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EFFICIENCY ENHANCEMENTS THROUGH THE USE OF MAGNETIC FIELD GRADIENT IN ORIENTATION MAGNETIC SEPARATION FOR THE REMOVAL OF POLLUTANTS BY MAGNETOTACTIC BACTERIA

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ABSTRACT

Orientation magnetic separation (OMS) represents a simple method that permits motile, field-susceptible magnetotactic bacteria (MTB) to be separated from water. Such an approach can be used to decontaminate polluted water through uptake of contaminants by the bacteria and their subsequent removal by the application of magnetic fields. In OMS, a separation channel through which an MTB culture is flowing is subjected to a magnetic field perpendicular to the flow direction. The bacteria "sense" the magnetic field, orientating themselves parallel to the field lines and then swim to the channel sides where they accumulate.

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The fluid flow through such a standard separation channel has been shown to cause dislodgement of accumulated bacteria. To reduce this effect, a new approach has been developed utilizing magnetic gradients to retain the bacteria at the walls of the separator. A study comparing the operation of a standard channel separator with three new designs containing nickel wire matrices has been carried out. The resultant separation efficiencies and the effect on separation of varying both the flow rate and the applied magnetic field are described. The new separators enhance the separation efficiency by up to 300% over the standard separator.

Key Words: Biomagnetism; Orientation magnetic separation; Magnetotactic bacteria

INTRODUCTION

In nature, magnetotactic bacteria (MTB) orientate themselves with respect to the local geomagnetic field to avoid swimming upwards, towards high dissolved oxygen regions in ponds and lakes. This magnetotactic ability[1] is achieved by the generation of a magnetic dipole within the bacteria that aligns with the earth's magnetic field (~ 0.05 mT). The magnetic dipole is formed from a chain of membrane-bounded magnetic crystals called magnetosomes.[2] Generally, magnetosomes are formed of magnetite (Fe_3O_4) but some strains of MTB form iron sulfide crystals, such as greigite (Fe_3S_4) and pyrite (FeS_2).[3–5] In the Northern Hemisphere the majority of MTB in a culture are "north seeking" and so swim towards the South pole of a permanent magnet.[6] However, the "south seeking" fraction of a culture is significant and so recovery of both the north and south seeking bacteria is important for a separation process to be successful.

The studied MTB strain has been found to accumulate a variety of heavy metals and is particularly suited to the accumulation of metals present at low concentrations (ppm or below) such as radioisotopes in waste streams.[7] Previous work by the authors[8,9] has shown that MTB can be successfully removed from water by harnessing their magnetic properties in a process termed orientation magnetic separation (OMS). This technique can in principle, therefore, form the basis of a decontamination process for certain wastewaters.

In such a process, a channel separator is subjected to a magnetic field perpendicular to the flow of MTB. The bacteria, through the presence of magnetite crystals within their structure, sense the field and orientate themselves to swim along magnetic field lines to the channel walls where they accumulate. To achieve separation using this technique, MTB must be both motile and magnetic field susceptible. Certain metallic ions can render MTB nonmotile and so OMS can only be considered for specific waste streams.

In two respects therefore, OMS contrasts with the established technique of high gradient magnetic separation (HGMS). In OMS, motile bacteria are required but the applied magnetic field is used only to align the bacteria and not to magnetically attract and retain them as in the case of HGMS. Laboratory studies have shown that other strains of bacteria such as desulfovibrio can be more efficiently recovered using HGMS than MTB.[10]

The OMS approach has led to the development of a laboratory-scale standard "channel separator," where metal-loaded MTB accumulate within a low flow velocity region ("still area") at the channel sides (Fig. 1).

The probability of capture of a bacterium as it enters the separator is dependent on its flagella migration speed ($\sim 40\,\mu$m sec^{-1}) and its position relative to the side walls of the separator. Statistical analysis of the velocity distribution within the bacteria culture has enabled the performance of the separator to be predicted. A homogeneous magnetic field of 0.13 T was applied across the standard separator to orientate the bacteria to swim in the required direction. Test work using the standard separator[11] showed that the performance of the channel separator was typically less than half that predicted by theory.

