Influence of iron valency on the magnetic susceptibility of a microbially produced iron sulphide.

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Abstract. Microbial iron sulphide is well known as an adsorbent for the treatment of metallic ion polluted wastewater. Under certain culture conditions a highly magnetic iron sulphide can be produced which would enable a low cost biomagnetic separation process to be developed. This paper illustrates that by raising the ferrous content of a ferrous – ferric sulphate rich medium the magnetic susceptibility of the iron sulphide produced is increased.

1. Introduction
Microbial biomass is frequently used for the bioremediation of heavy metal polluted waters as the biomass is capable of absorbing, adsorbing and in some cases precipitating the metals out of solution [1]. Sulphate reducing bacteria (SRB) are a genera of dissimilatory bacteria which utilise sulphate as a terminal electron acceptor and perform dissimilatory reduction of sulphate ions ($SO_4^{2-}$) to hydrogen sulphide ($H_2S$) gas [2]. The hydrogen sulphide produced by the SRB as part of their metabolic processes is capable of reacting with metal cations to form stable sulphide precipitates. In an iron rich environment the SRB form an iron sulphide, $Fe_xS_y$ (where for example $x=1$, $y=1$ in the case of iron (II) sulphide), which, under certain bacterial growth conditions can be magnetic. The magnetic $Fe_xS_y$ forms an extracellular coating on the bacteria, which is a good adsorbent due to its exceptionally high surface area to volume ratio [3].

The adsorption capacity of microbially produced $Fe_xS_y$ has been extensively researched as an alternative for heavy metal recovery and has been actively implemented and tested on common wastewater cations and industrial acid mine drainage [1,4]. Additional work on its suitability for biomagnetic separation has indicated that the bacteria are an ideal candidate [5]. It is however necessary to produce a highly magnetic $Fe_xS_y$ in order to achieve efficient, low cost magnetic separation recovery. Ideally, the magnetic properties of the sulphide would be such that separators based on permanent magnets could be used.

2. The production of a magnetic iron sulphide
2.1. Bacteria source
Salt marsh sediment containing a mixed culture of non magnetic SRB was obtained from a previously used sampling location in Hampshire, UK [6]. The sediment was collected from within the top 15cm of the marsh sediment and used to inoculate a 2L culture vessel containing a modified Freke and Tate
Postgate C growth medium [2] under non sterile conditions at 30°C. The medium is as follows: (per L with distilled water), 3.22g FeSO\(_4\), 7H\(_2\)O, 0.58g Fe\(_2\)(SO\(_4\))\(_3\).2H\(_2\)O, 0.07g (NH\(_4\))\(_2\)SO\(_4\), 0.5g KH\(_2\)PO\(_4\), 4.5g Na\(_2\)SO\(_4\) (anhydrous), 0.06g CaCl\(_2\).2H\(_2\)O, 0.06g MgSO\(_4\).7H\(_2\)O, 5ml sodium lactate @70% w/v and adjusted to approximately pH 6.7 using sodium hydroxide.

200 ml of salt marsh sediment was added to 1300 ml of culture medium and the culture vessel was mechanically stirred for 5 minutes. After 3 days of batch culture the presence of black iron sulphide particles could be seen on the inner walls of the culture vessel. By day 14 the culture medium was completely black with an odour of hydrogen sulphide detectable in the headspace of the culture vessel.

The magnetic susceptibility of the iron sulphide produced by day 14 was measured at 4x10\(^{-4}\)SI units (dry weight equiv.) using an automatic magnetic susceptibility balance [8]. To measure the dry weight equivalent magnetic susceptibility of the sulphide produced the following steps were undertaken: (i) 3 x 20 ml samples were extracted from the culture vessel and centrifuged at 2200 rpm for 15 mins. (ii) The supernatant was decanted and the recovered sludge measured for magnetic susceptibility, \(\chi\) by volume. (iii) The samples were then anaerobically dried to determine the moisture content to enable the calculation of the dry weight equivalent magnetic susceptibility.

2.2. Producing a highly magnetic iron sulphide

The bacteria were continuously cultured in the culture vessel (chemostat) at a dilution rate of 0.028hr\(^{-1}\). A 2T electromagnet with a tapered magnetic pole was used to generate a magnetic gradient across a 250 ml measuring cylinder filled with iron sulphide concentrate collected from the culture vessel. The magnetic fraction, accumulated at the magnetic pole was pipetted and then used as an inoculum for a new culture vessel.

An 11day (264hr) switched culture cycle was introduced to help refine the magnetic culture. The mixed culture cycle consisted of alternating batch-continuous phases, wherein the chemostats were operated in batch for 5days (120hr) and then run in continuous culture for 6 days (144hr) using the modified growth medium at a dilution rate of 0.028hr\(^{-1}\). The magnetic susceptibility was observed to reach a maximum during the batch phase of the 11 day culture cycle, with a typical value of 80x10\(^{-4}\)SI units [9]. An additional culture reactor was operated in continuous mode only, at a dilution rate of 0.015hr\(^{-1}\). In this case no increase in magnetic susceptibility was observed.

Magnetic saturations curves were determined for a selection of samples collected at various stages in the culture cycle. These measurements were conducted using a vibrating sample magnetometer, operated using a direct applied field between 0 and 1 Tesla.

