore biosynthesis- that was found either increased or diminished (with different variation rates) to the increase of iron concentration (in different concentration ranges), depending on the culture medium composition [11-15]. One needs to keep in mind that ferric iron salts, highly insoluble, are internalized only following reduction reaction assured by certain enzymes present in some microorganism membrane while ferrous ions are much better uptaken. So, in the study below we have investigated the effects of both types of iron ions delivered either from ferrous or ferric salts as well from magnetite based ferrofluid.

2. Experimental

The ferrofluid utilized in the frame of the present experimental project was prepared accordingly to Cotae [12] from magnetite stabilized with sodium oleate, ferrophase particles having the physical diameter ranging between 6.0 and 22.5 nm with an average value of 16.4 nm. Ferrofluid concentration in the culture medium was adjusted by consecutive dilutions resulting in iron concentration ranging between 2.2 microg/l and 75.0 microg/l. (2.2 - 4.5 - 9.0 - 18.0 - 36.0 - 72.0 microg/l). Similarly, aqueous solutions of iron salts were adjusted (ferric chloride, ferrous chloride and ferrous sulfate) to get the same iron concentrations. This chosen iron concentration range includes the usual iron concentration for MRI having as contrast agent the magnetic fluids, i.e. 10 microg/l. Biological specimens of wild strains of *Pseudomonas aeruginosa* isolated from hospital patients with digestive diseases have been grown in sterile glass tubes with standard liquid culture medium (nutritive broth) supplemented with aqueous ferrofluid or aqueous solutions of iron salts having equivalent concentrations.

Bacterial cell density in the inoculums was nephelometrically controlled using a Shimadzu spectrophotometer and calibration curve. Ten *P. aeruginosa* strains have been tested, the measurements being performed after 24 hours incubation at 35.0 ^{0} Celsius. Turbidimetric measurement at 520 nm was carried out in quartz cells 10 mm width, using Shimadzu device and distilled water as blanc sample. The experiment was repeated five times, the average values distributions being

represented in the form of box-plots. Statistical significance of the differences between the median values corresponding to the control and respectively to the iron loaded samples was assessed applying t-test (pair type, two tailed).

3. Results

In Fig. 1 the graph of light extinctions provided by turbidimetric measurements in ferrofluid samples is presented. Some variability in the response of the ten tested microbial strains is revealed by the boxes length corresponding to the distribution curves; but no exceptional large or small values were noticed. Similar data were extracted from the measurements carried out in ferrous sulfate samples (fig. 2) containing the same iron concentrations but only in the ferrous form; however more pronounced stimulatory effect is suggested by the shifting toward higher values of the light extinction boxes observable for the iron concentrations of 4.5 - 9.0 and 18.0 microg/l ($p<0.01$).

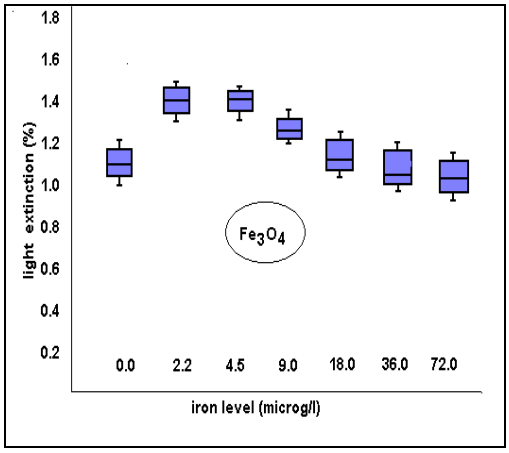

Fig.1. P. aeruginosa response to magnetite from ferrofluid dilutions

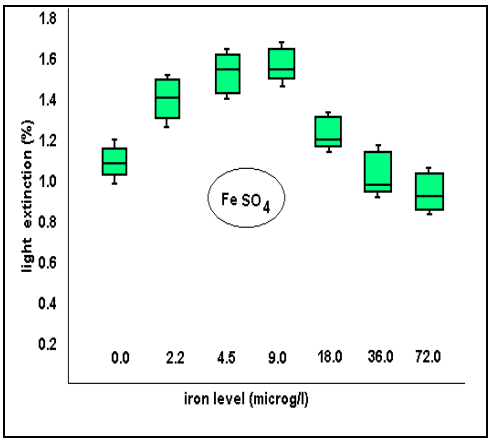

Fig. 2. P. aeruginosa response to ferrous sulfate

When compared to the control, the median values corresponding to the two highest iron concentrations tested within this experiment appear to be shifted to smaller values but the differences are not significant from statistic viewpoint. In the case of ferrous chloride samples the distribution curves, i.e. the box-plots from fig.3, they are slightly larger what can be related to the higher range of the bacterial strains sensitivity while some asymmetry can also be seen (the median position is asymmetric inside the boxes and the tails are not equal anymore).

The stimulatory effect of iron upon the microorganism growth is lower as the boxes are placed to smaller values in the light extinction scale; specific response can be seen for the highest iron concentrations where the box medians are situated under that of the control with statistic significance $p<0.05$.