It was observed that as the number of accumulated bacteria within the separator rose, an increasing fraction became "washed off" from the sidewalls and returned to the fluid passing through the channel. Eventually, a balance was achieved between the number of bacteria entering and leaving the separator limiting the efficiency of the separator, as shown schematically in Fig. 2.

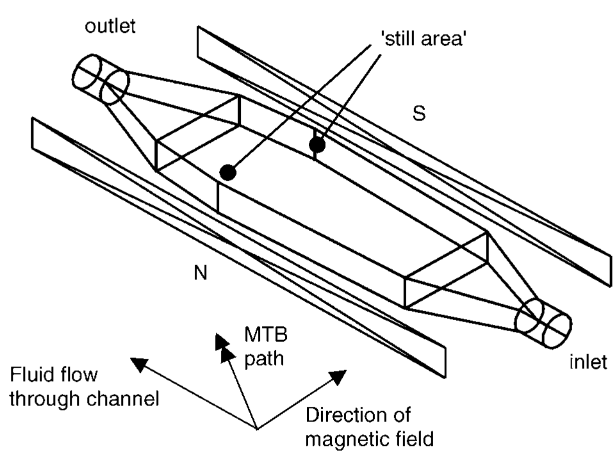

Figure 1. The principle of OMS as applied to a channel separator.

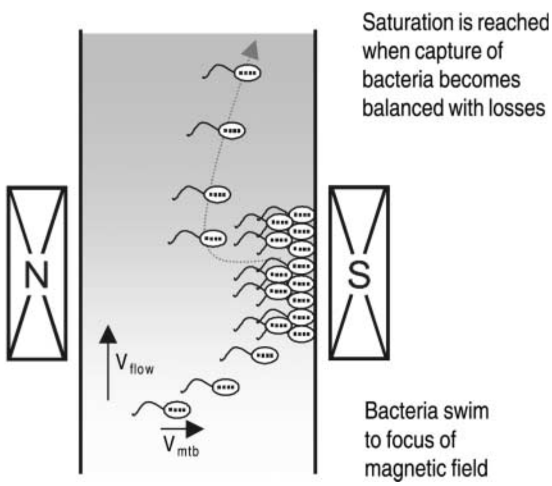

Figure 2. Schematic illustration of the process of MTB accumulation and wash off in a channel separator.

Figure 3 shows the density of motile, field susceptible MTB leaving a standard channel separator over a 7.5 hr period during which 500 cm^3 of culture was processed at a flow through rate of 65 $cm^3\,hr^{-1}$. Initially there was little or no wash off, and so, at this low flow rate, virtually all the motile, field susceptible MTB were captured. As the experiment progressed, the number of bacteria being held within the separator rose. Accordingly the rate at which accumulated bacteria became washed off and passed out of the separator rose. When the separator reached capacity, the rate of capture of bacteria became balanced by wash-off losses. This occurred after approximately 150 min, when 160 cm^3 of culture had passed through the separator.

The wash-off effect is amplified at high flow rates. In order to achieve a reduction in wash off and therefore, an enhancement in separator efficiency, magnetic gradients were employed. Such gradients were generated by the introduction of metallic wires within the separator, which, when combined with the externally applied magnetic field, produced an additional retention force on the bacteria. This paper describes a study of such hybrid separators.

EXPERIMENTAL CONSIDERATIONS

Magnetospirillia MTB were isolated from tidal salt marshes near Portsmouth, Hampshire, UK for this study. A typical spirillum bacterium was

