SIDE EFFECTS OF MAGNETIC FLUID BIOMEDICAL UTILIZATION ON HUMAN MICROFLORA

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Abstract. The effect of colloidal magnetite nanoparticles upon the growth of some bacteria identified recently as pathogens of the human body was studied aiming to reveal the possible side effects of ferrofluid aliquots residual after medical utilization. The stimulatory effect of small ferrofluid levels on the *Serratia mercescens* development was emphasized by means of turbidimetric assay and biomass measurements. For the highest ferrofluid concentration – 100 μL/L – the biomass accumulation at 30 hours was of about 1.5 higher than for the control sample while at 78 hours the biomass was almost double in comparison to the control. Turbidity level in the cell culture presents almost constant increase up to about 25% between 36 and 84 hours for ferrofluid concentrations of 80 and 100 μL/L.

Key words: *Serratia marcescens*, colloidal iron oxides, cell division, turbidimetric assay, mathematical correlation, microscope investigation.

INTRODUCTION

The influence of iron ions on the bacterial metabolism was underlined in various scientific reports developed considering that iron is the fourth element in the Earth crust and represents an essential substance for most organisms species. Despite its abundance on earth, there is practically no free iron available for bacteria whatever biotope they colonize since the oxidized form is insoluble while the reduced form is highly toxic for most macromolecular systems. More, it is generally sequestrated by iron- and heme-carrier proteins of biological systems [7]. Correspondingly to various biotopes bacteria have developed multiple iron acquisition systems. The iron homeostasis is essential to the bacteria multiplication and virulence. In [3], the first evidence of siderophore-mediated iron acquisition by streptococcus species was reported. Iron transport and utilization in *Bacillus megaterium* were examined in [2] where it was suggested that transport of ferric hydroxamates may occur by a facilitated diffusion-type process. In [6], it was shown that *Escherichia coli* and *Bacillus megaterium* grew rapidly without

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significant production of soluble siderophores in a defined iron sufficient medium. In [1] the iron metabolism in *Serratia marcescens* was studied; it was shown that *Serratia marcescens* expresses an unconventional iron (III) transport system, the uptake of Fe^{3+} occurring in the absence of an iron (III)-solubilizing siderophore, of an outer membrane receptor protein, and of the proteins involved in outer membrane transport. In this paper the authors report the result of their investigation on the putative side effects of relatively low contents of ferrofluids remained for some time in the human body after NMR investigation with ferrofluid as contrast agent or after tumor therapy by means of magnetically targeted drug delivery.

MATERIALS AND METHODS

The magnetite nanoparticles prepared according to Massart's method [5] were delivered by means of water ferrofluid stabilized with tetramethyl ammonium hydroxide (TMA); ferrophase volume fraction was of 3.5%, the saturation magnetization of 12.5 kA/m and the physical diameter was ranging between 5 and 20 nm (with average value of 12.1 nm); the concentrations were of 20, 40, 60, 80, and 100 μL/L (ferrofluid in the culture medium). Correspondingly, iron oxide concentrations ranged between 0.80 and 420 μg/L. An incubator thermostatic room (37.0±0.5 ºC) was used for the growth of bacterial control samples and magnetic fluid treated samples. The experimental investigation (following magnetic nanoparticles addition) lasted for 90 hours – the age of the initial cultures being of 18 hours. The cell density dynamics was investigated by means of turbidimetric assay of bacteria samples grown in glass tubes with liquid nutritive broth; the spectrophotometric device was a Metertek type apparatus (light extinction measured at 560 nm). Biomass accumulation was determined using a centrifugation device at 5,000 cyc/min and an analytic balance with 10^{-5} g accuracy. Bacteria cells morphology was monitored by microscopy of cell colonies developed on agarized culture medium based on nutritive broth analysis (Nikon type device being utilized). Five repetitions of the whole experiment were accomplished in identical initial conditions.

RESULTS AND DISCUSSION

The dynamics of bacteria biomass in the control sample, i.e. in the lack of magnetic fluid, presents an increase during the first 54 hours of incubation followed by considerable diminution, associated to the limitation of the growth resources in the tubes with culture medium (Fig. 1) toward the end of the observation period. In the presence of the ferrofluid aliquots the maximum of biomass accumulation appears earlier, at about 30 hours of incubation, with gradual increase to the increase of ferrofluid concentration. More, there is a second

maximum toward 78–84 hours of growth. For the highest ferrofluid concentration – 100 μL/L – the biomass accumulation at 30 hours is of about 1.5 times higher than that of the control sample while at 78 hours the biomass is almost double in comparison to the control.

