Magnetite nanoparticles for biosensor model based on bacteria fluorescence

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Fluorescence emission of pyoverdine – the siderophore synthesized by iron scavenger bacteria – was studied using *in vitro* cultures of *Pseudomonas aeruginosa* with the aim to design a biosensor system for liquid sample iron loading. Diluted suspensions of colloidal magnetite nanoparticles were supplied in the culture medium (10 microl/l and 100 microl/l) to simulate magnetic loading with iron oxides of either environmental waters or human body fluids. The electromagnetic exposure to radiofrequency waves of bacterial samples grown in the presence of magnetic nanoparticles was also carried out. Cell density diminution but fluorescence stimulation following 10 microl/l ferrofluid addition and simultaneous exposure to radiofrequency waves was evidenced. The inhibitory influence of 100 microl/l ferrofluid combined with RF exposure was evidenced by fluorescence data. Mathematical model was proposed to approach quantitatively the dynamics of cell density and fluorescence emission in relation with the consumption of magnetite nanoparticle supplied medium. The biosensor scheme was shaped based on the response to iron loading of bacterial sample fluorescence. [DOI: 10.2971/jeos.2009.09024]

Keywords: colloidal magnetite, iron chelate, iron scavenger bacteria

1 INTRODUCTION

Fluorescence phenomena in the living world were evidenced by using sensitive photo-detectors, able to record slight emissions of visible electromagnetic radiation generated at the level of various cells and tissues: seeds, roots and leaves of most plant species, neurons, liver or skin cells of numerous animals as well as in microorganism cells – fungi and bacteria. Among fluorescent bacteria there are certain *Pseudomonas* species characterized by the ability of iron uptake and chelating in the form of pyoverdine – a siderophore with blue greenish fluorescence [1], that can be further internalized by the plant roots for example. The internalization of ferrous iron is accompanied by oxidization reactions since ferric iron is preferred by cell energetic.

The issue of *Pseudomonas* bacteria incorporation into biosensor systems was investigated considering environmental sources of microorganisms. Temple et al., [2] have designed an iron biosensor (namely an iron-regulated promoter) following engineering *Pseudomonas* cells that colonize tree tissues in order to estimate the relative abundance of iron on some tree blossoms. Joyner and Lindow [3] developed an iron biosensor based on a *Pseudomonas syringae* mutant that exhibited iron-dependent fluorescence when iron was supplied to the growth media. Based on *Pseudomonas putida* Perry et al, [4] reported the design of a sensor system that responded in a rapid time with altered light emission when it was exposed to increasing levels of toxic metal ions: Cu(II), Zn, Pb, and Cd. Both *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*, although producing structurally different pyoverdines, have demonstrated highly efficient cross-reactions when tested for pyoverdine-mediated iron uptake.

In the next the authors focused on *Pseudomonas aeruginosa* since it is characterized by various ecological nishes including human body so that the iron detection could be not only of environmental but also of medical interest.

The magnetic contamination of human body fluids and cells may result by iron loading with fine ferromagnetic particles from water, air and soil, but also following medical procedures based on magnetic fluids administration for diagnostic (NMR with magnetic fluids) or therapy (magnetic carriers for drug targeting, hyperthermia for cancer treatment); we notice that in the medical context the magnetic contamination often overlaps the electromagnetic exposure.

Received September 30, 2008; published June 01, 2009 ISSN 1990-2573

2 MATERIALS AND METHODS

The aqueous magnetic suspensions of colloidal magnetite were derived by diluting a water based magnetic fluid prepared from iron oxide cores (FeII-FeIII system) stabilized with tetramethylamonium hydroxide shell [5], ferrophase having the average physical diameter of 7.94 nm, volume fraction of 2.05% and saturation magnetization of 123.30 Gs. Colloidal iron supply into the microorganism culture medium was ensured by adding adequate aliquots of magnetic fluid dilutions – 10 microl/l and respectively 100 microl/l – achieving nanoparticles concentrations of 10^{-14} – 10^{-15} cm^{-3}.