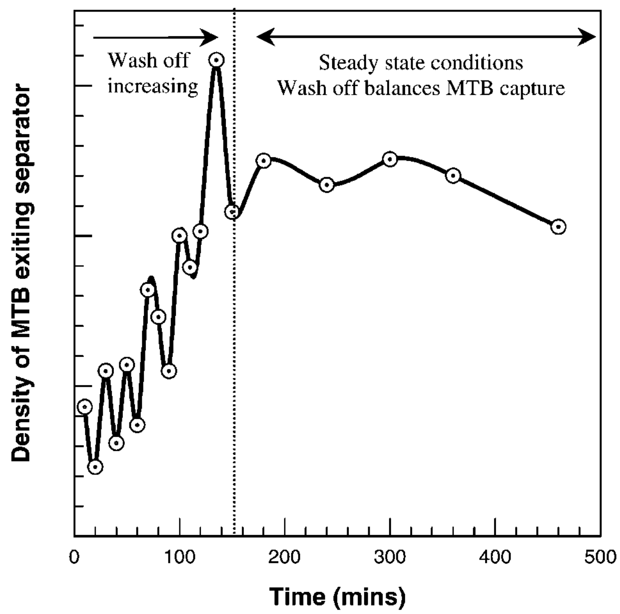

Figure 3. Variation in density of motile, magnetic field susceptible MTB exiting a channel separator with separator operation time. Balance between wash off and capture is reached after 150 min. The number of bacteria retained in the separator remained constant after this time.

found to be 4–6 μm long and 0.5 μm in width. Transmission electron microscopy showed that a chain of cubo-octahedral, magnetite magnetosomes was present along the line of length of the bacteria, typically consisting of 20 magnetosomes in a chain, each ~40 nm in diameter.

The various separators constructed for this study are shown in Fig. 4. Separator dimensions were 130 mm long, 30 mm wide, and 10 mm deep, and held 37 cm^3 of fluid. The end of the separators opposite to the entry flow were widened to 44 mm across, 60 mm along their length to provide the low flow, still area.

The ordered pin hybrid separator (Fig. 4d) consisted of a series of 250 μm diameter nickel pins mounted vertically along the sidewalls and down the center of the separator. When magnetized, each of these wires generated a magnetic gradient that resulted in a force on the bacteria as they approached the wire. Hence, at the proximity of the wire, high force-induced sites were generated for MTB accumulation. Furthermore, the central series of wires provided shorter pathways in the flow for MTB to reach the capture sites.

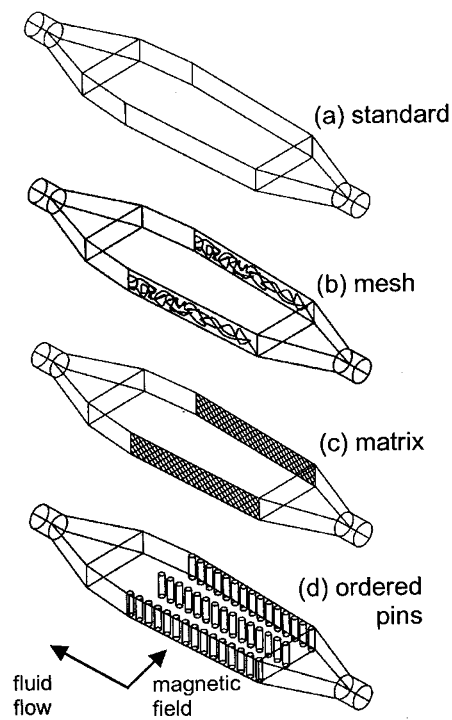

Figure 4. Schematic diagram showing the various channel separators used. (a) Standard, (b) random wire ($\phi = 8.3\,\mu$m), (c) matrix ($\phi = 50\,\mu$m), and (d) pin separators ($\phi = 250\,\mu$m).

Two additional hybrid separators were constructed with finer wires anchored to the sides of the separator, which produce higher magnetic gradients and larger retention forces. Figure 4c shows the matrix hybrid separator, in which 70 mm lengths of nickel matrix (wire diameter 50 μm) were anchored to the side walls of the separator. The random wire hybrid separator was constructed in a similar way but with a random distribution of 8.3 μm nickel wires anchored to the sidewalls (Fig. 4b).

Small-scale experiments were undertaken using 300 cm^3 volume of MTB cultures, which were allowed to flow through each of the separators under a range of applied magnetic fields (0.05, 0.09, and 0.13 T) and flow rates (130, 260, and 390 $cm^3\,hr^{-1}$). The cultures were produced from 10 days batch growth of MTB, incubated at 30°C using a standard growth medium[12] for MTB developed by Blakemore. The MTB were cultured in 200 cm^3 sealed, cylindrical containers with 10% headspace of air to enable magnetosome promoting, micro-aerobic conditions to establish within the culture vessel. To determine the performance of each separator, the initial density of motile, magnetic field susceptible MTB entering the separator was measured. Similarly, at the end of each experiment, the density of accumulated MTB within the separator was determined. Densities were assessed by counting the number of motile, field susceptible bacteria under an optical microscope coupled to an image processing system,[13] a typical density of bacteria is in the range 10^8–10^9 MTB cm^{-3}. The physical volume of the separator, 37 cm^3 (or 12% of the total volume treated), was taken into account when separator performance was evaluated. This volume is termed the "dead volume" representing the MTB fraction that would be present in the separator at the end of an experiment even if no capture had occurred.

