Bacterial adsorption onto thin Fe_3O_4 magnetic nanofilms

F. Ansari, R. Horvath, A. Aref, and J.J. Ramsden

Microsystems & Nanotechnology Centre, Cranfield University, Bedfordshire, MK43 0AL, UK,
f.ansari@cranfield.ac.uk

ABSTRACT

We have recently found that biodesulfurization activity is enhanced if bacteria are decorated with ferrous nanoparticles. In order to develop this further, we investigated the adhesion of magnetic nanoparticles to the surface of bacteria, by depositing a thin layer of Fe_3O_4 magnetic nanoparticles onto a $Si(Ti)O_2$ waveguide surface. The kinetics of adsorption of *Rhodococcus erythropolis* IGTS8 on the fabricated nanofilm was measured under controlled conditions using optical waveguide lightmode spectroscopy (OWLS), with which the number of deposited particles and bacteria could be accurately calculated. We found that it was very difficult to coat optical waveguides with bare magnetite nanoparticles, because of their aggregation. This problem could be solved by precoating the nanoparticles with poly ethylene glycol (PEG). On the other hand, this coating apparently prevented the adhesion of the bacteria to the magnetite.

Keywords: magnetic nanoparticles; optical waveguide; adsorption; bacteria; poly ethylene glycol

1 INTRODUCTION

A range of bacteria have been shown to be able to remove sulfur from organic compounds that commonly exist in petroleum. The most extensively investigated microorganisms belong to the genus *Rhodococcus*, which has shown reasonably high activity and stability for removing sulfur from organic compounds, but this activity is unlikely to be sufficient for commercial applications. We found previously [1] that bacteria decorated with Fe_3O_4 magnetic nanoparticles had a higher desulfurization activity compared to the nondecorated cells, encouraging further development of bacteria for sulfur removal and designing new biodesulfurization processes.

Iron oxide nanoparticles have attracted considerable attention during the last decade and they have been of great interest in many important technological applications. The use of magnetite nanoparticles in clinical medicine is important and has considerable promise for applications in the biomedical and diagnostic fields [4, 5]. Also magnetic nanoparticles may help to resolve many separations problems. They are attractive in separation applications because the activity of the particles can be tailored by modifying surface coatings on the nanoparticles. The nanoscale particles afford very high surface areas without the use of porous absorbents and can be recovered for reuse [1]. The adsorbents are easily separated by utilizing the magnetic interaction between the nanoparticle and an external applied magnetic field. These hybrid adsorbents offer the advantages of homogeneous and heterogeneous reaction systems combined.

All of these applications require magnetic nanoparticles that can be easily conjugated with biomolecules. The most widely used magnetic nanoparticles for biomedical applications are magnetite (Fe_3O_4) and related oxides, which are chemically stable (in contrast to nanoparticles of pure Fe metal), nontoxic, noncarcinogenic and have useful magnetic properties [6]. There has been a great deal of recent progress in their synthesis.

Obviously, for the particle to be successfully used to decorate bacteria, they need to be well dispersed prior to mixing them with the bacteria.

There are number of techniques used to measure the amount of mass deposited onto a surface in a few nanometers thin layer; among them optical and acoustic methods are widely used to obtain continuous measurement of nanoparticle adsorption on variously modified surfaces and to study the deposition process and the structure of thin films [3, 7, 8]. Optical waveguide lightmode spectroscopy (OWLS) is based on the confinement of light in a high refractive index layer [2], and from the most sensitive so-called monomode waveguides, two parameters can be independently determined: The refractive index and the thickness of the deposited film for nanoparticles (size $\leq \lambda/10$) the uniform thin film approximation is valid. Assuming a constant refractive index within the adsorbed layer gives access to these two parameters from which the amount of adsorbed material can be derived. Several experiments have been carried out with layers of adsorbed proteins, macromolecules, or nanoparticles [3, 7, 9, and 10]. In the present contribution OWLS is used to study the surface deposition of magnetic nanoparticles and the adsorption of bacteria on these magnetic nanofilms.

2 EXPERIMENTAL

Poly ethylene glycol (PEG) was from Sigma Aldrich, ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ferric chloride

hexahydrate ($FeCl_3 \cdot 6H_2O$), and all other chemicals were from Fisher Scientific (UK). *Rhodococcus erythropolis* IGTS8 (ATCC 53968) was from American Type Culture Collections (Virginia, USA). Water used was purified by ion exchange and reverse osmosis (ELGA-option3B, Elga Water Ltd, UK) For the experiments, the fabricated nanoparticles were suspended in the ultrapure water (100 μg/mL).