Electromagnetic exposure to radiofrequency waves (RF) was carried out for various time durations (2-4-6-8-14-20 hours) within a transverse electromagnetic cell able to deliver about 0.6 mW/cm^2 power density at 400 MHz frequency [6]. Magnetic measurements were carried out using a magnetometer device with Hall probe.

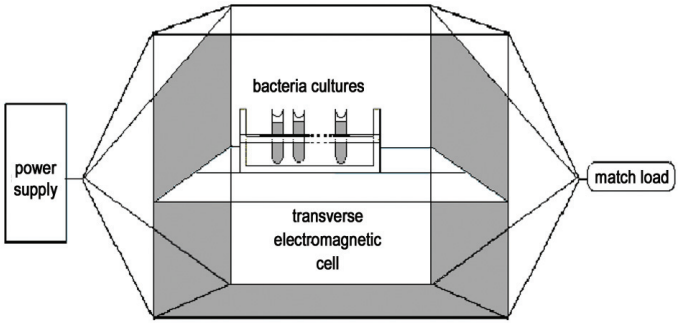

FIG. 1 Electromagnetic exposure cell at radiofrequency.

Wild strain of *Pseudomonas aeruginosa* was isolated from hospital patient with digestive diseases; adequate aliquots of 18 hours aged cells were inoculated in sterile glass tubes containing liquid broth culture medium (nutritive broth based on meat extract from OXOID, with 6.5 pH) supplemented with magnetic fluid dilutions. Bacterial inoculum density at 560 nm was turbidimetrically assayed using Meterteck device and calibration curve.

The turbidimetric measurements of bacteria growth were carried out at 24 hours after the inoculation – during this time the temperature being monitored for all controls and test tubes ($24.0 \pm 0.5^{\circ}C$). Then all the tubes were thermally treated at $100.0^{\circ}C$ into STERICELL room and centrifuged for 15 minutes at 3.500 cycles/min. The assay of siderophore accumulation was carried out by fluorescence investigation upon the supernatant liquid containing bacterial pyoverdine – released following the culture cell thermolysis (Perkin Elmer spectrofluorimeter, excitation light of 300 nm wavelength; fluorescence quenching being avoided by 1:10 dilution) The experiment was repeated five times, the same bacterial strain being used as iron loading probe. Statistical significance of the differences between control and iron loaded sample was assessed applying *t*-test (pair type, two tailed).

3 RESULTS

Fluorescence emission – induced by 300 nm excitation light – was characterized by wide band having the maximum at about 410 nm.

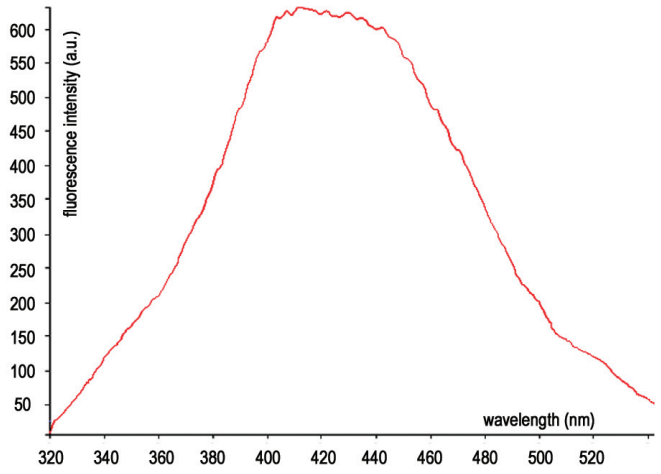

FIG. 2 Fluorescence band of bacterial pyoverdine (a.u. = arbitrary units)

The experimental data evidencing fluorescence variation in iron loaded bacterial samples are given in Figure 3. We mention that separate addition of magnetic fluid components (magnetite, tetramethyl ammonium hydroxide, water) did not result in detectable changes of fluorescent emission. Linear dependence on the RF-exposure time was noticed: positive variation for lowest magnetic fluid concentration (10 microl/l) as well as in the lack of magnetic fluid (Figure 3(a)) and respectively negative variation (Figure 3(b)) for highest concentration of magnetic fluid (100 microl/l) tested in this experiment. The turbidimetric data processing led to the cell density curves presented in Figure 4. There is a general tendency of increasing to the increase of the RF-exposure time (Figure 4(a)) with remarkable saturation effect for relatively high level of magnetic fluid (Figure 4(b)).

