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Development of a highly magnetic iron sulphide for metal uptake and magnetic separation

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Abstract

Microbial iron sulphide (FeS) is a well-known absorbent for heavy metals which has the potential to be used in biomagnetic separation. This paper illustrates that highly magnetic FeS can be produced from bioreactors which are continually switched between batch and continuous culture modes. Cadmium metal uptake studies highlight the sulphide absorbent properties of the FeS produced.

Keywords: Magnetic separation; Sulphate-reducing bacteria; Bacteria; FeS; Biomagnetic separation; Absorption of heavy metals; Cadmium uptake; Bioreactor

1. Introduction

Heavy metals occur naturally within the environment, and, at low concentrations, are utilised by microbes for metabolic processes [1]. Increased anthropogenic usage of these metals has consequently led to an influx into the environment which cannot be adequately sequestered by natural biosorption processes. As a result, improved methods of metal recovery are of paramount importance within the industries that contribute substantially to their release.

The ability of bacteria to act as an efficient heavy metal absorbent through the formation of insoluble metal sulphides has been long established [2,3]. For example, Watson and Ellwood [4] utilised laboratory-cultured sulphate-reducing bacteria (SRB) on mercury-contaminated effluent. The bacteria were successfully able to reduce the mercury concentration to less than a quarter of its original concentration on a single application. The potential of SRB to produce highly magnetic sulphides was discovered by accident by Freke and Tate in 1961 [5] while investigating the iron adsorption properties of the *Desulphovibrio* spp. bacteria. The Freke and Tate study used a non-sterile, continuous culture of a mixed population of naturally occurring strains of SRB. The culture rig was switched to batch mode on weekends and it was this switching that was observed to result in the production of a highly magnetic sulphide. The significance of the highly

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magnetic properties of the iron sulphide was not fully appreciated at that time as the work predated the development of magnetic separation. Watson, Bahaj and co-workers studied biomagnetic separation, the magnetic separation of biological carriers of pollutants (normally bacteria), that were either inherently magnetic or "seeded" to produce a magnetic biosorbent [6]. Studies of SRB using both microscopic (single wire cell) [7,8] and prototype separator sizes (e.g. vortex separation using a superconductor magnet) [9,10] showed that the bacteria were ideally suited for biomagnetic separation.

To maximise the separation efficiency, and hence minimise process cost, it is important to produce as highly magnetic a biosorbent as possible [11]. This paper revisits the original Freke and Tate work to try and understand the growth conditions required to achieve a highly magnetic iron sulphide. Foremost among the growth culture parameters considered, is the requirement to switch between batch and continuous culture conditions within the bioreactors to produce a magnetic product.

2. Experimental

2.1. Culture of magnetic biosorbent

A mixed culture of SRB was obtained from a tidal salt marsh in Hampshire, UK. Sediment was collected from between 5 and 15 cm below the surface of a large salt marsh pan. For the purposes of generating a magnetic culture, 1000 ml of a standard Postgate medium C [12] was modified with the aid of the Freke and Tate growth medium [5] (in gl^{-1}), 3.22 $FeSO_4 \cdot 7H_2O$, 0.58 $Fe_2(SO_4)_3 \cdot 2H_2O$, 0.07 $(NH_4)_2SO_4$, 0.5 KH_2PO_4, 4.5 Na_2SO_4 (anhydrous), 0.06 $CaCl_2 \cdot 2H_2O$, 0.06 $MgSO_4 \cdot 7H_2O$, 5 ml sodium lactate @ 70% w/v and adjusted to approximated pH 6.7 using sodium hydroxide. This modified Postgate C medium was then inoculated with 200 ml of unfiltered sediment from the tidal salt marsh pan in a 1.5 l bioreactor. The bioreactor was maintained at 300 °C with periodic gentle mixing until the bioreactor became opaque (14 days) following the generation of iron sulphide. There was no attempt to create sterile growth conditions or to maintain an oxygen-free air space in the bioreactor. The bioreactor was then switched to a continuous mode with an initial dilution rate of 0.028 reactor volumes per hour (i.e. 1.5 l of fresh medium is supplied to the 1.5 l bioreactor every 36 h).