RESULTS AND DISCUSSION

The separator performance was determined by measuring the relevant initial and final parameters of the experiments, taking into account the dead volume contribution as given by Eqs. (1) and (2):

$$\eta_t = \eta_m - (V_{dead}/V_{total})(100 - \eta_m) \quad (1)$$

$$\eta_m = 100 \times [(MTB_{initial\ density} - MTB_{final\ density})/MTB_{initial\ density}] \quad (2)$$

where η_m is the measured value for the separator efficiency (as a percentage, %), and η_t is the efficiency of the separator after the dead volume is taken into account (as a percentage, %), (V_{dead}/V_{total}) is the dead volume contribution, V_{total} is the total volume treated, and V_{dead} is the volume present in the separator before the start of the experiment. A fraction of the bacteria within the dead volume would have potentially been captured by the system. To account for this effect, a factor of $(100 - \eta_m)$ is introduced. The level of mechanical entrapment that occurred in each of the separators was assessed by applying a rotating magnetic field to the separator.[14] Such a field constrained the bacteria to swim through the separator in a helical path avoiding contact and capture on the walls. At all the flow rates investigated, mechanical entrapment for all separators was found to be approximately 5%.

The performance of the 50 μm matrix separator with respect to the flow rate and applied magnetic field is shown in Fig. 5. When comparing these results with the standard separator, it can be seen that the hybrid matrix separator yields a significant increase in performance. Under the highest magnetic field and flow rate combination, the matrix separator recovered 27% of the bacteria, compared to 11% for the standard separator under the same conditions. The highest percentage recovery in each of the separators was achieved at the lowest flow rate and highest applied magnetic field tested (130 cm^3 hr^{-1}, 0.13 T). In the case of the

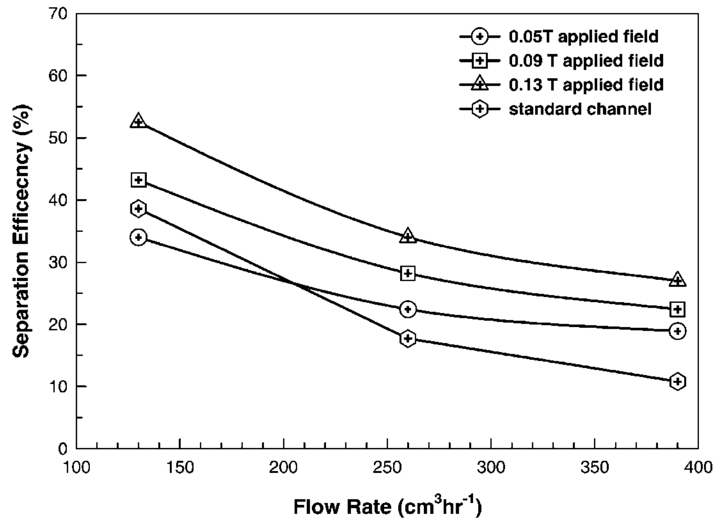

Figure 5. Separation efficiency of 50 μm wire matrix and standard separators as a function of flow rate.

matrix separator, this corresponded to the recovery of 53% of MTB compared to 39% using the standard separator.

There is a clear correlation between the applied magnetic field and level of recovery, indicating the role of magnetic gradients generated by the matrix, resulting in higher retention of the accumulated bacteria. This dependence can be highlighted by considering the magnetic force that retains a bacterium on a wire as described by:[15]

$$F_{\mathrm{Mag}} \propto (M_{\mathrm{s}}B/a) \times m_{\mathrm{MTB}}$$

where F_{Mag} is the retention force, M_{s} is the magnetization of the wire, B is the applied magnetic field, a is the wire radius, and m_{MTB} is the magnetic moment of the bacterium. The equation shows that the retention force is independent of the size of the bacterium but directly related to the magnetic moment resulting from the magnetosomes present within the bacterium.

