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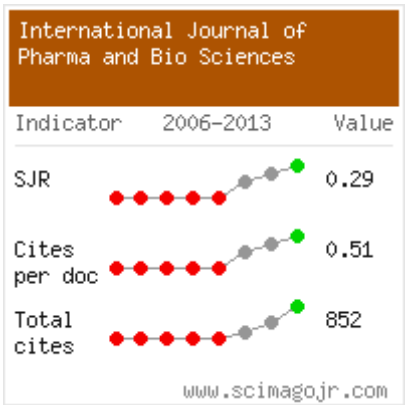

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MAGNETOSOMES SYNTHESIZED BY MAGNETOTACTIC BACTERIA *STENOTROPHOMONAS SPP.* STRAIN RP-8 FOR MAGNETIC HYPERTHERMIA

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ABSTRACT

Magnetosomes isolated from magnetotactic bacteria *Stenotrophomonas spp.* strain RP-8 was tested for magnetic fluid hyperthermia. Strain RP-8 cultivated in optimized magnetospirillum growth medium with microaerophilic condition under permanent magnetic field and magnetosomes were extracted by sonication. Transmission electron microscopic observation reveals the presence of magnetosomes whereas alternative gradient magnetometer (AGM) analyzed the magnetic measurement. The maximum Specific Absorption Rate (SAR) of magnetosomes is shown as 337.30 W/g at 502 Oe magnetic fields. In the present investigation, the magnetic properties and heating efficiency of magnetosomes extracted from strain RP-8 was applied for magnetic fluid hyperthermia.

KEYWORDS: **Hyperthermia, Magnetotactic bacteria, Specific Absorption Rate, Magnetosomes.**

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1. INTRODUCTION

The magnetic properties of magnetosome are of interdisciplinary interest because magnetosomes are potential carriers of magnetization as well as novel nano-biomaterials in biotechnological and biomedical applications.[1,2] Nanotechnology plays an important role in the field of biomedical application such as drugdelivery[3].Magnetic Fluid Hyperthermia (MFH) is one of the promising tool in treating cancer because cancer tumor cells are killed when heated to temperature of 42^0C however normal cells survive at this temperature.[4] The use of nanomagnet for hyperthermia has great significance in the field of nanobiomedicine. Magnetosomes are produced by the process of biomineralization in magnetotactic bacteria. Most MTB species or strain exclusively produces either magnetite or gregite magnetosomes and exhibit better heating effect under the applied field as well as the lipid membrane covering over the crystals provides better biocompatibility[5]. These magnetosomes and magnetite crystals play highly significant role for magnetic properties. Thus, magnetosomes have attracted greater attention as a natural biological magnetic material in medicine [7]. The magnetotactic bacteria *Stenotrophomonas spp* strain RP- 8, represent an excellent model for biosynthesis and is an upthrust area in cancer treatment[6]. In the present report, the magnetic properties and heating efficiency of magnetosome were evaluated and being tested for magnetic fluid hyperthermia application. Magnetic properties of strain RP-8 have been evaluated and it predicted that magnetosomes from magnetotactic bacteria *Stenotrophomonas spp.* could be considered as potential candidate in hyperthermia treatment.

2. MATERIALS AND METHODS

2.1 Collection, Enrichment, Isolation and Cultivation of strain RP-8

MTB bearing sediment sample (one lit.) was collected from the upper sediment layer of oxic-anoxic zone of lake sediment and enriched in modified magnetospirillum growth medium (MSGM). Further the enriched cells were purified by the capillary race track (CRT) and magnetic assessment technique[8, 9]. The isolated culture designed as RP-8 (Nucleotide Accession No.KC514104) inoculated in modified MSGM with microaerophilic condition under the permanent magnetic field at 28^0C for 96 h. The optimal growth medium for cell was modified containing 100 mL of lake water sediment extract, 0.37 gm of tartaric acid, 0.37 gm of succinic acid, 0.12 gm of sodium nitrate, 0.05 gm of sodium acetate, 0.035 gm of ascorbic acid, 5 mL mineral solution, 5 mL vitamin solution, 1.0 gm of peptone, 5 mL of 0.001 M ferric quinate, 0.5 gm of yeast extract, 0.1 gm of sodium chloride, 0.1 gm of sodium thioglycolate (All medium preparation reagents were purchased from Himedia, Mumbai, India) and 900 mL of filtered lake water which was purified by passing through a 0.45µm membrane filter[10].