Following an initial 4 weeks of continuous culture, a simple test using a permanent magnet showed that only a fraction of the iron sulphide produced by the continuous culture mode bioreactor was magnetic. The magnetic fraction of the microbial iron sulphide produced was recovered using an open gradient magnetic separator. Five hundred millilitre of concentrated iron sludge was placed in a measuring cylinder adjacent to the magnetic pole of a 2 T Helmholtz coil electromagnet, of which only one coil was energised. The magnetic fraction accumulated adjacent to the pole was recovered using a pipette. This recovered magnetic fraction was then used as a seed for a second, 'magnetic seed' bioreactor which was allowed to sit in batch for 14 days, prior to switching to continuous culture mode. The original and magnetic seed bioreactors were then run in parallel for a period of 6 months. The culture in both bioreactors remained stable, with the magnetic seed bioreactor maintaining the production of a more magnetic iron sulphide.

The contents of the magnetic seed bioreactor were then split in half to create two identical bioreactors running in parallel. The study compared the magnetic product of these two bioreactors running in parallel but under different growth conditions. The first bioreactor, termed 'continuous control' (CC), remained in continuous culture mode throughout the tests at a dilution rate of 0.028. The second bioreactor, termed 'switch batch-continuous' (SBC), was subjected to a 5 day batch: 6 day continuous growth sequence. During the 6 day continuous growth sequence, the dilution rate was identical to that of the CC-bioreactor, leading to an averaged dilution rate over an 11 day cycle of 0.015. The dilution rate of the CC-bioreactor was reduced to this level for the last 2 weeks of the test period (days 40–59 of the test period).

2.2. Magnetic susceptibility measurement of biosorbent

The magnetic susceptibility of the absorbent was measured as that of a wet sludge. 4×20 ml samples were extracted from each bioreactor on a daily basis and centrifuged at 2200 rpm for 15 min. The supernatant was decanted and the sludge extracted from three of the samples and used to measure, in triplicate, the magnetic susceptibility by volume, χ, of the biologically produced iron sulphide using an MSB-Auto (Sherwood Scientific Ltd). The fourth sample was stored at 4 °C as a future reference. The three samples were then dried to determine the moisture content to enable the calculation of the dry weight 'equivalent' magnetic susceptibility. To measure the dry weight susceptibility of the microbial iron sulphide using an MSB-Auto requires the drying of the wet sludge collected from approximately 1 l of the bioreactor product. This represents two-thirds of the reactor volume and would therefore necessitate sample collection over a 24 h period. Such a sample would not be representative of a point in time in the culture cycle and so could not be compared with the wet sludge measurements.

2.3. Microbial adsorption

The heavy metal adsorption properties of the biologically produced sulphide were assessed using a metal ion (cadmium) which is known to produce an insoluble sulphide. Batch adsorption experiments were conducted in 100 ml vials sealed with butyl rubber stoppers (Sigma/Supelco) in a temperature-controlled room (25 °C). Twenty millilitre of sample absorbent was extracted from each bioreactor and centrifuged for 15 min at 2200 rpm. The supernatant was decanted and the residue sludge (0.35 g≡0.018 g dry weight equivalent) was added to 100 ml of the cadmium salt [$Cd(NO_3)_2 \cdot 4H_2O$] prepared at various concentrations (500, 150 and 50 ppm). Control tests showed no evidence of cadmium absorption onto the test vial glassware.

In order to optimise adsorption by ensuring good contact between the biological iron sulphide and the metal solution, the samples were maintained in a constant state of suspension by agitating the samples on roller beds (Stuart Scientific Roller/Mixer SRT2). Sampling was conducted at regular intervals for a period of 90 min. The reaction flask was allowed to stand prior to sampling to allow time for a clear supernatant to develop. Fifty microlitre aliquot samples of the supernatant were taken and diluted in 4.95 ml of Milli-Q (Millipore Ltd) water and the soluble cadmium concentration in the supernatant was measured using a Varian AA200 atomic absorption spectrometer.