2.1 Fe_3O_4 Magnetic Nanoparticles

Magnetic nanoparticles coated with PEG were prepared and characterized as reported previously [11]. Briefly, a solution of a mixture of $Fe(NO_3)_3$ and $FeSO_4$ in an equimolar ratio (0.9 M : 0.9 M) was prepared and an equal volume of a solution of 20% (w/v) poly ethylene glycol in distilled water was then mixed with the iron solution, and kept at room temperature under nitrogen for 30 min. Sodium hydroxide was then dissolved in 125 mL of distilled water to achieve the desired concentration (0.5 M). The basic solution was purged with nitrogen and then heated in a mantle. When the reaction temperature reached 65 °C, the solution of iron salts was added dropwise to the basic solution. Black precipitates formed immediately upon addition of the iron salt solution. The reaction mixture was mixed vigorously for 30 minutes. The aggregates were then removed by centrifugation for 5 minutes at 3000 g. The supernatant was discarded by decantation and the particles were rinsed three times with approximately 150 mL of distilled and deoxygenated water by centrifugation at 3000 g for 5 min to remove excess ions in the suspension. Finally the particles were rinsed with approximately 100 mL of 0.01 M HCl to neutralize them. The particles were then collected, and were dried in an oven at 80 °C overnight. The magnetic nanoparticle concentration was expressed in terms of dry weight per volume of suspension medium.

In order to demonstrate that our synthetic route admits a fine control of the particle characterization, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were used to characterize the particles (Fig. 1).

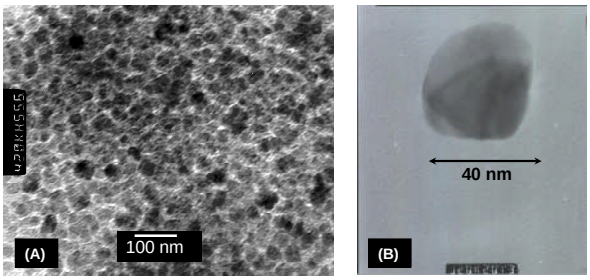

Fig. 1. (A) TEM image of a 40 nm Fe_3O_4 particle. (B) is a high resolution image of one the synthesized particles.

2.2 Bacteria Preparation

The cultures were grown until the mid-exponential growth phase in liquid medium and then centrifuged (Hettick-EBA 20) at 6000 rpm for 15 min. The supernatant was discarded and the cell pellets were washed twice with Ringer's solution. The cells were then resuspended in the same solution to A_{600} = 1.0 and used as a stock solution on the day of harvesting. A typical microbe is shown in Fig. 2.

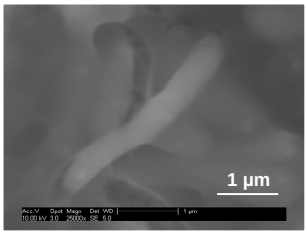

Fig. 2. SEM image of *Rhodococcus erythropolis* IGTS8. (dimension ≈ 3500 nm x 500 nm).

2.3 Waveguide Sensor

Fig. 3 shows a standard planar optical waveguide of $Si(Ti)O_2$ (thickness $d_F \sim 200$ nm and refractive index $n_F \sim 1.8$) with an incorporated shallow grating coupler (depth – 10 nm, grating constant Λ = 416.6 nm), obtained from MicroVacuum, Budapest (type 2400).

Before starting an experiment, the waveguides were kept in water overnight to avoid drift in the effective refractive index during the experiments. (Drift is attributed to the gradual filling of the porous waveguiding film with liquid [12].)

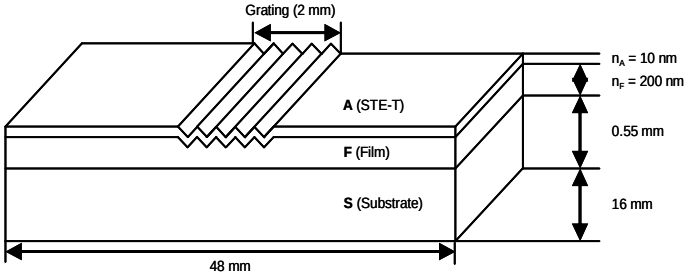

Fig. 3. A schematic diagram of the optical waveguide grating coupler sensor chip.

2.4 Optical Measurement

A flow-through cuvette (dimensions: cross-sectional area A = 1.7 mm^2, radius R = 1 mm, distance x from inlet to centre of measuring spot = 3.5 mm) was sealed to the surface of the prepared waveguide, and the whole assembly mounted in the measuring head of an BIOS-1 integrated optics scanner (ASI, Switzerland).

The effective refractive index of guided modes can be very conveniently determined if the waveguide incorporates a diffraction grating. An external beam of light couples into the waveguide provided its angle of incidence α onto the grating satisfies the resonance condition. The angles α at which the incoupled power is at a maximum were determined for the zeroth transverse electric (TE) and transverse magnetic (TM) modes using the BIOS-1 goniometer scanning device, which is capable of microradian precision, hence allowing the effective refractive indices to be determined with a precision of a few ppm ($\pm 10^{-6}$).

2.5 Particle & Bacterial Deposition

A fresh suspension of Fe_3O_4 sol at a concentration of 10 μg/mL in water was made up immediately before starting an experiment, mixing gently to avoid bubbles. The stock solution of bacteria were washed twice with water and the cells were then resuspended in water to $A_{600} = 1.0$.

