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Developments in micro- and nano-defects detection using bacterial cells

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ABSTRACT

This paper describes improvements to the Nondestructive Testing (NDT) technique recently proposed, based on the use of bacterial cell suspensions to identify micro- and nano-surface defects. New bacterial strains were used with magnetic fields to improve bacteria mobility. Different materials and defect morphologies were tested, including nanoindentation defects, micro-powder injection moulding components and micro-laser welding. Nanoindentations with 0.6 μm depth and 5.3 μm side length were successfully detected. Bacterial cells allow identifying different topographic attributes of the surfaces, such as roughness. Cracks of about 0.5 μm wide and 10 μm depth in a reference test block Type 1 were successfully detected.

1. Introduction

Recently, a new NDT technique based on bacterial cell suspensions was proposed to detect micro-surface defects [1]. The technique aimed to explore the *intentionality* and the *life* attributes of bacterial cells (physiological and morphological), the most relevant being their small dimensions, high penetration capacity, motility, adherence, fluorescence and response to electric and magnetic fields [2]. In this previous work, the main assumptions of the technique were experimentally validated, namely: the bacterial cells preferentially enter the defects, remain inside these after the removal stage, can be observed by the use of fluorescent compounds and thus, defects can be detected. Furthermore, a revelation stage can be performed by applying a culture medium to promote bacterial growth inside the defects, facilitating their visualization by the naked eye.

The proposed technique showed to be effective in detecting surface defects with a very low depth to area ratio, produced by Vickers microhardness indentation. A detection limit was established for the materials under test: 4.3 μm depth in aluminium, 2.9 μm in steel and 6.8 μm in copper. The technique envisages the detection of micro-surface defects common to occur, amongst others, in: microfabrication [3], orthodontics [4], optical components (including optical fibbers) [5], mould making industries [6], and solar cells [7]. The technique is totally innocuous and safe to operators, since only non-pathogenic bacteria are used, and the authors discuss this issue in [1].

Other NDT techniques are presently being studied by different research groups, based on nanofluids [8], light scattering and image analysis [9], electromagnetic [10,11] and ultrasound [12] with the same purpose.

Since the validation and preliminary results described in the previous paper [1], some relevant new questions and practical challenges have been identified. This paper reports the developments on the bacterial cell NDT technique, including the use of new bacterial strains and the application of magnetic fields to improve bacteria mobility. Dedicated equipment was developed and also instrumented in order to create and characterize the magnetic fields. Different materials and defect morphologies were tested, including application to industrial components. A comparison with dye penetrant technique was performed to assess the applicability of the proposed technique in standard defects.

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2. Application of electric and magnetic fields

Nearly all bacterial cells present a negative surface charge but some bacterial strains have the ability to change the surface charge to positive values under certain conditions [13]. Others present iron particles in their cytoplasm, enabling them to respond to magnetic fields [14]. Unlike the dye penetrant methodology which is based on viscosity and capillarity, electric or magnetic properties of bacteria can be used to improve their intrinsic motility. Cell migration into defects during the penetration stage may thus be improved by the application of an appropriate field. Fig. 1 schematically depicts the application of a magnetic (Fig. 1a) and an electric (Fig. 1b) field.

These electric or magnetic fields can be applied at different intensities, originating forces on the bacteria that are proportional to the field intensity. The fields can be also oriented in different directions to move bacteria (i) deep into defects, when they are applied perpendicularly to the surface, or (ii) to favour a wide spread of bacteria over the surface, when applied tangentially, so that bacteria are available on the putative defect sites. By applying an alternate magnetic field with a specific frequency, a stirring effect can be promoted on bacteria, increasing mobility and thus the probability of having bacteria available on the different defect locations. Certain frequencies can maximize the amplitude of bacteria movement, depending on the cell mass, the viscous damping effect and the applied force. A certain analogy can be established to magnetic particle NDT technique.

A simple dynamic model of bacteria behaviour under electric or magnetic fields can be derived from the scheme presented in Fig. 1c. This model is based on vectorial Newtonian mechanics with the following major assumptions: (i) only one bacteria with a spherical shape is considered in the medium, disregarding interactions between particles; (ii) the applied fields do not interact with the medium components; (iii) other forces and interactions besides dumping particle/medium are neglected; (iv) there is no interaction between the particle and the material to be inspected. Some of these assumptions do not address intrinsic relevant phenomena but the results allow the estimation of the overall dynamic behaviour of bacteria.