2.2 Extraction of magnetosomes from strain RP-8

For preparation of samples of magnetosomes, the cells were harvested by centrifugation at 4000 rpm for 20 min, and the supernatant was discarded. Then the cells were resuspended in 10 ml of Tris HCl buffer (pH 7.4) and sonicated at 30 W for 60 min. The permanent magnet was used to separate the magnetosomes, the solution was finally washed thrice and the sample was suspended in phosphate buffer saline[11].

2.3 Microscopic observation and elemental analysis

For TEM observation the cell pellet was fixed in a mixture of 2 % para-formaldehyde and 2.5 %glutaraldehyde in buffer for 2-3 h at 4° C for 10 min. This processed cell pellet was used for gridpreparation and then analyzed through TEM [11,12]. For iron analysis, cell mass was digested bysulphuric acid, perchloric acid and nitric acid (9:10:1), popularly known as tri-acid wet digestion method. Iron measurements were taken with Atomic Absorption Spectroscopy (AAS) [13].

2.4 Magnetic measurements, Induction Heating Studies and measurement of SAR

To characterize the magnetic properties of the magnetosomes an alternative gradient

magnetometer (AGM, Model MicromagTm2900) was used. For that, 25 µL suspensions of magnetosomes were deposited on silica substrate then dried and placed inside an AGM. The magnetic parameters Hc, Ms, Mr and Mr/Ms are estimated from the hysteresis loop. The temperature rise measurement of magnetosomes was measured by an induction heating unit (Easy Heat 8310, Ambrella; UK) with a 6 cm diameter (4 turns) heating coil by applying an AC magnetic field of 335.2 to 502 mT at a fixed frequency of 265 kHz. 1 mg/ml concentration of magnetosomes suspended in phosphate buffer saline was taken for the induction heating experiment. The specific absorption rate (SAR) of magnetosomes extracted from strain RP-8 was determined [14].Heat dissipation by magnetic nanoparticles under an AC magnetic field is measured in the terms of SAR (Wg -1). It can be expressed as,

$$SAR = C(\frac{dT}{dt})\frac{1}{m_{mgn}} \qquad (2)$$

Where C = specific is the heat capacity of suspension = 4.186 J (g ° c) -1 (DT/dt) = initial slope of temperature versus time graph, ms = mass of the suspension, and mm = mass of magnetic material in suspension.

3.RESULTS AND DISCUSSION

3.1 Magnetosomes for magnetic fluid hyperthermia

Gram negative rods of RP-8 cell contain magnetosomes inside the cell (Fig.1). The result of AAS revealed that the iron content of strain RP-8 was 9.270 ppm.

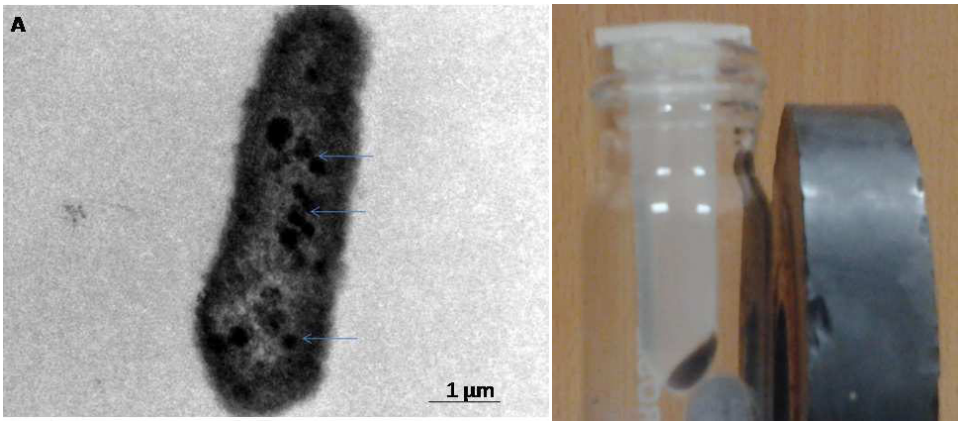

Figure 1
(A) TEM image of RP -8 cells with magnetosomes (indicated with arrow) And magnetic separation of magnetosomes.