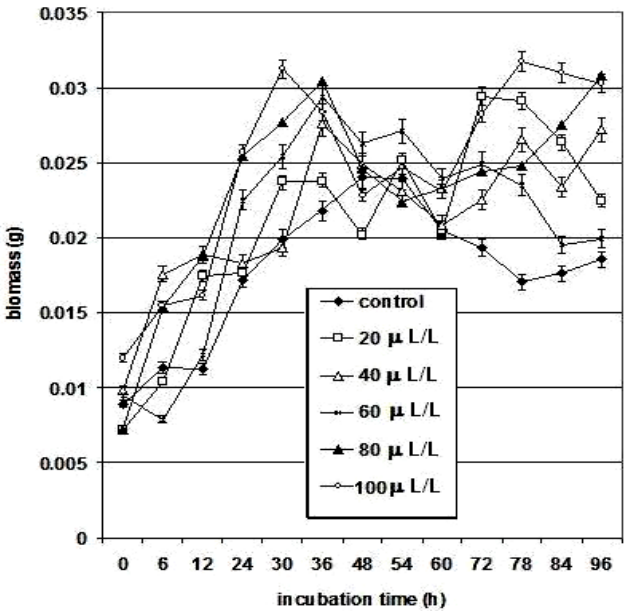

Fig. 1. The dynamics of *S. marcescens* biomass during the incubation in the presence of ferrofluid aliquots.

Standard deviations ranged between 2.5 and 6.5%. Turbidity level in the control bacteria sample (Fig. 2) exhibits first rapid increase with a peak at 30 hours and a diminution after 72 hours of growth. For the highest ferrofluid concentration there is an almost continuous increase of cell density terminated with a diminution tendency after 78 hours. Standard deviations ranged between 3.5 and 5.5%. As obvious the two measured parameters led to distinct families of curves, suggesting, in the first place that biomass dynamics is determined not only by cell density – related to the turbidity level – but also by cell unitary mass. In the presence of the ferrofluid a diminution of the biomass was noticed between 36 and 60 hours of growth when probably not all the bacteria descendant from the initial generation continued to multiply – cell division being probably temporary inhibited. The remaining cells still resulted in increased biomass accumulation toward the termination of the observation period (84 hours) either due to the increase of individual cell mass or/and due to the acceleration of the division rate. In both hypotheses it seems that the ferrofluid aliquots have mostly a stimulatory influence on the bacteria development, the final biomass being twice higher for the

concentration of 100 μL/L than for the control sample. However, proportional dependence between biomass and ferrofluid concentration could be noticed only during the first 30 hours from the inoculation – when the biomass appeared increased with up to 50% in comparison to the control micropopulation.

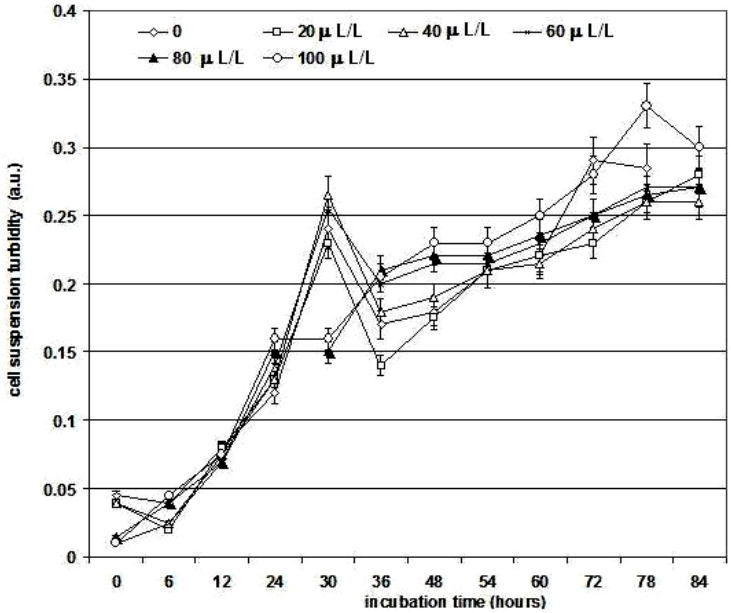

Fig. 2. The turbidity of bacterial cell suspension (arbitrary units) in *S. marcescens* cultivated with ferrofluid aliquots.