3. Results and discussion

3.1. Effect of culture conditions on the magnetic susceptibility of microbial iron sulphide

Prior to commencement of the magnetic susceptibility culture tests, both bioreactors were run in continuous mode. The magnetic susceptibility of microbial iron sulphide from both bioreactors was identical during this period with a typical value of 3.5×10^{-4} SI units. This is highlighted in Fig. 1 as the time period before day '0' on the x-axis. At day-0 (highlighted as 'A' in Fig. 1), the SBC-bioreactor was run in the alternate batch continuous mode described below:

Days 0−4 batch mode,

Days 5−10 continuous mode (dilution rate of 0.028).

The magnetic susceptibility of the microbial iron sulphide from both bioreactors was measured on a daily basis. The magnetic susceptibility of the CC-bioreactor remained consistent between 3×10^{-4} and 6×10^{-4} over the 40 days of the test when the bioreactor was at a dilution rate of 0.028 (between 'A' and 'C' in Fig. 1). During this period the SBC-bioreactor underwent three of the 11 day period culture cycles. The magnetic susceptibility was observed to rise when the culture was switched from continuous to batch mode. The peak magnetic susceptibility occurred between 3 and 4 days of batch culture (labelled B_1, B_2, B_3 and B_4 in Fig. 1). When the system was returned to continuous mode, a lower magnetic susceptibility

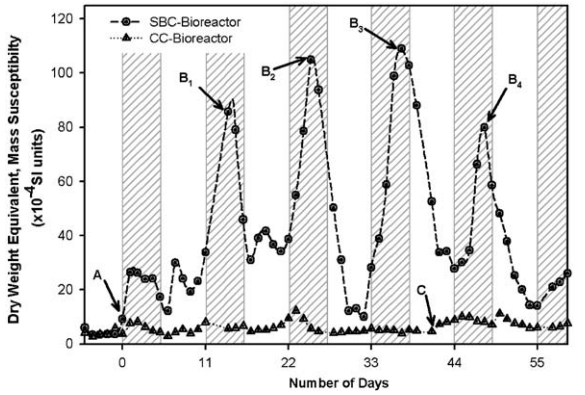

Fig. 1. Magnetic susceptibility of FeS biosorbent produced by the continuous control (CC) and switch batch continuous (SBC) bioreactors: 'A' initial switch to batch of SBC bioreactor, B_{1-4} peak susceptibility during batch culture phase, 'C' reduced dilution rate (0.015) applied to CC bioreactor.

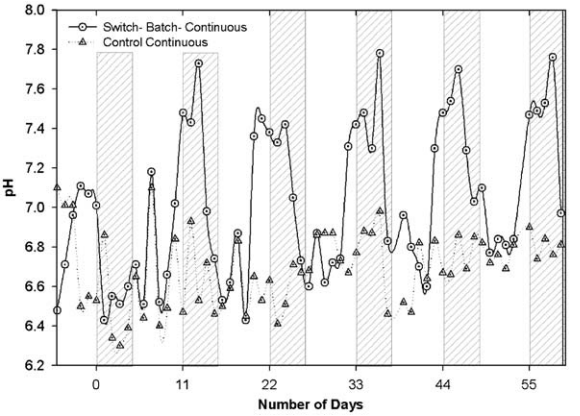

Fig. 2. Variation in bioreactor pH of both the switch batch continuous (SBC) and continuous control (CC) reactors over the 65 day test period.

iron sulphide was produced. Throughout the test period, the magnetic susceptibility of the SBC-bioreactor was consistently higher than that of the CC-bioreactor.

After 40 days ('C' in Fig. 1), the dilution rate of the CC-bioreactor was reduced to 0.015. Under this condition, the volume throughput of both reactors over an 11 day period was the same. No change in the magnetic susceptibility of the CC-bioreactor was observed over the subsequent 18 days.

In the time period between A and C, the CC-bioreactor produced 40 l of biosorbent with an average magnetic susceptibility of 5×10^{-4} SI units. In contrast, the SBC-bioreactor produced 20 l of biosorbent with an average susceptibility of 40×40^{-4} SI units, an order of magnitude higher than that quoted in previous studies [4,10,13].