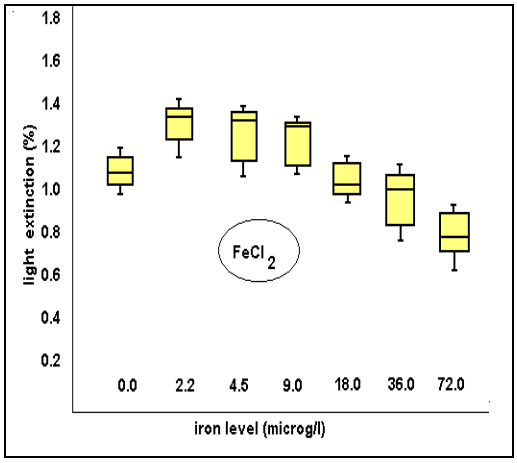

Fig. 3. P. aeruginosa response to ferrous chloride

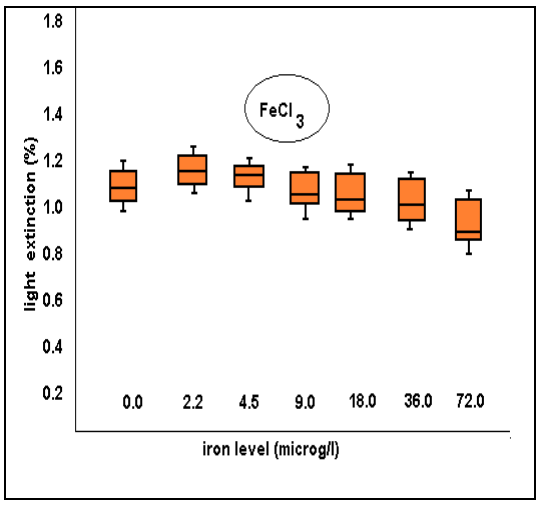

Fig. 4. P. aeruginosa response to ferric chloride

These results suggest certain inhibitory influence –in comparison to the ferrous sulfate data -probably due to of the chloride ions added together with the ferrous iron ones. In fig.4 the bacteria response to ferric chloride addition is presented. The stimulatory effect of iron ions is hardly

visible only for the smallest concentration (2.2 microg/l) while for other samples (4.5 – 9.0 – 18.0 - 36.0 microg/l) no significant changes were recorded; negative response of *P. aeruginosa* strains seems to be induced by the highest iron concentration (72.0 microg/l) with $p<0.05$.

4. Discussion

It seems that iron has generally a benefic effect on the bacteria growth mainly for relatively low concentrations in the culture medium which is concordant with the feature of iron scavenger of *P. aeruginosa*, known for its ability of iron uptake under the form of complex combinations. Previous reports [16-17] revealed the pyoverdine fluorescence increasing in the presence of iron ions, when delivered in the form of colloidal magnetite from diluted ferrofluids. The results presented inhere evidenced the highest stimulatory effect of ferrous sulfate on the bacteria cell density in the culture medium; similar positive influence was found in the case of colloidal magnetite (ferrofluid samples) while diminished availability of ferric ions from ferric chloride was also emphasized. The comparison of ferric and ferrous chlorides –on a side – and ferrous chloride and sulfate – on the other side - suggested that chloride ions (Fig. 5) could have some contribution to the decrease of the positive response of *P. aeruginosa* to relatively low iron ions concentrations. Concentrations over 10microg/l appear to induce some significant negative influence on the cell density in the cases of ferric and ferrous chlorides sustaining the hypothesis that chloride ions might have concurrent effects with the ions ones. This study leads to the conclusion that, from the medical viewpoint it seems that ferrofluid administration for diagnosis or therapy purposes is not able to induce stimulatory effects on the *P. aeruginosa* contaminants of these ones are present into the patient tissues since no significant effects on the microorganism growth were detected at iron levels over 9.0 microg/l.

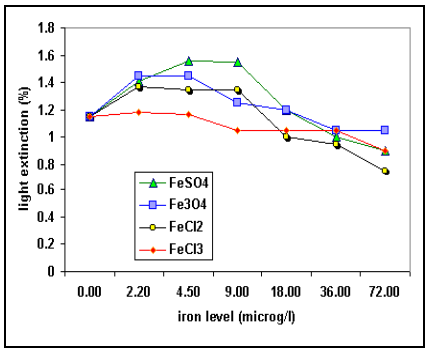

Fig. 5. The value of median within the distribution of light extinction versus iron level

However, in other situations, relatively low iron concentrations may interfere with these bacteria having stimulatory influence on their metabolism –which may be of both medical and environmental interest. Further investigations need to be carried out aiming to get complementary data regarding the impact of iron on some microorganism. Special attention seems to be required in the case of human body iron loading from various sources (regarding environment pollution) since the siderophore - synthesizing bacteria behave as iron scavengers.

5. Conclusions

Significant stimulatory effect of relatively low iron levels (2.2 - 4.5- 9.0 microg/l) upon the bacteria growth was found following turbidimetric measurements of the cell density in *P. aeruginosa* strains cultures supplied with colloidal magnetite, ferrous sulfate and ferrous chlorides. For higher concentrations (18.0 - 36.0 - 72.0 microg/l) either non-detectable response or inhibitory effects were evidenced. For ferric ions supplied from ferric chloride now stimulatory influence was revealed on *P. aeruginosa* bacteria in concordance with the known unavailability of ferric ions to the microorganism metabolism.

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