The correlation between biomass and turbidimetric data was analyzed (Fig. 3). According to the criterion of the best correlation coefficient, the trend lines chosen as representative for ferrofluid influence on *S. marcescens* growth are the logarithmic ones (for ferrofluid concentrations of 20–40–60–80–100 μL/L) and respectively polynomial ones (control samples) – Table 1.

Table 1

Quantitative approximation of experimental data correlation

Sample (μL/L)	Fitting equation (y = the biomass; x = turbidity level of cell suspension)	Correlation coefficient
20	$y = 0.0075 \ln x + 0.0359$	0.890
40	$y = 0.0048 \ln x + 0.0308$	0.860
60	$y = 0.006 \ln x + 0.0334$	0.892
80	$y = 0.006\ 1\text{n}\ x + 0.0349$	0.893
100	$y = 0.0055 \ln x + 0.035$	0.846
0	$y = -0.4972\ x^2 + 0.1991\ x + 0.0018$	0.896

According to above graphs (Fig. 3) the increase of the biomass tends to saturate to the increase of cell suspension turbidity in the presence of ferrofluid (logarithmic curves) while in the ferrofluid lack the biomass decreasing was evidenced toward to the end of the observation time (second order polynomial).

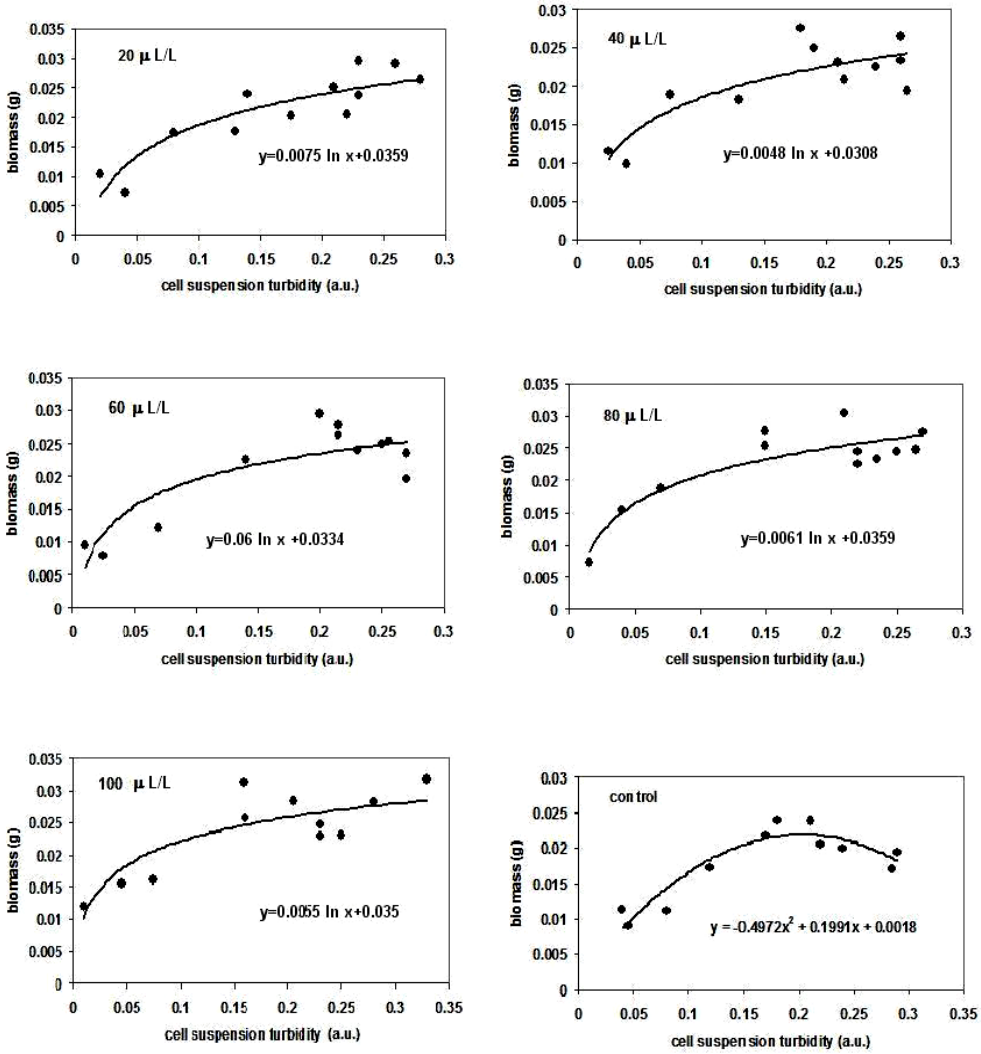

Fig. 3. Mathematical approximation of experimental measurement data (y = the biomass; x = turbidity level of cell suspension).

The data provided by turbidimetric measurements revealed distinct behavior of bacteria micropopulation at 30 hours for 80 and 100 µL/L ferrofluid concentrations where the peak exhibited by the control sample and the smaller

ferrofluid concentrations is cut. Light scattering seems to be reduced though biomass was increased – probably that higher cells not only scatter but also absorb light. It is possible that for smaller ferrofluid concentrations, when the cell size is not high enough, the turbidity remains almost the same as for the control sample. We presume that latter, when the second increase of biomass occurs, the acceleration of the cell division sustains the intensification of light scattering especially for the highest ferrofluid concentration, even there are also present giant cells.