Liquids were drawn through the cuvette using a peristaltic pump at a rate of 0.4 mL/s. Temperature was monitored by a thermocouple inserted into the cuvette outlet. Typical adsorption experiments were started by pumping pure water through the cuvette. When the goniometer readings, taken every 10–15 s, showed a stable baseline, flow was then switched to the Fe_3O_4 sol in water. After adsorption approached a plateau, the magnetic Fe_3O_4 solution was replaced by pure water to ascertain whether there was any desorption of the particles from the surface. After a plateau was again reached, the suspension of bacteria in pure water was pumped through the cuvette.

3 RESULTS

The particles can easily form aggregates and settle due to van der Waals interaction between them polymer-coated particles are monodisperse and have a higher magnetic moment than those prepared from co-percipitation of iron salts in basic aqueous solution [13, 14]. The nature of the coating polymer is of primary importance, not only to prevent particle aggregation, but also to control their relaxivities in biofluids [14]. Hydrophobic magnetic nanoparticles can be rendered water-soluble with amphiphilic ligands, the hydrophobic part of the ligand being retained on their surface. This is achieved through the adsorption of amphiphilic polymers that contain both a hydrophobic segment (mostly hydrocarbon) and a hydrophilic segment; such as poly ethylene glycol (PEG).

We ran experiments to reveal the effect of PEG deposition with nonPEG-coated and PEG-coated particles. In Fig. 4 we show typical raw data (effective refractive indices of magnetic Fe_3O_4 nanoparticles versus time on the $Si(Ti)O_2$ sensor chip for a 10 μg/mL solution of magnetic nanoparticles in water).

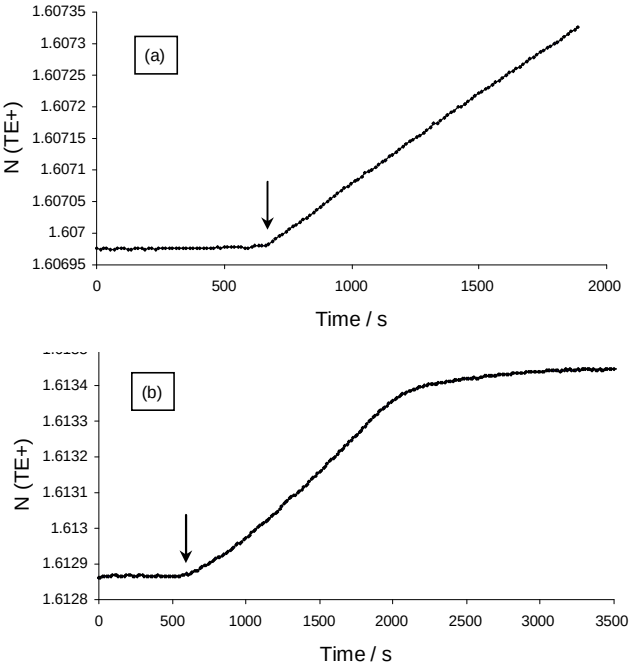

Fig. 4. Experimental deposition of non-PEG-coated (a) and PEG-coated (b) magnetic nanoparticles on the surface of the waveguide. The initial injection of nanoparticles is marked by the arrows. The star in (a) makes where the measurement had to be discontinued due to excessive noise.

The aggregation of the noncoated particles resulted in massive aggregates deposited on the waveguide, which increased the noise so much the measurement had to be discontinued (Fig. 4 (a)). In contrast, the PEG-coated magnetic nanoparticles form thin and well-defined films. Experiments were initiated by establishing a baseline and then injecting the nanoparticles. These nanoparticles are more favorable in their diffusion and binding kinetics characteristics [15]. After around 30 min we observed saturation of nanoparticles on the surface of the waveguide. The experiments were continued with bacteria. Fig. 5 shows the adsorption of bacteria on the film of magnetic nanoparticles.

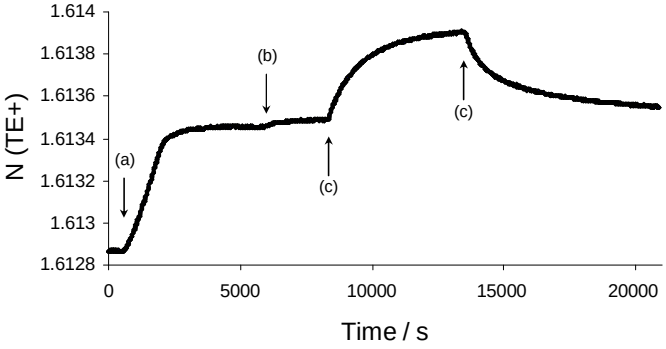

Fig. 5. Deposition of magnetic nanoparticles (initiated at

(a)), pure water (initiated at (b)), bacteria (initiated at (c)), pure water (initiated at (d)).

4 DISCUSSION & CONLUSIONS

Uncoated magnetite nanoparticles from giant aggregates and cannot be used to assemble thin films[1].

PEG coating allows excellent thin films to be deposited, which are highly adherent to silica-titania. On the other hand, bacteria are adsorbed reversibly on these films, suggesting that PEG-coated magnetite is not useful for decorating bacteria, but may be useful as an antibacterial coating.

ACKNOWLEDGMENT

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