4 DISCUSSION

Analyzing Figure 3 one can see that iron loading induced the enhancing (with up to 50%) of the sample fluorescence either in the lack of RF exposure (RF exposure time equal to 0) or for any other exposure time (between 2 and 20 hours) – except for 14 and 20 hours corresponding to 100 microl/l magnetic fluid, where *t*-test application showed non-significant changes comparatively to the control (for all the other samples $p < 0.05$). It seems that iron oxide availability is able to stimulate the biosynthesis of the fluorescent pigment based on iron chelates so that fluorescence measurement in bacterial samples grown in the presence of colloidal magnetite aliquots could underlie the iron loading biosensor. The system seems to be sensitive to magnetite traces – provided from natural or artificial sources – for any of the values of the RF-exposure time, i.e. either in the lack or the presence of electromagnetic exposure. The slope (equal to 3.72) of the strait line approaching the data corresponding to 10 microl/l magnetic fluid is about twice than for the iron free samples (1.54) while for 100 microl/l the slope

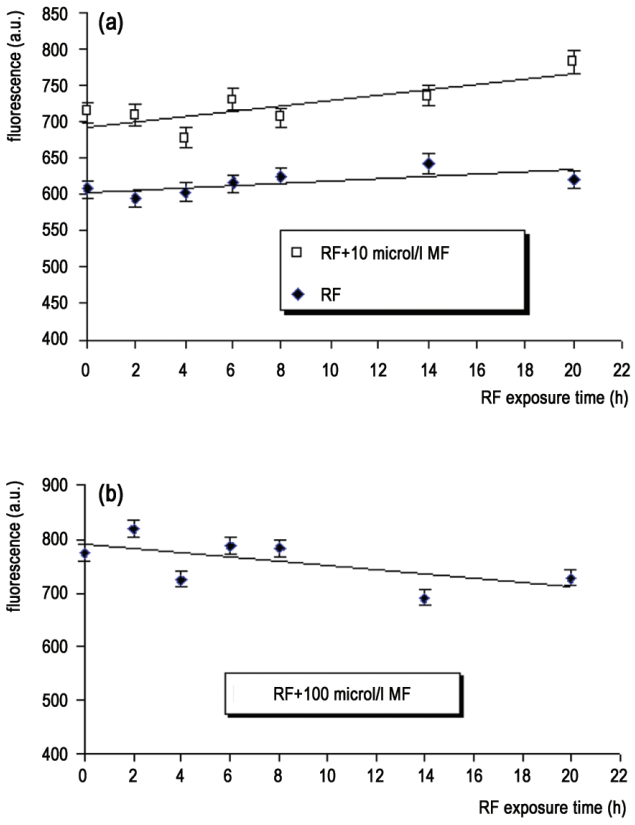

FIG. 3 Fluorescence data in RF – (a) exposed samples: 0 and 10 microl/l magnetic fluid content – (b) exposed samples: 100 microl/l magnetic fluid content.