3.2 Analysis of magnetic measurement

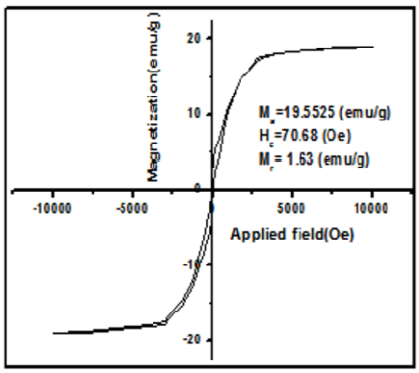

Figure 2
M-H curve of magnetosomes extracted from strain RP-8

The measurement of the hysteresis loop of the magnetosomes was taken by AGM at room temperature (Fig 2). The magnetization (M), remanence (Mr), and coercivity (Hc) obtained was 19.55 emu/g, 70.68 Oe and1.63 emu/g respectively.

3.3 Induction heating studies and specific absorption rate (SAR)

The heating efficiency of magnetosomes sample was evaluated under different magnetic field conditions from 335.2 to 502 Oe at fixed frequency 265 kHz for 30 min with the concentration of 1 mg/mL in phosphate buffer saline. In fig.4 the temperature kinetic curve, it is observed that heat generation magnetosomes required for hyperthermia reach within a short time span for applied field 419 and 502 Oe. The values of sufficiently high temperature observed for magnetosomes was 35.95, 43.4 and 49.32 under applied field of 335.2, 419 and 502 Oe respectively. It is observed that 335.2 Oe magnetic field is inadequate for magnetosomes to reach the hyperthermia.SAR of magnetosomes for variable magnetic fields 335.2, 419,502 Oe was found to be 140, 291.63,337.30W/g respectively. The maximum SAR value observed was 337.30 W/g at 502 Oe.

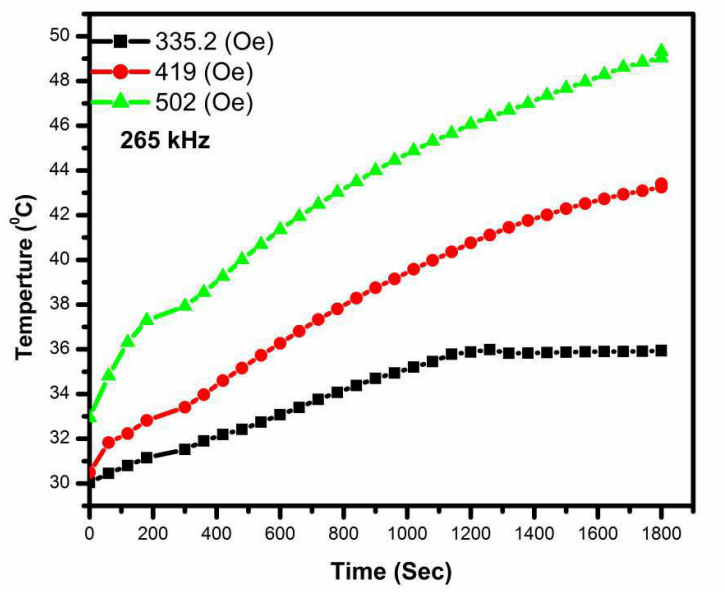

Figure.3
Induction heating studies of magnetosomes at variable magnetic field

3. CONCLUSION

In this present study, magnetosomes extracted from strain RP-8 reach to temperature required for magnetic fluid hyperthermia at 419 and 502 Oe. The value of sufficiently high temperature rises from 35 to 49^0C. The heating efficiency of magnetosomes of strain RP-8 increases with increasing magnetic field .It was also found that magnetic field around 335 Oe was unsuitable for hyperthermia. Versatile characteristics of *Stenotrophomonas spp* strain RP- 8 with magnetosomes can be used as a heat mediator for magnetic fluid hyperthermia.

CONFLICT OF INTEREST

Conflict of Interest declared none.

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