To generate higher retention forces the 50 μm matrix was replaced by a random distribution of 8.3 μm wires. The performance of the separator with respect to the flow rate and applied magnetic field is shown in Fig. 6. The retention force,[3] F_{Mag}, generated by the wire mesh is approximately six times that of the matrix under the same conditions. This is illustrated in the results where the performance of the random wire separator exceeds that of the matrix under all test conditions. This is despite the fact that the matrix separator has a larger surface

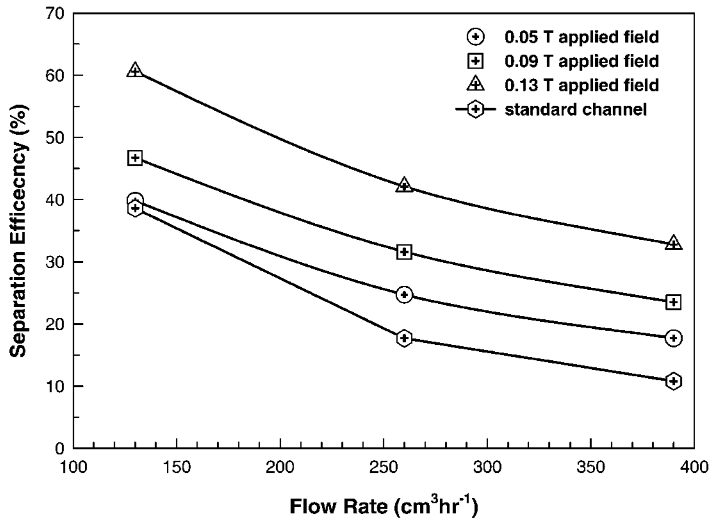

Figure 6. Separation efficiency of 8.3-μm random wire and standard separators as a function of flow rate.

area of wires and so potentially offers more sites for MTB accumulation. At the highest flow rate used ($390\,cm^3\,hr^{-1}$), the random wire separator achieved a three-fold increase in MTB recovery over the standard separator which does not use wires and so does not produce a retention force ($F_{Mag} = 0$). Separator efficiency is enhanced with increasing magnetic field intensity (Fig. 6) with the largest improvement in performance seen at the highest flow rate.

The ordered pin separator (Fig. 4d) was observed to have a better capture efficiency than the standard separator, particularly at the higher flow rates. However, the increase in efficiency was much smaller than that of the previously discussed hybrid separators. For a given flow rate, however, increasing the applied magnetic field caused no significant change in MTB recovery. This indicates that the benefit of the separator comes from the proximity of capturing sites coupled with the enlarged still area. The large diameter of the wire used in the separator resulted in very weak magnetic retention forces on the accumulated bacteria that did not contribute to retention of the biomass.

CONCLUSIONS

The ordered pin separator showed that improved separation can be achieved by providing closer magnetic sites where MTB can accumulate. The central row of pins in the separator effectively halved the width of the separator, reducing the distance MTB had to swim to reach a capture site, so increasing the probability of capture. Furthermore, this work has shown that a marked improvement in performance can be achieved by the use of magnetic gradients to retain the accumulated biomass. In particular, at the highest flow rate tested, separator performance was increased by a factor of three. In terms of "harvesting" motile, magnetic field susceptible MTB from a continuous culture system,[14] the use of a high flow rate using a random wire separator provides the best recovery conditions. For example, a 50% increase in the number of bacteria captured per unit time can be achieved by operating the random wire separator at a flow rate of $390\,cm^3\,hr^{-1}$ (33% recovery), compared with processing at $130\,cm^3\,hr^{-1}$ (61% recovery). This contrasts with the standard channel separator, where the maximum number of bacteria captured per unit time is achieved at the lowest flow rate.

In the hybrid OMS system discussed in this paper, the intensity of the magnetic field used was sufficiently low to enable the use of cheap, permanent magnets in a process environment. The high gradient enhancements could be harnessed in a cost effective manner without altering the fundamental simplicity of the OMS process.

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