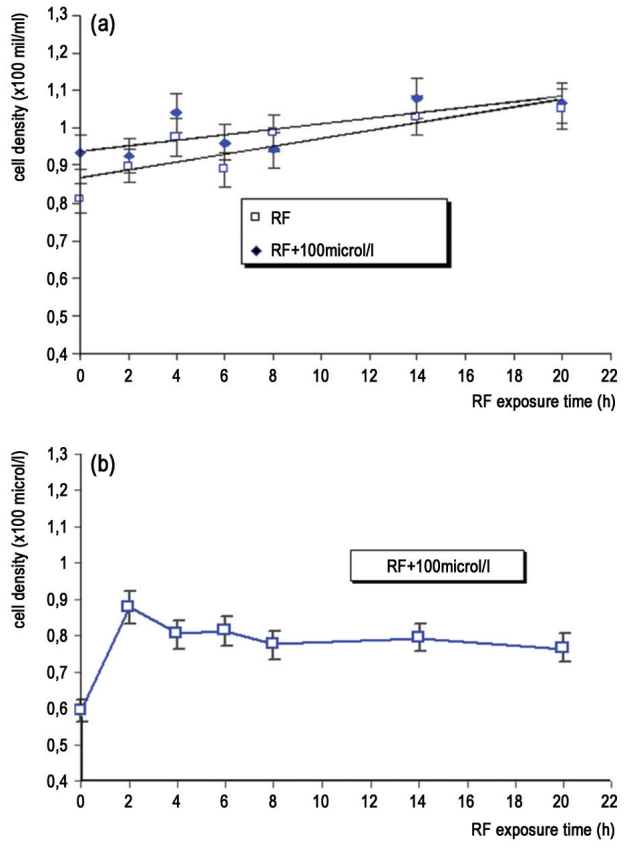

FIG. 4 Cell density for RF – (a) exposed samples: 0 and 10 microl/l magnetic fluid content – (b) exposed samples: 100 microl/l magnetic fluid content

absolute value is approximately the same as for 10 microl/l but with negative sign (-3.95).

To see if only the dynamics of fluorescent siderophore biosynthesis is influenced by the increased magnetic nanoparticle content or the bacterial cell proliferation could be also influenced, the cell density was investigated by turbidimetric data recording. The linear approach has revealed much smaller line slopes (0.007 for zero magnetic fluid and respectively 0.010 for 10 microl/l) than in the case of fluorescence measurements so that cell density seems to be not recommendable for biosensor working mode. More, in the case of 100 microl/l, there is visible tendency of cell density diminution in time after the maximum reaching for 2 hours exposure time. All changes were statistically significant ($p < 0.05$) when compared to the control. It results that there is no necessarily parallel evolution when the fluorescent pigment synthesis and the bacteria cell multiplication are taken into account.

The magnetic fluid concentrations tested in the frame of this experiment were similar to those that were shown to induce graduate effects in the case of other microbiological samples [7, 8] – the correspondent iron oxide levels being known to have biological effects on blood cells, for instance [9, 10].

The iron ions effects on the bacteria metabolism are evidently of bio-chemical nature – the magnetic feature being related only by the most frequently encountered physical form of iron ions in the nature – the magnetite. The iron scavenger feature of *Pseudomonas* bacteria refers to the pronounced tendency of iron ions uptake when this element is vanishing in the environment, suggesting that too much iron could determine the diminution of pyoverdine accumulation or even the appearance of some toxic effects.

Regarding, the biological effects of low power density RF waves, those are much more complicate to discuss; the thermal effects – generally known as cell growth stimulators – are not possible in the present experiment, since the exposures were carried out at less than 1 mW/cm^2. So, only the non-thermal influences are supposed to occur, triggering complex synergic biochemical processes that may result in cellular changes observable at the level of cell proliferation or/and at the level of pyoverdine biosynthesis or others. In Figure 5 the block scheme of the proposed biosensor system is presented. The *P. aeruginosa* based biosensor for iron loading detection is intended to carry out fluorescence measurements on fixed wavelength of 410 nm (in relation with a fluorescence calibration curve) in biological specimens as well as in environ-

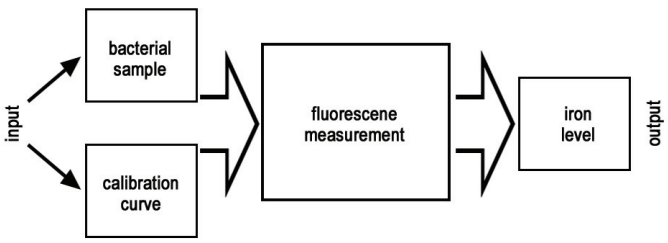

FIG. 5 The biosensor system scheme.

mental samples, the computer system comparing the raw data with a calibration curve to deliver the iron concentration.

Further experimental improvement is planned in order to increase the sensitivity of this detection method (i.e. the slope of the curve giving the fluorescence intensity versus iron oxide concentration) as well as the amplitude of the iron concentration range. In this respect new wild bacterial *P. aeruginosa* strains are to be tested while the composition of the culture medium is intended to be adjusted too, by adding new protein and/or mineral components able to stimulate the bacterial metabolism in yielding pyoverdine in the presence of extended concentration of iron from various sources.