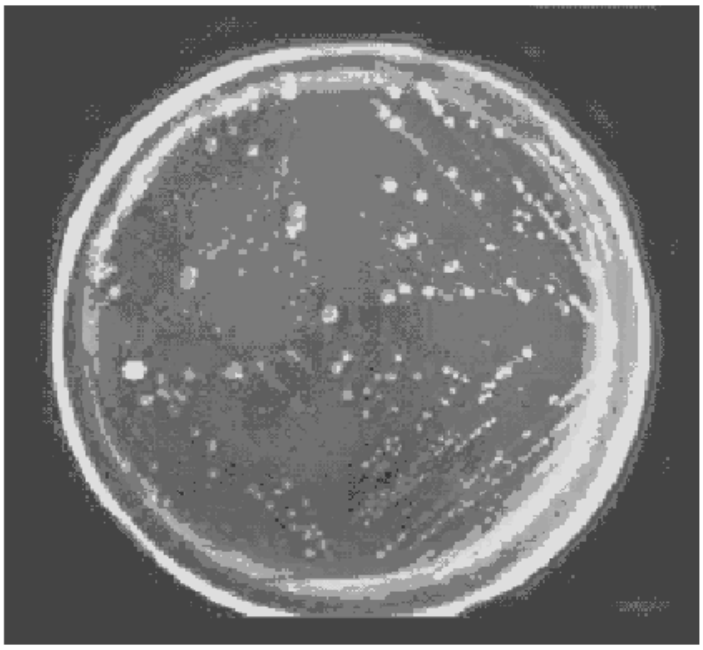

Fig. 4. Microscopic investigation revealed 2–3 times higher bacteria among normal ones in the samples developed in the ferrofluid presence.

The stimulatory effect of ferrofluid small levels on the individual bacteria size seems to be confirmed by the microscopic investigation that revealed the presence of larger cells beginning with 30 hours of growth (Fig. 4). The peculiar increase of biomass in the bacteria culture medium supplied with relatively high ferrofluid concentration was reported by us for *E. coli* and *S. aureus* [4] at 12–24 hours of growth; it was underlined that different bacteria strains could give different responses to the same external constraints. In order to get new data regarding the influence of ferrofluid aliquots on bacteria growth, deeper investigations are planned considering that low levels of microorganisms usually can be found in the human body and their virulence depends on the iron content of the growth medium.

CONCLUSION

It was evidenced that *Serratia marcescens* growth could be influenced by colloidal iron oxides supplied in the form of ferrofluid aliquots in the culture medium. Biomass measurement revealed atypical dynamics curves suggesting the ferrofluid effect on the cell division rate. Turbidity data evidenced peculiar behavior of bacteria growth at 30 hours of incubation in the presence of relatively high ferrofluid concentration. Microscopy investigation showed giant cells grown in the presence of colloidal iron oxide particles supplied by means of ferrofluid aliquots. The main biomedical issue concerns the side effects of ferrofluid traces remained in the human body following medical utilization.

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