The pH measurement profile for the duration of the experiment (Fig. 2) closely follows that of the magnetic susceptibility plots. The periods of highest pH (>7) for the SBC-bioreactor coincide with the batch phase of the culture period. This correlation is probably as a result of the bacteria altering the internal conditions of the reactor as a result of their metabolic processes. The continuous influx of pH-regulated medium to the CC-bioreactor prohibited great fluctuations of the culture conditions.

3.2. Cadmium metal adsorption

The relationship between absorbance and contact time was measured over a 2 h period for a range of cadmium concentrations (50, 150 and 500 ppm). The absorption profiles showed a similar trend of rapid initial uptake followed by a gradual slowing to a saturation level regardless of the initial concentration tested (Fig. 3). The percentage of Cd absorbed per gram dry weight equivalent of wet absorbent over a 90 min period with respect to the initial concentrations of 50, 150 and 500 ppm is shown. The uptake of Cd by the high magnetic bioreactor (SBC) and control reactor (CC) is also shown. At all three concentrations studied, the less magnetic biosorbent showed a higher metal uptake. At 500 ppm, a 20% reduction of Cd in solution is achieved. This corresponds to 0.56 g of Cd absorbed per gram dry weight equivalent of microbial iron sulphide. At 150 ppm initial Cd concentration, a 30% reduction is achieved (0.26 g Cd/g dry weight equivalent absorbent), and at 50 ppm a 75% reduction (0.21 g Cd/g dry weight equivalent absorbent) is achieved. The uptake of Cd per unit mass of absorbent is less at a lower concentration; this indicates that not all the absorbance sites have been occupied at these concentrations, i.e. the limiting factor for uptake is the solution concentration establishing equilibrium and not the availability of absorbance sites.

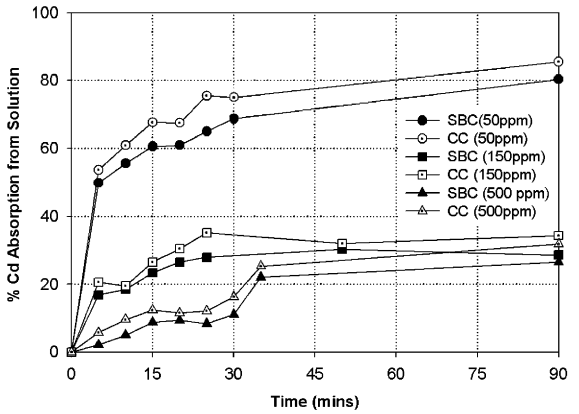

Fig. 3. Percent adsorption of cadmium from solution by wet microbial FeS at 50, 150 and 500 ppm. Curves for both switch batch continuous (SBC) and continuous control (CC) biosorbents are shown. Concentration of dry weight equivalent of absorbent used was 0.18 g/l in all tests.

4. Conclusion

The results of the culturing experiments show that it is the active switching of the bioreactor growth conditions from continuous to batch mode that provides the appropriate conditions for the production of a highly magnetic microbial iron sulphide. This process generates a magnetic sulphide which is on an average 8 times more magnetic than that produced under reduced dilution rate conditions. The most magnetic material was produced during the batch growth phase when the internal conditions of the culture vessel were altered as a result of the bacterial metabolic processes.

The batch adsorption experiments have shown that the biological sulphides produced can successfully remove Cd metal from solution (50–500 ppm range). Cadmium absorption is a strong indicator of the likelihood of absorption by the iron sulphide of a wider range of metals which form insoluble sulphides such as Ag, Pb, Hg, Zn and Sn. The less magnetic iron sulphide produced by the CC-bioreactor was found to have higher absorbance than the SBC iron sulphide. However, this difference was typically less than 10% and so is insignificant compared to the difference in magnetic susceptibility between the two iron sulphides (~8 times). From a metal uptake and magnetic separation perspective, the highly magnetic sulphide produced using the SBC-bioreactor offers the potential for low-cost processing of waste streams. Separation should be achievable using permanent magnets resulting in low process cost when compared to superconducting separators. This work is currently investigating the possibility of maximising the magnetic susceptibility of the iron sulphide produced through optimisation of the relative length of the batch and continuous phases of the SBC-bioreactor.

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