5 MATHEMATICAL MODEL

The theory of the mechanism underlying the obtained results is basically a non-linear approach of the fluorescent pigment synthesis in the present of iron oxide traces. Also, the cell density level – result of cell multiplication in the presence of magnetite – is supposed to depend non-linearly on the iron consumption rate while the rate of magnetite uptake is assumed to depend on the cell density as well as on the cell ability of magnetite internalization. More, the graphical curves evidenced by means of the measurement methods applied within the experimental study suggested the choosing of the mathematical equation system considering mass-action type kinetics in a well-mixed volume as applied – for instance – in the case of other biological phenomena such as the acute inflammatory response to an infectious pathogen [11].

Considering the ubiquitous need of iron of living bodies, the iron scavenger feature of *P. aeruginosa* cells and the bacteria dynamics in limited volume of nutritive medium the next equation system was proposed to describe the evolution of RF exposed bacterial samples, the main variables, all non-negative, being noted with x – the fluorescent pigment accumulation rate; y – the iron consumption rate; z – the cell density increasing rate while the independent temporal variable is assimilated to the time of RF exposure.

$$dx/dt = k_1 x(1-x) - k_{12}xy \tag{1}$$

$$dy/dt = k_{21}xy(1-y) + y(z-1) - zy^2 \tag{2}$$

$$dz/dt = k_{23}\{1 + \tanh[(y-\theta)/w]\} - k_3 z \tag{3}$$

where k_{ij} are the time constants ($i, j = 1, 2, 3$).

The equation system proposed for the description of the magnetite effect on the cell proliferation and pyoverdine synthesis involves the following:

- in Eq. (1): the rate of fluorescent pigment accumulation, x, is increasing when a significant level of pigment already exists, this being the consequence of the fact that an already significant cell number exists in the considered volume – so dx/dt depend linearly on the term $k_1 x$; but, to the increase of cell density level the iron resources are diminished so that, implicitly, the rate of new amounts of pyoverdine production is lowered-consequently dx/dt diminishes rapidly with x^2; the feature of iron scavenger of the studied bacteria involves that for relatively high iron level the fluorescent pigment accumulation diminishes – so dx/dt is diminishing for increased values of y;
- similarly, in Eq. (2): the iron consumption rate, dy/dt, is increasing for increased levels of z (the cell density) and implicitly, for significant amount of synthesized pyoverdine (the terms in yz and xy); for relatively high level of iron uptaken the rate of the process is controlled by the negative terms in y^2;
- in Eq. (3): the rate of cell density increasing is saturating for increasing level of iron in the environment as expression of the iron scavenger feature of the considered bacteria species, so that dz/dt depends sigmoidally on y by means of $\tanh(y)$ and inversely proportional on z; θ and w represent the activation threshold and respectively the activation width.

The solutions of the equation system reflect the dynamical tendencies suggested by the experimental data for some particular values of the system parameters and initial conditions. The RF-time exposures no longer than 22 hours are considered for comparison to the experimental curves. Two distinct behavioral situation could be found: (a) the fluorescence increasing simultaneously with the cell density – though following different curves – which is similar to the experimental case of relatively low content of magnetite nanoparticles as well as to the case of magnetite loading lacking; (b) fluorescence diminution versus cell density increasing with saturation tendency, as in the experimental case or relatively high content of magnetite loading (100 microl/l).

In Figure 6 the theoretical dynamics corresponding to the situation (a) is presented. for $k_{12} = 30$; $k_1 = 3$; $k_{21} = 0.4$; $k_{23} = 15$; $k^3 = 1$; $x(0) = 0.01$; $y(0) = 0.05$ and $z(0) = 0.539$ while in Figure 7 the situation (b) is described for $k_{12} = 30$; $k_1 = 3$; $k_{21} = 25$; $k_{23} = 15$; $k_3 = 1$; $x(0) = 0.2$; $y(0) = 0.05$ and $z(0) = 0.539$.