The model considers a bacterial cell as a spherical particle with a mass, m [kg], a Stokes radius, r [m], and an electric charge, q [C], or a content in iron (vol% Fe). The bacteria is inside a fluid with a density, ρ [kg/m^3], and a dynamic viscosity, μ [N s/m^2]. The fluid is placed on a sample surface, and the complete set is subjected to an electric field, E [V/m] or a magnetic flux density, B [T] along x direction, after the x–y referential depicted in Fig. 1c. Under these conditions the bacteria is subjected to an electric or magnetic force, F_e [N] (Eq. (1)) and F_m [N] (Eq. (2)), respectively. In addition, according to the Stokes law, a drag force, F_d [N] (Eq. (3)) opposes to the applied one, as well as an inertial force, F_i [N] (Eq. (4)).

$$\vec{F}_e = \vec{E}\cdot q \tag{1}$$

$$\vec{F}_m = \frac{\mu_r V}{\mu_0}\cdot B\cdot\frac{dB}{dx} \tag{2}$$

$$\vec{F}_d = 6\cdot\pi\cdot r\cdot\mu\cdot\frac{\partial x}{\partial t} \tag{3}$$

$$\vec{F}_i = m\cdot\frac{\partial^2 x}{\partial t^2} \tag{4}$$

The balance of these forces on a single particle can be expressed by Eq. (5), where $c = 6\cdot\pi\cdot r\cdot\mu$ is a drag constant, F [N] is the electric or the magnetic force, multiplied by a factor that considers the intensity variation along time, t [s], of the fields with an applied frequency ω [rad/s]. This second-order linear ordinary differential equation has a certain analogy with the equation of the forced vibration with damping. Eq. (6) gives the solution of this equation, that is, the position of a particle as a function of time. Considering the values of the involved variables, some terms of this equation have a negligible contribution to the result, and therefore, it is reasonable to describe the dynamic behaviour of the bacteria according to Eq. (7). It can be noticed that, according to this dynamic model, the distance travelled by the bacteria is proportional to the applied force (electric or magnetic), and inversely proportional to the drag constant and the frequency. Moreover, for the above considered values, resonance phenomena does not occur.

$$m\cdot\frac{\partial^2 x}{\partial t^2} + c\cdot\frac{\partial x}{\partial t} = F\cdot\sin(\omega t) \tag{5}$$

$$x(t) = \frac{c^2\cdot F\cdot(1-\cos(\omega t)) + m^2\cdot F\cdot\omega^2\left(1-e^{\frac{-c\cdot t}{m}}\right) - c\cdot m\cdot F\cdot\omega\cdot\sin(\omega t)}{c^3\cdot\omega + c\cdot m^2\cdot\omega^3} \tag{6}$$

$$x(t) \approx \frac{F\cdot(1-\cos(\omega t))}{c\cdot\omega} \tag{7}$$

The solution of Eq. (6) can be represented graphically (Fig. 2), allowing to assess the bacteria position along with time, for different frequencies. This way, the following values were considered: $r = 0.5\times10^{-6}$ m, $m = 5.3\times10^{-16}$ kg (considering the water density), %Fe = 0.1% (estimated), magnetic flux density $B = 40$ mT (400 G) and $dB/dx = -13.2$ T/m (measured). Although this is a theoretical simulation using fairly reasonable values, from Fig. 2 it is noticeable that there is a considerable movement amplitude due to the magnetic field which is more pronounced for low frequencies. For instance, for a frequency of 1 Hz, the amplitude of the movement is ca. 20 μm. These results were considered in the experimental tests described in Section 5, and therefore, a frequency of 1 Hz was adopted.

3. Functional prototype

A customized functional prototype was developed to apply and measure the magnetic fields in both vertical (Z) and horizontal (Y)

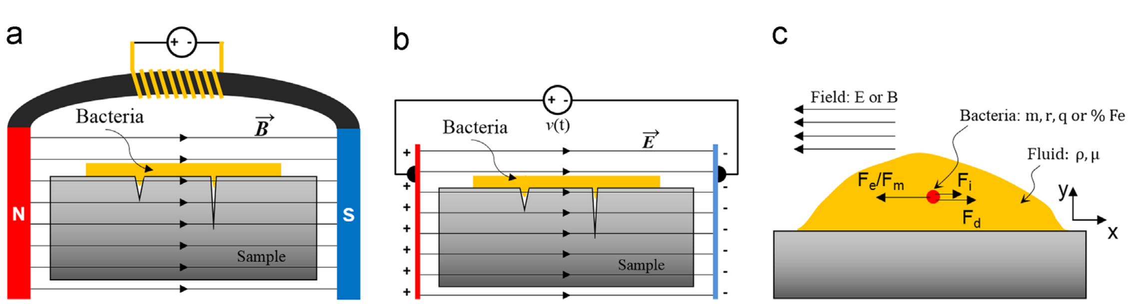

Fig. 1. Schematic representation of the application of magnetic (a) and electric (b) fields and simplified system of forces acting on a bacteria cell under these fields (c).