2.3. Optimising the highly magnetic iron sulphide

The described 11 day mixed culture cycle was employed with uniform culture conditions across three bioreactors. The original modified growth medium, an iron rich mix (650ppm Fe\(^{2+}\): 150ppm Fe\(^{3+}\)) was modified by varying the relative concentrations of Fe\(^{2+}\):Fe\(^{3+}\) and introduced to three bioreactors over a period of three complete eleven day culture cycles (test period duration of 33days).

The three bioreactors were run as follows:

1) 650ppm Fe\(^{2+}\): 150ppm Fe\(^{3+}\) - as per Freke and Tate and other authors, the ‘control’
2) 500ppm Fe\(^{2+}\): 300ppm Fe\(^{3+}\)
3) 750ppm Fe\(^{2+}\): 50ppm Fe\(^{3+}\)

3. Results and Discussion

A comparison of the magnetic susceptibility with respect to the reference concentration (650:150 ppm, Fe\(^{2+}\): Fe\(^{3+}\)) was conducted over the culture period duration of 33days. The average of 3 daily magnetic susceptibility readings from each bioreactor is shown in Figure 1. Prior to day 0 (annotated ‘A’), the official start of the test period, the three bioreactors were all operated in continuous culture mode using the reference concentration (650:150 ppm, Fe\(^{2+}\): Fe\(^{3+}\)). Less than 5% variance in the magnetic susceptibility of the sulphide produced was recorded during this time which is indicative of the similarity of the bioreactors. Over the course of the test period the magnetic susceptibility in all three
bioreactors was observed to follow a cyclic pattern; it gradually increased during the batch phase with the peak magnetic susceptibility being measured near the end of the batch phase, day 5 (labelled B₁, B₂, C₁ and D₁). When switched back to the continuous phase the magnetic susceptibility was observed to gradually decrease with time. No significant changes in magnetic susceptibility were observed until the start of the second 11 day cycle. Magnetic susceptibility peaks are therefore quoted as averages of the second and third cycles.

Figure 1. Magnetic susceptibility of microbial iron sulphide produced from three bioreactors fed with different Fe²⁺: Fe³⁺ ratios over three, eleven day mixed batch-continuous culture cycles. Peak magnetic susceptibilities are observed during the batch phase (hatched area) of the culture cycle.

Figure 2. Saturation magnetisation curve of a dried FeₓSᵧ sample taken during the continuous phase of the eleven day mixed culture cycle.

Figure 3. Saturation magnetisation curve of a dried FeₓSᵧ sample taken during the batch phase of the eleven day mixed culture cycle.

The 500ppm: 300ppm (Fe²⁺:Fe³⁺) bioreactor produced the least magnetic material with an average peak susceptibility of 7x10⁻⁴ SI units (average of C₃ and D₃). The ‘control’ bioreactor using the modified growth medium 650ppm: 150ppm recorded an averaged peak susceptibility of 28x10⁻⁴ SI units (average of C₂ and D₂), 4 times more magnetic than 500ppm: 300ppm bioreactor. The 750ppm:
50ppm bioreactor recorded the highest average peak susceptibility, $58 \times 10^{-4}$ SI units (average of C1 and D1), approximately double that of the reference bioreactor.

The peak magnetic susceptibility achieved using the modified culture medium 750ppm: 50ppm (Fe$^{2+}$:Fe$^{3+}$) is similar to that quoted in previous work [9], produced using the control Fe$^{2+}$:Fe$^{3+}$ ratio of 650:150. The bioreactor is a non-sterile, mixed culture system and so the bacterial population (suite of bacteria types and relative concentrations) is continually changing. It is therefore only really possible to make comparative assessments as to the optimum Fe$^{2+}$:Fe$^{3+}$ concentration when the same bacteria population is used initially. The conclusion that can be made on this basis is that the modified culture medium 750ppm: 50ppm (Fe$^{2+}$:Fe$^{3+}$) would be expected to produce a higher magnetic susceptibility Fe$_x$S$_y$ product for the majority of sulphate reducing bacteria culture distributions.

Saturation magnetisation curves performed on samples taken randomly from the reference bioreactor (650ppm:150ppm) during the initial production of a highly magnetic Fe$_x$S$_y$, at each phase of the culture cycle confirmed that the magnetic susceptibility of the material produced near the peak is more than 4 times higher than that quoted previously [10]. Figure 2 and 3 show the magnetic susceptibility of samples taken during the batch and continuous phase of the mixed culture cycle.

Above 0.2T the magnetic response of the batch phase Fe$_x$S$_y$ is linear with the applied field (saturation magnetisation). The continuous phase produced Fe$_x$S$_y$ has a different magnetisation curve requiring a 0.3T applied field to reach the linear response. This clearly indicates a different Fe$_x$S$_y$ structure material has been produced. The magnetic susceptibility balance used for the lab magnetic susceptibility measurement operated at 0.4T applied field, with the saturation magnetisation region of both the batch and continuous phase produced Fe$_x$S$_y$.

4. Conclusion

The study shows that iron valency does influence the magnetic susceptibility of the Fe$_x$S$_y$ produced. A high ratio of Fe$^{2+}$:Fe$^{3+}$ (750ppm: 50ppm) produces a highly magnetic sulphide which is approximately twice as magnetic as its control reference inoculating culture (650ppm: 150ppm). There is scope for further refinement of the Fe$^{2+}$:Fe$^{3+}$ ratio to yield even higher susceptibility Fe$_x$S$_y$ product.

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5. References

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