The qualitative comparison of theoretical results from Figure 6 with the experimental data let us see that the curve of fluorescence exhibits more rapid positive variation than the cell density one – in accord with the slopes values of the linear approaches from above graphs – while the rate of nutrients uptake slowly decreases; the experimental project however do not offer the possibility of measuring the iron uptake dynamics – we only can intuitively say that there is no necessarily proportional dependence between iron internalization in the bacterial cells and the fluorescent pigment synthesis since other metabolic processes could also require iron ions – such as certain respiratory enzyme biosynthesis.

For RF-exposure times longer than 22 hours – that could not be tested because of the bacterial samples limited growth environment, both fluorescence and cell density seem to saturate related to the diminution of available nutrients.

In Figure 7 the fluorescence rapid diminution is visible simultaneously with the monotone increase of cell density curve but the saturation tendency of the latest appears only for time du-

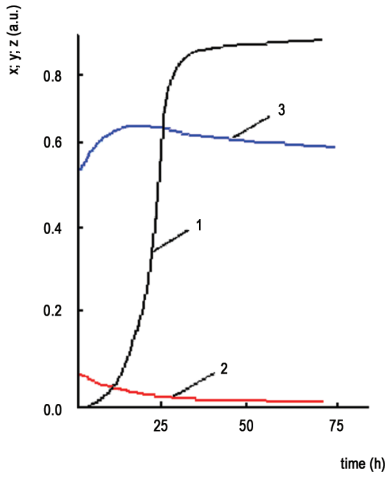

FIG. 6 The theoretical dynamics describing the cases of fluorescence and cell density increasing (a): (1) - fluorescent pigment, (2) - iron consumption, (3) - cell density.

rations longer than the longest RF-exposure time considered in the frame of the experimental project.

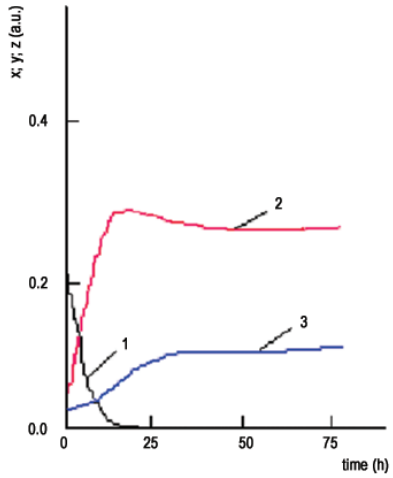

FIG. 7 The theoretical dynamics describing the cases of fluorescence diminution but cell density increasing (b): (1) - fluorescent pigment, (2) - iron consumption, (3) - cell density

The iron consumption reaches a maximum during fluorescence diminution which could be related to the iron scavenger feature of the *Pseudomonas* bacteria: after the rapid internalization of certain amount of iron ions the biosynthesis of fluorescent pyoverdine is reducing even if remarkable iron content still remains in the culture medium.

5.1 The main limits of the proposed model

By applying the above proposed model there was not found the computational possibility – in situation (a) – to make the difference between the bacteria cell responses for low iron content and the total lack of iron loading that was evidenced during experiments.

Model limitation is obvious also first from the fluorescence curve in (b) situation (Figure 7) – that zeroed for times shorter than 22 hours – what is not possible since pyoverdine content never vanish. Also, the cell density curve saturates much later than after 22 hours which is not concordant with the experiment.

6 CONCLUSIONS

Wild strain of *P. aeruginosa*, withdrawn from human body biological specimen was found sensitive to magnetite loading under RF wave exposure so that a biosensor scheme was proposed as useful in the detection of iron ions presence in the body liquids. The bacteria sensitivity to colloidal magnetite is determined by its intrinsic feature of iron scavenger so that the dynamics of the fluorescent siderophore synthesis is not necessarily parallel to that of the cell density. Fluorescence intensity was increased with up to 50% the slopes of experimental data being higher than those of cell density with two orders of magnitude.

Mathematical modeling proposed to describe the observed evolutions provides some qualitative similitude with the experimental data being able to reveal the peculiar feature of the fluorescence emission in the case of relatively high content of iron but not explaining the cellular mechanisms underlying the bacteria based biosensor.

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